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Structural insights into ligand recognition and activation of angiotensin receptors

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Abstract

G protein-coupled angiotensin II receptors, AT_1R and AT_2R, are integral components of the renin–angiotensin system (RAS) that regulates blood pressure and fluid balance in humans. While AT_1R is a well-established target of angiotensin receptor blockers (ARBs) for managing hypertension and a prime system for studying biased signaling, AT_2R has been recognized as a promising target against neuropathic pain and lung fibrosis. In this review, we discuss how recent structural advances illuminate ligand-binding modes and subtype selectivity, shared and distinct features of the receptors, their transducer-coupling patterns, and downstream signaling responses. We also underscore the key ATR aspects that require further studies to fully appreciate the mechanistic framework that fine-tunes their cellular and physiological functions, providing untapped potential for drug discovery.

Background and introduction to angiotensin receptors

Angiotensin II (AngII) (see Glossary) is a peptide hormone that plays a major role in the RAS via acting on AT_1R and AT_2R subtypes, which belong to the superfamily of G protein-coupled receptors (GPCRs) (Figure 1) [1]. AT_1R is expressed in many different cell types and signals via classical G_q-mediated pathways to elicit various responses related to regulation of blood pressure, electrolyte and water balance, and renal function [1]. Small-molecule **angiotensin receptors blockers** (ARBs), including losartan, candesartan, telmisartan, eprosartan, valsartan, irbesartan, olmesartan, and azilsartan, are clinically used

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as highly effective antihypertensive drugs [2]. AT_2R has typically low expression in adults, which can be upregulated during pathology, and does not signal via canonical G protein pathways; thus, its signaling mechanisms and function remain controversial [3]. In general, AT_2R counterbalances the action of AT_1R, while also offering tissue and cell protective function, for which it has been considered as a promising target against lung fibrosis [4]. An experimental AT_2R-selective agonist C21 has recently entered phase II clinical trials for the treatment against lung damage in patients infected with COVID-19 (Clinical Trial Number[i] NCT04452435). Additionally, antagonizing AT_2R receptor using selective inhibitor EMA-401 has been shown effective for reducing neuropathic pain [5].

Among the two subtypes of angiotensin receptors, AT_1R is a prototypical GPCR that signals by engaging with two intracellular transducers, heterotrimeric G proteins and versatile scaffolding proteins called β-arrestins (βarr). In recent years, it has been shown that for some GPCRs either specific mutations [6,7] or ligands can selectively tilt the balance of engagement either towards G protein or βarr axis [8]; a phenomenon widely referred to as biased signaling [9,10]. Therapeutic efficacy of biased ligands is increasingly appreciated for their ability to reduce side effects usually associated with balanced ligands [11,12]. Therefore, understanding the underlying mechanisms and structural rearrangements in GPCRs, induced by biased ligands, is essential for designing the next generation of safer drugs. AT_1R has been widely studied to understand the structural mechanism of bias. In fact, βarr-biased AT_1R ligands result in improved cardiac performance even after exerting antihypertensive effects, making them preferred alternatives to clinically used ARBs [13].

During the past several years, many breakthrough studies shed light on molecular mechanisms related to function of both angiotensin receptors. In this review, we primarily focus on structural aspects of ligand-receptor interaction with an emphasis on **subtype selectivity**, receptor activation, and **biased agonism**. We underscore the insights obtained by high-resolution crystal structures of AT_1R and AT_2R in complex with antagonists, agonists, and biased ligands (Table 1), and discuss how this information improved our current understanding of receptor activation and signaling mechanisms.

Structural snapshot of ARB binding

Initial insights into the overall AT_1R structure and AngII binding emerged from homology modeling based on methionine proximity assay data [14]. The first high-resolution room-temperature crystal structure of AT_1R in complex with a small molecule antagonist ZD7155 was obtained by serial femtosecond crystallography with an X-ray free electron laser (XFEL) (Table 1) [15]. The structure revealed canonical GPCR heptahelical transmembrane (7TM) architecture with an extracellular N terminus, three intracellular loops (ICLs), and three extracellular loops (ECLs) connecting seven transmembrane helices (TM1–7), followed by a short amphiphilic helix 8 (H8), and an intracellular C terminus. Two disulfide bonds stabilize conformations of the N terminus as well as the ECL2 that adopts a β-hairpin secondary structure as in most peptide receptors.

Resources

[i] https://clinicaltrials.gov/ct2/show/NCT04452435

Bound to an antagonist, the receptor was captured in a distinct inactive state, based on the orientation of microswitches critical for activation and the conformations of TM5, TM6, and TM7. All residues within the conserved **allosteric** sodium-binding site in class A GPCRs [16] remain intact in AT_1R except for the substitution of $Ser^{7.46}$ [17] to Asn, which likely disrupts sodium binding but instead stabilizes the inactive state by forming two hydrogen bonds with $Asn111^{3.35}$. In the absence of sodium ions, $Asn111^{3.35}$Ala mutation showed 300-fold higher affinity for AngII [18]. Another potential inactive state lock is formed by the DRY motif residue $Arg126^{3.50}$ and $Asn235^{6.30}$.

