Molecular insights into hERG potassium channel blockade by lubeluzole

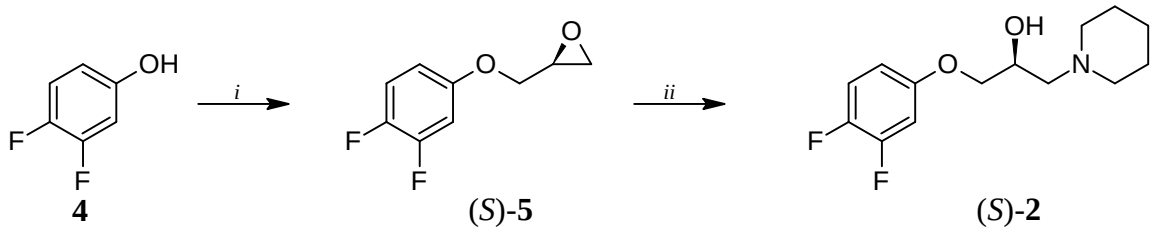
Supporting Information

Materials and Methods

Synthesis of lubeluzole, its enantiomer, and corresponding moieties

Chemistry

Lubeluzole [(*S*)-**1**], its enantiomer [(*R*)-**1**], and *N*-methyl-*N*-piperidin-4-yl-1,3-benzothiazol-2-amine (**3**) [Fig. 1A] were prepared as reported in the literature [Cavalluzzi *et al.*, 2013]. Compound **3** was then converted into its hydrochloride salt by treatment with a few drops of 2 M HCl and azeotropically removing water. (–)-(*S*)-1-(3,4-Difluorophenoxy)-3-(piperidin-1-yl)propan-2-ol [(–)-(*S*)-**2**] was obtained starting from 3,4-difluorophenol (**4**) which was reacted with (2*S*)-glycidyl tosylate to give the oxirane (*S*)-**5** which was in turn subjected to a ring-opening reaction with piperidine to give the desired product [Scheme 1].



4 (*S*)-**5** (*S*)-**2**

Scheme 1. *Reagents and conditions: (i) (2S)-glycidyl tosylate, NaH 60%, dry DMF, room temperature; (ii) piperidine, Yb(OTf)$_3$, dry CH_2Cl_2, room temperature.*

(+)-(*S*)-1-(3,4-Difluorophenoxy)methyl]oxirane [(+)-(*S*)-5].

A solution of 3,4-difluorophenol (**4**, 0.500 g, 3.8 mmol) in dry DMF (20 mL) was added dropwise to a suspension of NaH (60%) (0.34 g, 8.5 mmol) in dry DMF (20 mL) at room temperature, under nitrogen atmosphere. After 30 min, a solution of (2*S*)-glycidyl tosylate (0.895 g, 3.9 mmol) in dry DMF (20 mL) was slowly added dropwise and the mixture was stirred at room temperature for 24 h. Then, the mixture was quenched with saturated NH_4Cl, diluted with H_2O, and extracted with Et_2O. The combined organic phases were washed with saturated aqueous $NaHCO_3$ and dried over anhydrous Na_2SO_4. The solvent was removed *in vacuo*, and the residue was purified by flash chromatography ($CHCl_3$/petroleum ether, 9:1) to give 0.63 g of a yellow oil: quantitative yield; $[\alpha]_D^{20}$ = +5.2 (*c* 1, $CHCl_3$). Spectrometric and spectroscopic data were in agreement with those reported in the literature for the racemate [Liu *et al.*, 2001; Bruno *et al.*, 2006].

(–)-(*S*)-1-(3,4-Difluorophenoxy)-3-(piperidin-1-yl)propan-2-ol [(–)-(*S*)-2].

Prepared as reported in the literature for the racemate.Yield: 77%, yellowish oil; $[\alpha]_D^{20}$ = –22.7 (*c* 2, $CHCl_3$). The corresponding hydrochloride was obtained dissolving the free amine in anhydrous Et_2O and treating with gaseous HCl for a few seconds. Yield: 14%; mp 126–127 °C (abs EtOH/Et_2O). Anal. Calcd for ($C_{14}H_{19}F_2NO_2 \cdot HCl$): C, 54.64; H, 6.55; N, 4.55. Found: C, 54.41; H, 6.45; N, 4.63. Spectrometric and spectroscopic data were in agreement with those reported in the literature for the racemate [Desaphy *et al.*, 2013].

