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ORIGINAL PAPER

Molecular modeling study of isoindolines as L-type Ca^{2+} channel blockers by docking calculations

Teresa Mancilla-Percino · José Correa-Basurto · José Trujillo-Ferrara · Fernando R. Ramos-Morales · Mario E. Acosta Hernández · Jesús S. Cruz-Sánchez · Margarita Saavedra-Vélez

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Abstract Two series of isoindolines **1**(**a**–**g**) and **2**(**a**–**g**) were found by docking calculations to be possible L-type Ca^{2+} channel (LCC) blockers. The theoretical 3-D model of the outer vestibule and the selective filter of the LCC was provided by Professor Lipkind; this model consists of transmembrane segments S5 and S6 and P-loops contributed by each of four repeats (I, II, III, and IV) of Ca_v 1.2. Therefore, two well-known LCC blockers, nifedipine **3** and (*R*)-ethosuccinimide **4** were also evaluated, and their binding sites on the LCC were identified and compared with those obtained for **1**(**a**–**g**) and **2**(**a**–**g**). Analysis of the results shows that the target compounds tested probably could be LCC blockers, since they interact with or near the glutamic acid residues Glu393, Glu736, Glu1145 and Glu1446 (the EEEE locus), which belong to the LCC selectivity region. The ΔG values for all of the Ca^{2+} channel ligands are between−10.78 and −3.67 (kcal mol^{-1}), showing that LCC-**1b**, -**1e** and -**1f** complexes are more stable than the other compounds tested. Therefore, theoretically calculated dissociation constants K_d (μM) were obtained for all compounds. Comparing these values reveals that compounds **1b** (0.0244 μM), **1e** (0.0176 μM) and **1f** (0.0125 μM) exhibit more affinity for the LCC than the other compounds. This screening shows that the two series of isoindolines probably could act as LCC blockers.

Keywords L-type protein Ca^{2+} channel · Docking · Epilepsy · Isoindolines · α-Amino acids

T. Mancilla-Percino (✉)
Departamento de Química, Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional,
Apartado Postal 14-740, C.P. 07000 México D.F., México
e-mail: tmancill@cinvestav.mx

J. Correa-Basurto · J. Trujillo-Ferrara
Instituto Politécnico Nacional, Escuela Superior de Medicina, Sección de Estudios de Posgrado e Investigación, Departamento de Bioquímica, Departamento de Farmacología, Plan de San Luis y Díaz Mirón s/n Col. Casco de Santo Tomás, Delegación Miguel Hidalgo,
C.P. 11340 México D.F., México

F. R. Ramos-Morales · M. E. Acosta Hernández
Unidad de Servicios de Apoyo en Resolución Analítica, Universidad Veracruzana,
Circuito Gonzalo Aguirre Beltrán s/n Zona Universitaria,
C.P. 91000 Xalapa, Ver. México

J. S. Cruz-Sánchez
Instituto de Ciencias Básicas, Universidad Veracruzana,
Circuito Gonzalo Aguirre Beltrán s/n Zona Universitaria,
C.P. 91000 Xalapa, Ver. México

M. Saavedra-Vélez
Facultad de Química Farmacéutica Biológica, Universidad Veracruzana,
Circuito Gonzalo Aguirre Beltrán s/n Zona Universitaria,
C.P. 91000 Xalapa, Ver. México

Introduction

Voltage-operated Ca^{2+} channels play an important role in the control of intracellular Ca^{2+} concentration and are fundamental for stimulating the liberation of neurotransmitters and activating several intracellular proteins [1]. The anomalous functioning of calcium channels can cause a variety of diseases, such as epilepsy, which is the most common chronic brain disorder that disturbs human behavior [2]. In this context, a variety of drugs have been investigated as Ca^{2+} channel blockers [3–7]. On the other hand, some isoindolines are important intermediates for the synthesis of novel multidrug resistance reversal agents [8]; they have also shown anti-inflammatory [9, 10] and diuretic

[11, 12] activities. Therefore, they have been used to treat coronary vessel diseases [13, 14], and have been evaluated as alpha-adrenergic and adrenergic neuron blocking agents [15]. Due to our interest in the synthesis of 2-substituted isoindoline derivatives of α-amino acids [16], as well as in their biological evaluation [17, 18], we attempted a theoretical study of the isoindolines **1**(**a**–**g**) and **2**(**a**–**g**) (see Fig. 1) that utilized docking calculations to investigate if they could act as L-type Ca^{2+} channel (LCC) blockers; the LCC model was provided by Professor Lipkind [19]. Docking calculations provide evidence of the nature of the interaction between the ligands and the protein, as well as the affinity sites between them, so it is possible to predict the feasibility of the therapeutic use of these isoindolines. To check the results of this theoretical study, nifedipine **3** and ethosuccinimide **4** (Fig. 2), which are well-known LCC antagonists [3–7], were also evaluated by identifying their binding sites on the LCC and comparing them with those of **1**(**a**–**g**) and **2**(**a**–**g**).

