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Rational Design, Synthesis, and *in-silico* Evaluation of Homologous Local Anesthetic Compounds as TASK-1 Channel Blockers †

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Abstract: Advances in different technological and scientific fields have led to the development of tools that allow the design of drugs in a rational way, using defined therapeutic targets, through simulations that offer a molecular view of the ligand-receptor interactions, giving precise information for the design and synthesis of new compounds. Ion channels are of great relevance as therapeutic targets since they play roles in different pathologies. Several ion channels are expressed in the atria and constitute a therapeutic target for the treatment of atrial fibrillation (AF), the most common type of arrhythmia and an important risk factor for an increase in cerebrovascular accidents. The action potential (AP) of a cardiomyocyte is initiated by depolarization of the membrane through the inflow of sodium (Na+). The repolarization currents are carried out by different potassium (K+) channels. Background currents of TASK-1 channels can also contribute to AP and TASK-1 channel blockers could become innovative strategies against AF. The compounds used in this study will be based on local anesthetic (LAs)-type compounds that have been shown to be TASK-1 channel blockers such as lidocaine, ropivacaine and bupivacaine and have antiarrhythmic capacity, becoming potentially effective drugs for the treatment of AF. The main objective of this study is, based on the common characteristics of LAs, to propose the synthesis of analogues of LAs and evaluate them *in-silico* as TASK-1 channel blockers.

Keywords: Ion channels; TASK-1; atrial fibrillation; local anesthetics

1. Introduction

K_2P channels are involved in the control of resting membrane potential, hormonal secretion and the amplitude, frequency and duration of the action potential (AP) [1], regulating cellular excitability by conducting K^+ ions through the plasma membrane [2,3]. 15 K_2P channels in humans have been identified and classified into 6 subfamilies [2]. The TASK subfamily is composed from three members: TASK-1 (KCNK3); TASK-3 (KCNK9) and TASK-5 (KCNK15). High levels of TASK-1 expression have

been identified in peripheral tissues such as carotid bodies, in the heart atrium and in neuroepithelial bodies of the lung [3]. K_2P potassium channel blockade causes action potential (AP) prolongation and has recently been proposed as a new antiarrhythmic strategy [4,5].

Atrial fibrillation (AF) is a very common type of cardiac arrhythmia and is an important cause of embolic stroke, heart failure, and cardiovascular morbidity [6,7]. Output rectifier Kv1.5 channels, which are highly expressed in human atria but not in ventricles [8,9], are mainly responsible for the ultrafast potassium output current (IKur) and determine the duration of atrial AP, regulating repolarization, having great interest as a target for atrial antiarrhythmic drugs because it causes atrial but not ventricular repolarization. However, it has been proposed that IKur blockade may not be sufficient to suppress AF [9].

Other ion channels that generate atrial AP include the two-domain pore channel (K2P) TASK-1 [10], specifically expressed in the atria. In recent years, it has been established that known Kv1.5 blockers, used against AF and/or obstructive sleep apnea, such as A1899 blocker, modulate TASK-1 channels [11–14]. The higher affinity of these blockers for TASK-1 channels suggests that TASK-1 could be an unrecognized molecular target of Kv1.5 blockers, effective in AF [11]. In the case of local anesthetics (LAs), it has been observed that bupivacaine and ropivacaine, which block Kv1.5 [12,14–17], also act as blockers in TASK-1. In addition, LA lidocaine blockade has been reported on the TASK-1 channel [13,18] (Figure 1).

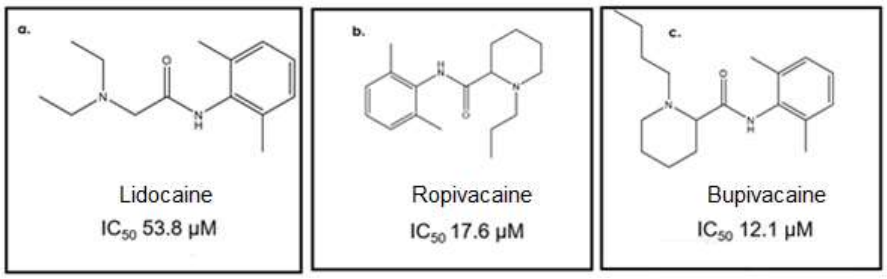


Figure 1. IC_{50} values reported for TASK-1 channel blockade by LAs in HEK-293 cell line. (**a**) Lidocaine. (**b**) Ropivacaine (**c**) Bupivacaine (13, 12).

