Selectivity is species-dependent: characterization of standard agonists and antagonists at human, rat and mouse adenosine receptors

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Table 1 The gene and protein accession numbers of the adenosine receptors in human, rat and mouse.

	Gene accession number	**Protein accession number**
mA_1	NM_001008533	NP_001008533.1
rA_1	NM_017155.2	NP_058851.2
hA_1	NM_000674	NP_000665.1
mA_{2A}	NM_009630	NP_033760.2
rA_{2A}	NM_053294.3	NP_445746.3
hA_{2A}	NM_001278497	NP_001265426.1
mA_{2B}	NM_007413	NP_031439.2
rA_{2B}	NM_017161.1	NP_058857.1
hA_{2B}	NM_000676.2	NP_000667.1
mA_3	NM_009631.3	NP_033761.2
rA_3	NM_012896	NP_037028.2
hA_3	NM_000677.3	NP_000668.1

Table 2 Primer sequences and restriction enzymes used for amplifying the cDNAs of murine adenosine receptors

Primer	Sequence 5'-3'	Gene bank reference
f-mA_1-EcoRI	GAGACGGAATTCATGCCGCCGTACATCTCGGC	NM_001008533
r-mA_1-BamHI	CCTACTAGGATCCCTAGTCATCAGCTTTCTCCTCTG	
f-mA_{2A}-MfeI	GAGACGCAATTGGATGGGCTCCTCGGTGTACATC	NM_009630
r-mA_{2A}-XhoI	CTTACTACTCGAGTCAGGAAGGGGCAAACTCTGAAG	
f-mA_{2B}-EcoRI	GAGACGGAATTCATGCAGCTAGAGACGCAAGACG	NM_007413
r-mA_{2B}-BamHI	CTTACTAGGATCCTCATAAGCCCAGACTGAGAGTAG	
f-mA_3-NotI	GTGACAGCGGCCGCATGGAAGCCGACAACACCAC	NM_009631.3
r-mA_3-EcoRI	CTTACTAGAATTCTTACTCAGTAGTCTGTTCCATG	
f-rA_1-NotI	GTGACAGCGGCCGCATGCCGCCCTACATCTCGGC	NM_017155.2
r-rA_1-EcoRI	CTTACTAGAATTCCTAGTCCTCAGCTTTCTCCTC	
f-rA_{2A}-NotI	GTGACAGCGGCCGCATGGGCTCCTCGGTGTACATC	NM_053294.3
r-rA_{2A}-AgeI	CTTACTAACCGGTTCAGGAAGGGGCAAACTCTGAAG	
f-rA_{2B}-EcoRI	GAGACGGAATTCATGCAGCTAGAGACGCAGGAC	NM_017161.1
r-rA_{2B}-BamHI	CTTACTAGGATCCTCACAAGCTCAGACTGAAAGTTG	

f-rA_3-EcoRI	GAGACGGAATTCATGAAAGCCAACAATACCACG AC	NM_012896
r-rA_3-XhoI	CTTGCAGTCTCGAGCTACTCAGTAGTCTGTTCAA GG	

Table 3 Primer sequences used for amplifying the cDNAs of rat adenosine receptors A_{2B} and A_3 from genomic DNA

Primer	Sequence 5'-3'
f-rA_{2B}	ATGCAGCTAGAGACGCAGGA
r-rA_{2B}-Exon1	CTGAGCGGGACGCGAATG
f-rA_{2B}-Exon2	GTATAAAGGTTTGGTCACTGGAA
r-A_{2B}	TCACAAGCTCAGACTGAAAGTTG
f-rA_3	ATGAAAGCCAACAATACCACGAC
r-rA_3-Exon1	ACTGTCAGCTTGACTCGCAGGTAT
f-rA_3-Exon2	CAGATATAGAACGGTTACCACTCAAAG
r-rA_3	CTACTCAGTAGTCTGTTCAAGGTTTG
r-rA_3-Overlap-Ex1	TGGTAACCGTTCTATATCTGACTGTCAGCTTGACTCG CAG
f-rA_3-Overlap-Ex2	CTGCGAGTCAAGCTGACAGTCAGATATAGAACGGTT ACC

