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REVIEW ARTICLE

Function and structure of bradykinin receptor 2 for drug discovery

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Type 2 bradykinin receptor (B2R) is an essential G protein-coupled receptor (GPCR) that regulates the cardiovascular system as a vasodepressor. Dysfunction of B2R is also closely related to cancers and hereditary angioedema (HAE). Although several B2R agonists and antagonists have been developed, icatibant is the only B2R antagonist clinically used for treating HAE. The recently determined structures of B2R have provided molecular insights into the functions and regulation of B2R, which shed light on structure-based drug design for the treatment of B2R-related diseases. In this review, we summarize the structure and function of B2R in relation to drug discovery and discuss future research directions to elucidate the remaining unknown functions of B2R dimerization.

Keywords: type 2 bradykinin receptor; G protein-coupled receptor; functions; structures; drug discovery

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INTRODUCTION

The kallikrein–kinin system (KKS) plays essential roles in maintaining the homeostasis of the cardiovascular system [1]. The KKS consists of kallikreins, kininogens, kinins, kininases, and kinin receptors [2]. There are 15 tissue kallikrein genes, *KLK1–KLK15*, and one plasma kallikrein gene in the human genome [3]. Kallikreins belong to the serine protease family and are widely distributed in the kidneys, pancreas, salivary glands, and plasma [4]. Two splice variants of kininogens are expressed in the liver: high-molecular-weight kininogen (HK, MW: 72 kDa) and low-molecular-weight kininogen (LK, MW: 48 kDa) [5]. Kinins are short-lived peptides originating from kininogens, and the nonapeptide bradykinin (RPPGFSPFR) is liberated from HK with the help of plasma kallikrein, while the decapeptide kallidin (KRPPGFSPFR) is liberated from LK by tissue kallikrein [6] (Fig. 1). The C-terminal arginines of bradykinin and kallidin can be cleaved by carboxypeptidases N and M into des-Arg^9-bradykinin and des-Arg^{10}-kallidin [6]. Kinins function as vasodilators by interacting with kinin receptors on the cell membrane [7]. Kinin receptors belong to the G protein-coupled receptor (GPCR) superfamily, with two subtypes, type 1 and type 2 bradykinin receptors (B1R and B2R) [8]. Although B1R and B2R show 32% sequence identity, they exhibit very different expression and ligand selectivity [9]. B2R is ubiquitously expressed in physiological and pathological conditions, while B1R is expressed rarely in normal tissues [10]. In particular, B1R is supposed to function as an essential mediator in oxidative stress and inflammation [11]. Bradykinin shows higher affinity for B2R than for B1R [12]. In contrast, kinins lacking C-terminal arginines, such as des-Arg^9-bradykinin and des-Arg^{10}-kallidin, preferentially bind B1R over B2R [12]. The mechanisms of kinin selectivity on receptors lie in the interactions between the C-termini of kinins and receptors [9]. After activating the receptors, kinins are degraded by kininases, such as angiotensin-converting enzymes (ACEs) and neutral endopeptidase (NEP), to terminate their functions [13].

In the human genome, the gene encoding B2R (*BDKRB2*) is located on chromosome 14q32, and the B2R protein contains 391 residues [14]. The N-terminus of B2R contains Met1 to Asn57, while the C-terminus ranges from Gln352 to Gln391 [15]. There are three putative glycosylation sites at Asn30, Asn39, and Asn207 and a palmitoylation site at Cys351 [16]. In addition, several serines and threonines are predicted to be phosphorylated by G protein-coupled receptor kinases (GRKs) [17].

B2R FUNCTIONS

B2R is activated upon the binding of endogenous agonists on the extracellular side, and in combination with the conformational transitions, G proteins can occupy the intracellular cleft surrounded by TMs2-7 [18, 19]. B2R mainly mediates the G_q signaling pathway, but in some cases, it also couples to G_i, G_s, and $G_{12/13}$ [20–22]. In endothelial cells, G_q proteins couple to B2R upon receptor activation and then disassociate into the $G\alpha_q$ subunit and $G\beta\gamma$ heterodimers [3] (Fig. 1). $G\alpha_q$ induces the activation of phospholipase C (PLC), the cleavage of phosphatidylinositol (4,5) bisphosphate (PIP_2), and the liberation of Ca^{2+} from endoplasmic reticulum (ER) [23, 24]. Increasing the level of intracellular Ca^{2+} promotes the activation of calcineurin and endothelial nitric oxide synthase (eNOS) and finally increases the concentration of nitric oxide (NO) in vessels [25]. Ca^{2+} also enhances the phosphorylation of phospholipase A2 (PLA_2) and triggers the release of prostaglandins [3] (Fig. 1).

Both NO and prostaglandins are vasodilators and are effective in lowering blood pressure [3, 26]. In addition to regulating the circulation, activated B2R also induces a transient increase in

