Structural Modification and Biological Evaluation of Dmt[1]-DALDA Analogues

European Opioid Conference

Novoa Alexandre[1], Van Dorpe Sylvia[2], Wynendaele Evelien[2], Chung Nga N.[3], Lemieux Carole[3], Schiller Peter W.[3], Tourwé Dirk[1], De Spiegeleer Bart[2], Ballet Steven[1]

[1] ORGC, Department of Organic Chemistry, Vrije Universiteit Brussel, Brussels, Belgium
[2] Drug Quality & Registration (DruQuaR) group, Department of Pharmaceutical Analysis, Ghent University, Ghent, Belgium
[3] Laboratory of Chemical Biology and Peptide Research, Clinical Research Institute of Montreal, Montreal, QC, Canada

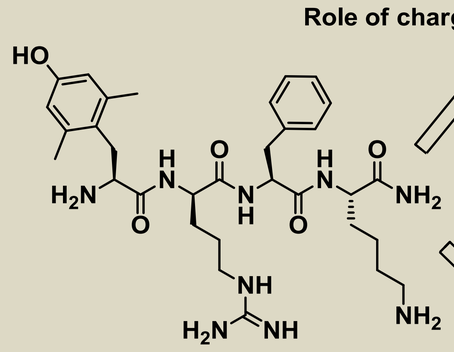
e-mail: anovoa@vub.ac.be

Introduction

The highly charged tetrapeptide Dmt-DALDA (Dmt-D-Arg-Phe-Lys-NH_2) has been previously identified as a potent μ-opioid receptor agonist and serves as a lead compound for the further development of novel therapeutic (peptidic) opioid analgesics.[1] Structural modifications of the peptide have been performed in order to determine the role of the side chain charges, N-methylation of the Phe[3]-Lys[4] amide bond, and the influence of a benzazepine conformational constraint[2] at the level of Phe[3].

All prepared compounds have been tested for their *in vitro* affinity and activity (GPI and MVD assays) and four of them for their *in vivo* tissue distribution and *in vitro* permeability (in- and efflux into and out of mouse brain and caco-2 test)

Dmt[1]-DALDA and analogues



Dmt1-DALDA 1

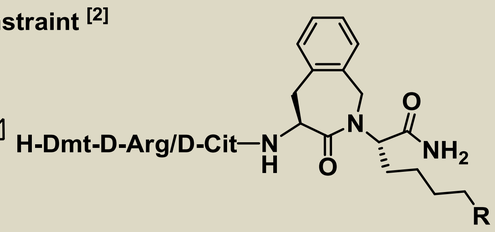
Role of charges

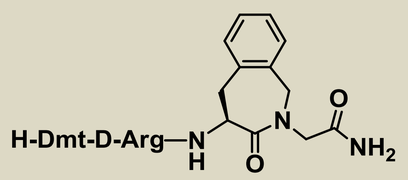
H-Dmt-D-Arg-Phe-Lys-NH_2 1
H-Dmt-D-Arg-Phe-Nle-NH_2 2
H-Dmt-D-Cit-Phe-Lys-NH_2 3
H-Dmt-D-Cit-Phe-Nle-NH_2 4

N-methylation

H-Dmt-D-Arg-Phe-NMeLys-NH_2 5
H-Dmt-D-Arg-Phe-NMeNle-NH_2 6
H-Dmt-D-Cit-Phe-NMeLys-NH_2 7
H-Dmt-D-Cit-Phe-NMeNle-NH_2 8

Introduction of conformational constraint [2]





H-Dmt-D-Arg-Aba-Lys-NH_2 9
H-Dmt-D-Arg-Aba-Nle-NH_2 10
H-Dmt-D-Cit-Aba-Lys-NH_2 11
H-Dmt-D-Cit-Aba-Nle-NH_2 12

