Discovery of a novel class of profen derivatives as FAAH inhibitors: MD and QM/MM studies of the binding mode and 3D SAR

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Fatty acid amide hydrolase (FAAH) is a serine hydrolase that catalyzes the deactivating hydrolysis of endocannabinoids. Endogenous FAAH substrates such as anandamide serve as key regulatory functions in the body and have been implicated in a variety of pathological conditions including pain, inflammation, sleep disorders, anxiety, depression and vascular hypertension, and there has been an increasing interest in the development of inhibitors of this enzyme. Recently, we have reported the binding mode in the FAAH rat structure of the two enantiomers of Flu-AM1 and Ibu-AM5[1]. Replacing isobutyl group of Ibu-AM5 with a trifluoromethylpyridinylamino moiety led to TPA5, which maintains the FAAH inhibitory activity. Here we investigate FAAH inhibition by TPA5 derivatives through computational methods, enzyme kinetics and SAR studies.

Design and Synthesis

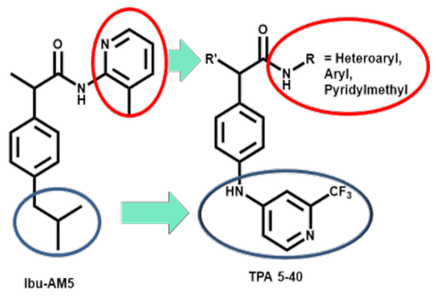
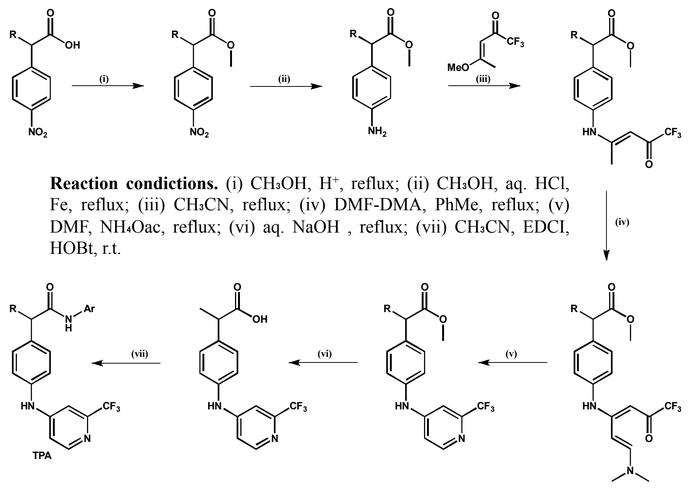


Figure 1. Ibu-AM5 FAAH inhibitory activity and TPA series design.

The 2-arylpropionamides **TPA 5-40** were prepared by an effective multistep synthesis.



Reaction condictions. (i) CH_3OH, H^+, reflux; (ii) CH_3OH, aq. HCl, Fe, reflux; (iii) CH_3CN, reflux; (iv) DMF-DMA, PhMe, reflux; (v) DMF, NH_4Oac, reflux; (vi) aq. NaOH , reflux; (vii) CH_3CN, EDCI, HOBt, r.t.

Scheme 1. Synthetic procedure for TPA series.

The2-arylpropionamides were tested for their inhibitory activity against FAAH using [^{3}H]AEA as substrate.

FAAH inhibition

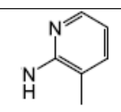
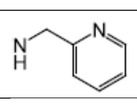
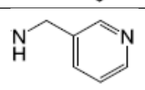
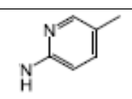
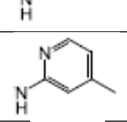
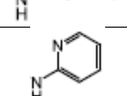
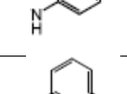
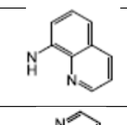
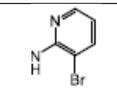
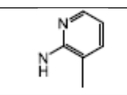
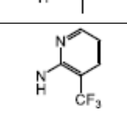
Compound	R	Ar	Max inhibition %	IC_{50} (μM)
TPA5	CH_3		100	0.59
TPA8	CH_3		100	23.0
TPA9	CH_3		100	32.0
TPA11	CH_3		68±4	11.0
TPA12	CH_3		100	4.0
TPA13	CH_3		93±3	12.0
TPA19	CH_3		75±7	4.3
TPA24	CH_3		100	0.13
TPA25	CH_3		100	0.10
TPA26	CH_3		100	0.33
TPA27	CH_3		100	0.058

