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Design synthesis and structure–activity relationship of 5-substituted (tetrahydronaphthalen-2-yl)methyl with *N*-phenyl-*N*-(piperidin-2-yl) propionamide derivatives as opioid ligands

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Abstract

Here, we report the design, synthesis and structure activity relationship of novel small molecule opioid ligands based on 5-amino substituted (tetrahydronaphthalen-2-yl)methyl moiety with *N*-phenyl-*N*-(piperidin-2-yl)propionamide derivatives. We synthesized various molecules including amino, amide and hydroxy substitution on the 5th position of the (tetrahydronaphthalen-2-yl)methyl moiety. In our further designs we replaced the (tetrahydronaphthalen-2-yl)methyl moiety with benzyl and phenethyl moiety. These *N*-phenyl-*N*-(piperidin-2-yl)propionamide analogues showed moderate to good binding affinities (850–4 nM) and were selective towards the μ opioid receptor over the δ opioid receptors. From the structure activity relationship studies, we found that a hydroxyl substitution at the 5th position of (tetrahydronaphthalen-2-yl)methyl group, ligands **19** and **20**, showed excellent binding affinities 4 and 5 nM, respectively, and 1000 fold selectivity towards the μ opioid relative to the delta opioid receptor. The ligand **19** showed potent agonist activities 75 ± 21 nM, and 190 ± 42 nM in the GPI and MVD assays. Surprisingly the fluoro analogue **20** showed good agonist activities in MVD assays 170 ± 42 nM, in contrast to its binding affinity results.

Keywords

Opioids; Opioid receptors; Pain; *N*-Phenyl-*N*-(piperidin-2-yl)propionamide

1. Introduction

Opioids are widely used in the treatment of moderate to severe pain. For the past 60 years, society has relied on opioid drugs (i.e., morphine and its analogues) to alleviate both acute and chronic neuropathic pain. Over hundred million people in the United States suffer from chronic and neuropathic pain.¹ The morphine structure was discovered in 1803 by the

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Supplementary data

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German pharmacist Serturmer, but only in the last 40 years researchers have made important progress in opioid drug discovery^{2–13} based on the natural endogenous opioid ligands. Including the molecularly cloned three opioid receptors (μ , δ and κ) and have a better understanding of mechanism of action, and the recent crystallographic analysis of μ and other opioid receptors.¹⁴ Unfortunately, current treatments for chronic pain are only partially effective, and many cause dangerous side effects. The traditional opioid drugs like morphine and fentanyl and their analogues while producing an analgesic effect, can be addictive (often abused), toxic at higher doses, and their clinical use is often limited because of the onset of adverse side effects. The μ receptor is the major receptor responsible for triggering both effects analgesia as well as its undesirable side effects.¹⁵ With this in mind, there is an obvious necessity to develop novel opioid ligands which are devoid of the side effects of present opioids. The search for novel analgesics has been a constant effort in opioid research, and has become a hot topic in medicinal chemistry. The plant compound morphine is the central drug molecule in the opioid research, and progressive deconstruction of morphine skeleton resulted in several new classes of opioid ligands such as morphinans, benzomorphans, 4-phenylpiperidines, 4-anilino piperidines, *N*-phenylpiperazines, and methadone-type compounds.² Among the major classes of opioid ligands, the 4-anilidopiperidines have represented the most powerful class of synthetic analgesics. Fentanyl¹⁶ is the prototype of the 4-anilidopiperidine class of synthetic opioid analgesics. The present article describes the design, synthesis and structure activity relationship studies of *N*-phenyl-*N*-(piperidin-2-yl)propionamide based opioid ligands which are closely relevant to the 4-anilidopiperidine analogues.

During the last decade our group has shown promising progress in synthesis of novel opioid ligands based on enkephalin analogues conjugated to the different sites of the 4-anilidopiperidine core and fentanyl molecule.^{17–19} In continuation efforts of our group in opioid research, recently we reported 5-substituted tetrahydronaphthalen-2-yl)methyl containing a 4-anilidopiperidine moiety as potent opioid ligands²⁰ and further we extended our design strategy by making conjugation of enkephalin analogues with 4-anilidopiperidine small molecules as novel bivalent ligands.²¹ Neurosearch²² and Sepracor Inc.²³ extensively explored the piperidinyl-4-yl-methyl and piperidinyl-3-yl-methyl with substituted phenethyl moiety and various heterocyclic compounds on the '*N*' piperidine as potential novel opioid ligands. They explored the fourth and third position of the piperidine ring connectivity to the *N*-phenylpropionamide group and other heterocycles to make different class of anilidopiperidine derivatives. The previous successful exploration of opioid research from our group and consideration of the above patent literature, have led us to explore novel opioid ligands based on the 5-substituted (tetrahydronaphthalen-2-yl)methyl moiety with *N*-phenyl-*N*-(piperidin-2-yl)propionamide derivatives.

2. Design principle

The rationale behind choosing the 5-substituted (tetrahydronaphthalen-2-yl)methyl moiety in the present design is to study the effect of an the additional hydrophobic cyclohexyl group with hydroxyl and amine functional groups and variation in the *N*-phenylpropionamide connectivity to the piperidine ring, and an additional methylene group on opioid binding and selectivity. In our designs we replaced the 5-amino substituted (tetrahydronaphthalen-2-

yl)methyl moiety with benzyl and phenethyl groups which are closely related to the fentanyl molecule except for the variation in piperidine ring connectivity to the *N*-phenylpropionamide and additional methylene group. However *N*-phenyl-*N*-(piperidin-2-yl)propionamide derivatives based opioid ligands are unexplored in the literature, as potential opioid ligands for their structure activity relationship and represents a promising new approach in the design of novel opioid ligands Fig. 1).

3. Synthesis

3.1. amine and amide substituted analogues (13–15)

The synthesis of compounds **13**, **14** and **15** began with the reductive amination of Boc-protected piperidine-2-carbaldehyde **1** with aniline and difluoroaniline (Scheme 1). The resulting compounds **4** and **5** were treated with propionyl chloride in presence of triethylamine in dichloromethane afforded the alkylated compounds **6** and **7**.