The high-affinity antagonist ZD7155, a precursor of the antihypertensive drug candesartan, binds deep in a large **orthosteric** ligand-binding pocket, interacting with residues from TM1-3, TM7, and ECL2 (Figure 2). The ligand is anchored by three key residues, $Arg167^{ECL2}$, $Tyr35^{1.39}$, and $Trp84^{2.60}$, which play essential roles but have not been previously implicated in binding of different ARBs. The positively charged $Arg167^{ECL2}$ forms ionic and hydrogen bonds with the acidic tetrazole and the naphthyridin-2-one groups of ZD7155, while $Tyr35^{1.39}$ and $Trp84^{2.60}$ form an additional hydrogen bond and a π-stacking interaction with the naphthyridin-2-one, respectively. Docking other common ARBs into the crystal structure along with site-directed mutagenesis study suggested that all these compounds bind in a similar pose as ZD7155 and interact with the same three key receptor residues. Their relative contributions to the total binding energy, however, vary for different compounds, which, due to their diverse chemical structures, are also engaged in additional interactions with other binding site residues. For example, the short alkyl tail present in several ARBs extends into the narrow hydrophobic pocket surrounded by $Tyr35^{1.39}$, $Phe77^{2.53}$, $Val108^{3.32}$, $Ile288^{7.39}$, and $Tyr292^{7.43}$.

It has been shown that small modifications of ligands can lead to changes in the ligand's mode of action despite retaining the same ligand binding mode. A follow-up study revealed the molecular basis for diverse pharmacological properties of olmesartan derivatives [18]. Substitution of the carboxyl group attached to the imidazole moiety of the inverse agonist olmesartan by the carbamoyl group turned it into a neutral antagonist, likely because of a switch of interaction from $Arg167^{ECL2}$ to $Tyr87^{2.63}$. Further addition of a 4-hydroxybenzyl group to the biphenyltetrazole moiety of olmesartan converted the ligand into a partial agonist. The 4-hydroxybenzyl group forms extensive interactions with the 'toggle switch' residue $Trp253^{6.48}$, implicated in the activation of many GPCRs [16], and with a cluster consisting of $Lys199^{5.42}$, $His256^{6.51}$, $Gln257^{6.52}$, and $Thr260^{6.55}$, which were previously identified to be important for the ligand-dependent activation of AT_1R [15].

Structural basis of AT_1R activation

Further understanding of endogenous ligand binding and receptor activation mechanisms arrived with crystal structures of AT_1R in complex with the endogenous peptide agonist AngII and its derivative [Sar^1,Ile^8]-angiotensin II (s-AngII) that acts as a partial agonist, which have been obtained in the presence of a conformation stabilizing nanobody AT110i1 mimicking receptor interaction with G protein (Table 1) [19–21].

The crystal structures revealed that AngII binds to AT_1R in an extended conformation, with its C terminus reaching deep inside the ligand-binding pocket and its N terminus pointing to the extra-cellular side, and forms both polar and nonpolar contacts with AT_1R residues (Figure 3), in agreement with previous homology-modeling studies [14]. The N termini of AT_1R and AngII, together with the β-hairpin of ECL2, form a twisted four-strand β sheet. As revealed by the crystal structures and confirmed by site-directed mutagenesis, $Tyr35^{1.39}$, $Trp84^{2.60}$, $Arg167^{ECL2}$, and $Lys199^{5.42}$ are involved in both ARB and peptide binding, however, $Phe182^{ECL2}$ and $Ile288^{7.39}$ interact only with peptides [19,20]. The last residue of AngII, Phe^{8}, is involved in triggering activation-related conformational changes in AT_1R by pulling TM5 inward via $Lys199^{5.42}$ that forms a salt bridge with the C terminus of AngII and by pushing on $Leu112^{3.34}$ with its bulky side chain. As a result, $Leu112^{3.34}$ changes its rotameric state to occupy the former position of $Tyr292^{7.43}$, which leads to rotation of TM3 around its axis. This rotation flips $Asn111^{3.35}$, breaking its hydrogen bonds with $Asn295^{7.46}$ and eliminating one of the major inactive state locks. Mutations of either of these two residues to Ala induce the constitutive activation of AT_1R [22], reinforcing the notion that both $Asn111^{3.35}$ and $Asn295^{7.46}$ stabilize the inactive conformation of AT_1R and play essential roles for the AT_1R activation.

The release of the inactive state lock results in a sequence of conformational changes that propagate towards the intracellular side of the receptor leading to an outward shift of TM6 by 11 Å along with an outward shift of TM5 and an inward shift of TM7. These TM shifts are stabilized by rearrangements of conserved microswitches, such as $Tyr302^{7.53}$ of the NPxxY motif that forms potential water-mediated hydrogen bond with $Tyr215^{5.58}$. Additionally, ICL2 that is unstructured in the inactive state of AT_1R adopts a short α helix, connecting the DRY motif residues $Arg126^{3.50}$ and $Asp125^{3.49}$ with $Arg137^{ICL2}$ and $Arg140^{ICL2}$, as well as with Asp112 from the conformation-stabilizing nanobody via an extensive ionic network. It has been observed that upon activation of class A GPCRs, $Arg^{3.50}$ switches from an ionic lock with $Asp^{3.49}$ to an extended conformation that can engage in interactions with the prolonged α5 helix of G protein [23]. In the active-like structures of AT_1R in complex with s-AngII and AngII, $Arg126^{3.50}$ remains in the inactive conformation, highlighting singularity of AT_1R activation pattern or potential artifact from using the nanobody. The main difference between the partial agonist s-AngII and the full agonist AngII is that the less bulky C-terminal residue Ile^{8} in s-AngII does not induce a TM3 rotation [19,20].

Structural insights into biased agonism

While biased signaling by AT_1R has been well established over a decade ago [13], its structural basis was poorly understood until recently. Initial insights were obtained in a study using double electron-electron resonance (DEER) spectroscopy, a pulsed electron paramagnetic resonance (EPR) technique, which can effectively measure the distribution of distances between two selected residues that are appropriately labeled [19]. The recent studies using the DEER approach on AT_1R system provided a map of global changes that occur in the receptor when it transitions from its basal state to a fully activated state (receptor bound to endogenous ligand AngII) as well as to other distinct conformations that AT_1R can sample upon binding to biased ligands of different efficacies. Expectedly, these

studies revealed an outward movement of TM6 away from the AT_1R core and an inward movement of TM7 upon receptor activation. Importantly, this approach also underscored the spectrum of conformational populations that the receptor samples both in ligand-bound and in ligand-free states. For example, about 60% of receptor population was found to exist in the open TM6 conformation, even in the presence of full agonist, AngII, suggesting the requirement for a transducer binding to fully enrich the active receptor population. Even in the ligand-free apo state, about 10% of the receptor population appears to sample active-like open conformation, which may explain the constitutive activity of AT_1R [21,24].

