A new era for the design of TRPV1 antagonists and agonists with the use of structural information and molecular docking of capsaicin-like compounds

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ABSTRACT
The design of TRPV1 antagonists and agonists has reached a new era since TRPV1 structures at near-atomic resolution are available. Today, the ligand-binding forms of several classical antagonists and agonists are known; therefore, the specific role of key TRPV1’s residues in binding of ligands can be elucidated. It is possible to place the well-defined pharmacophore of TRPV1 ligands, conforming by head, neck, and tail groups, in the right pocket regions of TRPV1. It will allow a more thorough use of molecular modelling methods to conduct more effective rational drug design protocols. In this work, important points about the interactions between TRPV1 and capsaicin-like compounds are spelled out, based on the known pharmacophore of the ligands and the already available TRPV1 structures. These points must be addressed to generate reliable poses of novel candidates and should be considered during the design of novel TRPV1 antagonists and agonists.

Introduction
Transient receptor potential vanilloid 1 (TRPV1) belongs to the vanilloid family of TRP (transient receptor potential) channels, relatives of voltage-gated potassium channels, and expressed in the central nervous system and the peripheral blood vessels1,2. It is activated by different mechanisms involving voltage, heat, and ligands, resulting in an increase in intracellular Ca2+ concentration and blood vessel constrictions3. TRPV1 has been identified as a promising therapeutic target to reduce pain perception4,5 and itch sensation under pathological conditions6. It is also involved in the regulation of several physiological and pathological processes; therefore, it has been also considered in the development of therapies against schizophrenia7, epilepsy8, diabetes9, ischaemia10, chronic cough11, etc.

Capsaicin, the primary pungent ingredient of chilli peppers, is the most characteristic exogenous chemical activator of TRPV1. Other exogenous agonists were identified such as olvanil12 and resiniferatoxin (RTX)13, and endogenous agonists have been also identified, such as anandamide14, N-oleylatedopamine15, etc. In capsaicin and natural capsaicin-like compounds, vanillyl was identified as an essential group to modulate TRPV1. Subsequently, a wide number of TRPV1 ligands have been synthesised that have other groups instead of vanillyl, such as pyrimidine16, 2-benzothiazolyl (3-fluoro-4-(hydroxymethyl)phenyl)propanamide21, etc.

The pharmacophores of capsaicin-like TRPV1 ligands
Capsaicin-like TRPV1 ligands have a well-defined pharmacophore, composed by a consecutive disposition of three chemical features: head, neck, and tail groups (Figure 1). Capsaicin-like TRPV1 ligands bind to a large pocket formed by transmembrane domains, where they adopt a "tail-up, head-down" configuration, placing the head close to the S4–S5 linker26. Head, neck, and tail, also designed as A-, B-, and C-regions, can be easily identified in capsaicin, where the vanillyl (4-hydroxy-3-methoxyphenyl) group is in the...
A-region, amide is in the B-region, and the lipophilic chain of the 8-methyl-6-nonenoic acid is in the C-region. It is possible to identify these pharmacophoric features in TRPV1 agonists and antagonists. For instance, the agonist MDR-652 contains the 3-fluoro-4-(hydroxymethyl)phenyl in the A-region, urea in the B-region, and 2-(tert-butyl)-4-(3-chlorophenyl)thiazole in the C-region. On the other hand, the antagonist capsazepine contains the 2,3,4,5-Tetrahydro-1H-2-benzazepine-7,8-diol in the A-region, thiourea in the B-region, and 4-chlorophenyl)ethyl in the C-region. Figure 2 shows examples of TRPV1 agonists and antagonists, where the pharmacophoric features are represented.

