# Nociceptin/Orphanin FQ Peptide Receptor as a Therapeutic Target for Obesity

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**Abstract:** Nociceptin/orphanin FQ (N/OFQ) peptide (NOP) receptor is a candidate target for novel therapeutics for a range of conditions, including obesity. This article reviews recent advances in understanding of N/OFQ's involvement in the regulation of food intake and appraises current developments in the rational design of antagonists aimed at the NOP receptor.

**Key Words:** Nociceptin/orphanin FQ, N/OFQ, nociceptin/orphanin FQ peptide (NOP) receptor, opioid receptor like-1 (ORL-1), opioid receptor-4 (OP4), kappa-type 3 opioid receptor (KOR-3), rat opioid receptor-C (ROR-C), obesity, food intake.

## INTRODUCTION

The increasing prevalence of obesity has exacted a toll on health and given rise to growing national economic pressures. According to the most recent National Health and Nutrition Examination Survey, approximately one-third of the United States adult population is obese, with a Body Mass Index > 30 (BMI = body weight (kg)/height ($m^2$)), and between 13 to 17% of children and teenagers measure above their ideal weight [1]. This trend of increasing obesity and its associated morbidity (hypertension, diabetes, heart disease and cancers, among others) is related to 120,000 excess deaths annualy [2].

Currently available therapies for obesity encompass lifestyle modification (through dietary manipulation, increased physical activity or altering behavior patterns relating to food and exercise), pharmacotherapy and surgical interventions. Unfortunately, dietary interventions are often associated with limited sustained weight loss, averaging a modest 2.1-6.6% reduction of initial body weight over a 4-5 year period [3]. The relative inefficacy of dietary interventions alone in achieving necessary long term weight loss is likely attributable to the complex interplay of behavioral, socio-cultural and genetic factors in the pathogenesis of obesity.

Common prescription-based obesity treatments may be categorized into those with peripheral actions, such as the intestinal lipase inhibitor orlistat (Xenical®, Roche), and those that influence brain neurochemistry. Compounds affecting brain neurotransmitters include monoamine and catecholamine modulators such as sibutramine (Meridia®, Abbot Laboratories) and sympathomimetic amines such as phentermine (Ionamin®, GlaxoSmithKline), mazindol (Sanorex®, Novartis), and clobenzorex (Rexigen® or Asenlix®, Aventis), Fig. (**1**).

Other compounds considered for the treatment of obesity are those which have been reported to reduce body weight. Examples of such compounds are the antidepressant sertraline (Zoloft®, Pfizer), the drug and alcohol dependence medication naltrexone (Revia®, Bristol-Myers Squibb and Contrave®, Orexigen (in combination with bupropion or Wellbutrin®)), and the glucose sensitizing medication metformin (Glucophage®, Bristol-Myers Squibb), Fig. (**2**) [4]. The US Food and Drug Administration Advisory Panel recently vetoed a recommendation for approval of the cannabinoid receptor antagonist rimonabant (Acomplia®, Sanofi-Aventis) for obesity treatment due to insufficient safety data, a compound approved for marketing for obesity by the European Medicines Agency (Fig. (**2**)) [5].

The clinical contraindications to the use of some of these approved and potential prescription-based treatments for weight loss include kidney, liver, heart disease, glaucoma or gallstone diseases which, collectively, preclude their prescription to many obese patients. Thus, there remains a pressing clinical need for novel pharmacotherapeutics with either greater efficacy or different safety profiles.

Understanding the neuronal mechanisms that regulate feeding may accelerate the development of more effective therapies with novel mechanisms of action. The cloning of the nociceptin/orphanin FQ peptide (NOP) receptor (also known as Opioid Receptor Like-1 (ORL-1); Opioid Receptor-4 (OP4); Kappa-type 3 opioid receptor (KOR-3); or Rat Opioid Receptor-C (ROR-C)) and its endogenous agonist N/OFQ, a 17-amino acid peptide, has provided researchers with a new drug discovery target [6-9]. NOP receptors are predominately located pre-synaptically, where they inhibit the release of various neurotransmitters, such as serotonin [10]. NOP receptors belong to the rhodopsin-like, 7-transmembrane (TM) domain, class A, G-protein coupled receptor (GPCR) superfamily consisting of ~1,000 proteins. To date, the high sequence homology of the NOP receptor with opioid receptors has focused the attention of the drug discovery field on the development of NOP receptor antagonists as putative anti-nociceptive agents. Much of the structural conservation of NOP receptor to opioid receptors is in the intracellular loops: all have similar lengths and patterns of hydrophobic/hydrophilic residues. However, despite considerable sequence identity in the TM domain (~61%) and extensive homology at the receptor and the ligand level,

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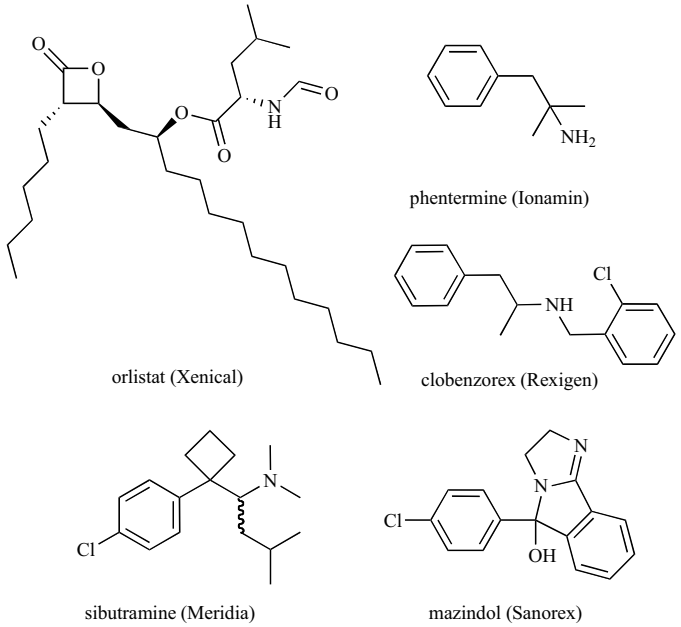


**Fig. (1).** Chemical structures of prescription-based obesity treatments.

these receptors lack corresponding pharmacological homology [11, 12].

## INVOLVEMENT OF N/OFQ IN FOOD INTAKE

### Central Distribution of NOP Receptor and N/OFQ (Fig. (3))

High levels of NOP receptor and N/OFQ expression throughout the brain suggest potential modulatory roles in physiological and behavioral processes such as ingestive behavior, locomotion, cognition, mood-related behaviors, cardiovascular regulation and nociception, to name but a few [7, 13-16]. Of particular interest is NOP receptor expression in regions implicated in food intake regulation, such as the paraventricular nucleus of the hypothalamus (PVH), arcuate nucleus of the hypothalamus (ARC), ventromedial hypothalamus (VMH), dorsomedial hypothalamic nucleus (DMH), lateral hypothalamic area (LHA) and dorsal raphe nucleus (DRN) [13, 17]. N/OFQ binding sites have also been reported to be widely distributed throughout the rat brain, with high to moderate binding in the VMH and DRN [18]. Studies assessing the localization of N/OFQ protein and mRNA also report high to moderate levels of expression in energy balance-related regions, such as the ARC, DMH and DRN [14]. This anatomical distribution of NOP receptor and N/OFQ lends circumstantial support to a role for this neuropeptide in the CNS regulation of food intake.

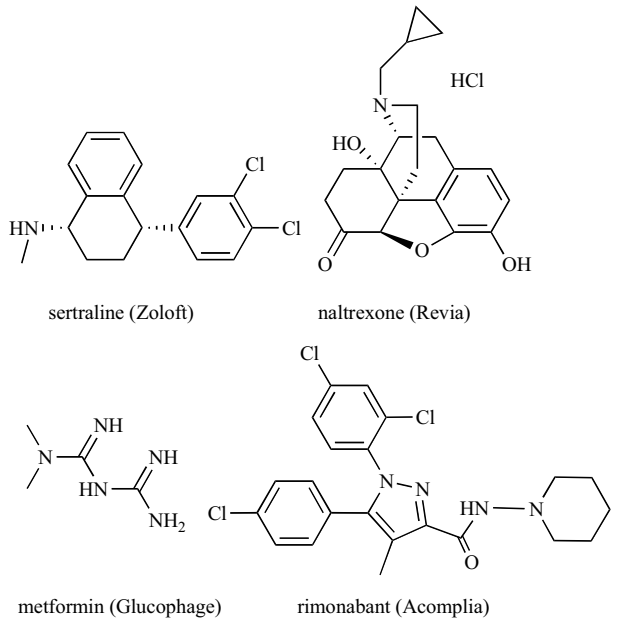


**Fig. (2).** Chemical structures of compounds reportedly associated with weight loss.

### Nutritional Regulation of Endogenous NOP Receptor and pro-N/OFQ

Consistent with the neuroanatomical localization of NOP receptors and N/OFQ in brain regions associated with energy balance, this pathway has been demonstrated to be nutritionally regulated. Specifically, under conditions of food deprivation/hunger, the NOP receptor is bilaterally and significantly down-regulated in the PVH and central nucleus of the amygdala (CeA), but not in the ARC or VMH, and only partially in the LHA of the rat [19]. The N/OFQ precursor pro-N/OFQ is down-regulated in response to a 16 hour fast in the amygdala, but not hypothalamus, of the rat [19]. These findings provide support that endogenous N/OFQ pathways play a physiological role in ingestive behavior.

