

Supporting Information

Best Experimental Strategies to Study L-Type Amino Acid

Transporter 1 (LAT1) Utilization by Ligands

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1. LC-MS/MS ionization parameters of compounds 1-15

Table S1. Ionization parameters for the LC-MS/MS methods of the studied compounds.

Compound	Precursor ion	Product ion(s)	Fragmentor Voltage (V)	Collision Energy (V)	Flow rate (mL/min)	Gradient (A:B)	LLOQ (nM)
1 → 1a *	514 (322.8)	301 (203.7)	120 (140)	46 (34)	0.2	90:10 →10:90	0.5 (10)
2	417	135	70	30	0.4	80:20 →20:80	0.05
3	417	135	60	20	0.4	80:20 →20:80	0.05
4 → KPF *	255	209	100	10	0.4	80:20 →20:80	0.1
5	371	177	60	16	0.3	90:10 →10:90	0.05
6	357	311	40	6	0.3	90:10 →10:90	0.05
7 → FA *	358 (193)	176 (134)	60 (100)	16 (16)	0.3	90:10 →10:90	0.05 (1.0)
8	301	181	100	11	0.3	95:5 →5:95	3.0
9	407	361	170	13	0.3	95:5 →5:95	1.0
10	369	323, 161	140	13	0.3	95:5 →5:95	1.0
11	393	347, 185	150	15	0.3	95:5 →5:95	1.0
12 → FLB *	408 (243)	362 (199)	175 (380)	15 (10)	0.5	95:5 →5:95	2.5 (2.5)
13 → IBU *	370 (205)	324, 161 (161)	150 (380)	15, 19 (5)	0.5	95:5 →5:95	2.5 (2.5)
14 → KPF *	255	209	100	10	0.4	80:20 →20:80	0.1
15 → NPX *	394 (229)	348, 185 (170)	150 (380)	15, 19 (10)	0.5	95:5 →5:95	2.5 (2.5)
Diclofenac (ISTD)	294	250	50	3	-	-	-
Labetalol (ISTD)	329	294, 162	70	10	-	-	-

* Compounds **4** and **14** were fully converted into ketoprofen (KPF), and compound **1** partly to its investigational perforin inhibitor **1a** and therefore, their transitions with a positive ion mode are reported only as KPF or as a prodrug and parent drug in parentheses. Compounds **7**, **12**, **13**, and **15** were also partly converted to their parent drugs (ferulic acid (FA), flurbiprofen (FLB), ibuprofen (IBU), and naproxen (NPX), respectively), whose transitions with a negative ion mode are reported below the studied LAT1-utilizing compounds in parentheses.

2. LC-MS/MS proteomic transitions for LAT1 peptides

Table S2. SRM/MRM transitions for absolute quantitative proteomics.

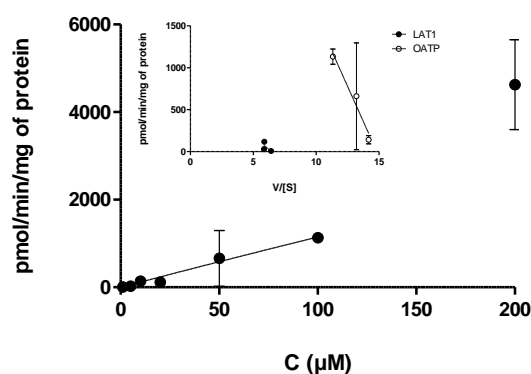
Protein	St/IS	Unique amino acid sequence	Retention time (min)	Transition number	MRM transitions (m/z)	
					Precursor ion (Q1)	Product ions (Q3)
LAT1	St	VQDAFAAAK	13.7	1	460.7	821.4
				2		578.3
				3		507.3
	IS	VQDAFAAAK*	13.7	1	464.8	829.4
				2		586.3
				3		515.3

