Current Literature June 10, 2017

Ruyin Cao – Wipf group

Structural basis for selectivity and diversity in angiotensin II receptors

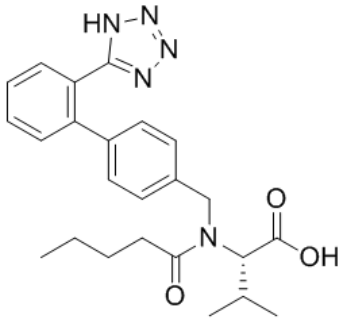
Zhang, H., Han, G.W., Batyuk, A., Ishchenko, A., White, K.L., Patel, N., Sadybekov, A., Zamlynny, B., Rudd, M.T., Hollenstein, K., Tolstikova, A., White, T.A., Hunter, M.S., Weierstall, U., Liu, W., Babaoglu, K., Moore, E.L., Katz, R.D., Shipman, J.M., Garcia-Calvo, M., Sharma, S., Sheth, P., Soisson, S.M., Stevens, R.C., Katritch, V., Cherezov, V.

Nature 2017, 544, 327-332

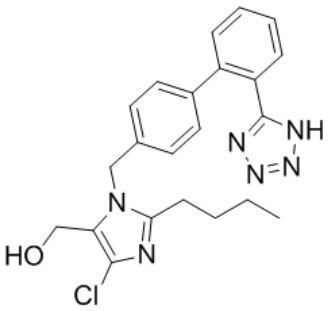
Well-characterized AT_1R

- Angiotensin II is a octapeptide hormone whose effects are mediated by two types of receptors, AT_1R and AT_2R.

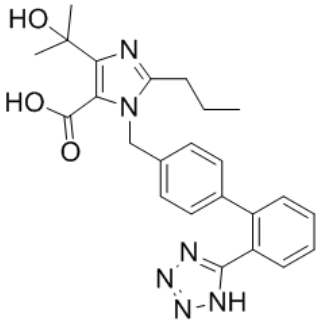
- Activation of AT_1R leads to vasoconstriction, aldosterone release that tend to elevate blood pressure and cause hypotrophy and hyperplasia. Several antagonists and inverse agonists of AT_1R have been approved for clinical use as anti-hypertensive drugs.



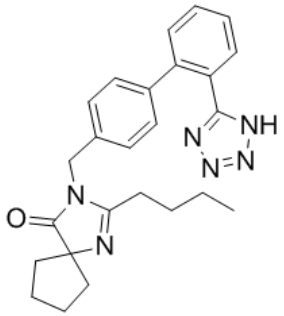
valsartan



losartan



olmesartan



irbesartan

Nature Reviews Drug Discovery 2002,1, 621-636

Enigmatic AT_2R as drug target

- Growing number of studies suggest that AT_2R signals primarily via non-canonical, G-protein and β -arrestin-independent pathways[1,2].

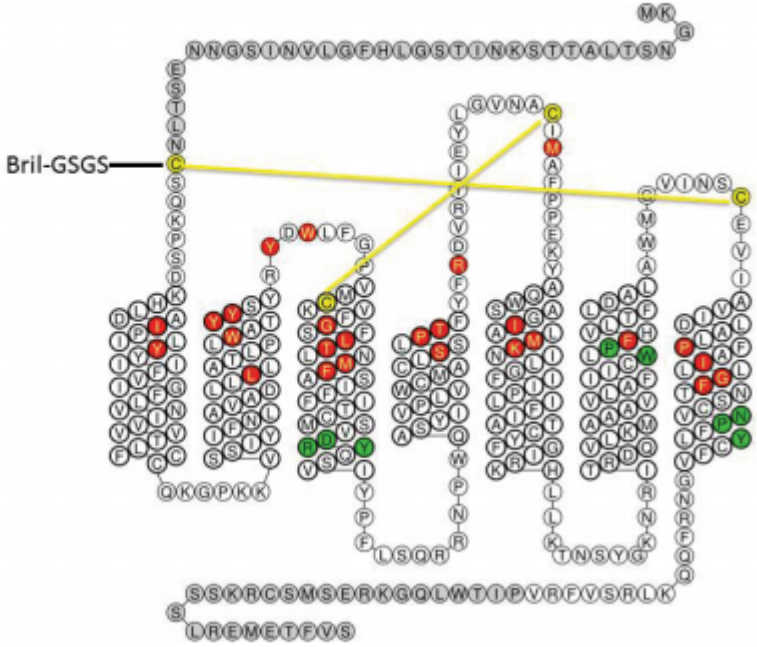
- AT_2R has been reported to counteract several AT_1R-mediated effects in the cardiovascular system. For example, in the vasculature, AT_2R has been suggested to counter-balance blood pressure increases exerted by AT_1R[3].

