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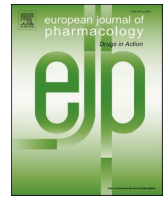
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Evolving views on the first two ligands of the angiotensin II type 2 receptor. From putative antagonists to potential agonists?

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ABSTRACT

The renin–angiotensin system is one of the most complex regulatory systems that controls multiple organ functions. One of its key components, angiotensin II (Ang II), stimulates two G-protein coupled class A receptors: the Ang II type 1 (AT1) receptor and the Ang II type 2 (AT2) receptor. While stimulation of the AT1 receptor causes G-protein-dependent signaling and arrestin recruitment, the AT2 receptor seems to have a constitutively active-like conformation and appears to act via G-protein-dependent and -independent pathways. Overstimulation of the AT1 receptor may lead to unwanted effects like inflammation and fibrosis. In contrast, stimulation of the AT2 receptor leads to opposite effects thus restoring the balance. However, the role of the AT2 receptor has become controversial due to beneficial effects of putative AT2 receptor antagonists. The two first synthetic AT2 receptor-selective ligands, peptide CGP42112 and small molecule PD123319, were initially both considered antagonists. CGP42112 was subsequently considered a partial agonist and it was recently demonstrated to be a full agonist. Based on the search-term PD123319 in Pubmed, 1652 studies have investigated putative AT2 receptor antagonist PD123319. Here, we put forward literature that shows beneficial effects of PD123319 alone, even at doses too low for antagonist efficacy. These beneficial effects appear compatible with agonist-like activity via the AT2 receptor. Taken together, a more consistent image of a therapeutic role of stimulated AT2 receptor emerges which may clarify current controversies.

1. The angiotensin II type 2 receptor

The renin-angiotensin system (RAS) has a major impact on a variety of serious diseases (Unger et al., 2015; Vargas et al., 2022). The protease renin is produced by the kidneys and cleaves the liver-derived protein angiotensinogen. This cleavage liberates the decapeptide angiotensin I (Angiotensin-(1–10)), which in turn can be cleaved by (combinations of) angiotensin converting enzyme (ACE), angiotensin converting enzyme-2 (ACE-2) and other peptidases yielding different angiotensin variants of which the octapeptide angiotensin II (Ang II) is the most important and most studied one. ACE converts Ang I into Ang II by removing the two carboxyterminal amino acids. Ang II stimulates both the angiotensin II type 1 (AT1) receptor and the angiotensin II type 2 (AT2) receptor. Overstimulation of the AT1 receptor may lead to vasoconstriction and adverse effects like inflammation and fibrosis (Fig. 1).

Stimulation of the AT1 receptor is thought to be counterbalanced by stimulation of receptors with protective effects (Li et al., 2017). Stimulation of the AT2 receptor by Ang II, Ang-(2–8), Ang-(1–9), Ang-(1–7)

(Bosnyak et al., 2011), or allosterically enhancing AT2 receptor activity by dipeptide Ang-(3–4) (Dias et al., 2017), generally leads to effects that are opposite to those resulting from AT1 receptor stimulation. In addition, stimulation of the Mas receptor (Santos et al., 2003) by Ang-(1–7) gives protective effects similar to those resulting from AT2 receptor stimulation. Furthermore, stimulation of Mas-related G-protein coupled receptor D by alamandine also leads to protective effects opposite to those from the AT1 receptor (Lautner et al., 2013).

Several G protein-dependent and -independent signaling pathways for the AT2 receptor appear to exist. AT2 receptor signaling can involve the Gi/o protein (Hansen et al., 2000; Hayashida et al., 1996; Kang et al., 1994), or a G-protein different from Gi/o (Buisson et al., 1995). Also, G-protein independent pathways have been reported (Brechler et al., 1994), as well as signaling via adaptor proteins that directly interact with the AT2 receptor (Horiuchi et al., 2012; Mogi et al., 2007; Nam-solleck et al., 2023a; Nouet et al., 2004; Reinemund, 2010). For a graphical schedule of AT2 receptor-linked pathways please see the excellent review of Bottari et al., (2015).

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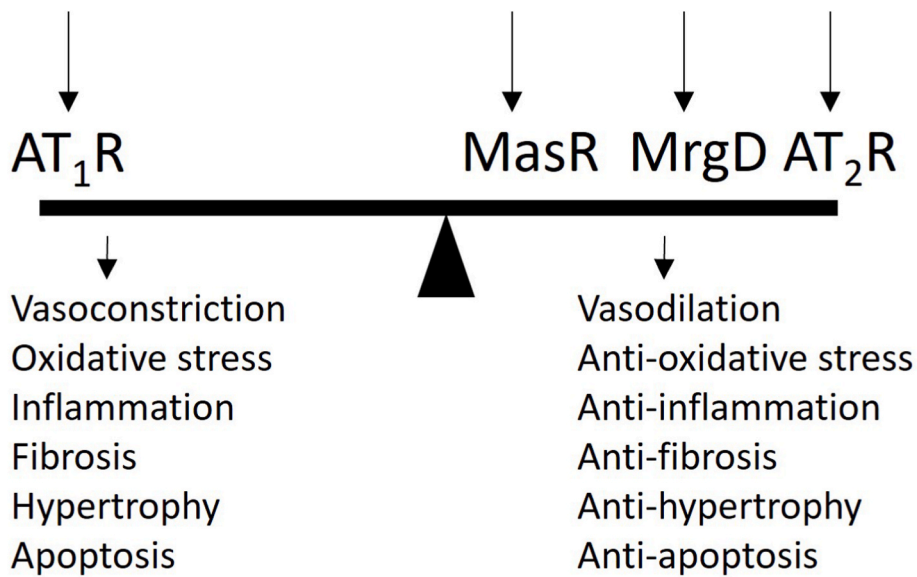


Fig. 1. Opposite biological effects of AT1 and AT2 receptor stimulation.

Ang II stimulates both the AT1 receptor and the AT2 receptor. Ang-(1–7) seems to stimulate Mas receptor and alamandine stimulates Mas-related G-protein coupled receptor D, indicated as MrgD. The adverse effects that result from (over)stimulation of AT1 receptor and the beneficial effects that result from stimulation of Mas receptor, Mas-related G-protein coupled receptor D and AT2 receptor are opposite and thus contribute to a balanced renin-angiotensin system.

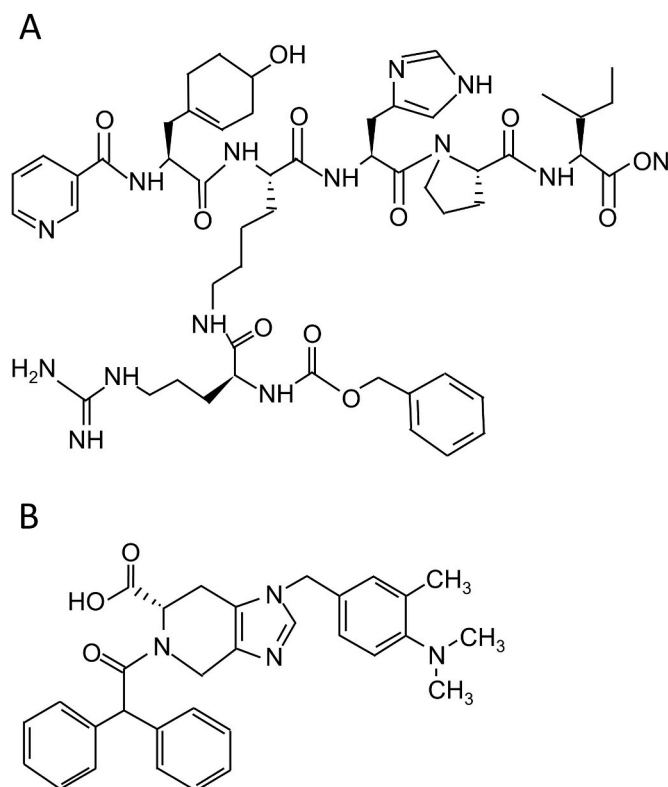


Fig. 2. Structures of CGP42112 (A) and PD123319 (B).

The two first AT2 receptor-selective ligands CGP42112 (Fig. 2A) and PD123319 (Fig. 2B) were both initially considered antagonists (reviewed in: Recarti and Bottari, 2015). The peptide CGP42112 was subsequently considered a partial agonist (Recarti and Bottari, 2015) and more recently firmly identified as a full agonist while comprehensively explaining the history of the molecule (Restrepo et al., 2022). The small molecules PD123319 and PD123177 were synthesized with the

intention of obtaining antagonists of the AT2 receptor and these compounds have been extensively studied (de Gasparo et al., 2015). Superposition of CGP42112 and PD123177 shows that the diphenyl system of PD123177 superimposes on the lipophilic pocket of isoleucine in CGP42112 (de Gasparo et al., 2015). Well-characterized AT2 receptor-specific agonists have been obtained such as small molecule C21 (Wan et al., 2004), lanthipeptide LP2 (Namsolleck et al., 2021a, 2023a, 2023b; Vilela et al., 2023; Wagenaar et al., 2013) and peptide NP-6A4 (Toedebusch et al., 2018).

