Supporting Information

Biophysical Mapping of the Adenosine A_{2A} Receptor

Andrei Zhukov, Stephen P. Andrews, James C. Errey, Nathan Robertson, Benjamin Tehan, Jonathon S. Mason, Fiona H. Marshall, Malcolm Weir & Miles Congreve*

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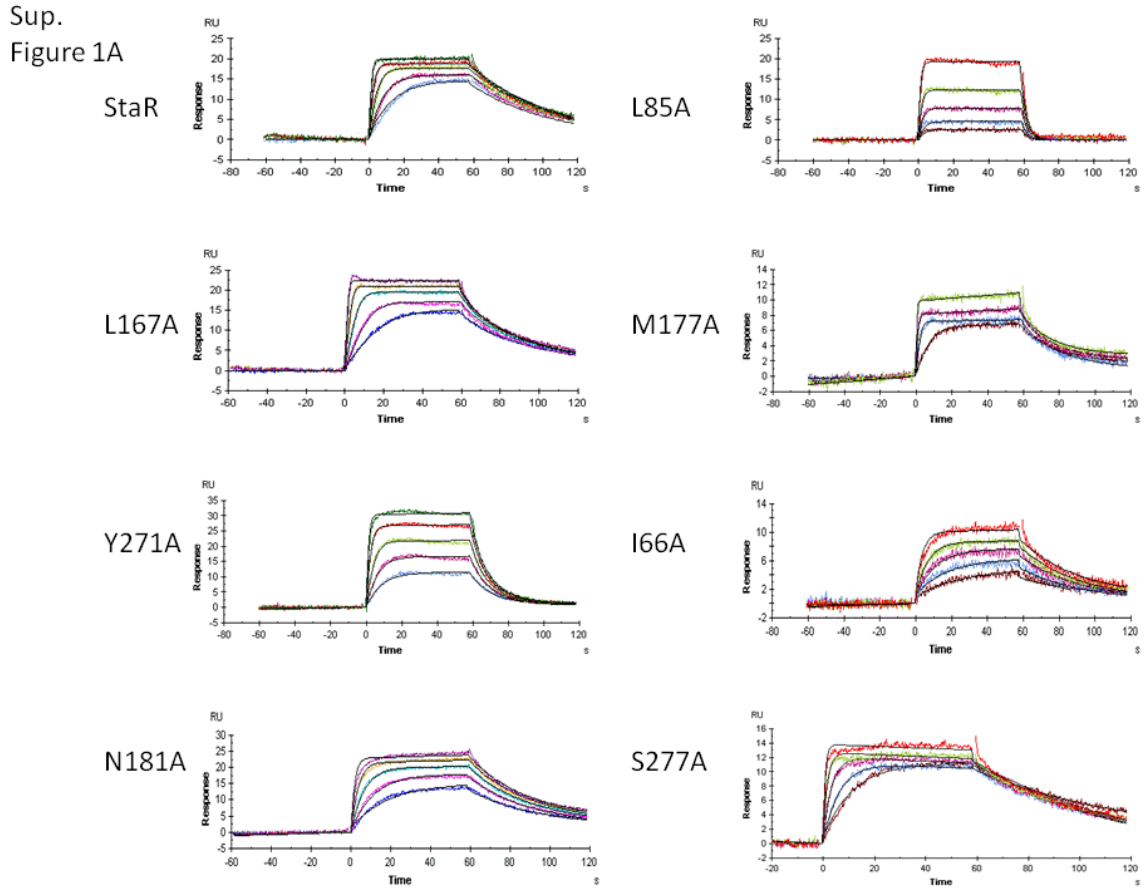
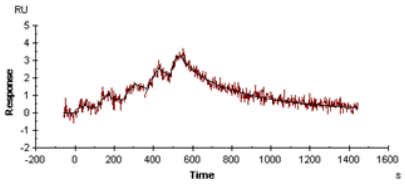
Figure S1 Sensorgrams of interaction of adenosine A_{2A} receptor StaR and binding site mutants with three compounds from different structural groups are presented in Supplementary Figures 1A, 1B and 1C. The proteins were immobilized on NTA chip and the ligands injected were at a series of concentrations in the single cycle or mulitcycle format at 10 ^{o}C. Presented are the raw data and fits to 1:1 interaction.

