Bioorganic & Medicinal Chemistry Letters 19 (2009) 6978–6980

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

A bivalent ligand (KMN-21) antagonist for μ/κ heterodimeric opioid receptors

Shijun Zhang, Ajay Yekkirala, Ye Tang, Philip S. Portoghese *

Department of Medicinal Chemistry, College of Pharmacy, University of Minnesota, Minneapolis, MN 55455, USA

ARTICLE INFO

Article history:
Received 7 September 2009
Revised 8 October 2009
Accepted 9 October 2009
Available online 4 November 2009

Keywords:
Opioid receptors
Dimerization
Bivalent ligand
Heterodimers

ABSTRACT

In an effort to develop antagonists for κ–μ opioid receptor heterodimers, a series of bivalent ligands **3**–**6** containing κ- and μ-antagonist pharmacophores were designed and synthesized. Evaluation of the series in HEK-293 cells revealed **4** (KMN-21) to selectively antagonize the activation of κ–μ heterodimers, suggesting possible bridging of receptors when the bivalent ligand spacer contains 21 atoms.

Published by Elsevier Ltd.

The opioid receptors belong to the superfamily of G-protein coupled receptors (GPCRs) and play important roles in pain perception and regulation. Three major types of opioid receptors including κ-, δ-, and μ-opioid receptors have been well characterized and cloned.[1] Numerous reports have demonstrated the dimerization/oligomerization of GPCRs,[2] and the existence of dimers for opioid receptors in cultured cells.[3–8] In addition, pharmacological evidence had suggested that multiple phenotypic κ-opioid receptor subtypes are related to heterodimeric opioid receptors. For example, κ–δ and κ–μ heterodimers have been linked to the κ_2 subtype.[7,9] Therefore it would be of value to develop chemical tools to assist in the pharmacological characterization of opioid receptor heterodimers.

In this regard, bivalent ligands have been developed as pharmacological tools to study the dimerization of opioid receptors.[10–14] Recently, we had reported two selective bivalent ligands KDN-21 and KDAN-18, that have suggested the association of δ_1 and κ_2 phenotypes with κ–δ heterodimers.[15,16] In view of the promising results from this bivalent ligand strategy and evidence showing the association of opioid receptors via homo- and hetero-dimerization,[4–8] we have extended the bivalent strategy to design tools for κ–μ heterodimers.

Here we report on the synthesis and in vitro biological characterization of a series of bivalent ligands **3**–**6** containing a κ-opioid antagonist pharmacophore 5′-guanidinonaltrindole (5′-GNTI) (**1**)[17] and a μ-opioid antagonist pharmacophore β-naltrexamine (β-NTX) (**2**)[18] linked through a spacer of varying length. The design rationale of these bivalent ligands is based on our previous studies of κ–δ bivalent ligands[15,16] and our desire to maintain a favorable hydrophilic and lipophilic balance coupled with flexibility. This included a spacer that contains (1) glycine units that maintain a favorable hydrophilic-lipophilic balance, (2) a succinyl unit that contributes to the flexibility for favorable interaction with heterodimers, and (3) an alkylamine moiety attached to pharmacophore **1** which permits variation of the spacer length by one atom increments (Fig. 1).

The synthetic protocol for **3**–**6** is shown in Schemes 1 and 2. Briefly, coupling reaction between carboxylic acid **7** and naltrexamine **8**, which were synthesized as reported[15,18], gave intermediate **9**, which on hydrolysis with TFA in dichloromethane afforded carboxylic acid intermediate **10** (Scheme 1). The reactions of monoprotected alkyldiamines **11**–**14** with KSCN followed by Cbz-protection yielded **15**–**18**, which upon condensation with **19** in the presence of $HgCl_2$ and Et_3N followed by deprotection, provided **20**–**23** in good

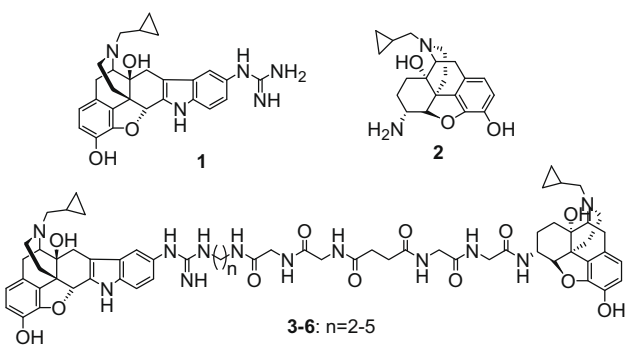
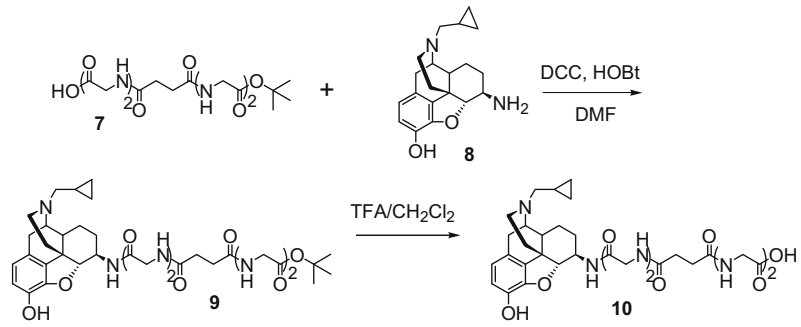