Fifteen K_2P channels in humans have been identified and classified into 6 subfamilies, according to sequence identity and functional characteristics: TWIK, TREK, TASK, TALK, THIK, and TRESK. The expression of K_2P channels has been detected in various tissues and plays an important role in excitable cells of the heart and brain (22). Structurally, K_2P channels contain two pores domain sequences (P1 and P2) *per* subunit, these channels assemble as dimers. Each subunit contains two outer transmembrane helices (M1 for P1 and M3 for P2), a pore helix, a selectivity filter signature sequence, and an inner transmembrane helix (M2 for P1 and M4 por P2) [2]. The M2 transmembrane segment is twisted by approximately 20°, this turn generates a fenestration in each subunit that consists of lateral passages that connect the pore with the hydrophobic cavity of the lipid bilayer [19].

In the case of bupivacaine that blocks the TASK-1 channel, it has been proposed that the binding site in TASK-1 could be below the helices of the pores, in the lateral fenestrations where it interacts with the residues of the second helix of the pore allosterically preventing K^+ flux activation [20]. It has been proposed that the binding site for bupivacaine may be "V" shaped in TASK-1, since the compound appears to interact with residues found on the upper side of the cavity, such as C110, M111, A114, in addition to I118, Q126, S127 in segment M2. T198 in Pore 2 (P2), V234, I235, G236, F238, L239 and N240 of the M4 segment. As well as T93 of M1-P1, L171 of M3 and F194 in M3-P2. This would place it laterally below the helices of the pores, in the lateral fenestrations (Figure 2).

As is stated before, it has been suggested that compounds with the ability to block TASK-1 channel could become an innovative strategy for the treatment of AF [21], as is the case of LAs. Because it is possible to define the chemical characteristics required for a ligand to be active against a certain target, for example, a functional group important for ligand-protein interactions, such as a hydrogen bond acceptor (HBA) group or a positive charge center [22,23], it arises the necessity to

propose the rational design of new compounds based on the common structural characteristics of LAs: lidocaine, bupivacaine and ropivacaine taking into account their ability to act as antiarrhythmic agents through the blockade of the TASK-1 channel [12–14,24–27].

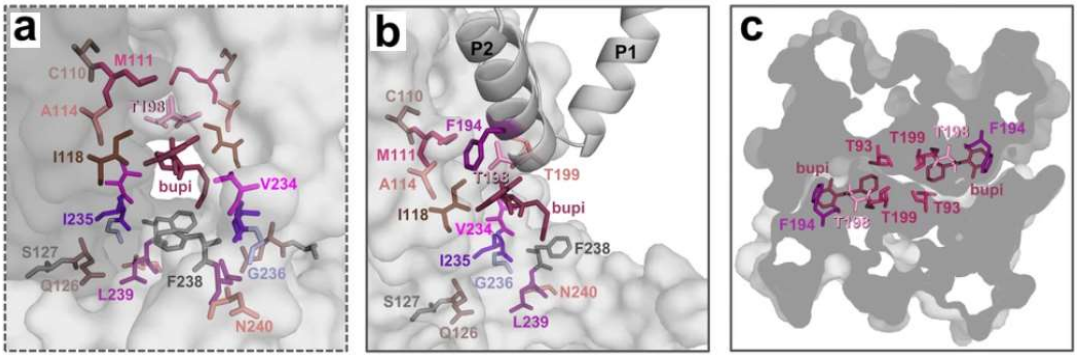


Figure 2. Homology model of the TASK-1 channel based on the TWIK-1 crystal (PDB: 3UKM). (**a**) Residues of the bupivacaine binding site in the M2 and M4 segments aligned with the lateral fenestrations. (**b**) Enlargement of bupivacaine interacting residues (**c**) Bupivacaine located below the second pore helix along with the binding residues at M2 and M4. Modified from Rinné et al., 2019 [20].