Analysis of receptor conformations in the presence of G protein- and βarr-biased ligands has started to illuminate some critical aspects of receptor structural changes that may be linked to preferred transducer coupling and distinct functional outcomes. For example, Gq-biased ligands, namely TRV055 and TRV056, appear to induce a less-pronounced outward movement of TM6, a subtle inward shift of TM7, and a TM5 movement towards TM6. βarr-biased ligands, such as TRV023, TRV026, TRV027, and TRV034, show significant diversity in conformational features that they impart to the receptor, although their overall functional responses are similar. These four ligands appear to induce different degrees of the outward TM6 movement in the receptor accompanied by additional differences in the TM5, TM7, and H8 regions (Figure 3) [19]. These intriguing observations based on the DEER spectroscopy measurements were further corroborated and expanded by crystal structures of the receptor in complex with different biased ligands (Table 1), and molecular dynamics simulation studies using these structural templates [19–21]. It was observed that βarr-biased agonists disrupt the two critical intermolecular interactions, that is, $Arg126^{3.50}$-$Asn235^{6.30}$ and $Asn111^{3.35}$-$Asn295^{7.46}$, differently than the natural agonist AngII does. For example, AngII induces inward movements of $Leu112^{3.36}$ and $Asn295^{7.46}$ and outward movements of $Asn111^{3.35}$, $Tyr292^{7.43}$, and $Asn298^{7.41}$, but in case of βarr-biased ligands, the reorientation of $Asn295^{7.46}$ appears to be sufficient to stabilize a receptor conformation able to accommodate βarrs [19–21]. Molecular dynamics simulation studies suggest that AT_1R can primarily sample two different active-like conformations: a canonical conformation and an alternative conformation. While the canonical conformation can accommodate both, the G_q α5 helix and the finger loop of βarr, the alternative conformation can only allow the docking of the βarr finger loop but not the G_q α5 helix [24]. It is important to note here that analogous conclusions were drawn from extensive earlier biochemical and simulation studies, even before the structural templates were available, which is certainly reassuring [25–29]. While the complete picture will emerge only when high-resolution structural snapshots of ternary complexes become available, these important studies certainly start to provide a structural mechanism for understanding biased agonism and pave the way for structure-based design of biased ligands [21,24].

Structural framework of subtype selectivity

Structural basis for selectivity and diversity between AT_1R and AT_2R has been illuminated by the structures of AT_2R in complex with small molecule ligands Comp1 (AT_2R selective) and Comp2 (dual AT_1R/AT_2R), derived from AT_1R antagonists (Table 1) [30]. Unexpectedly, the receptor bound to putative antagonists was crystallized in a distinct active-like state, based on the conformation of activation-related microswitches and positions of the

intracellular ends of TM5–7 (Figure 4A). However, in contrast to other active state GPCR structures, H8 flips over from its canonical orientation parallel to the membrane by ~130° to interact with the intracellular tips of TM5 and TM6, effectively blocking interactions with G proteins and βarrs, in agreement with reports that AT_2R does not signal through these canonical GPCR transducers [31]. Subsequent AT_2R structures determined in complex with the partial agonist s-AngII [27] and the endogenous agonist AngII [32,33] revealed no density for H8 in the s-AngII-bound structure and a more canonical orientation of H8 parallel to the lipid membrane in the AT_2R-AngII structure, suggesting that H8 potentially regulates AT_2R signaling. One caveat, however, is that the s-AngII structure has been obtained using the AT_2R construct with a BRIL partner fused in ICL3, which could interfere with the conformation of H8 observed in the structures with Comp1 and Comp2.

Because of the active-like AT_2R conformation and low conservation between the receptor sub-types (7 identical out of 13 residues in the ligand-binding site), the pocket shape for antagonist binding differs dramatically between AT_2R and AT_1R (Figure 4B). As a result, the dual antagonist Comp2 binds to AT_2R in a different conformation of its common scaffold compared with binding of ZD7155 and other ARBs to AT_1R. Despite the different binding pose, the residues equivalent to the main anchors of ARBs in AT_1R, Arg^{ECL2}, $Tyr^{1.39}$, and $Trp^{2.60}$, also provide critical interactions in AT_2R. Additionally, the tetrazole group of AT_2R compounds is engaged in new polar interactions with $Thr125^{3.33}$, $Thr178^{4.60}$, and $Lys215^{5.42}$. Further insights in selectivity were obtained by using molecular docking, site-directed mutagenesis, and structure–activity relationship (SAR) analysis [30]. Docking of these selective and dual ligands in crystal structures of AT_1R and AT_2R, as well as in models of the AT_1R active state and AT_2R inactive state, has demonstrated that, although the conformational state of AT_2R has little effect on binding, all tested compounds are incompatible with docking in the model of the AT_1R active state, corroborating the mode of action of these ligands as antagonists of AT_1R. Additionally, the obtained docking scores qualitatively reflect the relative affinities of the tested ligands at both receptors. The SAR analysis of compounds with a common quinazolinone–biphenyl–tetrazole scaffold suggests that R^1 derivatives are critical for AT_2R selectivity, while R^2 substituents define selectivity toward AT_1R (Figure 4C). The n-propyl group in the R^1 position fills hydrophobic subpockets in both receptors, which are composed of different residues. Smaller R^1 substituents (ethyl and methyl) induce a shift of the whole scaffold in AT_1R, resulting is less-optimal interactions and reduced affinity, while in AT_2R the size of the n-propyl moiety does not affect the ligand-binding pose. The aromatic substituents in R^2 position bind tightly to a hydrophobic subpocket in AT_2R, explaining a reduced binding affinity in case of smaller R^2 groups. Such a subpocket is absent in AT_1R, making it less sensitive to variations in the R^2 group size and allowing to accommodate larger R^2 substitutents.