### Administration of Exogenous N/OFQ and NOP Receptor Compounds

Central administration of exogenous N/OFQ consistently stimulates ingestive behavior in freely-feeding rats and

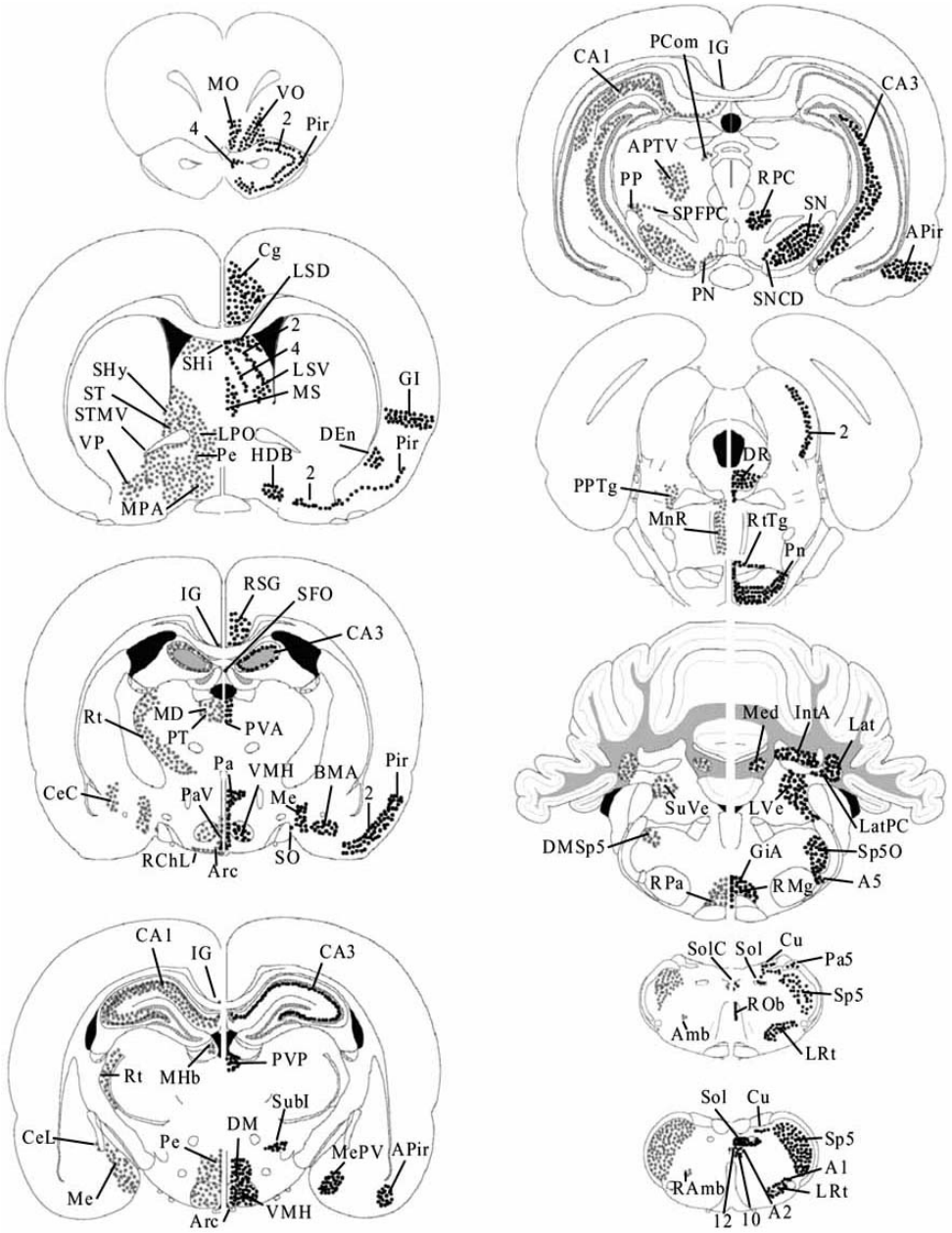


**Fig. (3).** Relative distribution of N/OFQ immunoreactivity (left hemisphere, gray stars indicate regions of high to moderate expression) and NOP receptor mRNA (right hemisphere, filled circles indicate regions of high to moderate expression) in rostral to caudal representative sections of the rat brain [13, 14]. Schematics of coronal brain sections displayed have been adapted from *The Rat Brain in Stereotaxic Coordinates* (5th Ed) by Paxinos and Watson [167]. Abbreviations are defined in list below.

chicks [19-27]. The orexigenic effect of N/OFQ may be prevented by pretreatment with NOP receptor antagonists [e.g. 24, 27]. Fragment N/OFQ(1-13)-$NH_2$, which has also been demonstrated to act as NOP receptor agonist in the amide form in other paradigms [28-30], likewise promotes food intake, whereas shorter sequences, such as N/OFQ(1-12)-$NH_2$ and N/OFQ(1-9)-$NH_2$, do not affect feeding [27].

The precursor peptide of N/OFQ, pro-N/OFQ, produces another cleavage product called nocistatin. Nocistatin acts as a functional antagonist of N/OFQ in multiple experimental settings, although its receptor and transduction pathways have not yet been fully clarified [31-33]. Nocistatin both attenuates N/OFQ-induced feeding and reduces deprivation-induced food intake [34]. Specific NOP receptor antagonists produce a similar profile. For example, the NOP receptor antagonists UFP-101 ([Nphe$^1$,Arg$^{14}$,Lys$^{15}$]-N/OFQ-$NH_2$) and NC-797 ((1R,2S)-**N**-amidino-2-[2-(4-chlorobenzoylamino)-6-methoxyquinazolin-4-yl]-aminocyclohexylamine dihydrochloride) prevent N/OFQ-induced orexigenic effects [35, 36]. Central treatment with antisense oligodeoxynucleotides directed against the NOP receptor also reduces N/OFQ-induced hyperphagia [23]. Likewise, N/OFQ was ineffective in stimulating feeding in NOP receptor null mice [24]. The

NOP receptor antagonist [Nphe$^1$]-N/OFQ(1-13)-$NH_2$ also inhibited deprivation-induced food intake [27]. Together, these data illustrate a consistent reduction in N/OFQ-induced feeding produced by pharmacological or genetic NOP receptor inactivation.

### Interaction with Energy Balance Pathways

It has been proposed that the endogenous NOP receptor agonist, N/OFQ, prolongs meal duration by inhibiting central pathways involved in meal termination [37, 38]. Indeed, N/OFQ or N/OFQ synthetic ligands have been reported to affect the activity of neurons within regions associated with satiety such as the PVH [39, 40], the ARC [37] and the DRN [41, 42]. Moreover, in conditions of hunger, expression of the $G_i$-coupled NOP receptor is reduced in the PVH and CeA, and partially in the LHA [19]. The inhibitory action of N/OFQ at a cellular level *via* the NOP receptor, the distribution of this orexigenic gene in brain satiety centers and the reduction of N/OFQ and NOP receptor expression in response to food deprivation, together provide circumstantial support for the hypothesis that N/OFQ stimulates feeding by inhibiting satiety pathways.

The PVH is a well-established brain region regulating energy balance as evidenced more than 30 years ago by the hyperphagia and obesity produced by PVH lesions [43]. While N/OFQ may act in this brain region to affect ingestive behavior, infusion of N/OFQ directly into the PVH does not significantly affect food intake [20]. However, ICV infusion of the pseudopeptide [Phe$^1\Psi$($CH_2$-NH)Gly$^2$]-N/OFQ(1-13)-$NH_2$ increases *c-fos* immunoreactivity (a marker of neuronal activation) in the PVH [39]. The neuropeptide oxytocin (OT) is expressed in the PVH and is involved in promoting satiety-induced cessation of food intake [44]. Olszewski *et al.* propose that N/OFQ may inhibit this OT satiety-associated pathway to affect feeding behavior [38].