Characterization of the functional role of the AT2 receptor is complicated by its (level of) expression on membranes of multiple organs, cell types and cytoplasmic located organelles, receptor activation, internalization and trafficking, the formation and dissociation of homo- and heterodimers on these membranes and the availability of ligands in cells and their compartments. The AT2 receptor is not only present on the plasma membrane but also on the mitochondrial membrane and on the nuclear envelope (Escobales et al., 2019). Opposing data were published on the internalization of the AT2 receptor from the plasma membrane in LLC-PK1 cells (Ferrão et al., 2017) and absence of the AT2 receptor internalization in human embryonal kidney 293 cells (Hein et al., 1997). The AT2 receptor can form constitutively active homodimers (Miura et al., 2005), but also heterodimers with the AT1 receptor (Anguiano-Robledo et al., 2007), bradykinin B2 receptor (Abadir et al., 2006), Mas receptor (Leonhardt et al., 2017; Patel and Hussain, 2018) and possibly the relaxin family peptide receptor 1 (Chow et al., 2014) and the insulin receptor (Nouet et al., 2004). Such heterodimerization may enhance or decrease the receptor response after its stimulation (Recarti and Bottari, 2015). Interestingly, dipeptide Ang-(3–4) induces the dissociation of AT1 - AT2 receptor dimers in renal proximal tubular cells of spontaneously hypertensive rats (Dias et al., 2014).

The AT2 receptor has also been suggested to be a direct inhibitor of the AT1 receptor (Abdalla et al., 2001). In general, the AT2 receptor is expressed at low levels except in fetal and neonatal tissues, but its expression is increased under pathological conditions. An exception is observed in human colorectal carcinoma cells in which AT2 receptor expression is decreased (Namsolleck et al., 2023a). The AT2 receptor-binding protein of 50 kDa (ATBP50) regulates the transport of the AT2 receptor to the cell membrane by binding to a specific motif within the cytoplasmic carboxy terminus of the AT2 receptor and

thereby enables the antiproliferative effects of the receptor (Mogi et al., 2007; Wruck et al., 2005). At least one AT₂ receptor agonist, NP-6A4, induces upregulation of its own expression (Toedebusch et al., 2018). AT₂ receptor expression levels are modulated by cross-talk via the AT₁ receptor (Savoia and Volpe, 2015). The AT₂ receptor may have constitutive activity and induces apoptosis even in the absence of Ang II stimulation (Miura and Karnik, 1999, 2000). Constitutive activity fits with the observed active-like conformation of the seven-transmembrane helical bundle (Zhang et al., 2017). The non-canonical positioning of the amphipathic helix VIII might block recruitment of intracellular G proteins and β -arrestins, and is consistent with the lack of AT₂ receptor signaling responses in standard cellular assays (Zhang et al., 2017). An open, active-like structure was also reported for the AT₂ receptor bound to the antagonist EMA401 with helix VIII blocking G-protein or β -arrestin recruitment (Perryman et al., 2022). In AT₂ receptor signal transduction upon Ang II and [Sar1, Ile8]-Ang II binding, G protein and β -arrestin signaling could not be verified by transforming growth factor shedding or β -arrestin recruitment assays (Asada et al., 2020). The AT₂ receptor appears to primarily signal via non-canonical and G protein- and β -arrestin-independent pathways (Guimond and Gallo-Payet, 2012; Porrello et al., 2009). Adaptor proteins of the AT₂ receptor, such as AT₂ receptor interacting protein which recruits the protein tyrosine phosphatase that contains an SH2 domain, and promyelocytic leukemia zinc finger protein may play a role in AT₂ receptor signaling (Horiuchi et al., 2012; Mogi et al., 2007; Namsolleck et al., 2023a; Nouet et al., 2004; Recarti and Bottari, 2015; Reinemund, 2010). Also other proteins may directly interact with the AT₂ receptor such as erb-b2 receptor tyrosine kinase 3 (ErbB3) (Knowle et al., 2000), connector enhancer of Ksr1 (CNK1) (Fritz and Radziwill, 2005), sodium-proton exchanger 6 (NHE6) (Pulakat et al., 2005) and tissue inhibitor of metalloproteinase 3 (TIMP3) (Kang et al., 2008). AT₂ receptor-mediated signaling has been comprehensively reviewed (Recarti and Bottari, 2015).

The crystal structures of the AT₂ receptor in complex with quinazolinone-biphenyltetrazole derivatives 1 and 2 have been reported (Zhang et al., 2017). However, it is not clear whether these ligands are agonists or antagonists. Also, the crystal structures of human AT₂ receptor in complex with the partial agonist [Sar1, Ile8]Ang II (Asada et al., 2018) and endogenous Ang II (Asada et al., 2020) have been elucidated. [Sar1, Ile8]Ang II interacts with both the core binding domain, which is a binding site of small-molecule ligands of AT₁ and AT₂ receptors (Zhang et al., 2017), and the extended binding domain, which is the allosteric modulator binding site of muscarinic acetylcholine receptor (Asada et al., 2018). An informative graphical comparison of the helical shifts in the AT₁ receptor from the inactive to the active state versus the AT₂ receptor with the noncanonical helix 8 position was recently published by Speck et al. (2022). The crystal structure of AT₂ receptor bound to putative antagonist EMA401 strongly confirms that the receptor is in an active-like conformation with helix 8 blocking G-protein or β -arrestin recruitment (Perryman et al., 2022).

Stimulation of the AT₂ receptor has therapeutic potential in many acute and chronic diseases and was studied extensively in multiple animal models (Unger et al., 2015). Also, multiple (putative) antagonists of the AT₂ receptor have been studied extensively, including PD123319, which, based on the search-term PD123319 in Pubmed, has been investigated in more than 1652 publications. PD123319 inhibited binding of Ang II to specific binding sites, but unequivocal functional blocking of the AT₂ receptor by PD123319 has not been demonstrated (Dudley et al., 1990; de Gasparo et al., 2015).

2. Functional effects of putative AT₂ receptor antagonists

Biological responses to receptor ligands in functional studies related to the RAS are complex and show in general a wide range of variation among experimental studies. This may be caused by the differential expression of receptors in multiple tissues and cells, receptor dimerization, ligand-specific AT₂ receptor pathways, other regulatory proteins of

Table 1

In vitro and *in vivo* systems with altered AT₂ receptor gene (*AT₂R*) expression indicate beneficial roles of AT₂R. Deletion/knockdown of *AT₂R* results in adverse effects. Overexpression of *AT₂R* results in beneficial effects.

Biological System	Modulated <i>AT₂R</i>	Biological effect	Reference
mice	<i>AT₂R</i>	Impaired drinking response to water deprivation; reduction in spontaneous movements; increased vasopressor response to Ang II	Hein et al. (1995)
mice	<i>AT₂R</i> ⁻	Increased blood pressure; increased sensitivity to pressor action by Ang II; increased AT ₁ R expression	Ichiki et al. (1995)
mice	<i>AT₂R</i> ⁻	Intracerebroventricular Ang II caused elevation of blood pressure	Li et al. (2003)
<i>in vitro</i> aorta rings	<i>AT₂R</i> ⁻	Enhanced vasoconstrictor response	Akishita et al. (1999)
rats	Knockdown <i>AT₂R</i>	Elevated blood pressure	Wang et al. (2004)
pregnant mice	<i>AT₂R</i> ⁻	Significantly increased mean arterial pressure during late gestation	Mirabito et al. (2014)
rats	Peripheral overexpression of <i>AT₂R</i>	Potential of the antihypertensive action of losartan	Li et al. (2007)
mice	<i>AT₂R</i> ⁻	Arterial thickening; perivascular fibrosis	Akishita et al. (2000)
mice	<i>AT₂R</i> ⁻	Adverse effects in cardiac remodeling	Wu et al. (2002); Oishi et al. (2003); Adachi et al. (2003); Ichihara et al. (2002)
rats	Overexpressed <i>AT₂R</i> in cardiac tissue	Prevention of cardiac hypertrophy in spontaneously hypertensive rats	Metcalfe et al. (2004)
mice	Overexpressed <i>AT₂R</i>	Benefits during post-MI remodeling via nitric oxide pathway; improved contractile function in adjacent noninfarcted myocardium	Bove et al. (2004); Bove et al., (2005)
<i>in vitro</i> myocytes; <i>in vivo</i> rats	Cardiac-selective overexpression of <i>AT₂R</i>	Protection of cardiac functions from ischemic injury	Qi et al. (2012)
mice	Overexpressed <i>AT₂R</i>	Reduced atherogenesis	Hu et al. (2008)
rats	<i>AT₂R</i> -over-expressing bone mesenchymal stem cells	Exosomes derived from <i>AT₂R</i> -over-expressing bone mesenchymal stem cells prevent restenosis	Zou et al. (2023)
mice	Smooth muscle cell specific overexpression of <i>AT₂R</i>	Stimulated bradykinin; enhanced nitric oxide/cGMP system	Tsutsumi et al. (1999)
mice	<i>AT₂R</i> ⁻	Exacerbated aortic disease compared with Marfan syndrome mice with <i>AT₂R</i>	Habashi et al. (2011)
mice	<i>AT₂R</i> ⁻	Reduced urinary sodium secretion	Siragy et al. (1999)
mice	<i>AT₂R</i> ⁻	Increased renal fibrosis; increased fibrocytes in bone marrow	Sakagawa et al., 2000
mice	<i>AT₂R</i> ⁻	Impaired nephrogenesis; impaired expression of Pax-2 and N-myc	Chen et al. (2008)
rats	Overexpressed <i>AT₂R</i>	Inhibition of cell proliferation in the outer adrenal cortex	Peters et al. (2012)

(continued on next page)

Table 1 (continued)

