OPIOID RECEPTOR ACTIVITY AND ANALGESIC POTENCY OF DPDPE PEPTIDE ANALOGUES CONTAINING A XYLENE BRIDGE

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INTRODUCTION

The cloning of opioid receptors has provided direct structural evidence of the concept of **"*multiple opioid receptors*"** as powerful tool for physiological and pharmacological evaluation of their roles in both normal and acute pain states.[1-2] Delta-opioid receptors (DOP) are appealing drug targets for pain relief, due to the lack of unwanted side effects and the strong antinociceptive activity showed by their selective agonists thus a **"smart" dual-acting opioid drug** should be designed to be a full agonist of DOP and only a partial agonist toward the μ-opioid receptors (MOR). Enkephalins are linear endogenous penta-peptides with high affinity for DOP regulating human nociception; numerous structural modifications have been explored during the last years to investigate the structure activity relationships (SAR) and to improve their selectivity;[3] H-Tyr-*c*[D-Pen-Gly-Phe-D-Pen]-OH (DPDPE) is the first synthetic prototype of highly selective constrained cyclic peptide for this receptor (Figure 1).

AIM AND SCOPE

In this work we propose the design, synthesis and biological evaluation of new cyclic DPDPE analogues **7a-c** containing *o*-, *m*-, *p*-xylene regioisomers respectively, as μ/δ mixed opioid receptor agonists, using *in vitro* and *in vivo* models to examine the antinociceptive activity. The *C*-terminal free carboxyl group in DPDPE improves the δ/μ selectivity,[4] then it has been maintained in our cyclic peptides. The cyclic peptides **7a-c** have been also investigated by molecular docking study, to discern the structural influences of xylene regioisomers on the molecular interactions at the DOP.

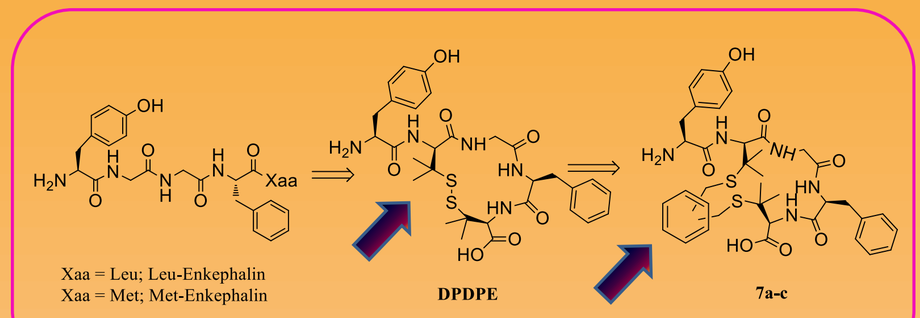
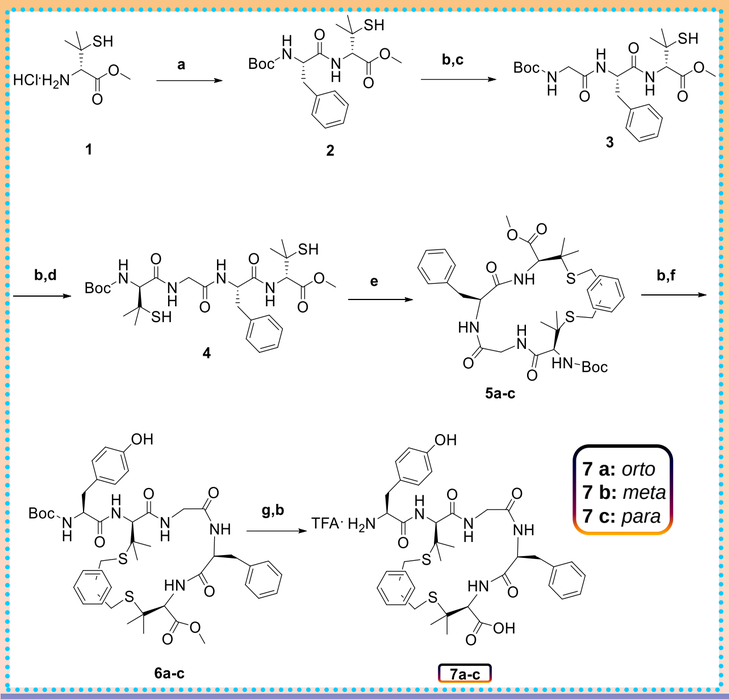


Figure 1. Cyclization strategy from Leu/Met-Enkephalins to gain compounds **7a-c**.