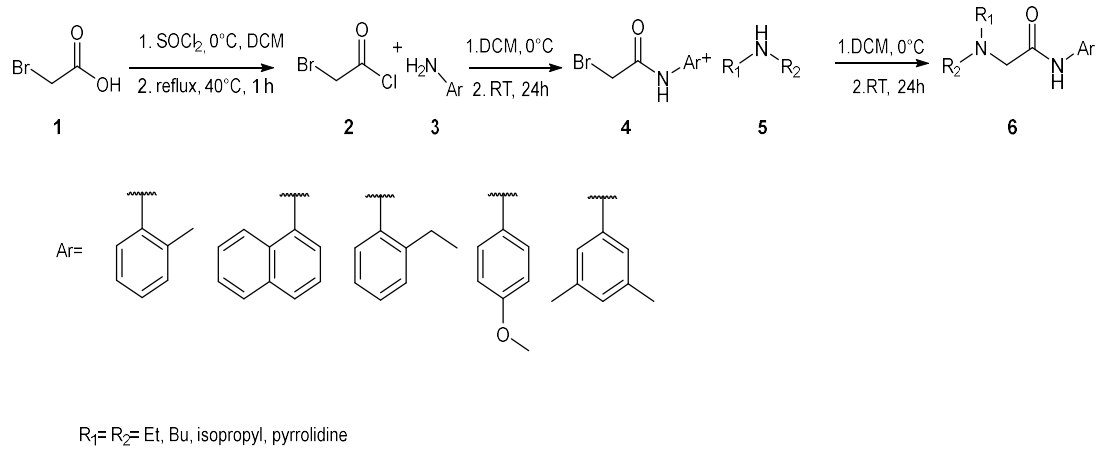
2. Methods

2.1. LAs Peers Design Phase

We proceeded to define the possible structures to be synthesized, based on: (a) main characteristics of LAs identified as blockers of the TASK-1 channel, taking into account the binding modes reported in the literature (Figure 2) and their structural common features. For this, the ALs pharmacophore was established using the "Phase" module of the Schrödinger 11.8 software, using lidocaine and the enantiomers of bupivacaine and ropivacaine as a reference. The ligands were drawn using the Maestro workspace, then they were refined using "LigPrep" and finally the hypothesis was generated considering the default criteria of Phase [28]. (b) Viability of the synthesis.

2.2. Synthesis Phase

With the above information, the organic synthesis was carried out, evaluating different methodologies to select the most suitable conditions for the synthesis. LAs homologues were synthesized using previously reported methodologies for amide synthesis and nucleophilic substitution reactions. So; the amidation reactions were done according to protocols described by Wang, Z. et al. [29] with modifications. In general terms, it was measured (1 mmol, 1 equiv.) of 2-bromo acetic acid (**1**) dissolved in 5mL of DCM at 0 °C and 5 mmol (5 equiv.) of thionyl chloride ($SOCl_2$) was added at 0 °C and maintained at reflux at 40 °C for 1h. The progress of the reaction was followed by TLC. Once obtained (**2**), it was reacted with different aromatic amines (**3**) (1.3 mmol, 1.3 equiv.), obtaining the intermediate (**4**) which in turn was reacted with aliphatic amines (1.2 mmol, 1.2 equiv.) (**5**) thus obtaining the desired compounds (**6**) (Scheme 1).



Scheme 1. Synthesis of LA homologous compounds.

2.3. In-Silico Evaluation Phase

Molecular coupling simulations of the homologous candidate compounds of the LAs were performed in the channel to analyze the most probable way of binding and their possible ligand-receptor interactions. Prior to the molecular coupling step, the structure of the TASK-1 protein was prepared, using the TASK-1 crystal (PDB: 6RV3) [30], in order to avoid steric hindrances. The possible forms of binding and interactions of the new synthesized molecules were established using the Glide program, incorporated in the Master Schrodinger 11.8 suite. From there, the poses of the molecules that had the best experimental interaction score (EIS) were selected, corresponding to a score based on the interaction with key residues reported in the previously reported bupivacaine binding site (19). To perform the docking, the molecules to be synthesized were built using ChemDraw and Maestro. Once the ligands were built, an optimization of the three-dimensional structure was carried out using the "LigPrep" module of the Schrödinger software, which can generate a series of optional structures according to the different ionization states, tautomers, stereochemistry, among others. Subsequently, the energy of the ligands was minimized using the "Macromodel" module included in Maestro Schrödinger 11.8, to obtain the ligands in their conformational states of basal energy.

