Bioorganic & Medicinal Chemistry Letters 24 (2014) 5219–5223

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Design, synthesis and evaluation of [^{3}H]PF-7191, a highly specific nociceptin opioid peptide (NOP) receptor radiotracer for in vivo receptor occupancy (RO) studies

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ARTICLE INFO

Article history:
Received 19 August 2014
Revised 19 September 2014
Accepted 24 September 2014
Available online 2 October 2014

Keywords:
NOP receptor
ORL1
Radiotracer
Receptor occupancy
PET

ABSTRACT

Herein we report the identification of (+)-*N*-(2-((1*H*-pyrazol-1-yl)methyl)-3-((1*R*,3*r*,5*S*)-6′-fluoro-8-azaspiro[bicyclo[3.2.1]octane-3,1′-isochroman]-8-yl)propyl)-*N*-[^{3}*H*]-methylacetamide {[^{3}H]PF-7191 [(+)-**11**]} as a promising radiotracer for the nociceptin opioid peptide (NOP) receptor. (+)-**11** demonstrated high NOP binding affinity (K_i = 0.1 nM), excellent selectivity over other opioid receptors (>1000×) and good brain permeability in rats ($C_{b,u}/C_{p,u}$ = 0.29). Subsequent characterization of [^{3}H](+)-**11** showed a high level of specific binding and a brain bio-distribution pattern consistent with known NOP receptor expression. Furthermore, the in vivo brain binding of [^{3}H](+)-**11** in rats was inhibited by a selective NOP receptor antagonist in a dose–responsive manner. This overall favorable profile indicated that [^{3}H](+)-**11** is a robust radiotracer for pre-clinical in vivo receptor occupancy (RO) measurements and a possible substrate for carbon-11 labeling for positron emission tomography (PET) imaging in higher species.

The nociceptin opioid peptide (NOP) receptor, formerly also known as opioid receptor-like 1 (ORL-1), was first identified in 1994.[1] It was widely considered as the 4th member of the opioid receptor family, sharing high sequence homology to μ, κ and δ opioid receptors. A heptadecapeptide, termed as nociceptin or orphanin FQ (N/OFQ),[2] was identified as the endogenous agonist for NOP receptors, which interestingly shows no appreciable affinity for other opioid receptor subtypes despite of its structural similarity to opioid peptide agonist dynorphin A. Like other opioid receptors, NOP receptors are widely expressed in the central nervous system (CNS) and peripheral organs. In the brain, NOP receptors are enriched in the cortex, hippocampus, hypothalamus and brain stem while expressed less in striatum and cerebellum.[3]

Pharmacologically, the NOP receptor is coupled to the activation of phospholipase C (PLC) and K^+ channels and the inhibition of adenylyl cyclase, with a main effect of inhibiting the release of key neurotransmitters, for example, acetylcholine, catecholamines, glutamate, etc. in neuronal tissues. Therefore there has been an interest in developing selective NOP antagonists as potential treatment for depression, anxiety, Parkinson's disease (PD) and obesity.[4] Within our group, we were interested in developing a selective NOP receptor radiotracer, specifically a [^{3}H] radiotracer that would allow rodent in vivo receptor occupancy (RO) measurements, which could then be used for effective lead characterization and prioritization, and ultimately clinical candidate selection. Development of a suitable radiotracer for in vivo NOP RO studies has been challenging due to its relatively low expression level (B_{max}) and high lipophilicity of literature leads. Prior to initiation of our effort, only one NOP antagonist PET radiotracer, [^{11}C]CPEB (**1**, Fig. 1), was reported which failed to demonstrate specific binding in vivo.[5] Since our effort was initiated, two additional positron emission tomography (PET) tracers, [^{11}C]-(*S*)-3-(2′-fluoro-4′,5-dihydrospiro[piperidine-4,7′-thieno-[2,3-*c*]pyran-1-yl)-2(2-fluorobenzyl)-*N*-methylpropanamide (**2**)[6] and [^{18}F]MRK-0911 (**3**)[7] have been reported, showing specific to non-specific ratios of 1.28 and ~2, respectively, in non-human primates (NHP).