H-Dmt-D-Arg-Aba-Gly-NH_2 13

Activity : μ agonist and δ agonist

Peptide	GPI (IC_{50}, nM)	Rel.pot.*	MVD (IC_{50}, nM)	Rel.pot.*	MOR (IC_{50}, nM)	Rel.binding*	DOR (IC_{50}, nM)	Rel.binding*	
H-Dmt-D-Arg-Phe-Lys-NH_2 **1**	**1.41**	**81**	**23.1**	**2.7**	**0.58**	**147**	**877**	**0.12**	side-chain charges are not essential for in vitro activity
H-Dmt-D-Arg-Phe-Nle-NH_2 **2**	**2.2**	**119**	**6.2**	**2.6**	**0.23**	**132.5**	**15.2**	**0.64**	
H-Dmt-D-Cit-Phe-Lys-NH_2 **3**	**0.31**	**791**	**1.4**	**9.64**	**0.57**	**52.4**	**50.0**	**0.25**	
H-Dmt-D-Cit-Phe-Nle-NH_2 **4**	0.89	203	4.7	4.12	0.79	28.1	3.98	1.46	
H-Dmt-D-Arg-Phe-NMeLys-NH_2 **5**	8.3	106	7.0	6.0	0.26	115.4	309	0.027	N-methylation is allowed for in vitro activity and potentially stabilizes the amide bond between Phe[3] and Lys[4]
H-Dmt-D-Arg-Phe-NMeNle-NH_2 **6**	4.35	45	1.5	9.3	0.26	135.8	16.0	0.51	
H-Dmt-D-Cit-Phe-NMeLys-NH_2 **7**	1.01	398	4.4	9.5	0.94	23.6	39.2	0.347	
H-Dmt-D-Cit-Phe-NMeNle-NH_2 **8**	0.65	434	1.5	1.0	2.92	11.3	5.03	1.94	
H-Dmt-D-Arg-Aba-Lys-NH_2 **9**	76.0	13	64 (IC_{25}, max50%)	0.67	0.605	50.97	38.56	0.276	the conformational constraint of the Phe residue by the Aba results in highly potent compounds, but is not compatible with the Lys side chain
H-Dmt-D-Arg-Aba-Nle-NH_2 **10**	1.45	327	3.1	5	0.432	82.28	7.46	1.24	
H-Dmt-D-Cit-Aba-Lys-NH_2 **11**	49.0	18.9	1000	30 % inh	2.618	8.547	56.91	0.108	
H-Dmt-D-Cit-Aba-Nle-NH_2 **12**	0.78 (IC_{35max} : 67 %)	643	1.16	17.5	0.931	38.64	3.176	2.1816	
H-Dmt-D-Arg-Aba-Gly-NH_2 13	**0.32**	**/**	**0.42**	**/**	**0.15**	**/**	**0.60**	**/**	**Superpotent μ/δ agonist**

***relative to Leu[5]-enkephalin (binding : MOR: 29.9 nM, DOR: 9.77 nM)**

Permeability

BBB penetration of ^{125}I-peptides[3]

Peptide	K_{in} (μl/(g×min))	Capillary fraction (%)	Parenchyma fraction (%)	k_{out} (min^{-1})
H-Dmt-D-Arg-Phe-Lys-NH_2 **1**	0.37 ± 0.08	23.12 ± 10.26	76.88 ± 10.26	0.058 ± 0.11
H-Dmt-D-Arg-Phe-Nle-NH_2 **2**	0.25 ± 0.25	32.97 ± 9.99	67.03 ± 9.99	0.039 ± 0.11
H-Dmt-D-Cit-Phe-Lys-NH_2 **3**	-0.31 ± 0.45	19.39 ± 15.81	80.61 ± 15.81	0.027 ± 0.022
H-Dmt-D-Arg-Aba-Gly-NH_2 **13**	0.45 ± 0.20	30.95 ± 19.75	69.05 ± 19.75	0.096 ± 0.046

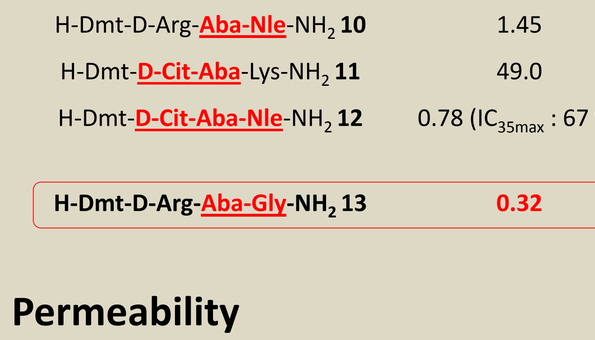
-Significant influx for peptide 1,2 and 13
-No efflux for peptides 1 and 2, while statistically significant efflux observed for 13

