Pergamon

SCIENCE DIRECT®

Bioorganic & Medicinal Chemistry Letters 13 (2003) 1585–1589

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

New Scaffolds in the Development of Mu Opioid-Receptor Ligands

Daniel Pagé,[a,*] Natalie Nguyen,[a] Sylvain Bernard,[a] Martin Coupal,[b] Mylène Gosselin,[b] Julie Lepage,[b] Lynda Adam[b] and William Brown[a]

[a]*Department of Chemistry, AstraZeneca R&D Montreal, 7171 Frederick-Banting, Saint-Laurent, Quebec, Canada H4S 1Z9*
[b]*Department of Molecular Pharmacology, AstraZeneca R&D Montreal, 7171 Frederick-Banting, Saint-Laurent, Quebec, Canada H4S 1Z9*

Received 2 December 2002; accepted 23 January 2003

Abstract—A new class of μ selective receptor antagonists has been developed using a combinatorial approach based on previously reported Dmt-Tic dipeptide ligands. Modified tetrahydroisoquinoline (Tiq) residues were reacted with different electrophiles in order to create novel molecules that would mimic the original dipeptide. A specific class of thioureas bearing basic pyrrolidine residues were shown to give good binding affinities. Further alkylation of the pyrrolidine ring with benzyl derivatives also proved to increase the μ binding affinity. In addition, it was demonstrated that μ binding was enhanced by the presence of polar groups around the benzyl ring having hydrogen-bonding character (donor/acceptor). This new class of ligands represents a novel scaffold in the development of opioid analogues.

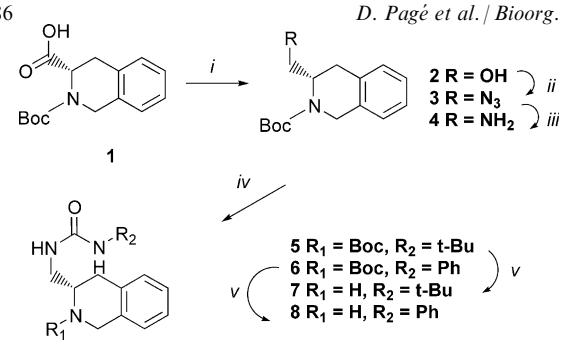
The development of selective agonists and antagonists toward the δ, μ and κ-opioid receptors has always been slowed down by the lack of knowledge about the geometry of their binding pocket. While most opioid agonists[1] have mainly been used in pain-relieving processes,[2] the opioid antagonists[3] could have potential applications in the treatment of different drug addictions, such as cocaine.[4] However, their complete mechanisms of action are not fully understood since the direct evaluation of the ligand-binding site interactions for such G-coupled protein receptors is rather complicated. One way in which to investigate the structure of these receptors is to look at their binding pocket with a specific class of ligands even though the structural diversity of the reported opioid ligands could suggest a relative degree of plasticity for these receptors.

One particular class of reported opioid ligands was that containing the Dmt-Tic[5] (Dmt: 2′,6′-dimethyltyrosine; Tic: 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid) pharmacophore. This synthetic dipeptide was the direct result of decades of studies around truncated enkephalin peptides. The introduction of the Dmt moiety in various opioid ligands produced different analogues with enhanced binding affinities toward mostly the δ and μ receptors.[5–7] Several modifications around this pharmacophore have been reported such as *N*-alkylations,[8] substitutions around the aromatic ring of Tic[9,10] or the replacement of Tic by various heteroaromatic residues,[11,12] which all produced altered pharmacological profiles for δ and μ. Recent studies involving C-terminus modifications demonstrated that the insertion of large hydrophobic residues could further alter the bioactivity of these ligands, producing ligands with dual activity profiles at the opioid receptors.[13–17] More specifically, previous work reported by our group[14] showed that the insertion of *t*-butyl or phenyl groups with a urea/thiourea linker at the C-terminus could result in ligands possessing δ or μ agonism.