Table 2. TPA series inhibition data. [a]Inhibition of 2 mmol·L^{-1} AEA hydrolysis in rat brain homogenates.

Computational studies

Docking were performed on the monomer of FAAH X-ray rat with Autodock 4.2 centring the box on the binding site of AEA (Fig. 2, violet spheres) the endogenous substrate of FAAH. The results showed that TPA5 binds to the cavity experimentally found for other competitive ligands,[2,3] adopting up to four different arrangements.

All-atom molecular dynamics (MD) refining of 100 ns were performed on each system using Amber12. Free energy calculations (MM/GBSA, MM/PBSA and SIE) and QM/MM were used to analyse the stability of the last 5 ns of the trajectories.

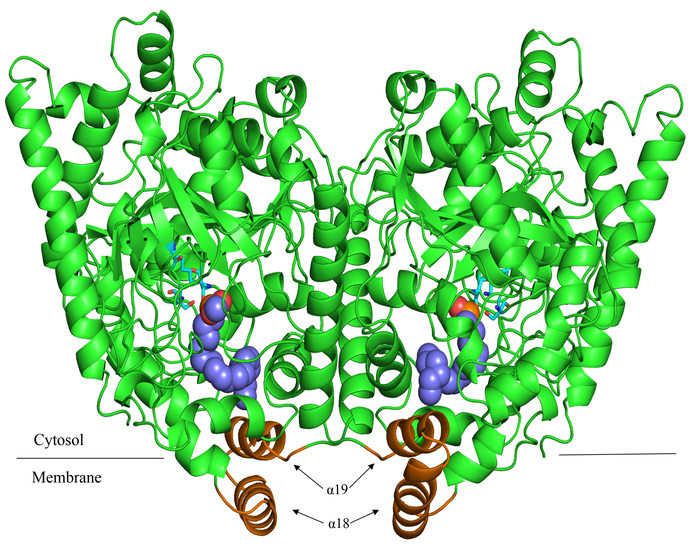


Figure 2. 3D structure and localization of FAAH and representation of the principal structural features: catalytic residues (cyan sticks), membrane interacting helices α18-α19 (orange cartoon) and anandamide analogue (violet spheres).

Docking scores, MD convergence and estimation of binding affinity strongly indicated the A1-binding mode as the preferred pose (Table 1).

	DOCKING				MD analysis						
ligand	mode	Pop%	AD score	MMGBSA	cl	Pop%	Time (ns)	MMGBSA	MMPBSA	SIE	QM/MM
TPA5	A1	50	-9,11	-59,46	0	97,8	95-100	-58,47	-15,76	-10,21	-78,05
	A2	30	-8,41	-49,65	a0	96,2	95-100	-48,04	-2,46	-9,29	-70,66
					b0	38,6	95-100	-48,64	5,26	-9,39	
	B1	9	-9,01	-49,18	a0	66,7	95-100	-45,79	3,03	-8,91	-59,25
					b0	75,1	95-100	-48,23	-2,65	-9,13	
	B2	3	-8,67	-50,68	a0	94,5	95-100	-42,41	3,00	-8,62	-63,6
					b0	48,7	95-100	-47,63	1,13	-9,29	

Table 1. Free energy calculation of the representative binding modes refined through MD runs of 100 ns. A1 mode showed convergence between monomers. In the cases of non-convergent binding mode, data are reported for the best cluster in the monomer A (a0) and B (b0). MMPBSA, MMGBSA, SIE and QM/MM free energy values are in kcal/mol.

