Give Boron a Chance: Boron Containing Compounds Reach Ionotropic and Metabotropic Transmembrane Receptors

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Abstract: The ligand-gated ion channels and seven transmembrane domain receptors are the greatest families of transmembrane receptors (TMR) expressed in mammalians and the major target of current available drugs. Recently, boron containing compounds (BCC) have shown capability of acting as ligands on these targets. This mini-review is focused on the description of BCC that target TMR which were evaluated under experimental models. The results in experimental models are related with the theoretical interaction studies of these ligands on the target proteins as 3D-models in order to explore the biological effects of BCC in molecular detail.

Keywords: Boron containing compound, 7TM receptor, channel receptor; drug development, synthesis, molecular recognition.

INTRODUCTION

Ionotropic receptors (ligand-gated ion channels) and metabotropic seven transmembrane domain receptors (7TM; also known as G-Protein Coupled Receptors, GPCR) together represent the great majority of transmembrane protein receptors expressed in mammals, and they are the major target of most currently available drugs [1,2]. Many strategies for improving the interaction between drugs and these receptors have been employed, finding ligands with high affinity and selectivity [2]. Among the ligands currently being sought for this purpose are boron containing compounds (BCC), and recent efforts are yielding a great quantity of new molecules [3].

BCC have been used as antiseptics, antibiotics, cosmetics and insecticides for more than a century [3-6]. In the last few decades BCC have been sought as a means of targeting biomolecules involved in cancer therapy, including non-specific target applications, such as Boron Neutron Capture Therapy [4,7,8], and direct selective action on some cancer targets [9].

Although some objections have been raised by researchers in relation to the toxicological profile of some BCC [10], experimental and clinical studies have shown that BCC can be administered in adult humans at high doses without toxic effects [10-13]. Moreover, some BCC are marketed today as proteosome inhibitors and antifungal drugs [4].

In the majority of currently known applications, BCC interact with an enzyme, and the presence of boron atom in some compounds has improved the ligand-enzyme interaction [6,14]. Recently, new BCC have been synthesized and tested as ligands on ionotropic or metabotropic transmembrane receptors (TMR). Several studies have shown some special characteristics of these ligands compared with similar compounds without a boron atom in their structures [4,6]. These features can give BCC some advantages, in terms of pharmacodynamics and pharmacokinetics, in drug development [4].

Nowadays, there is increasing interest in a detailed exploration of drug-receptor interactions at the atomic level. The growing availability of 3D models for TMR allows for new structural perspectives of the recognition of ligands by these receptors, which can lead to new proposed chemical structures and methodologies for improving ligand-TMR mediated effects [15,16]. Accordingly, the aim of the current contribution is to review the reports of BCC that target TMR, focusing on results from experimental models and insights from docking studies done with TMR-3D models.

BIOLOGICAL EFFECTS OF SOME BCC

To the best of our knowledge, sodium borate (also called borax) and boric acid were the first BCC for which biological activity was studied [10]. Today boronic acid, boronate and borane derivatives have all been shown to have effects in biological systems [3,4,17]. Moreover, the more than 20 complexes reported in the protein data bank are direct evidence that BCC can target enzymes or nuclear receptors [18]. However, in most studies the evidence of BCC-TMR interactions is only indirect, based on inferences from experimental results and clinical observations. Only in a few very recent studies have new approaches been applied that have the capability of providing direct evidence of interactions between BCC and TMR.

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EFFECTS OF BCC THAT INTERACT WITH IONOTROPIC RECEPTORS

Some reports have strongly suggested that there is an interaction of BCC with ionotropic channels, but direct experimental evidence is still lacking. In the current contribution we focus on such interactions, specifically those that disrupt the basal membrane potential or action potential mechanism and thus trigger consequences in elaborate and miscellaneous cellular pathways or processes, including muscle contraction, secretion, neuronal processing and transmission, fertilization, cell division, migration, differentiation, proliferation, metabolism and death [4,6].