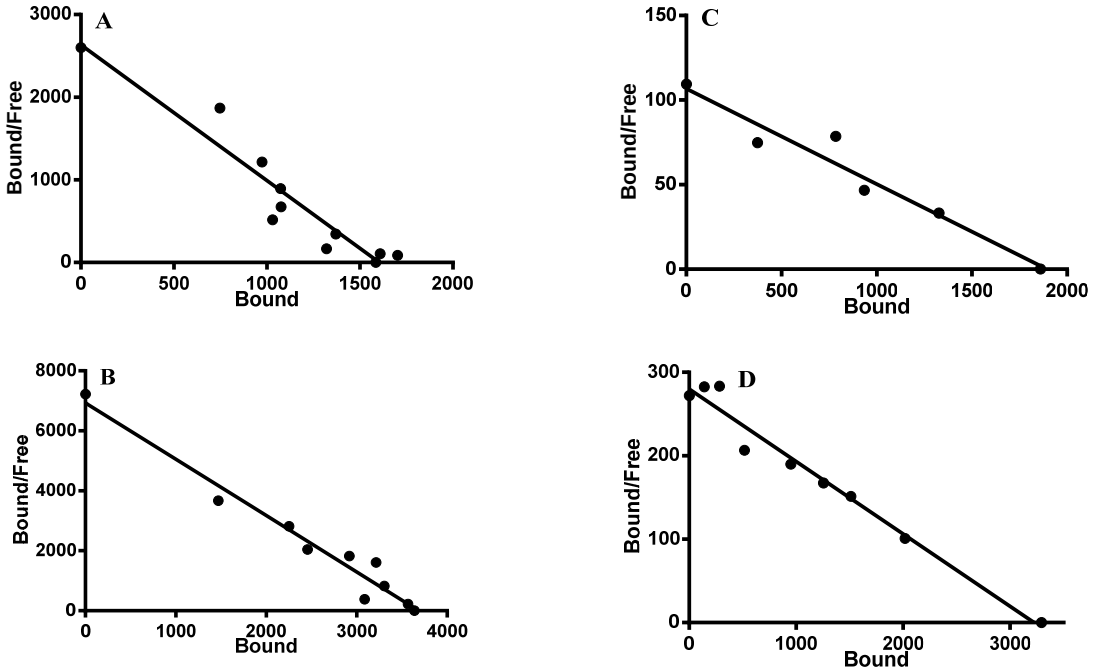

Fig. S1 Scatchard transformation of saturation binding assays at ARs stably expressed in recombinant CHO cells; (**A**) mA_1AR using [^{3}H]CCPA; (**B**) mA_1AR using [^{3}H]DPCPX; (**C**) $mA_{2A}AR$ using [^{3}H]CGS-21680; (**D**) $mA_{2A}AR$ using [^{3}H]MSX-2. Data are means of three independent saturation assays each performed in duplicates.