St – standard, IS – internal standard

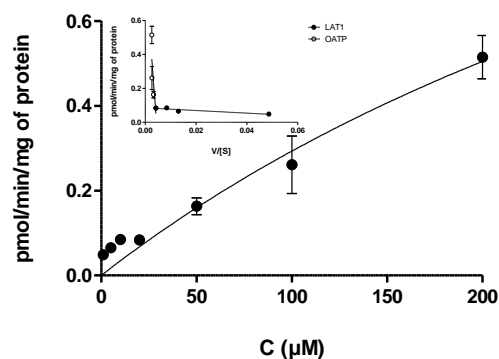
Bold letter with* denotes labelled arginine (R) or lysine (K) with a stable isotope ^{13}C and ^{15}N

3. Cellular uptake of compounds 1-15 into immortalized microglia (BV2 cells)

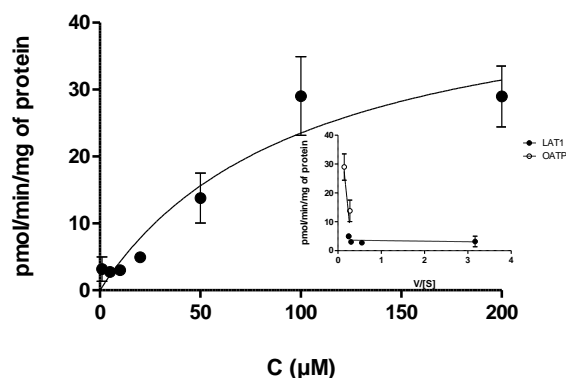
Compound 1



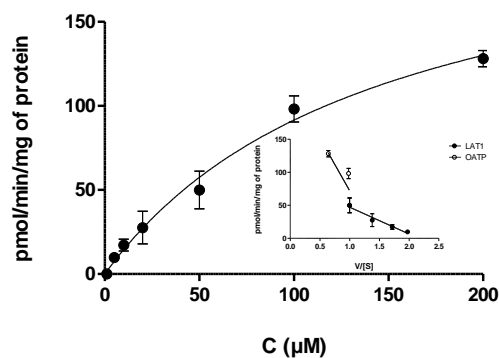
Compound 2