***N*-Methyl-*N*-piperidin-4-yl-1,3-benzothiazol-2-amine (3).** Prepared as reported in the literature [Cavalluzzi *et al.*, 2013]. Spectrometric and spectroscopic data were in agreement with the literature [Cavalluzzi *et al.*, 2013]. The corresponding hydrochloride was obtained dissolving the free amine in anhydrous Et_2O and treating with gaseous HCl for a few seconds. Yield: 78%; mp > 250 °C (abs EtOH/Et_2O). Anal. Calcd for $C_{13}H_{17}N_3S \cdot 3HCl \cdot 0.5EtOH$: C, 44.28; H, 6.10; N, 11.06. Found: C, 44.02; H, 5.79; N, 11.18.

Patch-clamp data analysis

The fractional block of I_{hERG} was determined using the equation:

$$\%block = \left(1 - \frac{I_{hERG(DRUG)}}{I_{hERG(CONTROL)}}\right) * 100$$

where $I_{hERG(CONTROL)}$ is the amplitude of I_{hERG} in the absence of the drug and $I_{hERG(DRUG)}$ is the I_{hERG} amplitude in the presence of the drug.

The relationship between drug concentration and I_{hERG} fractional block was determined by fitting data with the Hill-equation:

$$\%inhibition = \frac{100}{1 + \left(\frac{IC_{50}}{[DRUG]}\right)^{nH}}$$

where [DRUG], IC_{50} and n_H represent the concentration of drug, the drug concentration producing half-maximal inhibition of I_{hERG} and the Hill coefficient, respectively.

The normalized tail-current amplitude (I_n) was plotted versus test potential (V_t) and fitted with a Boltzmann function:

$$I_n = \frac{1}{\{1 + \exp[(V_{1/2} - V_t)/k]\}}$$

where $V_{1/2}$ is the voltage at which the hERG current is half-activated, and *k* is the slope factor.

Deactivation of hERG channels was quantified by fitting the time-dependent decay of I_{tail} to a mono- or bi-exponential function. To simplify the comparison between hERG current with or without lubeluzole, we fitted all the deactivation currents by a monoexponential function, as similarly done by [Wu *et al.*, 2016] for the drug ginsenoside Rg3.

Statistical analysis

Results were expressed as means ±, *n* represents the number of cells studied. Statistical significance was analysed using paired or unpaired *t*-tests or one/two-way analysis of variance (ANOVA), when appropriate (see Figure legends). A value of $P < 0.05$ was considered significant. Data were analyzed and fitted using the "Origin 8.1" software (OriginLab, Northampton, USA).

Molecular dynamics (MD) simulations

MD simulations were performed with Gromacs5.1.1 [Abraham et al., 2015] using the Amber99sb force field [Hornak et al., 2006]. The hERG WT and S631A channels were embedded into a palmitoyloleoylphosphatidylcholine (POPC) lipid bilayer and solvated with TIP3P water [Jorgensen et al., 1983]. K^+ ions were placed at selectivity filter sites S0, S2 and S4. Corrected monovalent ion Lennard-Jones parameters for the amber force field were used [Joung et al., 2008] Electrostatic interactions were calculated at a distance smaller than 1.0 nm, long-range electrostatic interactions were treated by the particle-mesh Ewald method at every step [Darden et al., 1993]. Lennard-Jones interactions were calculated with a cut off of 1.0 nm and the LINCS algorithm [Hess et al., 1997] was used to constrain bonds. Modeling hydrogens as virtual sites [Feenstra et al., 1999] allowed for an integration step of 4 fs. V-rescale was used to keep simulation temperature constant by coupling the protein, lipids and solvent (water and ions) separately to a temperature bath of 310 K. Likewise, the pressure was kept constant at 1 bar by using the Parrinello–Rahman barostat algorithm [Parinello et al., 1981] with a coupling constant of 1 ps. Prior to simulation, 5000 conjugate gradient energy-minimization steps were performed, followed by 5 ns of equilibrium simulation in which the protein atoms were restrained by a force constant of 1000 kJ mol^{-1} nm^{-2} to their initial position. Lipids, ions, and water were allowed to move freely during equilibration. Five times 100 ns MD simulations were performed for the WT channel as well as the S631A mutant channel.

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