A NOP receptor [^{3}H] radiotracer needs to meet a number of specific requirements in order to be viable in vivo. Structurally, it must be amenable to tritiation, typically requiring incorporation of three tritiums to offer sufficient specific activity for

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http://dx.doi.org/10.1016/j.bmcl.2014.09.069
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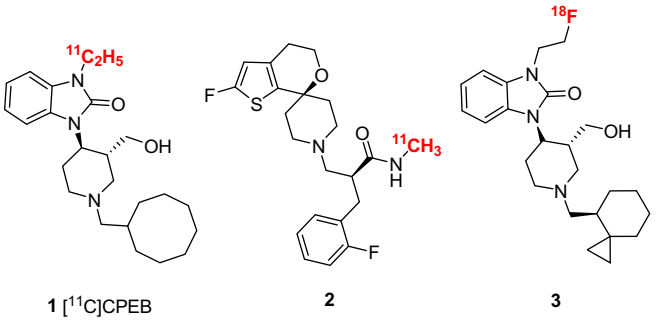

Figure 1. Known NOP receptor antagonist radiotracers.

in vivo studies (~75 Ci/mmol). Pharmacokinetic (PK)-wise, it needs to be brain permeable and has low non-specific binding (NSB) in brain tissues. Pharmacologically, it needs to be potent and selective towards the NOP receptor. The requisite binding affinity for a radiotracer is dictated by B_{max} with a ratio of $B_{max}/K_d > 10$ typically required.[8] The B_{max} of NOP receptor has been estimated at 237 fmol/mg protein in rat brain (~12 nM in the whole brain, assuming 50 mg protein/g wet tissue).[9] The B_{max} information on higher species, however, has been sparse. Only a single report on macaque brain exists, showing a less defined B_{max} estimation (>6.1 fmol/mg protein in receptor rich regions, for example, cortex, caudate and hippocampus).[10] Therefore, potency in the sub-nM range would likely be required to achieve in vivo engagement of NOP receptors.

Our group has previously disclosed a set of design and selection criteria for novel CNS PET tracer development.[11] Considering the similarity in property requirements between a PET tracer and a [^{3}H] radiotracer, we were keen to explore whether we could apply similar design principles for PET tracers in our in vivo [^{3}H] NOP radiotracer development effort. Specifically we targeted analogs with suitable physicochemical properties [CNS PET multi-parameter optimization (MPO) score > 3], good passive permeability [RR canine kidney (RRCK) > 5×10^{-6} cm/s][12] and low p-glycoprotein (P-gp) efflux [multidrug resistance (MDR) BA/AB < 2.5][13] for brain permeability[14] and appropriate fraction unbound in brain (Fu_b > 0.05)[15] to minimize the risk of NSB. With this set of parameters in mind, we started our effort by data-mining of our NOP antagonist chemical matter for viable tracer leads. We were particularly interested in the spirocyclic series previously developed by our Nagoya colleagues for its extensive SAR and overall excellent NOP receptor activities.[16] In-depth SAR analysis revealed intriguing trends on potency and selectivity. As shown in Figure 2A, switching the piperidine ring of **4** to a tropane ring led to ~5-fold increase in potency (**5** vs **4**). Further improvement in potency (14-fold) was achieved by placing a fluorine at the 4-position of the distal phenyl ring (**6** vs **5**), yielding compounds with sub-nM potency. The overall profile of **6** was attractive as it has many of the desirable attributes for a radiotracer lead, including a viable tritiation site (amide *N*-methyl), potent NOP binding affinity (K_i = 0.59 nM), high RRCK (>10), low MDR (<2.5) and favorable predicted Fu_b (>0.05). However, while it showed no appreciable potency for κ and δ opioid receptors (K_i > 10 μM), it was potent at μ opioid receptor (K_i = 5.9 nM), which we hypothesized would confound interpretation of in vivo RO. This prompted us to further investigate SAR specifically related to selectivity over μ opioid receptor. While the NOP potency was mainly influenced by the spiropiperidine portion of the molecules, we found that the μ opioid receptor selectivity could be modulated by varying the right hand *N*-alkyl portion (Fig. 2B). For example, reversing the amide connectivity (**8** vs **7**) significantly increased the NOP affinity while reducing μ opioid binding, leading to a net increase in selectivity from 1.4-fold