The ARC is another brain region critically linked to energy balance rich in N/OFQ fibers and NOP receptors [13, 14, 45]. Microinjections of N/OFQ directly into the ARC or VMH (which includes the ARC) produce a significant increase in food intake, thereby illustrating a functional role for the ARC in N/OFQ-induced feeding [20, 22]. The classic orexigenic neuropeptide Y (NPY) is expressed and nutritionally regulated in this region [45, 46]. However, it does not appear that NPY is a downstream target of N/OFQ [27]. Instead, evidence for an interaction between N/OFQ and the potent anorectic neuropeptide α-melanocyte-stimulating hormone (α-MSH) has been reported. Specifically, Bomberg *et al.* demonstrated that central administration of N/OFQ reduced feeding-induced neuronal activation of ARC α-MSH neurons [37]. These data suggest that N/OFQ may enhance feeding by inhibiting the activity of the ARC α-MSH neurons associated with hypophagia.

The DRN is a brainstem region that has been implicated in ingestive behavior and contains diffuse expression of the neurotransmitter serotonin [42]. NOP receptor ligands have been demonstrated to affect the activity of the central serotonergic systems [10, 47-50]. The strong inverse association between central serotonin levels and food intake has prompted investigation of this neurotransmitter for pharmaceutical obesity intervention [51-54]. Of interest, N/OFQ administered into the DRN reduces serotonin efflux [41], an effect which may decrease an inhibitory influence on feeding behavior. Further suggesting an interaction between N/OFQ and DRN serotonin neurons, the NOP receptor antagonist UFP-101 [55] effects were prevented by administration of a serotonin neurotoxin or reuptake inhibitor [56] or altered N/OFQ binding site density [42, 57].

Moreover, N/OFQ-induced activation of $K^+$ channels reduced neuronal excitability of DRN neurons [58]. Though N/OFQ-expressing raphe neurons have not been fully characterized, they do not appear to be co-expressed with other opioid peptide-expressing neurons [59]. Raphe NOP receptor-expressing neurons have also not been fully described, however, at least a subpopulation of these neurons co-express N/OFQ, thereby implying presynaptic modulation [60].

Injection of N/OFQ into the shell of the nucleus accumbens also produces a significant increase in food intake in rats [22]. A complete characterization of the chemical phenotype of NOP receptor expressing neurons in the hypothalamus, raphe nuclei, and nucleus accumbens has not yet been performed. Therefore, the underlying mechanisms of action of the endogenous NOP receptor agonist, N/OFQ, are not thoroughly understood. Nevertheless, evidence of an intricate interplay among N/OFQ and other neuromodulators affecting ingestive behavior has been reported [10, 37, 41, 42, 55-57].

## RATIONAL DESIGN OF NOP RECEPTOR ANTAGONISTS

A prevalent method of researching the receptor-ligand interaction in order to generate novel ligand structures has been the application of the "message-address" concept. A theory developed by Dr. Robert Schwyzer in the 1970s categorizes the conservation of the "message" sequence of a peptide ligand to a given peptide family and the "address" sequence to an individual receptor type [61]. The message component of a ligand activates the receptor while the address component fosters additional binding affinity without being crucial to the signal transduction process [62]. This concept has been widely applied toward ligand design for peptides and opioid receptor ligands [61, 63, 64]. It is important to note that structurally identical message peptide fragments assume different conformations in interacting with different receptor types or receptor subpopulations [63], thus limiting the success of this approach. Nevertheless, peptide ligands do serve as important tools for determining the receptor-ligand pharmacophores and the molecular mechanisms of ligand selectivity which, in turn, provide a basis for virtual screening of future drug leads as well as for rational drug design.

Novel GPCR ligand pharmacophores for NOP receptor selective ligands are often acquired *via* receptor homology modeling (based on x-ray structure of the inactive conformation of the first purified GPCR, rhodopsin [65, 66]), combinatorial chemistry approaches, or structure-activity relationship (SAR) analysis. The elucidation of the bioactive conformation of receptor selective opioid ligands relies heavily on the development of those structural models of opioid re-

ceptors and other GPCRs [67-70]. Structure-based design has been applied as one way of deciphering the essential structural elements required for receptor binding by ligands [71, 72].

Ligand SAR is a very useful tool to facilitate the identification of the essential features of a selective and potent compound. SAR on N/OFQ peptide sequence (FGGFTGARKSARKLANQ) has been extensively performed since its discovery. Like opioid receptor ligands, the full sequence of the endogenous ligand is not required for biological activity [73, 74]; N/OFQ(1-13) displays full agonism and high affinity for the NOP receptor, however, only in amide form [75]. Shorter N/OFQ fragments are inactive. The N-terminal tetrapeptide "message" of N/OFQ is required for activity. However, in contrast to opioid receptor ligands, N/OFQ Phenylalanine$^{1}$ residue is not obligatory for NOP receptor activation whereas the common Tyrosine$^{1}$ residue of opioid receptor agonists has been shown to be essential for activity [76, 77]. Tyrosine$^{1}$ interacts with the conserved Aspartate residue in TM3, Histidine in TM6, and Alanine in TM5 of the opioid receptors [66] and its recognition by the receptor triggers transition from inactive to active conformation [78, 79].

Similar to kappa opioid receptor ligands, the extended "address" moiety enriched by positively charged residues (such as Arginine$^{8}$, Arginine$^{12}$, Lysine$^{9}$, and Lysine$^{13}$ of N/OFQ) is a requirement for NOP receptor selective peptide ligands [80-82]. In addition, selective NOP receptor peptide ligands show increased protection from peptidases by amidation of the C-terminal [80].

In addition to peptide ligands, several selective non-peptide NOP receptor ligands have been described in the literature, which also share the major aspects of the NOP receptor ligand pharmacophore. The features of the NOP receptor ligand pharmacophore essential for high affinity and efficacy include: a central core ring containing the protonatable basic nitrogen, a heterocyclic moiety distal to the nitrogen by at least 3 carbons containing hydrogen bond acceptor group, and a lipophilic moiety on the basic nitrogen, Fig. (**4**) [83]. Non-peptide NOP receptor ligands have been classified into five major groups based on their chemical structure. They include morphinan-based ligands, benzimidazopiperidines, spiro-piperidines, aryl piperidines, and 4-amino-quinolines [84].

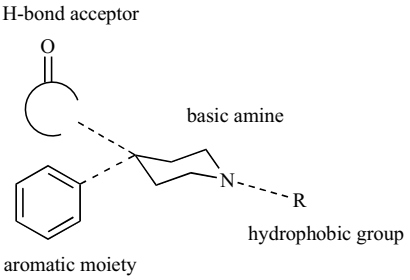


**Fig. (4).** 2-D NOP receptor ligand pharmacophore.

By definition, NOP receptor antagonists should block the pharmacological response induced by N/OFQ, without eliciting an effect by themselves (i.e. antagonists silence agonist-induced signaling) [85]. NOP inverse agonists, on the other hand, would exert opposite pharmacological effects to those of agonists [for review of concept, 86], though no such NOP receptor compounds have yet been described [34]. As expected, NOP receptor antagonists consistently and dose-dependently block N/OFQ-induced feeding [24, 27, 35, 36] and block a NOP receptor agonist discrimination cue in discrimination testing [87]. These data suggest that administered N/OFQ acts through the NOP receptor. The NOP receptor antagonist [Nphe$^{1}$]-N/OFQ(1-13)-$NH_2$ also blocks feeding induced in another paradigm, food deprivation [27]. Given that the expression of both NOP receptors and pro-N/OFQ changes in response to metabolic status [19], we speculate that endogenous N/OFQ is involved in inducing feeding/hunger following conditions of food deprivation. It should be pointed out, however, that currently available NOP receptor antagonists have not been reported to exert an effect on feeding on their own [24, 27, 36]. Therefore, these NOP receptor antagonists appear to reduce hyperphagia rather than induce hypophagia. The following sections review in detail the pharmacology of well characterized peptide and non-peptide NOP receptor antagonists.