Computational methods

The ligands were drawn using the Isis/Draw software [20]. Geometric pre-optimization of the ligands was carried out using HyperChem 6 software (version 6.0, Hypercube, Gainesville, FL, USA, http://www.hyper.com). The minimum energy structure of the ligands was obtained via density functional theory (DFT) calculations at the B3LYP/6-31G** level using Gaussian 98 software [21] running on a Pentium IV computer.

AutoDock (3.0.5) software was used for docking studies [22]. All docking simulations were carried out using the hybrid Lamarckian genetic algorithm [22], with an initial population of 100 randomly placed individuals and a maximum number of energy evaluations of 1.02×10^7. The resulting docked orientations within a root-mean square deviation of 0.5 Å were clustered together. The lowest energy cluster returned by AutoDock for each compound was used for further analysis. All other parameters were maintained at their default settings. All of the resulting

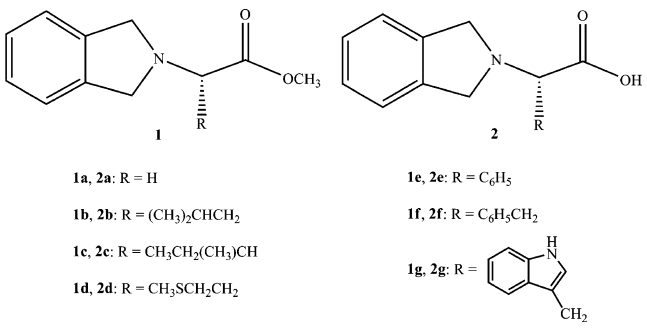

Fig. 1 Isoindoline series **1**(**a**–**g**) and **2**(**a**–**g**)

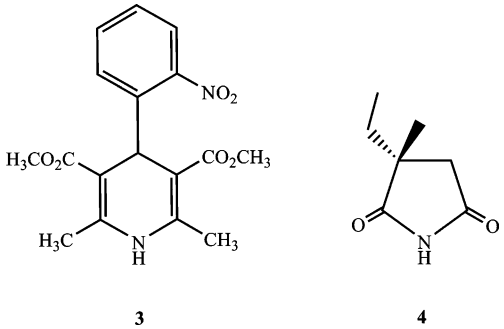

Fig. 2 Nifedipine **3** and (*R*)-ethosuccinimide **4**

docking visualizations were achieved using a visual molecular dynamics (VMD) program [23].

Results and discussion

The 3-D theoretical model of the LCC used to dock the isoindolines **1**(**a**–**g**), **2**(**a**–**g**) (Fig. 1), as well nifedipine **3** and (*R*)-ethosuccinimide **4** (Fig. 2) was created and provided by Professor Lipkind [19]. It consists of trans-membrane segments S5 and S6 and P-loops contributed by each of four repeats (I, II, III, and IV) of Ca_v 1.2.

It is known that LCC channels are highly sensitive to different chemical classes of organic Ca^{2+} channel blockers that are also called Ca^{2+} antagonists, such as 1,4-dihydropyridines (such as nifedipine), phenylalkylamines (PAAs) and benzothiazepines (BTZs) [24]. Different experimental approaches such as photoaffinity labeling, construction of a chimeric channel and mutagenesis have demonstrated that the IIIS6, IVS6 and IIIS5 segments interact with known LCC antagonists [25]. However, to our knowledge, there is less information available in relation to the IS5, II5, IS6, IIS6 segments [6].

Thus, in the present work we show the interactions of isoindolines **1**(**a**–**g**), **2**(**a**–**g**) as well **3** and **4** with the LCC, and then describe an analysis of the closest distances from the heteroatoms, carbons or hydrogens of all of the compounds tested to the atoms of LCC amino acid residues, in order to investigate the binding mode. Hence, only distances similar to or shorter than the sum of the van der Waals radius for the ligand atom and the hydrogen bond length of the protein were selected [26]. The results show that all of the isoindolines **1**(**a**–**g**), **2**(**a**–**g**) as well as **3** and **4** bind within the LCC, as summarized in Table 1.