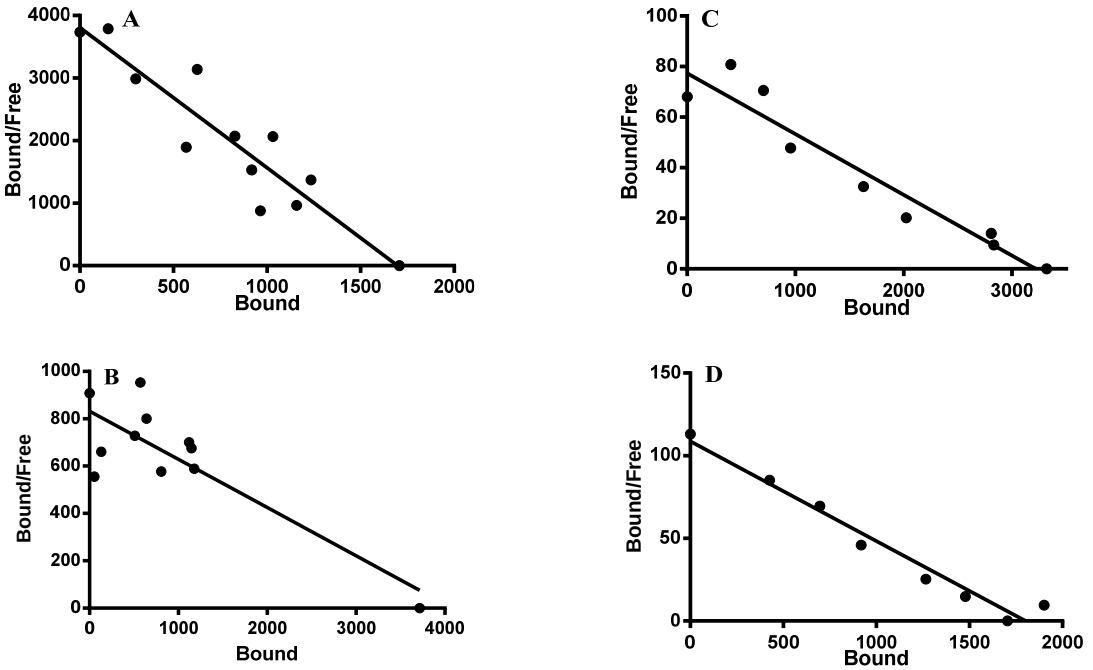

Fig S2 Scatchard transformation of saturation binding assays at ARs stably expressed in recombinant CHO cells; (**A**) $rA_{2B}AR$ using [^{3}H]PSB-603; (**B**) $mA_{2B}AR$ using [^{3}H]PSB-603; (**C**) rA_3AR using [^{3}H]NECA; (**D**) mA_3AR using [^{3}H]NECA. Data are means of three independent saturation assays each performed in duplicates.

```
mA1             MPPYISAFQAAYIGIEVLIALVSVPGNVLVIWAVKVNQALRDATFCFIVSLAVADVAVGA 60
rA1             MPPYISAFQAAYIGIEVLIALVSVPGNVLVIWAVKVNQALRDATFCFIVSLAVADVAVGA 60
hA1             MPPSISAFQAAYIGIEVLIALVSVPGNVLVIWAVKVNQALRDATFCFIVSLAVADVAVGA 60
                *** ************************************************************

mA1             LVIPLAILINIGPQTYFHTCLMVACPVLILTQSSILALLAIAVDRYLRVKIPLRYKTVVT 120
rA1             LVIPLAILINIGPQTYFHTCLMVACPVLILTQSSILALLAIAVDRYLRVKIPLRYKTVVT 120
hA1             LVIPLAILINIGPQTYFHTCLMVACPVLILTQSSILALLAIAVDRYLRVKIPLRYKMVVT 120
                ************************************************************ ***

mA1             QRRAAVAIAGCWILSLVVGLTPMFGWNNLSEVEQAWIANGSVGEPVIKCEFEKVISMEYM 180
rA1             QRRAAVAIAGCWILSLVVGLTPMFGWNNLSVVEQDWRANGSVGEPVIKCEFEKVISMEYM 180
hA1             PRRAAVAIAGCWILSFVVGLTPMFGWNNLSAVERAWAANGSMGEPVIKCEFEKVISMEYM 180
                 **************:**************  ** : * ****:*******************

mA1             VYFNFFVWVLPPLLLMVLIYLEVFYLIRKQLNKKVSASSGDPQKYYGKELKIAKSLALIL 240
rA1             VYFNFFVWVLPPLLLMVLIYLEVFYLIRKQLNKKVSASSGDPQKYYGKELKIAKSLALIL 240
hA1             VYFNFFVWVLPPLLLMVLIYLEVFYLIRKQLNKKVSASSGDPQKYYGKELKIAKSLALIL 240
                ************************************************************

mA1             FLFALSWLPLHILNCITLFCPTCQKPSILIYIAIFLTHGNSAMNPIVYAFRIHKFRVTFL 300
rA1             FLFALSWLPLHILNCITLFCPTCQKPSILIYIAIFLTHGNSAMNPIVYAFRIHKFRVTFL 300
hA1             FLFALSWLPLHILNCITLFCPSCHKPSILTYIAIFLTHGNSAMNPIVYAFRIQKFRVTFL 300
                **********************:*:***** **********************:*******

mA1             KIWNDHFRCQPKPPIEEDIPEEKADD 326
rA1             KIWNDHFRCQPKPPIDEDLPEEKAED 326
hA1             KIWNDHFRCQPAPPIDEDLPEERPDD 326
                *********** ***:**:***:.:*
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Fig. S3 The alignment of the A_1 adenosine receptor in mouse, rat and human. The transmembrane domains (TMs) are underlined in red. The different amino acids are in blue.

