Published in final edited form as:

Bioorg Med Chem Lett. 2013 September 15; 23(18): 5045–5048. doi:10.1016/j.bmcl.2013.07.043.

Structure activity relationship studies of 17-cyclopropylmethyl-3,14 β -dihydroxy-4,5 α -epoxy-6 α -(isoquinoline-3'-carboxamido)morphinan (NAQ) analogues as potent opioid receptor ligands: preliminary results on the role of electronic characteristics for affinity and function

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Abstract

17-Cyclopropylmethyl-3,14 -dihydroxy-4,5 -epoxy-6 -(isoquinoline-3 -carboxamido)morphinan (NAQ) was previously designed following the “message–address” concept and was identified as a potent and highly selective mu opioid receptor (MOR) ligand based on its pharmacological profile. We here report the preliminary structure activity relationship (SAR) studies of this novel lead compound. For the new ligands synthesized as NAQ analogues, their binding assay results showed that a longer spacer and a saturated ring system of the side chain were unfavorable for their MOR selectivity over the kappa and delta opioid receptors. In contrast, substitutions with different electronic properties at either 1 - or 4 -position of the isoquinoline ring of the side chain were generally acceptable for reasonable MOR selectivity. The majority of NAQ analogues retained low efficacy at the MOR compared to NAQ in the ³⁵S-GTP[S] binding assays while electron-withdrawing groups at 1 -position of the isoquinoline ring induced higher MOR stimulation than electron-donating groups did. In summary, the electronic characteristics of substituents at 1 - or 4 -position of the isoquinoline ring in NAQ seem to be critical and need to be further tuned up to achieve higher MOR selectivity and lower MOR stimulation.

Keywords

MOR; antagonists; NAQ; SAR

Naltrexone (Figure 1) has long been used for opioid addiction and alcoholism treatment.^{1, 2} It exerted its function mainly through blocking the mu opioid receptor (MOR).^{1, 2} Despite the demonstrated efficacy, its hepatotoxicity and inverse agonism on the MOR in opioid-

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Supplementary Information

Supplementary Information (chemical synthesis, compounds characterization, and biology evaluation) associated with this article can be found, in the online version, at.

dependent state compromised its clinical applications.^{3–5} Furthermore, naltrexone bound to the MOR and the kappa opioid receptor (KOR) with similar affinity.⁶ Naltrexone also possessed moderate efficacy at the KOR,⁷ which was proposed to counteract its therapeutic efficiency for addiction treatment.⁸

So far, a variety of molecules have been synthesized based on the skeleton of naltrexone. Most of the modifications happened at the C6-position by introducing a β -configuration substituent. These efforts provided a number of pharmacologically interesting compounds (Figure 1). Among them, 6 β -naltrexol is the major metabolite of naltrexone and it acted as a neutral MOR antagonist and a KOR inverse agonist.^{9–11} Deveau research group recently reported that diphenyl-6 β -naltrexamate carried improved MOR selectivity over both the delta opioid receptor (DOR) and KOR compared to 6 β -naltrexol.¹² β -funaltrexamine (β -FNA), N-naphthoyl- β -naltrexamine (NNTA), and N-isonicotinoyl- β -naltrexamine (NAP) were the amide derivatives of 6 β -naltrexamine. β -FNA was a MOR irreversible antagonist with reversible KOR agonist activity.¹³ NNTA selectively and potently activated the μ /kappa opioid receptor heteromers without inducing significant physical dependence and place preference in its ED₅₀ dose range.¹⁴ NAP acted as a peripheral MOR antagonist and significantly increased mouse gastrointestinal transit with an ED₅₀ around 0.0088 mg/kg. NAP also only displayed marginal withdrawing symptoms at a dose as high as 10 mg/kg.¹⁵

In contrast, only a few reports have described the 6 α -configuration derivatives of naltrexone.^{16–18} A thorough analysis of the binding data of these compounds revealed that the 6 α -isomers generally carried higher MOR selectivity over the KOR than the corresponding 6 β -isomers.^{16, 17} More recently, 17-cyclopropylmethyl-3,14 α -dihydroxy-4,5 α -epoxy-6 α -(isoquinoline-3-carboxamido)morphinan (NAQ, Figure 1) was found to be more efficacious and less susceptible to tolerance than naltrexone in reducing high concentration alcohol consumption in C57BL/6J mice by intermittent access.¹⁹ However, NAQ also acted as a DOR partial agonist with relative high efficacy and moderate potency in the [³⁵S]-GTP[γ -S] binding assays.²⁰ There have been reports that the DOR was associated with morphine dependence in mice.^{21–23} Thus a ligand without DOR agonism would be ideal for therapeutic purpose. To achieve that, we herein report the structure activity relationship (SAR) studies of NAQ, focusing on the spacer length between the epoxymorphinan skeleton and the isoquinoline ring (side chain), and the electronic properties of the side chain.