Compounds **1a**, **1b**, **3** and **4** bind within the LCC in the vicinity of domains IIS5 (661–669), IIS6 (763–773) and IIP (734–740), and **3** undergoes additional binding with the domain IIIS6 (1152–1156). Compound **1a** exhibits the following distances: in domain IIS6, the distance between the methyl group of OCH_3 and the side chain C of Leu766 is 3.09 Å, while it is 2.82 Å between the oxygen atom of the

Table 1 Compounds **1**(**a**–**g**), **2** (**a**–**g**), **3** and **4** within the LCC I–IV domains

Compound	IS5	IS6	IP	IIS5	IIS6	IIP	IIIS5	IIIS6	IIIP	IVS5	IVS6	IVP
1a				♦	♦	♦						
1b				♦	♦	♦						
1c	♦	♦	♦									
1d	♦	♦	♦									♦
1e		♦								♦	♦	♦
1f	♦	♦	♦									♦
1g	♦	♦	♦									
2a							♦		♦		♦	
2b	♦	♦	♦									
2c	♦	♦	♦									
2d	♦	♦	♦									♦
2e		♦								♦	♦	♦
2f										♦	♦	♦
2g	♦	♦	♦									♦
3				♦	♦	♦		♦				
4				♦	♦	♦						

OCH_3 group and the side chain C of Phe767. In the domain IIP, 2.60 Å separate the C=O group from the backbone O atom, and 2.60 Å separates the N and and the backbone C atom of Glu736, which belongs to the selective filter. Furthermore, 3.38 Å separates the oxygen atom of the C=O group from the backbone N atom of Trp738 (β-strand region). For **1b**, the distances in the domain IIS6 are: 3.69 Å between the N atom and the side chain CG, 3.43 Å between a carbon of the aromatic group of isoindoline and the side chain CE1, and 3.14 Å between the oxygen atom of the C=O group and the backbone O of Phe763, where the phenyl group of the amino acid residue is almost parallel to the ring of the isoindoline (Fig. 3). As depicted in Fig. 3, there is a distance of 3.07 Å between the methyl group from OCH_3 and the C of Glu736 (pink). Compound **3** exhibits, in domain IIS5, a distance of 2.74 Å between the N atom and the side chain O of Leu661; in domain IIP, there is a distance of 1.99 Å between the hydrogen atom of NH and the side chain O of Glu736; in domain IIS6, there is a distance of 3.14 Å between the oxygen atom of C=O and the side chain O of Gly770; in domain IIIS6, there is a distance of 3.32 Å between the N atom and the side chain O of Ile1153, and 2.79 Å separates the oxygen atom of the C=O group and the side chain C of Ile1156. Compound **4** shows the following distances: in domain IIS5, there is 2.85 Å between CD and the side chain O of Ile 665; in domain IIS6, there is a distance of 2.31 Å between CD and the side chain H of Leu766, and there is 3.56 Å between the N atom and the backbone O of Phe767. In domain IIP, there is a distance of 3.28 Å between the O atom and the backbone N of Glu736. Compounds **1a**, **3** and **4** show interactions with amino acid residue Glu736, which belongs to the selective filter, while **1b** is close to this.

Compounds **1c**, **1d**, **1f**, **1g**, **2b**, **2c**, **2d** and **2g** bind to LCC through the domains IS5 (279–290), IS6 (411–423) and IP (389–397), while **1d**, **1f**, **2d** and **2g** undergo additional binding with the domain IVP (1441–1449). Compound **1c** exhibits the following distances: in domain IP, there is 2.88 Å between the oxygen atom of the CH_3O group and the side chain O of Trp395 (β-strand region) and 3.59 Å between the methyl group of OCH_3 and the backbone N of Asp397 (β-strand region); in the domain IS6, there is 3.54 Å between N and the side chain C, and 3.24 Å between the oxygen atom of the C=O group and the side chain C of Phe415. Compound **1d** shows, in the domain IP, a distance of 2.68 Å between the oxygen atom of the CH_3O group and the backbone O of Trp395; in the domain IVP, there is a distance of 3.10 Å between the S atom and the side chain NH_2 of Arg1441. Compound **1f** shows, in the domain IP, distances of 2.84 Å between the N atom and the backbone O of Glu393 (pink), 2.91 Å between the oxygen atom of the C=O group and the backbone O, and 3.31 Å between the methyl of the OCH_3 group and the backbone C of Gly394. In the domain IS6, there is a distance of 3.35 Å between the oxygen atom of the CH_3O group and the side chain C of Leu418, whereas **1f** is close to the amino acid residue Glu1446 (pink), which belongs to the selective filter, and there is a distance of 5.93 Å between a carbon of the aromatic ring of isoindoline and CG of Glu1446 (Fig. 3). Compound **1g** exhibits the following distances in domain IP: 2.88 Å between the N atom and the side chain O of Glu393 and 3.47 Å between the methyl of OCH_3 and the side chain N of Trp395 (β-strand region). Compound **2b** shows the following distances: in domain IS5, there is a distance of 2.82 Å between the oxygen atom of the C=O group and the side chain CD2 of Leu290; in