1A, compound 1a; 0.125-2 µM.
1B, compound 2b; 10-160 nM.
1C, compound 3d; 10-160 nM.



Sup.
Figure 1B

StaR



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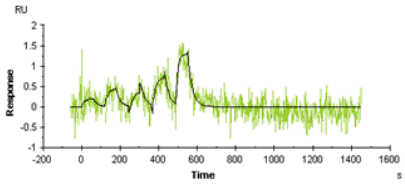

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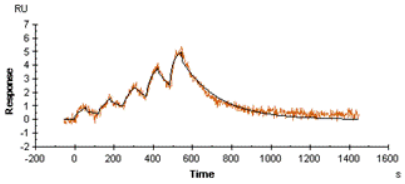

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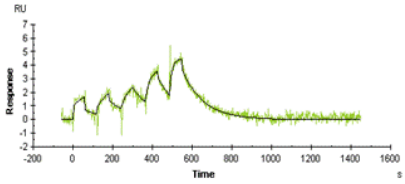

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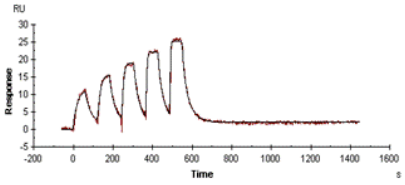

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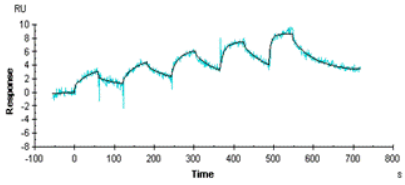

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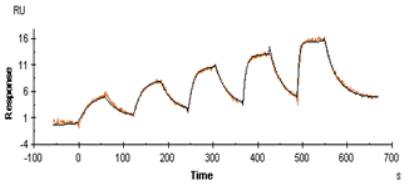

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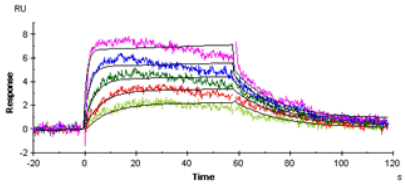
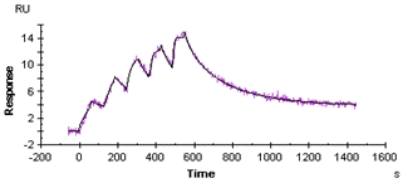


Sup.
Figure 1C

StaR



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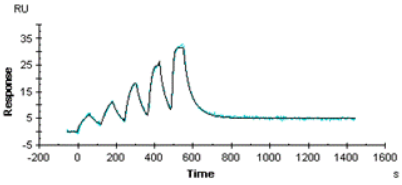

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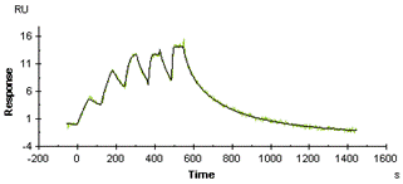

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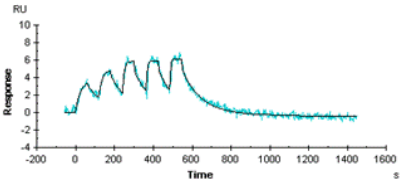

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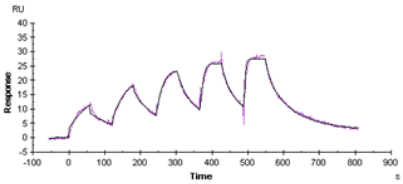