Among the first works suggesting interactions between a BCC and ionotropic receptors [19-21], Changeux *et al*. [19] reported the binding of *p*-trimethylammonium benzene diazonium fluoroborate (TDF) to acetylcholine ionotropic receptors as a tool for labeling the latter (Fig. **1**). It was suggested that this compound binds by means a covalent bond with the tyrosine, histidine and lysine side chains of this protein, which if true would give TDF the capability of behav-

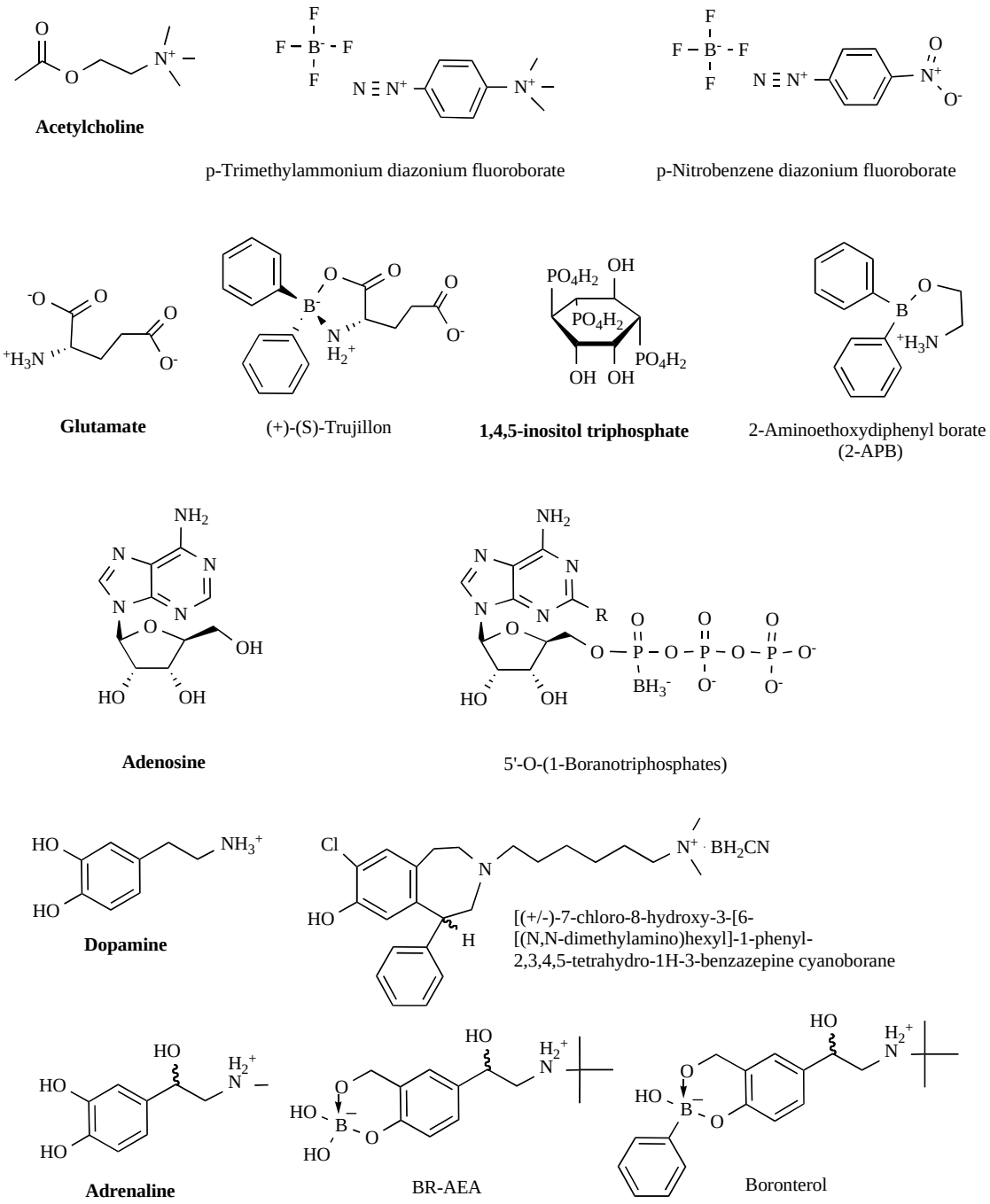

Fig. (1). Some boron containing compounds which have shown effects on ionotropic or metabotropic receptors and the endogenous ligands (labels in bold letters) of these receptors.

