Synthesis of a group of novel Xanomeline/77-LH-28-1 hybrid ligands and their FRET investigation at muscarinic acetylcholine receptor subtypes

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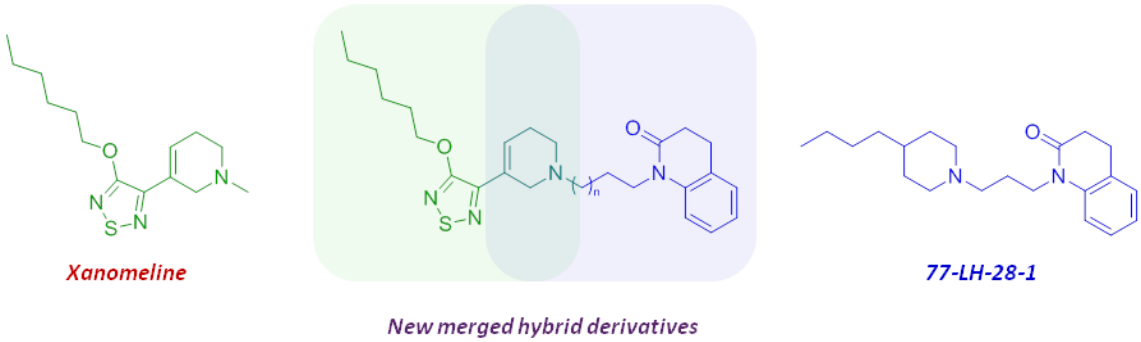
In connection with our interest in investigating novel rationally designed bitopic (i.e., orthosteric/allosteric) derivatives targeting muscarinic acetylcholine receptor (mAChR) subtypes (1,2,3), in this study we designed and synthesized a new set of ligands that integrate in the same molecular skeleton the pharmacophoric moieties of Xanomeline and of 77-LH-28-1 (1-[3-(4-butyl-1-piperidinyl)propyl]-3,4-dihydro-2(1*H*)-quinolinone). Xanomeline is a well-known M_1/M_4-preferring orthosteric agonist, which ameliorated cognitive impairments in Alzheimer's disease patients and showed activity in various models of schizophrenia, thus being potentially beneficial for treatment of positive, negative and cognitive symptoms (4). On the other hand, 77-LH-28-1 was characterized as an M_1-selective, positive allosteric modulator, thus representing an interesting pharmacological tool with cognition enhancing properties (5). As illustrated below, we planned the novel bipharmacophoric derivatives as merged structures, with the tetrahydropyridine nucleus of Xanomeline as the central core.



In the last years, different receptor sensors, based on the fluorescence resonance energy transfer (FRET), were generated for various G protein-coupled receptors, and represented a valuable tool to investigate real time receptor activation as well as ligand-receptor interactions. Recently, this analysis was performed also on a set of bitopic ligands designed for a selective interaction with M_1 mAChRs (6). Our preliminary results on the group of Xanomeline/77-LH-28-1 hybrid compounds indicate, for the M_1 sensor, a reproducible activation response, which depends on the linker length. Conversely, no FRET-related effect could be detected at the M_2 sensor. Thus, a critical spacer length of the hybrid compounds induces conformational changes with a degree of selectively for the M_1 muscarinic receptor. The synthesis and the results of pharmacological investigation will be presented and discussed.

References: 1. J. Antony, K. Kellershohn, M. Mohr-Andrä, A. Kebig, S. Prilla, M. Muth, E. Heller, T. Disingrini, C. Dallanoce et al., *FASEB J* **2009**, *23*, 442-450. 2. A. Bock, B. Chirinda, F. Krebs, R. Messerer, J. Bätz, M. Muth, C. Dallanoce et al., *Nat. Chem. Biol.* **2014**, 10, 18-20. 3. A. Bock, M. Bermudez, F. Krebs, C. Matera, B. Chirinda, D. Sydow, C. Dallanoce et al., *J. Biol. Chem.* **2016**, *291*, 16375-16389. 4. S. Barak, I. Weiner, *Int. J. Neuropsychoph.* **2011**, *14*, 1233-1246. 5. C. J. Langmead, N. E. Austin, C. L. Branch, J. T. Brown, K. A. Buchanan, C. H. Davies, I. T. Forbes et al., *Br. J. Pharmacol.* **2008**, *154*, 1104-1115. 6. R. Messerer, M. Kauk, D. Volpato, M. C. Alonso Canizal, J. Klöckner, U. Zabel, S. Nuber, C. Hoffmann, U. Holzgrabe, *ACS Chem. Biol.* **2017**, *12*, 833-843.