Removal of the *tert*-butoxycarbonyl group with 50% trifluoroacetic acid in dichloromethane afforded the compounds **8** and **9**, and followed by reaction with iodo compound **10** in the presence of potassium carbonate gave the Boc-protected analogues **11** and **12**, respectively, in good yield. The iodo compound **10** was synthesized according to the previously reported literature procedure.²⁴ The final ligands **13** and **14** were obtained on the removal of the *tert*-butoxycarbonyl group with 50% trifluoroacetic acid in dichloromethane. The final compounds were washed with diethyl ether two to three times to obtain the pure compounds. The amine trifluoroacetate derivative **13** was transformed into the corresponding acetamide derivative **15** by treating with acetyl chloride in the presence of triethylamine in dichloromethane.

3.2. Hydroxyl substituted analogues (19–20)

Scheme 2 outlines the procedures used to synthesize the hydroxyl substituted (tetrahydronaphthalen-2-yl)methyl analogues **19** and **20**. The iodo compound **16** was prepared according to the procedure previously reported by us.²⁰ The iodo compound **16** was treated with *N*-phenyl-*N*-(piperidin-2-ylmethyl)propionamide analogues **8** and **9** in the presence of potassium carbonate in acetone yielded the tetrahydropyranylated protected analogues **17** and **18**. The final hydroxy compounds **19** and **20** were obtained by the removal of tetrahydropyranyl group with pyridinium *p*-toluenesulfonate in methanol at reflux temperatures. The hydroxyl analogues **19** and **20** containing (*S*)-stereochemistry at the 5th position of the tetrahydronaphthyl group and other ligands (**13**, **14** and **15**) have (*R*)-stereochemistry.

3.3. Benzyl and phenethyl derivatives (23–24)

The ligands **23** and **24** were prepared according to the Scheme 3. The benzyl bromide and 1-phenethyl bromide were treated with *N*-phenyl-*N*-(piperidin-2-ylmethyl)propionamide **8** in the presence of potassium carbonate and dimethylformamide at 80 °C afforded the final ligands **23** and **24**, respectively, in good yields.

4. Structure activity relationship studies

Opioid binding affinities (see Table 1) of the novel *N*-phenyl-*N*-(piperidin-2-yl)propionamide analogues for the human δ -opioid receptor (hDOR) or the rat μ -opioid receptor (rMOR) were determined by radioligand competition binding analysis using DPDPE to label the δ -opioid receptor and DAMGO to label the μ -opioid receptor in cell membrane preparations from transfected cells that stably express the respective receptor type.²⁵ The functional bioactivity profiles of selected ligands (see Table 1) were determined in the MVD and GPI/LMMP smooth muscle preparations as described previously.²⁶ IC₅₀ values, relative potency estimates, and their associated errors were determined by fitting the data to the Hill equation by a computerized non-linear least-square method. The synthesized analogues in (Table 1) display a broad range of binding affinities for μ opioid receptors (880–4 nM), and to a lesser extent no affinity towards the δ opioid receptor. The ligands **13** and **15** having amine and amide substitution at 5th position of the (tetrahydronaphthalen-2yl)methyl group, and the ligand **14** containing the fluoro substitution on the *N*-phenyl group. Ligands **13** and **14** showed good binding affinity at μ opioid receptor 69 nM and 33 nM, respectively, and introduction of a 'F' on *N*-phenyl two fold increase the binding affinity. In case of ligand **15** amide substitution on 5th position of (tetrahydronaphthalen-2yl)methyl moiety lost the binding affinity in comparative to the ligands **13** and **14** having amino substitution.

In our further designs **19** and **20** we replaced the amino group with hydroxyl substitution at the 5th position of the (tetrahydronaphthalen-2yl)methyl group, and in ligand **20** additionally introduction of a two 'F's on *N*-phenyl group. These ligands **19** and **20** showed excellent and similar binding affinities (4 nM and 5 nM) at μ opioid receptors, respectively. The introduction fluorine on *N*-phenyl group does not have any influence on binding affinity. The μ opioid receptor binding affinities (4 nM and 5 nM) of **19** and **20** were more than 1000-fold lower than their respective δ receptor binding affinities, and these two compounds showed similar μ/δ K_i ratios (Table 1). The second series of ligands **23** and **24**, we replaced the 5-substituted (tetrahydronaphthalen-2yl)methyl group with benzyl and phenethyl moieties, and both these ligands showed almost similar binding affinities (71 nM and 82 nM) at μ opioid receptor and did not show binding affinity towards the δ opioid receptor (Table 1). The second series of ligands **23** and **24** are structurally relevant to the fentanyl molecule except for the, variation in the piperidine ring connectivity to the *N*-phenylpropionamide and an additional methylene group, which is highly potent 4-anilidopiperidine drug molecule.

Among these novel opioid ligands, the hydroxyl substituted ligands **19** and **20** showed potent agonist activities in MVD and GPI assays, and other analogues **13**, **14** and **23** exhibited moderate to weak opioid agonist activity and not correlated to with their binding affinities results. Even though the ligand **19** showed excellent binding affinity 4 nM and almost 1000 fold selectivity towards the μ opioid receptor, surprisingly the ligand **19** showed good agonist activity (186.5 ± 42.2 nM and 74.86 ± 21.22 nM) in both MVD and GPI assays, respectively. Similarly the fluoro analogue **20** showed excellent binding affinity and selectivity towards μ opioid receptor and in functional assay it's showed good agonist activity in MVD assay and weak opioid activity in GPI assay (169.4 ± 41.6 nM and 50.4% at

1 μ M). The binding affinity is not correlated with the MVD and GPI assay results. In vivo studies of compound **19** are currently in progress and will be a subject of a separate report.

5. Conclusions

A series of *N*-phenyl-*N*-(piperidin-2-yl)propionamide derivatives with amine, hydroxyl and amide substituents in 5th position of (tetrahydronaphthalen-2yl)methyl moiety were synthesized and pharmacologically evaluated. The ligands **19** and **20** with hydroxyl substitutions 5th position of (tetrahydronaphthalen-2yl)methyl group showed excellent binding affinities and 1000 fold selectivity towards the μ opioid receptor. The amine and amide (**13**, **14** and **23**) substituents showed moderate to good binding affinity towards the μ opioid receptor and these ligands showed very weak no binding affinity towards the δ opioid receptor. Along with very good binding affinities, the ligand **19** showed good agonist activities in both MVD and GPI assays. The fluoro analogue **20** showed good agonist activities in MVD assays which are not correlated with its binding affinity results. Thus, this is the first time that *N*-phenyl-*N*-(piperidin-2-yl)propionamide derivatives have been described as opioid ligands, so that these compounds may represent a new class of μ opioid receptor ligands.

