Electronic Supplementary Information (ESI) for:

Understanding the role of carbamate reactivity in fatty acid amide hydrolase inhibition by QM/MM mechanistic modelling

Alessio Lodola,*[a] Luigi Capoferri,[a] Silvia Rivara,[a] Ewa Chudyk,[b] Jitnapa Sirirak,[b] Edyta Dyguda-Kazimierowicz,[c] W. Andrzej Sokalski,[c] Mauro Mileni,[d] Giorgio Tarzia,[e] Daniele Piomelli,[f] Marco Mor,[a] and Adrian J. Mulholland*[b]

[a] *Dipartimento Farmaceutico, Università degli Studi di Parma, 43124 Parma, Italy. E-mail: Alessio.Lodola@unipr.it*

[b] *School of Chemistry, University of Bristol, Bristol, BS8 1TS, UK. E-mail: Adrian.Mulholland@bristol.ac.uk*

[c] *Department of Chemistry, Wrocław University of Technology, Wyb. Wyspianskiego 27, Wrocław, Poland.*

[d] *Department of Molecular Biology, The Scripps Research Institute, La Jolla, CA 92037, USA.*

[e] *Dipartimento di Scienze del Farmaco e della Salute, Università degli Studi di Urbino "Carlo Bo", 61029 Urbino, Italy.*

[f] *Department of Drug Discovery and Development, Italian Institute of Technology, 16163, Genova, Italy.*

Set up for QM/MM calculations

The FAAH-inhibitor complexes, obtained after 250 ps of stochastic boundary molecular dynamics simulation, were minimized to an energy gradient of 0.01 kcal mol^{-1} $Å^{-1}$ and employed for QM/MM reaction modelling. The system was partitioned according to the following scheme: the terminal methylamine of Lys142, the side chains of Ser217 and Ser241 and the whole structure of the inhibitors were treated at the PM3 semiempirical QM level,[1] while all the other atoms (7511 atoms) were treated with the CHARMM22 MM potential.[2] The covalent bonds at the boundary between the QM and MM regions were treated by introducing three 'HQ' link atoms,[3] which were included in the QM system (in total 62 quantum atoms for URB524, 63 for URB694 and 64 for URB618).

The QM/MM approach includes bonded and non-bonded interactions between the QM and MM systems, accounting for the electronic and steric effects exerted by the enzyme on the modelled reactions. Van der Waals and bonded interactions were described by MM terms, with standard CHARMM22 parameters[2] for the QM atoms.

Electrostatic interactions were treated by including the MM atomic charges (as atomic 'cores') in the Hamiltonian for the QM system.[3] A nonbonded cutoff of 12 Å was applied

using a group-based switching function to scale the electrostatic interactions smoothly down to zero over 8–12 Å. Atoms further than 14 Å from the Ser241 hydroxyl oxygen were kept fixed; with the exception of these boundary constraints, all the other atoms were free to move during the calculations.

The PM3-CHARMM22 potential was firstly applied to optimize the geometry of FAAH-carbamate inhibitor complexes, to an energy gradient of 0.01 kcal mol^{-1} $Å^{-1}$, and then to scan the potential energy surface (PES) of the carbamoylation reaction (divided into three main steps, as outlined in the main text), using the coordinate driving approach,[4] implemented in CHARMM (version 27b2, RESD command).[5]

This strategy, which has been shown to perform well for similar enzyme reactions,[6,7] allowed the identification of the minimum energy paths for Ser241 carbamoylation by the three inhibitors, and the location of (approximate) transition states, intermediates and products along the modelled pathways.

PM3-CHARMM22 structures of the reactants (Michaelis Complexes) and transition states (TS)s identified on the PESs were employed for DTSS calculations (see below).

Differential transition state Stabilization (DTSS) calculations

The catalytic activity of a given molecular environment, R, can be defined as lowering of the activation energy barrier, Δ, which is equivalent to the difference in interaction energy of R with the transition state, ΔE_{TS-R}, and substrate, ΔE_{S-R}:

$$\Delta = \Delta E_{TS-R} - \Delta E_{S-R} \qquad (Eq.\ 1)$$

Accordingly, transition state stabilization relative to substrates results in a negative value of Δ, giving rise to the catalytic effects (relative to the same reaction in the gas phase).[8,9] When calculated separately for particular components of the catalytic environment (e.g. enzyme active site residues), differential transition state stabilization (DTSS) energy provides a quantitative measure of the residue's contribution to lowering of the barrier.

