Supplementary Charts

Compound number	Name	CAS number	Structure
1	Diclofenac sodium	15307-29-6	
3	Phenazopyridine hydrochloride	136-40-3	
4	Flufenamic acid	530-78-9	F F N O
5	Aceclofenac	89796-99-6	
6	Dipyridamole	58-32-2	
7	Myricetin	529-44-2	
8	Sulfasalazine	599-79-1	
9	Thonzonium bromide	553-08-2	H_3C_0 N_{15} H_3C_0 H
10	Apigenin	520-36-5	но-С-С-С-С-С-С-С-С-С-С-С-С-С-С-С-С-С-С-С
12	Mefenamic acid	61-68-7	H ₃ C H ₃ HO O

Chart SD1. Structures of the 37 compounds selected as HTS hits for *Ca*FADS.

Compound number	Name	CAS number	Structure
13	Diflunisal	22494-42-4	
14	Benzethonium chloride	121-54-0	$H_{L}^{H,C} \xrightarrow{H,C} H_{L}^{C} \xrightarrow{H,C} H_$
16	Meclofenamic acid sodium salt monohydrate	6385-02-0	H_3C H_2C
18	Clomiphene citrate (Z,E)	50-41-9	$ \begin{array}{c} & & & \\ & & & \\ & & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ \end{array} \right) \xrightarrow{(n)}_{(n)} \left(\begin{array}{c} & & \\ & & \\ & & \\ \\ & & \\ \end{array} \right) \xrightarrow{(n)}_{(n)} \left(\begin{array}{c} & & \\ & & \\ & & \\ \\ & & \\ \end{array} \right) \xrightarrow{(n)}_{(n)} \left(\begin{array}{c} & & \\ & & \\ \\ & & \\ \end{array} \right) \xrightarrow{(n)}_{(n)} \left(\begin{array}{c} & & \\ & & \\ \\ & & \\ \end{array} \right) \xrightarrow{(n)}_{(n)} \left(\begin{array}{c} & & \\ & & \\ \\ & & \\ \end{array} \right) \xrightarrow{(n)}_{(n)} \left(\begin{array}{c} & & \\ & & \\ \\ & & \\ \end{array} \right) \xrightarrow{(n)}_{(n)} \left(\begin{array}{c} & & \\ & & \\ \\ & & \\ \end{array} \right) \xrightarrow{(n)}_{(n)} \left(\begin{array}{c} & & \\ & & \\ \\ & & \\ \end{array} \right) \xrightarrow{(n)}_{(n)} \left(\begin{array}{c} & & \\ & & \\ \\ & & \\ \end{array} \right) \xrightarrow{(n)}_{(n)} \left(\begin{array}{c} & & \\ & & \\ \\ & & \\ \end{array} \right) \xrightarrow{(n)}_{(n)} \left(\begin{array}{c} & & \\ & & \\ \\ & & \\ \end{array} \right) \xrightarrow{(n)}_{(n)} \left(\begin{array}{c} & & \\ & & \\ \end{array} \right) \xrightarrow{(n)}_{(n)} \left(\begin{array}{c} & & \\ & & \\ \end{array} \right) \xrightarrow{(n)}_{(n)} \left(\begin{array}{c} & & \\ & & \\ \end{array} \right) \xrightarrow{(n)}_{(n)} \left(\begin{array}{c} & & \\ & & \\ \end{array} \right) \xrightarrow{(n)}_{(n)} \left(\begin{array}{c} & & \\ & & \\ \end{array} \right) \xrightarrow{(n)}_{(n)} \left(\begin{array}{c} & & \\ & & \\ \end{array} \right) \xrightarrow{(n)}_{(n)} \left(\begin{array}{c} & & \\ & & \\ \end{array} \right) \xrightarrow{(n)}_{(n)} \left(\begin{array}{c} & & \\ & & \\ \end{array} \right) \xrightarrow{(n)}_{(n)} \left(\begin{array}{c} & & \\ & & \\ \end{array} \right) \xrightarrow{(n)}_{(n)} \left(\begin{array}{c} & & \\ & & \\ \end{array} \right) \xrightarrow{(n)}_{(n)} \left(\begin{array}{c} & & \\ & & \\ \end{array} \right) \xrightarrow{(n)}_{(n)} \left(\begin{array}{c} & & \\ & & \\ \end{array} \right) \xrightarrow{(n)}_{(n)} \left(\begin{array}{c} & & \\ & & \\ \end{array} \right) \xrightarrow{(n)}_{(n)} \left(\begin{array}{c} & & \\ & & \\ \end{array} \right) \xrightarrow{(n)}_{(n)} \left(\begin{array}{c} & & \\ & & \\ \end{array} \right) \xrightarrow{(n)}_{(n)} \left(\begin{array}{c} & & \\ & & \\ \end{array} \right) \xrightarrow{(n)}_{(n)} \left(\begin{array}{c} & & \\ & & \\ \end{array} \right) \xrightarrow{(n)} \left(\begin{array}{c} & & \\ & & \\ \end{array} \right) \xrightarrow{(n)} \left(\begin{array}{c} & & \\ & & \\ \end{array} \right) \xrightarrow{(n)} \left(\begin{array}{c} & & \\ & & \\ \end{array} \right) \xrightarrow{(n)} \left(\begin{array}{c} & & \\ & & \\ \end{array} \right) \xrightarrow{(n)} \left(\begin{array}{c} & & \\ & & \\ \end{array} \right) \xrightarrow{(n)} \left(\begin{array}{c} & & \\ & & \\ \end{array} \right) \xrightarrow{(n)} \left(\begin{array}{c} & & \\ & & \\ \end{array} \right) \xrightarrow{(n)} \left(\begin{array}{c} & & \\ & & \\ \end{array} \right) \xrightarrow{(n)} \left(\begin{array}{c} & & \\ & & \\ \end{array} \right) \xrightarrow{(n)} \left(\begin{array}{c} & & \\ & & \\ \end{array} \right) \xrightarrow{(n)} \left(\begin{array}{c} & & \\ & & \\ \end{array} \right) \xrightarrow{(n)} \left(\begin{array}{c} & & \\ & & \\ \end{array} \right) \xrightarrow{(n)} \left(\begin{array}{c} & & \\ & & \\ \end{array} \right) \xrightarrow{(n)} \left(\begin{array}{c} & & \\ \end{array} \right) \xrightarrow{(n)} \left(\begin{array}{c} & & \\ & & \\ \end{array} \right) \xrightarrow{(n)} \left(\begin{array}{c} & & \\ \end{array} \right) \xrightarrow{(n)} \left(\begin{array}$
22	Butoconazole nitrate	64872-77-1	
25	Quercetine dihydrate	6151-25-3	
28	Karakoline	39089-30-0	HO H Chiral HO H Chiral H, CH ₃ HO H H Chiral H, CH ₃ HO H H Chiral
29	Methyl- benzethonium chloride	25155-18-4	[+++C] a-
32	Diethylstilbestrol	56-53-1	HO CH ₃ OH
33	Alexidine dihydrochloride	1715-30-6	