Structures of TRPV1-ligand complexes

In 2008, Fernández-Ballester and Ferrer-Montiel modelled a full-length human TRPV1 (the cytosolic N-terminal, C-terminal, and membrane-spanning region) in the closed and open states by using available structural information in the ion channel field. These models were the first attempt to access the structural atomistic model of the TRPV1 channel and they were in agreement with a 19 Å structure of rat TRPV1 determined just that year by single particle electron cryomicroscopy. Despite these models tried to locate the molecular determinants of function in TRPV1, they were not accurate to investigate the interactions between TRPV1 and its ligands.

Previous to the determination of TRPV1 structures, mutagenesis studies allowed to identify residues that specify sensitivity to small vanilloid ligands, such as Y511, S512, and T550. In 2011, Lee et al. performed mutational analysis to evaluate the contribution of TRPV1 residues to ligand binding of capsaicin and RTX, and used this information to construct a tetrameric homology model of rat TRPV1. Then, they performed flexible docking analysis using this model and they found a good agreement with their mutation data. Over the next years, the same group used this model to propose the docked poses of different sulfonylamino-benzyl derivatives. This model used properly the structural information what was known at the time and provided clarification on various issues related to the interactions between TRPV1 and capsaicin-like ligands; however, it had some shortcomings. For instance, this model placed the residue K571 at the vanilloid pocket of the binding site, making a role of a hydrogen bond (HB) donor when ligands are bound, and did not identify the role of R557 and E570 in ligand binding. Previously, site-directed mutagenesis studies had indicated that these residues were involved in the transduction of the capsaicin-binding signal to the opening of the channel pore, with no specific information about their role in the binding of capsaicin.

Only after 2013, when Cao et al. determined the structures of rat TRPV1 by using single-particle cryo-microscopy, it was...
possible to prepare more reliable structural models of the complexes between TRPV1 and its ligands. The first TRPV1 structures, available in Protein Data Bank (PDB) with the codes 3J5P, 3J5Q, and 3J5R, allow to understand unique aspects of TRPV1 channel functions, including the interactions with capsaicin-like modulators. These authors reported structures that contain capsaicin and RTX in the binding site; however, they had a low resolution (3.8 and 4.2 Å), and they did not allow to clearly observe the chemical groups of the ligands and the role of several residues in the vanilloid binding site.

A few years later, in 2016, Gao et al. reported rat TRPV1 structures in lipid nanodiscs with single-particle electron cryo-microscopy at near-atomic resolution. They reported the apo state (PDB code 5IRZ) and liganded states forming complexes with RTX and capsazepine (5IRX and 5IS0, respectively) in the native bilayer environment. These structures clearly show the conformational changes induced by antagonist and agonist effects in TRPV1. Furthermore, they revealed the roles of the residues Y511, S512, T550, R557, and E570 proximal to the vanilloid pocket, and revealed details of the conformational changes of these residues when the TRPV1 channel is in the closed and open states (Figure 3). Specifically, these structures show that a cation-π interaction is formed between the residues R557 and Y554 (S4 helix) in the apo and antagonist-bound states (nonconductive form). But this interaction is disrupted in the agonist-bound state and R557 adopted another conformation forming an ionic lock with the residue E570 (S4-S5 linker), leading to the opening of the lower gate of TRPV1 (conductive form). This detailed structural information is extremely useful for the rational design of TRPV1 agonists and antagonists.

Structural details of the complexes between TRPV1 and capsaicin-like compounds

Over the last decades, the design of capsaicin-like TRPV1 ligands has been conducted by considering the pharmacophoric features head, neck, and tail, derived from the analysis of capsaicin (ligand-based design). However, after the determination of high-resolution structures of TRPV1 and its complexes, it is possible to describe the specific interactions between the pharmacophoric features of the ligands and the residues at the capsaicin binding site. The retrospective analysis of the specific chemical interactions between TRPV1 and its diverse ligands will be an essential step to go forward in a deeper knowledge of the structural aspects of this protein-ligand system. This analysis can be developed by...
constructing structural models, and by using computational molecular modelling and simulation methods that predict ligand-protein interactions and consider ligand and protein flexibilities. Learning from these models will be a necessary stage to assist the rational drug design of novel TRPV1 agonists and antagonists.