The syntheses of these NAQ analogues were achieved in two steps as reported previously (Scheme 1).¹⁶ Briefly, reductive amination of naltrexone with benzylamine and sodium borohydride followed by catalytic hydrogenation in the presence of hydrochloric acid furnished 6 α -naltrexamine dihydrochloride (6 α -NTA·2HCl) in a total yield of 79%.²⁴ A variety of substituted isoquinoline-3-carboxylic acids (see Supplementary Information) were then coupled to 6 α -NTA·2HCl via EDCI/HOBt method. After treatment with K₂CO₃ in methanol, NAQ analogues were obtained with moderate yields.

These new NAQ analogues were then screened in the *in vitro* opioid receptor binding assays and MOR [³⁵S]-GTP[γ -S] functional assays according to the procedures reported previously with minor revision (see Supplementary Information).^{6, 16} Naltrexone and NAQ were tested along under the same conditions for comparison (Table 1). All the NAQ analogues retained high binding affinity to the MOR within subnanomolar to nanomolar range. While they all bound to the MOR as potently as NAQ, it seemed that the electron-donating groups at the 4 α -position of the isoquinoline ring (compounds **4–6**) were favorable for MOR–ligand interactions whereas the less rigid ring system (**11**) was disadvantageous for MOR binding.

There was around 10-fold decrease in the KOR binding affinity for NAQ compared to that of naltrexone. The longer spacer (compounds **1–3**) moderately enhanced ligands' KOR binding affinity compared to NAQ. Electron-withdrawing groups at 4 -position of the isoquinoline ring (compound **7**) and saturated ring system (compound **11**) slightly improved their KOR binding affinity, whereas electron-donating groups showed reduced KOR binding affinity except for compound **6**. Regarding to their MOR selectivity, it thus seemed that a longer spacer, electron-withdrawing groups at 4 -position, or a saturated ring system yielded decreased MOR selectivity over the KOR while electron-withdrawing groups at 1 -position (compounds **8** and **10**), and electron-donating groups at both 1 - and 4 -positions (compounds **4–6**, and **9**) gave comparable or improved MOR selectivity over the KOR, relative to NAQ. Compound **4** showed the highest MOR selectivity over the KOR in this series of compounds (nearly 10 times higher than that of naltrexone).

NAQ showed similar binding affinity as naltrexone at the DOR. A longer spacer (compounds **1–3**) and a saturated ring system (compound **11**) significantly improved ligand DOR binding affinity (> 13 fold). Electron-donating groups at 4 -position of the isoquinoline ring (compounds **4–6**) also modestly augmented DOR affinity, whereas an electron-withdrawing group at 4 -position (compound **7**) and substitutions at 1 -position (compounds **8–10**), regardless of their electronic characteristics, were only marginally in favor of DOR binding, compared to NAQ. With respect to their MOR selectivity, it thus appeared that a longer spacer and a saturated ring system were devastating for MOR selectivity over the DOR, while substitutions with different electronic properties at either 1 - or 4 -position of the isoquinoline ring were tolerant with over 20-fold MOR selectivity over the DOR. Being the most selective one in this series of ligands, compound **10** displayed a similar MOR selectivity over the DOR to NAQ.

The majority of NAQ analogues demonstrated less than 30% of MOR stimulation in the ³⁵S-GTP[S] binding assay, except for compounds **4** and **10** (Table 1). No conclusive SAR could be drawn except that electron-withdrawing groups at 1 -position of the isoquinoline ring (compounds **8** and **10**) seemed to promote more MOR activation than an electron-donating group (compound **9**). Compound **3** with the longest spacer carried the lowest efficacy in this series of compounds whereas compound **4** showed the highest efficacy on the MOR. To be noticed, NAQ and all its analogues were 1 to 2 order of magnitudes less potent than naltrexone in the ³⁵S-GTP[S] binding assay.