- In the central nervous system, activation of AT_2R in nociceptive neurons is observed to induce neurite outgrowth and elongation[4].

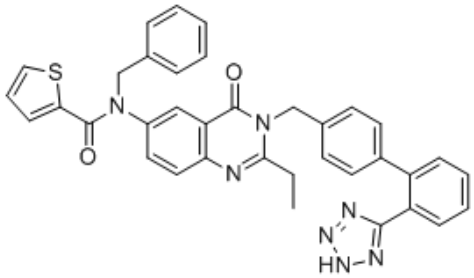
1. Font. Biosci. 2009, 14, 958-972
2. Font. Endocrinol. 2012, 3, 164
3. Sci. STKE 2003, 2003, pe16
4. Int. J. Hypertens. 2012, 2012,351758

Overview of this work

With the aim of understanding the structural basis for the functional role of AT2R receptor, Zhang et. al. determined crystal structures of engineered AT2R bound to two high-affinity ligands using X-ray free electron laser (XFEL).

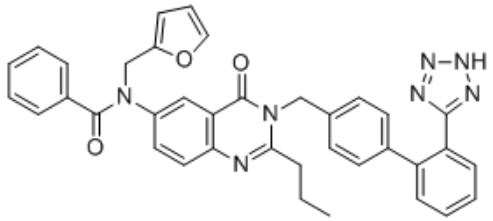


AT2R snake diagram



Compound 1
AT2R selective

PDBid: 5UNF (2.8 Å)
PDBid: 5UNG (2.8 Å)

