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PYRROLOOCTAHYDROISOQUINOLINES AS POTENT AND SELECTIVE δ OPIOID RECEPTOR LIGANDS: SAR ANALYSIS AND DOCKING STUDIES

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Abstract. Structure Activity Relationship and docking studies focused on the role of the non-aromatic δ address in a novel class of potent and selective δ ligands, pyrrolooctahydroisoquinolines, are discussed.

The presence of at least three types of opioid receptors,[1] named μ, κ and δ, in the central nervous system and periphery is now well established. Human μ, κ and δ opioid receptors have been cloned and shown to belong to the G protein-coupled receptor (GPCR) superfamily.[2] Their endogenous ligands are a family of opioid peptides involved in pain control, neuroendocrine physiology and affective behaviour.[3] Opiates currently in clinical use for treatment of pain, such as morphine, act through the μ receptor. However, their use is limited by severe side effects, including respiratory depression and constipation. With regard to the abuse liability of opiates, μ receptors are considered to be the primary site responsible for opiate addiction, whereas evidence suggests a lesser or possibly even no propensity of peptide δ agonists to cause dependence liability as well as other untoward side effects associated to the activation of μ receptors. On the other hand, the clinical use of κ receptor agonists is inhibited by their strong dysphoric and psychotomimetic effects.[4] Therefore, potent and selective non-peptidic δ agonists are needed to prove the concept that they may elicit effective analgesia without the unwanted side effects associated with the classical μ agonists.[5]

Several classes of non-peptidic δ ligands have been designed according to the "message-address" concept (Chart I).[6,7,8] This concept attributes the role of the opiate message to the Tyr^1 residue of the tetrapeptidic sequence of the endogenous opioid peptides (Tyr^1-Gly^2-Gly^3-Phe^4-X), whereas the δ address resides in the amino acid sequence which starts with Phe^4. The residues Gly^2-Gly^3 represent a spacer maintaining an appropriate distance between Tyr^1 and Phe^4. The presence of an aromatic moiety which acts as the δ address mimicking the Phe^4 of the endogenous opioid peptides is therefore a peculiarity of the first generation of non-peptidic δ selective ligands.[6,7] However, a structurally unrelated piperazine derivative, SNC 80, has been recently disclosed as a potent and selective δ opioid ligand.[8]

Recent studies focused on the role and structure of the δ address using SNC 80 and SB 205588[7] as templates, led to the identification of a novel class of potent and selective δ ligands featuring a carboxamido group as putative non-aromatic δ address (SB 219825 [**(-)-4a**], Ki δ = 0.95 nM; μ/δ = 136; κ/δ = 1410, Tab. 1).[9]

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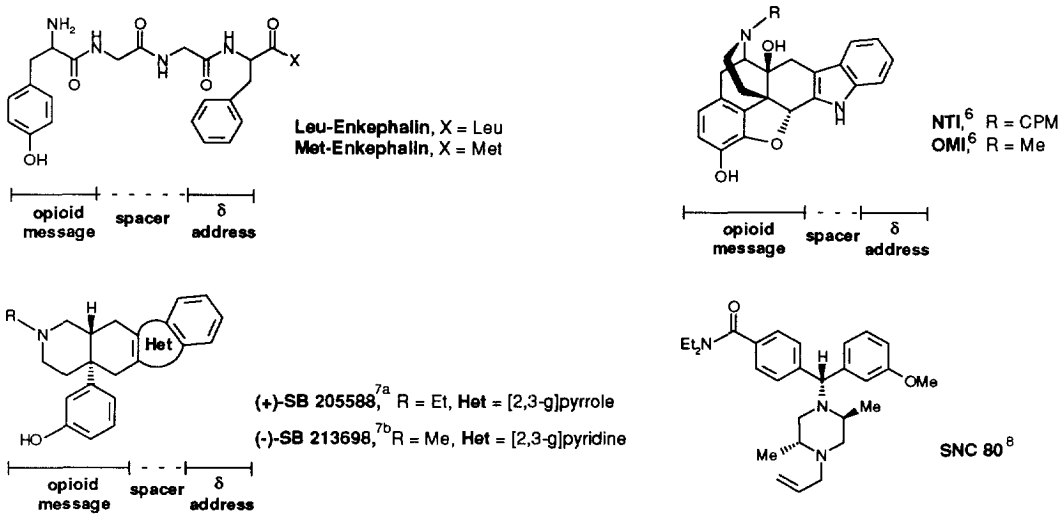
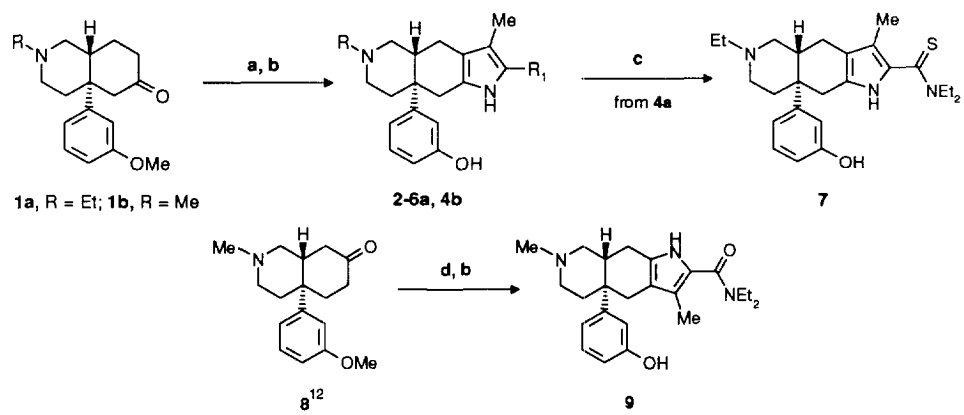
Chart I