In conclusion, a series of NAQ analogues as 6 -naltrexamine derivatives, were synthesized for structure activity relationship studies with respect to the spacer length between the epoxymorphinan skeleton and the isoquinoline ring (side chain), electronic characteristics of the side chain, and rigidity of the side chain. Opioid receptor binding assays showed that a longer spacer and a less rigid side chain were unfavorable for MOR selectivity over the KOR and the DOR, whereas the electronic properties of the side chain need to be further fine-tuned to achieve higher MOR selectivity and lower MOR efficacy. Nevertheless, the current study identified four NAQ analogues (compounds **4**, **5**, **9**, and **10**) with 10-fold selectivity over the KOR and the DOR, which was comparable to that of cyprodime²⁵. Among them, compound **4** resembled to buprenorphine²⁶ in the *in vitro* functional studies as a MOR partial agonist while carrying greatly improved MOR selectivity, which may be beneficial for drug abuse and addiction treatment. Further studies to evaluate these new ligands for their signaling pathway of MOR stimulation and/or inhibition, and their *in vivo* pharmacological profiles are underway.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We are grateful for Drs. Lee-Yuan Liu-Chen (Temple University) and Ping-Yee Law (University of Minnesota) for the generous gift of opioid receptor expressing CHO cell lines. Y.Y. thanks Irma B. Adams and Joanna C. Jacob for their technical assistance on the biological assays. The work was funded by PHS grants from NIH DA024022 (YZ).

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Figure 1.
Naltrexone and some of its C6-substituted derivatives.

Scheme 1.
Two-step synthetic route of NAQ analogues.

Table 1

Binding affinity, selectivity and MOR ^{35}S -GTP[S] functional assay results for NAQ analogues^a

Compd	R	K_i (nM)			Selectivity		MOR ^{35}S -GTP[S] Binding	
		μ	μ	μ	$/\mu$	$/\mu$	EC_{50} (nM)	% E_{max} of DAMGO
NTX	NA	0.33 ± 0.02	1.44 ± 0.11	143.5 ± 13.7	4.4	435	0.16 ± 0.04	5.4 ± 0.8
NAQ		1.11 ± 0.07	13.3 ± 1.1	161.9 ± 15.0	12	146	3.3 ± 0.4	20.8 ± 1.2
1		1.20 ± 0.04	1.10 ± 0.15	12.5 ± 0.7	0.9	10	4.6 ± 0.6	18.6 ± 1.1
2		0.68 ± 0.05	1.61 ± 0.04	8.4 ± 0.7	2.4	12	1.14 ± 0.11	27.38 ± 0.35
3		2.7 ± 1.4	0.61 ± 0.04	9.2 ± 0.4	0.2	3.4	14.1 ± 4.1	13.9 ± 1.9
4		0.55 ± 0.01	22.2 ± 2.1	33.9 ± 0.5	40	62	1.74 ± 0.13	51.0 ± 0.4
5		0.73 ± 0.07	18.3 ± 1.9	17.4 ± 1.8	25	24	1.23 ± 0.09	19.8 ± 0.8
6		0.45 ± 0.02	4.0 ± 0.4	32.8 ± 1.5	8.9	73	1.06 ± 0.03	20.5 ± 0.9
7		1.11 ± 0.06	5.1 ± 0.3	78.8 ± 0.7	4.6	71	6.0 ± 1.5	21.6 ± 0.8

Compd	R	K_i (nM)			Selectivity		MOR ^{35}S -GTP[S] Binding	
		μ	μ	μ	$/\mu$	$/\mu$	EC_{50} (nM)	% E_{max} of DAMGO
8		1.26 ± 0.04	10.8 ± 1.2	79.8 ± 2.4	8.6	63	2.62 ± 0.38	26.4 ± 0.9
9		2.1 ± 0.2	29.1 ± 0.7	117.5 ± 7.3	14	56	7.2 ± 0.5	22.2 ± 0.4
10		0.99 ± 0.07	10.1 ± 0.5	129.9 ± 9.6	10	131	3.32 ± 0.24	37.5 ± 0.7
11		2.9 ± 1.3	6.76 ± 0.54	9.3 ± 0.3	2.3	3.2	19.4 ± 10.3	24.8 ± 1.3

^aThe values were the means $\pm$ S.E.M. of four independent experiments. [^3H]Naloxone, [^3H]Naltrindole, and [^3H]diprenorphine were used to label the MOR, the DOR and the KOR, respectively. The percentage stimulation to DAMGO was the E_{max} of the compound compared to that of DAMGO (normalized to 100%). Naltrexone (NTX), and NAQ were tested along under the same conditions.