Supplementary Table 1. Predicted binding free energies using WaterMap and MM-GBSA for the series of triazolylpurine A2A adenosine antagonists including the relative enthalpy and entropy break down

Compounds	Substituent	Relative Affinity (kcal/mol)	WaterMap ΔΔG (kcal/mol)	WaterMap ΔΔG (kcal/mol)[b]	WaterMap ΔΔS (kcal/mol)	WaterMap ΔΔH (kcal/mol)
11	Hydrogen	0.0	0.0	0.0	0.0	0.0
25a	Methyl	0.3	0.2	0.2	-1.8	2
25e	Isopropyl	1.4	0.5	1.0	-3.0	3.5
25f	N-propyl	0.4	-0.7	0.3	-2.7	2
25b	N-butyl	-1.1	-3.5	-2.0	-6.8	3.3
25c	N-pentyl	-1.5	-5.0	-3.0	-9.7	4.7
25d	Phenethyl	-1.3	-3.8	-2.3	-6.1	2.3

Supplementary Experimental Procedures:

In order to reduce the positional variation of the common core during docking and to ensure a binding mode consistent with ZMA241385 for all compounds in the series, we applied hydrogen bond constraints to the side chain of Glu169 (EL2) and to the side chain of Asn253 (6.55). Other docking calculations were performed, such as constraining the core with or without the hydrogen bond constraints, to test the sensitivity of the scoring to the pose generation method. The poses and WaterMap energies were qualitatively the same; therefore we present only the results with the hydrogen bond constraints to Glu169 and Asn253. Docking without any of the constraints listed above resulted in less consistent poses and degradation in the WaterMap scoring.

WaterMap calculations consist of an all atom explicit solvent molecular dynamics simulation followed by a statistical thermodynamic analysis of water clusters (hydration sites) to compute enthalpy, entropy, and free energy of the hydration sites relative to bulk water. For the WaterMap calculations presented here, the protein was truncated to a region within 15 Å of the ligand and the resulting system was solvated in a TIP4P water box extending at least 5 Å in all directions. A restraint was applied to the protein heavy atoms and the system was relaxed with an initial minimization followed by a short molecular dynamics simulation heating the system from 10 K to 300 K. The binding site was then filled with additional water molecules using the *solvate_pocket* stage in WaterMap, which consists of 100,000 steps of grand canonical Monte Carlo (GCMC) simulation to populate the binding site with a realistic number of water molecules. A final pre-production simulation of 120 ps was run at 300 K. The production simulation was run for 2 ns at 300 K in the NTP ensemble. To test the sensitivity of the hydration site thermodynamics to the initial conditions, two additional calculations were run with: (1) fewer steps (50,000) of GCMC and (2) a longer production simulation run of 9 ns (as in Beuming et al.[24]). No significant differences were observed in either hydration site locations or thermodynamic quantities and therefore only the results from the default simulation are discussed here.

In WaterMap, the excess entropy is computed by numerically integrating a local expansion of spatial and orientational correlation functions[27] as implemented by Abel et al.[16] As an approximation, only contributions from the first order term of the expansion were included in the entropy calculation. The enthalpy is computed by averaging the

molecular mechanics energies of the water molecules in each hydration site over all frames of the molecule dynamics simulation. The WaterMap binding free energy for a ligand (ΔG) is computed as the sum of the excess entropy and enthalpy. Ligand scoring is achieved by summing the energies of each hydration site overlapped by the ligand. In the case of partial overlap with a hydration site, a fractional score is given based on the amount of overlap.

WaterMap only computes one part of the thermodynamics of binding (namely, the receptor desolvation); however, many of the other terms will roughly cancel when analyzing aliphatic substitutions on a fixed core, as in the series studied in this work. For example, the ligand desolvation penalty and electrostatic interactions will be roughly constant within a series of molecules that have a fixed core and aliphatic substitutions. Similarly, if all of the compounds fit in the binding site then the receptor reorganization/strain energy will be approximately the same, since additional strain in the receptor will not be needed to accommodate the ligand substitutions.