6. Experimental section

6.1. General considerations

All reactions were performed under N_2 unless otherwise noted. Melting points are uncorrected. All 1H NMR and ^{13}C NMR were recorded at 500 and 125 MHz, respectively, on a Bruker DRX 500. Chemical shifts were referred to TMS as internal standard in the case of $CDCl_3$ solution and to the residual proton signal of DMSO at 2.5 ppm in the case of DMSO- d_6 solution. The following abbreviations were used in reporting spectra: s = singlet, d = doublet, t = triplet, m = multiplet. Mass spectra were recorded on a Thermo Fisher LCQ ion trap mass spectrometer. High-resolution mass spectra (HRMS) were obtained using a Bruker Apex ion cyclotron resonance mass spectrometer in ESI+ mode. Unless otherwise mentioned, all of the solvents used were of LR grade. Usually, the flash chromatography was performed using 100–200 mesh silica gel. All of the organic extracts were dried over sodium sulfate after work up.

6.1.1. *tert*-Butyl 2-((phenylamino)methyl)piperidine-1-carboxylate (**4** Scheme 1)

—To a solution of *N*-Boc-piperidin-2-yl-formaldehyde **1** (0.8 g, 3.75 mmol) in 15 mL of dry dichloromethane was added aniline (0.51 mL, 5.62 mmol) and the mixture was stirred at room temperature for 30 min. Sodium triacetoxyborohydride (1.19 g, 5.62 mmol) was introduced in one portion, and the reaction mixture was stirred at room temperature overnight. The reaction mixture was diluted with dichloromethane (100 mL) and washed with 25 mL of aqueous potassium carbonate (2×25 mL) followed by brine (50 mL) and dried over anhydrous sodium sulfate. After the solvent was removed, the resultant oily residue was purified by a flash column chromatography (hexane/ethyl acetate) to afford *tert*-butyl 2-((phenylamino)methyl)piperidine-1-carboxylate **4** as a white colour solid (0.8 g, 80%); mp: 92–95 $^{\circ}C$. 1H NMR (499 MHz, Chloroform- d) δ 7.19–7.13 (m, 2H), 6.68 (m, 1H), 6.60–6.55 (m, 2H), 4.62–4.43 (m, 1H), 4.17–3.86 (m, 2H), 3.45 (t, J = 10.1 Hz, 1H),

3.18–3.01 (m, 1H), 2.79 (m, 1H), 1.77–1.59 (m, 5H), 1.57 (s, 1H), 1.45 (s, 9H). ^{13}C NMR (125 MHz, CDCl_3) δ 129.18, 116.96, 112.34, 79.72, 77.25, 77.20, 77.00, 76.75, 49.38, 28.42, 26.77, 25.36, 19.46.

6.1.2. *tert*-Butyl 2-(((3,4-difluorophenyl)amino)methyl)piperidine-1-carboxylate (5 Scheme 1)—Prepared as described for **4** from **1** (0.5 g, 2.34 mmol) and

3,4-difluoroaniline (0.45 g, 3.52 mmol) afforded the title compound as a light yellow colour solid (0.62 g, 81%); mp: 102–105 °C. ESI MS m/z (MH) $^+$ 327. ^1H NMR (499 MHz, Chloroform- d) δ 6.93 (m, 1H), 6.34 (m, 1H), 6.21 (m, 1H), 4.50 (s, 1H), 4.17–3.90 (m, 2H), 3.39 (m, 1H), 3.01 (m, 1H), 2.85–2.67 (m, 1H), 1.72–1.61 (m, 4H), 1.45 (s, 11H). ^{13}C NMR (125 MHz, CDCl_3) δ 151.86, 151.76, 149.91, 149.81, 145.23, 143.62, 143.52, 141.75, 141.64, 117.41, 117.40, 117.27, 117.25, 107.45, 107.43, 107.41, 107.38, 100.69, 100.53, 79.82, 79.81, 77.26, 77.00, 76.75, 49.18, 44.04, 39.12, 28.43, 28.35, 26.70, 26.69, 25.23, 25.18, 19.36.

6.1.3. *tert*-Butyl 2-((*N*-phenylpropionamido)methyl)piperidine-1-carboxylate (6 Scheme 1)—To a solution of *tert*-butyl 2-((phenylamino)methyl)piperidine-1-carboxylate **4** (0.85 g, 3.75 mmol) and triethylamine (1.0 mL, 9.37 mmol) in 15 mL of dry

dichloromethane was added propionyl chloride (0.31 mL, 4.68 mmol) drop wise at 0 °C. After being stirred at room temperature for 4 h, and the reaction mixture was diluted with dichloromethane (80 mL). The combined organic layers were washed with water and brine and dried over anhydrous sodium sulfate. After the solvent was removed, the resultant oily residue was purified by a flash column chromatography (hexane/ethyl acetate) to afford *tert*-butyl 2-((*N*-phenylpropionamido)methyl)piperidine-1-carboxylate **6** as a colour less liquid (0.8 g, 77%) MS (ESI) m/z (M+Na) $^+$: 369. ^1H NMR (499 MHz, Chloroform- d) δ 7.40 (dd, J = 8.4, 7.2 Hz, 2H), 7.37–7.29 (m, 2H), 7.22–7.08 (m, 1H), 4.41 (q, J = 19.3, 17.0 Hz, 2H), 3.94 (d, J = 13.7 Hz, 1H), 3.46–3.00 (m, 1H), 2.10–1.89 (m, 2H), 1.69–1.49 (m, 5H), 1.49–1.17 (m, 11H), 1.01 (t, J = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, CDCl_3) δ 173.78, 154.99, 142.93, 129.65, 127.79, 77.25, 77.00, 76.74, 48.17, 28.28, 27.76, 9.48.

6.1.4. *tert*-Butyl 2-((*N*-(3,4-difluorophenyl)propionamido)methyl)piperidine-1-carboxylate (7 Scheme 1)—Starting from **5** (0.62 g, 1.90 mmol) and propionyl chloride (0.19 mL, 2.28 mmol) and following the procedure described for **6**, compound **7** was obtained as a brown colour liquid (0.55 g, 75%). ESI MS m/z (MH) $^+$ 383. ^1H NMR (499 MHz, Chloroform- d) δ 7.20 (q, J = 9.0 Hz, 3H), 4.67–4.18 (m, 2H), 3.95 (d, J = 12.8 Hz, 1H), 3.27–3.02 (m, 1H), 2.10–1.88 (m, 2H), 1.73–1.51 (m, 5H), 1.51–1.22 (m, 11H), 1.03 (t, J = 7.5 Hz, 3H). ^{13}C NMR (126 MHz, CDCl_3) δ 173.48, 155.06, 151.36, 151.25, 150.91, 150.82, 149.35, 149.24, 148.82, 139.31, 139.25, 124.96, 118.01, 117.87, 79.25, 77.25, 77.20, 77.00, 76.74, 48.45, 48.01, 39.86, 28.33, 27.78, 27.25, 25.46, 19.56, 9.37.