Biological System	Modulated AT ₂ R	Biological effect	Reference
mice	AT ₂ R ⁻	Accelerated nephropathy in type I diabetes	Chang et al. (2011)
mice	AT ₂ R ⁻	Podocyte dysfunction and loss; enhanced focal segmental glomerulosclerosis; glomerular fibrosis; albuminuria	Liao et al. (2021); Liao et al. (2022)
mesangial cells	Overexpressed AT ₂ R	Protective effects of AT ₂ R after treatment with macrophage conditioned medium	Pawluczyk and Harris (2012)
rats	Overexpressed AT ₂ R	Protection of renal tubule mitochondria in diabetes	Micakovic et al. (2018)
mesenchymal stem cells	AT ₂ R ⁻	Accelerated adipogenesis	Matsushita et al. (2016)
rats	Epigenetically repressed AT ₂ R	β cell dysfunction and glucose intolerance	Kou et al. (2020)
mice	AT ₂ R ⁻	Anxiety-like behavior; lower body temperature; lower pain threshold, decreased level of brain beta endorphins	Okuyama et al. (1999); Watanabe et al. (1999); Sakagawa et al. (2000)
prostate cancer cells; xenografts in mice	Overexpressed AT ₂ R	Apoptosis; inhibition of tumor growth	Li et al. (2009); Li et al. (2016)
rat insulinoma cells	Overexpressed AT ₂ R	Apoptosis; impaired insulin secretion	Liu et al. (2015)
human prostate cancer cells	Overexpressed AT ₂ R	Up-regulation of 6 apoptosis-related genes	Pei et al. (2014)
bladder cancer cells	Overexpression of AT ₂ R	Apoptosis and inhibition of angiogenesis	Pei et al. (2017)
mice	mesenchymal stem cells overexpressing AT ₂ R	Increased cell migration to injured lung	Xu et al. (2018)

RAS, multiple pathophysiological processes wherein RAS is involved, complex biological systems for functional testing and a possible lack of specificity in ligand-receptor interaction.

2.1. Activities of PD123319

AT₂ receptor activation opposes AT₁ receptor actions, but also exerts effects beyond inhibitory cross-talk with AT₁ receptor signaling. This blurred the original picture of a clear difference between AT₁ receptor

stimulation potentially leading to unwanted effects and AT₂ receptor stimulation balancing the stimulation of the AT₁ receptor. Multiple studies with altered AT₂ receptor expression consistently and firmly demonstrate beneficial roles of the AT₂ receptor in various disorders including cardiovascular disease, nephropathy and metabolic disease (Table 1). The use of putative AT₂ receptor antagonists seems to have important therapeutic effects in *in vitro* and *in vivo* experimental models of diseases including neuropathic pain, cystic fibrosis, neonatal lung injury, ischemic heart injury, nephropathy, colitis, glioblastoma multiforme (Table 2: Cohen et al., 2007; Darrah et al., 2019; Kilic et al., 2019; Moulder et al., 2004; Perryman et al., 2022; Smith et al., 2013a; Wagenaar et al., 2014; Zizzo et al., 2020). A set of structurally similar AT₂ receptor antagonists, including PD123319, was initially used as analgesics (Table 2: Smith et al., 2013). One of these, EMA401, entered clinical trials for treating neuropathic pain. EMA400 produced analgesia in the chronic constriction injury of the sciatic nerve in a rat model of neuropathic pain already at a dose as low as 3 μg/kg. Receptor blockage usually requires excess of the antagonist over the (endogenous) agonist. To get a significant effect with an agonist temporary occupancy of only 5–20% receptors is sufficient, whereas with an antagonist occupancy of more than 50% of the receptors is needed (Muttenthaler et al., 2021). Furthermore, bolus administration of an agonist may have prolonged effects, whereas efficacy of antagonists requires constant blocking either by continuous infusion or by a very long T_{1/2} of the antagonist. At higher doses, 1–10 mg/kg, ligands similar to EMA400, including EMA200 (PD123319) and EMA300, also acted as analgesics and therefore probably have the same mode of action as the 3 μg/kg dose of EMA400. Taken together, the bolus dose of 3 μg/kg appears to be much too low for an antagonist to be effective, but is compatible with an agonistic mode of action. This is supported by observations in AT₂ receptor knockout mice which had a lower pain threshold and decreased level of brain beta endorphins (Table 1: Okuyama et al., 1999; Sakagawa et al., 2000; Watanabe et al., 1999). These findings indicate that the AT₂ receptor plays a beneficial role in reducing pain perception. Hence, an AT₂ receptor agonist may work as an analgesic, while an AT₂ receptor antagonist would not. Taken together and in view of the biological activity at low doses, these data consistently indicate that PD123319 and analogs probably act as agonists of the AT₂ receptor when exerting analgesic effects.

In rat pups with hyperoxia-induced lung injury (Table 2: Wagenaar et al., 2014), a rat model for bronchopulmonary dysplasia, administration of PD123319 had therapeutic effects. At a low dose of 0.1 mg/kg/d, PD123319 attenuated cardiopulmonary injury by reducing pulmonary inflammation and fibrosis and prevented pulmonary arterial hypertension-induced right ventricle hypertrophy (Wagenaar et al., 2014). These effects were very similar to those obtained with AT₂

Table 2

Overview of therapeutic effects in selected experimental studies of Ang II type 2 receptor ligands.

Molecule	System	Dosing	Results	Reference
EMA400	Chronic constriction injury rat model of neuropathic pain	0.003–0.3 mg/kg, intraperitoneal injection bolus dose	Analgesia	Smith et al. (2013a)
EMA200 (PD123319)	Idem	1–10 mg/kg, intraperitoneal injection bolus dose	Analgesia	Smith et al. (2013a)
PD123319	Cystic fibrosis mice	2 mg/kg/d subcutaneous injection bolus dose	Restored lung function comparable to wild type	Darrah et al. (2019)
PD123319	Rat model of neonatal chronic lung and heart disease	0.1 mg/kg/d, subcutaneous injection bolus dose	Reduced pulmonary hypertension and right heart disease	Wagenaar et al. (2014)
PD123319	Isolated rat heart	20 mg/kg, intraperitoneal injection of bolus dose	Beneficial effects on ischemic injury and oxidative damage	Kilic et al. (2019)
PD123319	Radiation-nephropathy rat-model	Infusion of 15 mg/kg/d	Delayed nephropathy	Moulder et al. (2004)
PD123319	Rat model of colitis	0.3–10 mg/kg/d, intraperitoneal injection bolus dose	Reduced oxidative stress and reduced inflammation; inhibit NF-κB activation	Zizzo et al. (2020)
PD123319	AT ₂ receptor -expressing GBM cells	1–30 μM	Reduced proliferation	Perryman et al. (2022)
EMA401	AT ₂ receptor -expressing GBM cells	1–30 μM	Reduced proliferation	Perryman et al. (2022)

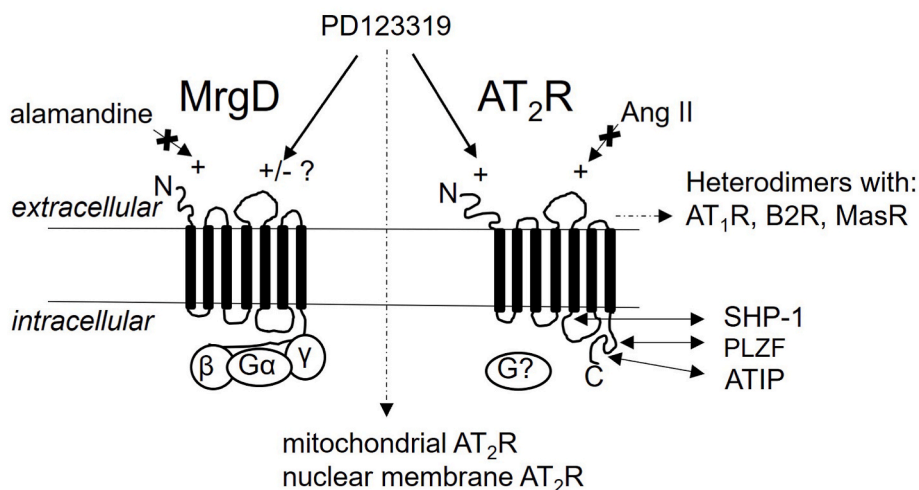


Fig. 3. PD123319 interacts with Mas-related receptor D and AT₂ receptor.

PD123319, and low dose of EMA400 (Table 2), appears to stimulate AT₂ receptor. PD123319 competes with alamandine for binding to Mas related G-protein coupled receptor D (MrgD) and with Ang II for binding to AT₂ receptor (Lautner et al., 2013). Mas-related G-protein coupled receptor D signals via classical coupled G-proteins/arrestin recruitment, whereas AT₂ receptor seems to signal via other effectors. AT₂ receptor-adaptor proteins, promyelocytic leukemia zinc finger protein (PLZF) and AT₂ receptor interacting protein (ATIP) which recruits the protein tyrosine phosphatase that contains an SH2 domain (SHP-1) are directly interacting with constitutively active AT₂ receptor and may play a role in signaling (Reinemund, 2010). Erb-b2 receptor tyrosine kinase 3 (ErbB3) (Knowle et al., 2000), Connector enhancer of Ksr1 (CNK1) (Fritz and Radziwill, 2005), sodium-proton exchanger 6 (NHE6) (Pulakat et al., 2005), and tissue inhibitor of metalloproteinase 3 (TIMP3) (Kang et al., 2008) also interact with AT₂ receptor. AT₂ receptor can form heterodimers with AT₁ receptor, bradykinin receptor (B2R) and Mas receptor (MasR).

receptor agonist LP2. Co-administration of AT₂ receptor agonist LP2 and 0.5 or 2 mg/kg/d PD123319 abolished all beneficial effects on right ventricular hypertrophy in experimental neonatal cardiopulmonary disease. The therapeutic activity of PD123319 alone, which is similar to that of AT₂ receptor agonist LP2, in combination with its relatively low dose, strongly suggests an agonistic mechanism.