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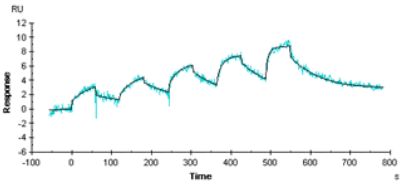

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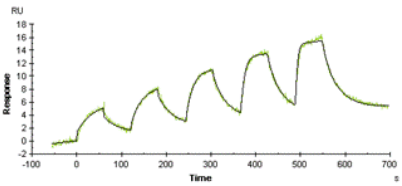

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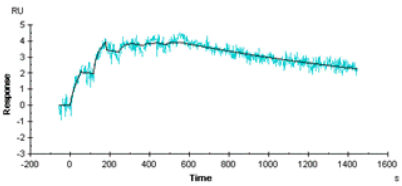


Figure S2 Representative data of the effect of binding site mutations on interaction kinetics of compounds ZM241385, 1a, 2b, and 3d. The association and dissociation rate constants k_{on} and k_{off} were obtained by fitting sensorgrams to a 1:1 interaction model. Panels on the right present the change of log k_{on} (above) and pk_{off} (below) caused by different mutations compared with the background StaR. Differences in binding affinity were generally derived from change in k_{off} rather than k_{on}. This is consistent with general observations for other systems. However, we can see that in some cases changes in k_{on} were important, and in further cases changes in k_{on} and k_{off} occur but cancel each other, meaning that overall affinity did not change. The data provided by the k_{on} and k_{off} values give additional insight into the energetics of binding of the various compound series and were used to aid in compound design during medicinal chemistry optimisation of series 3.

Sup.
Figure 2

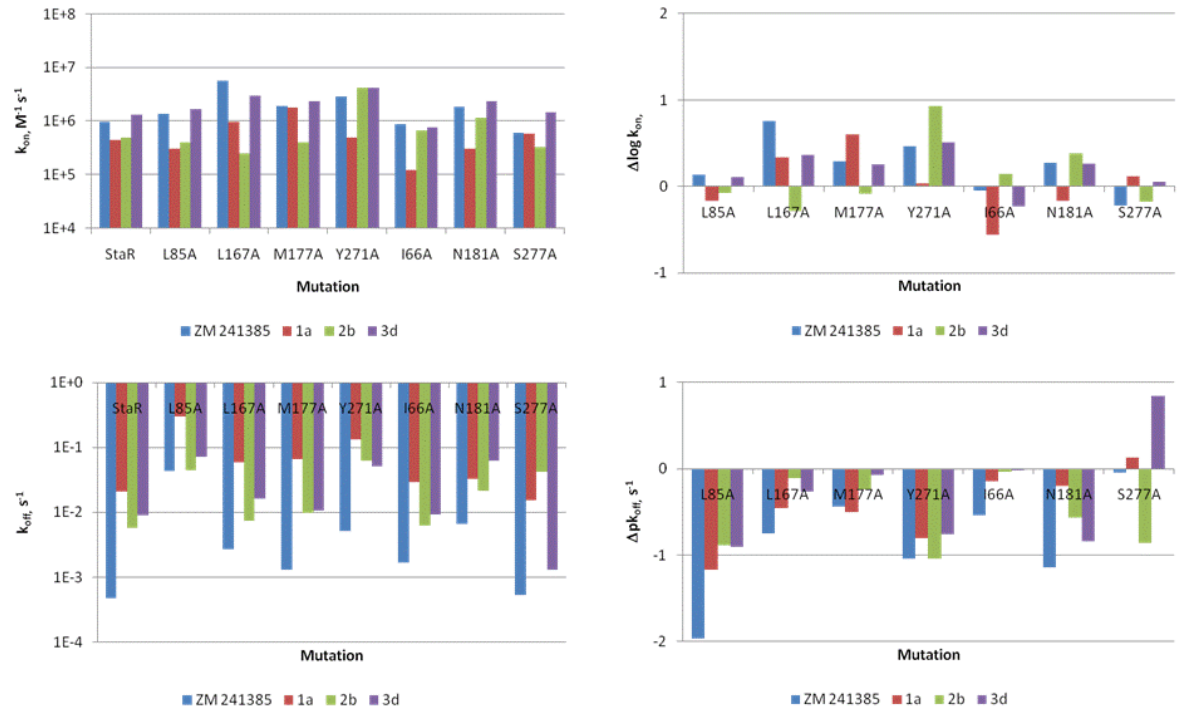


Table S1 Additional information for compound series 1 and 3 described in Table 1 of the main article.