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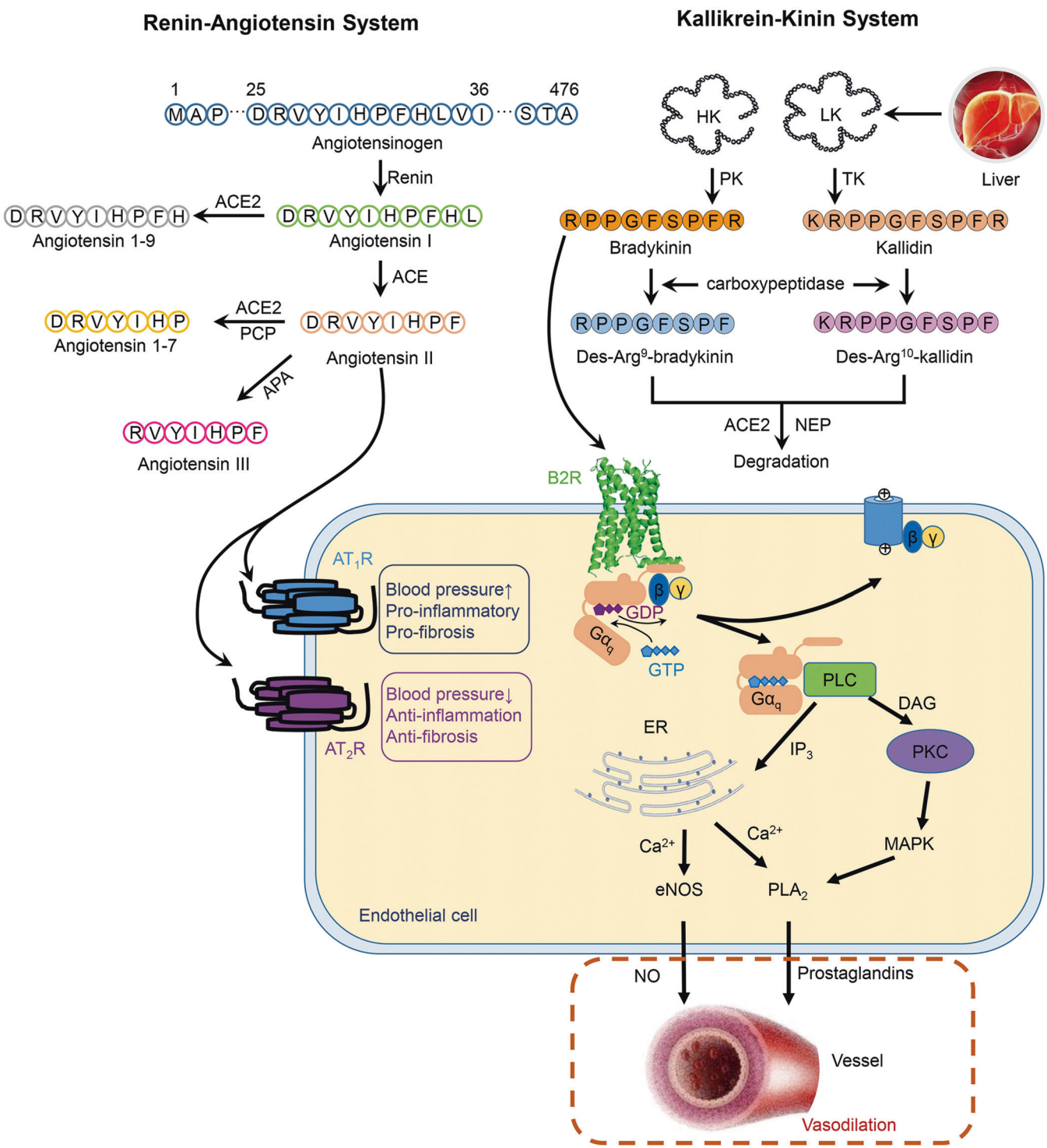

Fig. 1 The renin–angiotensin system and kallikrein–kinin system. In endothelial cells, upon bradykinin binding, GDP of the $G\alpha_q$ subunit is replaced by GTP, accompanying with the dissociation of $G\alpha_q$ and $G\beta\gamma$ subunits. $G\alpha_q$ activates PLC and calcium mobilization, and the increasing level of Ca^{2+} enhances the activities of eNOS and PLA_2, which promote the release of NO and prostaglandins, respectively, and lead to vasodilation. Angiotensin II could either bind to AT_1R to elevate the blood pressure, induce pro-inflammatory and pro-fibrosis activities, or bind AT_2R to reduce the blood pressure, perform anti-inflammation and anti-fibrosis activities (PCP prolyl carboxypeptidase, APA aminopeptidases A, PK plasma kallikrein, TK tissue kallikrein, DAG diacyl glycerol, PKC protein kinase C, MAPK mitogen-activated protein kinase).

endothelial permeability and the exudation of protein-rich fluid into the interstitium [27], which leads to inflammation. Additionally, B2R localizes to the sensory ganglia, dorsal horn, and peripheral nociceptors, and bradykinin activates B2R in the nervous system and evokes pain [28]. Dysfunction of B2R leads to cardiovascular diseases, such as hypertension, ventricular hypertrophy, and myocardosis [29, 30]. Studies on kallikrein-deficient mice show that the posterior wall and septum of the