Compound number	Name CAS numbe		Structure
35	Dienestrol	84-17-3	H ₃ C H ₃ C CH ₃ OH
37	Fluvastatin sodium salt	93957-55-2	CH CH CH CH CH CH CH CH CH CH CH CH CH CH CH CH CH CH CH CH
38	Raloxifene hydrochloride	82640-04-8	
39	Luteolin	491-70-3	HO, O, OH OH OH O
40	(+)-Isoproterenol (+)-bitartrate salt	54750-10-6	HO HO HO HO HO HO HO HO HO HO HO HO HO H
44	(-)-Eseroline fumarate salt	70310-73-5	HO H ₃ C Chiral N CH ₃ HO CH ₃ OH
46	Kaempferol	520-18-3	HO O OH OH OOH
47	Verteporfin	129497-78-5	

Supplementary Figures

Figure SD1. Similarity within members of the bacterial bifunctional FADS family. (A) Sequence alignment of *Ca*FADS (Q59263), *Spn*FADS (A0A0H2UPY5) and *Mt*FADS (NP_217302.1) obtained using CLUSTAL W (http://www.ebi.ac.uk/clustalw/) with default parameters. The consensus sequence is indicated on the top. Equivalent residues at each position show same color. Deep and light blue upper bars indicate regions for the FMNAT and RFK modules, respectively. (B) Structures of *Ca*FADS (PDB 2X0K), with the FMNAT and RFK modules in blue and light blue respectively, and of *Spn*FADS (PDB 3OP1), with the FMNAT and RFK modules in pink and light pink respectively. The reaction catalyzed by each module is next to the corresponding module. On the right it is shown the structural alignment showing an r.m.s.d. of 1.63 Å for 247 C α atoms. Structural figures were produced with PyMol (Delano 2002).