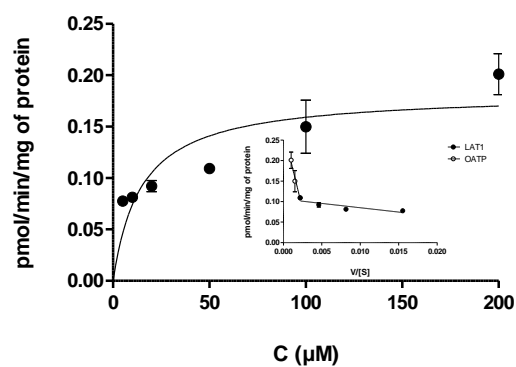
Compound 3



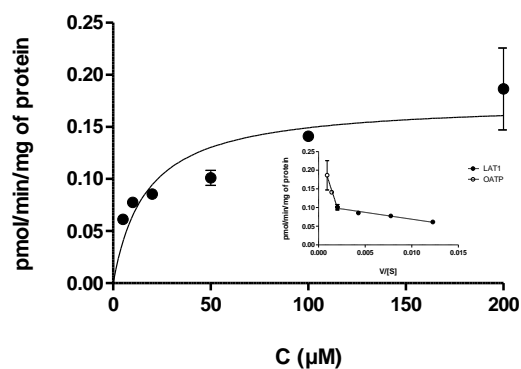
Compound 4



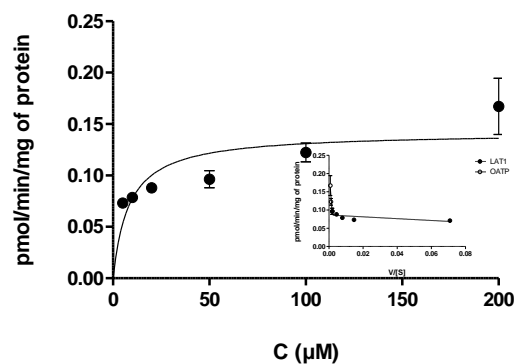
Compound 5



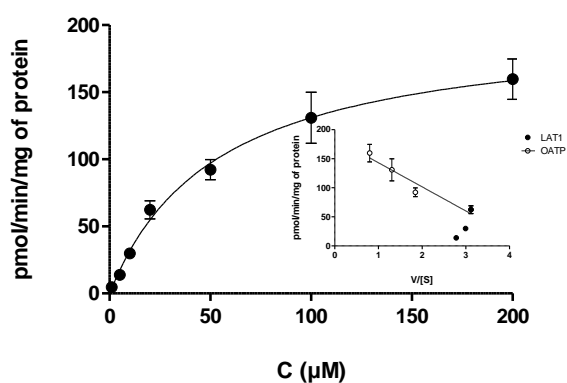
Compound 6



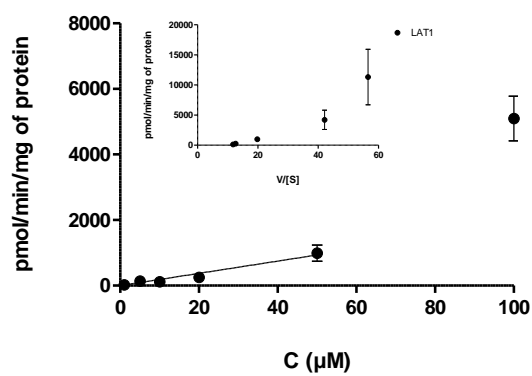
Compound 7



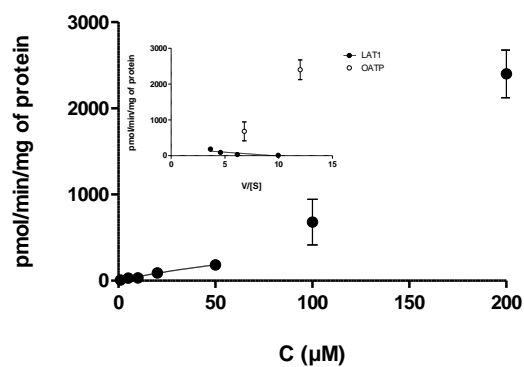
Compound 8



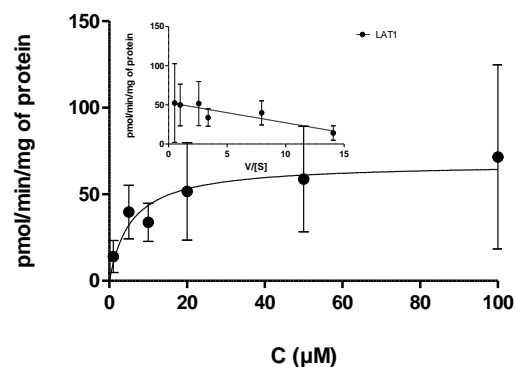
Compound 9