In contrast to distinct binding modes of small-molecule antagonists, the endogenous peptide agonist AngII binds to both receptors (Table 1) in a similar conformation with its C-terminal residue Phe^8 reaching deep inside the pocket, the five C-terminal residues Tyr^4-Phe^8 adopting a C-shaped conformation, and the N terminus stretching toward the extracellular opening of the pocket (Figure 4D). While the five C-terminal residues of AngII closely follow each other in both receptors with rmsd = 1.2 Å, the N-terminal residues deviate by as much as 3–4 Å. The most divergent Asp^1 of AngII forms several polar interactions with

Asp17$^{N\text{-term}}$ in AT_1R, but no interactions in AT_2R in agreement with a higher selectivity of Ang(2–8) towards AT_2R [34]. Arg^2 makes salt bridges with conserved Asp$^{6.58}$ and Asp$^{7.32}$ in both receptors; however, in AT_2R, its conformation is constrained by a stacking interaction with Trp283$^{6.62}$ (Gln267$^{6.62}$ in AT_1R), making the salt bridges less optimal. Accordingly, removal of two N-terminal residues, Ang(3–8), makes the peptide even more selective towards AT_2R [34]. Other marked differences include His^6 that forms a hydrogen bond with Tyr104$^{2.64}$ in AT_2R. In AT_1R, the equivalent residue Thr88$^{2.64}$ is too short for a hydrogen bond, and His^6 interacts with Asp281$^{7.32}$ instead. Mutation of His^6 to Tyr has been shown to dramatically increase peptide selectivity towards AT_2R, likely because of potential clashes between Tyr^6 and TM7 backbone in AT_1R [35].

Concluding remarks and future perspectives

In this review, we have discussed recent structural studies on angiotensin receptors to underscore the basis of receptor activation, subtype selectivity, and biased agonism. The recent surge of structural data on these receptors dramatically improved our understanding of ligand–receptor interaction and receptor activation; however, the next frontier is to visualize the receptors in complex with signal transducers, namely the Gq protein, βarrs, and others. Such structural snapshots combined with biochemical and biophysical characterizations and cellular studies, should further decipher the mechanistic framework of signaling in AT_2R and biased agonism in AT_1R.

Going forward, there are several key areas that require focused investigation to fully decipher the activation and signaling paradigms of the angiotensin receptor system (see Outstanding questions). For example, it would be interesting to see whether the results of AT_1R studies, one of the most well characterized receptors in terms of biased signaling, will be applicable to other GPCRs and provide a better conceptual framework for understanding biased agonism? Similarly, it remains to be seen if the growing structural information will guide the development of biased ligands and the design of follow up studies to better understand the mechanistic insights into biased signaling. AT_1R also presents a peculiar example, where the two isoforms of βarrs have distinct contributions in ERK1/2 MAP kinase activation. While βarr2 is supportive and its knockdown inhibits agonist-induced phosphorylation of ERK1/2, depletion of βarr1 has the opposite effect [36]. Thus, it remains to be explored if these isoform-specific functional outcomes are governed by distinct structural features of AT_1R-βarr1/2 complexes? In fact, recent studies have indeed started to provide some important structural clues into this functional diversity of βarr isoforms [37,38].

Although recent AT_2R crystal structures provided important insights into receptor subtype selectivity and offered initial clues on receptor activation, the mechanism of signal transduction by AT_2R remains poorly understood. We still lack information on the precise conformational changes involved in AT_2R activation, particularly with respect to receptor dynamics and potential regulation of signaling by H8. Addressing these questions will require dissecting AT_2R signaling mechanisms biochemically and using complementary methods, such as NMR, EPR, and single molecule fluorescence [39]. It also remains to be seen if additional crystal structures of AT_2R, for example, in complex with small molecule

drug candidates, the agonist C21 and the antagonist EMA401, may help to clarify molecular determinants of receptor activation and can be further leveraged for rational design of more efficient drugs. Finally, robust identification of AT_2R signal transducers and deciphering their structures in complex with AT_2R by X-ray crystallography or cryo-EM [40] should break the long-thought 'Enigma' code of AT_2R signaling [41].

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Highlight

Angiotensin receptors are some of the most-studied receptor systems to understand subtype selectivity of ligands and biased agonism from structural perspective.

Several crystal structures of AT_1R and AT_2R have allowed direct visualization of ligand binding at high resolution including those of ARBs and biased angiotensin II analogs.

Biophysical studies using EPR spectroscopy and molecular dynamics simulation have provided further insights into structural changes in the AT_1R that govern distinct transducer coupling and biased signaling.

Structural coverage of AT_1R and AT_2R now provide a previously lacking frame-work to design and characterize subtype-selective ligands and tailored biased ligands.

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Outstanding questions

How can the structural templates available now be leveraged to design subtype selective ligands and biased ligands?

What is the structural framework for differential transducer-coupling of AT_1R in response to agonist stimulation? For example, how does the structure of AT_1R differ between Gq- versus βarr-bound conformations?

How does ligand-bias at the receptor level manifests at the receptor–transducer coupling? For example, how does the structure of receptor–transducer complexes differ from each other in response to biased vs. unbiased ligands?

What is the role of H8 in signal transduction of AT_2R?