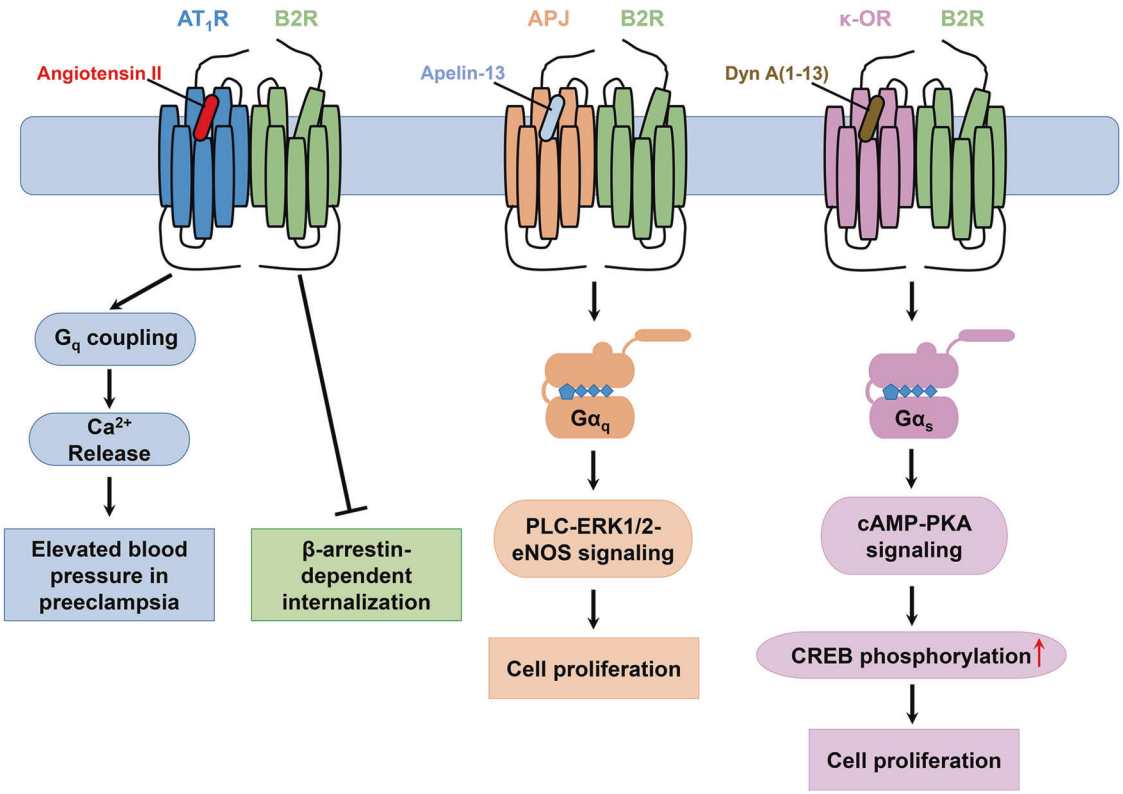

Fig. 2 Heterodimers of B2R with AT_1R, APJ, and κ-OR. AT_1R-B2R heterodimer enhances the Ca^{2+}-induced hypertension in preeclampsia and blocks the β-arrestin-mediated B2R internalization. APJ-B2R and κ-OR-B2R heterodimers promote cell proliferation through PLC-ERK1/2-eNOS and cAMP-PKA signaling pathways, respectively.

heart become thinner and that the heart tends to dilate, resulting in reduced left ventricular mass [31]. In addition, B2R is closely related to the development of renal diseases [32], respiratory diseases [33], neurological diseases [34], cancers [35, 36], and hereditary angioedema (HAE) [37]. In vitro and in vivo experiments have shown that peptide and nonpeptide B2R antagonists stimulate apoptosis in cancers through the activation of the MAP kinase pathway and the blockage of intracellular calcium mobilization activity, which activates the caspase pathway and leads to apoptosis [38]. HAE is an autosomal dominant inherited disease characterized by increased activation of the KKS [39]. HAE leads to edema in the limbs, fauces, gastrointestinal tract, and respiratory passage and results in asphyxiation [40].

The G_q signaling of B2R is terminated once GRKs bind to the receptor and phosphorylate specific serines and threonines at the C-terminus, such as Ser366, Thr369, Ser373, and Ser375 [17, 41]. Then, β-arrestins (β-arrestin1 and β-arrestin2) recognize the phosphorylation sites and promote the internalization of B2R with the help of clathrin and adaptor protein 2 (AP2) [42]. In endocytic vesicles, agonists and β-arrestin dissociate from B2R, and then receptors are recycled to the plasma membrane or degraded in lysosomes [43]. Based on the results of a B2R recycling experiment, B2R internalizes into endosomes with β-arrestin2 and is rapidly recycled to the plasma membrane [43]. Regarding the interactions between B2R and β-arrestins, the endocytosis level of B2R is mainly correlated with the β-arrestin2 level, while β-arrestin1 shows a compensatory behavior [44]. Previous results suggest that β-arrestin2 binds B2R with a deficient C-terminus and induces receptor internalization, although more weakly than for wild-type B2R, and the K342P mutation in helix 8 of B2R decreases signaling through β-arrestin2, indicating that helix 8 is important for B2R-β-arrestin2 coupling [44]. However, the results from β-arrestin1 show that it requires both helix 8 and the C-terminus [44]. Thus, β-arrestin1 mediates the phosphorylation-dependent internalization of B2R, while β-arrestin2 mediates both pathways [44].

The biological functions of B2R are determined not only by the actions of kinins but also by interactions with other GPCRs, such as angiotensin II type 1 receptor (AT_1R), apelin receptor (APJ), and κ-opioid receptor (κ-OR) (Fig. 2). Dimerization influences the agonist affinity, pharmacology, downstream signaling pathways, and trafficking of GPCRs [45]. AT_1R, functioning as a vasopressor, is a key component in the renin–angiotensin system, while B2R acts as a vasodepressor [46] (Fig. 1). AT_1R-B2R heterodimers reinforce Ca^{2+} signaling of AT_1R in preeclampsia and block the β-arrestin-dependent internalization of B2R [47]. APJ is widely expressed in the cardiovascular system. APJ-B2R heterodimers expressed in human umbilical vein endothelial cells (HUVECs) enhance the phosphorylation of eNOS and extracellular signal regulated kinases1/2 (ERK1/2), thus facilitating cell proliferation [48]. κ-OR is distributed in the nervous system, which is activated by dynorphin (Dyn) and participates in analgesia, fluid homeostasis, and anti-pruritic activity. κ-OR-B2R heterodimers enhance cell proliferation through G_s/cAMP/PKA pathways when Dyn A (1–13) binds κ-OR in the heterodimers [49].

B2R STRUCTURES

Several structures of B2R-G_q complexes have been recently solved using single-particle cryo-electron microscopy (cryo-EM) [18, 19] (Fig. 3a). B2R shares common structural features with other class A GPCRs, such as canonical seven-transmembrane helices (7TMs), a disulfide bond between TM3 and extracellular loop 2 (ECL2), and helix 8 lying parallel to the plasma membrane (Fig. 3b, c). ECL2 of B2R adopts a β-sheet conformation similar to that of AT_1R, angiotensin II type 2 receptor (AT_2R), and neurotensin receptor 1