The next step consisted in the preparation of the crystal of the TASK-1 protein, (PDB: 6RV3), this was carried out in the Schrödinger 11.8 software. Hydrogen atoms were added, ligands on the crystal that are not of interest for the present analysis were removed, and the energy of the three-dimensional structure was minimized. Once the ligands and the protein were minimized independently, the molecular coupling simulations were carried out with each of the proposed ligands. For the calculation of molecular coupling, the "IFD" (Induced Fit Docking) protocol was used, which uses the Glide software from the Schrödinger suite [31]. To carry out the molecular docking analysis, a single lateral protein fenestration was explored, since both subunits are symmetric. The exploration of the fenestration was performed considering the bupivacaine binding site residues. This protocol comprises four steps: A molecular coupling of the molecules at the defined site with smoothed potentials (considering the Van deer Waals radius of the atoms at half their value), then a sampling of the protein's side chains with each of the poses generated, then a re-coupling of the molecules in the new protein structures generated in the previous step and finally the assignment of the docking score considering the "GlideScore", energy and solvation terms through the "Prime" module of the software Schrödinger. Then, to the poses obtained with the IFD protocol the EIS score were assigned. To assign the EIS it was determined if each pose obtained was within a radius of 5 Å or less to any atom of the bupivacaine binding site residues. Poses that interact with a higher amount of residue will therefore have a higher EIS value.

3. Results and Discussion

The typical LAs drug has a tertiary amine hydrophilic domain, an intermediate linker containing an amide or an ester group, and a hydrophobic aromatic ring domain [32,33] (Figure 3). So; The classical substitution pattern on the aromatic ring of LAs includes; a methyl group (e.g., prilocaine), two methyl groups (e.g., lidocaine, bupivacaine, and ropivacaine), and sometimes three methyl groups (e.g., trimecaine, cyclomecaine, and pyromecaine). However, unlike the local anesthetic activity, it has been proposed that the antiarrhythmic properties of the compounds tolerate various types of substituents on the phenyl ring, obtaining homologous compounds with less toxicity than the classic ortho-methyl substituted (lidocaine, bupivacaine and ropivacaine) [14,27].

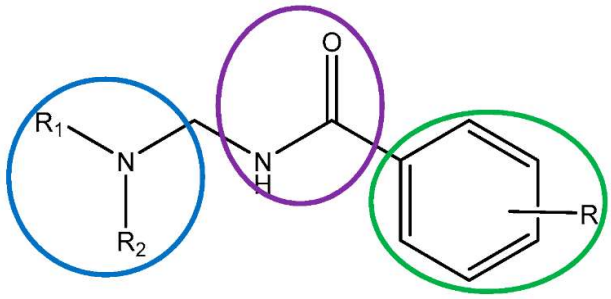


Figure 3. Basic structure of LAs, in blue, tertiary amine (hydrophilic domain), in purple, amide (intermediate linker) and in green substituted aromatic ring (hydrophobic domain).

Based on these characteristics, the pharmacophore was established (Figure 4) using Maestro Schrodinger 11.8, identifying the most important pharmacophore characteristics in Las.Thus, (1) a hydrophobic domain formed by the alkyl substituents of the aromatic domain was identified; (2) a hydrogen donor domain of the amide nitrogen; (3) a hydrogen acceptor, the carbonyl oxygen of the amide; (4) a cation-forming domain, the nitrogen of the tertiary amine. The ionizable amino group and the aromatic ring have been recognized as key fragments for blocking ion channels. For example, the aromatic amino acids of the receptor can interact with the ionizable amino group or the aromatic ring, involving specific interactions like cation-pi or aromatic-aromatic [34,35]. These interactions of the hydrophobic sites are decisive for the inhibition of the currents in the channels [18,35].

