Supporting Information

Discovery of Novel and Highly Selective Inhibitors of Calpain for the Treatment of Alzheimer´s Disease: 2-(3-Phenyl-1H-pyrazol-1-yl)nicotinamides

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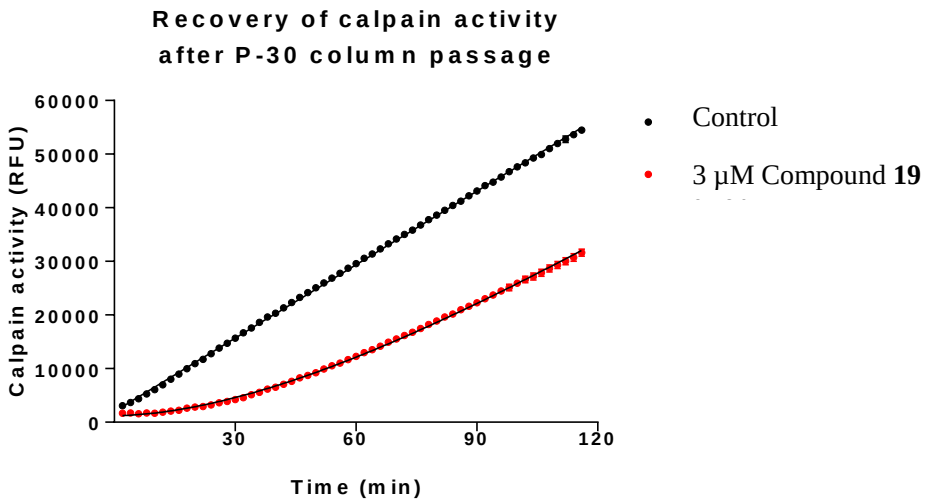
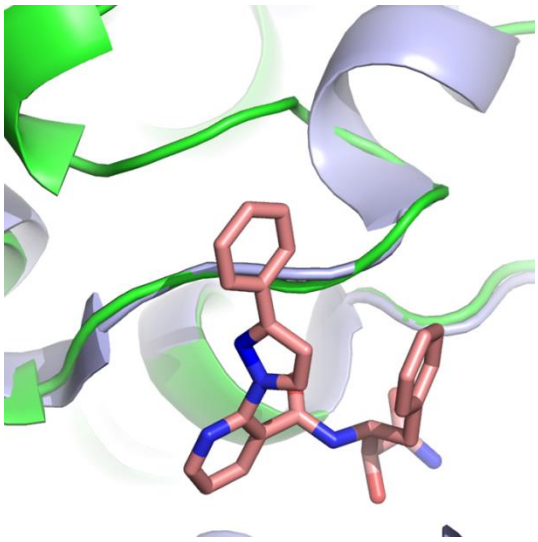
This supplementary information includes data on:

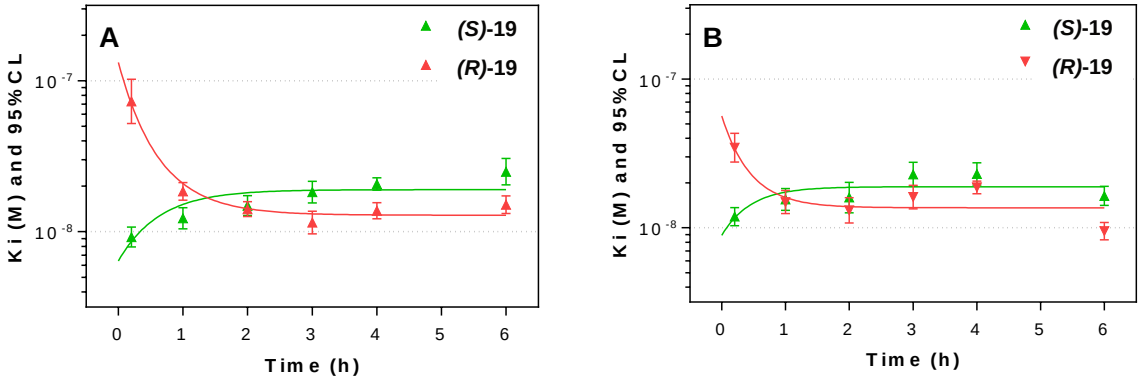
1. Recovery of calpain 1 activity after preincubation with compound **19** demonstrating reversible binding of ketoamide-based inhibitors.
2. Homology model of compound **19** in human calpain 1 and cathepsin K with close-up on the S3 site.
3. Racemisation of (*R*)- and (*S*)-**19** under physiological conditions at 37°C.
4. Profile of compound **19** in a CEREP panel of 74 receptor binding assays.
5. Profile of compound **19** the MDS EnzymeProfilingScreen of 82 enzymes.
6. Profile of compound **38** in a CEREP panel of 74 receptor binding assays.
7. Profile of compound **38** the MDS EnzymeProfilingScreen of 81 enzymes.
8. ^{1}H NMR and ^{13}C spectra for compounds **19** and **38.**
9. Effects of donepezil on REM sleep in rat.