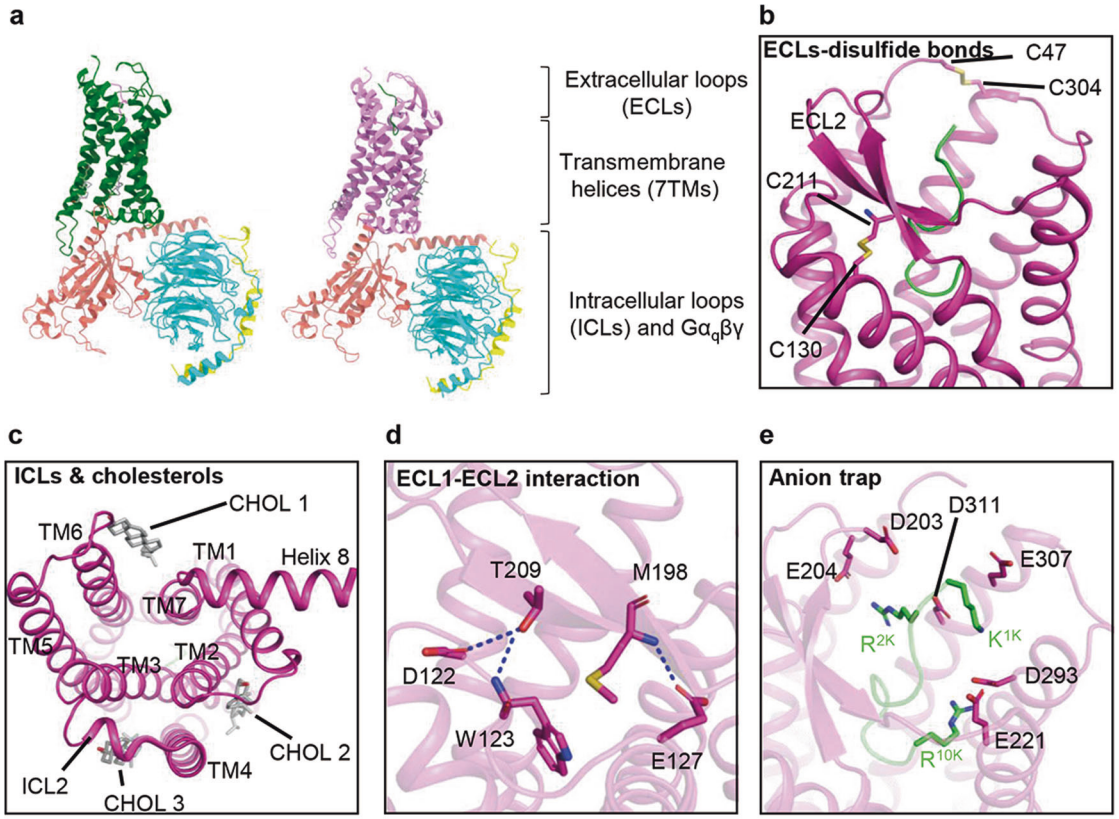

Fig. 3 B2R-G_q-bradykinin and B2R-G_q-kallidin structures. a Overall structures of bradykinin-bound B2R-G_q complex (PDB ID: 7F6H, B2R, green; bradykinin, violet; Gα, salmon; Gβ, cyan; Gγ, yellow) and kallidin-bound B2R-G_q complex (PDB ID: 7F6I, B2R, violet; kallidin, green). **b** ECL2 of B2R exhibits a β-sheet, with two disulfide bonds formed between C130-C211 and C47-C304. **c** At the intracellular side, ICL2 adopts a short α-helical conformation and three cholesterols (CHOL 1–3) lie in clefts between TM2–TM4, TM3–TM4, and TM6–TM7. **d** Hydrogen bonds (dash lines) formed between ECL1 and ECL2. **e** Anion trap formed by aspartic acids and glutamates locates at the entrance of ligand-binding pocket.

(NTSR1) [50–52] (Fig. 3b). The N/C-termini of B2R form short α-helices at P4-I7 and S366-S389, as predicted by Jpred [53], SOPMA [54], and Alphafold [55], which may facilitate the binding of peptide agonists and coupling of intracellular signaling proteins. In addition, the other disulfide bond between $C47^{N\text{-term}}$ and $C304^{7.25}$ (Ballesteros-Weinstein numbering [56]) locks the orthosteric binding pocket, and three structured cholesterols are observed at the intracellular clefts between TM2–TM4, TM3–TM4, and TM6–TM7 and probably regulate the B2R conformation in the allosteric mode [18] (Fig. 3b, c).

The funnel-like orthosteric binding pocket of B2R is covered by ECL2, which forms hydrogen bonds with D122, W123, and E127 in ECL1, functioning as a lid to decelerate the dissociation of peptide agonists [18] (Fig. 3d). Negatively charged residues, such as D203, E204, E221, D293, E307, and D311, are located at the entrance of the pocket, forming an anion trap to lock the positively charged peptide agonists in the pocket (Fig. 3e). Bradykinin and kallidin in the pocket adopt S-shaped conformations, with the N-termini extending to the extracellular side, while the C-termini insert into the bottom of the pocket. Pro3 of bradykinin (P^{3B}) or Pro4 of kallidin (P^{4K}) forms hydrogen bonds with I213 of B2R, while G^{4B} or G^{5K} forms an additional hydrogen bond with $R196^{4.64}$ (Fig. 4a). These two interactions anchor peptide agonists in the pocket, while intramolecular hydrogen bonds among G^{4B}/G^{5K}, S^{6B}/S^{7K}, and R^{9B}/R^{10K} stabilize the conformations of the peptide agonists [18]. Extensive polar, ionic, and hydrophobic interactions also contribute to agonist binding to B2R.

The structural superposition of des-Arg^{10}-kallidin-bound B1R and bradykinin-bound B2R shows similar conformations (Fig. 5a), while the interactions with Phe^9 of des-Arg^{10}-kallidin (F^{9DK}) and F^{8B}/ R^{9B} determine the peptide agonist selectivity between B1R and B2R. F^{9DK} is negatively charged and forms electrostatic interactions with $K118^{3.33}$ and $R202^{5.38}$ of B1R (Fig. 5b). However, F^{8B} is electroneutral and unable to contact the equivalent $S138^{3.33}$ and $T224^{5.38}$ of B2R (Fig. 5c). Furthermore, it is difficult to accommodate R^{9B} in the narrow gap surrounded by $R202^{5.38}$, $Y266^{6.51}$, and $E273^{6.58}$ of B1R, and steric hindrance occurs between R^{9B} and $R202^{5.38}$ of B1R [18, 19] (Fig. 5d). In contrast, no steric hindrance is observed between R^{9B} and $T224^{5.38}$, $F286^{6.51}$, or $D293^{6.58}$ of B2R (Fig. 5e). Thus, des-Arg^{10}-kallidin prefers B1R, and bradykinin prefers B2R.