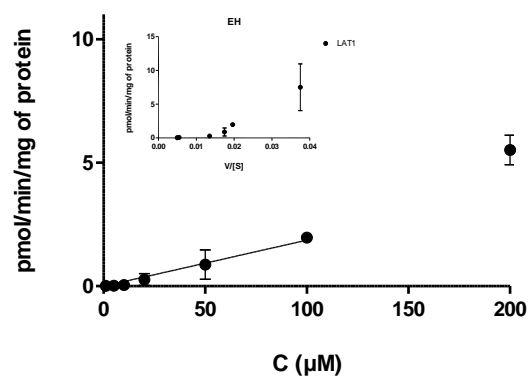
Compound 10



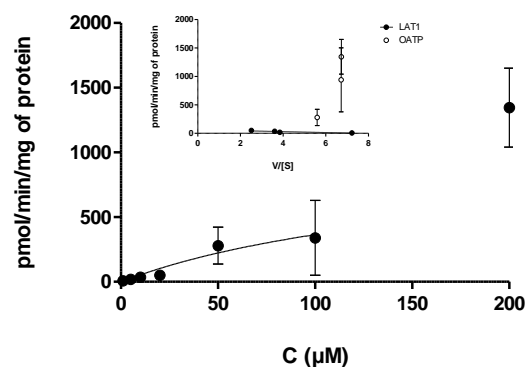
Compound 11



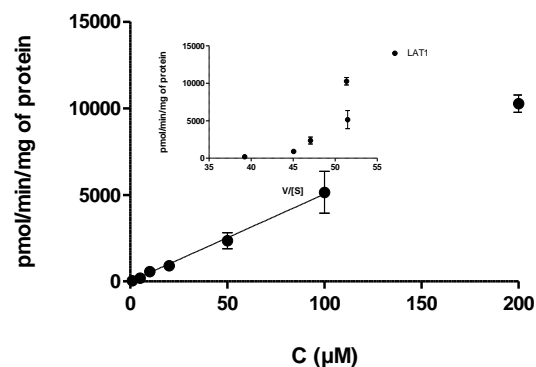
Compound 12



Compound 13



Compound 14



Compound 15

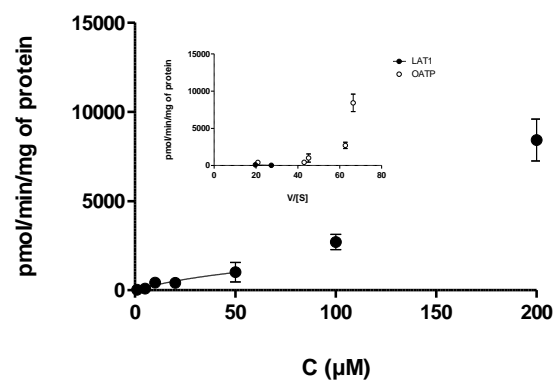


Figure S1. Cellular uptake of 1-200 μ M compounds **1-15** into immortalized microglia (BV2 cells) after 30 min incubation (mean \pm SD, n=3).