Table 1 compound ID	**Reference**
1a	example xviii, patent application US61/414767, earliest publication date 17 May 2012
1b	example xxi, patent application US61/414767, earliest publication date 17 May 2012
1c	example xxviii, patent application US61/414767, earliest publication date 17 May 2012
1d	example xxxii, patent application US61/414767, earliest publication date 17 May 2012
1e	1e is a fragment of example xxviii, patent application US61/414767, earliest publication date 17 May 2012
3a	example xxi, PCT/EP2011/051755, earliest publication date 5 August 2011
3b	example lxii, PCT/EP2011/051755, earliest publication date 5 August 2011
3c	example lxxvi, PCT/EP2011/051755, earliest publication date 5 August 2011
3d	example xcvii, PCT/EP2011/051755, earliest publication date 5 August 2011
3e	example cxiv, PCT/EP2011/051755, earliest publication date 5 August 2011
3f	example cclii, PCT/EP2011/051755, earliest publication date 5 August 2011
3g	example clxxxii, PCT/EP2011/051755, earliest publication date 5 August 2011
3h	example cclxxxv, PCT/EP2011/051755, earliest publication date 5 August 2011

Experimental details for Series 2

General experimental details:

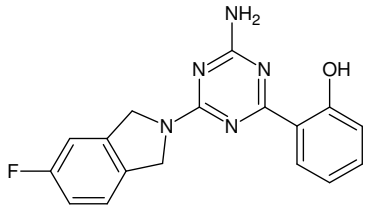
The chemistry delineated in this section was carried out by Oxygen Healthcare. Commercial reagents were used as received, without further purification. Room temperature refers to 25-27 ^{o}C. Chromatography refers to column chromatography performed using 60 - 120 mesh silica gel and executed under nitrogen pressure (flash chromatography) conditions. Thin layer chromatography (TLC) was run using the specified mobile phase and silica gel F254 as a stationary phase (Merck).

HPLC purities were measured under the following conditions: Instrument: Waters Alliance 2695. Column: Sunfire C-18, 250 x 4.6 mm, 5 µm, or equivalent. Gradient [time (min)/% solvent B in A]: 0.00/10, 9.00/90, 11.00/100, 20.00/100, 20.01/10, 25.00/10 (solvent A = 0.1% formic acid in water; solvent B = 0.1% formic acid in acetonitrile). 1 mL/min; detection wavelength specified for each compound in the detailed experimental section.

Mass spectroscopy (MS) was performed using electrospray ionization conditions on a Shimadzu 2010 EV.

LCMS purities were measured under the following conditions: Instrument: Waters Alliance 2795, Waters 2996 PDA detector and Micromass ZQ. Column: Waters X-Bridge C-18, 2.5micron, 2.1 x 20mm or Phenomenex Gemini-NX C-18, 3 micron, 2.0 x 30mm. Gradient [time (min)/solvent D in C (%)]: 0.00/2, 0.10/2, 8.40/95, 9.40/95, 9.50/2, 10.00/2 (solvent C = 1.58 g ammonium formate in 2.5 L water + 2.7 mL ammonia solution; solvent D = 2.5 L Acetonitrile + 132 mL (5%) solvent C + 2.7 mL ammonia solution). Injection volume 5 µL; UV detection 230 to 400 nM; column temperature 45 ^{o}C; 1.5 mL/min.