ing as an irreversible binding ligand in this context [19]. Later, studies confirmed that TDF and p-Nitrobenzene diazonium fluoroborate (NDF), act as potent inhibitors of the carbamylcholine-induced depolarization and of acetylcholinesterase by forming covalent bonds with the acetylcholine receptor and probably with acetylcholinesterase at the active site [22,23]. Also some NDF analogues have shown activity as reversible or irreversible acetylcholine receptor-inhibitors [22]. Moreover, specific cysteine and tyrosine residues have been implicated in recognition by mutational and binding studies [24,25]. In spite of these findings, the direct interaction of these compounds (and related compounds like p-N,N-dimethylamino.phenyldiazonium fluoroborate) with acetylcholine receptors is poorly understood.

Another known target for BCC is the inositol 1,4,5-triphosphate receptor (IP3R), an ionotropic channel located in the membrane of sarcoplasmic reticulum that increases the release of intracellular calcium [26]. The regulation of IP3R activity has been studied in relation to 2-Aminoethoxydiphenyl borate (2-APB), considered a membrane-permeable inhibitor of IP3R [26,27], well-known for its activity on transient receptor potential channels (TRP), which are non-selective cationic channels [27-30].

The capacity of 2-APB to inhibit the release of calcium affects the function of several systems: limitation of spontaneous activity in mouse embryonic stem cell-derived cardiomyocytes [31], inhibition of cardiomyocyte hypertrophy [32], and regulation of signaling pathways in cardiac fibroblasts [33]. Also, there are reports of the effects of this BCC on ionic channel regulation in rat neurons [34-36], on smooth muscle (or related) cells [37-39], on mouse pancreatic beta cells, and as an inhibitor of cell proliferation and apoptosis-induction of gastric cancer cells from malignant ascites [40].

Thus, there are potential applications of 2-APB in several medical areas. Recently, Xiao *et al.* have demonstrated the effect of 2-APB on IP3R in atrial muscle cell lines under conditions of ischemia or cholinergic activation. They have proposed 2-APB has potential application in some kinds of atrial fibrillation [41]. Additionally, Szatkowski *et al*. have described the antiproliferative effect of 2-APB on the MCF-7 human breast cancer epithelial cell line [42].

Other BCC, as phenylborinic acid, poly(arylhydroxyborane), and their respective esters, have been mentioned as regulators of intracellular calcium concentration. These compounds inhibit the release of calcium induced by inositol 1,4,5-triphosphate induced calcium release and/or capacitative Ca^{2+} entry into cells, thus controlling intracellular calcium concentration [43]. Also, aliphatic amine carboxyboranes, such as *n*-$C_{18}H_{37}(CH_3)_2NBH_2COOH$, the phosphine carboxyborane Ph_3PBH_2COOH, the copper(II) complex $Cu_2(Me_3NBH_2CO_2)_4.{}_2Me_3NBH_2CO_2H$ and tetraphenylboron were shown to reduce calcium efflux and to increase calcium influx into both the osteoblastic cells and the pup bones of mice, demonstrating the capacity of these compounds to act as preventive agents for osteoporosis. Even though the mechanism is not clear [44,45], the direct interaction with calcium channels and regulation of the production and release of chemical mediators initiating bone loss have been suggested [46,47].