Some modifications around the Dmt moiety of the pharmacophore have also been reported. A few reported substitutions around the Dmt (or Tyr) aromatic ring were shown to lead mostly in decreased activity.[18] Another more drastic change involved the substitution of the Dmt N-terminal amine by a methyl group[19,20] and other hydrophobic substituents[21] that, when incorporated into peptides, still managed to produce ligands with potent opioid antagonist properties. However, to our knowledge, no complete substitution of the Dmt moiety has been put forward yet. The introduction of different nuclei could be a starting point for the development of new types of opioid analogues and could give us further insight into the binding domain of opioid receptors. Incorporation of the C-terminus substituents mentioned above[14] could also benefit those ligands in

*Corresponding author. Tel.: +1-514-832-3200; fax: +1-514-832-3232; e-mail: daniel.page@astrazeneca.com; http://www.astrazeneca.ca/E/montreal

0960-894X/03/$ - see front matter © 2003 Elsevier Science Ltd. All rights reserved.
doi:10.1016/S0960-894X(03)00194-X

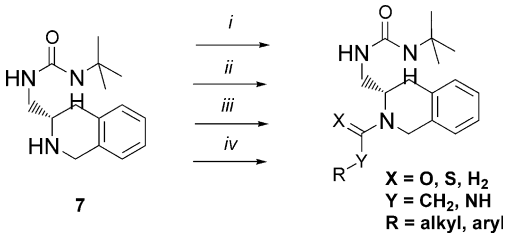


Scheme 1. (i) (a) Isobutyl chloroformate, NMM, DME, 0 °C, 5 min; (b) $NaBH_4$, H_2O, 0 °C, 15 min; (ii) PPh_3, DEAD, DPPA, THF, 0 °C to rt, 4 h; (iii) H_2, 10% Pd/C, MeOH, 12 h; (iv) RNCO, TEA, DCM, rt, 2 h; (v) 1M HCl/AcOH, rt, 1 h.

terms of increased binding affinities. Therefore, we report herein the synthesis and pharmacological characterization of a new series of ligands based on the original Dmt-Tic pharmacophore where C-terminus modified tetrahydroisoquinoline (Tiq) residues were reacted with different electrophiles used as potential Dmt replacements.

A combinatorial synthesis approach was used in order to generate an initial library of about 500 compounds. The C-terminus modified Tiq derivative could easily be prepared from commercially available Boc-L-Tic (**1**) (Scheme 1). The carboxylic acid was reduced to the corresponding alcohol (**2**) using a previously reported mixed anhydride procedure.[22] The alcohol (**2**) was then transformed to the azide (**3**) using standard Mitsunobu conditions. Catalytic hydrogenation afforded the corresponding amine (**4**), which was reacted directly with either *t*-butylisocyanate or phenyl isocyanate to obtain the Boc-protected urea derivatives **5** and **6**. Removal of the nitrogen protecting-group under acidic conditions afforded the final tetrahydroisoquinoline derivatives **7** and **8**.

The *t*-butyl Tiq analogue (**7**) was then reacted with a variety of commercially available electrophiles such as acids, acid chlorides, isocyanates, isothiocyanates and aldehydes to generate libraries of dipeptide analogues that included scaffolds such as aromatic, heteroaromatic, alkanes or cycloalkanes (Scheme 2). Most of the compounds generated this way could be obtained in >85% purity after simple washings and without any further purification. Their opioid receptor binding profiles were then determined. All tests were done at least in triplicate.



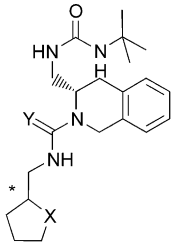
Scheme 2. (i) RCOCl, TEA, DCE, rt, 24 h; (ii) RCOOH, HATU, DIPEA, DMF, rt, 24 h; (iii) RNCO/RNCS, TEA, DCE, rt, 24 h; (iv) RCHO, THF, BH_3·pyr, rt, 24 h.

