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Insights on the role of boron containing moieties in the design of new potent and efficient agonists targeting the β2 adrenoceptor

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ABSTRACT

The development of β2 adrenoceptor (β_2AR) agonists is of increasing interest because of their wide-ranging applications in medicine, particularly for the treatment of pulmonary diseases. Regarding the relaxation of smooth muscle that lines airways of mammals, some boron-containing adducts have demonstrated greater potency and efficacy compared to well-known boron-free compounds. We herein report the design and synthesis as well as the chemical and pharmacological characterization of a new boron-containing compound: ((*R*)-6-((*S*)-2-(*tert*-butylammonio)-1-hydroxyethyl)-2-hydroxy-2-isobutyl-4*H*-benzo[*d*][1,3,2] dioxaborinin-2-uide). Compared to its precursor (salbutamol), this compound induced relaxation of smooth muscle in guinea pig tracheal rings with greater potency and efficacy ($EC_{50} \leqslant 28.02$ nM). Theoretical studies suggest the potential selectivity of this boron containing compound on the orthosteric site of beta adrenoceptors and/or signaling pathways, as well as the importance of the tetracoordinated boron atom in its structure for binding recognition properties.

The development of β2 adrenoceptor (β_2AR) agonists is an interesting task because of their wide-ranging applications in medicine. They are particularly useful for treating pulmonary diseases, and are also administered in cases of cardiovascular, central nervous system, immune and muscle disorders.[1]

β_2AR-agonists are among the drugs that have been shown in clinical practice to provide a significant improvement in the clinical manifestations of asthma and chronic obstructive pulmonary disease,[1,2] which are common causes of morbidity and disability in the world. Compared to well-known boron-free β_2AR-agonists, some boron-containing compounds (BCCs) have demonstrated higher potency and efficacy as relaxant agents of smooth muscle in airways of mammals.[3]

In addition, available drugs present several disadvantages, including their tendency to lose effectiveness after prolonged use. This reduced effect is apparently related to a decrease in the number of β_2ARs expressed on the cell surface.[3] An association has been established between this curtailed expression and biased-signaling in the arrestin pathways that is induced by compounds in cells expressing β_2ARs. Nevertheless, the mechanisms linked to this phenomenon are not well understood at the molecular level.[4]

There have been some clues in regard to the molecular details of the interaction between β_2ARs and their ligands that have implications for the design of new compounds with biased signaling.[3,5] For instance, it has been suggested that the activation of the arrestin pathway requires the interaction of compounds with residues outside of the orthosteric site of the targeted receptor. On the other hand, some authors point out the difficulty of establishing the active site.[5] In any case, it is clearly desirable to design selective agonists for β_2ARs, especially those with the ability to trigger biased signaling.[4,5]

In the present study we designed several compounds that may reach and activate β_2ARs, taking into account the observation in various reports that some residues in the fifth transmembrane domain (TM5) are involved in β_2AR activation.[6] The decision to develop BCCs owes itself not only to their proven high affinity for β_2ARs,[3] but also to their great ability to interact with key residues in the TM5 of these receptors. That is due to some in vitro and in silico experiments have suggested that Tyr199 and Ser203 in TM5 probably play a key role in β_2AR activation.[6]

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In general, the study of BCCs has been increasing in this century.[7] We have reported that BCCs which target β_2ARs share two features: they are derived from the same precursor molecule (salbutamol), and they were formed by linking a tetracoordinated boron atom to this precursor.[5] It has been suggested that the BCCs with these two features may be more stable in a physiological environment that those containing a tri-coordinated boron.[7]

Salbutamol, a partial agonist with β_2AR selectivity, has recently been suggested as an agonist for cyclic Adenosine monophosphate (*cAMP*) production, Extracellular signal-Regulated Kinases (ERK)1/2 activation, calcium release and receptor endocytosis, with the rank order of potency being ERK > cAMP > calcium > endocytosis.[8] A spread of more than 4 logarithmic units has been found between the most and least responsive signaling modalities. The aforementioned tetracoordinated boron atom is linked to this precursor by means of a chemical reaction in an alkaline medium with a non-polar solvent (Table 1). We have also analyzed (by means of in silico assays) the importance of the moieties linked to amine, the charges located in the proposed molecules, and the stereoisomeric forms derived from the placement of substituents at the β-carbon to amino.[3]