Specific structural details of how head, neck, and tail groups interact with the residues at the TRPV1 binding site can be described in detail. The binding site contains residues from two sequence units (chain A and B here). The head is placed inside the vanilloid pocket where the residues Y511, S512, R557, and E570 expose polar groups and can form HBs with ligands (Figure 4). The conformational changes between the position of these residues, when TRPV1 is in closed and open states (Figure 3), have effects in the pharmacophore of ligands with agonistic and antagonistic activities. For instance, in a recent report, a docking protocol by using the antagonistic-bound state of TRPV1 as receptor explains why a set of 5,5-diarylpentadienamides that contain isoquinoline or 3-hydroxy-3,4-dihydroquinolin-2(1H)-one as the head groups are antagonists, and not agonists. It was found that the head groups (both isoquinoline and 3-hydroxy-3,4-dihydroquinolin-2(1H)-one) have the exact geometry to establish HBs with the residues Y511, S512, and R557 in the closed conformation of TRPV1 and their geometry is not optimal to establish these interactions with these residues in the open TRPV1 conformation.

The neck is formed by polar groups that could form HBs with the side-chain hydroxyl groups of Y511 and T550 (Figure 4). These hydroxyl groups, separated by around 8 Å, define a polar region that connects the vanilloid pocket with a broad hydrophobic pocket. Since Y511 and T550 could establish specific HB interactions, they have important roles in the recognition of TRPV1 modulators.

Finally, the tail is formed by hydrophobic groups that are placed in a hydrophobic pocket formed by residues from the chains A and B of the TRPV1 structure. These residues are represented in Figure 4; it is important to note that rat and human TRPV1 capsaicin binding sites have only one different residue which is located in this pocket: M547 in rat TRPV1 is replaced by L547 in human TRPV1. Residues in this pocket do not form specific interactions, but modulate the potency of compounds from a same congeneric series. For instance, Saku et al. reported a series of N-(isoquinolin-5-yl)-2,4-pentadienenamides with IC₅₀ values between 0.072 and 950 nM (determined using the human TRPV1 receptor). These compounds have the same head and neck groups, and are differentiated only by their tail groups. Therefore, an accurate description of the interactions with the residues at this hydrophobic pocket should be needed to understand the differential activities of this congeneric series.

Methods

Docking of TRPV1 agonists and antagonists guided by available structural information

As discussed above, the recently launched rat TRPV1 structures forming complexes with capsaicin-like antagonists and agonists are reliable sources for generating structural models of TRPV1-

Figure 4. Interactions between ligands and the residues at the capsaicin binding site of rat TRPV1. As an example, the agonist RTX in the structure with PDB code 5IRX is represented as green balls and sticks. The residues of chains A and B of TRPV1 are represented as gray and yellow sticks, respectively. The residues involved in the interactions with the head, neck, and tail groups of the ligand are labelled with red, green, and blue boxes, respectively. The contributions of the residues to interactions with ligands are indicated in circles: hydrophobic H, polar P, hydrogen bond donor or acceptors HB.
ligand complexes by using protein-ligand docking methods. Previous identification of ligand characteristics (head, neck, and tail groups) are also important before applying these methods. Therefore, pharmacophore and geometric filters could be defined during docking calculations to obtain reliable models.

In this section, docking calculations of three series of compounds, named A, B, and C (Tables 1–3), were carried out. A flexible docking protocol was used to give examples of how molecular docking calculations can be performed to generate reliable models of TRPV1-ligand complexes. This protocol was achieved by using the Induced Fit Docking (IFD) method from Glide (Schrodinger LLC, New York, NY, USA, 2017).

The structures of the rat TRPV1 complexes with the agonist resiniferatoxin (5IRX) and with the antagonist capsazepine (5IS0) were analysed to verify that they adopt a tail-up, head-down configuration.