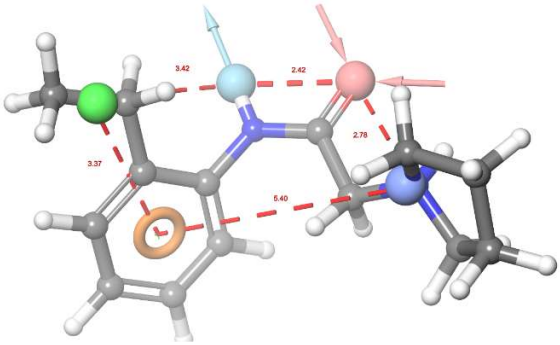


Figure 4. Proposed pharmacophore for LAs, in green: hydrophobic domain, orange: aromatic ring, light blue: hydrogen donor group, red: hydrogen acceptor and in blue: cationic domain.

Considering the common characteristics identified, compounds **1** to **9** were synthesized following the protocol of Wang, Z. et al. [29] with modifications, obtaining moderate to good yields. (Figure 5). Molecular coupling simulations were carried out, establishing the most stable ligand-receptor interactions. Different states were generated for each of the ligands, ten poses were generated in each case. Once these poses were obtained and taking into account the interacting residues for each pose in TASK-1 channel, the EIS value was calculated. EIS was determined according to how much the mutation of each residue influences the binding of bupivacaine, the higher the EIS value, it is more the influence of a certain residue and more important that residue is.

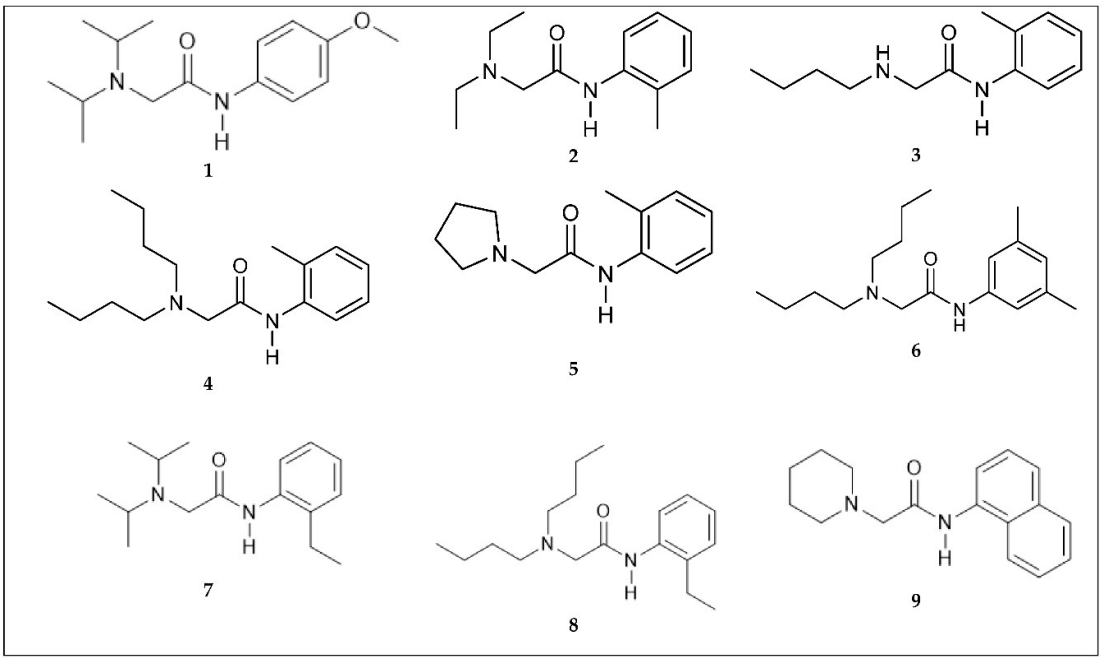


Figure 5. Synthesized LA homologous compounds.

Since it has been described by site directed mutagenesis that the bupivacaine binding site corresponds to amino acid residues located in the fenestrations of TASK-1 channel, a comparative analysis could be established the EIS values of bupivacaine *versus* the EIS value of each of the homologous compounds of LAs. In Table 1, the poses with the best scores of the compounds in their two possible states, protonated and neutral, in addition to bupivacaine, are arranged in descending order of EIS. In this analysis, it was found that most of the LAs homologues proposed here are oriented in a very similar way to bupivacaine, interacting with fenestration residues and, interestingly, locating towards the central cavity (see Figure 6). Consequently, their ESI values are much higher than that calculated for bupivacaine, since they can establish interactions with residues that have been analyzed to be of great importance for the blockade of TASK-1 by LAs, which could be promising, that is, these Results indicate that these compounds are possibly capable of interacting with the TASK-1 channel, forming interactions that will contribute to very stable ligand-receptor complexes.