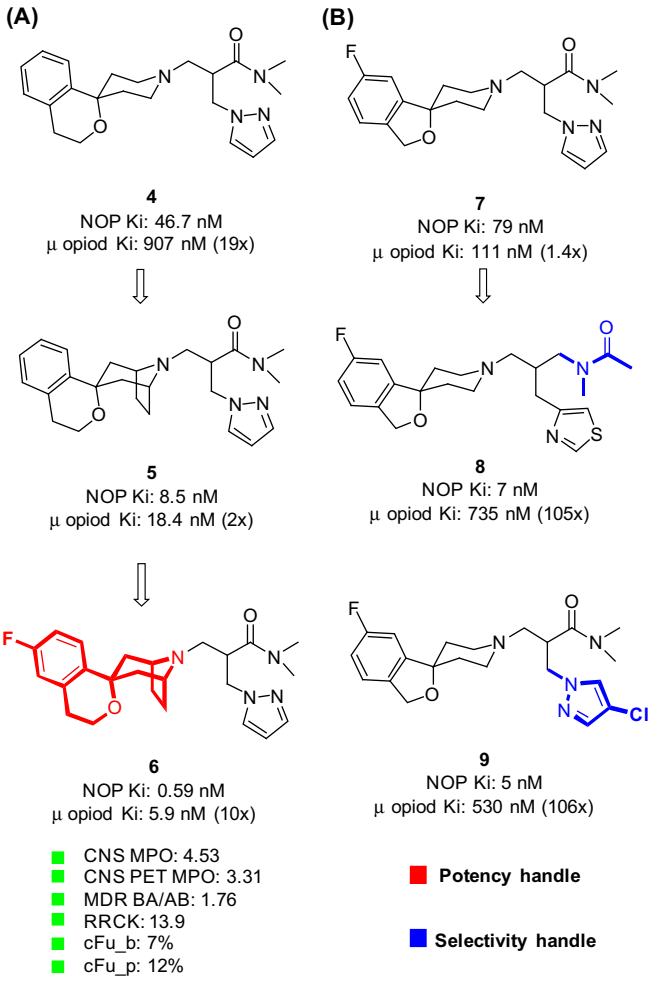

Figure 2. (A) historical SAR on NOP potency; (B) historical SAR on selectivity over μ opioid receptor.

to 105-fold. Alternatively, adding a substituent at the 4-position of the pendant pyrazole (**9** vs **7**) also yielded similar selectivity increase (1.4-fold to 106-fold).

Based on this information, we proposed a total of 6 analogs by combining the potency and selectivity handles identified from the SAR analysis. All analogs have an *N*-Me amide moiety as a potential tritiation site and could be divided into two structural classes based on the choice of their selectivity handles (Scheme 1). Analogs **10**–**12** utilized a reversed amide moiety for selectivity, with variations on the pendant R groups: a thiazole (**10**), an unsubstituted pyrazole (**11**), and 4-Cl pyrazole (**12**) with an intention to maximize selectivity by combining the two selectivity handles shown in Figure 1B. Analogs **13**–**15** utilized substituted heteroaryls (R groups) for selectivity, probing variations in heteroaryls (pyrazole for **13**, **14**; imidazole for **15**) and substituents (4-methyl for **13**, **15**; 4-chloro for **14**). Prior to the synthesis of any analogs, we prioritized this group of targets according to their physicochemical properties (CNS PET MPO) and absorption, distribution, metabolism and excretion (ADME) properties using in silico models.[17] As shown in Scheme 1, all six proposed analogs reside in the desirable physicochemical property space (CNS PET MPO >3). Analogs **10** and **15** were predicted to have high Pgp liability (cMDR BA/AB 5.32 and 8.95, respectively), suggesting a high risk of poor brain permeability. They were therefore not pursued despite their otherwise favorable property profile. In comparison, analogs **11**–**14** were predicted to have good passive permeability (cRRCK > 5) and low Pgp liability (cMDR BA/AB < 2.5), suggesting good brain permeability, among