Another ionotropic target for BCC has been described by our workgroup. We have obtained boron-containing amino acid derivatives, specifically glutamate analogues, among which are the stereoisomers of Trujillon (a BCC with a structure related to 2-APB; see Fig. **1**). These stereoisomers have been shown to increase spontaneous globus pallidus neuronal activity of the anesthetized rat. This capability is stereoselective, evidence by the fact that (+)-(S)-Trujillon is more effective than its enantiomer form [48]. Experimental results indicate that increased neuronal activity is mediated by NMDA-glutamate receptors (Fig. **2**) Furthermore, computational simulations show possible interactions of (+)-(S)-Trujillon with one of these glutamate receptors [49]. The binding mode is analyzed later in this review.

Yet another BCC-ionotropic receptor interaction was recently reported by Henderson *et al*., who studied the dose dependent decrease of calcium release from ryanodine receptor sensitive stores, and the regulation of cancer cell proliferation by boric acid (a BCC in current clinical abandonment). Moreover, they linked levels of boric acid in the blood with a decreased risk of prostate cancer [50].

EFFECTS OF BCC ON 7TM RECEPTORS

The study of the effects of the interaction between BCC ligands and 7TM receptors is more recent than that of the interaction of these compounds with ionotropic receptors. At beginning of this century Bezuglov *et al.* [51] developed boron containing derivatives of dopamine, serotonin and acetylcholine. Compared to the native neurotransmitters, these BCC have greater lipophilic properties, allowing them to more readily penetrate the blood-brain barrier and the cytoplasm of embryonic cells. Due to the fact that their effects were similar to those induced by arachidonic acid derivatives acting as agonists on serotonin 7TM receptors, or to antagonists on nicotinic acetylcholine ionotropic receptors, it was suggested that these BCC interact with intracellular receptor components of prenervous serotoninergic or cholinergic systems. However, the mechanisms that caused the effects mediated by these neurotransmitters were not clarified [51].

Fifteen years ago some BCC were designed as 7TM receptor ligands. Among these, [(+/-)-7-chloro-8-hydroxy-3-[6-[(N,N-dimethylamino)hexyl]-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine cyanoborane, a N-alkylaminobenzazepine, has shown activity as a D_1 dopamine receptor antagonist (*Ki* of 142 *nM* in a radiolabelled binding assay using [3H]-SCH 23390). However, the role of a boron atom in N-alkylaminobenzazepines is not necessarily important, as the boron-free *N,N* dimethylamino derivative had a *Ki* of 49 *nM* on the same receptor [52].

In purine receptors, another type of 7TM, Nahum *et al*. [53] reported that adenosine 5'-O-(1-boranotriphosphate) derivatives function as selective P2Y1 receptor agonists, demonstrating a diastereoselective phenomenon in activation [54].

Our workgroup has recently reported the effects of two BCC that are derivatives of salbutamol, a well-known β_2-adrenoceptor agonist [55,56]. The β_2-adrenoceptor is a 7TM implicated in the treatment of asthma and other pulmonary diseases. These two BCC have greater affinity than salbuta-