Supplemental Figure 1 Recovery of calpain 1 activity after preincubation with compound **19** and P-30 column passage.



Supplemental Figure 2 Model of compound **19** in human calpain 1 and cathepsin K with close-up on the S3 site. Shown is the protein backbone (calpain 1 in lightblue, cathepsin K in green).



Supplemental Figure 3 Racemisation of (R)- and (S)-19 under physiological conditions at 37°C. Compounds were spiked into buffer pH 7.4 (A) or hirudin-anticoagulated rat plasma (B) and calpain activity measured (Ki and 95% confidence limits).

Supplemental Table 1. Profile of **19** in CEREP panel of receptor binding assays at 10μM concentrations.

Assay	% Inhibition	Assay	% Inhibition
5-HT1A (h)	-9	GABA (non-selective)	5
5-HT1B	3	GAL1 (h)	7
5-HT2A (h)	13	GAL2 (h)	7
5-HT2C (h)	4	H1 (h)	-3
5-HT3 (h)	-11	H2 (h)	8
5-HT5A (h)	-19	K+V channel	3
5-HT6 (h)	-9	M1 (h)	32
5-HT7 (h)	-17	M2 (h)	3
A1 (h)	-7	M3 (h)	12
A2A (h)	0	M4 (h)	-7
A3 (h)	6	M5 (h)	1
Alpha 1	4	MC4 (h)	5
Alpha 2	5	MT1 (h)	9
AT1 (h)	2	Na+ channel (site 2)	-15
AT2 (h)	9	NE transporter (h)	6
B2 (h)	-19	NK1 (h)	-4
BB (non-selective)	-11	NK2 (h)	10
Beta 1 (h)	8	NK3 (h)	-3
Beta 2 (h)	-8	NT1 (h) (NTS1)	-9
BZD (peripheral)	95	Opiod delta 2	10
BZD (central)	6	Opiod kappa	36
Ca2+ channel (L, verapamil site)	-4	Opiod mu	4
CB1 (h)	12	ORL1 (h) (NOP)	-4
CCKA (h) (CCK1)	-4	P2X	1
CCKB (h) (CCK2)	-6	P2Y	17
CCR1 (h)	0	PACAP (PAC1) (h)	17
CGRP (h)	5	PCP	5
Cl- channel	8	PDGF	1
CXCR2 (h) (IL-8B)	-2	Sigma (non-selective)	5
D1 (h)	-2	SK+Ca channel	-1
D2S (h)	4	SST (non-selective)	0
D3 (h)	2	TNF-alpha (h)	-12
D4.4 (h)	3	TXA2/PGH2 (h) (TP)	12
D5 (h)	-22	V1a (h)	-8
DA transporter (h)	5	VIP1 (h) (VPAC1)	-7
ETA (h)	-1	Y1 (h)	-2
ETB (h)	11	Y2 (h)	-5

Supplemental Table 2. Profile of **19** in MDS EnzymeProfiling Screen at 10 μM concentrations.