Putative AT₂ receptor antagonist EMA401 and PD123319 appear effective against glioblastoma (Perryman et al., 2022). PD123319 inhibits the proliferation of AT₂ receptor-expressing cells in the absence of exogenous Ang II. Nevertheless, an AT₂ receptor [Y]6-Ang II agonist does not cause proliferation of U87 and GBM96 cell-lines (Perryman et al., 2022). In contrast to the beneficial effects of the putative antagonists of the AT₂ receptor in glioblastoma, LP2, an agonist of AT₂ receptor, inhibits growth of cell line-derived xenografts of glioblastoma in mice at a dose as low as 0.2 µg/kg/d (Namsolleck et al., 2021b). Importantly, multiple studies with modulated AT₂ receptor clearly demonstrate an anti-tumor role of AT₂ receptor (Table 1: Li et al. (2009); Li et al. (2016); Liu et al., 2015; Pei et al. (2014); Pei et al. (2017)). Hence, blocking AT₂ receptor is not expected to exert anti-tumor activity, whereas stimulating AT₂ receptor is. Therefore, AT₂ receptor agonist activity of PD123319 and analogs may explain their anti-tumor activity.

Many studies on cardiovascular systems with modulated AT₂ receptor expression provide a strong basis for beneficial roles of AT₂ receptor stimulation (Table 1: Adachi et al., 2003; Akishita et al., 1999, 2000; Bove et al., 2004, 2005; Habashi et al., 2011; Hein et al., Hu et al., 2008, 1995; Ichihara et al., 2002; Ichiki et al., 1995; Li et al., 2003; Li et al., 2007; Metcalfe et al., 2004; Mirabito et al., 2014; Oishi et al., 2003; Qi et al., 2012; Tsutsumi et al., 1999; Wang et al., 2004; Wu et al., 2002; Zou et al., 2023). Low dose of PD123319 alone reduced right ventricle hypertrophy in a pup model of acute lung injury (Wagenaar et al., 2014). Of note, in a study on ischemia-reperfusion injury and oxidative damage in isolated rat heart PD123319 had even more beneficial effects than the well-known highly effective AT₁ receptor antagonist losartan (Kilic et al., 2019), which could be well explained by agonistic effects via the AT₂ receptor of PD123319. Taken together, all these data strongly indicate that the beneficial effects of AT₂ receptor modulation by PD123319 may be caused by agonistic rather than

antagonistic effects.

2.2 Stimulatory effect of PD123319 on mitochondria

In contrast to peptide ligands, which only bind to their receptor on the cell surface, small molecule drugs may translocate across the plasma membrane to bind the AT₂ receptor on the membranes of organelles. This may explain the marked stimulation of mitochondrial respiration by PD123319 in Ang II-preconditioned rat hearts, resulting in activation of the respiratory chain and ATP production, thereby improving post-ischemic cardiac function (Escobales et al., 2019; Nuñez et al., 2018). Intracellular AT₁ receptor- and AT₂ receptor-linked mechanistic pathways of mitochondria-mediated cardioprotection by Ang II-preconditioning have been proposed (Escobales et al., 2019).

2.3 AT₂ receptor ligand PD123319 may interact with other receptors

Putative AT₂ receptor antagonist PD123319 has been reported to block some of the effects of Mas receptor agonist AVE-0991 and AT₂ receptor/Mas receptor agonist Ang-(1–7) (Raffai et al., 2011). Further research needs to clarify these results. More conclusive data have been reported on the interaction of PD123319 with Mas-related G-protein coupled receptor D. The heptapeptide alamandine is either formed by decarboxylation of the aspartate residue of Ang-(1–7) or by ACE-2 action on Angiotensin A, a D1A analogue of Ang II (Lautner et al., 2013; Liu et al., 2018; Qaradakhli et al., 2016). PD123319 inhibits the vasorelaxant action of alamandine in the aorta by displacing the binding of alamandine from its receptor (Li et al., 2017). Even in the absence of the AT₂ receptor in AT₂ receptor knockout mice, PD123319 inhibited the vasorelaxant action of alamandine and its binding to Mas-related G-protein coupled receptor D-transfected Chinese hamster ovary cells (Lautner et al., 2013).

3. Discussion

The AT₂ receptor is a highly important and special receptor which, at least in part, does not function via classical G-proteins or arrestin recruitment. Due to the lack of standard *in vitro* activity assays for this

receptor, the characterization of inhibitors/blockers and modulators is hampered. Allosteric sites have been reported for a wide range of G-protein coupled receptors (Congreve et al., 2017; Conn et al., 2014). As the AT₂ receptor appears to adapt an active-like conformation with constitutive activity, one would expect the induction of a clearly non-active conformation as a result of the interaction with a true receptor blocker, that not only antagonizes receptor binding but also inhibits receptor-mediated signaling. Taken together, data on examples wherein putative AT₂ receptor antagonists exert beneficial activities, even at 3 µg/kg for EMA400 (Table 2), indicate agonist activity. The data strongly suggest that a putative AT₂ receptor antagonist, like PD123319, may act at low doses as agonist, consistent with the suggested agonist activity of PD123319 (Zhou et al., 1993). This is supported by the observations in many *in vitro* and *in vivo* experimental disease models, summarized in Table 1, that increased AT₂ receptor expression results in beneficial pathophysiological effects and in which deletion/knockdown of the AT₂ receptor results in adverse effects. At higher doses agonism or antagonism may depend on aspects such as signaling pathways, desensitization, cell type-dependent receptor internalization (Ferrão et al., 2017; Hein et al., 1997), receptor heterodimerization and the used system. In addition, PD123319 appears to interfere with Mas-related G-protein coupled receptor D accessibility and Mas-related G-protein coupled receptor D-mediated activity (Fig. 3).

4. Conclusion

While beneficial effects of molecules that are putative AT₂ receptor antagonists may shed doubt on the therapeutic validity of stimulating AT₂ receptor, elucidation of agonistic action of these putative AT₂ receptor antagonists may restore therapeutic potential of specifically stimulating the AT₂ receptor in several serious diseases. In conclusion, in the absence of unequivocal molecular mechanistic data, existing literature data urge for the utmost prudence in the interpretation of results obtained with PD123319 and similar AT₂ receptor ligands.

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Declaration of competing interest

The authors declare that they have no conflict of interest.

Data availability

No data was used for the research described in the article.