Which transducers couple to AT_2R and how do they interact with the receptor?

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Glossary

Angiotensin II
a peptide hormone consisting of eight amino acids involved in regulation of blood pressure, water content, and sodium levels.

Angiotensin receptor blockers
antagonists and inverse agonists of AT_1R that stabilize an inactive conformation of the receptor, and are used clinically as antihypertensive drugs.

Biased agonism
the ability of ligands to preferentially trigger selective transducer-coupling and downstream signaling responses, that is, either heterotrimeric G proteins or βarrs.

Orthosteric and allosteric sites
the binding pocket occupied by the natural agonist is typically referred to as orthosteric, while other binding sites on the receptor are referred to as allosteric sites.

Subtype selectivity
the ability of ligands to selectively recognize and activate or inhibit a specific subtype of a given receptor.

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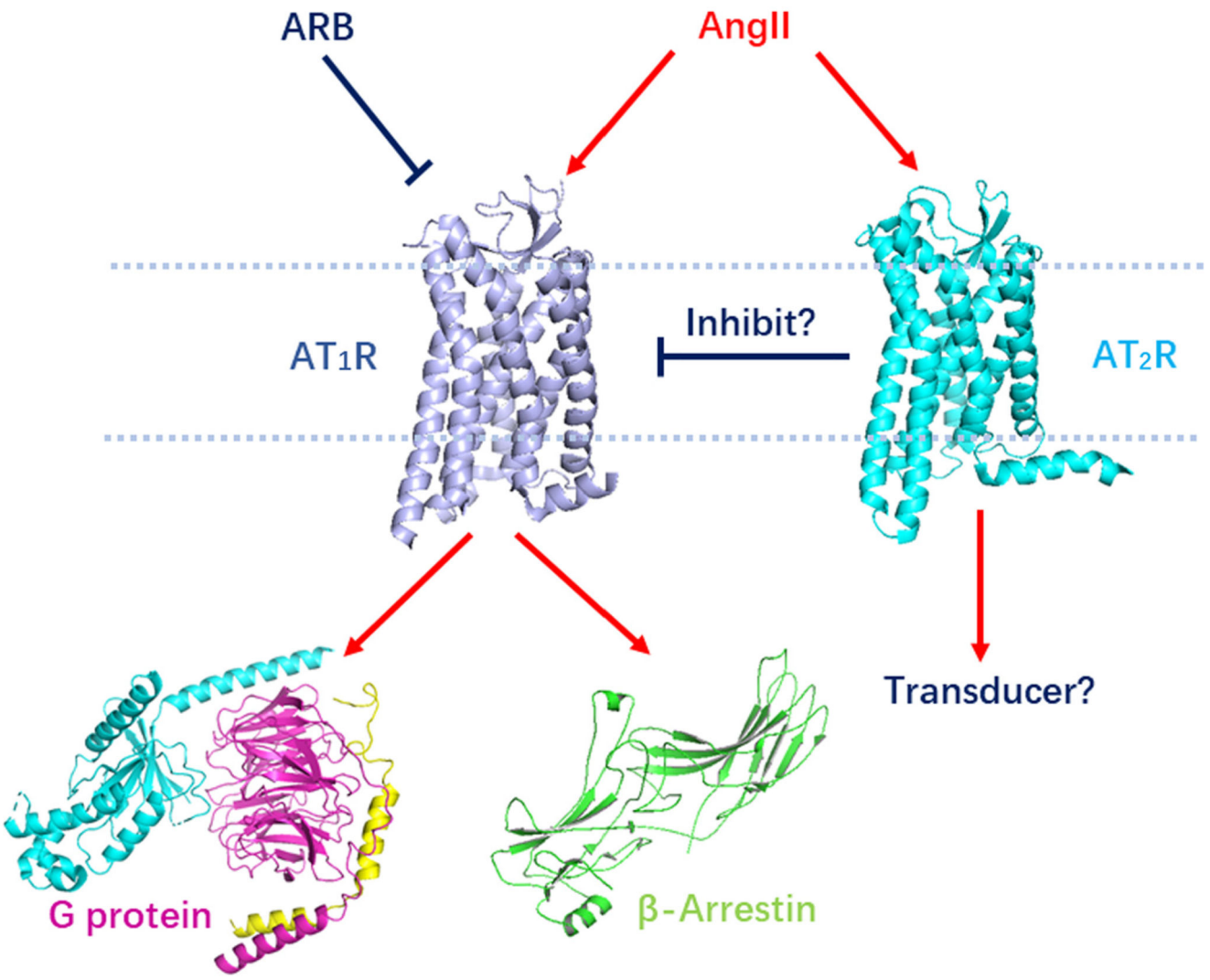

Figure 1. AT_1R/AT_2R signaling pathways.

AngII isthenatural ligand of AT_1R and AT_2R. AngII activates AT_1R that signals through Gq protein and β arrestin, while the transducer of AT_2R is still elusive. ARBs inhibit AT_1R by blocking the AngII binding, while AT_2R putatively inhibit AT_1R by forming inactive AT_1R–AT_2R heterodimer. Abbreviations: AngII, angiotensin II; ARB, angiotensin receptor blocker; AT_1R, angiotensin 1 receptor; AT_2R, angiotensin 2 receptor.