Results

Models of [4-(methylsulfonylamino)benzyl]thiourea analogues (series a and b) as TRPV1 antagonists

The TRPV1 antagonists from series A (Table 1) are chiral isomers, but their binding affinities were reported for the racemic mixtures. On the other hand, compounds from series B (Table 2) are also chiral isomers, and their binding affinities were reported for each stereo-isomer. Compounds from both series A and B

| R1 | R2 | Y | Chirality (C1) | Chirality (C2) | log(10^3/Ki) |
|----|----|---|---------------|---------------|-------------|
| B_5aR | H | H | 0 | R | – | 0.599 |
| B_3aS | H | H | 0 | S | – | 0.627 |
| B_19 | Me | H | 1 | R | R | 1.644 |
| B_20 | Me | H | 1 | S | R | 1.712 |
| B_21 | Me | H | 1 | R | S | –0.057 |
| B_22 | Me | H | 1 | S | S | 0.149 |
| B_25 | Me | 3,4-Me_2 | 1 | R | R | 1.818 |
| B_26 | Me | 3,4-Me_2 | 1 | S | S | 2.674 |
| B_29 | Me | 4-t-Bu | 1 | R | R | 1.747 |
| B_30 | Me | 4-t-Bu | 1 | S | S | 2.171 |
| B_33 | Me | 4-t-Bu | 0 | R | phenethyl | 0.377 |
| B_34 | Me | 4-t-Bu | 0 | S | R | 0.565 |

| R1 | R2 | Y | Chirality (C1) | Chirality (C2) | log(1/EC_{50}) |
|----|----|---|---------------|---------------|-------------|
| C_1a | H | 4-Cl-benzyl | 1.125 |
| C_1b | H | 4-Cl-phenethyl | 1.252 |
| C_1c | H | (E)-4-chlorostyryl | 0.387 |
| C_1d | H | (Z)-4-chlorostyryl | 1.387 |
| C_1e | H | 4-F-phenethyl | 0.886 |
| C_1f | H | 2,4-dichlorophenethyl | 0.886 |
| C_1g | H | tert-butylphenethyl | 0.319 |
| C_1h | H | 3-(4-chlorophenyl)propyl | 0.319 |
| C_1i | H | 4-(tert-butyl)benzyl | 0.292 |
| C_2a | –(CH_2)_3-NH_2 | 4-CI-phenethyl | 0.022 |
| C_2b | –(CH_2)_3-NH_2 | phenethyl | –0.435 |
| C_2c | –(CH_2)_3-NH_2 | 4-F-phenethyl | –0.362 |
| C_2d | –(CH_2)_3-NH_2 | 2,4-dichlorophenethyl | 0.495 |
| C_2e | –(CH_2)_3-NH_2 | 4-i-benzyl | –0.127 |
| C_2f | –(CH_2)_3-NH_2 | 4-(tert-butyl)phenethyl | –0.220 |
| C_2g | –(CH_2)_3-NH_2 | 4-(tert-butyl)benzyl | 0.770 |
| C_2h | –(CH_2)_3-NH_2 | 3,5-di-tert-butylbenzyl | 0.658 |
| C_2i | –(CH_2)_3-NH_2 | 4-(tert-butyl)phenethyl | 0.149 |
| C_3 | –(CH_2)_3-NH_2 | 4-Cl-phenethyl | –0.739 |
| C_4 | –(CH_2)_3-NH-CH_3 | 4-Cl-phenethyl | –0.847 |
| C_5 | –(CH_2)_3-N(CH_3)_2 | 4-Cl-phenethyl | –0.547 |
| C_8 | –(CH_2)_3-N-COCH_3 | 4-Cl-phenethyl | –0.710 |

Table 1. Structures of [4-(methylsulfonylamino)benzyl] thio ureas (series A) and their rat TRPV1 binding affinities (racemic mixtures) as log(10^3/Ki) (nM) values (reference34).