References

- Abadir, P.M., Periasamy, A., Carey, R.M., Siragy, H.M., 2006. Ang II type 2 receptor-bradykinin B2 receptor functional heterodimerization. *Hypertension* 48, 316–322. <https://doi.org/10.1161/01.HYP.0000228997.88162.a8>.
- AbdAlla, S., Lother, H., Abdel-tawab, A.M., Quitterer, U., 2001. The Ang II AT₂ receptor is an AT₁ receptor antagonist. *J. Biol. Chem.* 276, 39721–39726. <https://doi.org/10.1074/jbc.M105253200>.
- Adachi, Y., Saito, Y., Kishimoto, I., Harada, M., Kuwahara, K., Takahashi, N., et al., 2003. Ang II type 2 receptor deficiency exacerbates heart failure and reduces survival after acute myocardial infarction in mice. *Circulation* 107, 2406–2408. <https://doi.org/10.1161/01.CIR.0000072763.98069.B4>.
- Akishita, M., Yamada, H., Dzau, V.J., Horiuchi, M., 1999. Increased vasoconstrictor response of the mouse lacking Ang II type 2 receptor. *Biochem. Biophys. Res. Commun.* 261, 345–349. <https://doi.org/10.1006/bbrc.1999.1027>.
- Akishita, M., Iwai, M., Wu, L., Zhang, L., Ouchi, Y., Dzau, V.J., Horiuchi, M., 2000. Inhibitory effect of Ang II type 2 receptor on coronary arterial remodeling after aortic banding in mice. *Circulation* 102, 1684–1689. <https://doi.org/10.1161/01.cir.102.14.1684>.
- Anguiano-Robledo, L., Reyes-Melchor, P.A., Bobadilla-Lugo, R.A., Pérez-Alvarez, V.M., López-Sánchez, P., 2007. Renal angiotensin-II receptors expression changes in a model of preeclampsia. *Hypertens. Pregnancy* 26, 151–161. <https://doi.org/10.1080/10641950701252827>.
- Asada, H., Horita, S., Hirata, K., Shiroishi, M., Shiimura, Y., Iwanari, H., Hamakubo, T., Shimamura, T., Nomura, N., Kusano-Arai, O., Uemura, T., Suno, C., Kobayashi, T., So Iwata, S., 2018. Crystal structure of the human Ang II type 2 receptor bound to an Ang II analog. *Nat. Struct. Mol. Biol.* 25, 570–576. <https://doi.org/10.1038/s41594-018-0079-8>.
- Asada, H., Inoue, A., Kadji, F.M.N., Hirata, K., Shiimura, Y., Im, D., Shimamura, T., Nomura, N., Iwanari, H., Hamakubo, T., Kusano-Arai, O., Hisano, H., Uemura, T., Suno, C., Aoki, J., Iwata, S., 2020. The crystal structure of Ang II type 2 receptor with endogenous peptide hormone. *Structure* 28, 418–425. <https://doi.org/10.1016/j.str.2019.12.003>.
- Bosnyak, S., Jones, E.S., Christopoulos, A., Aguilar, M.-I., Thomas, W.G., Widdop, R.E., 2011. Relative affinity of angiotensin peptides and novel ligands at AT₁ and AT₂ receptors. *Clin. Sci.* 121, 297–303. <https://doi.org/10.1042/CS20110036>.
- Bove, C.M., Yang, Z., Gilson, W.D., Epstein, F.H., French, B.A., Berr, S.S., Bishop, S.P., Matsubara, H., Carey, R.M., Kramer, C.M., 2004. Nitric oxide mediates benefits of Ang II type 2 receptor overexpression during post-infarct remodeling. *Hypertension* 43 (3), 680–685. <https://doi.org/10.1161/01.HYP.0000115924.94236.91>.
- Bove, C.M., Gilson, W.D., Scott, C.D., Epstein, F.H., Yang, Z., Dimaria, J.M., Berr, S.S., French, B.A., Bishop, S.P., Kramer, C.M., 2005. The Ang II type 2 receptor and improved adjacent region function post-MI. *J. Cardiovasc. Magn. Reson.* 7 (2), 459–464. <https://doi.org/10.1081/jcmr-200053461>.
- Brechler, V., Reichlin, S., De Gasparo, M., Bottari, S.P., 1994. Ang II stimulates protein tyrosine phosphatase activity through a G-protein independent mechanism. *Recept. Channel* 2, 89–98.
- Buisson, B., Laflamme, L., Bottari, S.P., de Gasparo, M., Gallo-Payet, N., Payet, M.D., 1995. A G protein is involved in the angiotensin AT₂ receptor inhibition of the T-type calcium current in non-differentiated NG108-15 cells. *J. Biol. Chem.* 270, 1670–1674. <https://doi.org/10.1074/jbc.270.4.1670>.
- Chang, S.Y., Chen, Y.W., Chenier, I., Tran, S.M., Zhang, S.L., 2011. Ang II type II receptor deficiency accelerates the development of nephropathy in type I diabetes via oxidative stress and ACE2. *Exp. Diabetes Res.* 1–12, 2011, 521076. <https://doi.org/10.1155/2011/521076>.
- Chen, Y.-W., Tran, S., Chenier, I., Chan, J.S.D., Ingelfinger, J.R., Inagami, T., Zhang, S.-H., 2008. Deficiency of intrarenal Ang II type 2 receptor impairs paired homeo box-2 and N-myc expression during nephrogenesis. *Pediatr. Nephrol.* 23, 1769–1777. <https://doi.org/10.1007/s00467-008-0854-6>.
- Chow, B.S.M., Kocan, M., Bosnyak, S., Sarwar, M.B., Jones, E.S., Widdop, R.E., Summers, R.J., Bathgate, R.A.D., Hewitson, T.D., Samuel, C.S., 2014. Relaxin requires the Ang II type 2 receptor to abrogate renal interstitial fibrosis. *Kidney Int.* 86, 75–85. <https://doi.org/10.1038/ki.2013.518>.
- Cohen, E.P., Fish, B.L., Sharma, M., Li, X.A., Moulder, J.E., 2007. Role of the Ang II type-2 receptor in radiation nephropathy. *Transl. Res.* 150, 106–115. <https://doi.org/10.1016/j.trsl.2007.03.004>.
- Congreve, M., Oswald, C., Marshall, F.H., 2017. Applying structure-based drug design approaches to allosteric modulators of GPCRs. *Trends Pharmacol. Sci.* 38, 837–847. <https://doi.org/10.1016/j.tips.2017.05.010>.
- Conn, P.J., Lindsley, C.W., Meiler, J., Niswender, C.M., 2014. Opportunities and challenges in the discovery of allosteric modulators of GPCRs for treating CNS disorders. *Nat. Rev. Drug Discov.* 13, 692–698. <https://doi.org/10.1038/nrd4308>.
- Darrah, R.J., Jacono, F.J., Joshi, N., Mitchell, A.L., Sattar, A., Campanaro, C.K., Litman, P., Frey, J., Nethery, D.E., Barbato, E.S., Hodges, C.A., Corvol, H., Cutting, G. R., Knowles, M.R., Strug, L.J., Drumm, M.L., 2019. AGTR2 absence or antagonism prevents cystic fibrosis pulmonary manifestations. *J. Cyst. Fibros.* 18, 127–134. <https://doi.org/10.1016/j.jcf.2018.05.013>.
- de Gasparo, M., Whitebread, S., Criscione, L., Buehlmeier, P., Furet, P., 2015. The AT₂ receptor: historical perspective. P11–16. In: Unger, T., Steckelings, U., Dzau, V.J. (Eds.), *The Protective Arm of the Renin Angiotensin System: Functional Aspects and Therapeutic Implications*. Elsevier Inc. <https://doi.org/10.1016/B978-0-12-801364-9.00002-X>.
- Dias, J., Ferrão, F.M., Axelband, F., Carmona, A.K., Lara, L.S., Vieyra, A., 2014. ANG-(3–4) inhibits renal Na⁺–ATPase in hypertensive rats through a mechanism that involves dissociation of ANG II receptors, heterodimers, and PKA. *Am. J. Physiol. Ren. Physiol.* 306, F855–F856. <https://doi.org/10.1152/ajprenal.00488.2013>.
- Dias, J., Axelband, F., Lara, L.S., Muzi-Filho, H., Vieyra, A., 2017. Is angiotensin-(3–4) (Val-Tyr) the shortest Ang II-derived peptide, opening new vistas on the renin-angiotensin system? *J. Renin-Angiotensin-Aldosterone Syst. JRAAS* 18, 1470320316689338. <https://doi.org/10.1177/1470320316689338>.
- Dudley, D.T., Panek, R.L., Major, T.C., Lu, G.H., Bruns, R.F., Klinkefus, B.A., Hodges, J.C., Weishaar, R.E., 1990. Subclasses of Ang II binding sites and their functional significance. *Mol. Pharmacol.* 38, 370–377.
- Escobales, N., Nuñez, R.E., Javadov, S., 2019. Mitochondrial angiotensin receptors and cardioprotective pathways. *Am. J. Physiol. Heart Circ. Physiol.* 316, H1426–H1438. <https://doi.org/10.1152/ajpheart.00772.2018>.
- Ferrão, F.M., Cardoso, L.H.D., Drummond, H.A., Li, X.C., Zhuo, J.L., Gomes, D.S., Lara, L. S., Vieyra, A., Lowe, J., 2017. Luminal Ang II is internalized as a complex with Ang II type 1 receptor/Ang II type 2 receptor heterodimers to target endoplasmic reticulum in LLC-PK1 cells. *Am. J. Physiol. Ren. Physiol.* 313, F440–F449. <https://doi.org/10.1152/ajprenal.00261.2016>.