TPA5 resulted tightly packed between the catalytic triad and the ABP channel through a series of hydrogen bonds (Fig. 4A) and hydrophobic interactions with the apolar gorge of ABP (Fig. 4B). The methylpyridine moiety adopted a specific conformation, which seems a key requisite for FAAH inhibition for this class of compounds.

SAR on TPA5 derivatives supported A1-binding mode. In fact, the introduction of a methylene linker (TPA8, TPA9) avoid the right conformation of the methylpyridine moiety. Similarly, the absence or different position of the methyl group on the amidopyridine drop off the activity. (TPA11-13). Finally, TPA19 indicated that there is no space to accommodate larger groups.

The replacement of the methyl group in the amidopyridine ring by halogens (TPA24-27) increased the inhibitory activity. In particular, TPA27 showed a 10-fold enhancement in potency, but with a different mode of inhibition.

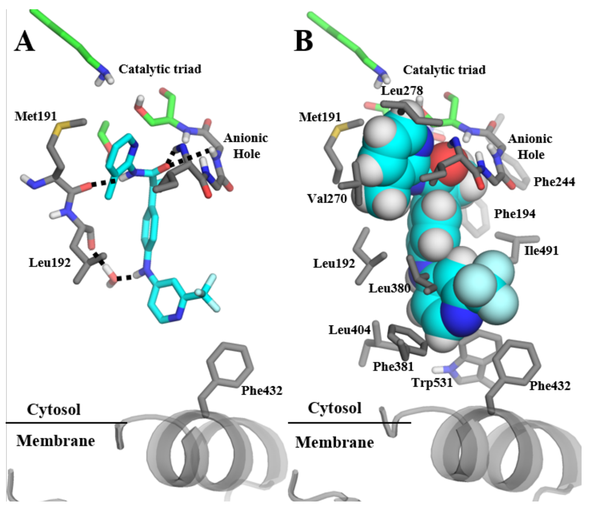
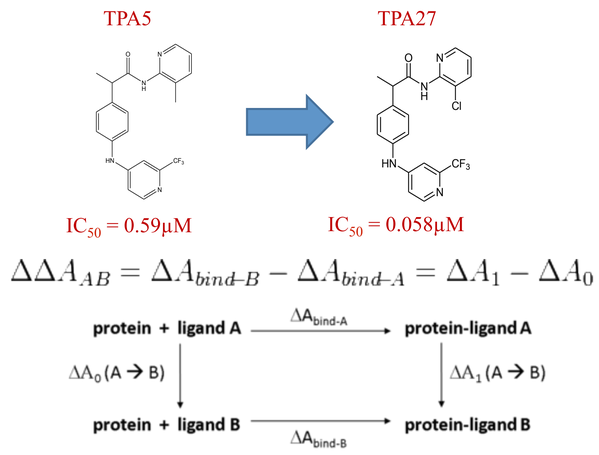


Figure 4. A1-mode of TPA5 refined after 100 ns of MD.



Scheme 2. The thermodynamic cycle used to compute the difference in binding free energy between TPA5 (ligand A) and TPA27 (ligand B).

Thermodynamic integration calculations were performed to study the effect of chlorine substitution, analysing the transformation of the methyl group on TPA5 in the chlorine atom in TPA27 and yielded a free energy difference of 0.3 kcal/mol. This result indicates a decrease of the TPA27 affinity for the competitive binding site, in agreement with the experimental finding that TPA27 follows essentially a non-competitive inhibition mechanism.

Mode of inhibition

TPA5 resulted a competitive inhibitor (Fig. 3B), showing little dependency upon preincubation time, which suggests a reversible inhibition (Fig. 3A).