## NOP RECEPTOR ANTAGONISTS – PEPTIDE LIGANDS (FIG. (5))

### [Phe$^{1}$Ψ($CH_2$-NH)Gly$^{2}$]-N/OFQ(1-13)-$NH_2$

N/OFQ and related sequences have been extensively used as a template for SAR studies [80, 88-90]. As a result, Calo *et al.* described the first potent peptide-based NOP receptor antagonist [Phe$^{1}$Ψ($CH_2$-NH)Gly$^{2}$]-N/OFQ(1-13)-$NH_2$ (PheΨ), as determined in radioligand binding assays and experiments on electrically evoked contraction of mouse vas deferens ($pA_2$ 6.75) and guinea pig ileum ($pA_2$ 7.02) [90, 91]. $pA_2$ refers to the negative logarithm to base 10 of the molar concentration of an antagonist that requires a 2-fold increase in agonist concentration necessary to elicit the original response. Since then, however, PheΨ has been evaluated in various tissues and presents agonist and antagonist efficacies depending on the tissue/system under study.

For example, application of PheΨ in assays utilizing rat cerebrocortical slice preparations as well as in cAMP accumulation assays revealed it to be a full agonist ($pEC_{50}$ 8.65) [92]. PheΨ has been shown to behave as a potent inhibitor of forskolin-stimulated accumulation of cAMP with $pEC_{50}$ of 8.12 in CHO cells stably expressing the NOP receptor [93]. PheΨ also inhibited $K^+$-evoked glutamate release in the cerebrocortical slice preparations ($pEC_{50}$ 7.59) but acted as a competitive antagonist in the rat vas deferens experiments ($pA_2$ 6.76) [92]. Intrathecal administration of PheΨ resulted in agonist effects on the nociceptive flexor reflexes in rats [94]. In rat locus coeruleus neuronal studies utilizing whole cell patch clamp techniques, PheΨ exhibited partial agonist activity at inhibiting calcium channel currents and opening $K^+$ channels ($pA_2$ of 7.6) [95].

The agonist effects of PheΨ were also observed *in vivo*. Inhibition of morphine analgesia in mice was comparable to that produced by N/OFQ [96, 97]. Electrophysiological recordings from dorsal horn neurons support centrally mediated PheΨ agonist effects [98]. Nociceptive responses *in vivo*

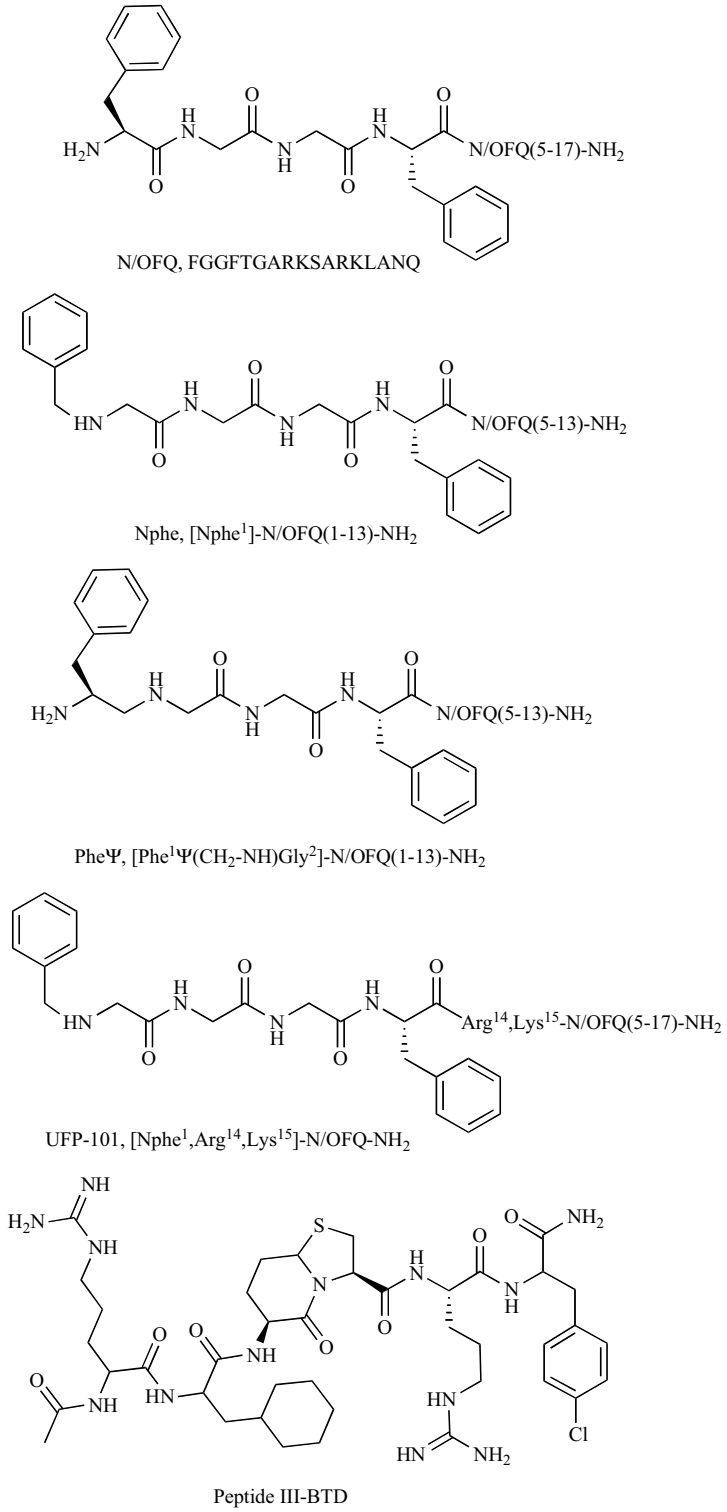


**Fig. (5).** N/OFQ and common peptide NOP receptor antagonists.

after ICV or intrathecal injection of PheΨ indicated agonist effects at supraspinal and spinal levels in the rat tail-flick test of nociception [99]. Further support for agonist effects of PheΨ comes from studies on the firing rate of rat preoptic area/anterior hypothalamic neurons [100] and on the activation of $K^+$ current in neurons in the PVH ($pEC_{50}$ 6.08) [101].

Evidence for lack of antagonism by PheΨ has also been reported in peripheral tissue experiments measuring contractions of the isolated proximal colon of the mouse ($pEC_{50}$ 8.22) and the distal colon of the rat ($pEC_{50}$ 8.5) [102]. Several reasons for the disparate pharmacodynamic profile of this peptide have been proposed. Receptor density, species, and experimental condition differences may account for the varied pharmacological actions of PheΨ seen at central and peripheral sites [99, 103, 104].

### [Nphe$^1$]-N/OFQ(1-13)-NH$_2$

Unlike PheΨ, [Nphe$^1$]-N/OFQ(1-13)-NH$_2$ (Nphe) demonstrates a clear antagonist profile in various experimental settings. In *in vitro* studies for example, Nphe displayed low potency but full antagonism at the NOP receptor in mouse colon tissue recordings of longitudinal isometric smooth muscle contraction ($pA_2$ 6) [105]. In rat cerebral cortex membranes, Nphe competitively inhibited N/OFQ-stimulated GTPγ$^{35}$S binding ($pA_2$ 7.76) [106]. Likewise, in cAMP accumulation studies in NOP expressing CHO cells and in electrically evoked contractions in isolated tissues of the mouse, rat and guinea pig, Nphe competitively antagonized the inhibitory effects of N/OFQ ($pA_2$ 6.0-6.4) [107]. Nphe also selectively antagonized N/OFQ effects in isolated organ baths, such as segments of the rabbit ileum ($pA_2$ 6.2) [108]. In brain slice assays, Nphe again displayed pure, selective and competitive antagonist properties at native NOP receptors using the patch-clamp recording technique in the midbrain ventrolateral periaqueductal gray (PAG), an area involved in opioid-induced supraspinal analgesia [109, 110]. Nphe also attenuated the $K^+$ current activated by N/OFQ concentration-dependently ($pA_2$ 6.64) [110].

Nphe is also active *in vivo*. Nphe completely abolished N/OFQ-stimulated feeding [27]. In addition, Nphe both reduced the amount and duration of feeding in response to a fast [27]. In nociception assays, Nphe prevented the pronociceptive and anti-morphine actions of ICV injected N/OFQ [107]. Moreover, Nphe potentiated morphine-induced analgesia [107, 111]. In a rat model relevant to neuropathic pain in humans, the spinal effects of Nphe in the chronic constriction injury of the sciatic nerve were analyzed. The results showed Nphe to fully antagonize N/OFQ but to lack effects on its own [112]. In other studies, selective antagonism of the spinally located NOP receptor has also been demonstrated [113, 114]. The effects of Nphe were further demonstrated to be mediated by NOP receptors as NOP receptor knockout mice lacked anti-nociceptive effects compared to the wild type mice [115]. The same study also revealed lack of tolerance development towards NOP receptor antagonists, unlike that seen with opioid receptor agonists [115]. N/OFQ's effects on cardiovascular function and cognition are also antagonized by Nphe [116, 117]. Specifically, Nphe antagonized N/OFQ-induced hypotension, bradycardia, and vasodilation in the rat [117]. In cognition assays, Nphe reversed N/OFQ-induced spatial learning and memory impairments in rats [118].