- Fritz, R.D., Radziwill, G., 2005. The scaffold protein CNK1 interacts with the AngII type 2 receptor. *Biochem. Biophys. Res. Commun.* 338, 1906–1912. <https://doi.org/10.1016/j.bbrc.2005.10.168>.
- Guimond, M.O., Gallo-Payet, N., 2012. How does angiotensin AT2 receptor activation help neuronal differentiation and improve neuronal pathological situations? *Front. Endocrinol.* 3 (164), 1–22. <https://doi.org/10.3389/fendo.2012.00164>.
- Habashi, J.P., Doyle, J.J., Holm, T.M., Aziz, H., Schoenhoff, F., Bedja, D., Chen, Y., Modiri, A.N., Judge, D.P., Dietz, H.C., 2011. Ang II type 2 receptor signaling attenuates aortic aneurysm in mice through ERK antagonism. *Science* 332, 361–365. <https://doi.org/10.1126/science.1192152>.
- Hansen, J.L., Servant, G., Baranski, T.J., Fujita, T., Iiri, T., Sheikh, S.P., 2000. Functional reconstitution of the Ang II type 2 receptor and Gi activation. *Circ. Res.* 87, 753–759. <https://doi.org/10.1161/01.RES.87.9.753>.
- Hayashida, W., Horiuchi, M., Dzau, V.J., 1996. Intracellular third loop domain of Ang II type-2 receptor - role in mediating signal transduction and cellular function. *J. Biol. Chem.* 271, 21985–92. <https://doi.org/10.1074/jbc.271.36.21985>.
- Hein, L., Barsh, G.S., Pratt, R.E., Dzau, V.J., Kobilka, B.K., 1995. Behavioural and cardiovascular effects of disrupting the Ang II type-2 receptor in mice. *Nature* 377, 744–747. <https://doi.org/10.1038/377744a0>.
- Hein, L., Meinel, L., Pratt, R.E., Dzau, V.J., Kobilka, B.K., 1997. Intracellular trafficking of Ang II and its AT1 and AT2 receptors: evidence for selective sorting of receptor and ligand. *Mol. Endocrinol.* 11, 1266–1277. <https://doi.org/10.1210/mend.11.9.9975>.
- Horiuchi, M., Iwanami, J., Mogi, M., 2012. Regulation of Ang II receptors beyond the classical pathway. *Clin. Sci.* 123, 193–203. <https://doi.org/10.1042/CS20110677>.
- Hu, C., Dandapat, A., Chen, J., Liu, Y., Hermonat, P.L., Carey, R.M., Mehta, J.L., 2008. Over-expression of Ang II type 2 receptor (agr2r) reduces atherosclerosis and modulates LOX-1, endothelial nitric oxide synthase and heme-oxygenase-1 expression. *Atherosclerosis* 199, 288–294. <https://doi.org/10.1016/j.atherosclerosis.2007.11.006>.
- Ichihara, S., Senbonmatsu, T., Price Jr., E., Ichiki, T., Gaffney, F.A., Inagami, T., 2002. Targeted deletion of Ang II type 2 receptor caused cardiac rupture after acute myocardial infarction. *Circulation* 106, 2244–2249. <https://doi.org/10.1161/01.cir.0000033826.52681.37>.
- Ichiki, T., Labosky, P.A., Shiota, C., Okuyama, S., Imagawa, Y., Fogo, A., Niimura, F., Ichikawa, I., Hogan, B.L., Inagami, T., 1995. Effects on blood pressure and exploratory behaviour of mice lacking Ang II type-2 receptor. *Nature* 377, 748–750. <https://doi.org/10.1038/377748a0>.
- Kang, J., Posner, P., Summers, C., 1994. Ang II type 2 receptor stimulation of neuronal K⁺ currents involves an inhibitory GTP binding protein. *Am. J. Physiol.* 267, C1389–C1397. <https://doi.org/10.1093/cvr/cv072>.
- Kang, K.-H., Park, S.-Y., Rho, S.B., Lee, J.-H., 2008. Tissue inhibitor of metalloproteinases-3 interacts with Ang II type 2 receptor and additionally inhibits angiogenesis. *Cardiovasc. Res.* 79, 150–160. <https://doi.org/10.1093/cvr/cv072>.
- Kilic, A., Ustunova, S., Usta, C., Bulut, H., Meral, I., Tansel, C.D., Gurevin, E.G., 2019. Ang II type 2 receptor blocker PD123319 has more beneficial effects than losartan on ischemia-reperfusion injury and oxidative damage in isolated rat heart. *Can. J. Physiol. Pharmacol.* 97, 1124–1131. <https://doi.org/10.1139/cjpp-2019-0076>.
- Knowle, D., Ahmed, S., Pulakat, L., 2000. Identification of an interaction between the Ang II receptor sub-type AT2 and the ErbB3 receptor, a member of the epidermal growth factor receptor family. *Regul. Pept.* 87, 73–82. [https://doi.org/10.1016/s0167-0115\(99\)00111-1](https://doi.org/10.1016/s0167-0115(99)00111-1).
- Kou, H., Gui, S., Dai, Y., Guo, Y., Wang, H., 2020. Epigenetic repression of AT2 receptor is involved in β cell dysfunction and glucose intolerance of adult female offspring rats exposed to dexamethasone prenatally. *Toxicol. Appl. Pharmacol.* 404, 115187. <https://doi.org/10.1016/j.taap.2020.115187>.
- Lautner, R.Q., Villela, D.C., Fraga-Silva, R.A., Silva, N., Verano-Braga, T., Costa-Fraga, F., Jankowski, J., Jankowski, A., Sousa, F., Alzamora, A., Soares, E., Barbosa, C., Kjeldsen, F., Oliveira, A., Braga, J., Savergnini, S., Maia, G., Peluso, A.B., Passos-Silva, D., Ferreira, A., Alves, F., Martins, A., Raizada, M., Paula, R., Motta-Santos, D., Klempin, F., Pimenta, A., Alenina, N., Sinisterra, R., Bader, M., Campagnole-Santos, M.J., Santos, R.A.S., 2013. Discovery and characterization of alamandine: a novel component of the renin-angiotensin system. *Circ. Res.* 112, 1104–1111. <https://doi.org/10.1161/CIRCRESAHA.113.301077>.
- Leonhardt, J., Villela, D.C., Teichmann, A., Münter, L.-M., Mayer, M.C., Mardahl, M., Kirsch, S., Namsolleck, P., Lucht, K., Benz, V., Alenina, N., Daniell, N., Horiuchi, M., Iwai, M., Multhaupt, G., Schülein, R., Bader, M., Santos, R.A., Unger, T., Steckelings, U.M., 2017. Evidence for heterodimerization and functional interaction of the angiotensin type 2 receptor and the receptor Mas. *Hypertension* 69, 1128–1135. <https://doi.org/10.1161/HYPERTENSIONAHA.116.08814>.
- Li, Z., Iwai, M., Wu, L., Shiuchi, T., Jinno, T., Cui, T.X., Horiuchi, M., 2003. Role of AT2 receptor in the brain in regulation of blood pressure and water intake. *Am. J. Physiol. Heart Circ. Physiol.* 284, H116–H121. <https://doi.org/10.1152/ajpheart.00515.2002>.
- Li, H., Gao, Y., Grobe, J.L., Raizada, M.K., Katovich, M.J., Summers, C., 2007. Potentiation of the antihypertensive action of losartan by peripheral overexpression of the ANG II type 2 receptor. *Am. J. Physiol. Heart Circ. Physiol.* 292, H727–H735. <https://doi.org/10.1152/ajpheart.00938.2006>.
- Li, H., Qi, Y., Li, C., Braseth, L.N., Gao, Y., Shabashvili, A.E., Katovich, M.J., Summers, C., 2009. Angiotensin type 2 receptor-mediated apoptosis of human prostate cancer cells. *Mol. Cancer Therapeut.* 8, 3255–3265. <https://doi.org/10.1158/1535-7163.MC.T-09-0237>.
- Li, J., Luo, J., Gu, D., Jie, F., Pei, N., Li, A., Chen, X., Zhang, Y., Du, H., Chen, B., Gu, W., Summers, C., Li, H., 2016. Adenovirus-mediated Ang II type 2 receptor overexpression inhibits tumor growth of prostate cancer in vivo. *J. Cancer* 7, 184–191. <https://doi.org/10.7150/jca.12841>.