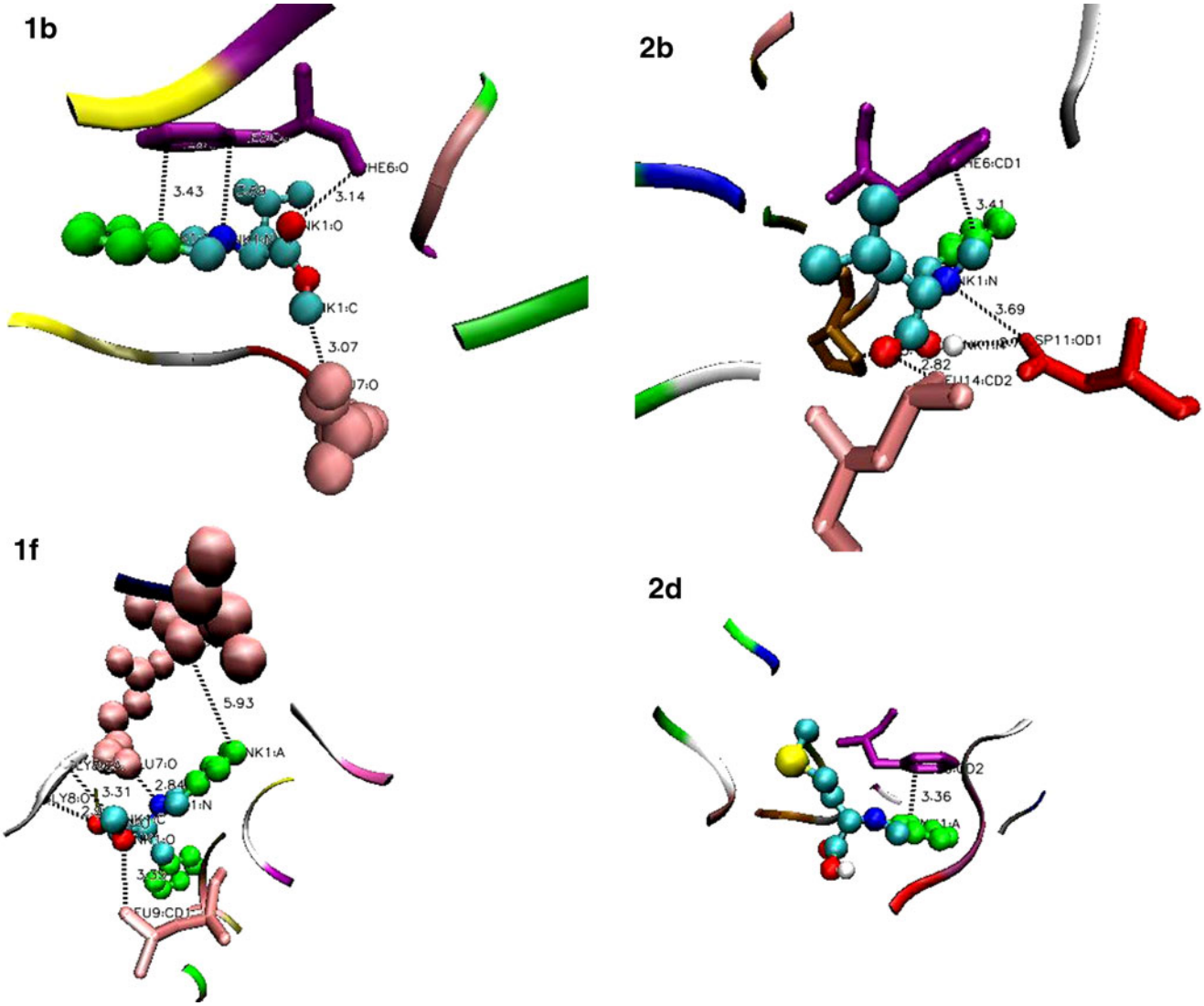

Fig. 3 Docked structures of **1b**, **1f**, **2b** and **2d** in the LCC; distances between atoms are indicated by *broken lines*

