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Synthesis, Opioid Receptor Binding, and Functional Activity of 5′-Substituted 17-Cyclopropylmethylpyrido[2′,3′:6,7]morphinans

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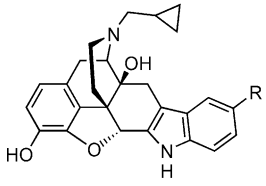
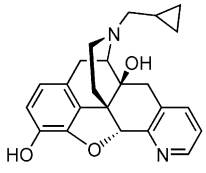
Abstract—A series of naltrexone-derived pyridomorphinans possessing various substituents at the 5′-position on the pyridine ring were synthesized and evaluated for opioid receptor binding in rodent brain membranes and functional activity in smooth muscle preparations. While the introduction of aromatic 1-pyrrolyl group (**6h**) improved the δ affinity and δ antagonist potency of the parent compound (**3**), the introduction of guanidine group (**6i**) transformed it to a κ selective ligand in opioid receptor binding and [^{35}S]GTP-γ-S functional assays.

Investigations on nonpeptide ligands arising from fusion of heteroaromatic systems to the 6,7-position of the C-ring of naltrexone led to the discovery of the indolomorphinan naltrindole (**1**) as opioid δ receptor selective antagonist ligand.[1,2] This ligand has been used widely as a pharmacological tool to study the biological effects resulting from the inhibition of the δ opioid system in vivo. Results from these studies indicate that δ antagonists may have broad potential as modulatory agents to prevent the tolerance and dependence side effects of μ analgesics such as morphine,[3] as immunosuppressants for preventing organ transplant rejections,[4] and as possible treatment agents for cocaine,[5] methamphetamine,[6] and alcohol abuse.[7]

The indolomorphinan unit present in **1** has been used as a template for appending various substituents at the indole moiety for modulating the binding selectivity and intrinsic activity at the δ, μ, and κ opioid receptors.[1,2,8–14] Of particular interest among such modifications is the introduction of amidine or guanidine groups at the 5′-position.[12–14] These modifications, in general, have been found to bring about a change in the profile from a δ selective ligand to that of a κ selective ligand. For example, the guanidino compound **2** has been reported to bind with high selectivity to the κ receptor and function as an antagonist in functional assays.[13] The preferential interaction of these ligands at the κ site has been attributed to the ability of the amidinoid groups to form ionic and hydrogen bonding interactions with the acidic Glu297 residue residing at the top of the TM6 of the κ receptor.[13]



1, R = H, NTI
2, R = NH-C(=NH)-NH$_2$, GNTI



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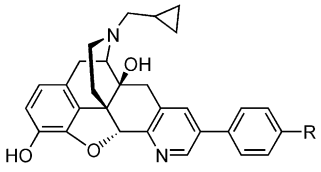

4, R = H
5, R = Cl

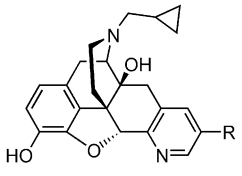

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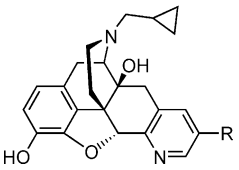
Table 1. Binding affinities at δ, μ and κ opioid receptors



Compd	R	K_i (nM) ± SEM			Selectivity ratio	
		δ[a]	μ[a]	κ[a]	μ/δ	κ/δ
6a	Br	1.2±0.13	15.5±1.0	55.7±7.0	12.9	46
6b	CN	4.5±0.28	16.0±1.8	33.9±2.0	3.6	7.5
6c	$CO_2C_2H_5$	4.2±0.27	37.0±3.4	9.6±0.93	8.8	2.3
6d	NO_2	5.5±0.67	17.5±2.0	92.0±12.8	3.2	17
6e	NH_2	8.0±0.3	12.8±0.93	12.0±1.2	1.6	1.5
6f	$NHCH_2C_6H_5$	1.3±0.09	15.0±0.8	6.7±0.84	11.5	5.2
6g	$CH_2NHC_6H_5$	1.8±0.07	6.0±0.2	19.0±0.6	3.3	10.6
6h	1-Pyrrolyl	0.64±0.08	19.0±1.0	12.2±0.6	30	19
6i	$NH(C{=}NH)NH_2$	18.7±1.2	26.0±4.3	0.70±0.12	1.4	0.037
3[b]	H	0.78±0.06	1.5±0.09	8.8±0.69	1.9	11
1[b]		0.41±0.09	99.0±4.6	35.8±4.0	241	87