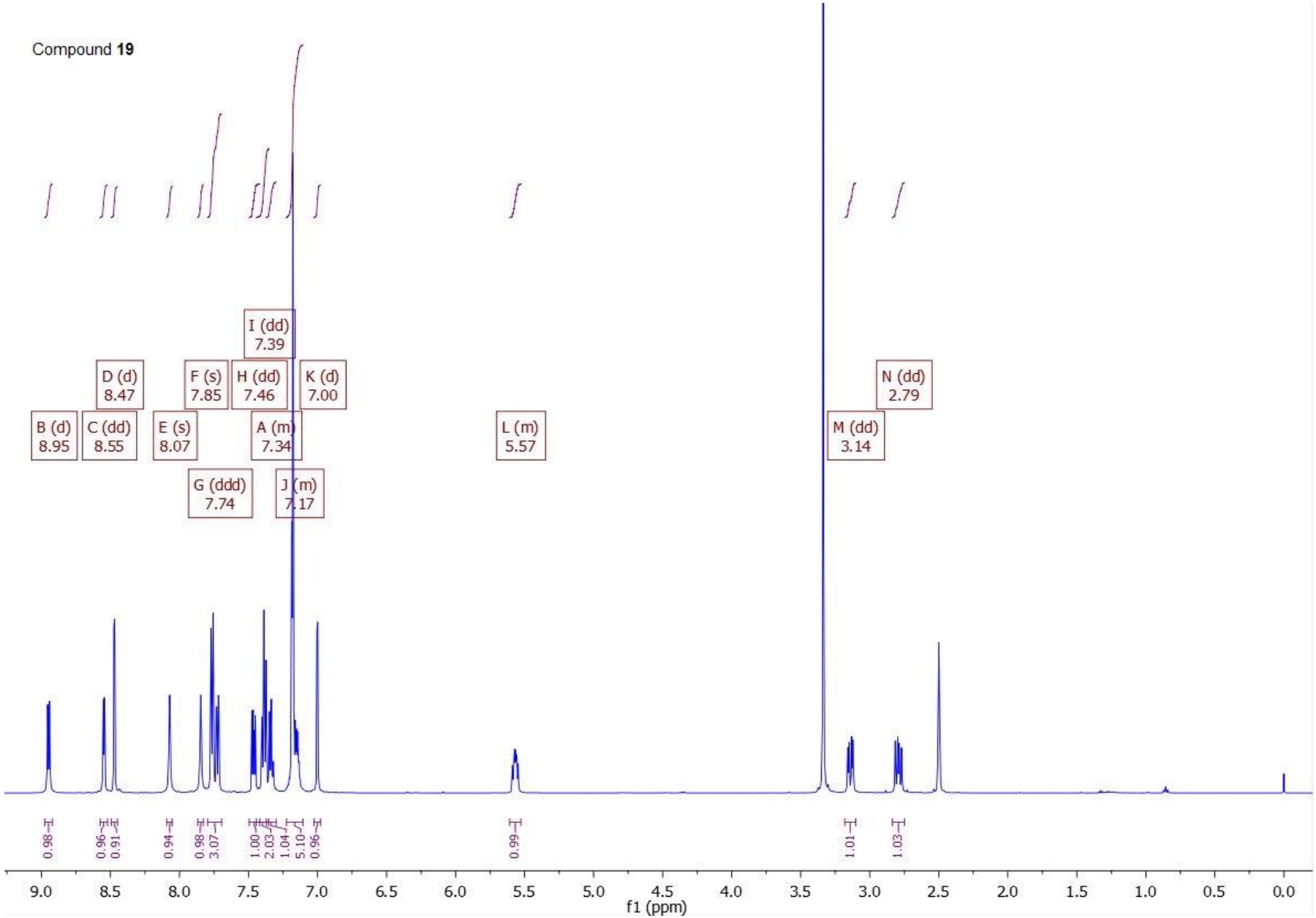
Assay	% Inhibition	Assay	% Inhibition
α–D-Glucosidase	-2	PDE4 (hu)	1
ATPase, Na+/K+	-2	PDE5 (hu)	-4
Carbonic Anhydrase (hu)	9	PDE6	-2
Carnitine Palmitoyltransferase	1	PLA2-II	2
Catechol-O-Methyltransferase	0	Proteasome (hu)	0
Choline Acetyltransferase (hu)	12	SRPK1 (hu)	10
Cholinesterase, Acetyl (hu)	13	AKT1 (hu)	-8
COX-1 (hu)	7	Ca2+/Calmodulin-Dep. II	23
COX-2 (hu)	-1	CAMK2D (hu)	24
CYP450, 19 (hu)	-1	CAMK4 (hu)	2
CYP450, 1A2 (hu)	1	DMPK (hu)	3
CYP450, 2C19 (hu)	-9	HIPK2 (hu)	-5
CYP450, 2C9 (hu)	-10	IKK-1 (hu)	-9
CYP450, 2D6 (hu)	2	IKK-2 (hu)	0
CYP450, 3A4 (hu)	-22	MAP2K1 (MEK1) (hu)	13
Sirtuin SIRT1 (hu)	-12	MAPK14 (p38α) (hu)	9
Sirtuin SIRT3 (hu)	5	MAPK3 (ERK1) (hu)	3
Free Radical Scavenger, SOD Mimetic	3	MAPKAPK5 (PRAK) (hu)	-1
Lipase (hu)	-6	PDK1 (hu)	-7
MAO-A (hu)	4	PHKG2 (hu)	4
MAO-B (hu)	5	PKD2 (hu)	1
Nitric Oxide Synthase, Endothelial	5	PRKCA (PKCα) (hu)	13
Nitric Oxide Synthase, Inducible	3	PRKCE (PKCε) (hu)	8
Nitric Oxide Synthase, Neuronal	6	STK17A (DRAK1) (hu)	1
Angiotensin Converting Enzyme	6	TSSK2 (STK22B) (hu)	14
Caspase 1 (hu)	1	PPP3CA (Calcineurin, PP2B) (hu)	-3
Chymotrypsin (hu)	16	ABL1 (ABL)	11
Cathepsin D (hu)	-1	EGF Receptor (hu)	0
Neutrophil Elastase 2 (hu)	0	EPHA4 (EphA4) (hu)	-9
Factor VIIa (hu)	-1	ERBB2 (HER2) (hu)	19
Factor Xa (hu)	-6	FGFR1 (hu)	1
HIV-1 Protease	11	FGFR2 (hu)	8
MMP-2 (hu)	-2	Fyn	6
MMP-3 (hu)	-5	Insulin Receptor (hu)	8
Neutral Endopeptidase (hu)	2	LCK (hu)	-2
Pepsin	5	TEK (hu)	8
Thrombin (hu)	4	ZA70 (ZAP-70) (hu)	-9
tPA (hu)	1	ACP1 (LMPTP-A) (hu)	2
Trypsin (hu)	-1	PTPN1 (PTP1B) (hu)	-2
TACE (hu)	-1	Sphingomyelinase, Acid (ASMase) (hu)	0
PDE3 (hu)	12	UDP Glucuronosyltransferase UGT1A1 (hu)	27