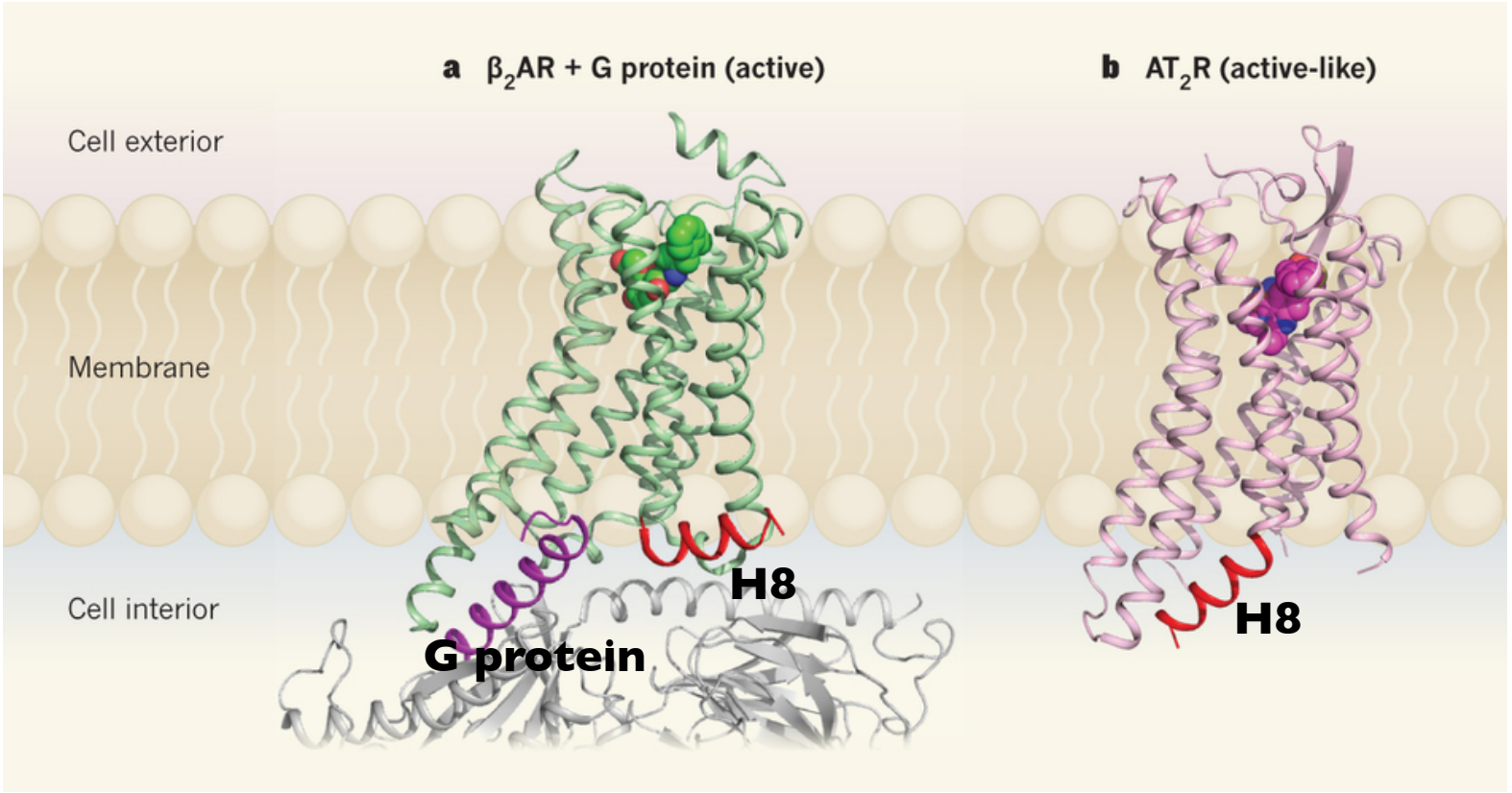


Compound 2
AT1R/AT2R dual active

PDBid: 5UNH (2.9 Å)

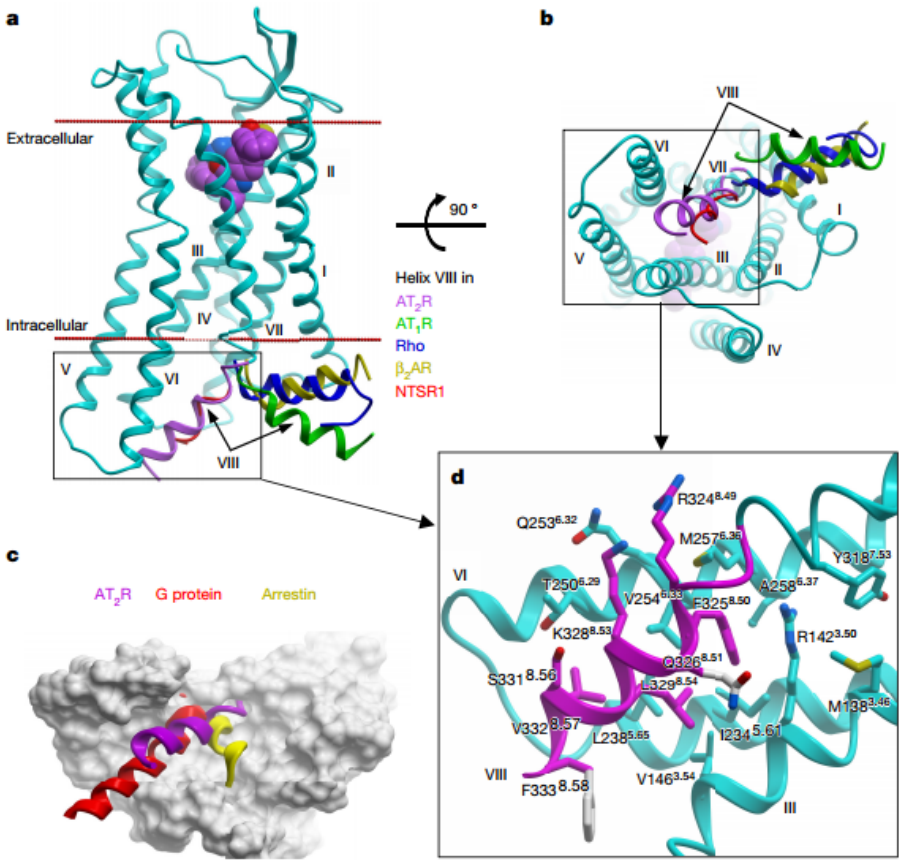
Highlight 1 of this work:

AT2R may be one receptor that blocks itself

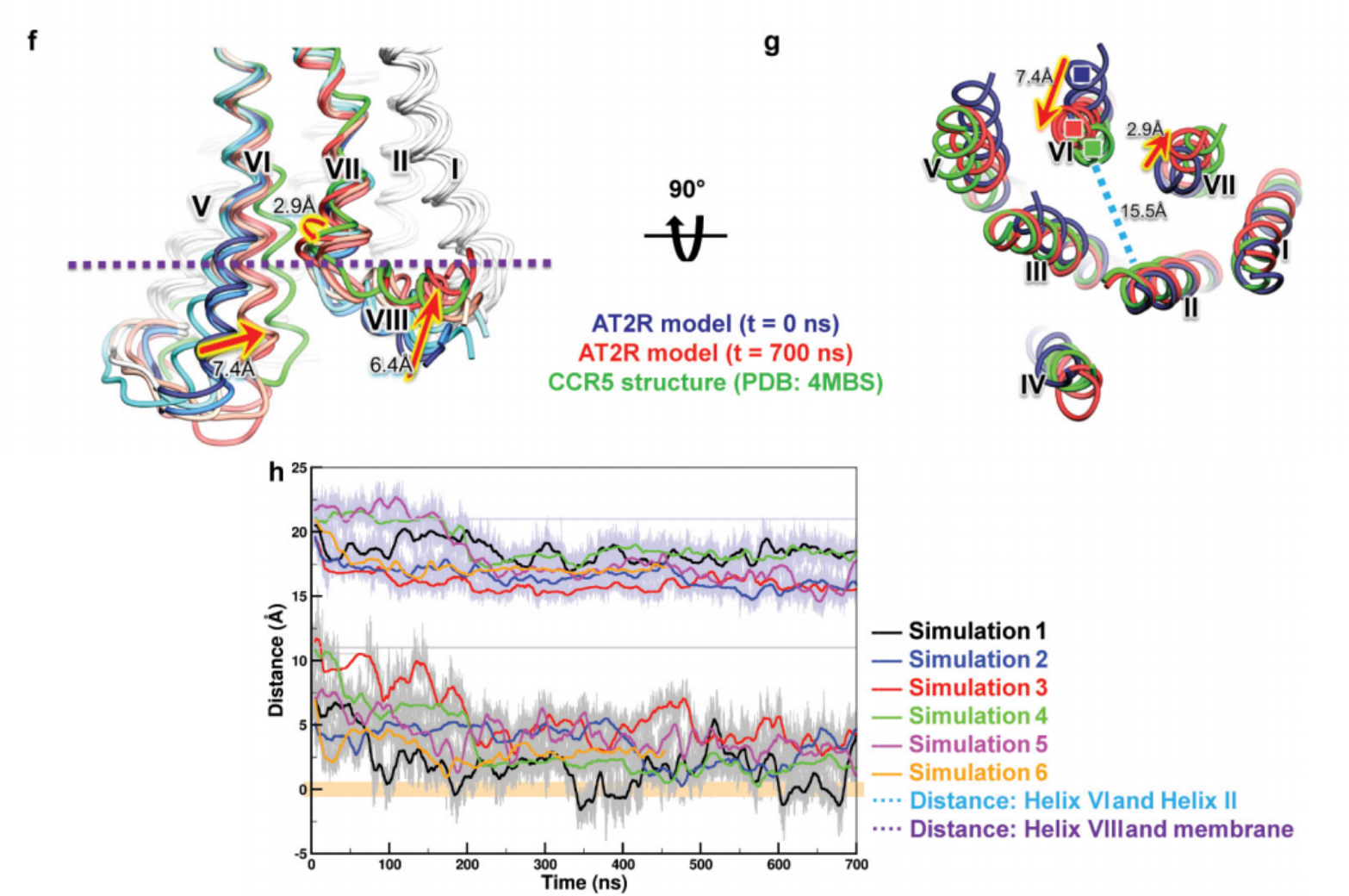


Structural comparison between active β2AR structure (PDBid: 3SN6) and active-like AT2R structure (PDBid: 5UNH)

H8 blocks putative G protein/ β -arrestin binding site



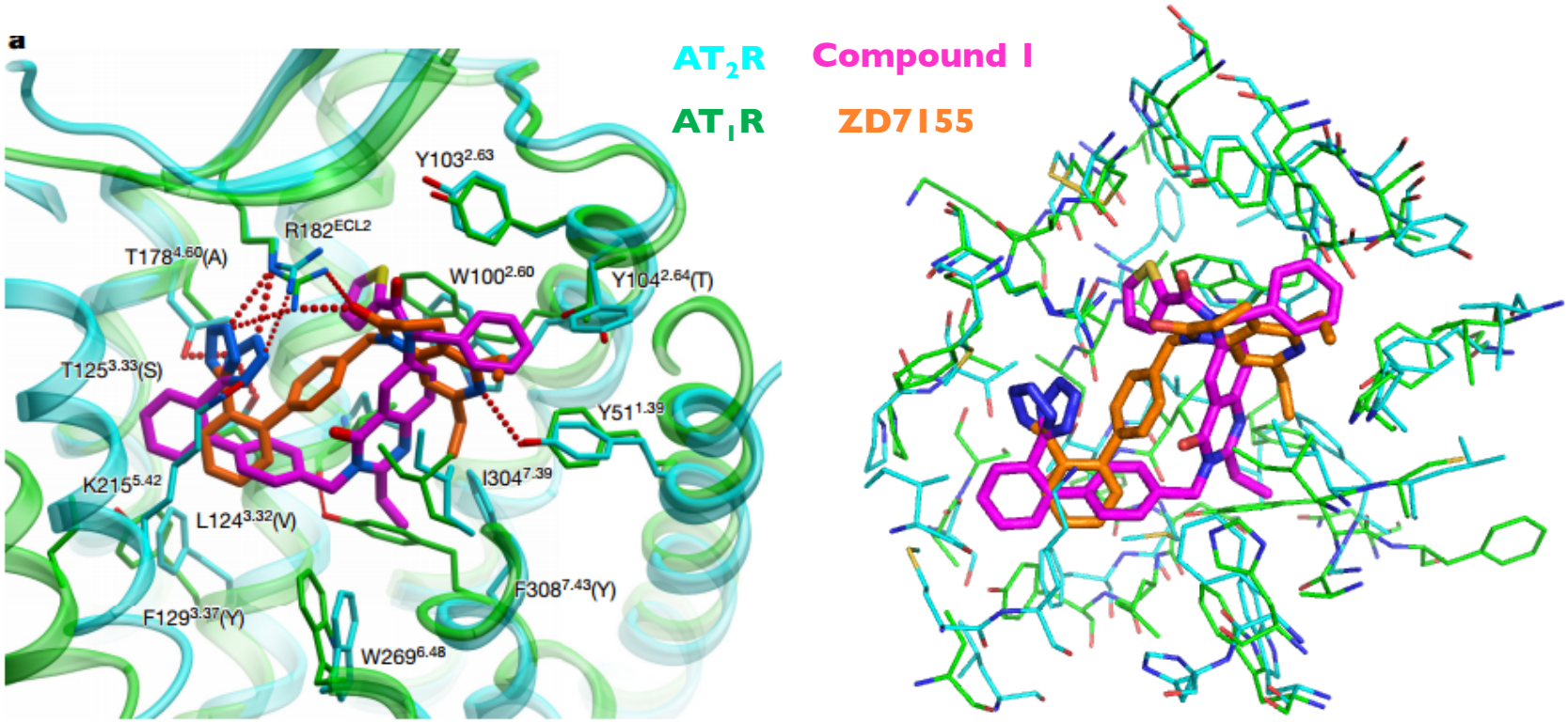
This non-canonical conformation of H8 is consistent with the lack of robust downstream signaling by AT2R as assessed by traditional G protein and β -arrestin assays.