At the B2R-G_q interface, the α5-helix of $Gα_q$ contributes the most contacts with B2R. $Y356^{G.H5.23}$ (CGN numbering [57]) of $Gα_q$ forms hydrogen bonds with $V151^{3.46}$, $D154^{3.49}$, and $R155^{3.50}$ of B2R (Fig. 4b). In addition, several pairs of polar interactions, namely, $R167^{ICL2}$-$R37^{G.hns1.02}$, $N254^{5.68}$-$Q350^{G.H5.17}$, and $R267^{6.32}$-$L358^{G.H5.25}$/ $V359^{G.H5.26}$, enhance the coupling between B2R and $Gα_q$. The intracellular loop 2 (ICL2) of B2R forms a short helix, positioning $M163^{ICL2}$ in the hydrophobic surface surrounded by F341, K345, I348, and S198 of $Gα_q$ (Fig. 4c).

In the inactive-state structures of muscarinic M_1 acetylcholine receptor (M_1R) [58] and histamine H_1 receptor (H_1R) [59], the conserved DRY motif at the intracellular tip of TM3 forms hydrogen bonds with the residues in TM6 and ICL2, stabilizing the closed conformation of the intracellular cavity. Additionally, $N^{7.49}$ of the conserved NPxxY motif forms hydrogen bonds with aspartic acids in TM2. In the active state B2R structure, F^{8B}/F^{9K} interacts with the conserved toggle switch $W283^{6.48}$, inducing the outward movement of $F279^{6.44}$ in the PIF motif and intracellular tip of TM6, which

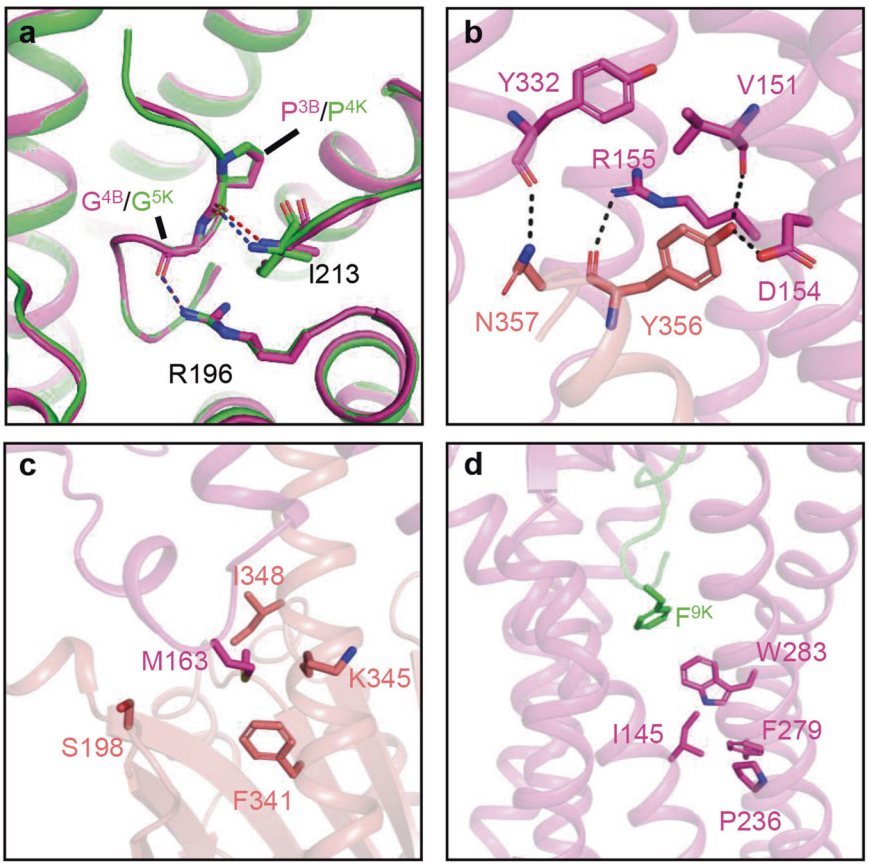

Fig. 4 Key residues participated in B2R ligand-binding and G_q coupling. a Binding poses of bradykinin (violet) and kallidin (green) in the pocket, key hydrogen bonds are formed between P^{3B}/P^{4K}-$I213^{ECL2}$ and G^{4B}/G^{5K}-$R196^{4.64}$. Hydrogen bonds are labeled as blue (bradykinin) and red (kallidin) dash lines (bradykinin-bound B2R, PDB ID: 7F6H, green; kallidin-bound B2R, PDB ID: 7F6I, violet). **b**, **c** Key hydrogen bonds (**b**) and hydrophobic interactions (**c**) involved in G_q coupling ($G\alpha_q$, salmon). **d** Conformations of toggle switch and PIF motif in kallidin-bound B2R structure.

breaks the hydrogen bonds between the DRY motif and TM6, as well as the NPxxY motif and TM2 (Fig. 4d). Then, the α5-helix of $G\alpha_q$ inserts into the intracellular cleft and couples to B2R by forming hydrogen bonds between DRY motif-$Y356^{G.H5.23}$ and NPxxY motif-$N357^{G.H5.24}$ for B2R activation (Fig. 4b).

B2R AGONISTS AND ANTAGONISTS

B2R is an essential drug target in maintaining the homeostasis of the cardiovascular system and relieving symptoms of edema and pain [60]. Due to the pro-inflammatory activity and widespread distribution of B2R in the central nervous system (CNS), the potent B2R-selective agonist labradimil (Arg-Pro-Hyp-Gly-Thi-Ser-Pro-Tyr(Me)-psi(CH_2NH)-Arg) is able to temporarily increase the permeability of the blood brain barrier (BBB) in the RG2 rat model of glioma, facilitating the entry of chemotherapeutics into the CNS to kill tumors in the brain [61]. The mechanisms underlying this phenomenon include the modification of vasculature characteristics around tumors, which prevents drug delivery to the tumor interstitium, as well as the release of nitric oxide and prostaglandin E2 to change the vascular physiology and morphology [62, 63].

