

## Supplementary Information

### Pyrazolo-triazolo-pyrimidine Scaffold as a Molecular Passepartout for the Pan-Recognition of the Human Adenosine Receptors

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**Table S1.** Sequence identity and similarity among hA<sub>1</sub>, hA<sub>2A</sub>, hA<sub>2B</sub> and hA<sub>3</sub> ARs. Full-sequence data are reported on the lower left (white background cells), and transmembrane helices (TM) data on the upper right (grey background cells).

	A1AR	A2A AR	A2B AR	A3 AR
A1AR	-	I 55%, S 70%	I 52%, S 70%	I 56%, S 77%
A2A AR	I 38%, S 48%	-	I 66%, S 82%	I 47%, S 66%
A2B AR	I 43%, S 59%	I 45%, S 56%	-	I 45%, S 64%
A3 AR	I 46%, S 65%	I 30%, S 45%	I 35%, 53%	-

Data were retrieved from the GPCRdb web site (<https://gpcrdb.org>).

**Figure S1**

P29275   AA2BR_H 4EIY_prep	..... MLETQDALYVALELVIAALSVAAGNVLCVAVGTANTLQTPNTNYFLVSLAAADVAVGLFAIPFAITISLGFCTDFYGGCLFLACFVLVLTQSSIFSLAVAVDRYLAI --XPIMGSSVYITVELAIAVLAAILGNVLVCWAVLNSNLQNVNIFYVVSLSAAADIAGVGLAIPFAITISLGFCAACHGCLFIACFVLVLTQSSIFSLAIAIDRYIAI
P29275   AA2BR_H 4EIY_prep	..... CVPLRYKSLVTGRARGVIAVLWVLAIFGIGLTPPLGWNSKDSATNCTEPWDGTTNESCCLVKCLFENVPMSYMYVFNFFGCVLPPLLIMLVIIKIFLVACRQLQ RIPLRYNGLVTGRAKGIIAICWVLSFAIGLTPMLGWN-----EGQVACLFEVDVPMNYMVYFNFFACVLPVLLMLGVYLRIFLAARRQLK
P29275   AA2BR_H 4EIY_prep	..... RTELM---DHSRTTLQREIHAAKSLAMIVGIFALCWLPHVAVNCVTLFQPAQGNPKWAMNMAILSHANSVVNPVIVAYRNRDFRYTFHKIISRYLLCQADVKS QMESQPLPGERARSTLQKEVHAAKSLAIVGLFALCWLPHIINCFTFFCPDCS-HAPLWLMYLAIVLSHTNSVNVNPFIVAYRIREFRQTFRKIIIRSHVX
P29275   AA2BR_H 4EIY_prep	..... GNGQAGVQPALGVGL

**Figure S1.** Sequence alignment between hA<sub>2B</sub> AR and hA<sub>2A</sub> AR, from structure 4EIY (after reconstruction of missing atoms and retro mutation to wild-type). Extracellular loop 2 was removed before the alignment. Alignment used for homology modeling.