Supplemental Table 3. Profile of **38** in CEREP panel of receptor binding assays at 10μM concentrations.

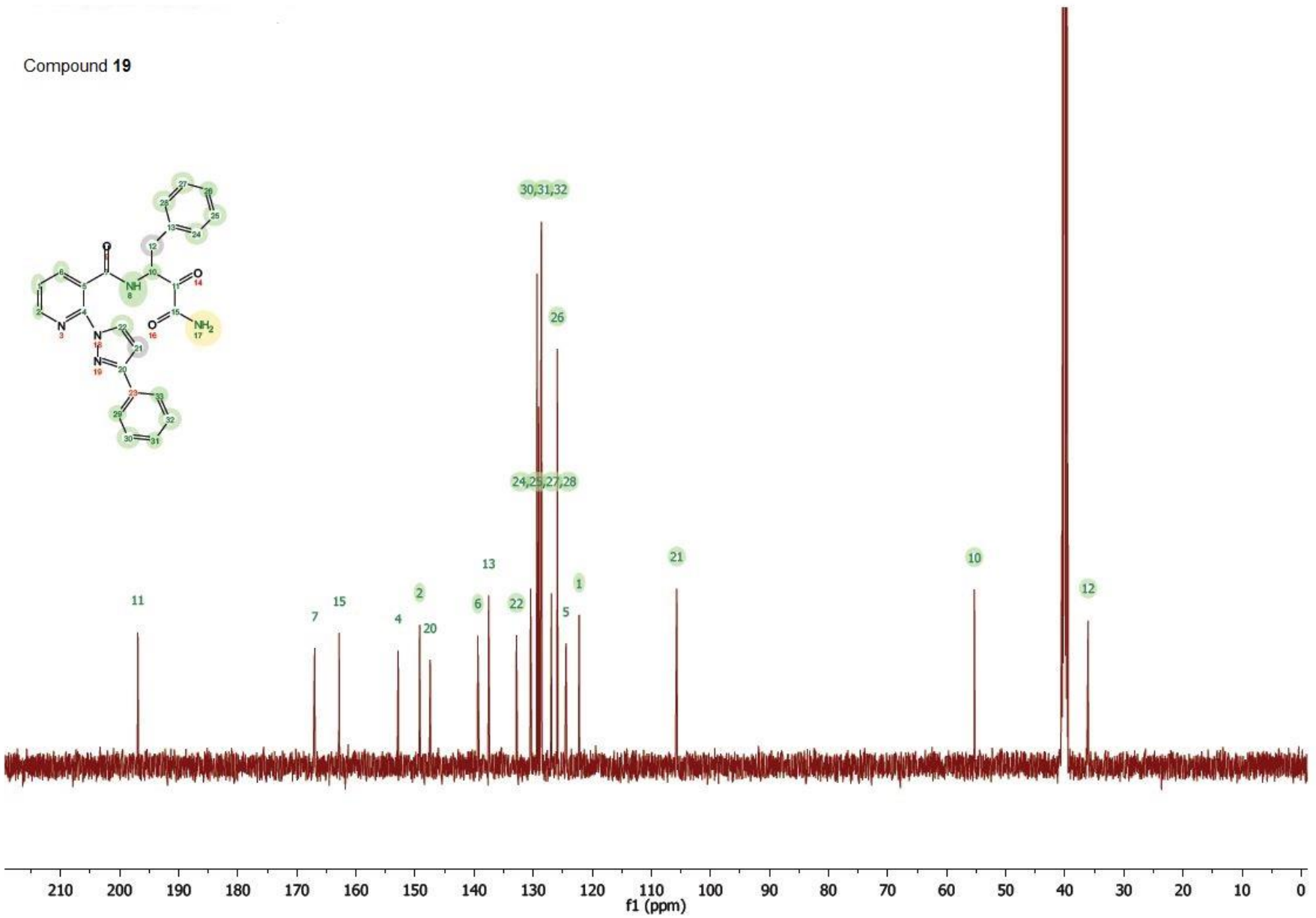
Assay	% Inhibition	Assay	% Inhibition
5-HT1A (h)	0	GABA (non-selective)	-4
5-HT1B	13	GAL1 (h)	-6
5-HT2A (h)	1	GAL2 (h)	-6
5-HT2C (h)	0	H1 (h)	1
5-HT3 (h)	5	H2 (h)	2
5-HT5A (h)	7	K+V channel	-3
5-HT6 (h)	1	M1 (h)	1
5-HT7 (h)	0	M2 (h)	4
A1 (h)	7	M3 (h)	-2
A2A (h)	5	M4 (h)	1
A3 (h)	13	M5 (h)	-1
Alpha 1	9	MC4 (h)	-1
Alpha 2	-5	MT1 (h)	5
AT1 (h)	-10	Na+ channel (site 2)	-3
AT2 (h)	-10	NE transporter (h)	-7
B2 (h)	-11	NK1 (h)	18
BB (non-selective)	-9	NK2 (h)	15
Beta 1 (h)	-10	NK3 (h)	-7
Beta 2 (h)	4	NT1 (h) (NTS1)	-6
BZD (peripheral)	-3	Opiod delta 2	-15
BZD (central)	93	Opiod kappa	5
Ca2+ channel (L, verapamil site)	8	Opiod mu	42
CB1 (h)	-12	ORL1 (h) (NOP)	0
CCKA (h) (CCK1)	8	P2X	0
CCKB (h) (CCK2)	9	P2Y	7
CCR1 (h)	-7	PACAP (PAC1) (h)	-21
CGRP (h)	9	PCP	-27
Cl- channel	-9	PDGF	-3
CXCR2 (h) (IL-8B)	10	Sigma (non-selective)	2
D1 (h)	-1	SK+Ca channel	4
D2S (h)	9	SST (non-selective)	-1
D3 (h)	-16	TNF-alpha (h)	0
D4.4 (h)	10	TXA2/PGH2 (h) (TP)	0
D5 (h)	-2	V1a (h)	20
DA transporter (h)	9	VIP1 (h) (VPAC1)	-3
ETA (h)	8	Y1 (h)	6
ETB (h)	8	Y2 (h)	-10