The development of B2R antagonists began in the 1960s. The first generation of B2R antagonists are bradykinin analogs that replace several residues with *D*-Phe, Hyp, or Thi, such as NPC-567 (*D*-Arg-Arg-Pro-Hyp-Gly-Phe-Ser-*D*-Phe-Phe-Arg) and NPC-349 (*D*-Arg-Arg-Pro-Hyp-Gly-Thi-Ser-*D*-Phe-Thi-Arg) [64] (Table 1). However, these agents show low affinity for B2R and are sensitive to peptidases, which hinder their clinical use. According to the results of solid-state NMR spectroscopy and molecular modeling [65, 66], the C-terminus of bradykinin may form a β-turn when binding to B2R. Several unnatural amino acids mimic the β-turn to strengthen the binding of antagonists and improve the subtype selectivity between B1R and B2R. The second generation of antagonists includes icatibant and NPC17731. Icatibant (*D*-Arg-Arg-Pro-Hyp-Gly-Thi-Ser-*D*-Tic-Oic-Arg) is a highly potent B2R antagonist for treating HAE [67] that was approved in 2011. Patients show poor compliance, however, as icatibant is administered by hypodermic injection. Thus, the exploitation of the third generation of B2R antagonists is imperative. Selective nonpeptide antagonists of B2R appeared in the 1990s, including Win64338 [68], FR173657 [69], bradyzide [70], anatibant [71], fasitibant [72], and JSM10292 [73] (Fig. 6). In addition to the convenience provided by oral administration, these nonpeptide antagonists are more resistant to metabolism and show fewer off-target effects [74, 75]. B-9430 (*D*-Arg-Arg-Pro-Hyp-Gly-Igl-Ser-*D*-Igl-Oic-Arg) is a derivative of bradykinin and binds to both human B1R and B2R with pIC_{50} values of 7.9 and 9.6, respectively [76]. In contrast, anatibant preferentially binds B2R over B1R [71]. Antagonists of B2R are promising therapeutics for relieving symptoms of inflammation, pain, and diabetes. In addition to icatibant, many other antagonists have been in clinical trials. Deltibant is a peptide antagonist for the treatment of pain, stroke, and severe brain injury [77]. Anatibant is helpful in the treatment of traumatic brain injury, while fasitibant is used for osteoarthritis. More information about these antagonists is summarized below.

Icatibant

Icatibant, also known as HOE-140, is a bradykinin analog with high affinity (K_i value of 0.064 nM) for human B2R [78]. In a phase 2 clinical study, 27 patients took intravenous prednisolone plus clemastine as the standard therapy protocol or subcutaneous

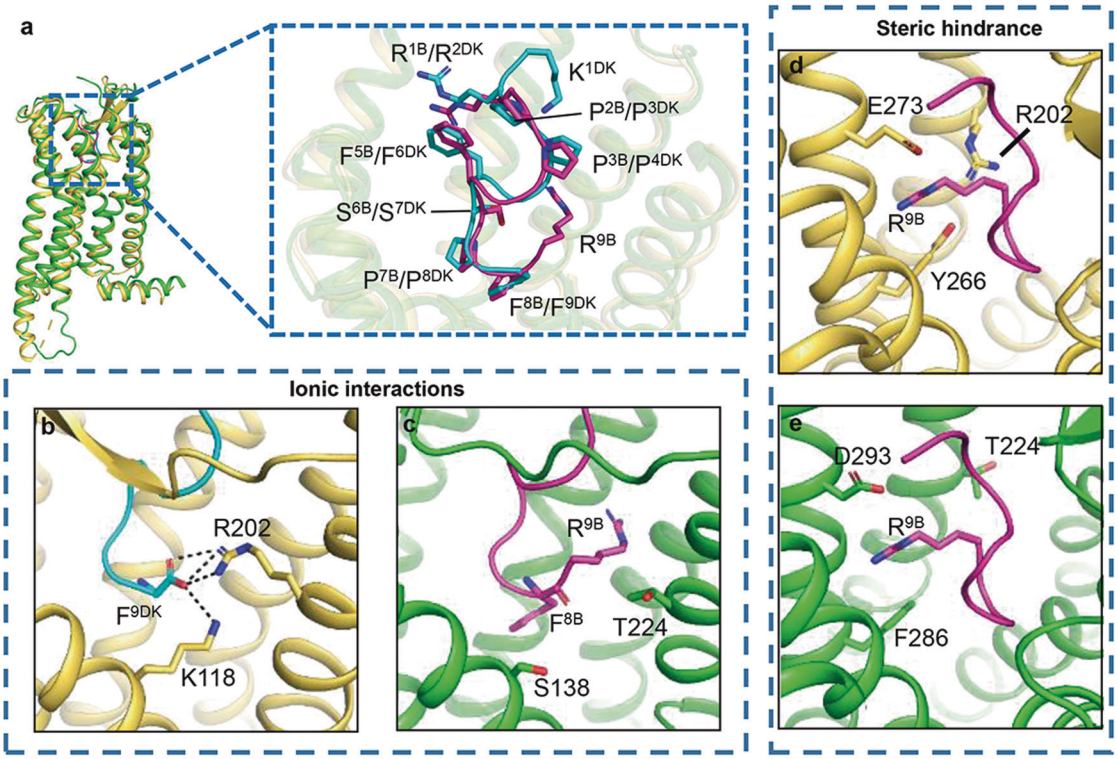

Fig. 5 Structural comparison between the binding pockets of B1R and B2R. a Structural comparison between B2R-bradykinin complex (PDB ID: 7F6H) and B1R-des-Arg^{10}-kallidin complex (PDB ID: 7EIB). **b–e** Ionic interactions between B1R and F^{9DK} as well as steric hindrance between ligand pocket of B1R and R^{9B} determine the preference of des-Arg^{10}-kallidin for B1R and bradykinin for B2R. (B1R, yellow-orange; B2R, green; des-Arg^{10}-kallidin, cyan; bradykinin, violet).

icatibant to analyze the efficiency of icatibant in ACE-inhibitor-induced angioedema by observing the median time of the resolution of edema [79]. The results indicate that the administration of icatibant shortened the median time by up to 19 h compared with the standard therapy. B2R is also an essential mediator of chronic bronchial asthma, and objective pulmonary function tests (PFTs) were carried out for patients with chronic asthma to evaluate the effects of icatibant [33]. After 4 weeks of treatment with icatibant, a 10% improvement in PFTs was achieved compared with the placebo group.

