European Journal of Medicinal Chemistry 155 (2018) 1–12

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Research paper

Binding mechanism investigations guiding the synthesis of novel condensed 1,4-dihydropyridine derivatives with L-/T-type calcium channel blocking activity

David Schaller [a], Miyase Gözde Gündüz [b, *], Fang Xiong Zhang [c], Gerald W. Zamponi [c], Gerhard Wolber [a]

[a] Department of Pharmaceutical and Medicinal Chemistry, Institute of Pharmacy, Freie Universität Berlin, Königin-Luise-Str. 2+4, Berlin, 14195, Germany
[b] Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Hacettepe University, Sıhhiye, Ankara, 06100, Turkey
[c] Department of Physiology & Pharmacology, Hotchkiss Brain Institute, University of Calgary, 3330 Hospital Drive NW, Calgary, T2N 4N1, Canada

ARTICLE INFO

Article history:
Received 13 April 2018
Received in revised form
14 May 2018
Accepted 20 May 2018
Available online 23 May 2018

Keywords:
1,4-dihydropyridine
Hexahydroquinoline
Calcium channel
Whole cell patch clamp
Binding mechanism
Molecular modelling

ABSTRACT

Nifedipine and isradipine are prominent examples of calcium channel blockers with a 1,4-dihydropyridine (DHP) scaffold. Although successfully used in clinics since decades for the treatment of hypertension, the binding mechanism to their target, the L-type voltage-gated calcium channel Cav1.2, is still incompletely understood. Recently, novel DHP derivatives with a condensed ring system have been discovered that show distinct selectivity profiles to different calcium channel subtypes. This property renders this DHP class as a promising tool to achieve selectivity towards distinct calcium channel subtypes. In this study, we identified a common binding mode for prominent DHPs nifedipine and isradipine using docking and pharmacophore analysis that is also able to explain the structure-activity relationship of a small subseries of DHP derivatives with a condensed ring system. These findings were used to guide the synthesis of twenty-two novel DHPs. An extensive characterization using ^{1}H NMR, ^{13}C NMR, mass spectra and elemental analysis was followed by whole cell patch clamp assays for analyzing activity at Cav1.2 and Cav3.2. Two compounds were identified with significant activity against Cav1.2. Additionally, we identified four compounds active against Cav3.2 of which three were selective over Cav1.2. Novel binding modes were analyzed using docking and pharmacophore analysis as well as molecular dynamics simulations.

1. Introduction

The different subtypes of the human voltage-gated calcium channel family are involved in essential physiological processes including neurotransmitter release, hormone secretion, excitation-transcription coupling and excitation-contraction coupling. Initial drug discovery campaigns focused on the L-type calcium channel Cav1.2 for the treatment of hypertension. Subsequently, several other types of calcium channel gained attention for their potential in treating epilepsy, pain and Parkinson's disease among others [1].

Although 1,4-dihydropyridines (DHPs) like nifedipine and isradipine (Fig. 1) have been successfully used as anti-hypertensive drugs for decades, the detailed structure-activity relationships between DHPs and their interaction sites within the DHP binding pocket of the L-type calcium channel Cav1.2 are still incompletely understood. Our current knowledge is primarily based on structure-function studies with chimeric proteins and site-directed mutagenesis (Fig. 2) [2]. Additionally, conformationally restrained DHP analogs helped us to explore the active conformation of DHPs (Fig. 1.) [3]. Ongoing efforts in exploiting the DHP scaffold yielded a novel compound class with a condensed ring system having distinct selectivity profiles to different calcium channel subtypes [4,5]. Of those, M4 was characterized extensively and turned out to be a broad-spectrum calcium channel inhibitor modulating L-, T-(Cav3.2) and N-type calcium channels with analgesic effects in mice (Fig. 1) [6]. These properties render this compound class a promising tool to study the fundamentals of achieving distinct selectivity profiles to calcium channel subtypes.

Studies aiming to elucidate the key interactions formed by DHPs to Cav1.2 depended on homology models that were generated

* Corresponding author.
E-mail address: miyasegunduz@yahoo.com (M.G. Gündüz).

https://doi.org/10.1016/j.ejmech.2018.05.032
0223-5234/

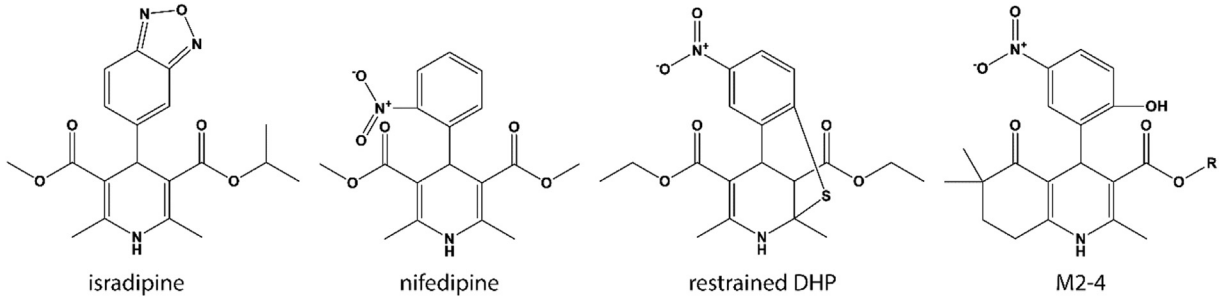

Fig. 1. Selected DHPs active at Cav1.2. M series was synthesized with different ester moieties R [4]: M2 - C_2H_5, M3 - $CH_2C_6H_5$, M4 - $CH_2CH(CH_3)_2$.

