DESIGN OF HIGH-AFFINITY LIGANDS FOR THE BENZOTHIADIAZINE ALLOSTERIC BINDING SITE OF THE AMPA RECEPTORS

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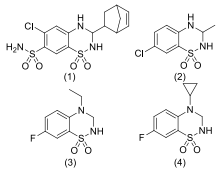
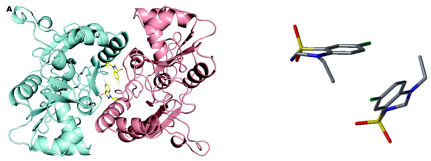
Introduction

L-glutamate is the major excitatory neurotransmitter in the mammalians central nervous system. This ligand is known to bind to metabotropic and ionotropic receptors. Among the latters, three subtypes have been identified: NMDA, AMPA and KA receptors. While an overstimulation of AMPA receptors may characterize neurological pathologies such as Hungtinton or Parkinson diseases, low AMPA signals may trigger some neurological disorders like cognitive deficit, schizophrenia, depression or ADHD. In this case, the use of AMPA positive allosteric modulators (AMPApams) seems an interesting approach. Indeed, they are expected to trigger less excitotoxicity phenomenons. The first compounds studied in this pharmacological class were cyclothiazide (1) and IDRA-21 (2). Over the past years, our team developed some potentiators belonging to 3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxides. Among the developed compounds emerge (3) and (4) [1][2].



State of the art

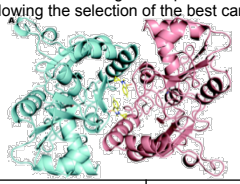
Compound (3) was cocrystallised with the ligand binding domain of a GluA2 subunit thanks to a collaboration with Pr Kastrup[3].
This study highlighted that two modulators bind in two contiguous sites at the dimer interface. These results suggested the design of dimeric benzothiadiazine 1,1-dioxides able to bind both sites.
The resulting dimers are expected to express better activity and affinity than their corresponding monomers.

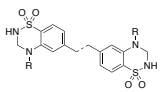


Modulator (3) cocrystallized with GluA2 LBD [3] and the relative position of the 2 ligands found in the allosteric binding pocket

Docking experiment

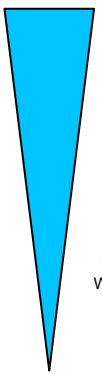
Based on the cristallographic data obtained with molecule (4) in the allosteric pocket, docking experiments were achieved by C. Bouckaert (NAMEDIC). This study confirmed in silico that the envisaged dimers could interact with the allosteric site. Moreover it gave a prediction of the binding energy of those dimers, thus allowing the selection of the best candidates.





Binding energy was calulated for each proposed dimer, and highlighted.

Structure	Binding energy (kcal/mol)
A	-55.6828
B	-54.8251
D	-53.2375
C	-45.4065



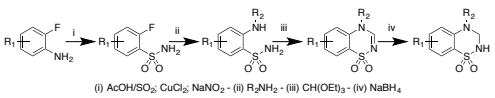
Providing best dimeric candidates based on these « rules »

Linker lengh:
Ethyl over propyl

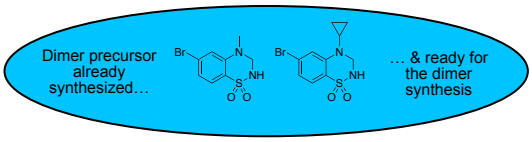
R substituents:
With Ethyl : equivalent
With Popyl : cPr over Me

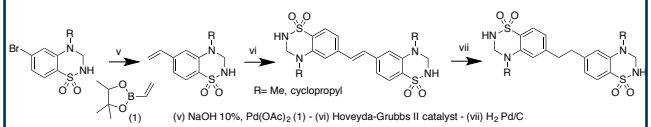
Synthesis

General pathway for the synthesis of benzothiadiazine dioxides



(i) $AcOH/SO_2$; $CuCl_2$; $NaNO_2$ - (ii) R_2NH_2 - (iii) $CH(OEt)_3$ - (iv) $NaBH_4$





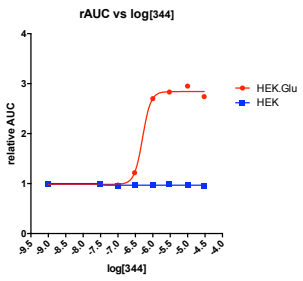
(1) (v) NaOH 10%, $Pd(OAc)_2$ (1) - (vi) Hoveyda-Grubbs II catalyst - (vii) H_2 Pd/C

Pharmacology

We first developed an electrophysiological assay in order to screen our new compounds. It quickly appeared that this strategy was too time consuming to be considered as a screening test.

Therefore, we have developed a fluorimetric assay on HEK293 cells stably expressing the GluA(2)/Q flop. This method seems to be more convenient to pursue our goal and can be used as a medium throuput screening, thus providing us the Ec50 of our modulators.

This figure shows the effect of molecule (4), our reference compound. The Ec50 recorded in our assay (0.66 µM) was found to be similar to that found in the literature (0.90 µM).



Conclusion

We have synthesized the first examples of dimers acting as putative AMPApams. In short term, we expect to obtain other novel dimer analogues predicted in the docking experiments. These molecules will be tested in order to validate or invalidate our hypothesis that dimeric compounds express higher affinity than monomers for the AMPA receptors. Some of those compounds will be sent to the University of Copenhagen for crystallographic studies on the GluA2 ligand binding domain. The crystallographic results may confirm the docking model, and thus the predictive power of this tool.

References

1 P. Francotte, et al; J. Med. Chem, 2007, 50, 3153-3157;
2 A.-B. Nørholm, P. Francotte, et al; J. Med. Chem, 2013; 56, 8736-8745;
3 C. Krintel et al; Biochem. J., 2012; 441: 173–178.