- Li, X.C., Zhang, J., Zhuo, J.L., 2017. The vasoprotective axes of the renin-angiotensin system: physiological relevance and therapeutic implications in cardiovascular, hypertensive and kidney diseases. *Pharmacol. Res.* 125, 21–38. <https://doi.org/10.1016/j.phrs.2017.06.005>.
- Liao, M.-C., Pang, Y.-C., Chang, S.-Y., Zhao, X.-P., Chenier, I., Ingelfinger, J.R., Chan, J.S.D., Zhang, S.-L., 2021. AT2R deficiency in mice accelerates podocyte dysfunction in diabetic progeny in a sex-dependent manner. *Diabetologia* 64, 2108–2121. <https://doi.org/10.1007/s00125-021-05483-7>.
- Liao, M.-C., Miyata, K.N., Chang, S.-Y., Zhao, X.-P., Lo, C.-S., El-Mortada, M.-A., Peng, J., Chenier, I., Yamashita, M., Ingelfinger, J.R., Chan, J.S.D., Zhang, S.-L., 2022. Ang II type-2-receptor stimulation ameliorates focal and segmental glomerulosclerosis in mice. *Clin. Sci.* 136, 715–731. <https://doi.org/10.1042/CS20220188>.
- Liu, M., Jing, D., Wang, Y., Liu, Y., Yin, S., 2015. Overexpression of Ang II type 2 receptor promotes apoptosis and impairs insulin secretion in rat insulinoma cells. *Mol. Cell. Biochem.* 400, 233–244. <https://doi.org/10.1007/s11010-014-2280-3>.
- Liu, C., Yang, C.-X., Chen, X.-R., Liu, B.-X., Li, Y., Wang, X.-Z., Sun, W., Li, P., Kong, X.-Q., 2018. Alamandine attenuates hypertension and cardiac hypertrophy in hypertensive rats. *Amino Acids* 50, 1071–1081. <https://doi.org/10.1007/s00726-018-2583-x>.
- Matsushita, K., Wu, Y., Pratt, R.E., Dzau, V.J., 2016. Deletion of Ang II type 2 receptor accelerates adipogenesis in murine mesenchymal stem cells via Wnt10b/beta-catenin signaling. *Lab. Invest.* 96, 909–917. <https://doi.org/10.1038/labinvest.2016.66>.
- Metcalfe, B.L., Huenteleman, M.J., Parilak, L.D., Taylor, D.G., Katovich, M.J., Knot, H.J., Summers, C., Raizada, M.K., 2004. Prevention of cardiac hypertrophy by Ang II type-2 receptor gene transfer. *Hypertension* 43, 1233–1238. <https://doi.org/10.1161/01.HYP.0000127563.14064.FD>.
- Micakovic, T., Papagiannarou, S., Clark, E., Kuzay, Y., Abramovic, K., Peters, J., Sticht, C., Volk, N., Fleming, T., Nawroth, P., Hammes, H.-P., Alenina, N., Gröne, H.-J., Hoffmann, S.C., 2018. The Ang II type 2 receptors protect renal tubule mitochondria in early stages of diabetes mellitus. *Kidney Int.* 94, 937–950. <https://doi.org/10.1016/j.kint.2018.06.006>.
- Mirabito, K.M., Hilliard, L.M., Wei, Z., Tikellis, C., Widdop, R.E., Vinh, A., Denton, K.M., 2014. Role of inflammation and the angiotensin type 2 receptor in the regulation of arterial pressure during pregnancy in mice. *Hypertension* 64, 626–631. <https://doi.org/10.1161/HYPERTENSIONAHA.114.03189>.
- Miura, S., Karnik, S.S., 1999. Ang II type 1 and type 2 receptors bind Ang II through different types of epitope recognition. *J. Hypertens.* 17, 397–404. <https://doi.org/10.1097/00004872-199917030-00013>.
- Miura, S., Karnik, S.S., 2000. Ligand-independent signals from Ang II type 2 receptor induce apoptosis. *EMBO J.* 19, 4026–4035. <https://doi.org/10.1093/emboj/19.15.4026>.
- Miura, S.-I., Karnik, S.S., Saku, K., 2005. Constitutively active homo-oligomeric Ang II type 2 receptor induces cell signaling independent of receptor conformation and ligand stimulation. *J. Biol. Chem.* 280, 18237–18244. <https://doi.org/10.1074/jbc.M500639200>.
- Mogi, M., Iwai, M., Horiuchi, M., 2007. Emerging concepts of regulation of Ang II receptors: new players and targets for traditional receptors. *Arterioscler. Thromb. Vasc. Biol.* 27, 2532–2539. <https://doi.org/10.1161/ATVBAHA.107.144154>.
- Moulder, J.E., Fish, B.L., Cohen, E.P., 2004. Impact of Ang II type 2 receptor blockade on experimental radiation nephropathy. *Radiat. Res.* 161, 312–317. <https://doi.org/10.1667/r3129>.
- Muttenthaler, M., King, G.F., Adams, D.J., Alewood, P.F., 2021. Trends in peptide drug discovery. *Nat. Rev. Drug Discov.* 20, 309–325. <https://doi.org/10.1038/s41573-020-00135-8>.
- Namsolleck, P., Richardson, A., Moll, G.N., Mescheder, A., 2021a. LP2, the first lanthipeptide GPCR agonist in a human pharmacokinetics and safety study. *Peptides* 136, 170468. <https://doi.org/10.1016/j.peptides.2020.170468>.
- Namsolleck, P., Kuipers, A., de Vries, L., Festuccia, C., 2021b. Angiotensin Type 2 (AT2) Receptor Agonists for Use in the Treatment of Cancer. WO2021023698 (Patent application).
- Namsolleck, P., de Vries, L., Moll, G.N., 2023a. LP2, a cyclic angiotensin-(1-7) analog extended with an N-terminal D-lysine, impairs growth of patient-derived xenografts of colorectal carcinoma in mice. *Peptides* 160, 170920. <https://doi.org/10.1016/j.peptides.2022.170920>.
- Namsolleck, P., Rodgers, K.E., Franklin, R., Moll, G.N., 2023b. LP2, a stable lanthipeptide derived from cAng-(1-7), exerts myeloprotective action in mice. *Eur. J. Haematol.* 110, 534–539. <https://doi.org/10.1111/ejh.13927>.
- Nouet, S., Amzallag, N., Li, J.-M., Louis, S., Seitz, I., Cui, T.-X., Alleaume, A.-M., Di Benedetto, M., Boden, C., Masson, M., Strosberg, A.D., Horiuchi, M., Couraud, P.-O., Nahmias, C., 2004. Trans-inactivation of receptor tyrosine kinases by novel Ang II AT2 receptor-interacting protein ATIP. *J. Biol. Chem.* 279, 28989–28997. <https://doi.org/10.1074/jbc.M403880200>.
- Núñez, R.E., Javadov, S., Escobales, N., 2018. Critical role of Ang II type 2 receptors in the control of mitochondrial and cardiac function in Ang II-preconditioned rat hearts. *Pflügers Archiv* 470, 1391–1403. <https://doi.org/10.1007/s00424-018-2153-9>.
- Oishi, Y., Ozono, R., Yano, Y., Teranishi, Y., Akishita, M., Horiuchi, M., Oshima, T., Kambe, M., 2003. Cardioprotective role of AT2 receptor in postinfarction left ventricular remodeling. *Hypertension* 41, 814–818. <https://doi.org/10.1161/01.HYP.0000048340.53100.43>.
- Okuyama, S., Sakagawa, T., Chaki, S., Imagawa, Y., Ichiki, T., Inagami, T., 1999. Anxiety-like behavior in mice lacking the Ang II type-2 receptor. *Brain Res.* 821, 150–159. [https://doi.org/10.1016/s0006-8993\(99\)01098-7](https://doi.org/10.1016/s0006-8993(99)01098-7).
- Patel, S., Hussain, T., 2018. Dimerization of AT2 and Mas receptors in control of blood pressure. *Curr. Hypertens. Rep.* 20, 41. <https://doi.org/10.1007/s11906-018-0845-3>.
- Pawluczuk, I.Z.A., Harris, K.P.G., 2012. Effect of angiotensin type 2 receptor overexpression on the rat mesangial cell fibrotic phenotype: effect of gender. *J. Renin-*