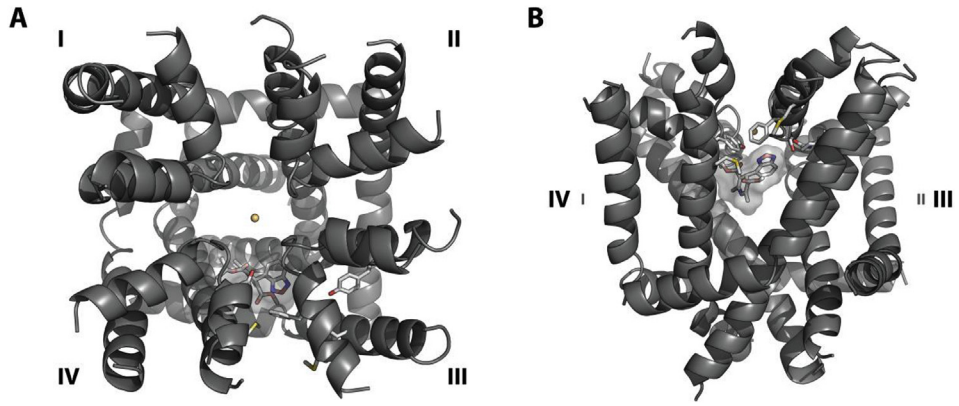

Fig. 2. Top view (**A**) and site view (**B**) of the homology model of rat Cav1.2 heterotetrameric pore domain. Residues that are known to affect DHP binding are depicted as well as the proposed binding pose of isradipine. The calcium ion in the selectivity filter is highlighted in yellow. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

using distantly related bacterial calcium channels [7] or potassium channels [8–11]. Recently, scientists were able for the first time to show the structural basis of DHP binding to an artificial but functional bacterial calcium channel [12]. But the authors conclude that a translation to the human Cav1.2 is not possible since the binding site is shifted and sequence similarity is rather low [12]. However, current advances in cryo-electron microscopy enabled researchers to generate near atomic resolution 3D structures of very challenging targets including the rabbit calcium channel Cav1.1 [13].

In this study, we aimed at rationalizing the binding mode of DHPs to Cav1.2. A homology model was created based on the recently released crystal structure of the closely related calcium channel Cav1.1. Docking and pharmacophore studies were employed to identify a common binding mode for the prominent DHPs isradipine and nifedipine, that could also be used to explain the structure-activity relationship of a DHP compound class with a condensed ring system. Twenty-two novel DHPs with a condensed ring system were synthesized and tested in-vitro using whole-cell patch clamp technique for the ability to block Cav1.2 to challenge our binding hypothesis. Additionally, compounds were tested for inhibition of Cav3.2 to gain an insight into the selectivity behavior of this compound class.

2. Results and discussion

2.1. Homology modelling

Using the voltage-gated calcium channel Cav1.1 (PDB: 3JBR) as template we developed a homology model of the pore domain of rat Cav1.2 (Fig. 2.). This domain forms a hetero-tetramer and DHPs are predicted to bind between subunit III and IV. Several residues that are known to affect DHP binding flank the proposed binding site in the described model [14–19]. These residues were used to characterize the binding pocket in the following docking studies.

2.2. Binding hypothesis for DHPs

Isradipine, nifedipine, a restrained DHP analog and compounds **M1-5** (Fig. 1) were flexibly docked into the homology model of rat Cav1.2 using GOLD 5.2 resulting in thirty diverse docking poses per ligand [20]. The docking poses of isradipine were used to generate structure-based pharmacophores in LigandScout 4.2 [21]. Subsequently, these pharmacophores were utilized to filter the docking poses of the other docked compounds. Out of thirty isradipine docking poses only one yielded a pharmacophore that was able to find docking poses of nifedipine, a restrained DHP analog and active compounds **M2-4**. This procedure resulted in the identification of a generalized binding mode for DHPs that is shared by all docked compounds and that may explain the structure-activity relationship of **M1-M5**. The interactions formed by isradipine involve two hydrogen bonds to SER1141 and TYR1489 and several hydrophobic interactions (Fig. 3A and B). Nifedipine shows a similar interaction pattern but misses a hydrophobic contact formed by the isopropyl ester of isradipine (Fig. 3C). The docking pose of the restrained DHP analog shows the same interactions as isradipine and supports a slightly distorted arrangement of the aromatic substituent to the DHP scaffold (Fig. 3D).

Using the isradipine pharmacophore described above we identified binding poses of **M1-5** that rationalize the structure-activity relationship of this compound series (Fig. 4, Fig. S1). **M2-5** show highly similar interactions compared to isradipine involving two hydrogen bonds to SER1141 and TYR1489 and several hydrophobic