domain IS6, there is a distance of 3.14 Å between the oxygen atom of the HO group and the side chain CB of Pro411; in domain IP, 2.75 Å separates the proton of the OH group and the side chain O of Asp397. However, the phenyl group of Phe415 is almost parallel to the aromatic ring of **2b** and is at a distance of 3.41 Å from it (Fig. 3). For **2c**, the following distances are observed in domain IP: 2.89 Å between the proton of the OH group and the backbone O of Trp395; 1.98 Å between the proton of the OH group and the side chain OD of Asp397. The interactions with these amino acid residues show that **2c** is in the inner hydrophobic core, and in domain IS6 the distances are: 3.51 Å between the N atom and the side chain CE1, and 3.29 Å between the oxygen atom of the C=O group and the side chain O of Phe415. For compound **2d**, the distances are as follows. In domain IS5, the distance between the S atom and the backbone N of Leu317 is 3.66 Å; in domain IP, the distance between OH and the side chain O of Asp397 is 1.88 Å; in domain IS6, the following distances are observed: 3.12 Å between the oxygen atom of the C=O group and the backbone N of Trp412, and 3.52 Å between the N atom and the side chain C of Phe415, where the phenyl group of this amino residue is almost parallel to the aromatic ring of **2d** and is at a distance of 3.36 Å from it (Fig. 3). For **2g**, the following distances are observed: in domain IP, there is a distance of 2.45 Å between the hydrogen atom of the NH group and the side chain CD1 of Trp395, and 2.98 Å separates the oxygen atom of the HO group from the side chain CG of Pro411; in domain IS6, 2.20 Å separates the hydrogen of the OH group and the side chain NE1 of Trp412, while 3.40 Å separates the N atom of HN and the side chain CB of Phe415.

Compounds **1c**, **1d**, **1g**, **2b**, **2c**, **2d** and **2g** bind in the β-strand region, which is part of the inner hydrophobic core,

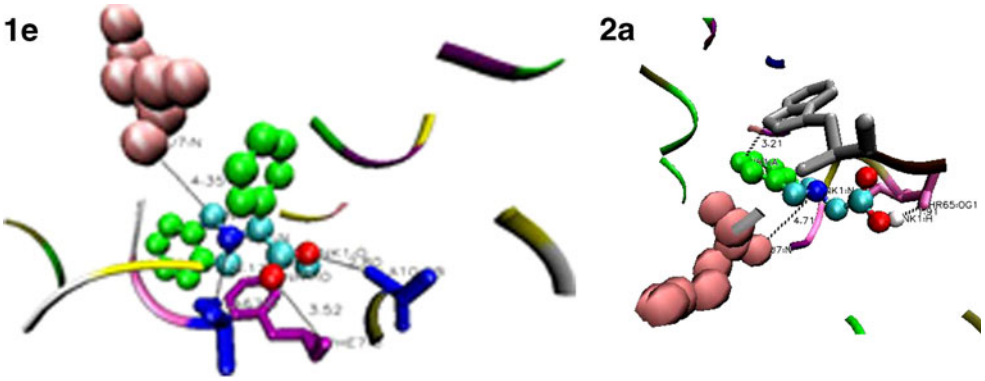

Fig. 4 Docked structures of **1e** and **2a** in the LCC; distances between atoms are indicated by *broken lines*

and compounds **1f** and **1g** exhibit interactions with Glu393, which belongs to the selective filter.