```
mA2A            ---MGSSVYIMVELAIAVLAILGNVLVCWAVWINSNLQNVTNFFVVSLAAADIAVGVLAI 57
rA2A            ---MGSSVYITVELAIAVLAILGNVLVCWAVWINSNLQNVTNFFVVSLAAADIAVGVLAI 57
hA2A            MPIMGSSVYITVELAIAVLAILGNVLVCWAVWLNSNLQNVTNYFVVSLAAADIAVGVLAI 60
                   ******* **********************:*********:*****************

mA2A            PFAITISTGFCAACHGCLFFACFVLVLTQSSIFSLLAIAIDRYIAIRIPLRYNGLVTGMR 117
rA2A            PFAITISTGFCAACHGCLFFACFVLVLTQSSIFSLLAIAIDRYIAIRIPLRYNGLVTGVR 117
hA2A            PFAITISTGFCAACHGCLFIACFVLVLTQSSIFSLLAIAIDRYIAIRIPLRYNGLVTGTR 120
                ********************:****************************************** *

mA2A            AKGIIAICWVLSFAIGLTPMLGWNNCSQ-KDE-NSTKTCGEGRVTCLFEDVVPMNYMVYY 175
rA2A            AKGIIAICWVLSFAIGLTPMLGWNNCSQ-KDG-NSTKTCGEGRVTCLFEDVVPMNYMVYY 175
hA2A            AKGIIAICWVLSFAIGLTPMLGWNNCGQPKEGKNHSQGCGEGQVACLFEDVVPMNYMVYF 180
                ***************************.* *:  * :: ****:*:***************:

mA2A            NFFAFVLLPLLLMLAIYLRIFLAARRQLKQMESQPLPGERTRSTLQKEVHAAKSLAIIVG 235
rA2A            NFFAFVLLPLLLMLAIYLRIFLAARRQLKQMESQPLPGERTRSTLQKEVHAAKSLAIIVG 235
hA2A            NFFACVLVPLLLMLGVYLRIFLAARRQLKQMESQPLPGERARSTLQKEVHAAKSLAIIVG 240
                **** **:******.:************************:****************** 

mA2A            LFALCWLPLHIINCFTFFCSTCQHAPPWLMYLAIILSHSNSVVNPFIYAYRIREFRQTFR 295
rA2A            LFALCWLPLHIINCFTFFCSTCRHAPPWLMYLAIILSHSNSVVNPFIYAYRIREFRQTFR 295
hA2A            LFALCWLPLHIINCFTFFCPDCSHAPLWLMYLAIVLSHTNSVVNPFIYAYRIREFRQTFR 300
                ******************. * *** *******:***:******************** 

mA2A            KIIRTHVLRRQEPFRAGGSSAWALAAHSTEGEQVSLRLNGHPLGVWANGSAPHSGRRPNG 355
rA2A            KIIRTHVLRRQEPFQAGGSSAWALAAHSTEGEQVSLRLNGHPLGVWANGSATHSGRRPNG 355
hA2A            KIIRSHVLRQQEPFKAAGTSARVLAAHGSDGEQVSLRLNGHPPGVWANGSAPHPERRPNG 360
                ****:****:****:*.*:**  .****.::************ ********.*. *****

mA2A            YTLGPGGGGSTQGSPG-----DVELLTQEHQ-EGQEHPGLGDHLAQGRVGTASWSSEFAP 409
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```
rA2A            YTLGLGGGGSAQGSPR-----DVELPTQERQ-EGQEHPGLRGHLVQARVGASSWSSEFAP 409
hA2A            YALGLVSGGSAQESQGNTGLPDVELLSHELKGVCPEPPGLDDPLAQDGAGVS-------- 412
                *:**  .***:* *       **** ::* :    * *** . *.*  .*.:

mA2A            S 410
rA2A            S 410
hA2A            -
```

Fig. S4 The alignment of the A_{2A} adenosine receptor in mouse, rat and human. The transmembrane domains (TMs) are underlined in red. The different amino acids are in blue.

