Rat α4β2		Human α4β2		Human α7	(Enant)-compound
Ki (nM)	EC_{50} (μM)	I_{max} (%ACh)		EC_{50} (μM)	I_{max} (%ACh)
(S)-Isoanatabine	650	0.91	78.8	45.1	76.1
(R)-Isoanatabine	198	0.32	103	51.9	31.2
(S)-Anatabine	282	0.79	39.8	33.0	103
(R)-Anatabine	114	0.48	17.2	41.1	90.3
(S)-Nicotine	5.6	0.21	84.3	56.5	91.7
(S)-Anabasine	1,100	7.91	76.0	18.4	100
Anabaseine	94	~12	~8	18	100

(R)-Isoanatabine (1) is 3-fold more potent than (S)-isoanatabine at α4β2 receptor, but its α7 I_{max} is only about half as great; (2) is a potent α4β2 agonist comparable to (S)-nicotine; (3) displays a much higher efficacy at the α4β2 receptor and a much lower I_{max} at α7 relative to anatabine; (4) relative to anabasine, is a 9-fold more potent α4β2 partial agonist but a 3-fold less efficacious α7 partial agonist; (5) Isoanatabine, relative to anabaseine, is a much more potent and efficacious α4β2 agonist. The 3,4 position double bond in isoanatabine and, to a lesser extent, the 4,5-position double bond in anatabine contribute greatly to their α4β2 agonist activities.

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1.19

Automated two-electrode voltage clamp for medium-throughput studies of ion channels with non-destructive sample analysis

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Electrophysiological recordings using patch clamp systems are important tools for the study of ion channels and receptors. Although conventional patch clamp delivers high-quality data, it does not allow a fast high-throughput screening of drugs on ion channels or receptors, and is mostly limited to record data of maximum 40 cells daily by a skilled scientist. Here, we present a new automated two-electrode voltage clamp, which allows a fast, reliable, and high-quality screening of up to 96 drugs in a single experiment. Our system is equipped with one 96-well plate, which contains the samples (or toxins) using minimal volumes (220 μl). A fact that is especially important when probing effects of samples that are only available in minute amounts such as toxins. Since the automate uses a non-destructive measurement with drugs stored in the 96-well plate, samples can be reused several times to evaluate their effects either at the same channel subtype or to another membrane protein. A second 96-well plate contains the injected oocytes from *Xenopus*. Oocytes are automatically loaded and poked and their properties assessed to determine the membrane quality. As the automate works fully unattended, measurements can be carried continuously over 24 h. Depending on the type of experiment, and therefore the chosen protocol, we can measure from few to hundreds of oocytes daily. The importance of such a tool is illustrated by characterizing several variants of the ImI and ImII toxins. This new system provides a medium-throughput screening platform and expands by orders of magnitudes the number of samples and cells that can be measured in a single day while offering, in addition, the capacity to work with very limited sample size.

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1.20

Novel α7 nAChRs ligands: From virtual screening to functional assays

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Progresses made in the understanding of the tridimensional structure of the nicotinic acetylcholine receptors (nAChRs) allows to exploit new strategies for the finding of novel molecules acting at this class of ligand gated channels. Taking advantage of the crystal structure of the acetylcholine binding protein (AChBP), in silico ligand design from the chemical universe data base (GDB) [1] and virtual screening was performed. This allowed the identification of novel molecules that should display selectivity for the α7 nAChRs. Selected virtual hits were synthesized and their functional properties assessed at human nAChRs expressed in *Xenopus* oocytes. Experimental protocols were designed to probe the putative agonist or antagonist activities of these molecules. 72,945 virtual ligands were investigated using docking (Autodock and Glide) and shape similarity to known α7 ligands (ROCS). Thirty-eight structures among the 10% top-scoring virtual hits were selected for their structural novelty and ease of synthesis, prepared by standard organic synthesis methods, and their properties analyzed. Most of the molecules displayed antagonist properties with IC_{50}'s in the low micromolar range. Thus while this strategy properly identifies ligands that interact with the receptor, further refinement of our model is required for the identification of selective agonists. Altogether these data illustrate the power of combining virtual chemistry, small scale synthesis and electrophysiological approaches.

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1.21

Homology models of the alpha7 acetylcholine receptor based upon bacterial receptors: Comparison of experimental and *in silico* derived data

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Unraveling the mechanistic link between agonist binding and ion permeation in ligand-gated channels remains a challenge for modern biophysics. The recent high-resolution crystallization of