Table 2. Structures of specific stereoisomers of [4-(methylsulfonylamino)benzyl] thioureas (series B) and their rat TRPV1 binding affinities as log(10^3/Ki) (nM) values (reference35).

Table 3. Structures of (3-methoxybenzyl)thioureas (series C) and their rat TRPV1 agonist activities as log(1/EC_{50}) (μM) values (reference25).
contain a 4-(methylsulfonylamino)benzyl as a head group. Their neck is the thiourea, and their tail is a series of bulky groups that contains a unique chiral centre with 3-pivaloyloxymethyl and phenyl (or phenylalkyl) substituents. Compounds from series B contain two chiral centres: the first one is at the methylene group of the benzyl fragment of 4-(methylsulfonylamino)benzyl, and the second one is at the bulky tail group.

The 4-(methylsulfonylamino)benzyl group is longer than the 2,3,4,5-Tetrahydro-1H-2-benzazepine-7,8-diol group of capsazepine. When docking calculations of compounds from series A and B inside the rigid protein of the PDB structure with code 5IS0 (containing the antagonist capsazepine) were carried out, no solutions were found adopting “tail-up, head-down” configurations. Therefore, a flexible docking protocol was performed, hoping to find adequate solutions.

The docking poses obtained for compounds from series A inside the binding pocket of rat TRPV1 are shown in Figure 5 and are included in the Sdf format as Supplementary Material (Section S1). These results show that adequate orientations were found for both R and S isomers (with the exception of the R isomer of A_21) by placing the head, neck, and tail groups at the zones of the TRPV1 represented in Figure 4. All the obtained docking poses were similar to the pose of capsazepine in the reference structure. HB interactions between compounds from series A and four amino acids in the TRPV1 binding pocket (S512, R557, Y511, and T550) were observed. The same orientations were observed for compounds from series B, which are represented in Figure 6 (left) and are included in the Sdf format as Supplementary Material (Section S2).

The most probable 4-(methylsulfonylamino)benzyl interactions, as the head group, were elucidated with the docking calculations reported here. The sulphonyl oxygens were hydrogen-bonded to R557 and S512 side-chain groups, respectively. The methyl group was oriented towards the S4-S5 linker and the phenyl formed a π-π stacking with Y511. These interactions were similar but not identical to the ones reported by Lee et al. in their models before the determination of the cryomicroscopy TRPV1 structure. In those models authors reported HBs between the 4-(methylsulfonylamino)benzyl group and G558, I564, or K571; however, these residues are not placed at the TRPV1’s binding site in the cryomicroscopy TRPV1 structure.

Therefore, the poses reported here for compounds from series A and B correct the flaws of earlier models, and explain why compounds that contain a 4-(methylsulfonylamino)benzyl group are antagonists. Previously, it was mentioned that salt bridge between R557 and E570 is not formed when TRPV1 is in a nonconductive TRPV1 conformation. The 4-(methylsulfonylamino)benzyl group contributes to prevent the formation of this salt bridge, since it interacts directly with R557 and places the methyl group at the space between R557 and E570 (Figures 5 and 6). On the other hand, the thiourea, as the neck group, was hydrogen-bonded to Y511 and T550 side-chain groups; meanwhile, the 3-pivaloyloxymethyl and phenyl (or phenylalkyl) substituents, which are the tail groups, occupy the large hydrophobic pocket formed between residues from chain A and B of TRPV1.