^{1}H-NMR spectra were recorded at 400 MHz on a Bruker instrument. Deuterated solvents were used as specified. Chemical shift values are expressed in parts per million, i.e. (δ)-values. The following abbreviations are used for the multiplicity for the NMR signals: s=singlet, b=broad, d=doublet, t=triplet, q=quartet, dd=doublet of doublets, dt=double of triplets, m=multiplet.



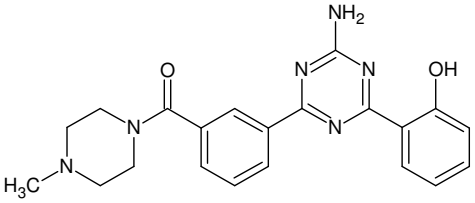
Preparation of 2-[4-amino-6-(5-fluoro-1,3-dihydro-2*H*-isoindol-2-yl)-1,3,5-triazin-2-yl]phenol **(2a)**

A solution of cyanuric chloride (5.0 g, 27.11 mmol) in THF (20 mL) was cooled to -10 °C. di*iso*propylethylamine (3.5 g, 27.11 mmol) was added drop-wise and the resulting mixture was stirred at -10 °C for 5 min. A solution of ammonia in THF (2.6 M, 35 mL) was then added drop-wise and the reaction was followed by TLC for one hour. After completion, the reaction mixture was concentrated *in vacuo*, diluted with water (50 mL) and extracted with ethyl acetate (3 x 100 mL). The

combined organic layers were dried over Na_2SO_4 and evaporated under reduced pressure. The crude residue was purified by gradient flash chromatography, eluting with up to 20% ethyl acetate in hexanes, affording 4,6-dichloro-1,3,5-triazin-2-amine (4.35 g, 78%).

A solution of 4,6-dichloro-1,3,5-triazin-2-amine (0.50 g, 3.03 mmol) in *iso*-propanol (5 mL) was cooled to 0 °C and treated with triethylamine (0.642 g, 6.36 mmol) and 5-fluoroisoindoline hydrochloride (0.410 g, 3.03 mmol). The resulting mixture was stirred at 0 °C for one hour with TLC monitoring. Upon completion of the reaction, the resulting precipitate was collected on a filter bed, washed with hexane and dried *in vacuo*. 4-Chloro-6-(5-fluoro-1,3-dihydro-2*H*-isoindol-2-yl)-1,3,5-triazin-2-amine (0.75 g, 50%) was used without further purification. HPLC: 99.10 % purity (280 nm); MS: 247.9 $[M]^+$ (ESI +ve).

4-Chloro-6-(5-fluoro-1,3-dihydro-2*H*-isoindol-2-yl)-1,3,5-triazin-2-amine (0.70 g, 2.52 mmol) and 2-hydroxyphenylboronic acid (0.382 g, 2.77 mmol) were mixed in 1,4-dioxane (10 mL), and treated with an aqueous solution of Na_2CO_3 (0.53 g, 5.0 mmol in 1 mL H_2O). The mixture was then de-gassed, treated with tetrakis(triphenylphosphine)palladium(0) (5 mol%) at room temperature for 5 mintues, and heated at 100 ^oC until the disappearance of starting materials was observed by TLC. The reaction was then cooled to room temperature and quenched with water (10 mL). The resulting mixture was extracted with EtOAc (3 x 10 mL) and the combined organics were dried over anhydrous sodium sulfate. The resulting solution was concentrated *in vacuo* and purified by gradient flash chromatography, eluting with mixtures of ethyl acetate and hexanes to afford 2-[4-amino-6-(5-fluoro-1,3-dihydro-2*H*-isoindol-2-yl)-1,3,5-triazin-2-yl]phenol (253 mg, 29%). HPLC: 12.60 min, 99.27% purity (215 nm); MS: 335 $[M]^+$ (ESI +ve); ^1H NMR: (400 MHz, $CDCl_3$) δ: 4.77-4.80 (d, 2H), 4.88-4.92 (d, 2H), 6.86-6.91 (m, 2H), 7.13-7.28 (m, 1H), 7.30-7.46 (s, 2H, $-NH_2$), 7.36-7.47 (m, 3H), 13.80 (s, 1H).