```
mA2B            MQLETQDALYVALELVIAALAVAGNVLVCAAVGASSALQTPTNYFLVSLATADVAVGLFA 60
rA2B            MQLETQDALYVALELVIAALAVAGNVLVCAAVGASSALQTPTNYFLVSLATADVAVGLFA 60
hA2B            MLLETQDALYVALELVIAALSVAGNVLVCAAVGTANTLQTPTNYFLVSLAAADVAVGLFA 60
                * ******************:************::.:**************:*********

mA2B            IPFAITISLGFCTDFHGCLFLACFVLVLTQSSIFSLLAVAVDRYLAIRVPLRYKGLVTGT 120
rA2B            IPFAITISLGFCTDFHSCLFLACFVLVLTQSSIFSLLAVAVDRYLAIRVPLRYKGLVTGT 120
hA2B            IPFAITISLGFCTDFYGCLFLACFVLVLTQSSIFSLLAVAVDRYLAICVPLRYKSLVTGT 120
                ****************:.****************************** ******.*****

mA2B            RARGIIAVLWVLAFGIGLTPFLGWNSKDSATSNCTELGDGIANKSCCPVTCLFENVVPMS 180
rA2B            RARGIIAVLWVLAFGIGLTPFLGWNSKDRATSNCTEPGDGITNKSCCPVKCLFENVVPMS 180
hA2B            RARGVIAVLWVLAFGIGLTPFLGWNSKDSATNNCTEPWDGTTNESCCLVKCLFENVVPMS 180
                ****:************************ **.****  ** :*:*** *.**********

mA2B            YMVYFNFFGCVLPPLLIMLVIYIKIFMVACKQLQRMELMDHSRTTLQREIHAAKSLAMIV 240
rA2B            YMVYFNFFGCVLPPLLIMMVIYIKIFMVACKQLQHMELMEHSRTTLQREIHAAKSLAMIV 240
hA2B            YMVYFNFFGCVLPPLLIMLVIYIKIFLVACRQLQRTELMDHSRTTLQREIHAAKSLAMIV 240
                ******************:*******:***:***: ***:********************

mA2B            GIFALCWLPVHAINCITLFHPALAKDKPKWVMNVAILLSHANSVVNPIVYAYRNRDFRYS 300
rA2B            GIFALCWLPVHAINCITLFHPALAKDKPKWVMNVAILLSHANSVVNPIVYAYRNRDFRYS 300
hA2B            GIFALCWLPVHAVNCVTLFQPAQGKNKPKWAMNMAILLSHANSVVNPIVYAYRNRDFRYT 300
                ************:**:***:**  .*:****.**:*************************:

mA2B            FHKIISRYVLCQAETKGGSGQAGAQSTLSLGL 332
rA2B            FHRIISRYVLCQTDTKGGSGQAGGQSTFSLSL 332
hA2B            FHKIISRYLLCQADVKSGNGQAGVQPALGVGL 332
                **:*****:***::.*.*.**** *.::.:.*
```

Fig. S5 The alignment of the A_{2B} adenosine receptor in mouse, rat and human. The transmembrane domains (TMs) are underlined in red. The different amino acids are in blue.

```
mA3             MEADN-TTETDWLNITYITMEAAIGLCAVVGNMLVIWVVKLNPTLRTTTFYFIVSLALAD 59
rA3             MKANNTTTSALWLQITYITMEAAIGLCAVVGNMLVIWVVKLNRTLRTTTFYFIVSLALAD 60
hA3             -MPNN-STALSLANVTYITMEIFIGLCAIVGNVLVICVVKLNPSLQTTTFYFIVSLALAD 58
                  .:* :*     ::******  *****:***:*** ***** :*:***************

mA3             IAVGVLVTPLAIAVSLQVKMHFYACLFMSCVLLIFTHASIMSLLAIAVDRYLRVKLTVRY 119
rA3             IAVGVLVIPLAIAVSLEVQMHFYACLFMSCVLLVFTHASIMSLLAIAVDRYLRVKLTVRY 120
hA3             IAVGVLVMPLAIVVSLGITIHFYSCLFMTCLLLIFTHASIMSLLAIAVDRYLRVKLTVRY 118
                ******* ****.*** : :***:****:*:**:*****************************