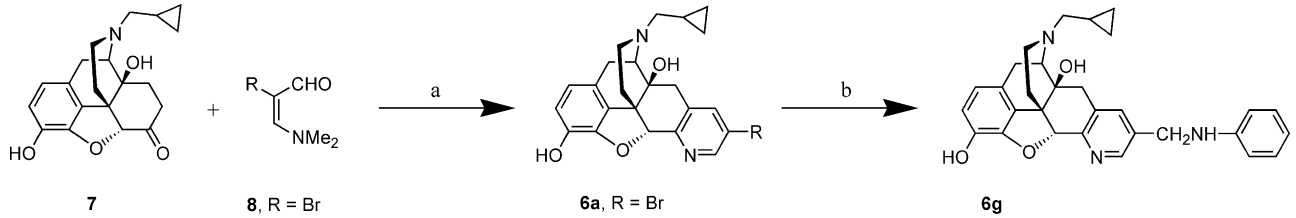
[a]Displaced ligands were [^{3}H]DADLE (δ), [^{3}H]DAMGO (μ), [^{3}H]U69,593 (κ).
[b]Data from ref 15.

In our earlier investigations on naltrexone-derived C-ring fused ligands, we found that the pyridomorphinan **3** displayed high affinity binding at the δ receptor ($K_i = 0.78$ nM).[15] The introduction of a phenyl group at the 5′-position (**4**) led to improvements in δ receptor binding selectivity and functional antagonist potency. Moreover, the 4-chlorophenyl analogue **5** was found to display the properties of a potent δ antagonist with μ agonist activity. On the basis of these findings it was of interest to explore the effect of various substituents on the pyridomorphinan template **3** in binding and intrinsic activity at the opioid receptors. Herein we report the synthesis and activity profile of the pyridomorphinans **6** (**6a–i**, Table 1) possessing various substituents at the 5′-position.