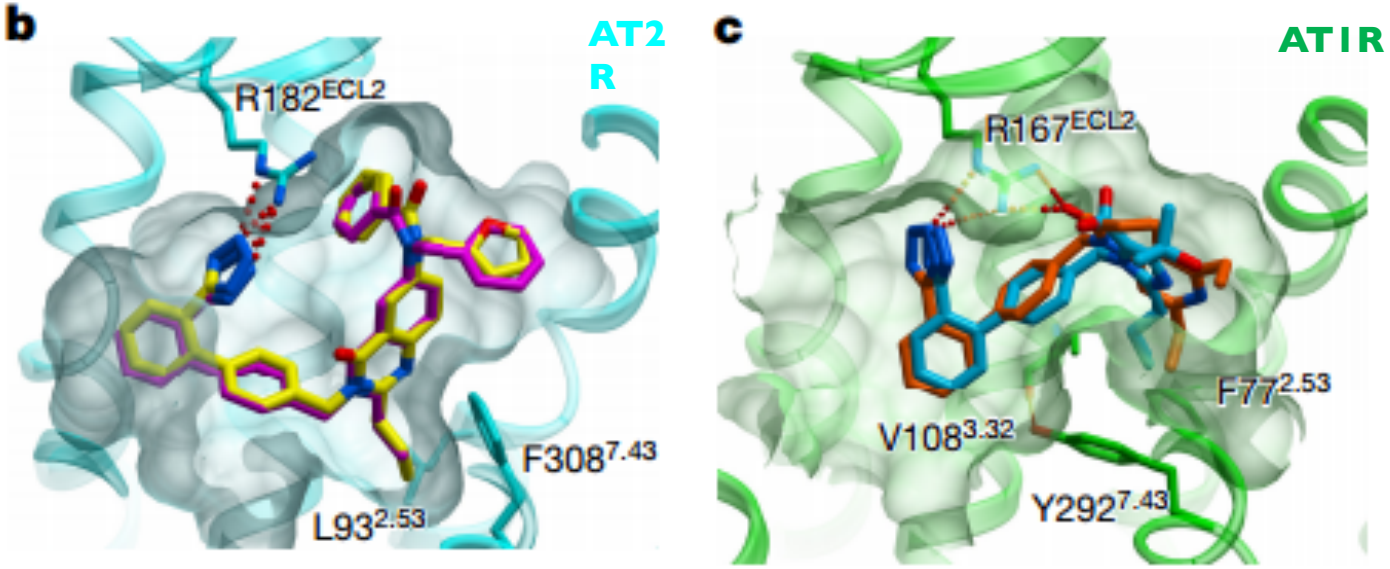
MD simulations indicate relaxation of H8 into canonical membrane-bound conformation is accompanied by an inward shift of the intracellular tip of H6 toward H2.