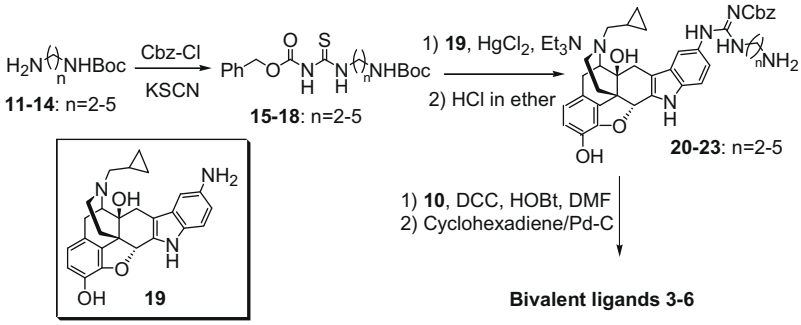
Figure 1. Designed bivalent ligands.

* Corresponding author. Tel.: +1 612 624 9174; fax: +1 202 513 8609.
E-mail address: porto001@umn.edu (P.S. Portoghese).

0960-894X/$ - see front matter Published by Elsevier Ltd.
doi:10.1016/j.bmcl.2009.10.045



Scheme 1. The synthetic route for the carboxylic acid intermediate **10**.



Scheme 2. The synthetic route for the designed bivalent ligands **3**–**6**.

yield. Finally, standard amide coupling reactions of **10** with **20**–**23** followed by catalytic hydrogenation afforded the desired bivalent ligands **3**–**6** (Scheme 2). The chemical structures of bivalent ligands were characterized and confirmed by ^{1}H NMR and Fast-atom bombardment mass spectra (FABMS) (ESI). The purity of designed ligands was determined by reverse HPLC (Acetonitrile/H_2O/TFA: 50/50/0.1 and MeOH/H_2O/TFA: 30/70/0.1) to be >98%.

After synthesis, the antagonist activities of **3**–**6** were evaluated by measuring inhibition of Ca^{2+} release in HEK-293 cells that stably express κ- and μ-opioid receptors singly or together (Fig. 2). A chimeric G-protein was transiently transfected in this assay for Ca^{2+} release.[19] The selective agonists used to evaluate the antagonism selectivity of the target compounds were U69, 593[20] (κ), and DAMGO (μ).[21] While all of the bivalent ligands **3**–**6** (1 μM concentration) antagonized Ca^{2+} release in cells containing singly expressed receptors, only bivalent ligand **4** (KMN-21) significantly antagonized both U69593- and DAMGO-induced Ca^{2+} release in cells containing coexpressed κ- and μ-opioid receptors. These data suggest that bivalent ligand **4** with a spacer length of 21 atoms engages κ–μ opioid heterodimers more efficiently than homodimers. In this regard, it is noteworthy that the κ–δ bivalent ligand antagonist, KDN-21, also contains a 21-atom spacer, suggesting common bridging modes to κ–μ and κ–δ heterodimeric receptors.[16] To further understand the interactions of bivalent ligand **4** with κ–μ heterodimers, additional studies would be required.

In summary, a series of bivalent ligands containing κ- and μ-opioid antagonist pharmacophores attached to variable length spacers were designed and synthesized as chemical tools to investigate κ–μ heterodimers. Biological evaluation in HEK-293 cells revealed that bivalent ligand **4** with 21 atoms in its spacer significantly antagonized Ca^{2+} release of activated κ- and μ-opioid receptors. Together with our previous results of different bivalent ligands,[16,17] the results further exemplifies the power of bivalent ligands as pharmacological tools in investigating the dimerization of opioid receptors in particular and GPCRs in general.