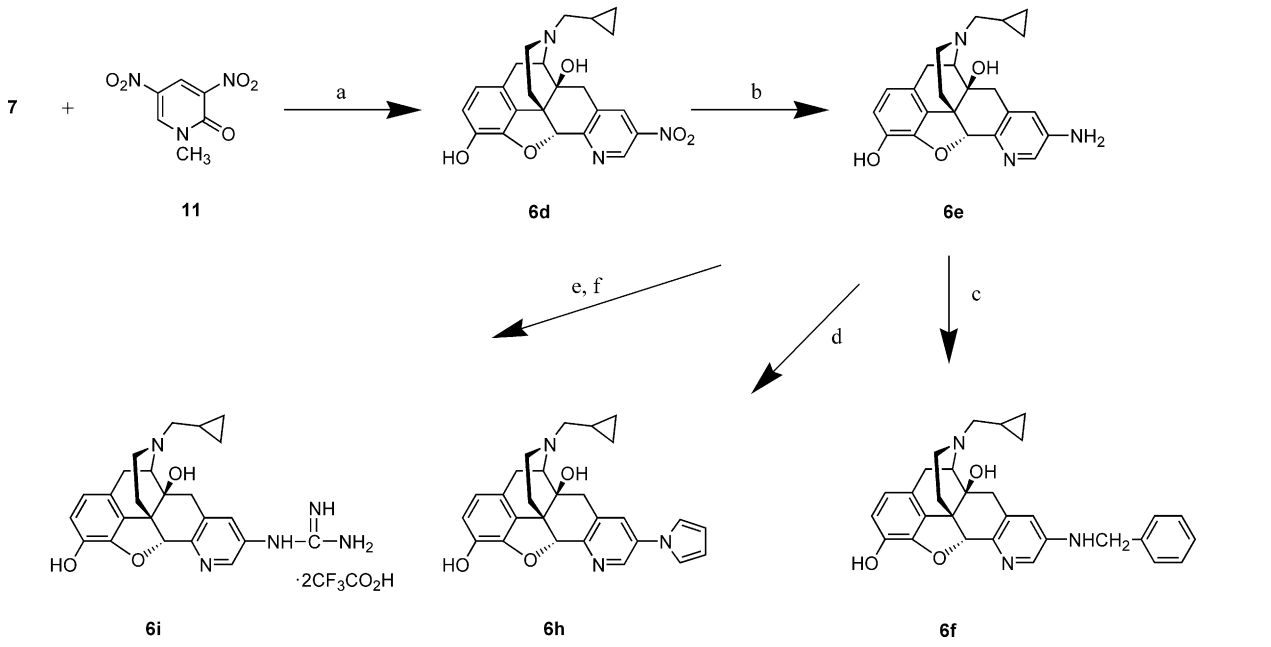
The bromo-, cyano- and carbethoxypyridines **6a–c** were synthesized by the pyridine annulation methodolgy[15] involving the condensation of naltrexone (**7**) with 2-bromo-[16] (**8**), 2-cyano-[17] (**9**) or 2-carbethoxy-3-(dimethylamino)acrolein (**10**)[18] in the presence of ammonium acetate in acetic acid (Scheme 1).[19] The nitropyridine **6d** was obtained by Tohda's method[20] by reacting naltrexone with 2,5-dinitro-1-methyl-2-pyridone (**11**) and methanolic ammonia. Reduction of the nitro group by catalytic hydrogenation over palladium on carbon gave the amine **6e**, which was converted to the benzylamine **6f** by reductive alkylation with benzaldehyde and sodium cyanoborohydride (Scheme 2). The isomeric anilinomethyl compound **6g** was synthesized from the nitrile **6b** by reductive amination with aniline (Scheme 1).[21] The amine **6e** served as the starting material for **6h** and **6i**. The pyrrole **6h** was prepared by the acid catalyzed condensation of **6e** with 2,5-dimethoxytetrahydrofuran.[22] Reaction of **6e** with 1,3-bis(*tert*-butoxycarbonyl)-2-methylthiopseudourea in the presence of mercury(II) chloride and triethylamine[13,23,24] yielded the BOC-protected guanidine intermediate which on treatment with trifluoroacetic acid gave the guanidine **6i** as the bis-trifluoroacetate salt.

The binding affinities of the target compounds **6a–i** for opioid receptors were determined using rat brain membranes for δ and μ receptors and guinea pig brain membranes for the κ receptors as described in the literature (Table 1).[11,15] Data for the unsubstituted pyridine **3** and naltrindole (**1**) are listed for comparison purposes. The affinity data for **6a**, as compared with **3**, indicates that the introduction of the bromine at the 5′-position does not significantly affect the binding affinity at the δ receptor. The bromine at the 5′-position, however, decreases the binding affinity at the μ (10-fold) and κ (6-fold) sites thus enhancing the δ receptor binding selectivity. The introduction of electron withdrawing cyano, ester or nitro group as well as the introduction of electron donating amino group all led to a modest decrease in binding affinity at the δ site. Conversion of the primary amine **6e** to the secondary benzylamine **6f** improved the δ affinity 6-fold. This improvement in the affinity at the δ site may be due to the presence of the aromatic phenyl group in the benzylamine moiety since the isomeric anilinomethyl compound **6g** also displayed δ affinity similar to that of **6f**. Of particular interest is the observation that conversion of the primary amino group in **6e** to the aromatic pyrrole (**6h**) unit capable of participating in π–π and cation–π interactions enhanced the affinity at the δ site (12-fold) and decreased the affinity at the μ site (1.5-fold). Thus, among the ligands studied, the pyrrolyl compound **6h** has the highest affinity at the δ receptor with the highest μ/δ binding selectivity ratio.