mA3             RTVTTQRRIWLFLGLCWLVSFLVGLTPMFGWNRKATLASSQNSSTLLCHFRSVVSLDYMV 179
rA3             RTVTTQRRIWLFLGLCWLVSFLVGLTPMFGWNRKVTLELSQNSSTLSCHFRSVVGLDYMV 180
hA3             KRVTTHRRIWLALGLCWLVSFLVGLTPMFGWNMKLTSEYHRNVTFLSCQFVSVMRMDYMV 178
                : ***:***** ******************** * *    :* : * *:* **: :****
```

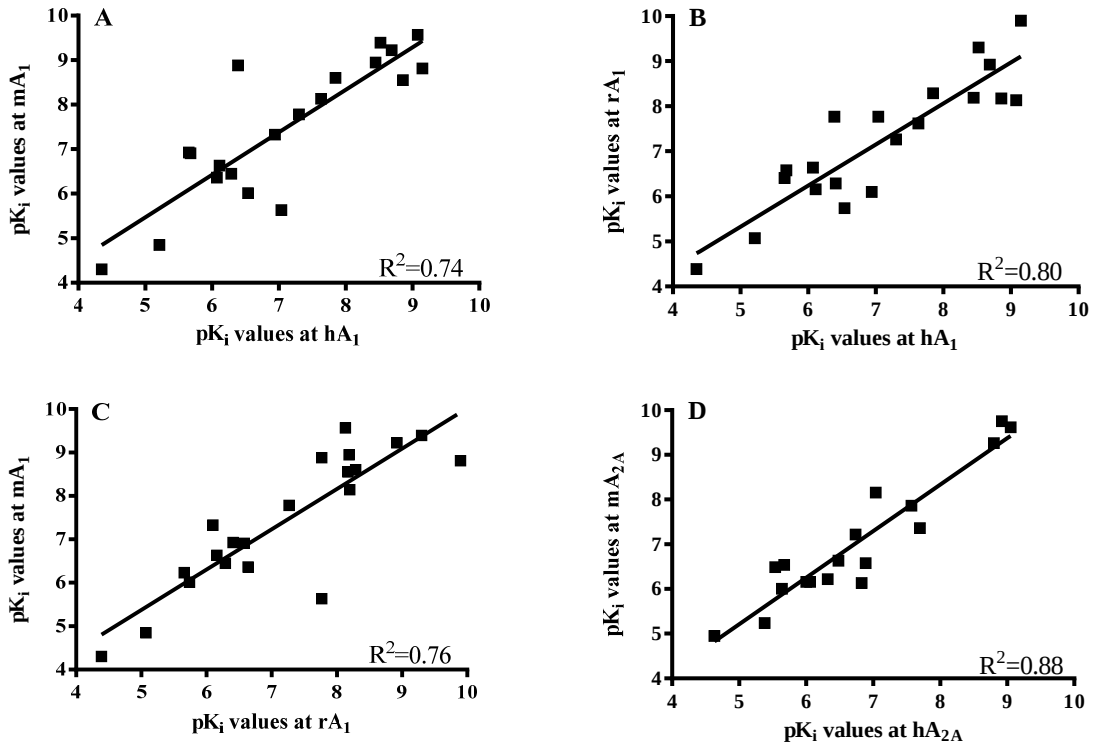
```
mA3             FFSFVTWILVPLVVMCVIYLDIFYIIRNKLSQNLSGFRETRAFYGREFKTAKSLFLVLFL 239
rA3             FFSFITWILIPLVVMCIIYLDIFYIIRNKLSQNLTGFRETRAFYGREFKTAKSLFLVLFL 240
hA3             YFSFLTWIFIPLVVMCAIYLDIFYIIRNKLSLNLSNSKETGAFYGREFKTAKSLFLVLFL 238
                :***:***::****** ************** **:. :** ********************

mA3             FALCWLPLSIINFVSYFDVKIPDVAMCLGILLSHANSMMNPIVYACKIKKFKETYFLILR 299
rA3             FALCWLPLSIINFVSYFNVKIPEIAMCLGILLSHANSMMNPIVYACKIKKFKETYFVILR 300
hA3             FALSWLPLSIINCIIYFNGEVPQLVLYMGILLSHANSMMNPIVYAYKIKKFKETYLLILK 298
                ***.******** : **: ::*:::: :***************** *********::**:

mA3             ALRLCQTSDSLDSNMEQTTE 319
rA3             ACRLCQTSDSLDSNLEQTTE 320
hA3             ACVVCHPSDSLDTSIEKNSE 318
                *  :*:.*****:.:*:.:*
```

Fig. S6 The alignment of the A_3 adenosine receptor in mouse, rat and human. The transmembrane domains (TMs) are underlined in red. The different amino acids are in blue.

Correlation of pK_i values

In order to determine the correlation coefficients, the available pK_i values of all the compounds (both from this work as well as from literature) were calculated and a linear regression of the pK_i values was conducted. The R^2 of the linear regression analysis is equal to the correlation coefficient. A compound was not taken into consideration if it was only screened (for example, if K_i value > 10 µM).

Correlation coefficients of the pK_i values at A_1 were found to be between 0.74 and 0.80, whereas the correlation coefficients of pK_i values at A_{2A} ranged between 0.83 and 0.88. Interestingly, the correlation between the three species is better at the A_{2A}AR than A_1AR despite their lower sequence identity. It was also counterintuitive that results for mouse and rat A_{2A}AR correlate slightly worse than mouse with human receptor. The curves and correlation coefficients are given in figure S7.



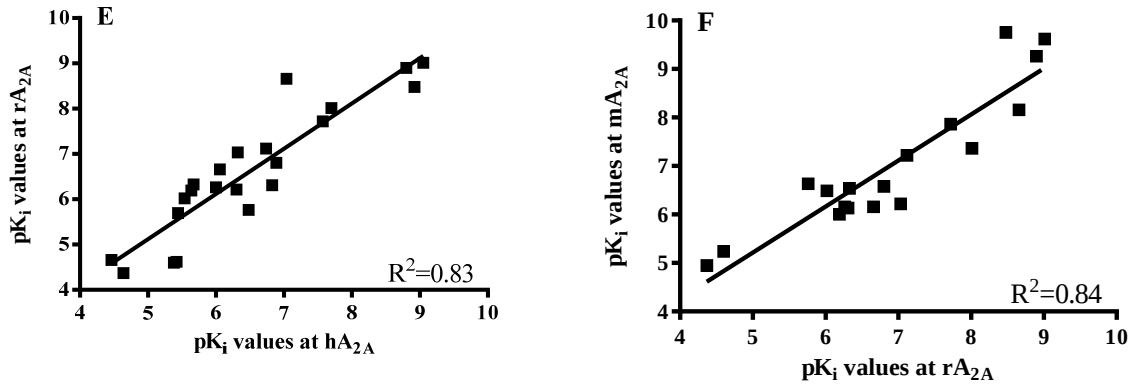
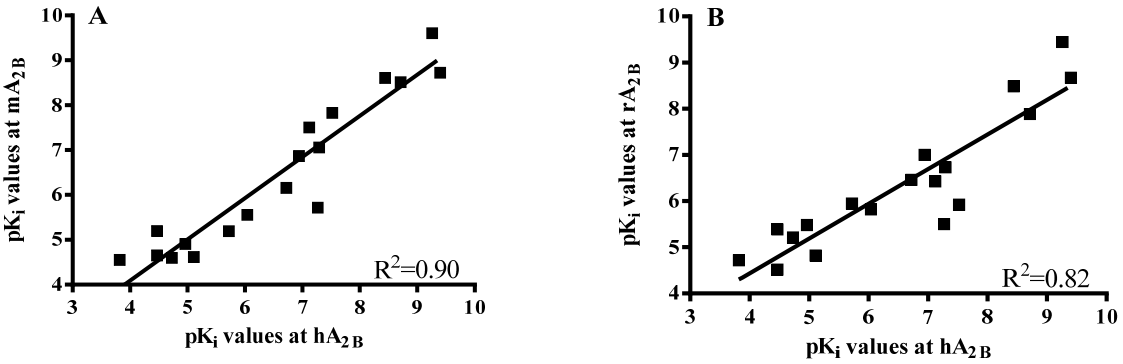


Fig. S7 1 Correlation of the pK_i values at A_1 and A_{2A} receptors in the human, rat and mouse.