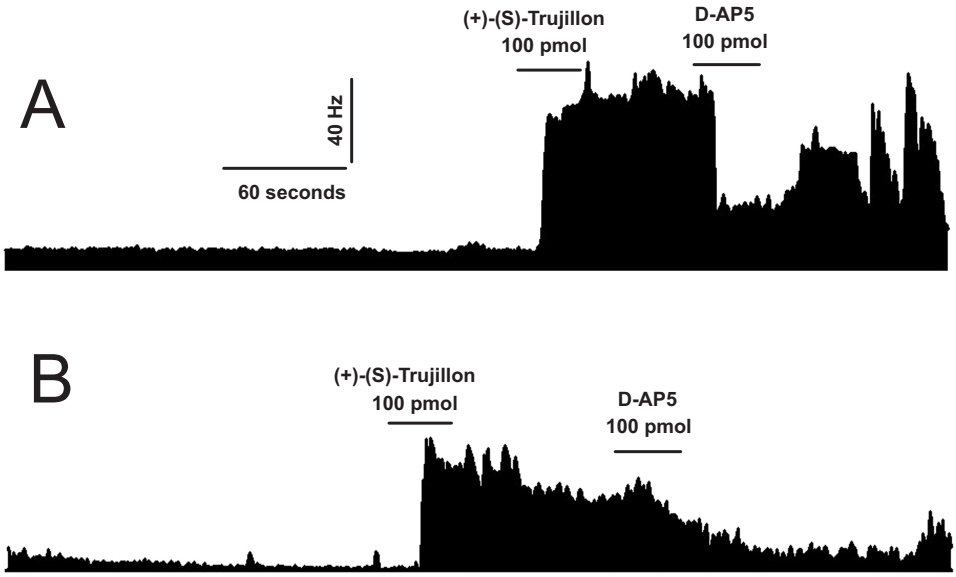

Fig. (2). Local application of (+)-(S)-Trujillon increases the basal firing of globus pallidus neurons by activating NMDA receptors. A and B show two spike rate histograms of pallidal neurons. D-AP5, an NMDA antagonist, diminished the increased in the spiking evoked by (+)-(S)-Trujillon.

mol on the guinea pig β_2-adrenoceptor, judging by the greater relaxing effect on guinea pig tracheal rings [55,56]. Moreover, one of these compounds has higher efficacy than salbutamol in bronchodilator action [56]. The direct interaction of these BCC with β_2-adrenoceptors is strongly by the fact that because propranolol and ICI118,551, which are well-known β_2-adrenoceptor antagonists, shift the BCC curves to right in a concentration-dependent manner [55,56].

Other synthesized BCC are derivatives of endogenous ligands for some 7TM, as choline, aminoacids or nucleosides. These derivatives have shown themselves to be effective antineoplastic-cytotoxic, anti-inflammatory and/or hypolipidemic agents, and their mechanisms of action have often been associated with the disruption of enzymatic activity in related-processes [57-59]. Also some fluorescent-BCC have been employed for identifying 7TM distribution in tissues [60].

A MOLECULAR MODELING OF THE BCC-TMR INTERACTION

Recent data allow us to study the BCC-TMR interactions. The X-ray crystal structures for some of these targets have been elucidated for both ionotropic and metabotropic receptors [61,62]. This information about family A of the metabotropic receptors could be particularly useful since are often considered as drug-targets. Another important source of data are homology modeling techniques, which allow for the construction of a specific receptor model based in the use of the available structures as templates.

Also, boron atom parameters for computational simulations have been established, and have been applied in some works in which the role of boron has been studied in targeting some enzymes [14].

Recently, we have explored the BCC-TMR interaction mode by applying the new data. Thus, we have found some BCC with greater affinity on the NMDA-glutamate and β_2-adrenoceptors than their respective precursors [48,49,55,56]. This greater affinity of BCC is related to the interaction between the boron atom (or atoms in gem position to boron) with the polar side chains of amino acids, the latter are often important in the recognition of endogenous ligands by 7TM [55].

For the glutamate –ionotropic- receptor, no direct interaction of the boron atom was visualized for one BCC (S-Trujillon), probably due to the hindrance effect of moieties linked to it [49]. The nearest residue was lysine located in the ligand binding core reported for glutamate in the NAMD-NR2 [62]. The amine of the lysine side chain and the tetracoordinated boron atom of S-Trujillon contain opposite charges which could be important in the increased affinity found theoretically and experimentally [49], see Fig. (**3**).

In the case of compounds targeting the β_2 –metabotropic-adrenoceptor, the importance of a boron atom is clear. Interactions of BCC compounds with conserved amino acids in the fifth transmembrane domain (serines and tyrosine residues implicated in receptor activation) of 7TM receptors [55] were key to affinity in theoretical simulations [55,56]. The atoms of the ligand and receptor that are involved in these interactions often had opposite partial electrostatic charges and/or formed hydrogen bonds [55,56]. We found a greater calculated affinity of BCC on other 7TM targets compared to similar structures without a boron atom (unpublished data). Thus, the addition of a boron atom in the compounds leds to its interaction with polar residues included in binding clusters of TMR, which appear to be advantageous for recognition, and in some cases for activating the receptors [55].