Interesting hits were obtained particularly in the thiourea class of compounds (Table 1). The presence of a 2-tetrahydrofuran (**9**) moiety resulted in improved binding affinities for all three opioid receptors. When the corresponding isomers (**10** and **11**) were synthesized, the *R* enantiomer was shown to bring the δ binding affinity to 53 nM, but the analogous urea derivative (**12**) lost substantial δ binding affinity. Subsequent replacement of the tetrahydrofuran by a pyrrolidine ring (**13**) increased the μ-binding affinity while slightly decreasing δ. Since this pyrrolidine moiety introduced a basic nitrogen, it appeared to be a more promising scaffold in terms of possible chemistry, and was therefore used as an anchor for the subsequent synthesis of our new class of Tiq derivatives.

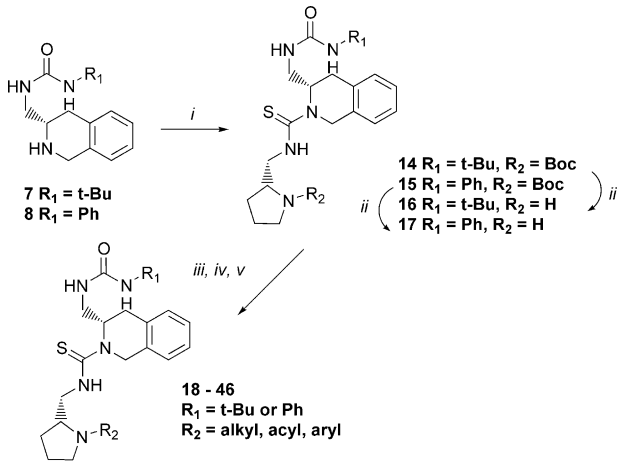
Different analogues were prepared according to Scheme 3. The C-terminus modified Tiq ureas **7** or **8** were reacted with the 2-Boc-pyrrolidine isothiocyanate that was generated in situ from the corresponding 2-*R*-(aminomethyl)-1-Boc-pyrrolidine and thiophosgene to form compounds **14** and **15**. Removal of the Boc groups resulted in amines **16** and **17**, which were then either alkylated or acylated to give products **18**–**24** (Table 2).

From these results, it was determined that any substitution on the pyrrolidine nitrogen was detrimental to δ binding but an aromatic substitution (**20**) increased μ-binding affinity (K_i = 23.2 nM). Furthermore, it appeared that the basic nitrogen was required to retain μ-binding affinity as derivatives **22** and **23** bearing the benzoate and benzyl urea moieties respectively, lost μ affinity. This observation is consistent with most of the previously reported opioid ligands. However, the δ and κ binding affinities were comparable to those observed for the receptor μ. It was observed that the chirality of the pyrrolidine did not seem to influence greatly the μ binding since the corresponding *S* isomer of **24** showed an almost identical μ affinity of 31.3 nM. These results prompted us to focus on this specific class as μ ligands. The Tiq derivative bearing a phenyl urea was chosen as a template since our previous work[14] revealed this group to be a preferred motif for μ-binding. This Tiq-pyrrolidine derivative was reacted with different aldehydes under reductive amination conditions, affording

Table 1. Binding affinities of generated hits to opioid receptors



Compd	X	Y	*	μ K_i (nM)	δ K_i (nM)	κ K_i (nM)
9	O	S	R/S	685±127	308±12	481±84
10	O	S	R	303±26	52.7±3.6	511±72
11	O	S	S	>1000	>1000	>1000
12	O	O	R	392±94	649±28	>1000
13	NH	S	R	85.5±5.4	159±19	>1000

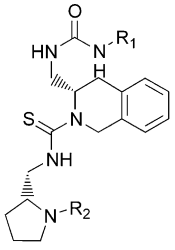


Scheme 3. (i) 2-*R*-(aminomethyl)-1-Boc-pyrrolidine, $CSCl_2$, DIPEA, rt, 1 h; (ii) 1 M HCl/AcOH, rt, 1 h; (iii) RCHO, $NaBH(OAc)_3$, AcOH, THF, rt, 12 h; (iv) RCOCl, TEA, DCM, rt, 1 h; (v) RNCO, TEA, DCM, rt, 1 h.

compounds **25–46** (Scheme 3).[23] The results are summarized in Table 3. The δ and κ binding affinities were included for compounds having K_i values for μ <10 nM in order to assess the relative selectivity.

