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Discovery of a new mexiletine-derived agonist of the hERG K^+ channel

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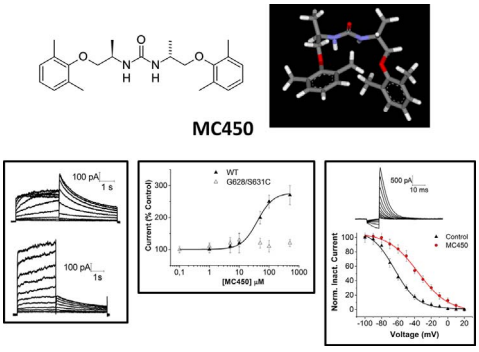
HIGHLIGHTS

- MC450 is a new mexiletine-derived agonist of hERG K^+ channel.
- MC450 increases the activation current of hERG K^+ channel with an EC_{50} of 41 ± 4 μM
- MC450 primarily acts on the inactivation mechanism of hERG channel
- The hERG double mutant G628C/S631C is insensitive to MC450.

GRAPHICAL ABSTRACT



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ABSTRACT

The human Ether-a-go-go Related Gene (hERG) potassium channel plays a central role in the rapid component (I_{Kr}) of cardiac action potential repolarization phase. A large number of structurally different compounds block hERG and cause a high risk of arrhythmias. Among the drugs that block hERG channel, a few compounds have been identified as hERG channel activators. Such compounds may be useful, at least in theory, for the treatment of long term QT syndrome.

Here we describe a new activator of hERG channel, named MC450. This compound is a symmetric urea, derived from (*R*)-mexiletine. Using patch-clamp recordings, we found that MC450 increased the activation current of hERG channel, with an EC_{50} of 41 ± 4 μM. Moreover MC450 caused a depolarizing shift in the voltage dependence of inactivation from − 64.1 ± 1.2 mV (control), to − 35.9 ± 1.4 mV, whereas it had no effect on the voltage dependence of activation. Furthermore, MC450 slowed current inactivation and the effect of MC450 was attenuated by the inactivation-impaired double mutant G628C/S631C.

1. Introduction

The hERG channel ($K_V11.1$) encodes the α-subunit of the I_{Kr} potassium channel [1,2]. Mutations in hERG gene can impair channel function thereby leading to long QT syndrome (LQTS), which consists of an abnormal prolongation of the time between the Q-wave and the T-wave of the heart's electrical cycle [3]. hERG channel may also mediate the 'acquired' (drug-induced) form of the LQTS syndrome [4]. Indeed, it is now known that structurally different drugs (e.g. Class III antiarrhythmics, antibiotics, antihistamines, antidepressants and anticancer agents) are able to block the hERG channel, causing often a concomitant risk of sudden death, as a side-effect [5]. As a consequence,

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hERG is considered one of the most important antitargets in the early drug discovery process [6,7].

Among the drugs that block hERG channel, a few compounds have been identified as hERG channel activators, during large-scale screening of chemical libraries [8–15]. On the basis of their molecular mechanism, hERG activators can be classified in two categories (Type 1 and 2): Type 1 activators slow the rate of channel deactivation. In contrast, Type 2 agonists act on the inactivation process of $K_V11.1$ channel [12]. However, many activators show both mechanisms of action and multiple binding sites. For this reason, we are still far from fully understanding the mechanism of binding of such compounds.

hERG activators, by increasing the outward potassium current during the ventricular action potential, may accelerate the action potential repolarization and reduce its duration. As a consequence, the discovery of hERG activators is important not only from a safety perspective, but also because these drugs can be proposed for the treatment of congenital forms of long QT syndrome [15,16].

Inherited LQTS is usually treated by administration of β-adrenergic receptor blockers and, when this therapy is inadequate, an implantable defibrillator or pacemaker may be adopted. Treatment of acute drug-induced LQTS consists of magnesium sulfate and discontinuing use of the offending drug. This treatment is often inadequate [8]. Drugs that activate hERG channels could help facing both chronic and acute LQTSs [17].

This work took inspiration from the following considerations. (i) A number of possible activators have been proposed and several of them present a urea scaffold [15]. In particular, the symmetric urea NS1643 (**1**, Fig. 1) was reported as a partial hERG agonist [8] whose activating effect would stem from dual opening/blocking activity. Further on, it was demonstrated that **1** binds the outer vestibule/pore entrance of the channel [14].

We hypothesized that the presence of two phenolic groups might be responsible for low membrane penetration of **1**. (ii) Mexiletine (**2**), a well-known class Ib antiarrhythmic [18] and antimyotonic drug [19], has been shown to exert cardioprotective activity in animal models through the opening of cardiac K^+-ATP channels [20,21]. At the same time, mexiletine is a low-affinity blocker of hERG channel [22,23]. Moreover, mexiletine is able to reverse the action potential prolongation in models of the long term QT syndrome [24–26]. Finally mexiletine may permeate cell membranes [27]. We speculated that linking two mexiletine units connecting their respective amine groups through a carbonyl group would have given the symmetric urea **3** resembling the structure of **1** with the possibility to act on the same binding site recognizing **1** and/or penetrate the cell membrane to reach further possible binding sites of hERG (Fig. 1).