6.1.5. 2-((*N*-Phenylpropionamido)methyl)piperidin-1-ium (8 Scheme 1)—To an ice-cold stirred solution of the **6** *tert*-butyl 2-((*N*-phenylpropionamido)methyl)piperidine-1-carboxylate (0.56 g, 1.61 mmol) in dichloromethane (4 mL) was added trifluoroacetic acid (4 mL). The resulting mixture was stirred for 2 h, and the solvent was stripped of under reduced pressure. To the resultant residue methanol was added couple of times and the

solvent was stripped off under reduced pressure and dried afforded the amine trifluoroacetate derivative **8** as a thick brown colour liquid (0.2 g, 74.9% of yield), MS (ESI) m/z (M+H)⁺: 247. ¹H NMR (499 MHz, DMSO-*d*₆) δ 8.70–8.61 (m, 1H), 8.59–8.47 (m, 1H), 7.52–7.41 (m, 4H), 7.41–7.36 (m, 1H), 4.10 (dd, J = 14.4, 8.5 Hz, 1H), 3.54 (dd, J = 14.4, 4.8 Hz, 1H), 3.34–3.23 (m, 1H), 3.14–3.01 (m, 1H), 2.85 (m, 1H), 2.11–1.89 (m, 2H), 1.77–1.64 (m, 3H), 1.64–1.49 (m, 1H), 1.44–1.33 (m, 2H). 0.90 (t, J = 7.4 Hz, 3H). ¹³C NMR (126 MHz, DMSO) δ 174.51, 159.61, 159.32, 159.04, 158.75, 142.82, 130.31, 128.90, 128.55, 119.90, 117.57, 115.25, 112.92, 55.39, 51.66, 44.82, 40.50, 40.42, 40.33, 40.26, 40.17, 40.09, 40.00, 39.92, 39.83, 39.67, 39.50, 27.70, 26.56, 22.11, 21.82, 9.54.

6.1.6. 2-((*N*-(3,4-Difluorophenyl)propionamido)methyl)piperidin-1-ium (**9**)

Scheme 1)—Starting from **7** (0.55 g, 1.43 mmol) and trifluoroacetic acid/dichloromethane (1:1) (8 mL) and following the procedure described for **8** and the compound **9** was obtained as a light brown colour low melting solid (0.57 g, 100% quantitative yield). ¹H NMR (499 MHz, DMSO-*d*₆) δ 8.65 (d, J = 10.9 Hz, 1H), 8.57–8.45 (m, 1H), 7.72–7.64 (m, 1H), 7.56 (m, 1H), 7.36 (m, 1H), 4.08 (dd, J = 14.6, 8.8 Hz, 1H), 3.50 (dd, J = 14.6, 4.5 Hz, 1H), 3.33–3.22 (m, 1H), 3.16–3.02 (m, 1H), 2.92–2.78 (m, 1H), 2.01 (m, 2H), 1.78–1.64 (m, 3H), 1.64–1.51 (m, 1H), 1.46–1.30 (m, 2H), 0.91 (t, J = 7.3 Hz, 3H). ¹³C NMR (126 MHz, DMSO) δ 174.41, 159.76, 159.49, 159.21, 158.94, 151.02, 150.91, 150.69, 150.59, 149.04, 148.94, 148.72, 148.62, 139.52, 139.49, 139.46, 139.43, 126.35, 120.39, 118.89, 118.74, 118.58, 118.05, 115.71, 113.36, 55.11, 51.71, 44.74, 40.50, 40.43, 40.33, 40.25, 40.17, 40.09, 40.00, 39.92, 39.83, 39.73, 39.67, 39.50, 27.66, 26.52, 22.08, 21.86, 9.37.

6.1.7. tert-Butyl ((1*R*)-6-((2-((*N*-phenylpropionamido)methyl)piperidin-1-yl)methyl)-1,2,3,4-tetrahydronaphthalen-1-yl)-carbamate (11** Scheme 1)**—To a solution of 2-((*N*-phenylpropionamido)methyl)piperidin-1-ium **8** (0.072 g, 0.25 mmol) potassium carbonate (0.089 g, 0.64 mmol) in acetone (8 mL) at 0 °C was added iodo compound **10** (0.1 g, 0.25 mmol). The resultant mixture was stirred for 4 h at room temperature. The reaction mixture was filtered through Whatman filter paper, and solvent was removed from the filtrate. The residue obtained upon evaporation of solvent was chromatographed over silica gel and eluted with 50% ethyl acetate/hexane to give the title compound **11** (0.08 g, 61%) as a light yellow colour solid. mp: 126–129 °C. MS (ESI) m/z (M+H)⁺: 506. ¹H NMR (499 MHz, Chloroform-*d*) δ 7.40–7.35 (m, 2H), 7.34–7.29 (m, 1H), 7.19–7.15 (m, 1H), 7.13 (m, 2H), 7.00–6.96 (m, 1H), 6.91–6.88 (m, 1H), 4.85–4.74 (m, 1H), 4.71 (d, J = 8.9 Hz, 1H), 4.04 (m, 1H), 3.92 (m, 1H), 3.80–3.69 (m, 1H), 3.35 (dd, J = 13.7, 4.2 Hz, 1H), 2.78–2.59 (m, 4H), 2.21 (m, 1H), 2.10–1.94 (m, 3H), 1.82–1.65 (m, 5H), 1.48 (s, 12H), 1.40–1.32 (m, 1H), 1.02 (t, J = 7.4 Hz, 3H). ¹³C NMR (126 MHz, CDCl₃) δ 173.74, 155.25, 142.93, 138.35, 136.81, 136.79, 135.32, 129.38, 128.86, 128.20, 128.17, 128.11, 128.10, 127.50, 126.34, 78.99, 77.25, 77.00, 76.74, 57.27, 57.19, 49.66, 48.80, 48.33, 30.43, 29.10, 28.31, 27.73, 27.40, 27.33, 23.98, 23.96, 21.88, 21.85, 19.81, 19.79, 9.48.