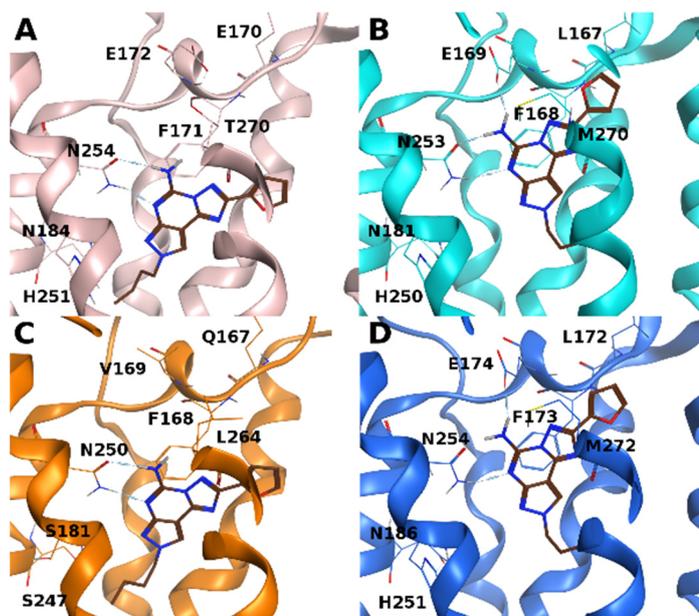
**Figure S2**

PODMS8   AA3R_H 5UEN_prep	..... MPNNSALSLANVTYITMEIFIGLCAIVGNVLVICVVKLNPSLQTTFFYFIVSLALADIAGVGLVMPPLAIVVSLGITIHFYSCLFMTCLLLIFTHASIMSLAIAVDRYL -----XISAFQAYIGIEVLIALVSPGNVLVIHAVKVNQALRDATFCFIVSLAVADVAVGLVPLAAILINIGPQTYFHTCLMVACPVLLITQSSILALLAIAVDRYL
PODMS8   AA3R_H 5UEN_prep	..... RVKLTVRYKRVVTHRRWLALGLCWLVSFLVGLTPMFGWN--MKLTSEYHRN---VFFLSCQFVSVMRMDYHVYFSFLTWIFPLVVMCAIYLDIFYIIRKLSLMLSN RVKIPLRYKRVVTPRRAAVAIAGCWILSFVVGGLTPMFGWNNLSAVERAWAANGSMGEPVIKCEFEKVISMEYHMYVFNFFVWVLPVLLMLVLIYLEVFIYLRKQLNKKVSA
PODMS8   AA3R_H 5UEN_prep	..... SK-ETGAFYGREPKTAKSLFVLFVLFALSWLPLSIINCIYFNG--EVPQLVLYMGILLSHANSMMNPVIVAYKIKKFKETYLLILKACVCHPSDSLDTSIERNSE SSGDPQRYYGKELKIAKSLALILFLFALSWLPLHILNCITLPCPSCHKPSILTYIAIFLTHGNSAMNPVIVAFRIQKFRVTFKLIWNDFRCQFX

**Figure S2.** Sequence alignment between hA<sub>3</sub> AR and hA<sub>1</sub> AR, from structure 5UEN (after reconstruction of missing atoms and retro mutation to wild-type). Alignment used for homology modeling.



**Figure S5**



**Figure S5.** Docking pose of compound **11** (brown) at hA<sub>1</sub> (pink, **A**), hA<sub>2A</sub> (cyan, **B**), hA<sub>3</sub> (orange, **C**), hA<sub>2B</sub> (blue, **D**) ARs structures. The following receptor 3D structures were employed: experimental X-ray for hA<sub>1</sub> (PDB IDs: 5UEN) and hA<sub>2A</sub> ARs (PDB IDs: 4E1Y), homology models for hA<sub>2B</sub> and hA<sub>3</sub> ARs (built on hA<sub>2A</sub> and hA<sub>1</sub>, respectively). A docking pose alternative to the ZM-241385-like one is displayed here.

**Video S1.**

Selected docking poses for compounds **1-12** (magenta) at hA<sub>1</sub> AR (pink) structure (PDB ID: 5UEN). Two alternative binding modes are shown for compounds **8, 9, 11** (z = ZM-241385-like; a = alternative). Compounds **8-12** were docked at the X-ray structure, compounds **1-7** were docked at the same structure refined by induced fit docking of compound **4**.

**Video S2.**

Selected docking poses for compounds **1-12** (magenta) at hA<sub>2A</sub> AR (cyan) structure (PDB ID: 4E1Y). Two alternative binding modes are shown for compounds **8, 9, 10, 11, 12** (z = ZM-241385-like; a = alternative). Compounds **8-12** were docked at the X-ray structure, compounds **1-7** were docked at the same structure refined by induced fit docking of compound **4**.

**Video S3.**

Selected docking poses for compounds **1-12** (magenta) at hA<sub>2B</sub> AR (blue) structure (model built using hA<sub>2A</sub> AR structure 4E1Y as template). Two alternative binding modes are shown for compounds **8, 9, 10, 11, 12** (z = ZM-241385-like; a = alternative). Compounds **8-12** were docked at the homology model, compounds **1-7** were docked at the same structure refined by induced fit docking of compound **4**.

**Video S4.**

Selected docking poses for compounds **1-12** (magenta) at hA<sub>3</sub> AR (orange) structure (model built using hA<sub>1</sub> AR structure 5UEN as template). Two alternative binding modes are shown for compounds **8, 9, 11, 12** (z = ZM-241385-like; a = alternative). Compounds **8-12** were docked at the homology model, compounds **1-7** were docked at the same structure refined by induced fit docking of compound **4**.