SAR studies testing Nphe analogs for agonist and antagonist activities in the mouse vas deferens demonstrated the critical importance of the aromatic ring of the N-terminal Phenylalanine residue. Enlargement or steric modification resulted in a loss of its antagonist potency (from $pK_B$ of 6.43 for Nphe to 5.30-5.86 for other analogs) [119]. Nphe's peptide nature may preclude interest in developing it further; however, Nphe provides a scaffold for the design of a new class of drugs to study food intake processes and their aberration.

### UFP-101

[Nphe$^1$,Arg$^{14}$,Lys$^{15}$]-N/OFQ-NH$_2$, UFP-101, was identified as a NOP receptor antagonist in the early 2000s [55]. This compound is an example of successful rational drug design by simple chemical modification measures. The Phenylalanine$^1$ side chain has been shifted from the C-alpha of residue 1 to the N-terminus nitrogen of N/OFQ which effectively eliminates agonist efficacy. Substitution of Leucine$^{14}$ and Alanine$^{15}$ to basic residues of Arginine$^{14}$ and Lysine$^{15}$ succeeded in achieving increased ligand potency and extended duration of action *in vivo* [35, 120]. UFP-101 has been assessed in a variety of pharmacological bioassays ranging from radioligand competition ($pK_i$ 10.24) and GTPγ$^{35}$S binding assays ($pA_2$ 9.1) [55], to cAMP accumulation ($pA_2$ 7.1) [55] and receptor internalization assays [121]. UFP-101 has been shown to prevent N/OFQ-induced $K^+$-dependent hyperpolarizing currents in various brain preparation assays ($pA_2$ 6.92) [56, 122]. Effects of UFP-101 on N/OFQ-mediated electrically-induced contractions in the rat and mouse vas deferens, and the guinea-pig ileum yielded $pA_2$ values of 7.30, 7.29, and 7.18, respectively [55]. Determination of neurotransmitter release was also measured. In the rat cerebral cortex synaptosomes preloaded with $^3$H-5-hydroxytryptamine or norepinephrine, UFP-101 displayed antagonist effects with a calculated $pA_2$ value of 7.3-7.7 [47, 48, 55, 123]. UFP-101 behaved as a pure and selective antagonist in those settings causing a rightward shift in the concentration response curves of all NOP receptor agonists studied [124].

UFP-101 was also assessed in spinal anti-nociception models *in vivo* by evaluating N/OFQ and UFP-101 in the mouse tail withdrawal assay and *in vitro* by assessing excitatory postsynaptic currents in laminae I and II of the mouse spinal cord dorsal horn with patch-clamp techniques [125]. In both settings UFP-101 dose-dependently prevented anti-nociceptive effects of N/OFQ ($pA_2$ ~6.44); an effect that was insensitive to pharmacological blockade of opioid receptors but sensitive to genetic inactivation of NOP receptors [125].

The anti-depressant-like properties of UFP-101 have also been assessed in the rodent. Like NOP receptor knockout mice, mice treated with UFP-101 had decreased immobility time in the forced swim test, thereby displaying anti-depressant-like behavior [126]. In rat brain slices, UFP-101 prevented N/OFQ-mediated $K^+$-dependent hyperpolarizing currents in locus coeruleus and DRN neurons, but was inactive by itself [56].

UFP-101 actions at the NOP receptor have also been evaluated in locomotor studies. This compound dose-dependently prevented the inhibitory effects of ICV NOP receptor agonists on spontaneous locomotion in mice while by itself not exerting any effects [55, 127]. Moreover, UFP-101 reduced haloperidol-induced akinesia measured by rat

performance in the rotarod test [168]. When injected in the PVH, UFP-101 also reversed N/OFQ-induced heart rate decrease and renal excretory responses [128].

Most interestingly, UFP-101 has been evaluated in feeding-related experimental paradigms. N/OFQ has been shown to affect gastric and intestinal motility [129-132] as well as gastric secretions [133]. Building on these results, Morini *et al.* have shown UFP-101 to reverse the protective effects of N/OFQ on ethanol-induced gastric lesions [134]. A recent report by Economidou *et al.* described in detail the antagonist effects of ICV injection of UFP-101 in freely feeding rats [36]. By itself, UFP-101 did not modify cumulative food intake, even at high doses, whereas it fully and dose-dependently prevented the orexigenic effect of ICV N/OFQ [36]. UFP-101 serves as another example of successful rational drug design.

### Peptide III-BTD

Another example of the NOP receptor peptide antagonist is Peptide III-BTD, L-Arg-D-Cha-BTD-D-Arg-D-Fcl (D-Cha, D-cyclohexylalanine; BTD, (3S,6S,9R)-2-oxo-3-amino-7-thia-1-aza-bicyclo[4.3.0]nonane-9-carboxylic acid; D-Fcl, *p*-chloro-D-phenylalanine). Peptide III-BTD is a non-selective NOP receptor antagonist ($pK_i$ 7.6) identified from a synthetic combinatorial library of beta-turn constrained peptides [135, 136]. This novel peptide ligand displays potent antagonism of N/OFQ in the GTP$\gamma^{35}$S binding assay ($pA_2$ 6.36), where it shifts the concentration response curve of N/OFQ by about 65 fold to the right [135]. Peptide III-BTD belongs to a small group of compounds with dual pharmacological identities; namely, it has been shown to act as a NOP receptor antagonist at low concentrations and as an opioid receptor agonist at concentrations 10-fold higher [135]. Combination of such efficacies leads to prevention of the development of tolerance to the analgesic properties of opioids [107, 137, 138]. The effect of this compound on ingestive behavior has not yet been reported.

### Summary

Peptide ligands are ubiquitous in biological systems and include hormones, neuropeptides, antigens, cytokines and growth factors [139]. Although peptides, as ligands, are useful tools in deciphering endogenous physiology of novel receptors, they display highly unfavorable pharmacological properties and are usually considered only a starting point in drug discovery and development efforts. In general, peptide ligands display poor bioavailability, limited duration of action, usually a lack of oral activity and protection from proteolysis, a risk of immunogenic effects, and a limited ability to cross cellular membranes. However, as cumbersome as they may seem, they offer potential lower toxicity, minimized drug-drug interaction, lower unspecific binding to non-targets, and increased biological and chemical diversity. Linear peptides have served as starting points for various detailed SAR studies leading toward design of peptidomimetics followed by non-peptide drugs with equally potent efficacies. For example, the successful design of a non-peptide somatostatin receptor subtype-2 agonist from the endogenous peptide ligand [140], the design of endothelin antagonists [141] or luteinizing hormone-releasing hormone antagonists [142] serve as excellent examples. Some of the methodologies applied in SAR iterations include truncations, deletions, substitutions, alanine and D-amino acid scans, as well as design of constrained peptide ligands for the determination of 3D bioactive conformation and pharmacophore models. The reader is pointed toward a review by Dr. Victor J. Hruby to further illuminate the issues relating to the design of peptide ligand agonists and antagonists [139].

Selective NOP receptor antagonists have been developed *via* modifications of the endogenous ligand or *via* modifications of leads from high-throughput screening. As described above, most NOP receptor peptide antagonists were generated from rational structure-activity studies on truncated N/OFQ peptide [80] (Table (**1**)). The introduction of a pseudopeptide bond ($CH_2$-NH) led to what was initially thought to be a full and competitive antagonist (PheΨ) for the NOP receptor [91]. Transposition of the $Phe^1$ sidechain from the α-carbon of $Phe^1$ to N-terminal nitrogen resulted in a design of a peptide with pure antagonist efficacy (Nphe) [107]. Further modifications, such as substitutions in the C-terminal portion of N/OFQ, resulted in a potent and longer acting antagonist with improved potency (UFP-101). The major advantage of utilizing peptide ligands is the ease of deciphering receptor's pharmacology and functional physiology. In the case of the NOP receptor peptide, ligands appear to be used more as tools rather than for drug design and discovery *per se*. Due to an increased ability to cross the blood-brain barrier, non-peptide analogs are typically sought for therapeutic utility.

## NOP RECEPTOR ANTAGONISTS – NON-PEPTIDE LIGANDS (FIG. (6))

Novel non-peptide ligands are usually generated either *via* synthetic methods (either *de novo* or based on endogenous peptide ligands), combinatorial library approaches, or are identified from natural sources. Several selective NOP receptor antagonists have been identified. Structures of these compounds are discussed below, depicted in Fig. (**6**), and biological data is summarized in Table (**2**).