From these results, it was shown that the introduction of polar groups, especially those with hydrogen bonding character (donor/acceptor) such as OMe and OH, greatly increased the binding affinities toward the μ receptor. Position 4 of the aromatic ring seemed specifically sensitive to hydrogen-bond donating groups, such as those in compounds **27** and **37** bearing phenol and *N*-acetate groups, which possessed binding affinities of 6.0 and 10.3 nM, respectively. The best *ortho* substituent was the OMe group (compound 28, $K_i = 8.8$ nM). Other aromatic systems such as naphthalene (**38**), furan (**39**), thiophene (**40**), and pyridine (**41**) did not greatly influence the binding. It seems that anything planar, but not too bulky, with an aromatic-type character can be accommodated in the binding pocket of the receptor. The best result was obtained with a combination 2-OMe and 4-OH groups (**46**), increasing the μ binding affinity to 1.1 nM.

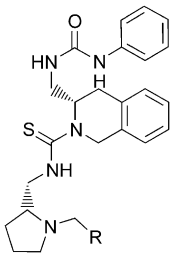
Table 2. Binding affinities of tetrahydroisoquinoline-pyrrolidine derivatives to opioid receptors



Compd	R_1[a]	R_2[a]	μ K_i (nM)	δ K_i (nM)	κ K_i (nM)
Dmt-Tic			945±211	1.8±0.6	>1000
14	*t*-Bu	Boc	>1000	330±41	>1000
16	*t*-Bu	H	85.5±5.4	159±19	>1000
17	Ph	H	38.2±4.7	554±12	310±21
18	*t*-Bu	Me	176±13	>1000	>1000
19	*t*-Bu	*t*-Bu	137±7	>1000	>1000
20	*t*-Bu	Bn	23.2±3.9	508±44	480±15
21	*t*-Bu	Ac	>1000	443±32	>1000
22	*t*-Bu	Bz	>1000	551±40	405±67
23	*t*-Bu	PhNHCO	395±55	>1000	>1000
24	Ph	Bn	31.1±3.8	205±22	303±39

[a]Abbreviations: Ac: acetyl; Bn: benzyl; Boc: *tert*-butoxycarbonyl; *t*-Bu: *tert*-butyl; Bz: benzoyl; Me: methyl; Ph: phenyl.

Table 3. Mu binding affinities of tetrahydroisoquinoline-pyrrolidine derivatives



Compd	R	μ K_i (nM)	δ K_i (nM)	κ K_i (nM)
DAMGO		0.53±0.01	290±82	>1000
24	Ph	31.1±3.7	—	—
25	2-OH-Ph	11.7±1.5	—	—
26	3-OH-Ph	5.2±0.9	140±19	194±52
27	4-OH-Ph	6.0±0.2	113±17	530±79
28	2-OMe-Ph	8.8±0.4	509±28	109±15
29	3-OMe-Ph	34.3±8.5	—	—
30	4-OMe-Ph	22.8±6.2	—	—
31	2-CN-Ph	14.8±3.3	—	—
32	3-CN-Ph	50.4±9.4	—	—
33	4-CN-Ph	33.7±7.8	—	—
34	2-F-Ph	40.5±6.7	—	—
35	3-F-Ph	56.0±10.1	—	—
36	4-F-Ph	26.2±4.6	—	—
37	4-NHAc-Ph	10.3±1.2	—	—
38	2-Naphthalene	62.8±9.4	—	—
39	3-Furan	22.7±1.3	—	—
40	3-Thiophene	24.0±3.9	—	—
41	2-Pyridine	31.1±6.0	—	—
42	2,3-di-OMe-Ph	2.7±0.8	113±12	39.5±12.5
43	2-OH, 3-OMe-Ph	6.8±1.8	263±19	543±140
44	3-OH, 4-OMe-Ph	17.7±4.2	—	—
45	3,4-di-OH-Ph	21.4±1.4	—	—
46	2-OMe, 4-OH-Ph	1.1±0.1	127±9	130±9