The guanidine compound **6i** was specifically designed to test whether the incorporation of the basic guanidine moiety as the κ address element will provide a κ selective pyridomorphinan in a manner similar to that observed in the indolomorphinan series of compounds.[13] It was gratifying to find that **6i** indeed displayed a remarkable



Scheme 1. Reagents and reaction conditions: (a) $AcONH_4$, AcOH, reflux; (b) **6b**, $PhNH_2$, H_2, Ra–Ni, AcOH, rt, 4 h.



Scheme 2. Reagents and reaction conditions: (a) NH_3/MeOH, 70 °C, 20 h; (b) H_2, 10% Pd/C, EtOH, 50 psi, 24 h; (c) PhCHO, $NaBH_3CN$, AcOH–MeOH, rt, 24 h; (d) 2,5-dimethoxytetrahydrofuran, AcOH, reflux, 20 min; (e) *t*-BocN=C(SMe)NH-*t*-Boc, $Hg(II)Cl_2$, Et_3N, DMF; (f) CF_3CO_2H, CH_2Cl_2, rt, 16 h.

change in the binding profile. This guanidinopyridomorphinan binds to the κ receptor with subnanomolar affinity ($K_i = 0.7$ nM) and is 26-fold selective for κ over δ receptors and 37-fold selective for κ over μ receptors. In binding assays using cloned receptors with [^{3}H]diprenorphine as the radioligand, the guanidinoindolomorphinan **2** has been reported to bind with an affinity of 0.18 nM at the κ receptor and to display δ/κ and μ/κ selectivity ratios of 257 and 125, respectively.[13] Although the affinity and κ selectivity of the pyridomorphinan **6i** are apparently lower than that of the indolomorphinan **2**, the observed shift in the binding profiles between **3** and **6i** parallels that observed between **1** and **2**.

All of the target compounds were evaluated in vitro for opioid agonist and antagonist activity on the electrically stimulated mouse vas-deferens (MVD) and guinea-pig ileal longitudinal muscle (GPI) preparations as previously described.[11,15] At 1 μM concentration, none of the tested compounds displayed any significant agonist activity in the MVD or GPI (maximum inhibition of electrically induced contraction was less than 14% in the MVD and less than 4% in the GPI). All of the ligands displayed antagonist profile of activity at the δ receptor in the MVD. The antagonist potencies of the compounds are listed in Table 2. While compounds **6a**, **6f–h** displayed moderate antagonist potency in the MVD ($K_e < 10$ nM), other compounds behaved as weak antagonists ($K_e > 10$ nM). Although there is no strict correlation between the binding potency at the δ site in the brain membranes (K_i values in Table 1) and the δ antagonist potency in the MVD smooth muscles (K_e values in Table 2), the K_e/K_i ratios for all of the compounds fall within the range of 1.7 (for **6g**) to 10.3 (for **6h**). With the exception of **6b**, all of the ligands displayed moderate to weak antagonist activity at the μ receptor in the GPI.

Table 2. Opioid antagonist potencies in the MVD and GPI smooth muscle preparations

Compd	Antagonist activity in the MVD K_e (nM)[a]	Antagonist activity in the GPI K_e (nM)[b]	Selectivity ratio GPI/MVD
6a	3.6±1.4	36±21	10
6b	44±4	>1000	>22
6c	20±2	87±34	4.4
6d	53±11	200±50	3.8
6e	25±4	48±9	1.9
6f	6.5±2.7	5.9±0.8	0.9
6g	3.0±0.3	2.7±0.3	0.9
6h	6.6±1.0	62±10	9.4
6i	170±40	500±200	2.9
3[c]	37±1	190±65	5.1
1[c]	0.53±0.18	43±3	81

[a]Determined using DPDPE as the agonist ligand for the δ receptor.
[b]Determined using PL-017 as the agonist ligand for the μ receptor.
[c]Data included for comparison.