Α									FMN	AT module
Conservation Bourd A200 MPTADS CalFADS	-384.21111 NRRRLAIVOR	TENOR OT O	21 4 p + r + + t r + T = 8 D + V + V + OWERCY - T + E D N + A V +		AT T. T. T. M.	HENRY CARACING	TEADATAINT	TH SPYNIELEL EFYHFDLFLH YFCSHCAQ L-RRAFLO		91 1×RECVIELV VODLOIZVEL ASSFELDAVL
Sper ADS NEFADS Cer ADS	VIDETEQTES VIDETEQTES VNPPITOPNK VIDETROLSO	TTADEFFATT		VIGANITES	NARGAROALI OKKYAEGLIK KAAGNVOILA BAAGTADGLA	101 97	des	SACEARA ISST	NIRGALLOCH NIRGALLOCH YINSCYDAOD THEFLOCED	VERAMARIES VNEAGRIIGA NVAAMEALDA VARANWALDA
Soverado Boverados Mérados Caeriados	PAYYAGAYYA FLASHOXYA HAVYAGYYA		TETANGERES TETANGERES TETANGERES TETANGERES	ATTAL ADDAY				THATFAURLE KAYTFOLISA MATTEARTO THEFTFOLIST	NTCVNITORN TVCKIVLOTT DICEPTLOND	ADLYGGRWAN ADLYGDHYAL ADLYGHDYRY
BurFADS MMADS CaFADE	YNA DH THOAT	AFRANKISLAN AFRANKSLAN AFRANKSLAN		NV8 DLLST TLLAGDVGAH	SHI RMA+ETTPLO	ALS RFK	module			



Figure SD2. Hit 24 as inhibitor of the FMNAT activity of *Ca*FADS. Michaelis-Menten plots at different concentrations of 24 and saturation (A) of ATP and (B) of FMN. Lineaweaver-Burk representations with global fit (C) to non-competitive inhibition model when ATP saturating and (D) to competitive inhibition model when FMN saturating. Reaction rates obtained in 20 mM PIPES, pH 7.0, 10 mM MgCl₂, 2.5 % DMSO, at 25 °C, with 15 μ M FMN and 10-450 μ M ATP (FMN saturating) or with 350 μ M ATP and 0.5-20 μ M FMN (ATP saturating) (n=3, mean \pm SD).



Figure SD3. Hit 31 as inhibitor of the FMNAT activity of *Ca*FADS. Michaelis-Menten plots at different concentrations of 31 and saturation (A) of ATP and (B) of FMN. (C) Lineaweaver-Burk representations with global fit to non-competitive inhibition model when ATP saturating, and (D) to competitive inhibition model when FMN saturating. Reaction rates obtained in 20 mM PIPES, pH 7.0, 10 mM MgCl₂, 2.5% DMSO, at 25 °C, with 15 μ M FMN and 10-450 μ M ATP (FMN saturating) or with 350 μ M ATP and 0.5-20 μ M FMN (ATP saturating) (n=3, mean \pm SD).



Figure SD4. Results from the docking analysis of the best five poses of the inhibitors (A) 24, (B) 27 (the five with almost identical orientation) and (C) 31 at the *Ca*FADS FMNAT site. Inhibitors, FMNAT module and RFK module are showed in purple, gray and green, respectively. The poses for 24 and 27 correspond to the docking performed for the first molecular binding at the FMNAT module (24₁ and 27₁).