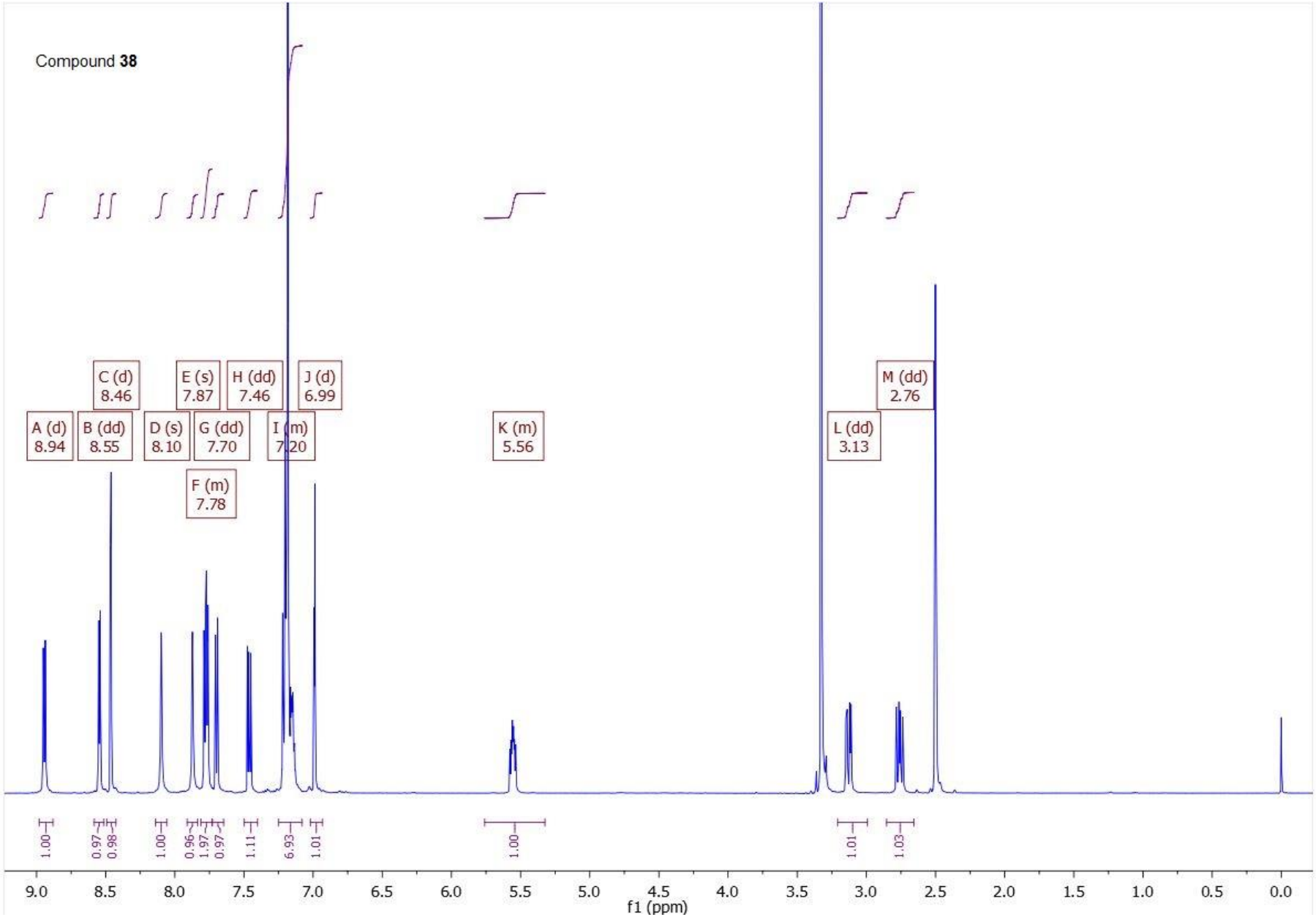
Supplemental Table 4. Profile of **38** in MDS EnzymeProfiling Screen at 10 µM concentrations.