Highlight 2 of this work:

Orthosteric binding sites of AT_1R and AT_2R are structurally different



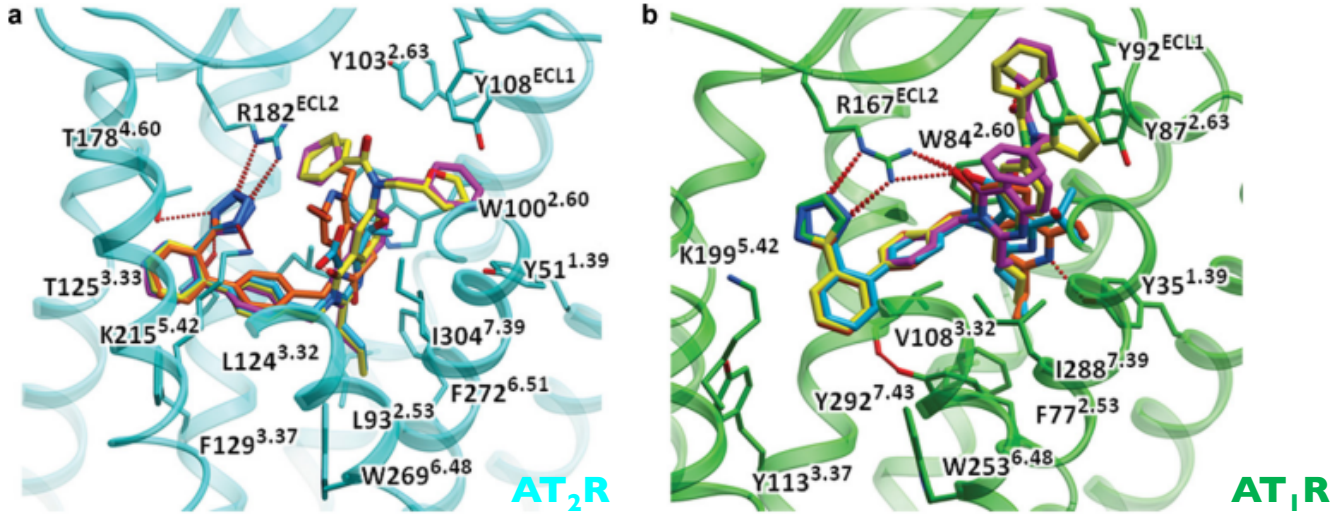
48% of residues within 6 Å of Compound 1 in AT_2R are not conserved in AT_1R



Compound	AT1R Ki (nM)	AT2R Ki (nM)
Compound 1	180	0.34
Compound 2	3.7	0.35
Olmesartan	5.3	N/A
ZD7155	3.0	N/A

AT1R-Olmesartan binding pose : 4ZUD (2.8 Å)
AT1R-ZD7155 binding pose: 4YAY (2.9 Å)