Table 1. Molecular coupling results for each of the synthesized LAs homologous compounds and their possible interactions with bupivacaine binding site in TASK-1 channel.

Compound	IFD Score	Pose	Interactions	Total Interactions	Total EIS
6_prot	−1052.16	7	A93, B93, B114, A118, B118, A126, A171, A194, A198, B198, A199, B199, A235, A236, A238, A239	16	91.85
6_neut	−1051.13	5	A93, B93, B111, B114, A118, B118, A126, A171, A194, A198, A199, B199, A235, A236, A238, A239	16	90.45
9_neut	−1052.34	8	A93, B93, B114, A118, B118, A126, A194, A198, A199, B199, A235, A236, A238, A239	14	85.42
8_neut	−1050.38	9	A93, B93, B111, B114, B118, A194, A198, A199, B199, A235, A236, A238, A239	13	82.14

5_prot	−1048.26	9	A93, B93, B114, B118, A194, A198, A199, B199, A235, A236, A238, A239	12	80.17
1_prot	−1051.92	3	A93, B93, B114, A118, B118, A126, A171, A194, A198, A199, A235, A236, A238, A239	14	78.26
3_prot	−1049.29	7	A93, B93, B114, A118, B118, A126, A171, A194, A198, A199, A235, A236, A238, A239	14	78.26
4_neut	−1051.27	1	A93, B93, B114, B118, A171, A194, A198, A199, A235, A236, A238, A239	12	73.01
3_neut	−1046.00	8	A93, B93, B114, B118, A126, A194, A198, A199, A235, A236, A238, A239	12	72.98
5_neut	−1050.12	7	A93, B93, B114, B118, A126, A194, A198, A199, A235, A236, A238, A239	12	72.98
7_prot	−1052.30	2	B93, B114, B118, A171, A194, A198, A199, A234, A235, A236, A238, A239	12	70.60
2_neut	−1049.67	10	A93, B93, B114, B118, A194, A198, A199, A235, A236, A238, A239	11	69.94
9_prot	−1053.36	6	A93, B93, B114, B118, A194, A198, A199, A235, A236, A238, A239	11	69.94
Bupivacaine	−1051.66	1	B93, B114, B118, A171, A194, A198, B198, A235, A236, A238, A239	11	62.78
2_prot	−1050.13	9	B93, B114, B118, A194, A198, A199, A235, A236, A238, A239	10	59.71
8_prot	−1051.78	4	B93, B111, B114, B118, A171, A194, A198, A199, A235, A238, A239	11	59.57
4_prot	−1050.99	1	B93, B114, B118, A171, A194, A198, A199, A235, A238, A239	10	57.61