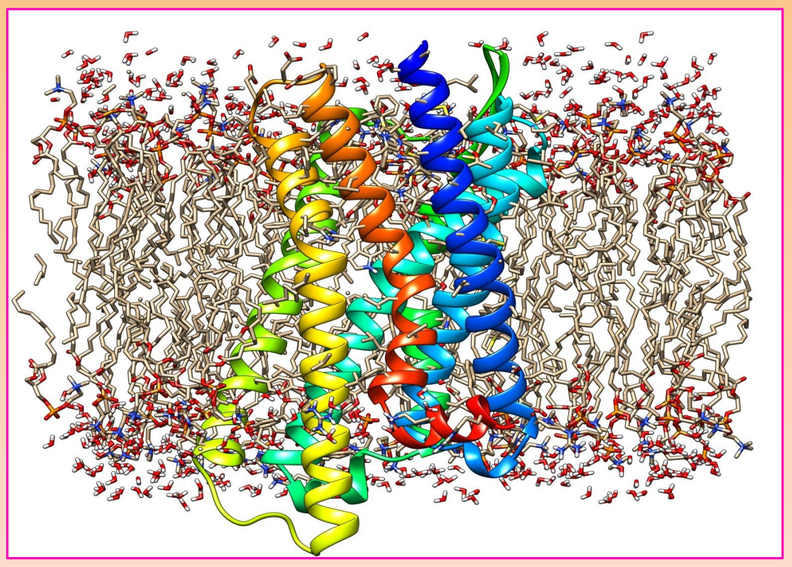
SYNTHESIS AND CHARACTERIZATION

A *side-chain-to-side-chain* cyclization involving the two thiols groups and three dibromo-xylene regioisomers has been performed. The xylene-type bridging reaction utilizes the exquisite reactivity of dibromo-xylene scaffolds toward free thiols groups of D-Pen residues on compound **4**, providing cyclic peptides containing two robust thioether bonds (**5a-c**). Compounds **7a-c** have been characterized as TFA salts by UPLC-MS and ^{1}H-NMR.



Scheme 1. *Reagents and Conditions*. (a) 1.1 equiv BocPhe-OH, 1.1 equiv EDC·HCl, 1.1 equiv HOBt anhydrous, 3.3 equiv DIPEA, DMF under N_2 atmosphere, r.t., overnight; (b) TFA/DCM = 1:1 under N_2 atmosphere, r.t., 1h; (c) 1.1 equiv BocGly-OH, 1.1 equiv EDC·HCl, 1.1 equiv HOBt anhydrous, 3.3 equiv DIPEA, DMF under N_2 atmosphere, r.t., overnight; (d) 1.1 equiv Boc(D)Pen-OH, 1.1 equiv EDC·HCl, 1.1 equiv HOBt anhydrous, 3.3 equiv DIPEA, DMF under N_2 atmosphere, r.t., overnight; (e) 2.1 equiv of *o*-dibromo-xylene, 6 days for **5a**, 1.3 equiv of *m*-dibromo-xylene, 4 days for **5b**, 2.1 equiv of *p*-dibromo-xylene, 6 days for **5c**, 2.6 equiv DIPEA in DMF under N_2 atmosphere, r.t.; (f) 1.1 equiv BocTyr-OH, 1.1 equiv EDC·HCl, 1.1 equiv HOBt anhydrous, 3.3 equiv DIPEA, DMF under N_2 atmosphere, r.t., overnight; (g) 4 equiv NaOH 1M in THF, 5h for **7a**, 2 equiv NaOH 1M in THF, 2h for **7b**, 3.5 equiv NaOH 1M in THF, 3h for **7c**, r.t.

MOLECULAR DYNAMICS SIMULATION



MD of compound **7a** on DOR by GROMACS 5.2: System built by CHARMM-GUI server with DPPC bilayer membrane, 310 K, t = 1 ns; CHIMERA UCFS for visualization.

MOLECULAR DOCKING STUDY

To explore the impact of xylene bridge incorporation on the biological activity profile, compounds **7a-c** and DPDPE were docked to the δ-opioid receptor (PDB:4RWD) using Glide embedded in Maestro 9.2 and employing the generated grid around 10Å from the crystallographic ligand H-Dmt-Tic-Phe-Phe-NH_2 (DIPP-NH_2).[5] The best docking pose for each ligand was selected basing on XP scoring function.