Compounds **6h** and **6i** were examined in the [^{35}S]GTP-γ-S functional assay in guinea pig caudate membranes as described previously.[25,26] The functional antagonist potencies of these compounds along with those of the standard δ antagonist NTI (**1**) and κ antagonist nor-BNI are given in Table 3. The data confirms that **6h** is a potent δ antagonist ($K_i = 0.4$ nM) whereas **6i** is a potent κ antagonist ($K_i = 1$ nM). The functional antagonist potency of **6h** at the δ receptor and **6i** at the κ receptor are 10-fold lower than that of the standard ligands NTI and nor-BNI respectively. However, the functional antagonist selectivity profile of **6h** and **6i** parallels that of NTI and nor-BNI and is in conformity with the selectivity observed in the binding assays.

Table 3. Antagonist activity of **6h**, **6i**, NTI (**1**) and nor-BNI on agonist stimulated [^{35}S]GTP-γ-S binding in guinea pig caudate membranes

Compd	Apparent functional K_i (nM ± SD)			μ/δ	κ/δ
	δ SNC-80[a]	μ DAMGO[b]	κ U69,593[c]		
6h	0.4 ± 0.03	4.2 ± 0.5	8.8 ± 0.5	11	22
6i	6.5 ± 0.7	6.1 ± 0.8	1.0 ± 0.09	0.9	0.2
1	0.04 ± 0.004	1.8 ± 0.3	6.0 ± 0.08	45	150
nor-BNI	10.2 ± 0.9	14.8 ± 1.3	0.12 ± 0.01	1.5	0.01

[a]Determined using 10 μM SNC-80 as the agonist ligand for the δ receptor.
[b]Determined using 10 μM DAMGO as the agonist ligand for the μ receptor.
[c]Determined using 10 μM U69,593 as the agonist ligand for the κ receptor.

The present study indicates that the binding affinity and the binding selectivity profile of ligands derived from the pyridomorphinan scaffold can be manipulated by introduction of suitable substituent groups at the 5′-position on the pyridine ring. While nonaromatic electron donating or withdrawing substituents impart only modest changes in the binding profile, the attachment of an aromatic system at this position enhances binding affinity at the δ receptor and improves binding selectivity for the δ receptor. Moreover, appending the basic guanidine group as a κ address element at the 5′-position brings about a change in binding selectivity from a δ selective ligand to a κ selective ligand. In the functional activity assays in vitro all of the target compounds studied retain an antagonist profile of activity. These results indicate that pyridomorphinan unit could be used as a template for the development of novel antagonist ligands with selectivity for the opioid δ or κ receptors through molecular manipulations involving the introduction of aromatic or basic substituents at the 5′-position.