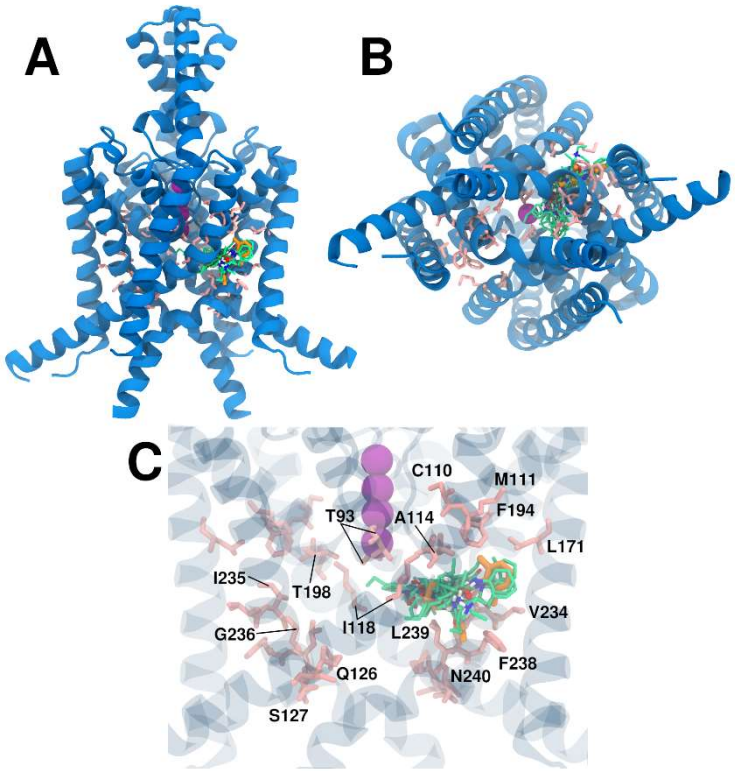


Figure 6. TASK-1 structure (PDB ID: 6RV3) (**A**) LAs homologues were docked in the lateral fenestrations in TASK-1 channel. (**B**) Top view of the channel. (**C**) Enlargement of LAs binding site. Residues of bupivacaine binding site are shown. They are located below the second pore helix, M2 and M4.

The ligand with the highest EIS is compound **6**, both in its protonated and neutral forms, with ESI values of 91.85 and 90.45, respectively. These values are much higher than the EIS value calculated for bupivacaine, which was 62.78. This is probably due to the fact that this ligand is located between the fenestration, a location similar to bupivacaine, and part of its structure is located towards the central cavity, interacting with several important residues described for the blockade of bupivacaine. This orientation then allows it to interact with debris from both the fenestrations and the central cavity (see Figure 7). Similarly the compounds 9_neut, 8_neut, 5_prot, 1_prot, 3_prot, 4_neut, 3_neut, 5_neut, 7_prot, 2_neut, and 9_prot have ESI values greater than the value calculated for bupivacaine. On the other hand, the compounds 2_prot, 8_prot and 4_prot have ESI values slightly lower than those calculated for bupivacaine, which suggests that these compounds could have a similar blocking effect on the TASK-1 channel.

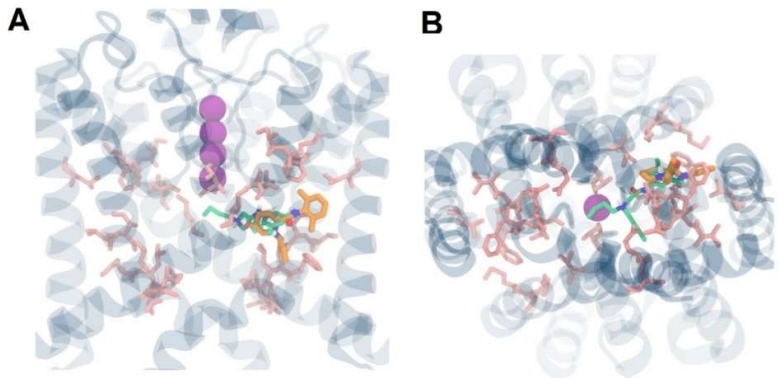


Figure 7. Comparison of the interaction of compound **6** (green) with bupivacaine (orange) in TASK-1 (**A**) View from the plane of the membrane (**B**) Intracellular view.

Some of the amino acids of the binding site for bupivacaine coincide with those of the binding site of A1899 [36], a classic TASK-1 blocker, such as I118 in M2 and I235, G236, L239 and N240 in M4. However, other novel amino acids, different from the A1899 binding site, were identified for bupivacaine: C110, M111, A114, Q126 and S127 in the M2, V234 and F238 segment in the M4, T198 segment of P2. Which makes the bupivacaine binding site distinctly different from A1899 binding site [20]. The binding site (BS) of the compounds described here present greater similarity with bupivacaine BS than with A1899 BS.

4. Conclusions

A rational design of LAs homologous compounds was carried out, based on the common structural chemical characteristics of LA, the identified pharmacophore, the reported binding site for bupivacaine in TASK-1 channel, and it was possible to establish that the binding site of these new compounds has similarities with bupivacaine BS. Such new compounds could be used in the treatment of AF through the modulation of the action potential.

Through molecular docking analysis it was possible to establish the most stable poses for each ligand with the channel, and by comparative analysis with the bupivacaine binding residues it was possible to calculate the EIS values obtaining higher values for the LAs homologues than for bupivacaine, which converted into interesting compounds as potential TASK-1 channel blockers.

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