Deltibant

As a B2R antagonist, deltibant has been confirmed to alleviate the depressor response of bradykinin in rats and rabbits. In addition, a single dose of deltibant could reverse the profound hypotensive response and low survival rate caused by LPS in piglet models of endotoxic shock [80]. To investigate the clinical effects of deltibant on traumatic brain injury (TBI), 11 of 20 candidate patients took deltibant, while the others received placebo [81]. After treatment with deltibant, patients faced less risk of brain swelling and cerebral edema.

Anatibant

Anatibant, previously known as LF 16-0687, displays a sub-nanomolar affinity to human B2R with a K_i value of 0.67 nM [71]. Anatibant is a selective B2R antagonist that hinders downstream G_q signaling and the production of inositol 1,4,5-triphosphate (IP_3). In vivo experiments performed in anesthetized rats showed that anatibant antagonizes bradykinin-induced edema [71]. In clinical trials, 25 patients suffering severe traumatic brain injury were selected to investigate the pharmacokinetics, safety, tolerability, and pharmacological effects of the anatibant [82]. TBI patients and healthy volunteers both tolerated the subcutaneous injection of 22.5 mg of anatibant, which reached therapeutic concentrations in the plasma within 2 h. BK1-5 is a metabolite of bradykinin, whose levels in plasma and cerebrospinal fluid increase excessively after trauma, which suggests that the active state B2R may participate in the progression of TBI. However, the competitive binding of anatibant at B2R may impede the activation of B2R and function as a therapeutic choice for TBI patients.

Fasitibant

Fasitibant, a nonpeptide B2R antagonist, shares similar chemical scaffolds with anatibant. The affinity of fasitibant to human B2R was measured through [^{3}H]bradykinin competition assays with a pK_i value of 10.3 [83]. In addition, fasitibant is more effective than anatibant in blocking G protein signals. In vivo studies were performed in rats pretreated with monosodium iodoacetate (MIA) as a knee joint osteoarthritis model and used to evaluate the effects of icatibant and fasitibant in relieving the sense of pain [84]. MIA treatment induces the upregulation of bradykinin activities and the production of prostaglandin E2. Compared to the analgesic effect of icatibant, fasitibant is more potent and long-lasting in eliminating the physiological effects of activated B2R.

The search for new scaffolds targeting B2R could be accelerated by structure-based drug design and virtual screening. According to the successful cases of μ-OR [85] and M_1R [86], commercially available lead-like compounds could be docked into the B2R structure. Compounds with the potential to interact with the key residues of B2R could be further optimized with diverse chemical moieties. After evaluation of the affinity, subtype selectivity, and pharmacological effects of compounds, the in vivo performance of the promising candidates could be further analyzed in animal or clinical trials.

In addition, allosteric modulators are expected to increase on-target selectivity and decrease side effects caused by off-target effects at other receptors [87]. Based on the B2R structures, there

Table 1. Pharmacological and clinical information of B2R agonists and antagonists.

Name	Sequence	Pharmacological property	Activity	Clinical Stage	Related diseases
Labradimil [78]	Arg-Pro-Hyp-Gly-Thi-Ser-Pro-Tyr(Me)-psi(CH_2NH)-Arg	Agonist	19 nM (K_i)	Phase III	Brain tumor
Deltibant [76]	*D*Arg-Arg-Pro-Hyp-Gly-Thi-Cys-*D*Phe-Leu-Arg | BSH | *D*Arg-Arg-Pro-Hyp-Gly-Thi-Cys-*D*Phe-Leu-Arg	Antagonist	–	Phase II	Traumatic brain injury
NPC-567 [109]	*D*-Arg-Arg-Pro-Hyp-Gly-Phe-Ser-*D*-Phe-Phe-Arg	Antagonist	120 nM (IC_{50})	–	–
NPC-349 [110]	*D*-Arg-Arg-Pro-Hyp-Gly-Thi-Ser-*D*-Phe-Thi-Arg	Antagonist	20.5 nM (K_i)	–	–
NPC17731 [110]	*D*-Arg-Arg-Pro-Hyp-Gly-Phe-Ser-[*D*Hype(transpropyl)]-Oic-Arg	Antagonist	0.180 nM (K_d)	–	–
Icatibant [78]	*D*-Arg-Arg-Pro-Hyp-Gly-Thi-Ser-*D*-Tic-Oic-Arg	Antagonist	0.064 nM (K_i)	Approved for sale	HAE & asthma
Anatibant [71]	–	Antagonist	0.67 nM (K_i)	Phase II	Traumatic brain injury
Fasitibant [83]	–	Antagonist	0.05 nM (K_i)	Phase II	Osteoarthritis
Win64338 [68]	–	Antagonist	64 nM (K_i)	–	Broncho-constriction
FR173657 [111]	–	Antagonist	8.9 nM (IC_{50})	–	Allergic diseases
Bradyzide [70]	–	Antagonist	772 nM (K_i)	–	Hyperalgesia
JSM10292 [112]	–	Antagonist	1.1 nM (IC_{50})	–	–

BSH hexamethylenebissuccinimide, *Hyp trans*-4-hydroxyproline, *Hype trans*-4-propoxyproline, *Tic* tetrahydroisoquinoline-3-carboxylic acid, *Oic* octahydroindole-2-carboxylic acid, *Thi* β-(2-thienyl)-alanine.

are putative sites for allosteric modulator binding, although the B2R structures bound to the allosteric modulators are needed to elucidate the allosteric mechanism.