The IFD method yielded a scoring-energy value for each compound from series A and B. It was analysed whether a correlation exists between these values and experimentally determined binding affinities. Compounds from series A have two isomers (R and S), but their activities were reported for the racemic mixtures. Possible correlations were explored for R isomers, for S isomers, and for selections that combine both isomers, but low correlations were found (R² < 0.3). This might be due to the fact that experimental Ki values for compounds from series A do not correspond to one isomer, but to the racemic mixture. On the other hand, compounds from series B have the Ki values for each stereo-isomer; therefore, it is possible, in theory, to explore more reliable correlations. A good correlation between the scoring-energy values and the experimental Ki values was found with a correlation coefficient of R² = 0.775 (Figure 6 right). This result confirms that the use of the PDB structure with code 5IS0 as starting coordinates for a flexible docking protocol is a good strategy for studying the structural features of the TRPV1 antagonists.
Ligand-induced fit of the binding pocket could lead to important conformational changes of some residues. During the docking of compounds from series A and B, the residues Y511, S512, L515, R557, and E570 had root mean square deviation (RMSD) values above 1.5 Å (using the structure with PDB code 5IS0 as reference) when interact with the head groups of ligands. Furthermore, the residues F587 and L669 had higher RMSD values to accommodate the bulky tail groups of the ligands, and T550 had an RMSD above 1.9 Å for establishing HB with the thiourea neck group.

Models of [4-(methylsulfonylamino)benzyl]thiourea analogues (series C) as TRPV1 agonists

Compounds from series C (Table 3) are agonists25. According to the head group, the series C can be separated in compounds C_1, which contain the 4-hydroxy-3-methoxybenzyl substituent, and compounds C_2, that contain the 4-(2-aminoethoxy)-3-methoxybenzyl substituent. Besides, compounds that contain modifications to the last substituent are also present (C_3, C_4, C_5, and C_8). Their neck is the thiourea, and their tail is a series of phenylalkyl substituents.

Docking calculations of compounds from series C inside the protein of the PDB structure with code 5IRX (containing the agonist RTX) were carried out by the flexible docking protocol described above. The docking poses obtained for compounds from series C inside the binding pocket of rat TRPV1 are shown in Figure 7 and are included in the Sdf format as Supplementary Material (Section S3). These results show that adequate orientations were found for the whole series by placing the head, neck, and tail groups at the zones of the TRPV1 represented in Figure 4. All the obtained docking poses were similar to the pose of RTX in the reference structure. The head groups of compounds C_1 were hydrogen bonded to the residues SS12 and R557, and their neck groups were hydrogen bonded to the residues Y511 and T550 (Figure 7A). Their tail groups were oriented to the pocket near L669 of the chain B, except for compounds C_1c (with a rigid (E)-4-chlorostyryl substituent) and C_1i (with a bulky 4-(tert-buty)benzyl substituent) that had the tail groups near F543 from chain A.

On the other hand, compounds C_2 (the series), C_3, C_4, C_5, and C_8 placed their phenyl groups of the head outside the vanilloid pocket, by forming HBs between NH2 of the ligand’s aminoocthoxystyryl and the side-chain OH of SS12, and between the ligand’s methoxy and side-chain OH of Y511 (Figure 7C and 7E). Their neck groups were hydrogen bonded to T550 and their tail groups were oriented to the hydrophobic pocket near the residue F543.

The most probable 4-hydroxy-3-methoxybenzyl interactions, as the head group of compounds C_1, were elucidated with the docking calculations reported here. Docking poses explain why 4-hydroxy-3-methoxybenzylthioureas are agonists. Previously, it was mentioned that salt bridge between R557 and E570 is formed when TRPV1 is in a conductive TRPV1 conformation. The 4-hydroxy-3-methoxybenzyl group allows the formation of this salt bridge, since it interacts directly with R557 (Figure 7A). Poses of compounds C_2, C_3, C_4, C_5, and C_8 show that 4-(2-aminoocty)oxy)-3-methoxybenzyl as head group, also allows the formation of the R557–E570 salt bridge (Figures 7D and 7E).