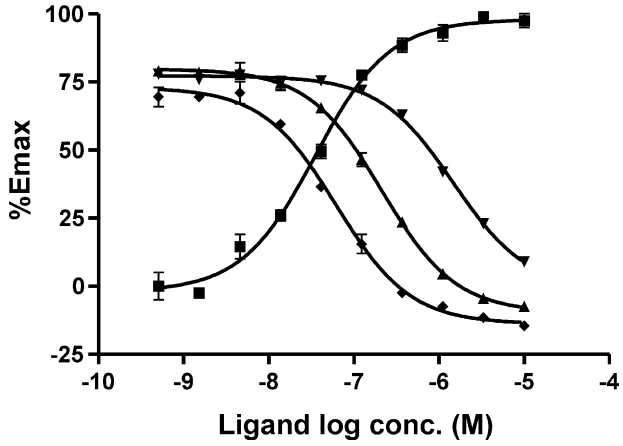


Figure 1. GTPγ[^{35}S] binding reversals of 500 nM of DAMGO by compound **26** (▲), compound **42** (▼) and compound **46** (◆). Full agonistic properties of DAMGO (■) are also shown and represent 100% relative E_{max}.

All the compounds having binding affinities less than 15 nM were tested in GTPγ[^{35}S] assays[24] to see whether they activated the μ receptor. However, no activation was observed for any of the compounds up to a concentration of 1μM. Compounds **26**, **42**, and **46** were therefore tested in competition assays against the μ agonist DAMGO (DAMGO: Tyr-DAla-Gly-[*N*Me-Phe]-NH$(CH_2)_2$-OH) to assess their potential antagonist effects (Fig. 1). They were shown to block the effect of DAMGO with K' values[25] of **21**, 126 and 6 nM, respectively.

These molecules represent new scaffolds in the development of μ opioid-receptor antagonists. It is noteworthy to mention that even though some partial μ-agonism was previously observed with this Tiq urea scaffold,[14] it is clear in this study that the compounds produced were antagonists, suggesting a different mode of binding. It has been previously demonstrated that subtle changes to these types of molecules could greatly alter their pharmacological profiles, converting them from antagonists to agonists. Work is currently underway in our laboratories to explore further the SAR of this series.

Acknowledgements

The authors are thankful to Dr. Ralf Schmidt and Dr. Chris Walpole for helpful discussions.