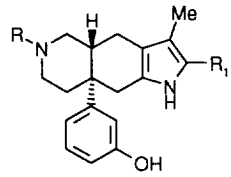
These findings, along with the very promising binding profile of the prototype SB 219825 **[(-)-4a]**, prompted the synthesis of novel derivatives as useful tools to study the influence of different non aromatic δ addresses on the affinity and selectivity for the δ opioid receptor. In this regard, the novel racemic pyrrolooctahydroisoquinolines **2-3a, 5-6a, 4b, 7** and **9** were synthesised[10] (Scheme 1) and tested in δ, μ and κ opioid binding assays.[11] The results are summarised in Table 1.

Scheme 1. Synthetic schemes for compounds **2-6a, 4b** and **9**

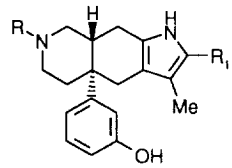


Reagents: (a) $MeCOC(NNHPh)R_1$, Zn, AcOH, AcONa, 60-100°C; (b) BBr_3, CH_2Cl_2, rt; (c) Lawesson's reagent, toluene, reflux; (d) $MeCOC(NNHPh)CONEt_2$, Zn, AcOH, AcONa, 60-100°C.

Table 1. Binding affinities of pyrrolooctahydroisoquinolines and standard compounds



2a-7



9

Compd	R	R_1	Binding Ki (nM)[a] δ	μ	κ
2a	Et	$CONH_2$	15.1±4.3	139±30	70.4±10.1
3a	Et	$CONMe_2$	6.8±1.0	580±56	>1000
4a	Et	$CONEt_2$	1.9±0.4	407±25	1298±120
(+)-4a	Et	$CONEt_2$	3535±310	>5000	>5000
(-)-4a	Et	$CONEt_2$	0.95±0.21	129±30	1340±490
5a	Et	$CON(i\text{-}Pr)_2$	2.6±0.1	57.9±8.0	>1000

Compd	R	R_1	Binding Ki (nM)[a] δ	μ	κ
6a	Et	COOEt	2.4±0.2	141±32	169.2±41
4b	Me	$CONEt_2$	2.1±0.1	93.2(n=2)	~500(n=2)
7	Et	$CSNEt_2$	1.5±0.4	42.0±4.9	449±94
9	Me	$CONEt_2$	3.0±0.5	89.4±19.8	>1000
(-)-SB 213698			0.23±0.04	59.6±1.2	312 ±30.0
NTI			0.46±0.03	15.5±2.6	9.5±2.8

[a]Experiments were in triplicate unless otherwise indicated in parentheses.