Mexiletine is generally used as the racemate. When urea **3** is concerned, four hypothetical forms should be considered: a *meso* form (RS), a racemate (R,R plus S,S in 50:50 ratio) and the isolated enantiomers (R,R and S,S). To obtain unbiased results, we started with the isolated enantiomer (*R*,*R*)-**4** (MC450), since it has been reported that hERG may display stereoselectivity of binding [28].

2. Materials and methods

2.1. Electrophysiology experiments

Patch-clamp studies were carried out in HEK293 cells trasfected with Herg1A, clone C5 (DI.V.A.L. Toscana srl, Italy). Electrophysiological recordings were performed using the whole-cell mode of the patch-clamp technique. The extracellular solution had the following composition: 140 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 2 mM $CaCl_2$, 10 mM Glucose, 10 mM HEPES, pH 7.4 with NaOH. The following intracellular solution was used: 130 mM KCl, 1 mM $MgCl_2$, 10 mM HEPES, 10 mM EGTA, 5 MgATP pH 7.2 with KOH. Solutions were applied to the cell via a gravity-fed perfusion system (VC-6 Six Channel Valve Controller, Warner Instruments), which allows solution change in the order of milliseconds. For recordings a Multiclamp 700 B amplifier (Molecular Devices, Inc., Sunnyvale, CA) and Digidata 1440 data acquisition board (Molecular Devices, Inc., Sunnyvale, CA) with pCLAMP 10 software (Molecular Devices, Inc., Sunnyvale, CA) were used.

2.2. Synthesis of MC450

Methyl (1′*R*,2*S*,3*R*,4*R*,5*R*,6*R*)-3,4,5-tri-*O*-acetyl-6-[2-(2,6-dimethylphenoxy)-1-methylethylaminocarbonyloxy]tetrahydro-2*H*-pyran-2-carboxylate (**5**, Scheme 1) was prepared as previously reported [29]. All chemicals were purchased from Sigma–Aldrich or Lancaster at the highest quality commercially available. Yield refers to purified product and is not optimized. The structures of the compounds were confirmed by routine spectrometric and spectroscopic analyses. Only spectra for compounds not previously described are given. $ESI^{+/-}$/MS/MS analyses were performed with an Agilent 1100 series LC-MSD trap system VL Workstation (Agilent, Palo Alto, CA, USA). Elemental analyses were performed with a Eurovector Euro EA 3000 analyzer. TLC analyses were performed on precoated silica gel on aluminum sheets (Kieselgel 60 F254, Merck).

1,3-Bis[(2*R*)-1-(2,6-dimethylphenoxy)propan-2-yl] urea [(*R*,*R*)-4, MC450]

To a cooled and stirred suspension of compound **5** (0.30 g, 0.56 mmol) in methanol (8 mL), 1 M NaOH (6 mL, 6.0 mmol) was added dropwise. The mixture was stirred in an ice bath for 30 min and

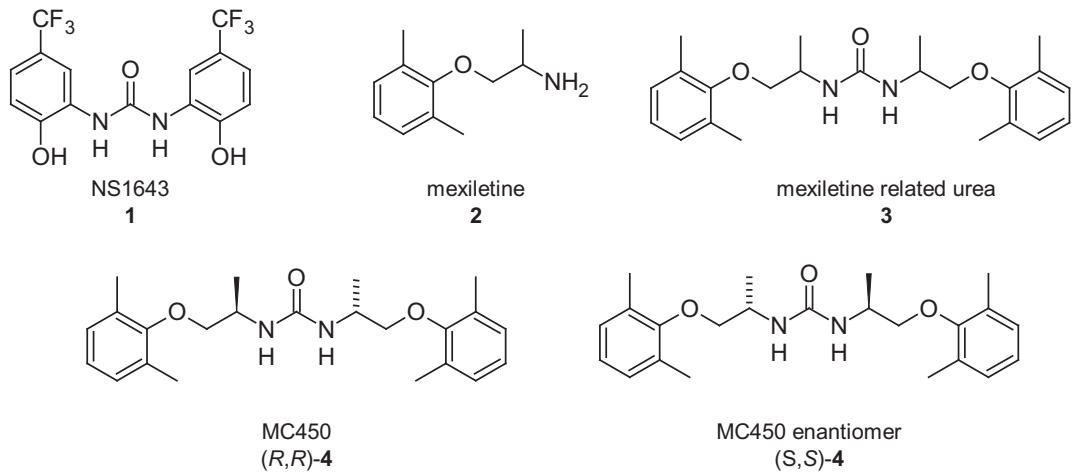

Fig. 1. Structures of the hERG agonist NS1643 (**1**), the antiarrhythmic mexiletine (**2**), and related ureas.