References and Notes

1. Kaczor, A.; Matosiuk, D. *Curr. Med. Chem.* **2002**, *9*, 1567.
2. Beedle, A. M.; Zamponi, G. W. *Drug Develop. Res.* **2002**, *54*, 118.
3. Kaczor, A.; Matosiuk, D. *Curr. Med. Chem.* **2002**, *9*, 1591.
4. Menkens, K.; Bilsky, E.; Wild, K.; Portoghese, P. S.; Reid, L.; Porreca, F. *Eur. J. Pharm.* **1992**, *219*, 345.
5. Lazarus, L. H.; Bryant, S. D.; Cooper, P. S.; Guerrini, R.; Balboni, G.; Salvadori, S. *Drug Discovery Today* **1998**, *3*, 284.
6. Bryant, S. D.; Salvadori, S.; Cooper, P. S.; Lazarus, L. H. *Trends Pharmacol. Sci.* **1998**, *19*, 42.
7. Schiller, P. W.; Weltrowska, G.; Berezowska, I.; Nguyen, T. M.-D.; Wilkes, B. C.; Lemieux, C.; Chung, N. N. *Biopolymers (Peptide Science)* **1999**, *51*, 411.
8. Salvadori, S.; Balboni, G.; Guerrini, R.; Tomatis, R.; Bianchi, C.; Bryant, S. D.; Cooper, P. S.; Lazarus, L. H. *J. Med. Chem.* **1997**, *40*, 3100.
9. Pagé, D.; McClory, A.; Mischki, T.; Schmidt, R.; Butterworth, J.; St-Onge, S.; Labarre, M.; Payza, K.; Brown, W. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 167.
10. Santagada, V.; Balboni, G.; Caliendo, G.; Guerrini, R.; Salvadori, S.; Bianchi, C.; Bryant, S. D.; Lazarus, L. H. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 2745.
11. Balboni, G.; Salvadori, S.; Guerrini, R.; Bianchi, C.; Santagada, V.; Calliendo, G.; Bryant, S. D.; Lazarus, L. H. *Peptides* **2000**, *21*, 1663.
12. Tourwé, D.; Cauwenberghe, S. V.; Vanommeslaeghe, K.; Mannekens, E.; Geerlings, P.; Toth, G.; Péter, A.; Csombos, J. In *Peptides: The Wave of the Future, Proceedings of the Second International and Seventeenth American Peptide Symposium*; Lebl, M., Houghten, R. A., Eds.; American Peptide Society: San Diego, 2001; p 683.
13. Salvadori, S.; Guerrini, R.; Balboni, G.; Bianchi, C.; Bryant, S. D.; Cooper, P. S.; Lazarus, L. H. *J. Med. Chem.* **1999**, *42*, 5010.
14. Pagé, D.; Naismith, A.; Schmidt, R.; Coupal, M.; Labarre, M.; Gosselin, M.; Bellemare, D.; Payza, K.; Brown, W. *J. Med. Chem.* **2001**, *44*, 2387.
15. Balboni, G.; Guerrini, R.; Salvadori, S.; Bianchi, C.; Rizzi, D.; Bryant, S. D.; Lazarus, L. H. *J. Med. Chem.* **2002**, *45*, 713.
16. Balboni, G.; Salvadori, S.; Guerrini, R.; Negri, L.; Giannini, E.; Jinsmaa, Y.; Bryant, S. D.; Lazarus, L. H. *J. Med. Chem.* **2002**, *45*, 5556.
17. Schiller, P. W.; Weltrowsak, G.; Bolewska-Pedyczak, E.; Nguyen, T. M.-D.; Lemieux, C.; Chung, N. N. In *Peptides 1996, Proceedings of the Twenty-Fourth European Peptide Symposium*; Ramage, R., Epton, R., Eds.; Mayflower Scientific: Kingstwinford, UK, 1998; p 785.
18. Santagada, V.; Caliendo, G.; Severino, B.; Perissutti, E.; Ceccarelli, F.; Giusti, L.; Mazzoni, M. R.; Salvadori, S.; Temussi, P. A. *J. Peptide Sci.* **2001**, *7*, 374.
19. Lu, Y.; Weltrowska, G.; Lemieux, C.; Chung, N. N.; Schiller, P. W. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 323.
20. Lu, Y.; Nguyen, T. M.-D.; Weltrowska, G.; Berezowska, I.; Lemieux, C.; Chung, N. N.; Schiller, P. W. *J. Med. Chem.* **2001**, *14*, 3048.