6.1.8. tert-Butyl ((1*R*)-6-((2-((*N*-(3,4-difluorophenyl)propionamido)methyl)piperidin-1-yl)methyl)-1,2,3,4-tetrahydronaphthalen-1-yl)carbamate (12** Scheme 1)**—Starting from **9** (0.19 g, 0.48

mmol) and iodo compound **10** (0.12 g, 0.32 mmol) and following the procedure described for **11**, compound **12** was obtained as a white colour solid (0.12 g, 68%). mp: 139–142 °C. MS (ESI) m/z (M+H)⁺: 542. ¹H NMR (499 MHz, Chloroform-*d*) δ 7.21 (dd, J = 7.6, 4.9 Hz, 1H), 7.19–7.11 (m, 1H), 7.00 (t, J = 7.3 Hz, 1H), 6.97–6.92 (m, 1H), 6.89 (s, 2H), 4.81 (d, J = 7.9 Hz, 1H), 4.76–4.65 (m, 1H), 3.92 (m, 2H), 3.72 (dd, J = 13.6, 4.4 Hz, 1H), 3.47–3.33 (m, 1H), 2.82–2.56 (m, 4H), 2.35–2.19 (m, 1H), 2.01 (q, J = 8.1 Hz, 3H), 1.79 (q, J = 7.8, 7.1 Hz, 3H), 1.74–1.58 (m, 2H), 1.48 (s, 13H), 1.03 (t, J = 7.3 Hz, 3H). ¹³C NMR (126 MHz, CDCl₃) δ 173.51, 155.40, 155.38, 149.19, 139.39, 138.40, 137.05, 135.67, 128.87, 128.39, 128.31, 126.38, 126.35, 124.70, 117.84, 117.70, 79.16, 77.26, 77.20, 77.00, 76.75, 57.05, 56.92, 56.87, 49.58, 48.88, 48.42, 30.54, 29.21, 28.40, 27.86, 26.91, 26.87, 23.57, 21.89, 19.94, 19.91, 9.45.

6.1.9. *N*-((1-(((*R*)-5-Amino-5,6,7,8-tetrahydronaphthalen-2-yl)methyl)piperidin-2-yl)methyl)-*N*-phenylpropionamide (13** Scheme 1)**—To an ice-cold stirred solution of the **11** (0.15 g, 0.29 mmol, 1 equiv) in dichloromethane (4 mL) was added trifluoroacetic acid (4 mL). The resulting mixture was stirred for 2 h, and the solvent was stripped of under reduced pressure and dried. The resultant residue was washed with diethyl ether a couple of times and dried afforded the pure amine trifluoroacetate derivative **13** as a light yellow colour solid (0.11 g, 73% of yield); mp: 145–148 °C. ESI MS m/z (MH)⁺ 406. HRMS [M+H]⁺ 406.28523 (theoretical 406.28529); ¹H NMR (499 MHz, DMSO-*d*₆) δ 11.12–10.89 (m, 1H), 8.60 (d, J = 5.9 Hz, 3H), 7.64–7.54 (m, 1H), 7.54–7.33 (m, 7H), 4.62 (m, 1H), 4.51–4.27 (m, 2H), 4.25–4.10 (m, 2H), 3.75–3.62 (m, 1H), 3.52–3.42 (m, 1H), 3.21 (bs, 1H), 3.06 (d, J = 13.0 Hz, 1H), 2.80–2.60 (m, 2H), 2.13–1.86 (m, 6H), 1.78–1.59 (m, 4H), 1.39–1.16 (m, 1H), 0.95–0.86 (m, 3H). ¹³C NMR (126 MHz, DMSO) δ 174.04, 142.71, 141.94, 138.51, 138.48, 138.32, 134.20, 132.75, 130.28, 130.21, 130.14, 130.10, 129.49, 129.45, 129.41, 129.26, 128.86, 128.80, 128.51, 73.29, 72.64, 71.00, 66.83, 61.08, 60.66, 55.45, 48.00, 47.97, 44.10, 40.61, 40.52, 40.45, 40.35, 40.28, 40.19, 40.11, 40.02, 39.94, 39.85, 39.69, 39.61, 39.52, 28.85, 27.79, 27.62, 27.60, 21.31, 18.65, 18.62, 9.80.

6.1.10. (1*R*)-6-((2-((*N*-(3,4-Difluorophenyl)propionamido)methyl)piperidin-1-yl)methyl)-1,2,3,4-tetrahydronaphthalen-1-aminium (14** Scheme 1)**—Starting from **12** (0.1 g, 0.18 mmol) and trifluoroacetic acid/dichloromethane (1:1 6 mL) and following the procedure described for **13**, compound **14** was obtained as a white colour solid (0.070 g, 70%); mp: 156–159 °C. ESI MS m/z 442 (MH)⁺. HRMS [M+H]⁺ 442.2663 (theoretical 442.2664); ¹H NMR (499 MHz, DMSO-*d*₆) δ 10.32–9.85 (m, 1H), 8.40 (s, 3H), 7.71–7.60 (m, 1H), 7.59–7.45 (m, 2H), 7.34 (m, 3H), 4.77–4.50 (m, 1H), 4.50–4.27 (m, 2H), 4.20–4.0 (m, 2H), 3.29–3.03 (m, 1H), 2.98–2.61 (m, 3H), 2.16–1.81 (m, 6H), 1.81–1.46 (m, 5H), 1.43–1.18 (m, 2H), 0.93 (p, J = 7.5, 6.7 Hz, 3H). ¹³C NMR (126 MHz, DMSO) δ 174.16, 174.09, 159.17, 158.92, 158.66, 158.41, 148.87, 139.13, 138.71, 134.30, 132.54, 132.44, 132.18, 131.90, 130.42, 130.25, 129.32, 129.25, 128.82, 128.72, 126.43, 126.03, 118.92, 118.76, 118.63, 118.49, 118.35, 116.26, 60.73, 55.55, 50.72, 49.21, 48.53, 48.07, 40.65, 40.59, 40.50, 40.43, 40.33, 40.26, 40.17, 40.09, 40.00, 39.83, 39.67, 39.50, 28.77, 27.76, 27.60, 27.55, 21.63, 20.88, 18.57, 18.53, 9.60, 9.58.