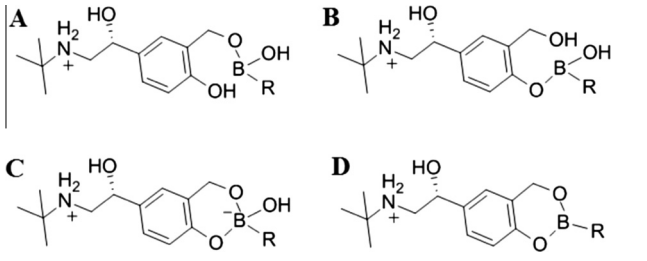
In the current contribution we built a 3D representation of several adducts formed by combining salbutamol and some boronic acids, the latter of which were recently tested as potential moieties with a low toxicity profile.[9] These BCCs could then be analyzed in terms of a diol-condensation between either a tri- or tetracoordinated boron in its structure and the hydroxyl groups of the catechol moiety, and also an interaction between the tricoordinated compounds and the substituent in para or ortho position in the phenyl ring relative to the *tert*-butyl-ethylamine moiety. The structural changes in the *tert*-butyl moiety of salbutamol were avoided because of previous observations (based on theoretical assays carried out by our workgroup) indicating that even minor changes disrupt the binding mode or affinity for ligands.[3]

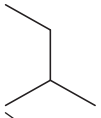
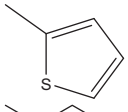
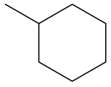
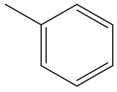
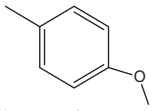
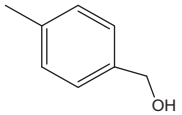
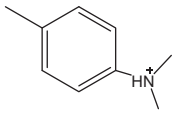
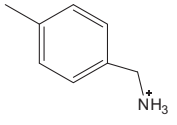
By using in silico assays the proposed structures were evaluated according to the affinity and binding mode with which they interacted on the recently reported three-dimensional (3-D) model of active guinea pig β_2AR. This 3-D model is useful for predicting affinity values, judging by the correlation of such values with those reported in vitro for well-known ligands.[10] In order to reach a greater approximation between predicted affinity and that found experimentally, we made an adjustment based on the fact that affinity values are underestimated in the β_2AR model.[10] The ligand–protein docking methodology was also useful for validating the estimating procedure and the proposed ligand–receptor interactions.

The in silico assays suggested the importance of certain moieties in the ligands and of interactions with specific residues in β_2ARs. For example, we observed greater affinity for compounds with a phenyl ring or with more compact moieties as a substituent of boronic acid (see Table 1). The compounds with bulkier substituents showed lower affinity for the β_2AR but conserved the ability to fit into the orthosteric binding site, which is in agreement with previous reports by our workgroup and others.[5,8,11] However, scant reports have been published on chemical modifications on this side of the catecholamine core (the substituents of the benzene ring)[12] compared to the extensive information available on the moieties in the amino-linked extreme.

We analyzed the possible adducts between *R*-salbutamol and boric acid as well as one of nine boronic acids. Among the forty compounds included in the in silico study (Fig. 1), we selected adducts between salbutamol and 2-methylpropyl boronic acid for a more detailed analysis due to their calculated high affinity on β_2ARs. Additionally, 2-methylpropyl boronic acid showed a relatively low toxicological profile.[9] Among these selected adducts, the best score (highest predicted affinity on β_2AR) was observed for the compound that has a tetracoordinated boron atom linked with two oxygen

Table 1
Chemical structures of the tested compounds



Compound	R
1	OH
2	CH_3
3	
4	
5	
6	
7	
8	
9	
10	

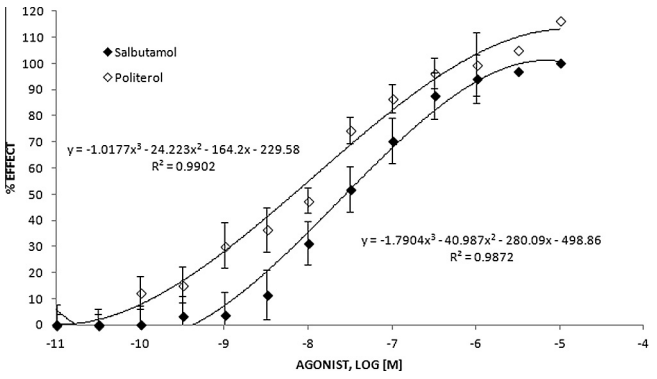


Figure 1. Calculated affinity by docking studies of 40 compounds tested on the 3-D model of a guinea pig β_2AR structure.