The structures of Michaelis complexes and transition states (TS)s for FAAH Ser241 carbamoylation by URB524, URB694, and URB618 inhibitors were taken from the QM/MM simulations described in the previous section and in the main text. To make the calculations feasible, FAAH active site models were built by selecting residues within 6 Å of the reaction centre (R) of the systems, defined as the centre of mass of the catalytic triad, formed by Lys142, Ser217 and Ser241. In addition to the catalytic triad members, the structures

(Michaelis complexes and their relative TSs) resulted thus composed by 16 amino acids, two water molecules, and a carbamate inhibitor. All broken bonds were saturated with hydrogen atoms (with positions optimized at the HF/6-31G level). Such an approach has been shown to give useful results in analysing enzyme-catalysed reactions, as reported in recent publications.[9,10]

Interaction energies were calculated in a pairwise fashion (i.e., for the reaction centre and each FAAH residue separately) at the MP2/6-31G(d) level of theory both for the Michaelis complexes (ΔE_{S-R}) and for the transition states (ΔE_{TS-R}). Basis set superposition error was accounted for by means of a counterpoise correction.[11]

Finally, the DTSS energy for each residue was obtained by applying equation 1 (Table S1 and main text).

It has been recently suggested that a water molecule interacting with *p*-OH or *p*-NH_2 groups might affect the inhibitor's potency,[12] so DTSS analysis for the URB694 and URB618 inhibitors was also performed for this particular water molecule (Wat627).

Table S1: Differential Transition State Stabilization (DTSS) energy at the MP2/6-31G(d) level of theory (all values in kcal/mol).

Residue	Inhibitor		
	URB524	URB694	URB618
Pro188	-0.18	-0.13	-0.22
Met191	-0.39	-0.57	0.47
Leu192	-0.46	-0.53	0.01
Ser193	0.34	0.34	0.31
Phe194	-0.32	-0.35	-0.15
Gly216	-0.65	-0.64	-0.23
Ser218	-4.33	-3.86	-4.44
Thr236	-7.91	-7.52	-7.47
Asp237	0.17	0.54	2.50
Ile238	-3.39	-2.99	-3.05
Gly239	-2.83	-3.29	-4.34
Gly240	-2.35	-2.23	-3.43
Arg243	-0.98	-1.21	-2.74
Wat355	0.48	0.07	0.09
Ile491	-0.61	-0.72	-0.83
Thr494	-0.16	-0.15	-0.28
Asn498	-0.21	-0.18	-0.23
Wat623	-1.37	-0.64	-1.36
sum	**-25.13**	**-24.04**	**-25.40**

References

[1] J. J. P. Stewart, *J. Comput. Chem*., 1989, **10**, 221.

[2] A. D. MacKerell, Jr., D. Bashford, M. Bellott, R. L. Dunbrack, Jr., J. D. Evanseck, M. J. Field, S. Fischer, J. Gao, H. Guo, S. Ha, D. Joseph-McCarthy, L. Kuchnir, K. Kuczera, F. T. K. Lau, C. Mattos, S. Michnick, T. Ngo, D. T. Nguyen, B. Prodhom, W. E. Reiher III, B. Roux, M. Schlenkrich, J. C. Smith, R. Stote, J. Straub, M. Watanabe, J. Wiórkiewicz-Kuczera, D. Yin and M. Karplus, *J. Phys. Chem. B*, 1998, **102**, 3586.

[3] M. J. Field, P. A. Bash and M. Karplus, *J. Comput. Chem.*, 1990, **11**, 700.

[4] K. P. Eurenius, D. C Chatfield,. B. R Brooks and M Hodoscek. *Int. J. Quantum Chem.* 1996, **60**, 1189.

[5] B. R. Brooks, R. E. Bruccoleri, B. D. Olafson, D. J. States, S. Swaminathan and M. Karplus, *J. Comput. Chem.,* 1983, **4**, 187.

[6] H. M. Senn and W. Thiel*, Angew. Chem. Int. Ed.,* 2009, **48**, 1198.

[7] M. W. van der Kamp, A. J. Mulholland, *Nat. Prod. Rep.* 2008, **25**, 1001

[8] W. A. Sokalski, *J. Mol. Catal.* 1985, **30**, 395.

[9] B. Szefczyk, A. J. Mulholland, K. E. Ranaghan and W. A. Sokalski, *J. Am. Chem. Soc*., 2004, **126**, 16148

[10] P. Szarek, E . Dyguda-Kazimierowicz, A. Tachibana and W. A. Sokalski, *J. Phys. Chem. B*, 2008, **112**, 11819.

[11] S. F. Boys and F. Bernardi, *Mol. Phys.* 1970, **19**, 553.

[12] G. Tarzia, A. Duranti, G. Gatti, G. Piersanti, A. Tontini, S. Rivara, A. Lodola, P. V. Plazzi, M. Mor, S. Kathuria and D. Piomelli, *ChemMedChem*, 2006, **1**, 130.