### J-113397

1-[(3*R*,4*R*)-1-cyclooctylmethyl-3-hydroxymethyl-4-piperidyl]-3-ethyl-1,3-dihydro-2*H*-benzimidazol-2-one (J-113397) was the first non-peptide NOP receptor antagonist identified from a chemical library screen by Banyu Pharmaceutical ($pK_i$ 8.7) [143]. It has been shown to selectively inhibit N/OFQ binding in CHO cells expressing NOP receptors, to effectively block N/OFQ-induced suppression of forskolin stimulated cAMP accumulation ($pA_2$ 7.6) [143, 144], and to dose-dependently inhibit N/OFQ stimulated GTP$\gamma^{35}$S binding ($pA_2$ 8.3) [144, 145]. Similar effects were observed in crude membrane preparations from the rat brain and spinal cord [146]. J-113397 competitively antagonized the contractile effects of N/OFQ in the mouse colon and the effect of N/OFQ in electrically stimulated preparations of mouse vas deferens ($pA_2$ 7.85), guinea pig ileum ($pA_2$ 7.75), and rat vas deferens ($pA_2$ 7.77) [147]. It also antagonized selectively and competitively N/OFQ induced activation of $K^+$ channels in rat periaqueductal gray slices ($pA_2$ 8.37) [148]. Subcutaneous administration of J-113397 caused inhibition of ICV

**Table 1. Summary of Biological Data on Peptide NOP Receptor Antagonists**

| Name | Receptor Binding | Functional Activity | Summary of Preparation |
|---|---|---|---|
| PheΨ | $pK_i$ 6.8 [89] | $pA_2$ 6.75 [91] | Electrically stimulated mouse vas deferens |
| | | $pA_2$ 7.02 [91] | Electrically stimulated guinea pig ileum |
| | | $pEC_{50}$ 8.65 [92] | cAMP accumulation assay in $CHO_{NOP}$* |
| | | $pEC_{50}$ 8.12 [93] | cAMP accumulation assay in $CHO_{NOP}$ |
| | | $pEC_{50}$ 7.59 [92] | $K^+$ evoked glutamate release |
| | | $pA_2$ 6.76 [92] | Electrically stimulated rat vas deferens |
| | | $pA_2$ 7.60 [95] | Whole cell patch clamp, inhibition of $Ca^{2+}$ and opening of $K^+$ channels |
| | | $pEC_{50}$ 6.08 [101] | Activation of $K^+$ current in PVH neurons |
| Nphe | $pK_i$ 8.4 [107] | $pA_2$ 6 [105] | Muscle contraction of mouse colon tissue |
| | | $pA_2$7.76 [106] | GTPγ$^{35}$S binding assay |
| | | $pA_2$ 6 [107] | cAMP accumulation assay in $CHO_{NOP}$ |
| | | $pA_2$ 6.04, 6.16, 6.4 [107] | Electrically stimulated tissue in mouse, rat and guinea pig, respectively |
| | | $pA_2$ 6.2 [108] | Electrically stimulated rabbit ileum |
| | | $pA_2$ 6.64 [110] | Attenuation of $K^+$ current |
| UFP-101 | $pK_i$ 10.24 [55] | $pA_2$ 9.1 [55] | GTPγ$^{35}$S binding assay |
| | | $pA_2$ 7.1 [55] | cAMP accumulation assay in $CHO_{NOP}$ |
| | | $pA_2$ 6.92 [122] | N/OFQ-dependent $K^+$ hyperpolarization |
| | | $pA_2$ 7.30, 7.29, 7.18 [55] | Electrically stimulated contraction in rat, mouse and guinea pig tissue, respectively |
| | | $pA_2$ 7.6 [55] | $^3$H-5HT release |
| | | $pA_2$ 6.44 [125] | patch clamp |
| peptide III-BTD | $pK_i$ 7.6 [135] | $pA_2$ 6.36 [135] | GTPγ$^{35}$S binding assay |

*Chinese hamster ovary cells expressing NOP receptor

$pEC_{50}$: the negative logarithm to base 10 of the molar concentration of an agonist that produces 50% of the maximal effect.

$pA_2$: the negative logarithm to base 10 of the antagonist molar concentration that makes it necessary to double the agonist concentration to elicit the original submaximal response. It has been calculated using the Gaddum–Schild equation.

N/OFQ-induced hyperalgesia in mice [144]. Moreover, J-113397 has been demonstrated to be highly selective for the NOP receptor over the opioid receptors [143, 144, 148].

Some of the pharmacological actions of J-113397 mediated *via* the NOP receptor include inhibition of hyperalgesia [144], anti-depressant properties as determined in the mouse forced swim test [149], and attenuation of morphine tolerance and dependence [150]. Enhancement of buprenorphine's anti-nociceptive effects [151] as well as reduction in susceptibility to kainite-induced seizure [152] has also been reported.

J-113397 increases the bioavailability of the neurotransmitter dopamine. Specifically, J-113397 elicited mesolimbic dopamine release [153] and has been shown to increase hedonic state when injected peripherally [154]. When injected intranigrally or given systemically, J-113397 significantly elevated striatal dopamine release and facilitated motor activity [123]. However, NOP receptor knockout studies suggest that the dopaminergic effects of J-113397 are not mediated by the NOP receptor [155]. Other studies support the significant effect produced by J-113397 on release of monoamines after intraperitoneal administration in awake rats [156]. A complete picture of the pharmacological effects of J-113397 may be obscured by the wide and nonspecific binding that has been observed in *in vivo* imaging studies [157].

## Trap-101

J-113397 is a difficult compound to synthesize and the challenging purification of its diastereomers hinders its commercial use. Removal of the two chiral centers *via* introduction of a C3-C4 double bond on the piperidine ring simplified the synthetic protocol whilst preserving the pharmacological efficacy. An achiral analog of J-113397 has been identified and assessed in NOP receptor pharmacological assays [158]. Trap-101, 1-(1-cyclooctylmethyl-5-hydroxymethyl-1,2,3,6-tetrahydro-pyridin-4-yl)-3-ethyl-1,3-dihydrobenzoimidazol-2-one, shows similar efficacy to the chiral J-113397 in the electrically stimulated mouse vas deferens functional assay ($pA_2$ 7.75) [158]. Moreover, receptor binding experiments confirmed high selectivity of this analog for the NOP receptor over the opioid receptors with $pK_i$ at NOP of 8.65 [158]. A series of GTPγ$^{35}$S binding experiments showed Trap-101 to be a competitive NOP receptor antagonist lacking activity *per se,* but concentration-dependently reducing the response to NOP receptor agonist, N/OFQ, with

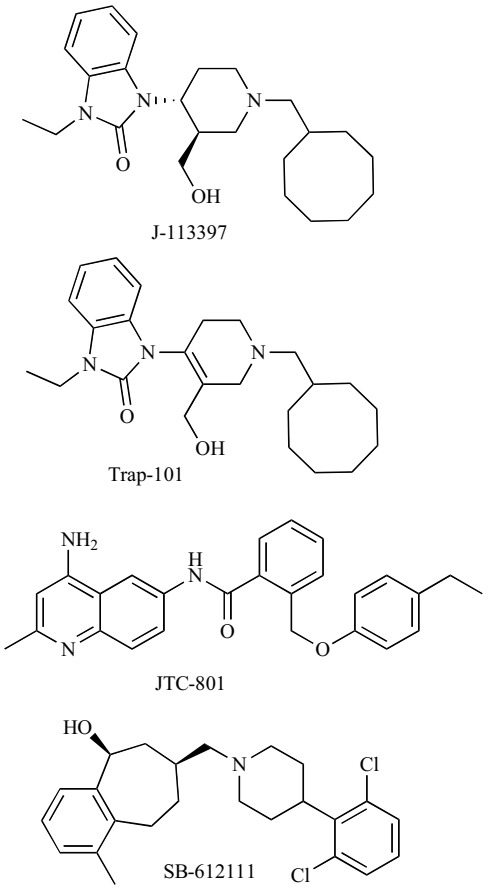


**Fig. (6).** Common non-peptide NOP receptor antagonists.

a $pA_2$ value of 8.55 [158]. Trap-101 has an attractive pharmacological profile and reports further describing its physiological effects *in vivo* will be of interest.