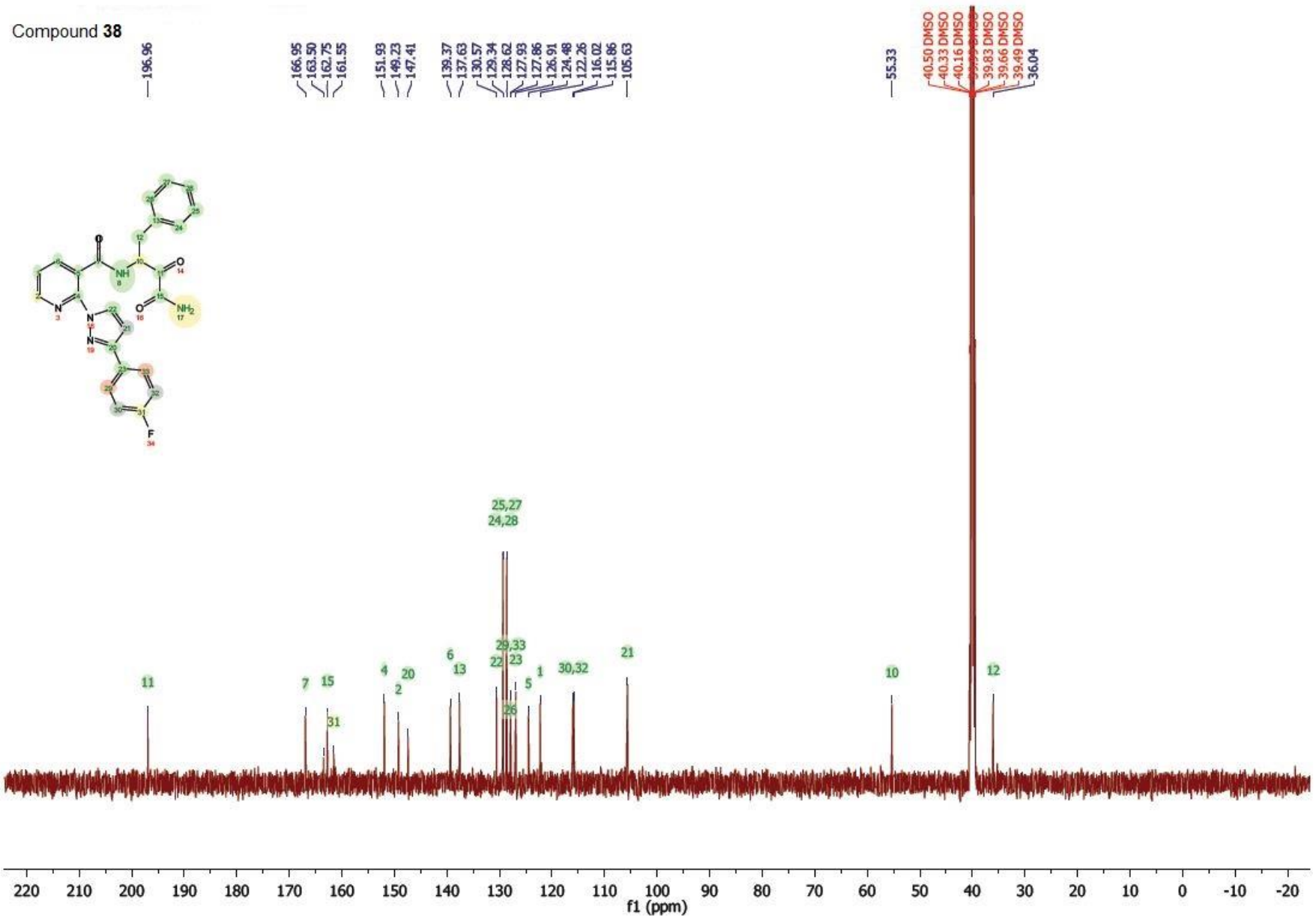
Assay	% Inhibition	Assay	% Inhibition
α–D-Glucosidase	7	PDE4 (hu)	-2
ATPase, Na+/K+	9	PDE5 (hu)	-2
Carbonic Anhydrase (hu)	13	PDE6	7
Carnitine Palmitoyltransferase 1	-4	PLA2-II	-8
Catechol-O-Methyltransferase (COMT)	23	Proteasome (hu)	-8
Cholinesterase, Acetyl (hu)		SRPK1 (hu)	13
COX-1 (hu)	6	AKT1 (hu)	9
COX-2 (hu)	-8	Ca2+/Calmodulin-Dep. II	-16
CYP450, 19 (hu)	27	CAMK2D (hu)	-13
CYP450, 1A2 (hu)	-1	CAMK4 (hu)	26
CYP450, 2C19 (hu)	22	DMPK (hu)	5
CYP450, 2C9 (hu)	1	HIPK2 (hu)	-5
CYP450, 2D6 (hu)	0	IKK-1 (hu)	-2
CYP450, 3A4 (hu)	-2	IKK-2 (hu)	15
Sirtuin SIRT1 (hu)	0	MAP2K1 (MEK1) (hu)	0
Sirtuin SIRT3 (hu)	-15	MAPK14 (p38α) (hu)	3
Free Radical Scavenger, SOD Mimetic	3	MAPK3 (ERK1) (hu)	-1
Lipase (hu)	0	MAPKAPK5 (PRAK) (hu)	-5
MAO-A (hu)	2	PDK1 (hu)	-2
MAO-B (hu)	2	PHKG2 (hu)	20
Nitric Oxide Synthase, Endothelial	3	PKD2 (hu)	-14