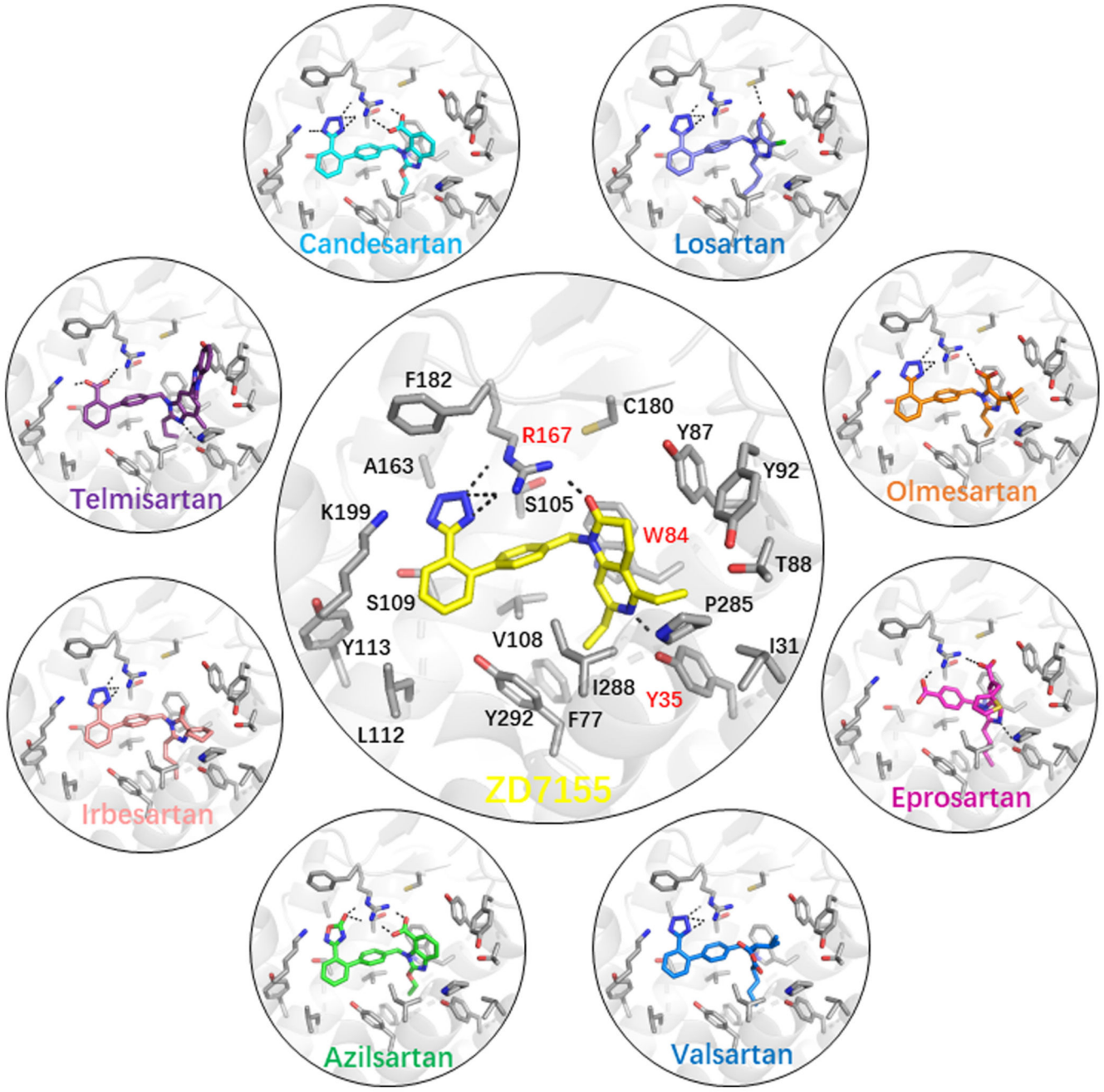

Figure 2. Binding of angiotensin receptor blockers (ARBs) to angiotensin 1 receptor (AT_1R).

The crystal structure of AT_1R in complex with ZD7135 (central large circle) revealed key anchoring interactions of the ligand with three critical residues $Arg167^{ECL2}$, $Tyr35^{1.39}$, and $Trp84^{2.60}$, as labelled in red. Docking of various ARBs in the AT_1R crystal structure (small circles) demonstrated that they bind in the orthosteric ligand-binding pocket in similar poses by forming extensive interactions with the same three anchoring residues as well as with other common and specific residues. All ligand-receptor hydrogen bonds and ionic interactions are shown as broken lines.