6.1.11. *N*-((1-(((*R*)-5-Acetamido-5,6,7,8-tetrahydronaphthalen-2-yl)methyl)piperidin-2-yl)methyl)-*N*-phenylpropionamide (15 Scheme 1)—To a solution of the amine trifluoroacetate derivative **13** *tert*-butyl ((1*R*)-6-((2-((*N*-phenylpropionamido)methyl)piperidin-1-yl) methyl)-1,2,3,4-tetrahydronaphthalen-1-yl)carbamate (0.16 g, 0.30 mmol) in dry dichloromethane (8 mL) at 0 °C under argon atmosphere was added triethylamine (0.12 mL, 0.92 mmol) followed by acetyl chloride (0.028 mL, 0.40 mmol) drop wise. The resultant reaction mixture was stirred at room temperature for 2 h. The reaction mixture was diluted with dichloromethane (50 mL) and washed with water and brine and dried over anhydrous sodium sulfate. The residue obtained upon evaporation of solvent was chromatographed over silica gel and eluted with 70% ethyl acetate/hexane to give the title compound acetamido derivative **15** (0.1 g, 72%) as a light yellow colour thick liquid. ESI MS m/z (MH)⁺ 448. HRMS [M+H]⁺ 448.2959 (theoretical 448.2958); ¹H NMR (499 MHz, Chloroform-*d*) δ 7.41–7.35 (m, 2H), 7.34–7.29 (m, 1H), 7.14 (m, 2H), 6.95 (d, *J* = 8.8 Hz, 2H), 6.83 (dd, *J* = 7.7, 4.8 Hz, 1H), 5.78 (m, 1H), 3.98 (m, 2H), 3.73 (d, *J* = 13.8 Hz, 1H), 3.35 (d, *J* = 13.8 Hz, 1H), 2.87–2.60 (m, 4H), 2.41–2.10 (m, 5H), 2.10–1.95 (m, 4H), 1.85–1.63 (m, 3H), 1.49–1.30 (m, 4H), 1.02 (t, *J* = 7.4 Hz, 3H). ¹³C NMR (126 MHz, CDCl₃) δ 174.15, 174.14, 173.89, 143.05, 143.02, 138.03, 136.88, 136.87, 134.59, 129.52, 129.38, 129.33, 128.20, 127.61, 126.83, 124.67, 77.25, 77.20, 77.00, 76.96, 76.74, 57.63, 57.18, 54.06, 54.02, 49.78, 49.70, 49.11, 49.01, 29.38, 28.92, 27.85, 27.57, 26.99, 24.01, 22.95, 22.94, 22.02, 21.99, 9.57.

6.1.12. *N*-Phenyl-*N*-((1-(((5*S*)-5-((tetrahydro-2*H*-pyran-2-yl)oxy)-5,6,7,8-tetrahydronaphthalen-2-yl)methyl)piperidin-2-yl)methyl) propionamide (17 Scheme 2)—Starting from **8** (0.18 g, 0.50 mmol) and iodo compound **16** (0.12 g, 0.33 mmol) and following the procedure described for **11**, compound **17** was obtained as a colour less liquid (0.11 g, 68%). ESI MS m/z (MH)⁺ 491. ¹H NMR (499 MHz, Chloroform-*d*) δ 7.36 (q, *J* = 7.4 Hz, 2H), 7.30 (t, *J* = 7.4 Hz, 1H), 7.16–7.09 (m, 3H), 7.00 (dd, *J* = 14.4, 8.0 Hz, 1H), 6.92 (d, *J* = 8.5 Hz, 1H), 4.92–4.74 (m, 1H), 4.72–4.60 (m, 1H), 4.10–3.92 (m, 3H), 3.79–3.68 (m, 1H), 3.63–3.51 (m, 1H), 3.36 (d, *J* = 13.7 Hz, 1H), 2.82–2.54 (m, 4H), 2.29–2.13 (m, 1H), 2.11–1.80 (m, 6H), 1.80–1.31 (m, 12H), 1.03 (t, *J* = 7.4 Hz, 3H). ¹³C NMR (126 MHz, CDCl₃) δ 173.88, 143.06, 138.86, 137.22, 137.09, 135.34, 135.08, 129.49, 129.17, 129.07, 128.80, 128.71, 128.68, 128.22, 127.57, 126.27, 125.82, 98.95, 98.93, 95.33, 95.28, 77.25, 77.20, 77.00, 76.89, 76.75, 73.75, 73.72, 70.59, 70.56, 62.67, 62.65, 62.52, 62.50, 57.65, 57.60, 57.55, 57.50, 49.76, 49.14, 31.11, 31.09, 30.67, 30.29, 29.66, 29.27, 28.92, 27.88, 27.65, 27.61, 27.58, 25.58, 25.56, 24.10, 24.07, 22.08, 22.05, 19.87, 19.86, 19.62, 19.60, 19.04, 19.01, 18.89, 18.88, 9.61.

6.1.13. *N*-(3,4-Difluorophenyl)-*N*-((1-(((5*S*)-5-((tetrahydro-2*H*-pyran-2-yl)oxy)-5,6,7,8-tetrahydronaphthalen-2-yl)methyl)piperidin-2-yl)methyl)propionamide (18 Scheme 2)—Starting from **9** (0.22 g, 0.56 mmol) and iodo compound **16** (0.15 g, 0.40 mmol) and following the procedure described for **11**, compound **18** was obtained as a colour less liquid (0.15 g, 71%). MS (ESI) m/z (M+H)⁺: 527. ¹H NMR (499 MHz, Chloroform-*d*, 1:1 diastereomeric ratio) δ 7.37 (dd, *J* = 7.9, 3.9 Hz, 0.5H), 7.19–7.10 (m, 1.5H), 7.05–6.95 (m, 2H), 6.91 (dt, *J* = 11.3, 3.0 Hz, 1H), 6.87–6.79 (m, 1H), 4.92–4.87 (m, 0.5H), 4.86–4.82 (m, 0.5H), 4.80 (t, *J* = 4.9 Hz, 0.5H), 4.69 (t, *J*

= 4.8 Hz, 0.5H), 4.09–3.92 (m, 2H), 3.87 (m, 1H), 3.72 (dd, J = 13.6, 2.9 Hz, 1H), 3.70–3.60 (m, 1H), 3.40 (d, J = 13.6 Hz, 1H), 2.80–2.70 (m, 2H), 2.69–2.60 (m, 2H), 2.27 (m, 1H), 2.08–1.95 (m, 3H), 1.95–1.81 (m, 2H), 1.80–1.35 (m, 13H), 1.03 (t, J = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, CDCl_3) δ 173.57, 151.27, 151.16, 149.26, 149.16, 148.59, 139.40, 138.68, 138.42, 137.36, 137.24, 135.58, 135.36, 129.29, 129.05, 128.79, 128.75, 126.26, 125.81, 124.77, 117.91, 117.79, 117.67, 99.10, 99.09, 95.43, 95.36, 77.26, 77.21, 77.00, 76.93, 76.75, 73.85, 70.58, 70.55, 62.69, 62.62, 62.56, 57.25, 57.10, 57.04, 56.88, 49.62, 49.10, 48.98, 31.13, 31.11, 31.10, 30.69, 29.69, 29.30, 29.27, 28.96, 28.94, 27.90, 27.66, 27.63, 26.98, 26.93, 25.58, 23.60, 22.02, 21.95, 19.86, 19.69, 19.63, 19.07, 19.05, 18.87, 9.50.