Ligand Cross docking in (in)active AT_1R/AT_2R structures



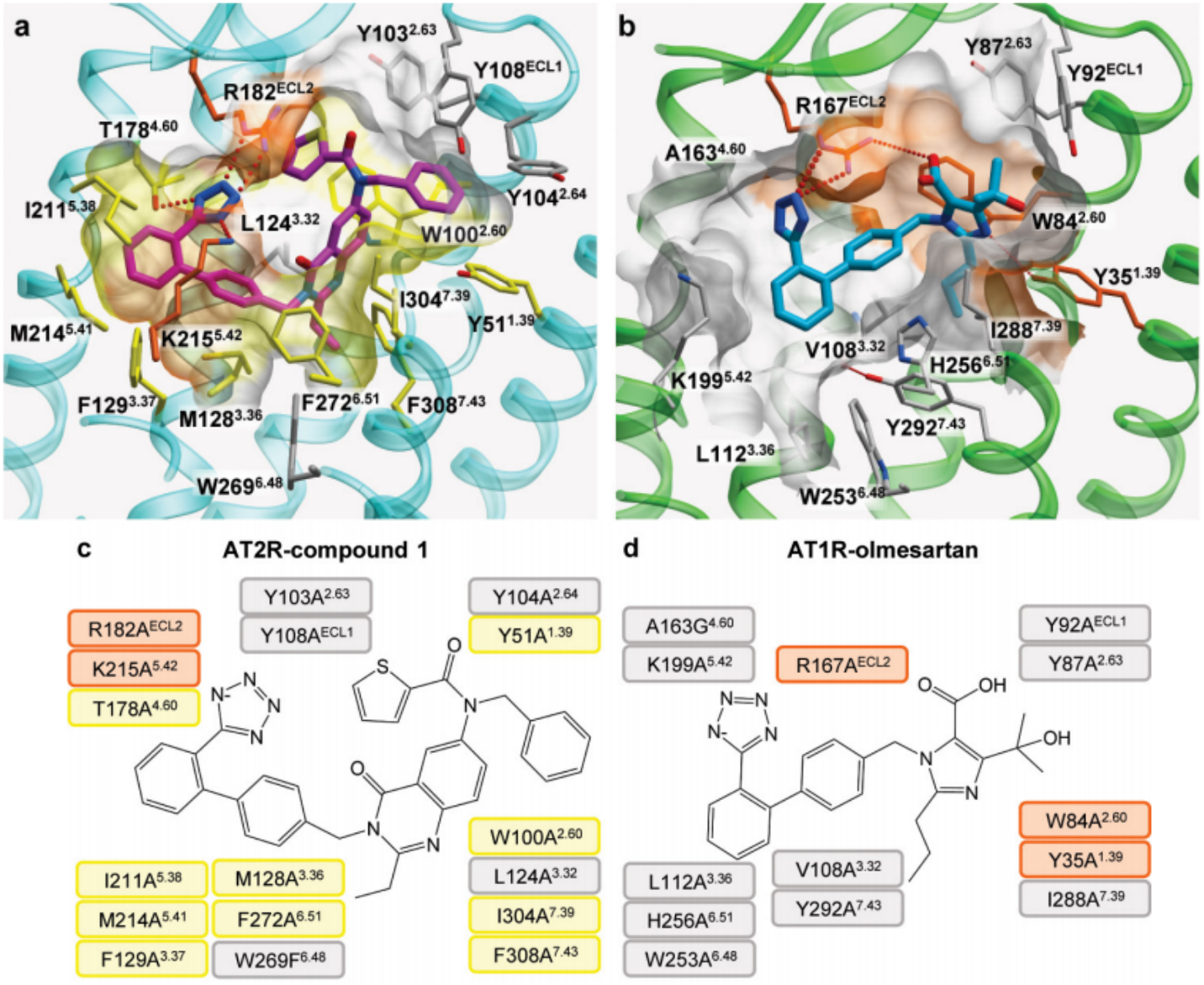
Compound	Inactive AT_1R docking score (kJ/mol)	Active AT_1R docking score (kJ/mol)	Inactive AT_2R docking score (kJ/mol)	Active AT_2R docking score (kJ/mol)
Compound 1	-30	-21	-44	-39
Compound 2	-33	N/B	-43	-41
Olmesartan	-33	N/B	-27	-31
ZD7155	-36	-19	-19	-19

Homology models

Ligand-binding pocket mutations validate structures

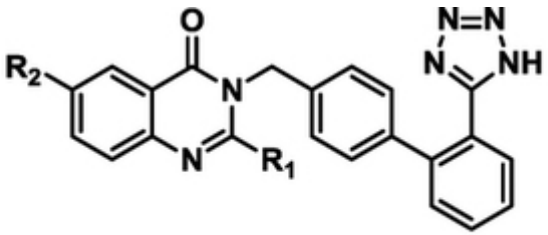
Mutation	logKd, AngII	logKi, Cpd 1	logKi, Cpd 2
Wild type	-9.32 ± 0.25 (n=5)	-9.15 ± 0.12 (n=3)	-9.43 ± 0.13 (n=3)
Y51A	-8.25 ± 0.09 (n=3)	-7.90 ± 0.08 (n=3)	-7.65 ± 0.09 (n=3)
W100A	-8.91 ± 0.09 (n=3)	-7.92 ± 0.08 (n=3)	-8.27 ± 0.07 (n=3)
Y103A	-9.45 ± 0.19 (n=2)	-8.65 ± 0.10 (n=3)	-8.94 ± 0.12 (n=3)
Y103F	-8.76 ± 0.13 (n=3)	-7.81 ± 0.08 (n=3)	-8.15 ± 0.10 (n=4)
Y104A	-9.35 ± 0.18 (n=3)	-8.62 ± 0.08 (n=3)	-9.09 ± 0.07 (n=3)
Y108A	-9.13 ± 0.16 (n=2)	-8.58 ± 0.09 (n=3)	-8.81 ± 0.12 (n=3)
L124A	-9.49 ± 0.29 (n=2)	-8.53 ± 0.05 (n=3)	-8.73 ± 0.07 (n=3)
M128A	-8.74 ± 0.08 (n=3)	-7.60 ± 0.06 (n=3)	-7.68 ± 0.07 (n=3)
F129A	-8.89 ± 0.11 (n=3)	-7.94 ± 0.10 (n=3)	-7.99 ± 0.11 (n=3)
T178A	-9.55 ± 0.03 (n=4)	-8.57 ± 0.07 (n=4)	-8.57 ± 0.08 (n=4)
R182A	No binding	N/A	N/A
R182K	-9.31 ± 0.20 (n=3)	-7.71 ± 0.08 (n=3)	-8.17 ± 0.08 (n=3)
I211A	-9.40 ± 0.05 (n=3)	-8.03 ± 0.08 (n=3)	-8.12 ± 0.08 (n=3)
M214A	-9.24 ± 0.16 (n=2)	-8.08 ± 0.09 (n=3)	-8.40 ± 0.13 (n=3)
K215A	No Binding	N/A	N/A
K215Q	No Binding	N/A	N/A
W269F	-9.77 ± 0.08 (n=3)	-8.50 ± 0.14 (n=3)	-9.29 ± 0.13 (n=3)
F272A	-9.35 ± 0.21 (n=3)	-7.94 ± 0.11 (n=3)	-7.71 ± 0.08 (n=3)
I304A	-9.40 ± 0.29 (n=2)	-7.69 ± 0.06 (n=2)	-7.41 ± 0.05 (n=2)
F308A	-9.45 ± 0.31 (n=3)	-7.92 ± 0.07 (n=3)	-7.36 ± 0.06 (n=3)