Preparation of {3-[4-amino-6-(2-hydroxyphenyl)-1,3,5-triazin-2-yl]phenyl}(4-methylpiperazin-1-yl)methanone **(2b)**

4,6-Dichloro-1,3,5-triazin-2-amine (25.0 g, 152.4 mmol) and 2-(benzyloxy)phenylboronic acid (33.0 g, 144.8 mmol), were mixed in 1,4-dioxane (250 mL), and treated with an aqueous solution of Na_2CO_3 (40.0 g, 378.78 mmol in 40 mL H_2O). The mixture was then de-gassed, treated with tetrakis triphenylphosphine palladium (3.50 g, 3.03 mmol) at room temperature for 5 minutes, and heated at 70 ^oC for 3 hours at which time the disappearance of starting materials was observed by TLC. The reaction was then cooled to room temperature and quenched with water (300 mL). The resulting mixture was extracted with EtOAc (3 x 300 mL) and the combined organics were dried over anhydrous sodium sulfate. The resulting solution was concentrated *in vacuo* and purified by gradient

flash chromatography, eluting with mixtures of ethyl acetate and hexanes to afford 4-[2-(benzyloxy)phenyl]-6-chloro-1,3,5-triazin-2-amine (10.9 g, 23%).

4-[2-(Benzyloxy)phenyl]-6-chloro-1,3,5-triazin-2-amine (0.50 g, 1.60 mmol) and 3-(4-methylpiperazine-1-carbonyl)phenylboronic acid hydrochloride (0.67 g, 2.40 mmol) were mixed in 1,4-dioxane (5 mL), and treated with an aqueous solution of Na_2CO_3 (0.51 g, 4.8 mmol in 0.5 mL H_2O). The mixture was then de-gassed, treated with tetrakis(triphenylphosphine)palladium(0) (5 mol%) at room temperature for 5 minutes, and heated at 90 ºC for 16 hours. The reaction was then cooled to room temperature and quenched with water (10 mL). The resulting mixture was extracted with EtOAc (3 x 10 mL) and the combined organics were dried over anhydrous sodium sulfate. The resulting solution was concentrated *in vacuo* and purified by gradient flash chromatography, eluting with mixtures of ethyl acetate and hexanes to afford {3-[4-amino-6-(2-(benzyloxy)phenyl)-1,3,5-triazin-2-yl]phenyl}(4-methylpiperazin-1-yl)methanone (crude yield: 1.00 g, 78%, used without further purification).

Crude {3-[4-amino-6-(2-(benzyloxy)phenyl)-1,3,5-triazin-2-yl]phenyl}(4-methylpiperazin-1-yl)methanone (0.10 g, 0.21 mmol) was dissolved in ethyl acetate (0.5 mL) and the resulting solution was treated with palladium hydroxide on carbon (0.10 g, 0.70 mmol) and 1,4-cyclohexadiene (0.17 g, 2.10 mmol). The resulting mixture was heated in microwave reactor at 140 ºC for 1 hour then filtered and washed with ethyl acetate (25 mL). The filtrate was evaporated under reduced pressure and the product was purified by preparative TLC, affording the title compound, {3-[4-amino-6-(2-hydroxyphenyl)-1,3,5-triazin-2-yl]phenyl}(4-methylpiperazin-1-yl)methanone (50 mg, 51 %). LCMS: 3.32 min, 100% purity, m/z 391.3 $[M+H]^+$ (ESI+); NMR: (400 MHz, DMSO) δ: 2.19 (m, 3H), 2.30-2.60 (m, 4H), 3.25 (m, 2H), 3.64 (m, 2H), 6.96 (m, 2H), 7.45 (m, 1H), 7.65 (m, 2H), 8.06 (s, 1H), 8.17 (s, 1H), 8.37 (s, 1H), 8.45 (m, 2H), 13.29 (s, 1H).