- Angiotensin-Aldosterone Syst. JRAAS 13, 221–231. <https://doi:10.1177/1470320311432185>.
- Pei, N., Jie, F., Luo, J., Wan, R., Zhang, Y., Chen, X., Liang, Z., Du, H., Li, A., Chen, B., Zhang, Y., Summers, C., Li, J., Gu, W., Li, H., 2014. Gene expression profiling associated with Ang II type 2 receptor-induced apoptosis in human prostate cancer cells. *PLoS One* 9, e92253. <https://doi:10.1371/journal.pone.0092253>.
- Pei, N., Mao, Y., Wan, P., Chen, X., Li, A., Chen, H., Li, J., Wan, R., Zhang, Y., Du, H., Chen, B., Jiang, G., Xia, M., Summers, C., Hu, G., Gu, D., Li, H., 2017. Ang II type 2 receptor promotes apoptosis and inhibits angiogenesis in bladder cancer. *J. Exp. Clin. Cancer Res.* 36, 77. <https://doi:10.1186/s13046-017-0542-0>.
- Perryman, R., Renziehausen, A., Shaye, H., Kostagianni, A.D., Tsiailanis, A.D., Thorne, T., Chatziathanasiadou, M.V., Sivolapenko, G.B., El Mubarak, M.A., Han, G. W., Zarzycka, B., Katritch, V., Lebon, G., Lo Nigro, C., Lattanzio, L., Morse, S.V., Choi, J.J., O'Neill, K., Kanaki, Z., Klinakis, A., Crook, T., Cherezov, V., Tzakos, A.G., Syed, N., 2022. Inhibition of the Ang II type 2 receptor is a novel therapeutic strategy for glioblastoma. *Proc. Natl. Acad. Sci. U.S.A.* 119, e2116289119. <https://doi:10.1073/pnas.2116289119>.
- Peters, B., Podlich, D., Ritter, M., Müller, A., Wanka, H., Maser-Gluth, C., Seitz, C., de Boni, L., Maier, E., Gretz, N., Peters, J., Hoffmann, S.C., 2012. A new transgenic rat model overexpressing the Ang II type 2 receptor provides evidence for inhibition of cell proliferation in the outer adrenal cortex. *Am. J. Physiol. Endocrinol. Metab.* 302, E1044–E1054. <https://doi:10.1152/ajpendo.00080.2011>.
- Porrello, E.R., Delbridge, L.M.D., Walter, T., 2009. The Ang II type 2 (AT2) receptor: an enigmatic seven transmembrane receptor. *Front. Biosci.* 14, 958–972. <https://doi:10.2741/3289>.
- Pulakat, L., Cooper, S., Knowle, D., Mandavia, C., Bruhl, S., Hetrick, M., Gavini, N., 2005. Ligand-dependent complex formation between the Ang II receptor subtype AT2 and Na⁺/H⁺ exchanger NHE6 in mammalian cells. *Peptides* 26, 863–873. <https://doi:10.1016/j.peptides.2004.12.015>.
- Qaradakhli, T., Apostolopoulos, V., Zulli, A., 2016. Angiotensin (1-7) and alamdandine: similarities and differences. *Pharmacol. Res.* 111, 820–826. <https://doi:10.1016/j.phrs.2016.07.025>.
- Qi, Y., Li, H., Shenoy, V., Li, Q., Wong, F., Zhang, L., Raizada, M.K., Summers, C., Katovich, M.J., 2012. Moderate cardiac-selective overexpression of Ang II type 2 receptor protects cardiac functions from ischaemic injury. *Exp. Physiol.* 97, 89–101. <https://doi:10.1113/expphysiol.2011.060763>.
- Raffai, G., Durand, M.J., Lombard, J.H., 2011. Acute and chronic angiotensin-(1-7) restores vasodilation and reduces oxidative stress in mesenteric arteries of salt-fed rats. *Am. J. Physiol. Heart Circ. Physiol.* 301, H1341–H1352. <https://doi:10.1152/ajpheart.00202.2011>.
- Recarti, C., Bottari, S., 2015. AT2 receptor signaling: solved and unsolved. In: Unger, T., Steckelings, U., Santos, R.A. (Eds.), (2015) The Protective Arm of the Renin Angiotensin System: Functional Aspects and Therapeutic Implications. Elsevier Inc, pp. 23–33. <https://doi:10.1016/B978-0-12-801364-9.00004-3>.
- Reinemund, J., 2010. Die transkriptionelle regulation des angiotensin-AT2-rezeptorgens und des Ang II type 2 receptor-binding-protein-gens. *Prozesswissenschaften der Technischen Universität, Berlin*, pp. 1–135.
- Restrepo, Y.M., Noto, N.M., Speth, R.C., 2022. CGP42112: the full AT2 receptor agonist and its role in the renin-angiotensin-aldosterone system: no longer misunderstood. *Clin. Sci.* 136, 1513–1533. <https://doi:10.1042/CS20220261>.
- Sakagawa, T., Okuyama, S., Kawashima, N., Hozumi, S., Nakagawasai, O., Tadano, T., Kisara, K., Ichiki, T., Inagami, T., 2000. Pain threshold, learning and formation of brain edema in mice lacking the Ang II type 2 receptor. *Life Sci.* 67, 2577–2585. [https://doi:10.1016/S0024-3205\(00\)00841-9](https://doi:10.1016/S0024-3205(00)00841-9).
- Santos, R.A.S., Simoes e Silva, A.C., Maric, C., Silva, D.M.R., Machado, R.P., de Buhr, I., Heringer-Walther, S., Pinheiro, S.V.B., Lopes, M.T., Bader, M., Mendes, E.P., Lemos, V.S., Campagnole-Santos, M.-J., Schultheiss, H.-P., Speth, R., Walthers, T., 2003. Angiotensin-(1-7) is an endogenous ligand for the G-protein-coupled receptor Mas. *Proc. Natl. Acad. Sci. U.S.A.* 100, 8258–8263. <https://doi:10.1073/pnas.1432869100>.
- Savoia, C., Volpe, M., 2015. Ang II type 1 receptor–Ang II type 2 receptor Cross Talk. In: Unger, T., Steckelings, U., Santos, R.A. (Eds.), 2015. The Protective Arm of the Renin Angiotensin System: Functional Aspects and Therapeutic Implications. Elsevier Inc, pp. 35–39.
- Siragy, H.M., Inagami, T., Ichiki, T., Carey, R.M., 1999. Sustained hypersensitivity to Ang II and its mechanism in mice lacking the subtype-2 (AT2) angiotensin receptor. *Proc. Natl. Acad. Sci. U.S.A.* 96, 6506–6510. <https://doi:10.1073/pnas.96.11.6506>.
- Smith, M.T., Wyse, B.D., Edwards, S.R., 2013. Small molecule Ang II type 2 receptor (AT2R) antagonists as novel analgesics for neuropathic pain: comparative pharmacokinetics, radioligand binding, and efficacy in rats. *Pain Med.* 14, 692–05. <https://doi:10.1111/pme.12063>.
- Speck, D., Kleinau, G., Szczepek, M., Kwiatkowski, D., Catar, R., Philippe, A., Scheerer, P., 2022. Angiotensin and endothelin receptor structures with implications for signaling regulation and pharmacological targeting. *Front. Endocrinol.* 13, 880002. <https://doi:10.3389/fendo.2022.880002>.
- Toedebusch, R., Belenchia, A., Pulakat, L., 2018. Cell-specific protective signaling induced by the novel Ang II type 2 receptor-agonist NP-6A4 on human endothelial and smooth muscle cells. *Front. Pharmacol.* 9, 928. <https://doi:10.3389/fphar.2018.00928>.
- Tsutsumi, Y., Matsubara, H., Masaki, H., Kurihara, H., Murasawa, S., Takai, S., Miyazaki, M., Nozawa, Y., Ozono, R., Nakagawa, K., Miwa, T., Kawada, N., Mori, Y., Shibasaki, Y., Tanaka, Y., Fujiyama, S., Koyama, Y., Fujiyama, A., Takahashi, H., Iwasaka, T., 1999. Ang II type 2 receptor overexpression activates the vascular kinin system and causes vasodilation. *J. Clin. Invest.* 104, 925–935. <https://doi:10.1172/JCI7886>.
- Unger, T., Steckelings, U., Santos, R.A. (Eds.), 2015. The Protective Arm of the Renin Angiotensin System: Functional Aspects and Therapeutic Implications. Elsevier Inc, pp. 23–33. <https://doi:10.1016/B978-0-12-801364-9.00004-3>.
- Vargas Vargas, R.A., Millán, J.M.V., Bonilla, E.F., 2022. Renin-angiotensin system: basic and clinical aspects - a general perspective. *Endocrinol. Diabetes Nutr.* 69, 52–62. <https://doi:10.1016/j.endien.2022.01.005>.
- Villela, D.C., Namsolleck, P., Reichetzer, C., Moll, G.N., 2023. AT2 receptor agonist LP2 restores respiratory function in a rat model of bleomycin-induced lung remodeling. *Peptides* 170, 171106. <https://doi:10.1016/j.peptides.2023.171106>.
- Wagenaar, G.T.M., Laghmani, E.H., Fiddler, M., Sengers, R.M.A., de Visser, Y.P., de Vries, L., Rink, R., Roks, A.J.M., Folkerts, G., Walther, F.J., 2013. Agonists of MAS oncogene and Ang II type 2 receptors attenuate cardiopulmonary disease in rats with neonatal hyperoxia-induced lung injury. *Am. J. Physiol. Lung Cell Mol. Physiol.* 305, L341–L351. <https://doi:10.1152/ajplung.00360.2012>.
- Wagenaar, G.T.M., Sengers, R.M.A., Laghmani, E.H., Chen, X., Lindeboom, M.P.H.A., Roks, A.J.M., Folkerts, G., Walther, F.J., 2014. Ang II type 2 receptor ligand PD123319 attenuates hyperoxia-induced lung and heart injury at a low dose in newborn rats. *Am. J. Physiol. Lung Cell Mol. Physiol.* 307, L261–L272. <https://doi:10.1152/ajplung.00345.2013>.
- Wan, Y., Wallinder, C., Plouffe, B., Beaudry, H., A. K., Wu, X., Johansson, B., Holm, M., Botoros, M., Karlén, A., Pettersson, A., Nyberg, F., Fändriks, L., Gallo-Payet, N., Hallberg, A., Alterman, M., 2004. Design, synthesis, and biological evaluation of the first selective nonpeptide AT2 receptor agonist. *J. Med. Chem.* 47, 5995–08. <https://doi:10.1021/jm049715t>.
- Wang, H., Gallinat, S., Li, H.W., Summers, C., Raizada, M.K., Katovich, M.J., 2004. Elevated blood pressure in normotensive rats produced by 'knockdown' of the angiotensin type 2 receptor. *Exp. Physiol.* 89, 313–322. <https://doi:10.1113/expphysiol.2004.027359>.
- Watanabe, T., Hashimoto, M., Okuyama, S., Inagami, T., Nakamura, S., 1999. Effects of targeted disruption of the mouse Ang II type 2 receptor gene on stress-induced hyperthermia. *J. Physiol.* 515 (Pt 3), 881–885. <https://doi:10.1111/j.1469-7793.1999.881ab.x>.
- Wruck, C.J., Funke-Kaiser, H., Pufe, T., Kusserow, H., Menk, M., Schefe, J.H., Kruse, M. L., Stoll, M., Unger, T., 2005. Regulation of transport of the angiotensin AT2 receptor by a novel membrane-associated Golgi protein. *Arterioscler. Thromb. Vasc. Biol.* 25, 57–64. <https://doi:10.1161/01.ATV.0000150662.51436.14>.
- Wu, L., Iwai, M., Nakagami, H., Chen, R., Suzuki, J., Akishita, M., Gasparo, M., Horiuchi, M., 2002. Effect of Ang II type 1 receptor blockade on cardiac remodeling in Ang II type 2 receptor null mice. *Arterioscler. Thromb. Vasc. Biol.* 22, 49–54. <https://doi:10.1161/hq0102.102277>.
- Xu, X.-P., Huang, L.-L., Hu, S.-L., Han, J.-B., He, H.-L., Xu, J.-Y., Xie, J.-F., Liu, A.-R., Liu, S.-Q., Liu, L., Huang, Y.-Z., Guo, F.-M., Yang, Y., Qiu, H.B., 2018. Genetic modification of mesenchymal stem cells overexpressing Ang II type 2 receptor increases cell migration to injured lung in LPS-induced acute lung injury mice. *Stem Cells Transl. Med.* 7, 721–730. <https://doi:10.1002/sctm.17-0279>.
- Zhang, H., Han, G.W., Batyuk, A., Ishchenko, A., White, K.L., Patel, N., Sadybekov, A., Zamylny, B., Rudd, M.T., Hollenstein, K., Tolstikova, A., White, T.A., Hunter, M.S., Weierstall, U., Liu, W., Babaoglu, K., Moore, E.L., Katz, R.D., Shipman, J.M., Garcia-Calvo, M., Sharma, S., Sheth, P., Soisson, S.M., Stevens, R.C., Katritch, V., Cherezov, V., 2017. Structural basis for selectivity and diversity in Ang II receptors. *Nature* 544, 327–332. <https://doi:10.1038/nature22035>.
- Zhou, J., Ernsberger, P., Douglas, J.C., 1993. A novel angiotensin receptor subtype in rat mesangium. Coupling to adenylyl cyclase. *Hypertension* 21, 1035–1038. <https://doi:10.1161/01.hyp.21.6.1035>.
- Zizzo, M.G., Caldara, G., Bellanca, A., Nuzzo, D., Di Carlo, M., Serio, R., 2020. PD123319, Ang II type II receptor antagonist, inhibits oxidative stress and inflammation in 2, 4-dinitrobenzene sulfonic acid-induced colitis in rat and ameliorates colonic contractility. *Inflammopharmacology* 28, 187–199. <https://doi:10.1007/s10787-019-00619-z>.
- Zou, X., Liao, Y., Liu, Z., Xu, X., Sun, W., Qin, H., Wang, H., Liu, J., Jing, T., 2023. Exosomes derived from AT2R-overexpressing BMSC prevent restenosis after carotid artery injury by attenuating the injury-induced neointimal hyperplasia. *J. Cardiovasc. Transl. Res.* 16, 112–126. <https://doi.org/10.1007/s12265-022-10293-2>.