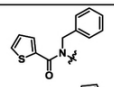
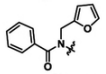
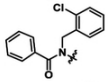
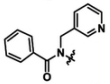
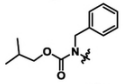
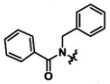
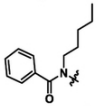
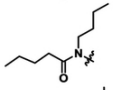
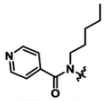
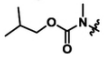
Effects of single residue mutations in the AT_2R ligand-binding pocket on the ligand binding affinities


a
Y103^2.63
Y108^ECL1
R182^ECL2
T178^4.60
Y104^2.64
I211^5.38
L124^3.32
W100^2.60
I304^7.39
Y51^1.39
M214^5.41
K215^5.42
F129^3.37
M128^3.36
F272^6.51
F308^7.43
W269^6.48
b
Y87^2.63
Y92^ECL1
R167^ECL2
A163^4.60
W84^2.60
Y35^1.39
I288^7.39
V108^3.32
H256^6.51
K199^5.42
Y292^7.43
L112^3.36
W253^6.48
c
AT2R-compound 1
Y103A^2.63
Y104A^2.64
R182A^ECL2
Y108A^ECL1
Y51A^1.39
K215A^5.42
T178A^4.60
W100A^2.60
L124A^3.32
I211A^5.38
M128A^3.36
I304A^7.39
M214A^5.41
F272A^6.51
F308A^7.43
F129A^3.37
W269F^6.48
d
AT1R-olmesartan
A163G^4.60
Y92A^ECL1
K199A^5.42
R167A^ECL2
Y87A^2.63
W84A^2.60
Y35A^1.39
L112A^3.36
V108A^3.32
I288A^7.39
H256A^6.51
Y292A^7.43
W253A^6.48

SAR of compound 1 analogues

R1 substituent is crucial for high AT_2R selectivity
R2 substituent is crucial for high AT_1R selectivity



Compound	R_1	R_2	AT_2R K_i (nM)*	AT_1R K_i (nM)*	AT_2R fold selectivity
1	Ethyl		0.34 ± 0.06	184 ± 50	530x
2	*n*-Propyl		0.35 ± 0.05	3.72 ± 0.03	11x
3	Methyl		1.7 ± 0.4	700 ± 200	410x
4	*n*-Propyl		0.65 ± 0.01	1.8 ± 0.2	2.8x
5			11.5 ± 0.5	37 ± 3	3.2x
6			120 ± 50	450 ± 20	3.8x
7	*n*-Propyl		1.7 ± 0.5	10.4 ± 1.7	6.1x
8	*n*-Propyl		10.9 ± 0.1	9.90 ± 0.01	0.9x
9	*n*-Propyl		1,790 ± 150	1.6 ± 0.1	0.001x
10	*n*-Propyl		4.9 ± 0.2	12.9 ± 2.8	2.6x
11	*n*-Propyl		4.1 ±0.8	6.7 ± 0.9	1.6x
12	*n*-Propyl		18.8 ± 0.1	17.3 ± 4.3	0.9x
13	*n*-Propyl		2,990 ± 80	16.7 ± 10.4	0.006x
14	Methyl		5,300 ± 2,400	360 ± 80	0.07x

Conclusion

- The non-canonical conformation of H8 in the active-like AT2R might provide potential explanations for its poor coupling to G proteins and β-arrestins. H8 may play a dual role in the modulation of AT2R function. On the one hand, upon adopting the X-ray conformation it may stabilize the active-like state while repressing the activity and signaling. On the other hand, upon switching to membrane-bound conformation, H8 can support the recruitment of G proteins and β-arrestins.

- Both AT1R and AT2R are important drug targets. Blockade of AT1R has anti-hypertensive effects while modulation of AT2R could be useful for cardioprotection and neuropathic pain. The differently shaped ligand binding pockets of the two receptors may open the avenue to design selective ligands for individual receptor target.