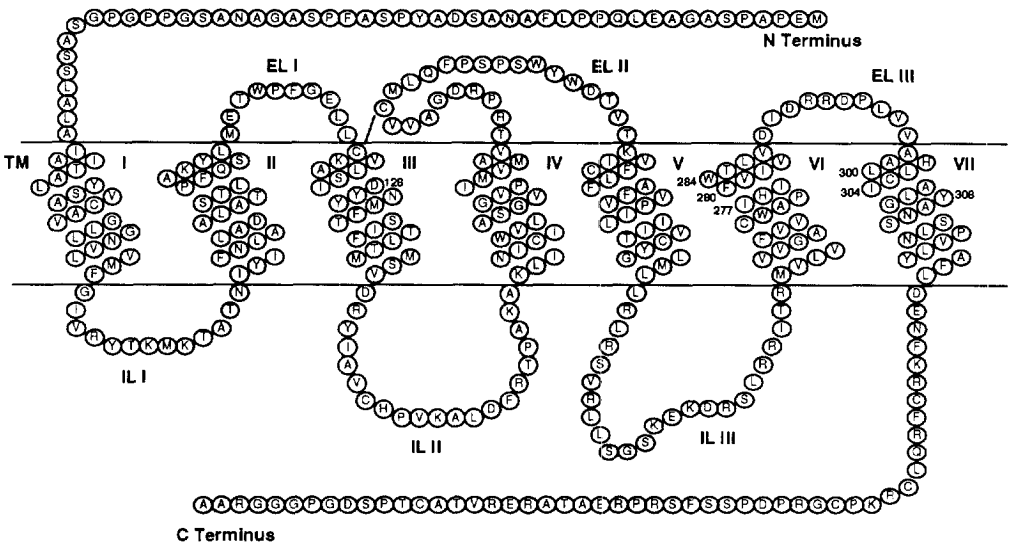
Studies regarding the bulkiness of the carboxamido δ address revealed that small groups such as R_1 = $CONH_2$ and R_1 = $CONMe_2$ caused 8- and 3-fold decrease in δ opioid receptor affinity, respectively, along with a decrease in the μ/δ selectivity (*cf.* **2a** and **3a**, *vs* **4a**). On the other hand, a large lipophilic substituent, i.e. R_1 = $CON(i\text{-}Pr)_2$, was well tolerated (*cf.* **5a** *vs* **4a**), suggesting that the interaction between the amidic moiety and the δ receptor is mainly lipophilic and that there are no adverse steric effects. Subsequent studies were focused on the structural requirements of the non-aromatic δ address by replacing the amidic group with ester and thioamide bioisosters. Both **6a** (R_1 = COOEt) and **7** (R_1 = $CSNEt_2$) maintained a good affinity for the δ receptor although they were somewhat less δ selective. These results indicated that other lipophilic groups may substitute for the original diethylamido moiety without affecting significantly the interaction with the δ opioid receptor.

Delta opioid receptor (DOR) modelling and docking experiments were performed to rationalise the interaction of the aromatic and non-aromatic δ addresses with the receptor. A 3-D model of the transmembrane domains (TMs) of DOR (human, mouse and rat TMs regions show 100% identity), identified as seven highly conserved hydrophobic regions connected by less conserved hydrophilic loops (Figure 1), was built using bacteriorhodopsin[13] as a template according to the methods described in the literature.[14] Energy minimisation was conducted using the Weiner and Kollman united atoms force field[15] in AMBER 4.1.[16,17]

Manual interactive docking experiments were performed using SB 213698 (Figure 2) and SB 219825 (Figure 3). The main recognition points between the "opiate message" of the above molecules and the receptor were: i) an electrostatic interaction between Asp 128 (TM3) and the protonated nitrogen of the ligands and ii) a hydrogen bond between His 278 (TM6) and the phenolic hydroxy group of the molecules under study. Site-

directed mutagenesis data on Asp 128, although controversial, confirmed that this residue plays a role in ligand binding in all three opioid receptors[18]. Point mutation of His 278 showed it to be important for binding at the μ receptor[19].

Figure 1. Serpentine model of human δ opioid receptor



Previous docking experiments[20] revealed the presence of a hydrophobic binding pocket at the extracellular end side of TM6 and TM7 that can accommodate the aromatic δ address of NTI. Ile 277 (TM6), Phe 280 (TM6), Trp 284 (TM6), Leu 300 (TM7), Ile 304 (TM7) and Tyr 308 (TM7) were identified as key residues constituting this binding cavity. In particular, Trp 284 is specific to the δ receptor while charged residues i.e. Lys and Glu are present in the same position of the μ and κ receptors, respectively. The hydrophilic nature of these amino acids could negatively affect the binding of δ ligands, that showed a lipophilic moiety in this "address region". Analysis of μ/δ chimeric receptors[21] along with site-direct mutagenesis studies[21] demonstrated that Trp 284 is important both for high affinity binding and for δ selectivity.