Considering IFD scoring-energy values for compounds from series C, analyses of possible correlations with experimentally determined binding affinities were performed. A good correlation between the scoring-energy values and the experimental log(1/EC50) values was found for compounds C_1 (nine compounds) with a correlation coefficient of R2 = 0.638 (Figure 7B). A good correlation was also found for compounds C_2 (nine compounds) with a correlation coefficient of R2 = 0.661 (Figure 7D). Finally, a good correlation was also found for the whole series C, including compounds C_1, C_2, C_3, C_4, C_5, and C_8 (twenty-two compounds) with a correlation coefficient of R2 = 0.788 (Figure 7F). This result confirms that the use of the PDB structure with code 5IRX as starting coordinates for a flexible docking protocol is a good strategy for studying the structural features of the TRPV1 agonists.
Conclusions

The design of novel capsaicin-like TRPV1 antagonists and agonists has been moving to a new era in which the structural information should play a preponderant role. Access to structures of antagonist-and agonist-bound states of TRPV1 in PDB will lead to a more rational design. Specific structural features have been provided to medicinal chemists involved in the design of TRPV1 modulators, and they should take full advantage of the new available information with the help of molecular modelling methods, as

![Figure 7. Structural models of vanillyl thioureas as TRPV1 agonists. (Left) Docking poses. (Right) Plot of Docking Energy values against biological activity represented as the log (1/EC_{50}) values. (A) poses of 4-Hydroxy-3-methoxybenzyl thioureas C_{1a}–C_{1i}, (B) Plot of Docking Energy values against biological activities for C_{1a}–C_{1i}, (C) poses of 4-(2-Aminoethoxy)-3-methoxybenzyl thioureas C_{2a}–C_{2j}, (D) Plot of Docking Energy values against biological activities for C_{2a}–C_{2j}, (E) poses of compounds C_{3}, C_{4}, C_{5}, and C_{8}, (F) Plot of Docking Energy values against biological activities for C_{1a}–C_{1i}, C_{2a}–C_{2j}, C_{3}, C_{4}, C_{5}, and C_{8}. The vanillyl thioureas are represented as green sticks. The chains A and B of TRPV1 are in gray and yellow cartoon representations, respectively. Relevant residues of TRPV1 in the binding site are in stick representation.](image-url)

Conclusions

The design of novel capsaicin-like TRPV1 antagonists and agonists has been moving to a new era in which the structural information should play a preponderant role. Access to structures of antagonist-and agonist-bound states of TRPV1 in PDB will lead to a more rational design. Specific structural features have been provided to medicinal chemists involved in the design of TRPV1 modulators, and they should take full advantage of the new available information with the help of molecular modelling methods, as
done in successful studies of other biological targets. After the availability of rat TRPV1 structures at near-atomic resolution, several works reported novel TRPV1 agonists or antagonists including docking calculations. These works determined the orientations of selected candidates; and there are no recent reports with comparisons between docking poses of congeneric compounds.

Here, several specific structural characteristics, related to capsaicin-like TRPV1 antagonists and agonists, are discussed to draw the attention of medicinal chemists. Capsaicin-like TRPV1 modulators have a pharmacophore composed by head, neck, and tail groups, and these groups are placed at specific subsites at the capsaicin binding site. The available structural information will allow to construct more reliable models between TRPV1 and ligands that inactivate or activate ion conduction. However, it is important to use this information in such a way as to provide more comprehensive details, i.e. more convincing knowledge of the interactions between the pharmacophore of the novel ligands and the key residues at subsites adopting conformations in non-conductive or conductive states.

As a brief conclusion, the intention in this work is not to criticize the lack of the use of structural models and computer-aided molecular design methods in the design of TRPV1 modulators, but to emphasize that nonconductive and conductive models are available since a few years ago, and pharmacophoric characteristics of capsaicin-like antagonists and agonists have been revealed since many years ago, and the use of all this information together will contribute to a deeper understanding of the interactions between novel molecules and TRPV1. Indeed, in this new era, the use of structural information and molecular docking, if used properly, will cause a remarkable leap forward in the design of more potent TRPV1 modulators.

**Disclosure statement**

No potential conflict of interest was reported by the author(s).

**Funding**

This research was funded by FONDECYT Regular grant number 1210138

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