Journal of Alzheimer's Disease 35 (2013) 1–11
IOS Press

Supplementary Data

Aftins Increase Amyloid-β_{42}, Lower Amyloid-β_{38}, and Do Not Alter Amyloid-β_{40} Extracellular Production *in vitro*: Toward a Chemical Model of Alzheimer's Disease?

Arnaud Hochard[a,b], Nassima Oumata[a], Karima Bettayeb[c], Olfa Gloulou[d], Xavier Fant[b], Emilie Durieu[a,b], Nelly Buron[e], Mathieu Porceddu[e], Annie Borgne-Sanchez[e], Hervé Galons[a,d], Marc Flajolet[c] and Laurent Meijer[a,*]
[a]*ManRos Therapeutics, Centre de Perharidy, Roscoff, Bretagne, France*
[b]*CNRS, USR3151, Station Biologique, Roscoff, Bretagne, France*
[c]*Laboratory of Molecular and Cellular Neuroscience, The Rockefeller University, New York, NY, USA*
[d]*Laboratoire de Chimie Organique 2, CNRS, UMR 8601, Université Paris-Descartes, Paris, France*
[e]*Mitologics SAS, Hôpital Robert Debré, 48, Boulevard Sérurier, Paris, France*

CHEMISTRY

Preparation of Aftins-1 (3) and -2 (4)

The synthesis of Aftins MRT2-309 (2), Aftin-1 (3) and Aftin-2 (4) is depicted in Scheme 1.

N6-Benzyl-N6-methyl–9H-purine-2,6-diamine (2)

N-Methylbenzylamine and NEt_3 were added to a solution of 2-amino-6-chloropurine in *n*-butanol. The mixture was stirred at 85°C for 6 h. After cooling to 20°C, Aftin-2 separated upon filtration of the precipitate. Yield 74%, mp 130°C. ^{1}H-NMR ($CDCl_3$) δ ppm: 3.35 (brs 3H); 4.75 and 5.25 (2 brs, CH_2); 7.35 (m, 5H, C_6H_5); 7.65 (s, 1H, 8-H).

*Correspondence to: Laurent Meijer, ManRos Therapeutics, Centre de Perharidy, 29680 Roscoff, Bretagne, France. Tel.: +33 6 08 60 58 34; E-mail: meijer@manros-therapeutics.com.

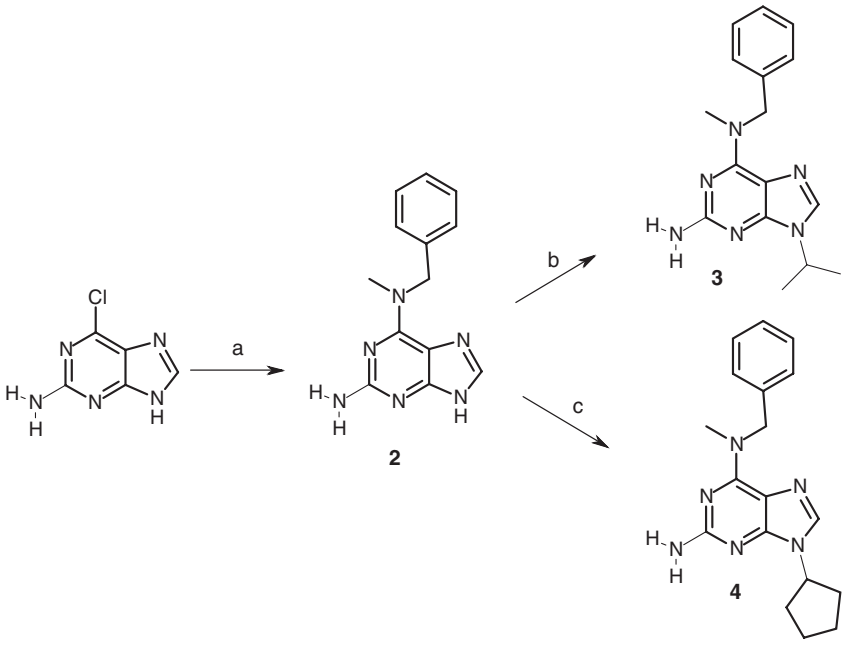
N6-Benzyl–9-isopropyl-N6-methyl-purine-2,6 -diamine (Aftin-1) (3)

Yield 72%, mp 110°C. ^{1}H-NMR ($CDCl_3$) δ ppm: 1.45 (d, 6H, CH $(CH_3)_2$); 3.30 (brs, CH_3N); 4.51 (brs CH_2); 4.62 (hept, 1H, CH $(CH_3)_2$); 5.23 (brs, NH_2).7.2 (m, 5H, C_6H_5) 7.48 (s, 1H, 8-H). ^{13}C-NMR 22.74 ($CHCH_3$); 35.83 (brs, CH_3N); 45.76 (CH); 53.26 (CH_2N); 115.25 (CH); 127.07 (CH); 127.65 (CH); 128.49 (CH) 129.05; 133.53 (CH); 138.33 (CH); 152.54 (CH); 155.27 (CH); 159.08 (CH).