FUTURE DIRECTIONS

B2R is a key regulator in the KKS, and dysfunction of B2R leads to cardiovascular diseases, neurological diseases, hereditary angioedema and cancers [60]; thus, B2R has been an ideal target for drug discovery for several decades, but only icatibant is approved for clinical use [68, 71, 72, 78, 88]. It is quite challenging and time-consuming to explore new chemical entities without structural information on the target. The stereochemical requirements for B2R antagonist binding have been revealed by molecular dynamics simulations, molecular modeling, and solid-state NMR spectroscopy; however, the detailed receptor–ligand interactions remained elusive [89, 90]. With the recently determined B1R and B2R structures, the molecular mechanisms of ligand binding, subtype selectivity, receptor activation, and G protein coupling have been elucidated and are expected to accelerate the structure-based design of novel B2R agonists. Nevertheless, the active state B2R structures seem not to be ideal for antagonist design, especially for nonpeptide antagonists. Peptide antagonists may adopt different binding poses with different interactions to hinder the conformational changes necessary for B2R activation. The key pharmacophores for nonpeptide antagonists are still elusive based on the current agonist-bound structures and need to be identified from the B2R-antagonist structures.

The binding sites for allosteric modulators have been revealed in several GPCR structures. In the $P2Y_1R$ structure, the antagonist BPTU binds to the interface between $P2Y_1R$ and the lipid bilayer through hydrophobic interactions [91]. It blocks the movements of TM2 and TM3 required for $P2Y_1R$ activation. The CCR9-selective antagonist vercirnon occupies the intracellular cavity that binds G proteins, preventing CCR9 activation and G protein coupling [92]. The extracellular domain (ECD) is quite suitable as an allosteric regulatory site for class C GPCRs [87]. Structures have revealed that positive and negative allosteric modulators can be accommodated in the extracellular vestibule or the bottom of the orthosteric binding site [93–100]. However, no obvious vestibule or large ECD exists in the B2R structures, no lipidic ligand targeting B2R has been reported previously, and the orthosteric binding pocket of B2R is relatively shallow [18]. The intracellular cavity might be a potential binding site for negative allosteric modulators to block the downstream release of NO and prostaglandins.

GPCR heterodimers exhibit different physiological roles from monomers. B2R is associated with many GPCRs, such as AT_1R, κ-OR, APJ, and dopamine D2R, that regulate blood pressure, cell proliferation, and neutrophil adhesion to endothelial cells [46, 48, 49, 101, 102]. However, no structure of B2R heterodimers has been determined. Several heterodimeric structures of class C GPCRs have been revealed, such as metabotropic glutamate receptors (mGluRs) [103] and $GABA_B$ receptors [104]. In both structures, the transmembrane helices provide hydrophobic interactions for dimerization, while G proteins couple to only one monomer. In the $GABA_B$ structure, the positive allosteric modulator BHFF resides within the crevice between monomers.

Increasing evidence has indicated that B2R is related to the development of the COVID-19 pandemic and is a potential drug target for related disorders. SARS-CoV-2 uses angiotensin-converting enzyme 2 (ACE2) for its infection, which is widely distributed in lung alveolar cells and degrades des-Arg^9-bradykinin [105]. The loss of ACE2 during infection leads to the accumulation of

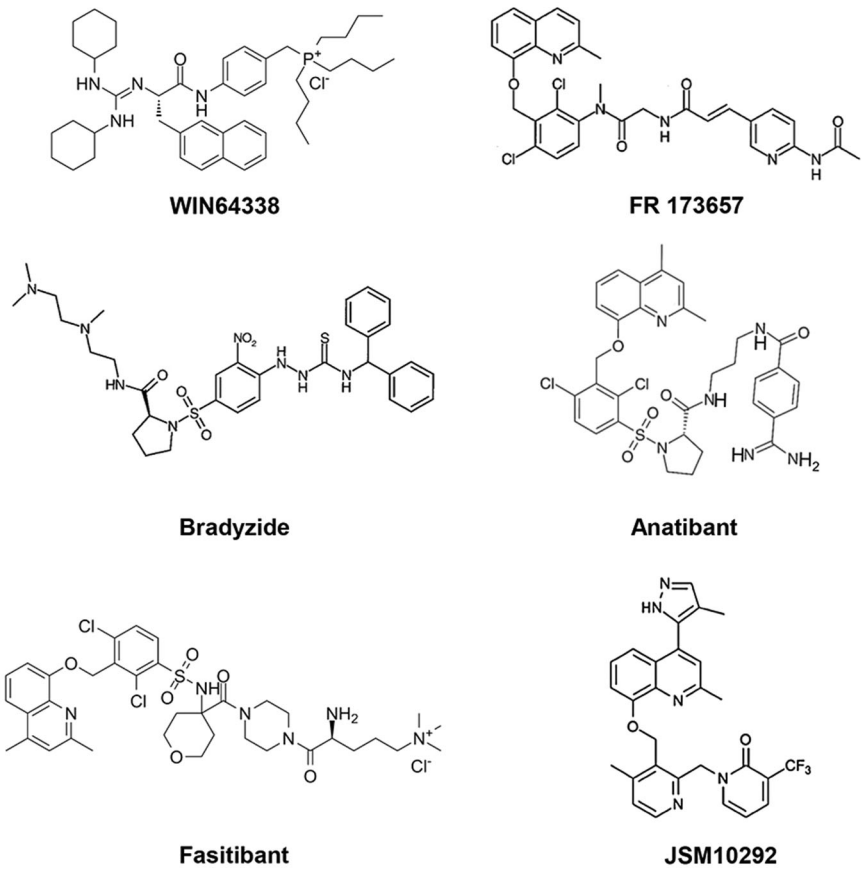

Fig. 6 The third generation of B2R antagonists. Chemical structures of WIN64338, FR 173657, Bradyzide, Anatibant, Fasitibant, and JSM10292.

des-Arg^9-bradykinin and bradykinin and the upregulation of B2R activities in the lungs as well as pulmonary angioedema [106]. Eighty-nine percent of COVID-19 patients administered icatibant showed a 3 L/min reduction in oxygen supplementation after 24 h, while the proportion was 17% in the control group [107]. The clinical application of icatibant and the development of new B2R antagonists are thus needed to treat pulmonary angioedema and suppress thromboinflammation [108].

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ADDITIONAL INFORMATION

Competing interests: The authors declare no competing interests.

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