### JTC-801

A series of N-(4-amino-2-methylquinolin-6-yl)-2-(4-ethylphenoxymethyl)-benzamide monohydrochloride (JTC-801) analogs have been evaluated in SAR studies. Substitution in the 3-position of the quinoline was demonstrated to be an important structural requirement as bulky lipophilic or electron withdrawing groups decreased the binding affinity [159]. Also, lack of substitution on the $NH_2$ is a strict requirement for increased affinity at the NOP receptor [160] which is also aided by the 3-methyl substitution that enhances $NH_2$ basicity [159]. Fluoro derivatives of JTC-801 displayed lower binding affinity than ethyl compounds suggesting increased tolerance for para-phenolic substitutions due to lack of involvement of that side chain in direct interaction with receptor residues [159].

*In vitro*, JTC-801 ($pK_i$ 7.35) completely antagonized N/OFQ-induced suppression of forskolin stimulated accumulation of cyclic AMP ($pA_2$ 5.5) [161]. JTC-801 has also been assessed in pain modulation experiments [162]. *In vivo*, intravenous or oral administration completely antagonized N/OFQ-induced allodynia in mice [161]. The effect of JTC-801 on neuropathic pain induced by L5 spinal nerve transection in mice has also been investigated. Oral administration of JTC-801 alleviated thermal hyperalgesia in a dose-dependent manner [163]. A reduction in *c-fos* immunoreactivity has been observed in the dorsal horn of the spinal cord of rat after systemic treatment with JTC-801 blocking N/OFQ-induced allodynia and hyperalgesia [164]. Due to low selectivity profile, *in vivo* results can not be interpreted as solely due to NOP receptor antagonism [161]. The effect of JTC-801 in feeding paradigms has not yet been reported.

### SB-612111

In *in vitro* radioligand binding ($pK_i$ 9.48), GTP$\gamma^{35}$S binding ($pA_2$ 9.7), and cAMP accumulation ($pA_2$ 8.63) assays, (-)-*cis*-1-methyl-7-[[4-(2,6-dichlorophenyl)piperidin-1-yl]methyl]-6,7,8,9-tetrahydro-5*H*benzocyclohepten-5-ol (SB-

**Table 2. Summary of Biological Data on Non-Peptide NOP Receptor Antagonists**

| Name | Receptor Binding | Functional Activity | Preparation |
|---|---|---|---|
| J-113397 | $pK_i$ 8.7 [143] | $pA_2$ 7.6 [143,144]<br>$pA_2$ 8.3 [147, 148]<br>$pA_2$ 7.85, 7.75, 7.77 [147]<br>$pA_2$ 8.37 [148] | cAMP accumulation in $CHO_{NOP}$<br>GTP$\gamma^{35}$S binding assay<br>Electrical stimulated mouse vas deferens, guinea pig ileum and rat vas deferens, respectively<br>Attenuation of $K^+$ current |
| Trap-101 | $pK_i$ 8.65 [158] | $pA_2$ 7.75 [158]<br>$pA_2$ 8.55 [158] | Electrical stimulation of mouse vas deferens<br>GTP$\gamma^{35}$S binding assay |
| JTC-801 | $pK_i$ 7.35 [161] | $pA_2$ 5.5 [161] | cAMP accumulation in $HeLa_{NOP}$* |
| SB-612111 | $pK_i$ 9.48 [165,166] | $pA_2$ 9.7 [165,166]<br>$pA_2$ 8.63 [165,166]<br>$pA_2$ 8.5, 8.2, 8.4 [166] | GTP$\gamma^{35}$S binding assay<br>cAMP accumulation in $CHO_{NOP}$<br>Electrically stimulated preparation of mouse and rat vas deferens and guinea pig ileum, respectively |

*HeLa cells expressing NOP receptor

**Table 3. Recent Patents in NOP Receptor Antagonist Design**

| Patent # | Title | Inventors | Assignee(s) | Application Date |
|---|---|---|---|---|
| US 7,220,751 | Quinazoline derivatives and drugs | Okano Masahiko; Mori Kazuya | Nippon Shinyaku Co., Ltd. (Japan) | November 1, 2001 |
| US 7,202,259 | Therapeutic agents useful for treating pain (substituted 4-tetrazolyl-4-phenylpiperidine) | Chen Zhengming | Euro-Celtique S.A. (Luxembourg) | November 13, 2003 |
| US 7,192,964 | 4-oxoimidazolidine-2-spiropiperidine derivatives | Hashimoto Masaya, Okamoto Osamu | Banyu Pharmaceutical Co., Ltd. (Japan) | July 18, 2002 |
| US 7,183,436 | Substituted 4-aminocyclohexanols | Sundermann Bernd, Hennies Hagen-Heinrich, Englberger Werner, Wnendt Stephan | Gruenenthal GmbH (Germany) | January 16, 2004 |
| US 7,125,877 | Benzimidazole derivatives | Kobayashi Kensuke, Takahashi Hirobumi, Kawamoto Hiroshi, Kato Tetsuya, Itoh Satoru, Yoshizumi Takashi, Okamoto Osamu | Banyu Pharmaceutical Co., Ltd. (Japan) | May 14, 2003 |
| US 7,081,463 | Hydroxy alkyl substituted 1,3,8-triazaspiro[4.5] decan-4-one derivatives useful for the treatment of ORL-1 receptor mediated disorders | Battista Kathleen, Bignan Gilles, Connolly Peter J., Reitz Allen B., Ross Tina Morgan, Scott Malcolm, Middleton Steven A., Orsini Michael | Janssen Pharmaceutica N.V. (Belgium) | September 5, 2003 |
| US 7,049,287 | Nociceptin-based analgesics (compounds share a general formula of Arg-Tyr-Tyr-Arg-Trp-Arg) | Judd Amrit K. | Synvax, Inc. (USA) | October 9, 2002 |
| US 6,995,168 | Triazaspiro compounds useful for treating or preventing pain | Chen Zhengming, Vicotry Sam | Euro-Celtique S.A. (Luxembourg) | May 29, 2003 |
| US 6,969,724 | Compounds (derivatives of substituted 6,7,8,9-tetrahydro-5H-benzocyclohepten-5-ol) | Barlocco Daniela, Cignarella Giorgio, Giardina Giuseppe Arnaldo Maria, Grugni Mario, Ronzoni Silvano | SmithKline Beecham-SpA (UK) | December 19, 2002 |
| 6,969,712 | Benzimidazole derivatives | Okamoto Osamu, Kawamoto Hiroshi, Kobayashi Kensuke, Itoh Satoru, Kato Tetsuya, Yamamoto Izumi, Iwasawa Yoshikazu | Banyu Pharmaceutical Co., Ltd. (Japan) | November 14, 2001 |
| US 6,903,094 | Amide derivatives and nociceptin antagonists | Shinkai Hisashi, Ito Takao, Yamada Hideki | Japan Tabacco, Inc. (Japan) | May 10, 2002 |
| US 6,872,733 | Benzimidazolone compounds | Goehring R. Richard, Chen Zhengming, Vicotry Sam, Kyle Donald | Euro-Celtique S.A. (Luxembourg) | April 18, 2002 |
| US 6,869,960 | N-substituted spiropiperidine compounds as ligands for ORL-1 receptor | Ito Fumitaka, Koike Hiroki, Morita Asato | Pfizer Inc. (NY, USA) | January 23, 2003 |
| US 6,867,222 | Nociceptin analogs (substituted piperidinyl-1,3-dihydro-2H-benzimidazole) | Sun Qun, Goehring R. Richard, Kyle Donald, Chen Zhengming, Vicotry Sam, Whitehead John | Euro-Celtique, S.A. (Luxembourg) | April 18, 2002 |
| US 6,861,421 | Nociceptin analogs (substituted piperidinyl-2,1,3-benzothiadiazin-2,2-dione | Goehring R. Richard, Chen Zhengming, Whitehead John, Gharagozloo Parviz, Vicotry Sam, Kyle Donald | Euro-Celtique S.A (Luxembourg) | April 18, 2002 |

**(Table 3. Contd….)**

| Patent # | Title | Inventors | Assignee(s) | Application Date |
|---|---|---|---|---|
| US 6,828,440 | Spiroindene and spiroindane compounds | Goehring R. Richard, Vicotry Sam, Kyle Donald | Euro-Celtique, S.A. (Luxembourg) | April 18, 2002 |
| US 6,794,389 | Quinazoline derivatives and drugs | Okana Masahiko, Mori Kazuya | Nippon Shinyaku Co., Ltd. (Japan) | September 25, 2002 |
| US 6,686,370 | Triazospiro compounds having nociceptin receptor affinity | Kyle Donald, Goehring R. Richard | Euro-Celtique S.A. (Luxembourg) | December 6, 2000 |
| US 6,635,653 | Spiropyrazole compounds | Goehring R. Richard, Lee Gary, Gharagozloo Parviz, Vicotry Sam, Kyle Donald | Euro-Celtique S.A. (Luxembourg) | April 18, 2002 |
| US 6,476,044 | Use of morphine derivatives as medicaments for the treatment of neuropathic problems | Wnendt Stephan, Strassburger Wolfgang, Buschmann Helmut, Reiss-Mueller Elke, Krueger Thomas | Gruenenthal GmbH (Germany) | February 15, 2002 |
| US 6,410,561 | Amide derivatives and nociceptin antagonists (substituted N-(quinolyl)-2-[methyl]benzamide hydrochloride | Shinkai Hisashi, Ito Takao, Yamada Hideki | Japan Tobacco Inc. (Japan) | September 22, 2000 |
| US 5,834,478 | Morphinan hydroxamic acid compounds | Ito Fumitaka | Pfizer Inc. (NY, USA) | September 12, 1997 |

Source: United States Patents and Trademark Office. The status of each application is slightly different from country to country. For further details, contact USPTO, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450, USA. Tel: 1 (800) 7896-9199 (http://www.uspto.gov/patft/index.html).