6.1.14. *N*-((1-(((*S*)-5-Hydroxy-5,6,7,8-tetrahydronaphthalen-2-yl)methyl)piperidin-2-yl)methyl)-*N*-phenylpropionamide (19 Scheme 2)—To a solution of **17** *N*-phenyl-*N*-((1-(((*S*)-5-((tetrahydro-2*H*-pyran-2-yl)oxy)-5,6,7,8-tetrahydronaphthalen-2-yl)methyl)piperidin-2-yl)methyl)propionamide (0.13 g, 0.26 mmol) in dry methanol (10 mL) under argon atmosphere was added pyridinium *p*-toluenesulfonate (0.019 g, 0.07 mmol) and the resulting mixture was heated to reflux temperature for 3 h. The reaction mixture was allowed to cool to room temperature and the solvent was evaporated under reduced pressure. The residue obtained upon evaporation of solvent was chromatographed over silica gel and eluted with 6% methanol/dichloromethane to give the title product **19** as a white colour sticky solid (0.06 g, 56% yield) ESI MS m/z (MH)⁺ 407. HRMS [M+H]⁺ 407.2691 (theoretical 407.2693); ^1H NMR (499 MHz, Chloroform-*d*) δ 7.38 (t, J = 7.5 Hz, 2H), 7.29 (dd, J = 28.7, 6.4 Hz, 2H), 7.14 (d, J = 7.6 Hz, 2H), 7.03 (d, J = 7.9 Hz, 1H), 6.94 (s, 1H), 4.75 (d, J = 4.7 Hz, 1H), 3.99 (m, 2H), 3.75 (d, J = 13.7 Hz, 1H), 3.37 (d, J = 13.8 Hz, 1H), 2.86–2.57 (m, 4H), 2.21 (dd, J = 12.7, 6.2 Hz, 1H), 2.12–1.82 (m, 4H), 1.72 (m, 5H), 1.55–1.20 (m, 4H), 1.03 (t, J = 7.5 Hz, 3H). ^{13}C NMR (126 MHz, CDCl_3) δ 173.98, 143.09, 137.17, 136.74, 129.53, 129.05, 128.37, 128.24, 127.63, 126.54, 77.25, 77.00, 76.82, 76.75, 67.93, 57.65, 57.45, 49.82, 49.14, 32.32, 29.25, 27.89, 27.59, 24.07, 22.05, 18.79, 18.77, 9.61.

6.1.15. *N*-(3,4-Difluorophenyl)-*N*-((1-(((*S*)-5-hydroxy-5,6,7,8-tetrahydronaphthalen-2-yl)methyl)piperidin-2-yl)methyl)propionamide (20 Scheme 2)—Starting from **18** (0.13 g, 0.24 mmol, 1 equiv) and pyridinium *p*-toluenesulfonate (62 mg, 0.40 mmol) and following the procedure described for **19**, compound **20** was obtained as a white colour flaky solid (65 mg, 60% yield). Mp: 110–112 °C. MS (ESI) m/z (M+H)⁺: 443 (MH)⁺. HRMS [M+H]⁺ 443.2503 (theoretical 443.2493); ^1H NMR (499 MHz, Chloroform-*d*) δ 7.30 (dd, J = 7.8, 3.3 Hz, 1H), 7.15 (q, J = 9.0 Hz, 1H), 7.04 (m, 1H), 6.99–6.91 (m, 2H), 6.91–6.85 (m, 1H), 4.75 (t, J = 4.8 Hz, 1H), 4.00–3.84 (m, 2H), 3.74 (d, J = 13.7 Hz, 1H), 3.41 (d, J = 13.7 Hz, 1H), 2.82–2.59 (m, 4H), 2.35–2.21 (m, 1H), 2.07–1.84 (m, 4H), 1.84–1.62 (m, 5H), 1.56–1.34 (m, 4H), 1.03 (t, J = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, CDCl_3) δ 173.59, 151.20, 151.10, 149.20, 149.09, 139.36, 138.77, 137.36, 136.80, 128.90, 128.41, 128.38, 126.41, 124.71, 117.87, 117.81, 117.73, 117.67, 77.26, 77.20, 77.00, 76.75, 67.86, 57.15, 56.95, 56.84, 49.61, 48.95, 48.86, 32.31, 29.23, 27.86, 26.87, 23.57, 23.53, 21.90, 21.87, 18.80, 18.78, 9.46.

6.1.16. *N*-((1-Benzylpiperidin-2-yl)methyl)-*N*-phenylpropionamide (23 Scheme

3)—To a solution of **8** 2-((*N*-phenylpropionamido)methyl)piperidin-1-ium (0.1 g, 0.35 mmol), in dry DMF (6 mL) under argon atmosphere was added potassium carbonate (0.14 g, 1.06 mmol), followed by benzyl bromide (0.12 mL, 1.06 mmol). The resulting mixture was stirred at 80 °C for 5 h. The reaction mixture was allowed to cool to room temperature and the solvent was evaporated under reduced pressure. The residue obtained upon evaporation of solvent was diluted with ethyl acetate (60 mL) washed with water and brine and dried over sodium sulfate. After the solvent was removed, the resultant oily residue was purified by a flash column chromatography (hexane/ethyl acetate) to afford *N*-((1-benzylpiperidin-2-yl)methyl)-*N*-phenylpropionamide **23** as light brown colour thick liquid (0.07 g, 65% yield) ESI MS m/z (MH)⁺. 337; HRMS [M+H]⁺ 337.2273 (theoretical 337.2274); ¹H NMR (499 MHz, Chloroform-*d*) δ 7.40–7.35 (m, 2H), 7.34–7.29 (m, 1H), 7.24–7.16 (m, 5H), 7.15–7.11 (m, 2H), 4.03 (dd, J = 13.5, 8.2 Hz, 1H), 3.96 (dd, J = 13.5, 4.7 Hz, 1H), 3.79 (d, J = 13.8 Hz, 1H), 3.43 (d, J = 13.8 Hz, 1H), 2.78–2.63 (m, 2H), 2.23 (m, 1H), 2.03 (q, J = 7.4 Hz, 2H), 1.78–1.63 (m, 2H), 1.52–1.32 (m, 4H), 1.03 (t, J = 7.4 Hz, 3H). ¹³C NMR (126 MHz, CDCl₃) δ 173.88, 143.05, 139.63, 129.50, 128.49, 128.20, 127.95, 127.58, 126.47, 77.25, 77.00, 76.74, 57.70, 57.46, 49.68, 48.95, 27.84, 27.61, 24.13, 21.96, 9.56.