4. Cellular uptake of compounds **1-15** in the absence and presence of LAT1-inhibitor

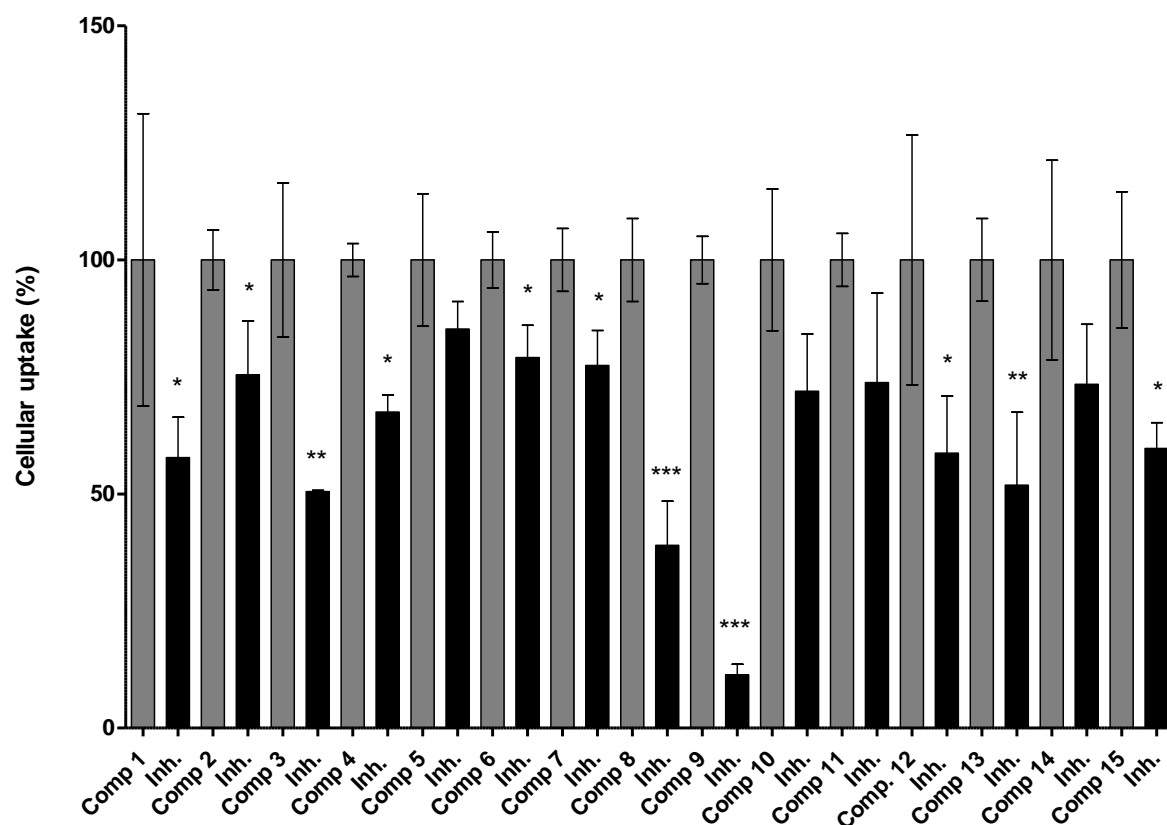
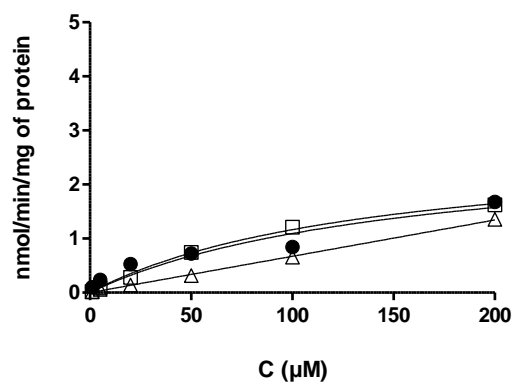


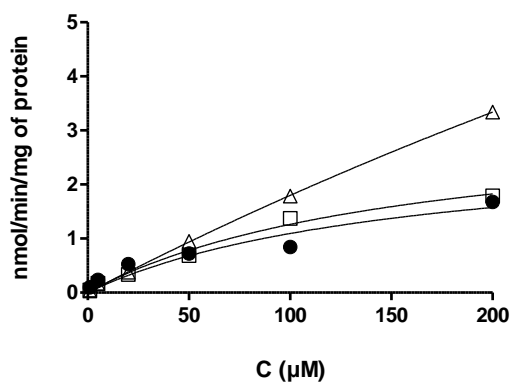
Figure S2. Cellular uptake of 100 μ M compounds **1-15** into immortalized microglia (BV2 cells) with and without LAT1-inhibitor (KMH-233) after 30 min incubation (mean \pm SD, n=3).