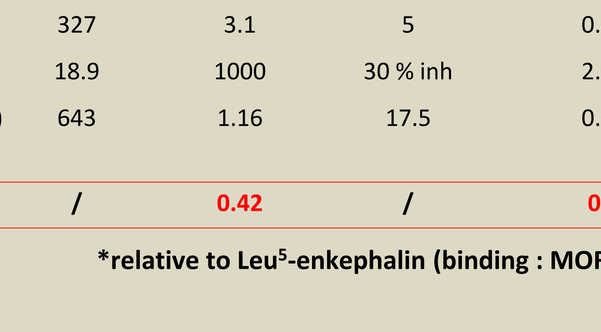
CACO-2 test

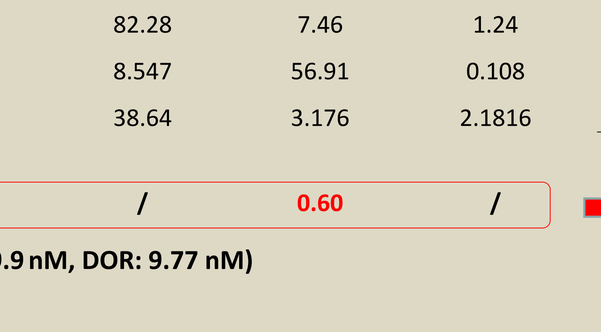
Peptide	Chem. Properties MW / Log P / Log D (7.4)/Log S tPSA / nD / nA / nRot	A-B perm. (10^{-6} cm/s)	A-B recover (%)	B-A perm. (10^{-6} cm/s)	B-A recover (%)
H-Dmt-D-Arg-Phe-Lys-NH_2 **1**	639.8 / 0.4 / -0.4 / -6.6 264.6 / 10 / 8 /20	<0.1	89	<0.1	95
H-Dmt-D-Arg-Phe-Nle-NH_2 **2**	624.8 / 1.9 / 1.1 / -7.0 238.5 / 9 / 7 /19	<0.4	101	<0.5	89
H-Dmt-D-Cit-Phe-Lys-NH_2 **3**	640.8 / 0.4 / -0.4 / -7.4 257.8 / 9 / 8 /19	<0.6	85	<0.8	111

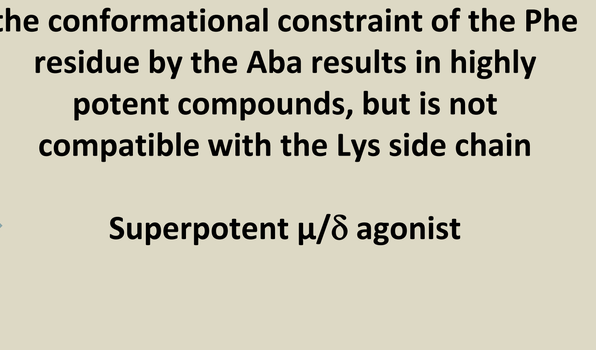
the CACO-2 assay predicts a limited transport through the intestinal membrane

Relative concentrations in the different tissues : MTR experiment









-Significative accumulation in liver for H-Dmt-D-Arg-Phe-Nle-NH_2 2
- No specific accumulation for the three others peptides

Conclusions

A. Side chain charges and/or N-methylation are not essential for in vitro affinity and activity
B. Significant influx into the mouse brain for peptide 1, 2 and 13 demonstrated by the K_{in} values and the parenchyma fraction
C. The CACO-2 test predict a limited transport through the intestinal membrane (GI → blood).

References

[1] a) Zhao K., Luo G., Zhao G.-M., Schiller P.W., Szeto H. H., *J. Pharmacol. Exp. Ther.,* **2002**, Vol. 304, p 425-432.
b) Shimoyama M., Szeto H.H., Schiller P.W., Tagaito Y., Tokairin H., Eun C.M., Shimoyama N., *Pharmacology,* **2008**, *83*, p 33-37
[2] a) Ballet S., Frycia A., Piron J., Chung N.N., Schiller P.W., Kosson P., Lipkowski A.W., Tourwe D., *J. Peptide Res.*, **2005**, 66, p 222-230
b) Van Rompaey K., Van den Eynde I., De Kimpe N., Tourwe D., *Tetrahedron*, **2003**, 59, p 4421-4432
[3] Van Dorpe S., Adriaens A., Polis I., Peremans K., Van Bocxlaer J., De Spiegeleer B., *Peptides*, **2010**, 31, p 1390-1399

Acknowledgements

We thank the Research Foundation-Flanders (FWO) and the Ministère du Développement Economique, de l'Innovation et de l'Exportation du Québec (MDEIE) for the financial support.

A.N. would like to personally thank the Organizing Committee for the travel grant.