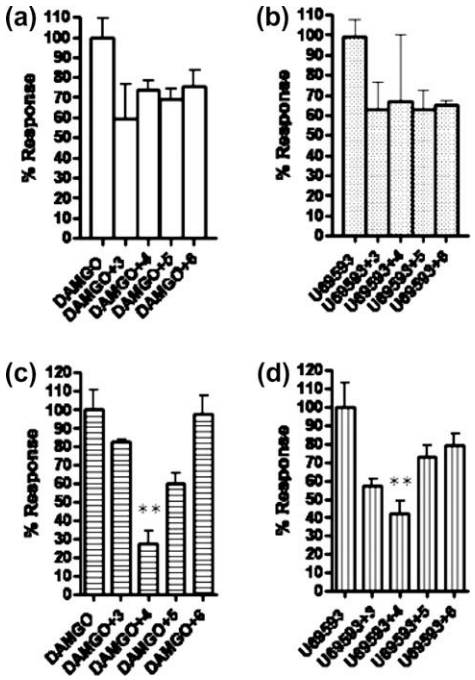


Figure 2. Compound **4** (KMN-21) is an antagonist in HEK-293 cells coexpressing μ- and κ-opioid receptors. Intracellular calcium release experiments were performed using appropriate agonist ligands and compounds **3**–**6** in HEK-293 cells stably expressing (a) mu, (b) kappa, (c and d) mu/kappa opioid receptors. Cells were transiently transfected with chimeric G-protein, Δ6-Gqi4-myr (200 ng for every 40,000 cells) and pre-incubated with antagonist compounds **3**–**6** (1 μM) and dye from calcium release kit (Molecular Devices). Intracellular calcium release was measured in a Flexstation apparatus (Molecular Devices) using DAMGO or U69593 (1 μM). Experiments were performed in triplicate ($n = 4$) and significance was measured using ANOVA (** = $p < 0.01$).

Acknowledgments

We thank Mike Powers for capable technical assistance. This research is supported by grant DA01533 from National Institute on Drug Abuse.

References and notes

1. Dhawan, B. N.; Cesselin, F.; Raghubir, R.; Reisine, T.; Bradley, P. B.; Portoghese, P. S. *Pharmacol. Rev.* **1996**, *48*, 567.
2. Angers, S.; Salahpur, A.; Bouvier, M. *Annu. Rev. Pharmacol. Toxicol.* **2002**, *42*, 409.
3. Levac, B. A.; O'Dowd, B. F.; George, S. R. *Curr. Opin. Pharmacol.* **2002**, *2*, 76.
4. Rios, C. D.; Jordan, B. A.; Gomes, I.; Devi, L. A. *Pharmacol. Ther.* **2001**, *92*, 71.
5. Portoghese, P. S.; Lunzer, M. M. *Eur. J. Pharmacol.* **2003**, *467*, 233.
6. Gomes, I.; Jordan, B. A.; Trapaidze, N.; Nagy, V.; Devi, L. A. *J. Neurosci.* **2000**, *20*, RC110.
7. Jordan, B. A.; Devi, L. A. *Nature* **1999**, *399*, 697.
8. Wang, D.; Sun, X.; Bohn, L. M.; Sadee, W. *Mol. Pharmacol.* **2005**, *67*, 2173.
9. Simonin, F.; Slowe, S.; Becker, J. A.; Matthes, H. W.; Filliol, D.; Chluba, J.; Kitchen, I.; Kieffer, B. L. *Eur. J. Pharmacol.* **2001**, *414*, 189.
10. Portoghese, P. S.; Ronsisvalle, G.; Larson, D. L.; Yim, C. B.; Sayre, L. M.; Takemori, A. E. *Life Sci.* **1982**, *31*, 1283.
11. Erez, M.; Takemori, A. E.; Portoghese, P. S. *J. Med. Chem.* **1982**, *25*, 847.
12. Portoghese, P. S.; Larson, D. L.; Yim, C. B.; Sayre, L. M.; Ronsisvalle, G.; Lipkowski, A. W.; Takemori, A. E.; Rice, K. C.; Tam, S. W. *J. Med. Chem.* **1985**, *28*, 1140.
13. Portoghese, P. S.; Larson, D. L.; Sayre, L. M.; Yim, C. B.; Ronsisvalle, G.; Tam, S. W.; Takemori, A. E. *J. Med. Chem.* **1986**, *29*, 1855.
14. Portoghese, P. S.; Ronsisvalle, G.; Larson, D. L.; Takemori, A. E. *J. Med. Chem.* **1986**, *29*, 1650.
15. Bhushan, R. G.; Sharma, S. K.; Xie, Z. H.; Daniels, D. J.; Portoghese, P. S. *J. Med. Chem.* **2004**, *47*, 2969.
16. Daniels, D. J.; Kulkarni, A.; Xie, Z. H.; Bhushan, R. G.; Portoghese, P. S. *J. Med. Chem.* **2005**, *48*, 1713.
17. Jones, R. M.; Portoghese, P. S. *Eur. J. Pharmacol.* **2000**, *396*, 49.
18. Sayre, L. M.; Portoghese, P. S. *J. Org. Chem.* **1980**, *45*, 3366.
19. Waldhoer, M.; Fong, J.; Jones, R. M.; Lunzer, M. M.; Sharma, S. K.; Kostenis, E.; Portoghese, P. S.; Whistler, J. L. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 9050.
20. Lahti, R. A.; Miekelson, M. M.; Mccall, J. M.; Von Voightlander, P. F. *Eur. J. Pharmacol.* **1985**, *109*, 281.
21. Handa, B. K.; Lane, A. C.; Lord, J. A.; Morgan, B. A.; Rance, M. J.; Smith, C. F. *Eur. J. Pharmacol.* **1981**, *70*, 531.