5. Cellular uptake of [14 C]-L-leucine after incubation of compounds 1-15

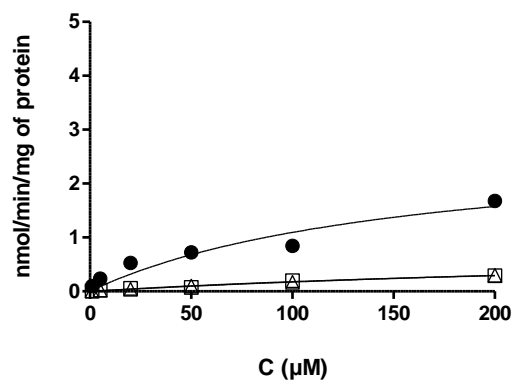
Compound 1



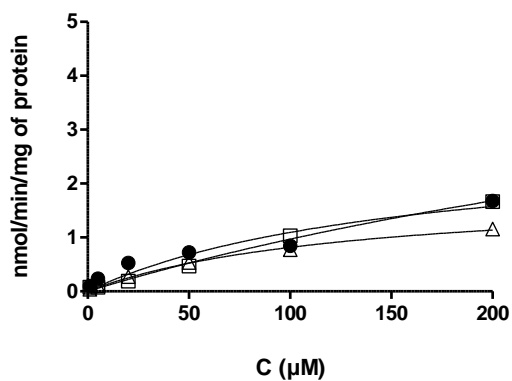
Compound 2



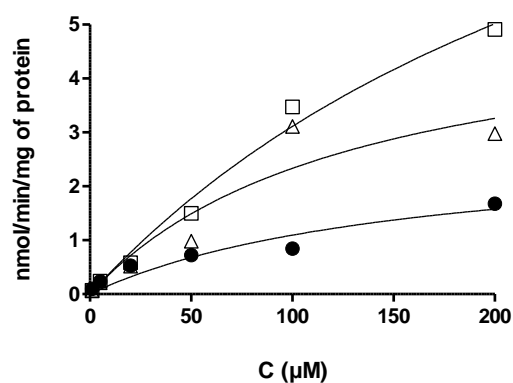
Compound 3



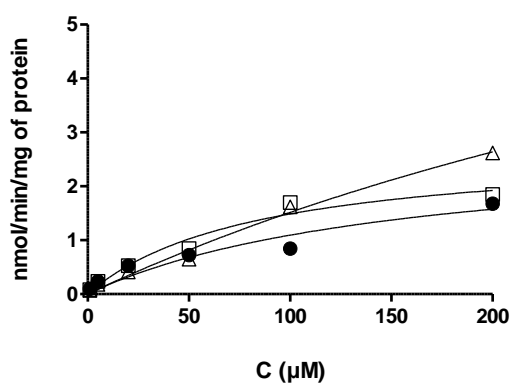
Compound 4



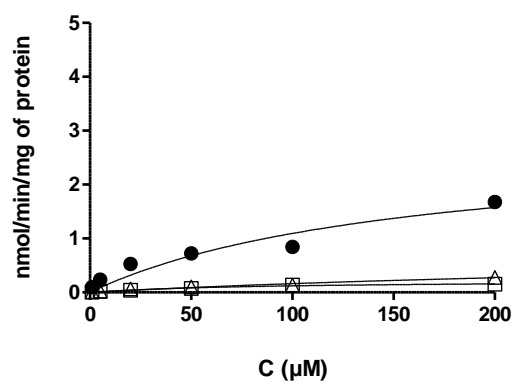
Compound 5



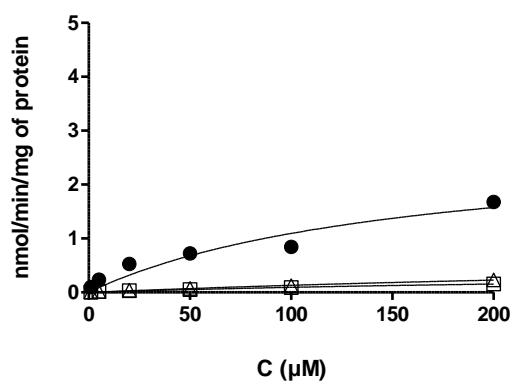
Compound 6



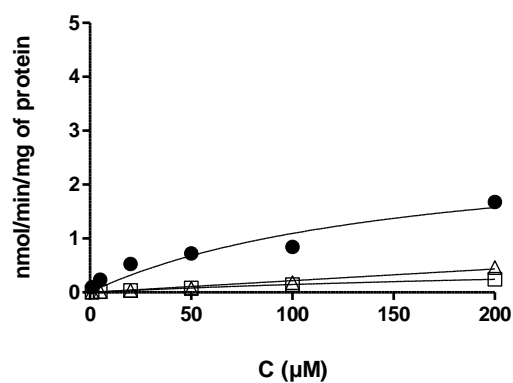