6.1.17. *N*-((1-Phenethylpiperidin-2-yl)methyl)-*N*-phenylpropionamide (24

Scheme 3)—Starting from **8** (0.1 g, 0.27 mmol) and 2-phenylethylbromide **22** (0.05 mL, 0.41 mmol) and following the procedure described for **23**, compound **24** was obtained as a light brown colour liquid (0.065 g, 67%). ESI MS m/z (MH)⁺ 351. HRMS [M+H]⁺ 351.2432 (theoretical 351.2430); ¹H NMR (499 MHz, Chloroform-*d*) δ 7.42–7.37 (m, 2H), 7.34–7.29 (m, 1H), 7.27–7.22 (m, 2H), 7.16 (m, 3H), 7.09–7.05 (m, 2H), 4.03 (dd, J = 13.4, 8.9 Hz, 1H), 3.83 (dd, J = 13.4, 4.3 Hz, 1H), 2.82 (m, 1H), 2.67 (m, 5H), 2.44 (m, 1H), 2.04 (q, J = 7.4 Hz, 2H), 1.72 (m, 2H), 1.60–1.47 (m, 2H), 1.47–1.32 (m, 2H), 1.03 (t, J = 7.4 Hz, 3H). ¹³C NMR (126 MHz, CDCl₃) δ 174.04, 143.24, 140.58, 129.56, 128.70, 128.25, 128.22, 127.68, 125.80, 77.25, 77.20, 77.00, 76.74, 56.78, 55.61, 50.37, 48.83, 33.13, 27.91, 27.81, 24.59, 22.16, 9.60.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

GPCR	G protein-coupled receptor
MOR	Mu opioid receptor
DOR	delta opioid receptor
Boc	<i>tert</i> -butyloxycarbonyl

NaOtBu	sodium <i>tert</i> -butoxide
Et₃N	triethylamine
HCl	hydrochloric acid
BH₃-SMe₂	borane dimethylsulfide
DHP	dihydropyran
PPTS	pyridinium <i>p</i> -toluenesulfonate
Ph₃P	triphenylphosphine
Na(OAc)₃BH	sodium triacetoxyborohydride
DCE	dichloroethane
DIPEA	<i>N,N</i> -diisopropylethylamine
ACN	acetonitrile
DCM	dichloromethane
rt	room temperature
CHO	Chinese hamster ovary
D-ALEA	[D-Ala ² ,Leu ⁵]enkephalin amide
hDOR	human δ opioid receptor
DPDPE	c[D-Pen ² ,DPen ⁵]enkephalin
D-AMGO	[D-Ala ² ,NMePhe ⁴ ,Gly ⁵ -ol]enkephalin
Dmt	2,6-dimethyltyrosine
Tyr	tyrosine
GPI	guinea pig isolated ileum
LMMP	longitudinal muscle with myenteric plexus
rMOR	rat μ opioid receptor
MVD	mouse vas deferens
RP-HPLC	reverse phase high performance liquid chromatography
HRMS	high resolution mass spectrometry
SAR	structure–activity relationship
TFA	trifluoroacetic acid
TLC	thin layer chromatography

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Figure 1.
Design principle of novel opioid ligands.

Scheme 1.

Preparation of amine and amide substituted analogues. Reagents and conditions: (a) $\text{Na}(\text{OAc})_3\text{BH}$, Na_2SO_4 , DCM, rt; (b) propionyl chloride, Et_3N , DCM, rt; (c) 50% trifluoroacetic acid in DCM 0 °C–rt, 2 h; (d) K_2CO_3 , acetone, rt, 4 h; (e) acetyl chloride, Et_3N , DCM, rt.

Scheme 2.

Preparation of hydroxyl substituted analogues. Reagents and conditions: (a) K_2CO_3 , acetone, 4 h, rt; (b) PPTS, MeOH, reflux temperature, 4 h.

Scheme 3.

Preparation of benzyl and phenethyl derivatives. Reagents and conditions: (a) K_2CO_3 , DMF, 80 °C, 5 h.

Table 1

Binding affinities and in vitro opioid activity profiles of amine, amide and hydroxyl substituted analogues 13-24

Compd	Binding K_i (nM)				K_i ratio μ/δ	MVD (δ) ^c	GPI (μ) ^c
	Log IC ₅₀ ^b	MOR (μ) ^a	Log IC ₅₀ ^b	DOR (δ) ^a			
13	-6.83 ± 0.09	69	-4.92 ± 0.09	5200	1/79	18% at 1 μ M	1005 ± 58
14	-7.25 ± 0.09	33	-4.72 ± 0.70	8950	1/271	22% at 1 μ M	10% at 1 μ M
15	-5.70 ± 0.16	880	-4.87 ± 0.05	5900	1/7	nd	nd
19	-8.16 ± 0.06	4	-5.11 ± 0.13	3702	1/925	186 ± 42.2	74.86 ± 21.22
20	-7.89 ± 0.12	5	-5.41 ± 0.13	3700	1/736	169 ± 41.6	50.4% at 1 μ M
23	-6.49 ± 0.14	140	-4.95 ± 0.12	4100	1/81	36.2% at 1 μ M	29.8% at 1 μ M
24	-6.85 ± 0.04	82	-4.91 ± 0.10	5800	1/71	nd	nd
Fentanyl		5.9		570		9.4 ± 4.0	3.4 ± 0.4

^aCompetition analyses against radiolabeled ligand ([³H]DPDPE for DOR δ [³H]DAMGO for μ) were carried out using rat brain membranes.

^bLogarithmic values determined from the nonlinear regression analysis of data collected from at least two independent experiments.

^cConcentration at 50% inhibition of muscle contraction at electrically stimulated isolated tissues.