612111) behaved as a potent and selective NOP receptor antagonist [165, 166]. Experiments on SB-612111's effects on electrically stimulated mouse ($pA_2$ 8.5) and rat ($pA_2$ 8.2) vas deferens as well as on guinea pig ileum ($pA_2$ 8.4) have suggested longer and stronger interactions with the NOP receptor compared to J-113397 [166].

*In vivo*, SB-612111 blocked N/OFQ's effects on food intake [24]. Specifically, ICV N/OFQ injection produced a significant hyperphagic effect that was abolished with SB-612111 treatment in freely feeding mice [24]. However, SB-612111 did not attenuate 17 hour food deprivation-induced feeding [24]. SB-612111 also prevented N/OFQ's effect on supraspinal nociception, spinal nociception, and mood-related responses [165]. In summary, SB-612111 may become an attractive tool to investigate the food intake regulatory mechanism of the NOP receptor system.

## CURRENT DEVELOPMENTS

In addition to academically driven structure-activity based ligand development, several pharmaceutical companies are pursuing the development of potent NOP receptor antagonists. Indications cover most physiological effects of the NOP receptor, very broadly ranging from analgesics, anti-inflammatories, diuretics, anesthetics, neuroprotective agents, anti-tussives, anti-asthmatics, anti-epileptics, anti-convulsants, anti-hypertensives, anti-depressants, anxiolytics, agents for hearing regulation, modulation of locomotor activity, learning and memory, neurotransmitter and hormone release, kidney function modulators, and agents to control sodium excretion. Anti-obesity and appetite controlling indication has been listed as well (for example, patents #7,220,751; #7,192,964; #7,183,436; #7,081,463; #6,969,712; #6,794,389). The steady growth of patent applications reflects the excitement in the field of the NOP receptor ligand design.

As previously mentioned, non-peptide NOP receptor ligands fall into five major categories based on their chemical structure. The recent patent applications for NOP receptor antagonists, listed in Table (**3**), illustrate this variety of non-peptide structures with high affinity to the NOP receptor. The structural diversity of NOP ligands varies, from N/OFQ structure based compounds (#7,049,287) and morphinan-based derivatives (#6,476,044; #5,834,478), to quinolines and quinazoline derivatives (#7,220,751; #6,903,094; #6,794,389; #6,410,561), benzimidazole (#7,125,877; #6,969,712; #6,872,733; #6,867,722), spiropyrazole, spiropiperidine and triazaspiro compounds (#7,192,964; #7,081,463; #6,995,168; #6,869,960; #6,686,370; #6,635,653) as well as aminocyclohexanol compounds (#7,183,436). Other patents describe compounds with phenylpiperidine (#7,202,259), benzocycloheptenol (#6,969,724), benzothiadiazindione (#6,861,421), as well as spiroindene and spiroindane moieties (#6,828,440).

## FUTURE PROSPECTS

The surge in the prevalence of obesity is currently the focus of intense scientific, medical, and public attention, and novel pharmacotherapies are being pursued. As described, a growing body of experimental evidence has implicated the NOP receptor in central neural pathways mediating ingestive behaviors. Critically, activation of the NOP receptor by its endogenous agonist, N/OFQ, increases food intake, an effect blocked by NOP receptor antagonists. The considerable effort exercised by medicinal chemists has provided synthetic

agents which may be used as valuable tools for the elucidation of the underlying pathways involved in the mechanism of action of N/OFQ on food intake. Significant progress made by neuroscientists has broadened our understanding of the involvement of N/OFQ in the regulation of food intake processes. These tools, together with the different *in vivo* models utilized, suggest that the NOP receptor system may be a promising therapeutic target for the treatment of ingestive behavior and related disorders.

## ABBREVIATIONS

10 = Dorsal motor nucleus of the vagal nerve

12 = Hypoglossal nucleus

2 = Layer 2 cortex

4 = Layer 4 cortex

A1 = A1 noradrenaline cells

A2 = A2 noradrenaline cells

A5 = A5 noradrenaline cells

Amb = Ambiguus nucleus

APir = Amygalopiriform transition area

APTV = Anterior pretecal nucleus, ventral part

ARC = Arcuate nucleus of the hypothalamus

BMA = Basomedial amygdaloid nucleus, anterior part

CA1 = Field CA1 of the hippocampus

CA3 = Field CA3 of the hippocampus

CeA = Central nucleus of the amygdala

Cg = Cingulated gyrus

Cu = Cuneate nucleus

DEn = Dorsal endopiriform nucleus

DMH = Dorsomedial hypothalamic nucleus

DMSp5 = Dorsomedial spinal trigeminal nucleus

DRN = Dorsal raphe nucleus

GI = Granular insular cortex

GiA = Gigantocellular reticular nucleus, alpha

HDB = Nucleus of the horizontal limb of the diagonal band of Broca

IG = Indusium griseum

IntA = Interposed cerebellar nucleus, anterior part

Lat = Lateral (dentate) cerebellar nucleus

LatPC = Lateral cerebellar nucleus, parvicellular part

LHA = Lateral hypothalamic area

LPO = Lateral preoptic area

LRt = Lateral reticular nucleus

LSD = Lateral septal nucleus, dorsal

LSV = Lateral septal nucleus, ventral

LVe = Lateral vestibular nucleus

MD = Mediodorsal thalamic nucleus

Me = Medial amygdaloid nucleus

MePV = Medial amygdaloid nucleus, posteroventral

Med = Medial (fastigial) cerebellar nucleus

MHb = Medial habenular nucleus

MnR = Median raphe nucleus

MO = Medial orbital cortex

MPA = Medial preoptic area

MS = Medial septal nucleus

PaV = Paraventricular hypothalamic nucleus, ventral part

Pa5 = Paratrigeminal nucleus

PCom = Nucleus of the posterior commissure

Pe = Periventricular hypothalamic nucleus

Pir = Piriform cortex

PN = Paranigral nucleus of the VTA

Pn = Pontine nuclei

PP = Peripeduncular nucleus

PPTg = Pedunculopontine tegmental nucleus

PT = Paratenial thalamic nucleus

PVA = Paraventricular thalamic nucleus, anterior

PVP = Paraventricular thalamic nucleus, posterior

PVH = Paraventricular nucleus of the hypothalamus

RAmb = Retroambiguus nucleus

RChL = Retrochiasmatic area, lateral

RMg = Raphe magnus nucleus

ROb = Raphe obscurus nucleus

RPa = Raphe pallidus nucleus

RPC = Red nucleus, parvicellular

RSG = Retrosplenial granular cortex

Rt = Reticular thalamic nucleus

RtTg = Reticulotegmental nucleus of the pons

SFO = Subfornical organ

SHi = Septohippocampal nucleus

SHy = Septohypothalamic nucleus

SN = Substantia nigra

SNCD = Substantia nigra, compact part, dorsal tier

SO = Supraoptic nucleus

Sol = Nucleus of the solitary tract

SolC = Nucleus of the solitary tract, commissural

Sp5 = Spinal trigeminal nucleus

Sp5O = Spinal trigeminal nucleus, oral

SPFPC = Subparafascicular thalamic nucleus, parvicellular part

ST = Bed nucleus of the stria terminalis

STMV = Bed nucleus of the stria terminalis, medial division, ventral part

SubI = Subincertal nucleus

SuVe = Superior vestibular nucleus

VMH = Ventromedial nucleus of the hypothalamus

VO = Ventral orbital cortex

VP = Ventral pallidum

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