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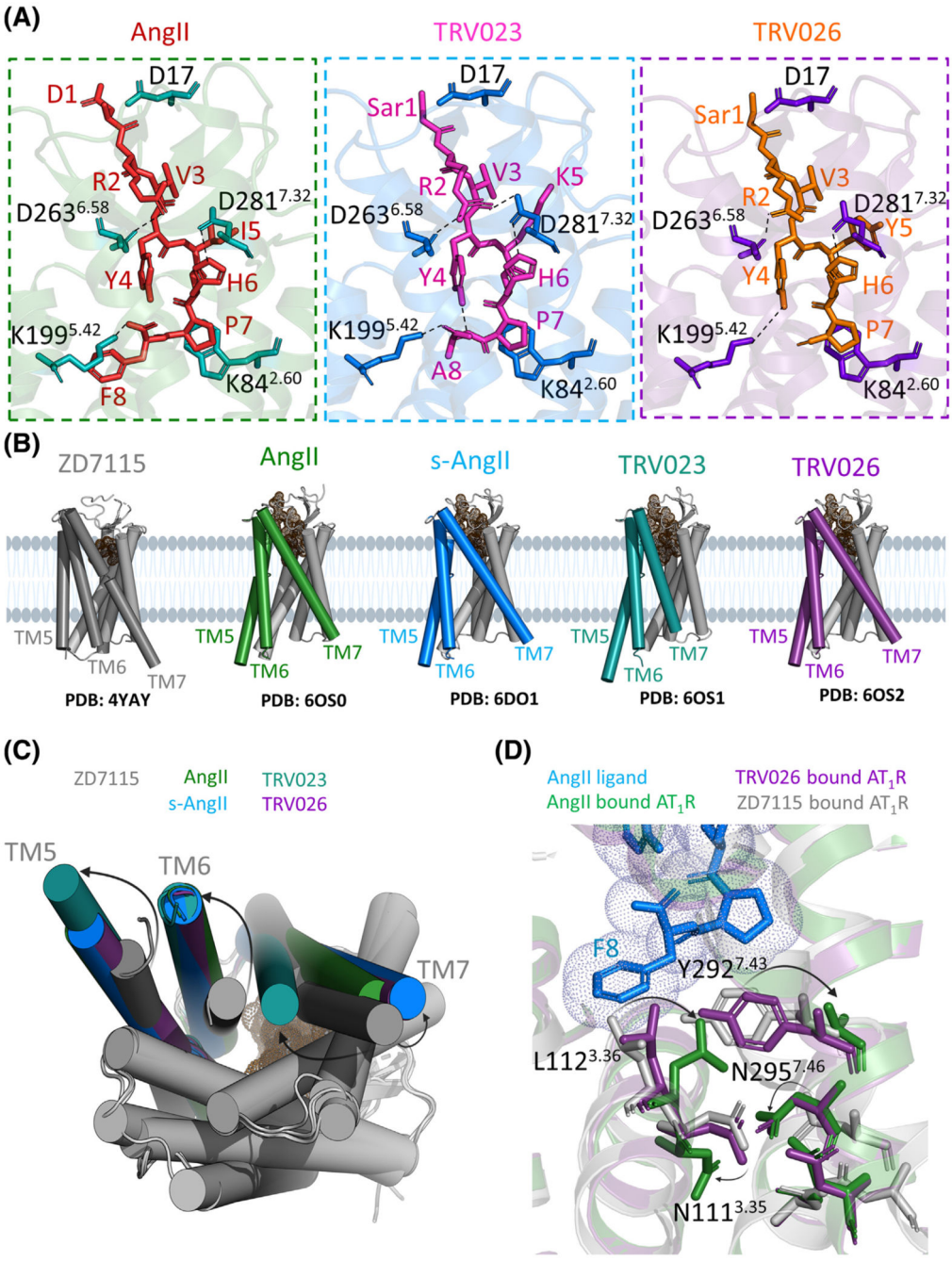

Figure 3. Molecular mechanisms of AT_1R activation and biased signaling.

(A) Structural details of peptide ligand binding to AT_1R: endogenous agonist AngII (PDB ID 6OS0) and βarr-biased ligands TRV023 (PDB ID 6OS1) and TRV026 (PDB ID 60S2). The structures show key residues interacting with respective peptides. Overall, all peptide ligands show similar placement of their peptide backbone in the receptor orthosteric pocket and similar interactions with key residues including D17, $K84^{2.60}$, $D263^{6.58}$, and $D281^{7.32}$. (B) Agonist binding induces large conformational changes in TM5, TM6, and TM7 as highlighted in specific colors for different ligands. (C) An overlay of AT_1R structures bound

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to different ligands viewed from the intracellular side. Importantly, TM6 and TM5 move out of the receptor core whereas TM7 moves inward compared to AT_1R bound to antagonist ZD7115 (PDB ID 4YAY). For agonists, AngII and s-AngII, TM6 shows maximal movement, while TM5 moves the least. In case of biased agonists, TRV023 and TRV026, it is TM7 that shows the highest diversity in its movement pattern, which is distinct for each biased agonist. For TRV023, TM7 shows maximal inward movement, whereas for other ligands it drifts away in the opposite direction at different magnitudes. (D) Conformational switches induced by ligand binding. The full endogenous agonist AngII (blue sticks) induces an inward movement of $L112^{3.36}$ and $N295^{7.46}$ and an outward movement of $N111^{3.35}$ and $Y292^{7.43}$ (green sticks) with respect to their conformations in AT_1R-ZD7155 (grey sticks). Surprisingly, in case of TRV026 (purple sticks), $L112^{3.36}$, $N111^{3.35}$, and $Y292^{7.43}$ show very little if any conformational changes compared to AT_1R-ZD7155 residues (gray sticks). It is the reorientation of $N295^{7.46}$ that is critical and seems to be sufficient to put AT_1R in a conformation that can accommodate βarr as seen in the AT_1R crystal structures bound to various βarr-biased agonists. Abbreviations: AngII, angiotensin II; AT_1R, angiotensin 1 receptor; βarr, β arrestin; s-AngII, [Sar^1,Ile^8]-angiotensin II; TM, transmembrane helix.