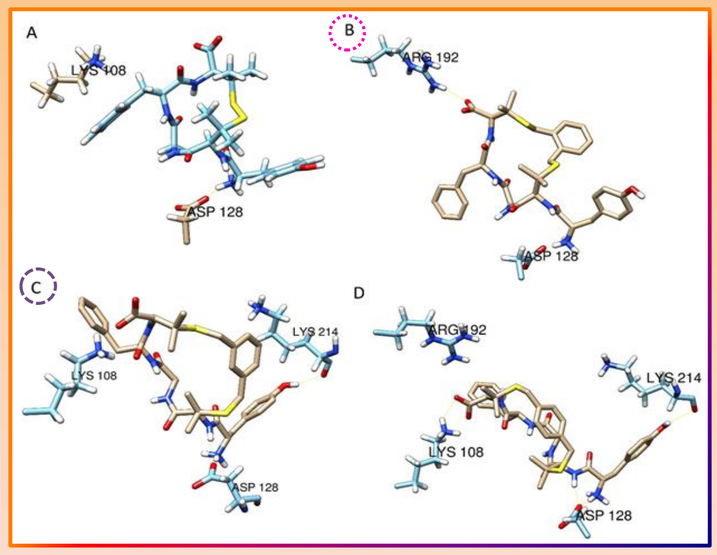


Figure 2. Best docking poses of DPDPE (A), compound **7a** (B), compound **7b** (C) and compound **7c** (D) at the DOR (only the involved residues are depicted).

The interaction between residue Asp128 and the amino terminus of each ligand is the most important and was preserved in all 5 models. The interaction with Arg192 has been also maintained in **7a** and **7c**, but **7b** gained the best docking score and its best docking pose is stabilized by hydrogen bonds with Asp128, Lys214 and a saline bridge with Lys108. The xylene bridge enclosed in **7a-c** is topically very similar to the Tic moiety in DIPP-NH_2.[5]

IN VITRO BINDING ASSAYS

All the cyclic compounds **7a-c** showed good binding profile for DOR, although they displayed a lower affinity than DPDPE (Figure 3). However, they showed a considerable higher affinity for MOR compared to DPDPE, indicating that the structural modifications on the parent compound significantly reduced its δ-opioid receptor selectivity (Table 1). This behavior closely resembles that of small cycle enkephalin analogues containing a thiourea bridge.[6] In particular, cyclic peptide **7a** had the best K_i value for DOR and MOR (Table 1).

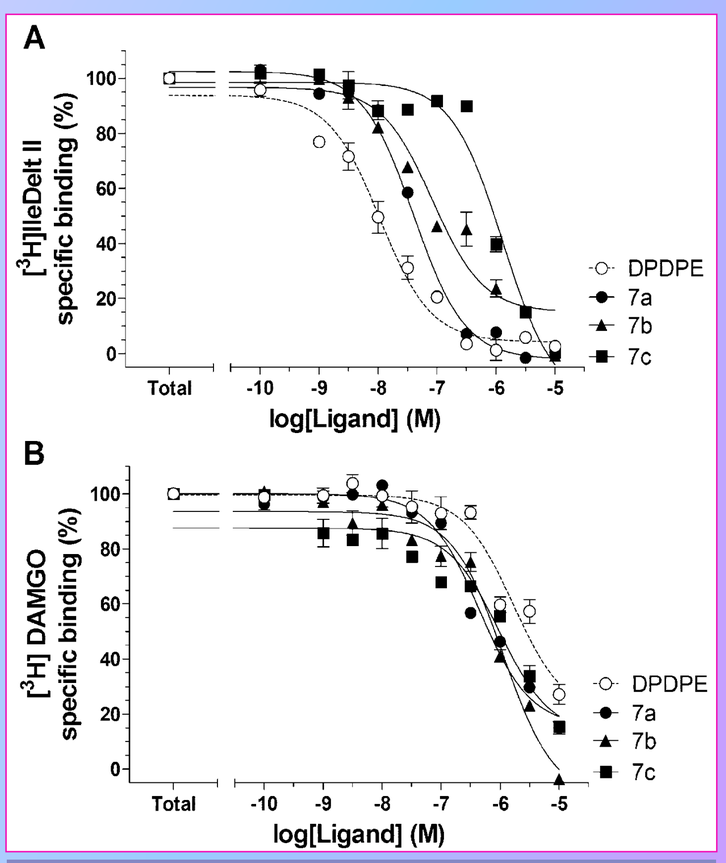


Figure 3. The δ and μ opioid receptors binding properties of **7a-c** compared to DPDPE in radioligand competition binding experiments. Figures represent the specific binding of [^{3}H]IleDelt II (**A**) and [^{3}H]DAMGO (**B**) in percentage in the presence of increasing concentrations (10^{-10}-10^{-5} M) of the indicated unlabeled ligands in rat brain membrane homogenates. "Total" on the x-axis indicates the total specific binding of the radioligand, which is measured in the absence of the unlabeled compounds. The level of total specific binding was defined as 100%. Points represent means ± S.E.M. for at least three experiments performed in duplicate.