Compound 7



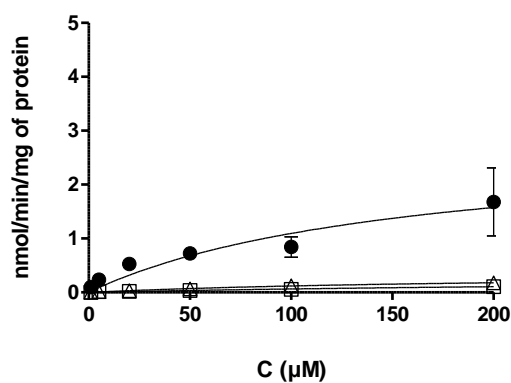
Compound 8



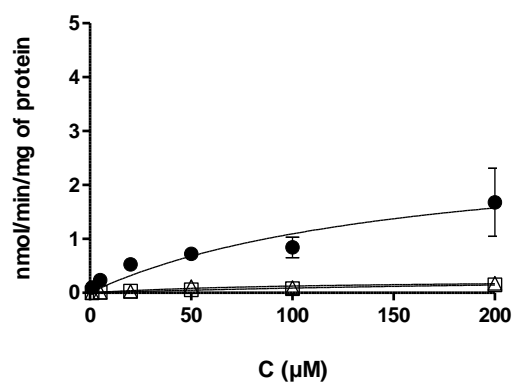
Compound 9



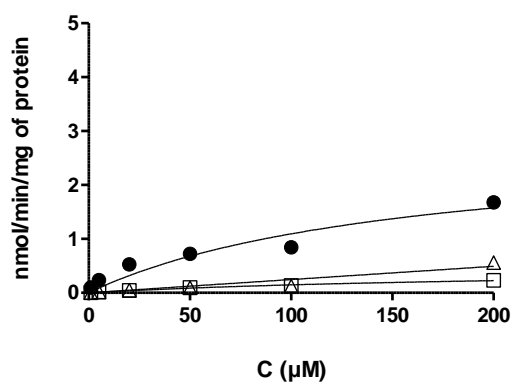
Compound 10



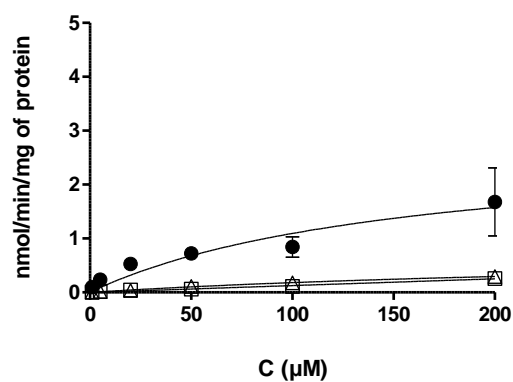
Compound 11



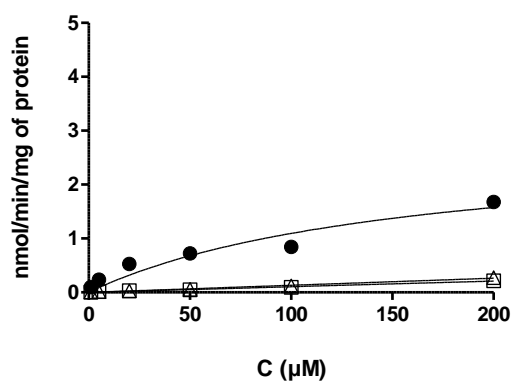
Compound 12



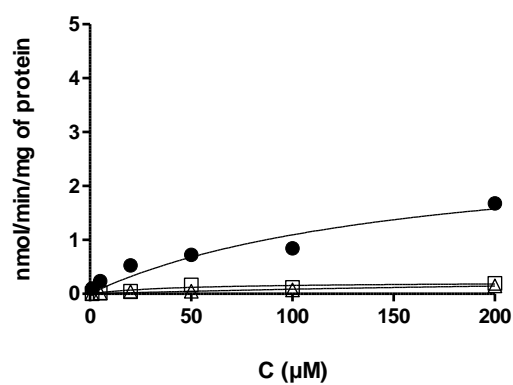
Compound 13



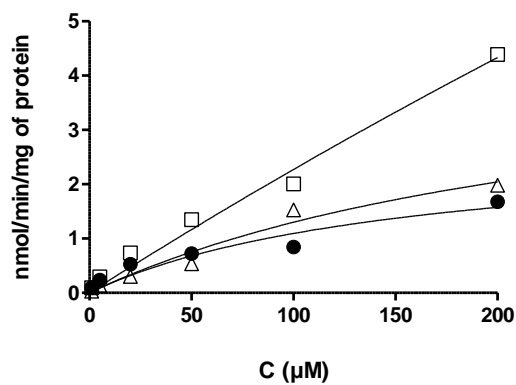
Compound 14



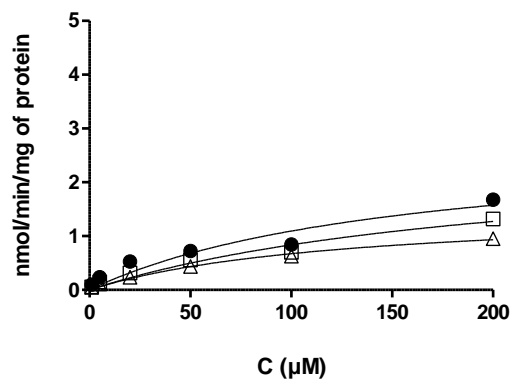