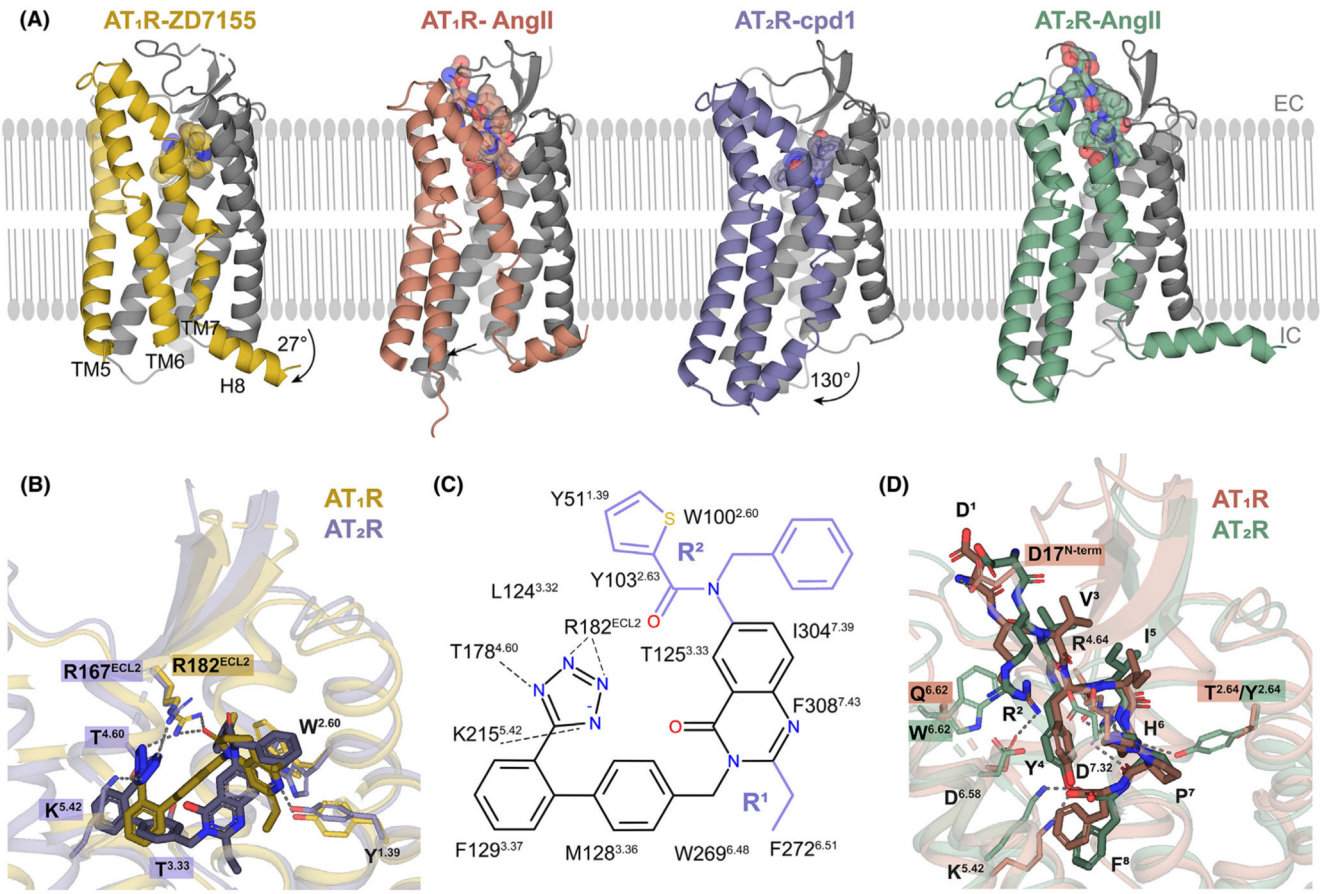

Figure 4. Structural details of AT_1R/AT_2R selectivity.

(A) Overall conformations of AT_1R bound to antagonist ZD7155 (yellow, PDB ID 4YAY) and to endogenous agonist AngII (red, PDB ID 6OS0) and of AT_2R bound to small molecule antagonists Compound 1 (purple, PDB ID 5UNG) and to endogenous agonist AngII (green, PDB ID 6JOD). Largest conformational changes are observed for TM5–7 and H8, highlighted in different colors. (B) Distinct binding modes for small-molecule antagonist binding to AT_1R (yellow, PDB ID 4YAY) versus AT_2R (purple, PDB ID 5UNG). (C) Schematic diagram of AT_2R interactions with a small-molecule antagonist. Chemical structure of Compound 1 contains a common quinazolinone–biphenyl–tetrazole structure–activity relationship scaffold with two substituent groups, R^1 and R^2, colored in purple. (D) Comparison of AngII binding to AT_1R (red, PDB ID 6OS0) versus AT_2R (green, PDB ID 6JOD). Abbreviations: AngII, angiotensin II; AT_1R, angiotensin 1 receptor; AT_2R, angiotensin 2 receptor; TM, transmembrane helix.

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Table 1

Available crystal structures of AT_1R and AT_2R

	Receptor subtype	PDB ID	Resolution, Å	Ligand	Ligand type	Receptor State	Fusion partner	Complex partner	Refs
1	AT_1R	4YAY	2.9	ZD7155	Small-molecule antagonist	inactive	N-term BRIL		[15]
2	AT_1R	4ZUD	2.8	Olmesartan	Small-molecule antagonist	inactive	N-term BRIL		[18]
3	AT_1R	6DO1	2.9	s-AngII	Peptide partial agonist	active	ICL3 BRIL	Nanobody AT110i1	[20]
4	AT_1R	6OS2	2.7	TRV026	Peptide-biased agonist	active	ICL3 BRIL	Nanobody AT110i1le	[21]
5	AT_1R	6OS1	2.8	TRV023	Peptide-biased agonist	active	ICL3 BRIL	Nanobody AT110i1le	[21]
6	AT_1R	6OS0	2.9	AngII	Peptide endogenous agonist	active	ICL3 BRIL	Nanobody AT110i1	[21]
7	AT_2R	5UNG	2.8	Comp 1	Small-molecule antagonist, AT_2R selective	active-like	N-term BRIL		[30]
8	AT_2R	5UNF	2.8	Comp 1	Small-molecule antagonist, AT_2R selective	active-like	N-term BRIL		[30]
9	AT_2R	5UNH	2.9	Comp 2	Small-molecule antagonist, dual AT_1R/AT_2R	active-like	N-term BRIL		[30]
10	AT_2R	5XJM	3.2	s-AngII	Peptide partial agonist	active	ICL3 BRIL	Fab 4A03	[32]
11	AT_2R	6JOD	3.2	AngII	Peptide endogenous agonist	active	N-term BRIL	Fab 4A03	[33]
12	AT_2R	7C6A	3.4	s-AngII	Peptide partial agonist	active	ICL3 BRIL	Fab SRP2070	[42]