This advantage could be related to the similarities and differences between boron and carbon. Important similarities include the nearness of boron to carbon in the periodic table as well as the boron capability to form compounds of an ap-

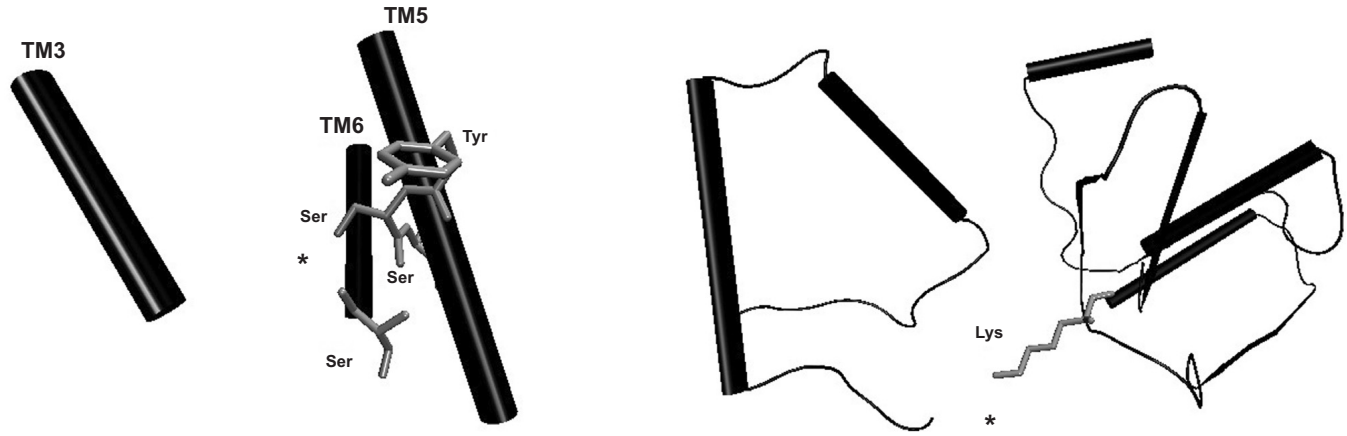

Fig. (3). Interactions of BCC on transmembrane receptors. Well-known segments involved in ligand recognition of the metabotropic human D_2 Dopamine receptor (left) and the NMDA-NR2A glutamate ionotropic receptor (right) in cartoon representation. Amino acids which have shown interaction with boron atoms (the observed position is marked with an asterisk) of some BCC are in bond label representation.

propriate size for targeting TMR-binding sites [4]. Additionally, there are similarities (and some differences) in the geometry of boron and carbon [63].

In contrast, the cluster organization of BCC could give them advantage over carbon-based compounds [63,64]. Whereas, carbon is often in four-coordinated form in biological compounds and drugs, boron is found as a trivalent/tetravalent metalloid. The three/four-coordinated form could be advantageous in relation to the basic or acid properties of the binding pocket surface in the targeted receptor. This different coordinated boron atom is relative easy to identify by ^{11}B Nuclear Magnetic Resonance, where the three-coordinated (acid) forms have a shift ranking from 14.2 to 22.5 ppm [65], and the four-coordinated (basic) forms have a shift ranking 0 to 5.94 ppm [48,55,56,66-68].

Also, while carbon forms a wide range of organic compounds, boron forms oxides and salts, and thus behaves similar to a metal. However, as with non-metals, it also forms acids (such as boric acid, H_3BO_3) [4]. Nevertheless, unlike a metal it has strong affinity for electrons owing to its vacant π-orbital. This makes boron, and BCC, electron-deficient, which leads to rather unusual structures. In some of these structures the boron atom in a four-coordinated form shows a negative (electron rich, see Fig. **4**) partial charge, which could be important in the non-covalent interactions with side chains of residues that are conserved in the binding pockets of TMR [69].