Nitric Oxide Synthase, Inducible	3	PRKCA (PKCα) (hu)	-4
Nitric Oxide Synthase, Neuronal	3	PRKCE (PKCε) (hu)	0
Angiotensin Converting Enzyme	3	STK17A (DRAK1) (hu)	8
Caspase 1 (hu)	-8	TSSK2 (STK22B) (hu)	-6
Chymotrypsin (hu)	0	PPP3CA (Calcineurin, PP2B) (hu)	5
Cathepsin D (hu)	18	ABL1 (ABL)	-10
Neutrophil Elastase 2 (hu)	0	EGF Receptor (hu)	13
Factor VIIa (hu)	2	EPHA4 (EphA4) (hu)	-4
Factor Xa (hu)	0	ERBB2 (HER2) (hu)	-4
HIV-1 Protease	2	FGFR1 (hu)	1
MMP-2 (hu)	-5	FGFR2 (hu)	-6
MMP-3 (hu)	-1	Fyn	2
Neutral Endopeptidase (hu)	0	Insulin Receptor (hu)	14
Pepsin	6	LCK (hu)	1
Thrombin (hu)	24	TEK (hu)	0
Tissue Plasminogen Activator tPA (hu)	13	ZA70 (ZAP-70) (hu)	-10
Trypsin (hu)	4	ACP1 (LMPTP-A) (hu)	4
TACE (hu)	4	PTPN1 (PTP1B) (hu)	-3
PDE3 (hu)	0	Sphingomyelinase, Acid (ASMase) (hu)	0
	14	UDP Glucuronosyltransferase UGT1A1 (hu)	22