21. Schiller, P. W.; Lu, Y.; Weltrowska, G.; Berezowska, I.; Wilkes, B. C.; Nguyen, T. M.-D.; Chung, N. N.; Lemieux, C. In *Peptides: The Wave of the Future, Proceedings of the Second International and Seventeenth American Peptide Symposium*; Lebl, M., Houghten, R. A., Eds.; American Peptide Society: San Diego, 2001; pp 676–678.
22. Rodriguez, M.; Llinares, M.; Doulut, S.; Heitz, A.; Martinez, J. *Tetrahedron Lett.* **1991**, *32*, 923.
23. All products were purified by reversed phase HPLC on Luna C-18 column (250×21.2 mm) using a gradient of 30–80% CH_3CN/H_2O containing 0.1% TFA and were isolated as their corresponding TFA salts in 50–65% yields. All products gave satisfactory analytical characterization showing purity >95% as determined by HPLC using a Zorbax C-18 column (λ=215, 254 and 280 nm). Selected analytical characterization: Compound **26**: ^{1}H NMR (400 MHz, CD_3OD) δ 7.30–6.80 (m, 13H), 4.78 (m, 1H), 4.51 (d, J=12.69 Hz, 1H), 4.16–3.92 (m, 5H), 3.42 (m, 1H), 3.20–3.08 (m, 3H), 2.96–2.92 (m, 1H), 2.25 (m, 1H), 1.96 (m, 3H); MS (MH+): 530.2. Anal. calcd (%) for $C_{30}H_{35}N_5O_2S$; C, 56.85; H, 5.35; N, 10.11; Found(%): C, 56.87; H, 5.34; N, 10.30; HPLC k′: 4.35. Compound **42**: ^{1}H NMR (400 MHz, CD_3OD) δ 7.29–6.95 (m, 12H), 4.80 (m, 1H), 4.51 (d, J=12.30 Hz, 1H), 4.26–4.16 (m, 2H), 3.93 (m, 1H), 3.85 (s, 3H), 3.79 (s, 3H), 3.75 (m, 1H), 3.52 (m, 1H), 3.21 (m, 1H), 3.10 (dd, J=5.08, 15.62 Hz, 1H), 2.95 (d, J=16.01 Hz, 1H), 2.25 (m, 1H), 1.97 (m, 3H); MS (MH+): 574.3. Anal. calcd (%) for $C_{32}H_{39}N_5O_3S$: C, 56.30; H, 5.62; N, 9.43; Found (%): C, 56.31; H, 5.53; N, 9.49; HPLC k′: 5.77. Compound **46**: ^{1}H NMR (400 MHz, CD_3OD) δ 7.31 (m, 3H), 7.25–7.19 (m, 9H), 6.98 (m, 1H), 6.41 (s, 1H), 6.35 (dd, J=1.36, 8.10 Hz, 1H), 4.75 (d, J=15.82 Hz, 1H), 4.36 (m, 1H), 4.17 (m, 2H), 3.96 (m, 1H), 3.78 (s, 3H), 3.74 (m, 1H), 3.48 (m, 1H), 3.20 (m, 1H), 3.12 (dd, J=5.08, 15.62 Hz, 1H), 2.95 (d, J=15.43 Hz, 1H), 2.26 (m, 1H), 1.98 (m, 3H); MS (MH+): 560.2. Anal. calcd (%) for $C_{31}H_{37}N_5O_3S$: C, 54.57; H, 5.21; N, 9.25; Found(%): C, 54.56; H, 5.21; N, 9.08; HPLC k′: 3.41.
24. The reversal of DAMGO-induced stimulation of GTPγ[^{35}S] binding was used to assay the antagonist properties of the compounds. Membranes were combined with approximately 0.2 nM GTPγ[^{35}S], 500 nM of DAMGO, and various concentrations of the antagonist compounds. The assay was performed in 50 mM Hepes, 20 mM NaOH, pH 7.4, 5 mM $MgCl_2$, 100 mM NaCl, 1 mM EDTA, 1 mM DTT, 0.1% BSA, 15 μM GDP. After 1 h, the bound radioactivity was determined by filtration. Control and stimulated GTPγ[^{35}S] binding was determined in absence and presence of 30 μM of

DAMGO. Values of DAMGO EC_{50} and E_{max} were obtained from a 4-parameter logistic curve fits of percent stimulated GTPγ[^{35}S] binding vs log(molar ligand), solving for EC_{50}, E_{min}, E_{max} and hill slope.

25. K′ values were determined for each antagonist using the Cheng and Prusoff (*Biochem. Pharmacol.* **1973**, *22*, 3099) equation:

$$\frac{IC_{50Antagonist}}{1 + ([Agonist\ used]/EC_{50}\ Agonist)}.$$