	Ki		*Ratio*
Cpds.	[^{3}H]IleDelt II (δ)	[^{3}H]DAMGO (μ)	μ/δ
DPDPE	4.5 nM	438.1 nM	97.3
7a	**16.9 nM**	**115 nM**	**6.7**
7b	**34.9 nM**	**292.2 nM**	**8.3**
7c	546 nM	249 nM	0.5

Table 1. The affinity values (K_i) and μ/δ selectivity ratios of **7a-c** compounds and DPDPE in [^{3}H]IleDelt II and [^{3}H]DAMGO competition binding assays in rat brain membrane homogenates. The K_i values were calculated based on the competition binding curves seen in Figure 3.

IN VIVO ANTINOCICEPTIVE TESTS

Antinociception assays have been also carried out to investigate the *in vivo* potential activity of the newly designed compounds. From the tail flick (TF) and hot plate (HP) tests we observed that compound **7b** exerted a potent analgesic effect over 60% MPE, ranging from 15 to 60 minutes, after i.c.v. administration; this result has been also confirmed by formalin test (Figure 4).

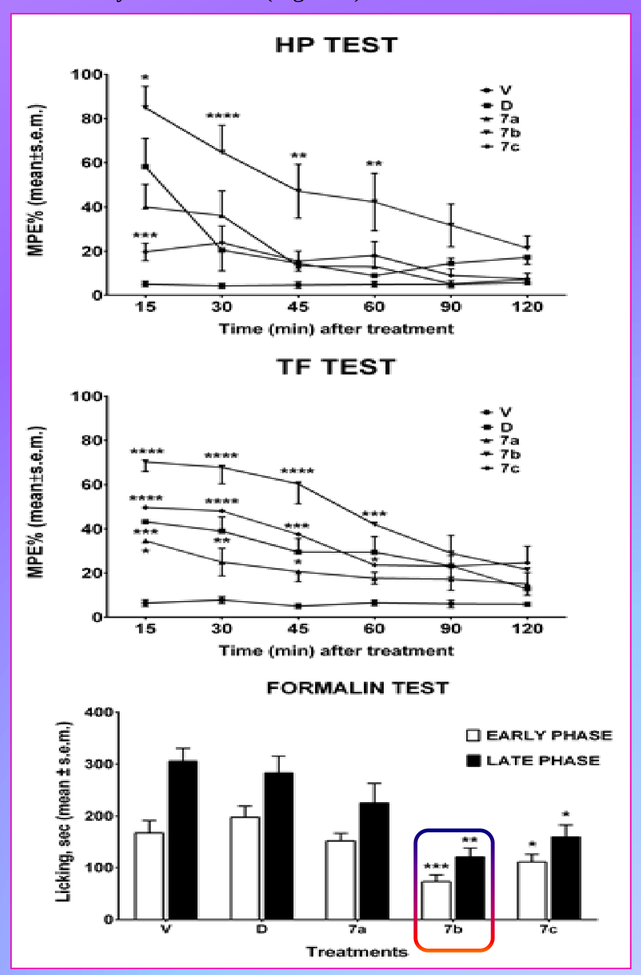


Figure 4. TF, HP and FT assays on **7a-c**. V is for vehicle, D is for DPDPE. In the HP and TF test, drugs were injected i.c.v. at 23 nmol/mouse. In the formalin test, drugs were administered s.c. at 150 nmol/mouse, 15 minutes before formalin. **** is for P<0.0001, *** is for p<0.001, ** is for p<0.01, * is for p<0.05 *vs* V. N=8-10.

CONCLUSIONS

- Compound **7b** incorporating *m*-xylene bridge represents an exquisite example of well-balanced equilibrium between δ-opioid receptor affinity, μ/δ selectivity and analgesic potency.
- The novel compounds induced robust and long-lasting antinociceptive effects both after central and local peripheral administration.
- Our data validate the hypothesis that the design of novel chemical entities specifically targeted at known receptors will provide vantages for pain-relief in many pathological situations.

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