Supporting Tables

Table SP1. Apparent kinetic parameters for the FMNAT activity of *Ca*FADS at different concentrations of the 24, 27 and 31 inhibitors. Data obtained at 25°C in 20 mM PIPES, pH 7.0, 10 mM MgCl₂, 2.5% DMSO.

	Saturat	ing ATP		Saturat	ting FMN			
[24]	k _{cat}	K_m	[24]	k_{cat}	K_m			
(µM)	(min ⁻¹)	(µM)	(µM)	(\min^{-1})	(µM)			
0	34 ± 3.8	16.3 ± 3.2	0	22.4 ± 0.7	29.4 ± 4.9			
0.12	$24.3\pm4.1\ensuremath{^{\mathrm{a}}}$	14.0 ± 3.9	0.04	22.5 ± 4.8	11.5 ± 0.7 a			
0.2	9.8 ± 1.3 $^{\mathrm{a}}$	10.6 ± 2.4	0.12	$7.2\pm0.4^{\rm \ a}$	$42.1\pm3.9^{\rm \ a}$			
0.4	5.6 ± 0.6 a	$4.3\pm1.0^{\rm \ a}$	0.2	$4.3\pm0.4^{\rm \ a}$	35.8 ± 3.2			
0.6	5.7 ± 1.3 ^a	11.1 ± 3.9	0.4	1.8 ± 0.1 a	12.0 ± 3.6 ^a			
	Saturat	ing ATP		Satura	ting FMN			
[27]	k_{cat}	K_m	[27]	k_{cat}	K_m			
(µM)	(\min^{-1})	(µM)	(µM)	(min ⁻¹)	(µM)			
0	34 ± 3.8 ^a	16.3 ± 3.2	0	22.4 ± 0.7	29.4 ± 4.9			
0.05	$21.6\pm2.9^{\rm \ a}$	10.1 ± 2.9	0.1	22.8 ± 1.3	59.7 ± 5.7 $^{\rm a}$			
0.1	16.1 ± 1.7 $^{\mathrm{a}}$	7.7 ± 2.1	0.2	22.2 ± 1.4	$48.2\pm5.6^{\rm \ a}$			
0.2	15.1 ± 1.5 ^a	13.5 ± 2.6	0.4	18.1 ± 1.6	51.8 ± 5.5 ^a			
0.75	$10.0\pm0.8^{\rm \ a}$	12.8 ± 2.1	0.75	17.1 ± 3.3	$51.5\pm4.9^{\rm \ a}$			
1	$6.5\pm0.2^{\rm \ a}$	15.0 ± 2.0	1	$12.5\pm0.5~^{\rm a}$	40.9 ± 5.7			
	Saturat	ing ATP		Satura	ting FMN			
[31]	k _{cat}	K_m	[31]	k_{cat}	K_m			
(µM)	(min ⁻¹)	(µM)	(µM)	(min ⁻¹)	(µM)			
0	34 ± 3.8	16.3 ± 3.2	0	22.4 ± 0.7	29.4 ± 4.9			
1	$24.6\pm2.9^{\rm \ a}$	13.3 ± 3.1	4	$19.7\pm1.0^{\mathrm{a}}$	43.3 ± 7.9			
4	$18.1\pm1.4^{\mathrm{a}}$	6.8 ± 1.3	5	17.3 ± 1.1 ^a	47.5 ± 5.7 $^{\mathrm{a}}$			
5	6.4 ± 1.2^{a}	24.4 ± 7.0	7	16.9 ± 0.6^{a}	79.1 ± 8.7 $^{\rm a}$			
7	3.0 ± 0.4 a	13.3 ± 3.6	10	15.3 ± 1.3 $^{\rm a}$	102.9 ± 24.2 $^{\rm a}$			
10	3.0 ± 0.4 a	12.3 ± 3.6	12	$13.8 \pm 1.0^{\text{ a}}$	92.1 ± 14.9 ^a			
^a Values showing statistically significant differences, $p < 0.002$, from the values in the								

absence of compounds, as determined by the one-way ANOVA test (n=3, confidence interval 95%).

Delano, W. L. (2002). "They PyMOL molecular graphics system." <u>DeLano Scientific, San</u> <u>Carlos, CA, USA</u>: <u>http://www.pymol.org</u>.