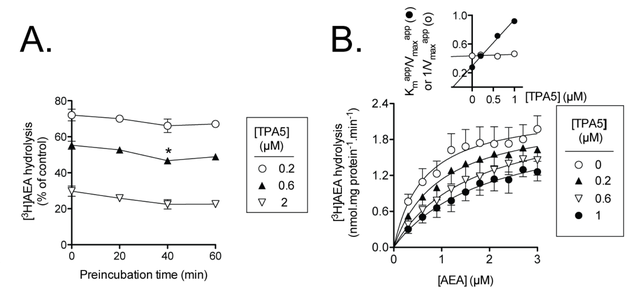


Figure 3. Mode of inhibition of rat brain FAAH by TPA5. Panel A: homogenates were preincubated with the compounds for the times shown prior to addition of 0.5 μM [^{3}H]AEA and assay for FAAH activity (means and s.e.m., n=3-4) Panel B: AEA hydrolysis at the substrate and inhibitor concentrations shown (means and s.e.m, n=3).

Inhibition kinetics experiments showed that TPA27, unlike TPA5, behaves as non-competitive inhibitor. Dilution experiments (Fig. 5C) revealed that TPA27 is a reversible inhibitor while time-dependency experiments excluded the possibility to have a tight binder of FAAH (Fig. 5A).

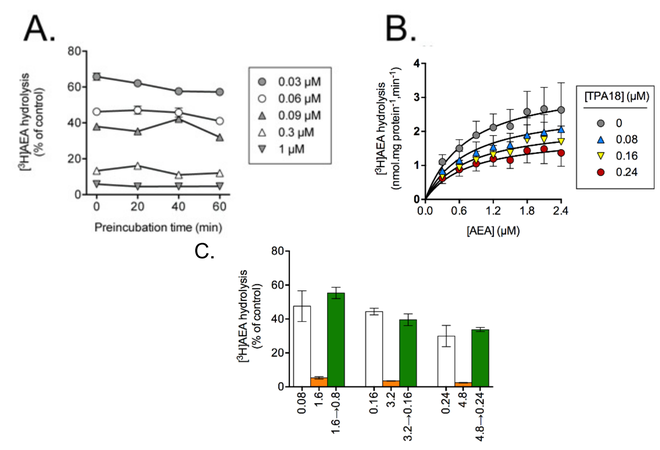


Figure 5. Mode of inhibition of rat brain FAAH by TPA27. A) homogenates were preincubated with the compounds for the times shown prior to addition of 0.5 μM [^{3}H]AEA and assay for FAAH activity (means and s.e.m., n=3-4). B) AEA hydrolysis at the substrate and inhibitor concentrations shown (means and s.e.m, n=3). C) dilution experiments.

Conclusions

Replacing of the isobutyl group of Ibu-AM5 with a trifluoromethylpyridinamino moiety led **TPA5** that maintains the FAAH inhibitory activity and shows pure competitive inhibition kinetic.

Computational studies, exploiting QM/MM and free energy approaches, allowed us to identify the binding mode of TPA5 in a cavity experimentally found for other competitive inhibitors.

Among TPA5 derivatives, the compound TPA27 exhibited a 10-fold enhancement in the inhibitory profile against FAAH and is the first non-competitive reversible FAAH inhibitor. Kinetic experiments highlighted how small substitutions in the compounds belonging to the series of TPA derivatives, should dramatically affect the inhibition mechanism and binding preferences of these inhibitors.

References:

1. Karlsson J, Morgillo CM, Deplano A, Smaldone G, Pedone E, Luque FJ, Svensson M, Novellino E, Congiu C, Onnis V, Catalanotti B, Fowler CJ. Interaction of the N-(3-Methylpyridin-2-yl)amide Derivatives of Flurbiprofen and Ibuprofen with FAAH: Enantiomeric Selectivity and Binding Mode. *PLoS One*. 2015 Nov 13;10(11):e0142711
2. Bracey MH, Hanson MA, Masuda KR, Stevens RC, Cravatt BF. Structural adaptations in a membrane enzyme that terminates endocannabinoid signaling. *Science*. 2002 Nov 29; 298(5599):1793-6.
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