Compounds **1e** and **2e** bind to the same site, between the domains IS6 (420, 423), IVS5 (1375, 1378, 1379, 1382), IVS6 (1459, 1462, 1463, 1466) and IVP (1442–1446), so **1e** shows a distance of 2.80 Å between the oxygen atom of the CH_3O group and the side chain C of Ala1405 (IVS5), while **1e** and **2e** undergo interactions with Phe1402 (IVS5) and Ala1443 (IVP). Therefore, for **1e**, the distance between the N atom and the backbone O of Phe1402 is 3.17 Å and it is 2.74 Å between the oxygen atom of the C=O group and the backbone O of Ala1443; for **2e**, the distances are 2.33 Å between the hydrogen atom of the OH group and the backbone O of Phe1402 and 2.74 Å between the oxygen atom of the C=O group and the backbone O of Ala1443. Therefore, **1e** and **2e** (Fig. 4) bind close to Glu1446 (pink), which belongs to the selective filter. For **1e**, the distance between a carbon atom in the five-membered ring of isoindoline and the N atom of Glu1446 is 4.35 Å. Compound **2a** binds to IIIS5 (1044, 1047), IIIP (1140–1148), IIIS6 (1148, 1151, 1152, 1155) and IVS6 (1456, 1460), and there is a distance of 1.91 Å between the hydrogen of the OH group and the side chain O of Thr1140, and 3.21 Å between a carbon of the aromatic group and the side chain NE1 of Trp1147. Since **2a** binds to IIIP, it is close to Glu1145 (pink), which belongs to the selective filter, and there is a distance of 4.71 Å between the N atom of isoindoline and the side chain N of Glu1147 (Fig. 4). Isoindoline **2f** binds between IVS5 (1378, 1382), IVS6 (1459, 1463, 1463, 1466) and IVP (1421–1423), and presents the following distances: 3.27 Å between the oxygen of the HO group and the side chain C of Met1409, and 1.96 Å between the proton from the OH group and the side chain O of Phe1486.

ΔG (kcal mol^{-1}) values were obtained for all of the LCC ligands, and they are summarized in Table 2. The ΔG values show that all of the Ca^{2+} channel–isoindoline complexes, except for **2d**, are more stable than the Ca^{2+} channel–niphedipine and –(*R*)-ethosuccinimide complexes, and that Ca^{2+} channel–**1b**, –**1e**, and –**1f** complexes are more stable than the others. Regarding the theoretical dissociation constant K_d (μM) values (Table 2), compounds **1b** (0.0244 μM), **1e** (0.0176 μM) and **1f** (0.0125 μM) showed greater affinities to the Ca^{2+} channel than the other compounds. These results show that all of the compounds **1**(**a**–**g**) and **2**(**a**–**g**), except for **2d**, could be better or similar channel Ca^{2+} inhibitors than the compounds used for reference purposes.

Conclusions

In this contribution we report the results of a docking study of two series of isoindolines, **1**(**a**–**g**) and **2**(**a**–**g**), as possible LCC blockers. Compounds **1a**, **3** and **4** show interactions with amino acid residue Glu736, which belongs to the selective filter, while **1b** is close to this. Compounds **1c**, **1d**, **1g**, **2b**, **2c**, **2d** and **2g** bind in the β-strand region, which is part of the inner hydrophobic core. Compounds **1f** and **1g** exhibit interactions with residue Glu393, and **If** is close to

Table 2 ΔG (kcal mol^{-1}) and K_d (μM) values for compounds **1** to **4**

Compound	**1a**	**1b**	**1c**	**1d**	**1e**	**1f**	**1g**
ΔG	−8.55	−10.38	−8.15	−6.97	−10.57	−10.78	−8.53
K_d	0.54	0.0244	1.06	7.78	0.0176	0.0125	0.552
Compound	**2a**	**2b**	**2c**	**2d**	**2e**	**2f**	**2g**
ΔG	−7.33	−7.31	−7.38	−3.67	−7.81	−7.82	−6.31
K_d	4.28	4.32	3.89	2034.00	1.90	1.84	2.35
Compound	**3**	**4**					
ΔG	−4.13	−5.91					
K_d	939.00	46.70					

Glu1446, and both of these residues belong to the selective filter. Compounds **1e** and **2e** interact with residue Glu1446.

Isoindolines **1a** and **1b** bind to the LCC at the same site as compounds **3** and **4**, used for reference purposes. The distances between several atoms of all compounds and some atoms of the amino acid residues of the LCC were obtained. The data show that most of these compounds bind to the IS5, IIS5, IS6 and IIS6 segments of Ca_v 1.2. To our knowledge, less information is available on these segments than the others.

The ΔG values were obtained for all compounds. The Ca^{2+} channel–**1b**, –**1e**, –**1f** complexes were found to be more stable, and to consequently show greater affinity (K_d) for the LCC, than the others. These results show that all of the compounds **1**(**a**–**g**) and **2**(**a**–**g**), except for **2d**, could be better or similar Ca^{2+} channel inhibitors than the compounds used for reference purposes.

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