Also, we should keep in mind that these features of boron are not only advantageous when targeting TMRs, but also in relation to intracellular targets such as enzymes, on which BCC frequently act as inhibitors [3,4,6]. Based on crystal complexes that have been obtained, the role of boron appears to implicate both non-covalent and covalent interactions with (charged or uncharged) polar residues of proteins [70-72].

So, several functions are disrupted by BCC-enzyme interactions, among these those related to anabolism or catabolism [6]. We have described a possible mechanism of catabolism-evasion by BCC that target metabotropic receptors,

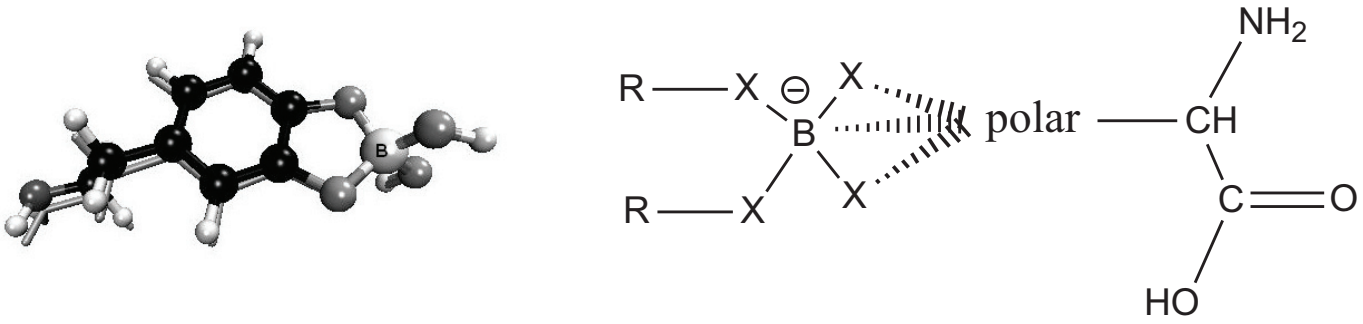

Fig. (4). The superimposition of two theoretically optimized (by the semi-empirical method AM1) putative dopamine and acid adducts shows the similar role and spatial disposition of boron and carbon in four-coordinated form (isosteric forms, on the left) and a common mechanism of interaction proposed between four-coordinated boron and amino acid of TMR is depicted on the right.

where there is an interaction with polar amino acids outside of the catalytic site of some enzymes [73]. However, the enzyme-mediated catabolism of boron-carbon bonds has not been clearly proved. These features could prove long-action in some BCC targeting TMR.

Finally, the unique hydrophobic behavior of some BCC should be emphasized (often greater than carbon containing compounds equivalents) [4]. This hydrophobic behavior has been employed in drug design in order to reach citosolic targets [74-77]. On the other hand, the hydrophilic behavior of some other BCC (including those BCC with the tetracoordinated boron and/or hydrophilic moieties exposed) have led to their proposed inclusion in liposomal formulations in order to increase their bioavailability or improve their capacity to reach intracellular targets [78].

In conclusion, in the present contribution we present data showing the utility of BCC as ligands for transmembrane receptors, which are in turn the most attractive targets for the pharmaceutical industry. Some features of BCC could give them potential advantages over the drugs currently available. The pharmacodynamic, pharmacokinetic and toxicological profiles of these compounds should be completed for consolidating an opportunity in drug development and to evaluate their usefulness.

CONFLICT OF INTEREST

None declared.

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ABBREVIATIONS

2-APB	=	2-Aminoethoxydiphenyl borate
3D	=	Three dimensional
7TM	=	Seven transmembrane domains receptor
BCC	=	Boron containing compounds
GPCR	=	G protein coupled receptor
IP3R	=	inositol 1,4,5-triphosphate receptor
NAMD-NR2	=	Nicotine Amide Methyl D-Aspartate glutamate receptor 2
TMR	=	Ionotropic and Metabotropic Transmembrane receptors

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