A: correlation between mouse and human at A_1AR, **B**: correlation between rat and human at A_1AR, **C**: correlation between rat and mouse at A_1AR, **D**: correlation between mouse and human at $A_{2A}AR$, **E**: correlation between rat and human at $A_{2A}AR$, **F**: correlation between rat and mouse at $A_{2A}AR$.

The correlation coefficients of the pK_i values at A_{2B} were also high, ranging between 0.82 and 0.90. The correlation between the species at A_3AR is not expected to be high since the genetic divergence is high. Many antagonists were not considered because they were not active at A_3AR. The correlation coefficient between mouse and human was only 0.52 but if we considered only A_3 agonists the coefficient will increase to 0.93 (data not shown). Rat correlates better with human than mouse with coefficient of 0.83, whereas rat and mouse correlate less with a correlation coefficient of 0.73 as shown in figure S8.



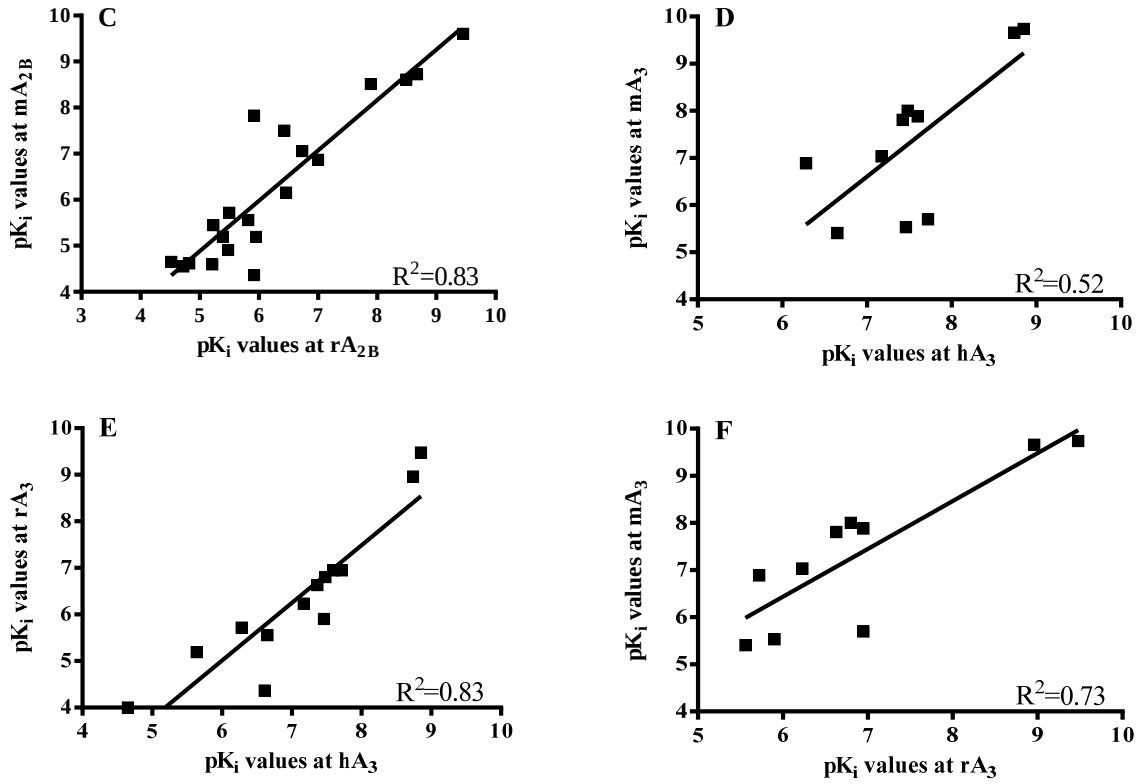


Fig. S8 Correlation of the pK_i values at A_1 and A_{2A} receptors in the human, rat and mouse.

A: correlation between mouse and human at A_{2B}AR, **B**: correlation between rat and human at A_{2B}AR,

C: correlation between rat and mouse at A_{2B}AR, **D**: correlation between mouse and human at A_3AR,

E: correlation between rat and human at A_3AR, **F**: correlation between rat and mouse at A_3AR.