The above identified hydrophobic pocket can receive both the aromatic and the non-aromatic δ addresses. Although definitive conclusions on specific interactions cannot be drawn from the proposed crude model, the following hypotheses can be made: *i*) Trp 284 might be involved in a π–π interaction with the benzene ring of SB 213698; *ii*) a hydrogen bond between the indolic NH and the carbonylic oxygen of SB 219825 cannot be excluded; however, the high affinity of compound **7**, bearing a $CSNEt_2$ address, with higher lipophilicity but reduced capability to form H-bond in respect to the $CONEt_2$, suggests that lipophilicity may overwhelm H-bond stabilization in conferring δ affinity; *iii*) the pyrrolic NH of SB 219825 does not seem to be involved in any

interaction with the receptor; the validity of this observation is confirmed by the high δ affinity of compound **9** in which the carboxamido address lies in the same region of space as in SB 219825 but the NH points to an opposite direction; these results support the hypothesis of the role of the pyrrole ring as a "spacer".

Collectively, results of our docking studies based on the "message-address" concept are in good agreement with mutagenesis data, and with the SAR analysis on the pyrrolooctahydroisoquinolines, which underlined the importance of lipophilic substituents in the δ address region.

In summary, SARs of a novel class of potent and selective δ ligands, the pyrrolooctahydroisoquinolines, featuring non-aromatic δ addresses have been reported. A model of the transmembrane regions of the δ opioid receptor has been constructed. Interactive docking of molecules from this novel chemical class has provided a binding model which agrees with the SARs and might explain their affinity and selectivity for the δ receptor.

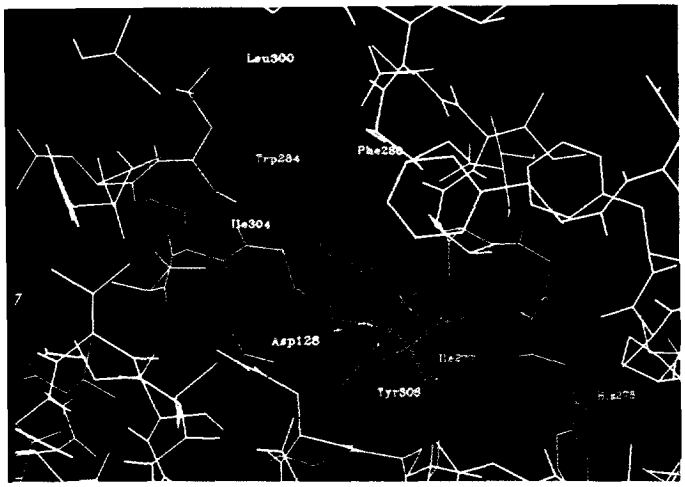


Figure 2.[22] SB 213698 (cyan) docked into the δ opioid receptor (extracellular view). Amino acid residues of the receptor interacting with the ligand are coloured in orange and labelled. Van der Waals surfaces are represented for residues constituting the hydrophobic binding pocket which accommodates the δ address.

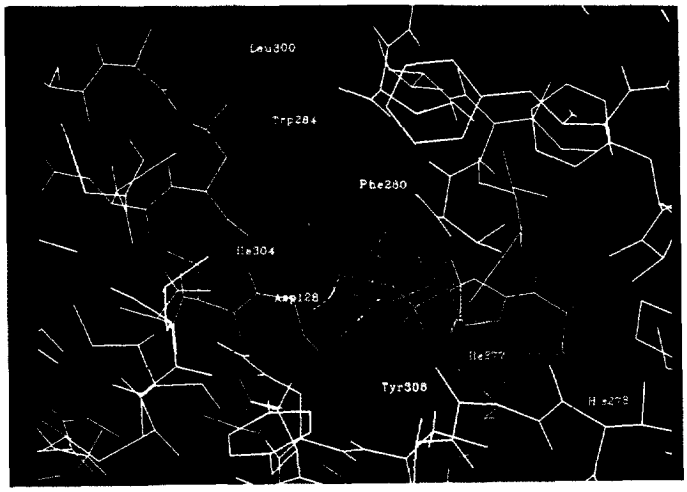


Figure 3.[22] SB 219825 (cyan) docked into the δ opioid receptor (extracellular view). Amino acid residues of the receptor interacting with the ligand are coloured in orange and labelled. Van der Waals surfaces are represented for residues constituting the hydrophobic binding pocket which accommodates the δ address.

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