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References and Notes

1. Portoghese, P. S.; Sultana, M.; Nagase, H.; Takemori, A. E. *J. Med. Chem.* **1988**, *31*, 281.
2. Portoghese, P. S.; Sultana, M.; Takemori, A. E. *J. Med. Chem.* **1990**, *33*, 1714.
3. Abdelhamid, E. E.; Sultana, M.; Portoghese, P. S.; Takemori, A. E. *J. Pharmacol. Exp. Ther.* **1991**, *258*, 299.
4. Arakawa, K.; Akami, T.; Okamoto, M.; Akioka, K.; Nakai, I.; Oka, T.; Nagase, H. *Transplant. Proc.* **1993**, *25*, 738.
5. Menkens, K.; Bilsky, E. J.; Wild, K. D.; Portoghese, P. S.; Reid, L. D.; Porreca, F. *Eur. J. Pharmacol.* **1992**, *219*, 345.
6. Suzuki, T.; Mori, T.; Tsuji, M.; Nagase, H. *Life Sci.* **1994**, *55*, PL339.
7. June, H. L.; McCane, S. R.; Zink, R. W.; Portoghese, P. S.; Li, T. K.; Froehlich, J. C. *Psychopharmacology* **1999**, *147*, 81.
8. Portoghese, P. S.; Sultana, M.; Nelson, W. L.; Klein, P.; Takemori, A. E. *J. Med. Chem.* **1992**, *35*, 4086.
9. Portoghese, P. S.; Farouz-Grant, F.; Sultana, M.; Takemori, A. E. *J. Med. Chem.* **1995**, *38*, 402.
10. Korlipara, V. L.; Takemori, A. E.; Portoghese, P. S. *J. Med. Chem.* **1994**, *37*, 1882.
11. Ananthan, S.; Johnson, C. A.; Carter, R. L.; Clayton, S. D.; Rice, K. C.; Xu, H.; Davis, P.; Porreca, F.; Rothman, R. B. *J. Med. Chem.* **1998**, *41*, 2872.
12. Olmsted, S. L.; Takemori, A. E.; Portoghese, P. S. *J. Med. Chem.* **1993**, *36*, 179.
13. Stevens, W. C., Jr.; Jones, R. M.; Subramanian, G.; Metzger, T. G.; Ferguson, D. M.; Portoghese, P. S. *J. Med. Chem.* **2000**, *43*, 2759.
14. Jales, A. R.; Husbands, S. M.; Lewis, J. W. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 2259.
15. Ananthan, S.; Kezar III, H. S.; Carter, R. L.; Saini, S. K.; Rice, K. C.; Wells, J. L.; Davis, P.; Xu, H.; Dersch, C. M.; Bilsky, E. J.; Porreca, F.; Rothman, R. B. *J. Med. Chem.* **1999**, *42*, 3527.
16. Gais, H.-J.; Hafner, K.; Neuenschwander, M. *Helv. Chim. Acta* **1969**, *52*, 2641.
17. Reichardt, C.; Kermer, W.-D. *Synthesis* **1970**, 538.
18. Arnold, Z. *Coll. Czech. Chem. Commun.* **1961**, *26*, 3051.
19. All new target compounds were characterized by ^{1}H NMR, mass spectral data, and elemental analysis.
20. Tohda, Y.; Eiraku, M.; Nakagawa, T.; Usami, Y.; Ariga, M.; Kawashima, T.; Tani, K.; Watanabe, H.; Mori, Y. *Bull. Chem. Soc. Jpn.* **1990**, *63*, 2820.
21. Rosowsky, A.; Mota, C. E.; Wright, J. E.; Queener, S. F. *J. Med. Chem.* **1994**, *37*, 4522.
22. Campiani, G.; Nacci, V.; Fiorini, I.; De Filippis, M. P.; Garofalo, A.; Greco, G.; Novellino, E.; Altamura, S.; Di Renzo, L. *J. Med. Chem.* **1996**, *39*, 2672.
23. Kim, K. S.; Qian, L. *Tetrahedron Lett.* **1993**, *34*, 7677.
24. Ho, K.-C.; Sun, C.-M. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1517.
25. Partilla, J. S.; Carroll, F. I.; Thomas, J. B.; Rice, K. C.; Zimmerman, D. M.; Rothman, R. B. *Analgesia* **1999**, *4*, 27.
26. Thomas, J. B.; Atkinson, R. N.; Rothman, R. B.; Fix, S. E.; Mascarella, S. W.; Vinson, N. A.; Xu, H.; Dersch, C. M.; Lu, Y.-F.; Cantrell, B. E.; Zimmerman, D. M.; Carroll, F. I. *J. Med. Chem.* **2001**, *44*, 2687.