Compound 19
B (d) 8.95
C (dd) 8.55
D (d) 8.47
E (s) 8.07
F (s) 7.85
G (ddd) 7.74
H (dd) 7.46
I (dd) 7.39
J (m) 7.17
K (d) 7.00
A (m) 7.34
L (m) 5.57
M (dd) 3.14
N (dd) 2.79
0.98
0.96
0.91
0.94
0.98
3.07
1.00
2.03
1.04
5.10
0.96
0.99
1.01
1.03
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

Compound **19**


30,31,32
26
24,25,27,28
13
21
10
1
12
11
15
7
4
2
20
6
22
5
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


Compound 38
A (d) 8.94
B (dd) 8.55
C (d) 8.46
D (s) 8.10
E (s) 7.87
F (m) 7.78
G (dd) 7.70
H (dd) 7.46
I (m) 7.20
J (d) 6.99
K (m) 5.56
L (dd) 3.13
M (dd) 2.76
1.00
0.97
0.98
1.00
0.96
1.97
0.97
1.11
6.93
1.01
1.00
1.01
1.03
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)


Compound 38
196.96
166.95
163.50
162.75
161.55
151.93
149.23
147.41
139.37
137.63
130.57
129.34
128.62
127.93
127.86
126.91
124.48
122.26
116.02
115.86
105.63
55.33
40.50 DMSO
40.33 DMSO
40.16 DMSO
39.83 DMSO
39.66 DMSO
39.49 DMSO
36.04
25,27
24,28
29,33
22
23
26
11
7
15
31
4
2
20
6
13
1
5
30,32
21
10
12
f1 (ppm)

The effects of the AChE inhibitor donepezil on REM sleep pattern in rat are summarized in **Supplemental Table 5**. Whereas the increase in REM time reaches statistical significance, the parameter latency to REM only shows a trend to decrease due to relatively high variability between individual data.

Supplemental Table 5: Summary of dose-dependent effects of donepezil on rat sleep. All parameters are expressed as % of vehicle controls (mean ± SEM). (* $p<0.05$ from control)

Donepezil (mg/kg)	3
REM time	116 ± 8*
Latency to REM	88 ± 13
Total sleep time	102 ± 3