Compound 15



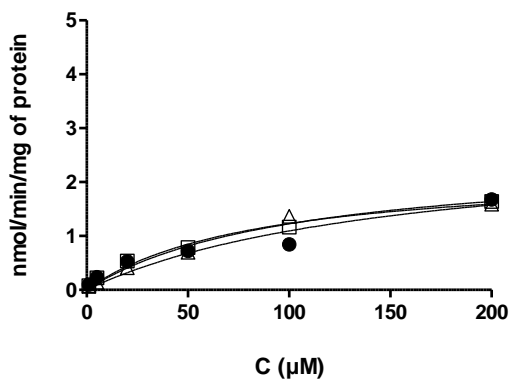
Thyroxin



Dexamethasone



Phenobarbital



L-Glutamine

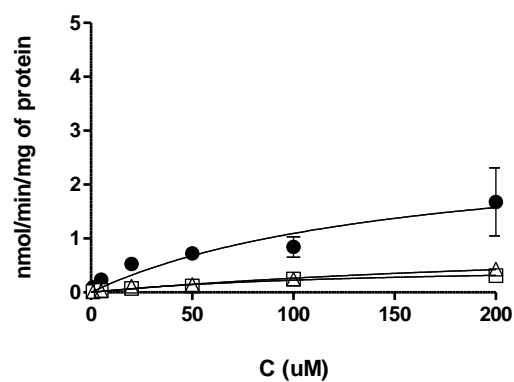


Figure S3. Cellular uptake of 1-400 μM [^{14}C]-L-leucine (● filled circles) into immortalized microglia (BV2 cells) after 10 min incubation (□ open squares) or 3 h incubation (Δ open triangles) of compounds **1-15**, thyroxine, dexamethasone, phenobarbital, or L-glutamine (mean \pm SD, n=3).