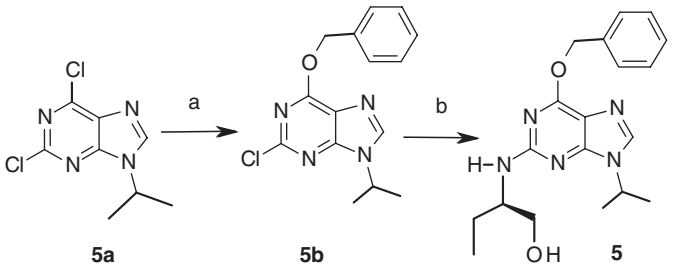
N6-Benzyl–9-cyclopentyl-N6-methyl-purine-2,6 -diamine (Aftin-2) (4)

Mp 160°C. ^{1}H NMR ($CDCl_3$) 1.70 (m, 2H, cyclopentyl); 1.83 (m, 4H, cyclopentyl); 2.16 (m, 2H, cyclopentyl); 3.28 (brs, 3H, CH_3); 4.61 (brs, 2H, CH_2-C_6H_5); 4.73 (quint, 1H, cyclopentyl); 4.61 (brs, 2H, CH_2); 5.21 (brs, NH_2); 7.25 (m, 5H); 7.46 (s, 1H).

ISSN 1387-2877/13/$27.50



Scheme 1. Reagents and conditions. a) N-methylbenzylamine, N $(Et)_3$, in n-butanol, 85°C; b) *iso*-propylbromide, K_2CO_3, DMSO 16–18°C; c) 2-bromocyclopentane, K_2CO_3, DMSO 16–18°C.



Scheme 2. Reagents and conditions. a) NaH, benzylic alcohol; b) (R)-2-aminobutanol, 140°C.

Preparation of Aftin-3 (5)

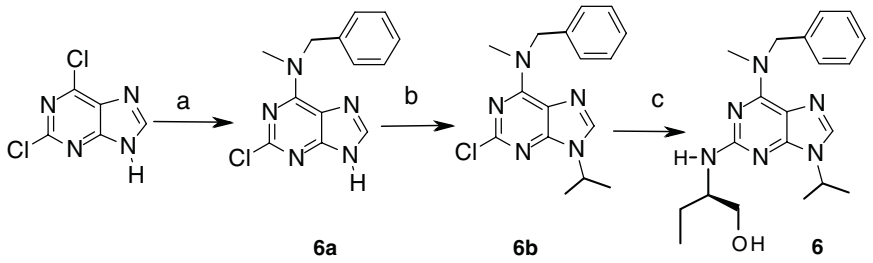
The synthesis of Aftin-3 (5) is presented in Scheme 2. Compound 5a was prepared as described [1].

6-Benzyloxy-2-chloro-9-isopropyl-purine (5b)

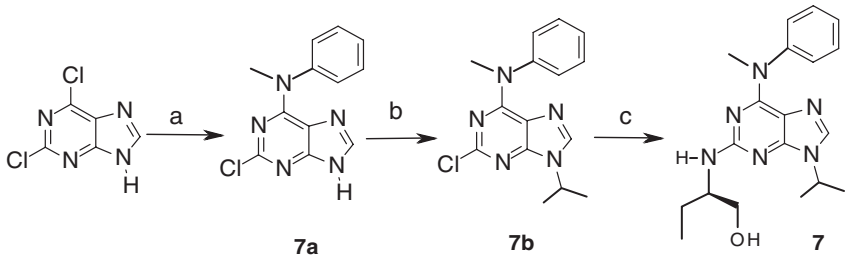
THF (20 mL) was slowly added under nitrogen to NaH 60% (1.70 g; 54.6 mmol) dispersion in mineral oil cooled by an external bath. Benzyl alcohol (2 mL, 18.5 mmol) in THF (10 mL) was added dropwise at 0°C. After 15 min stirring at the same temperature, 2, 6-dichloro-9-*iso*-propylpurine, 5a (3.64 g, 15.8 mmol) in 20 mL THF was slowly added. After 12 h stirring 20°C, the mixture was cooled at 0°C and 100 mL 2M NH_4Cl solution was slowly added. The mixture was extracted by AcOEt (3×20 mL) and the organic layer was washed with water (2×20 mL). After drying (Na_2SO_4) 5b crystallized upon concentration of the solution. ^{1}H-NMR ($CDCl_3$) δ ppm: 1.63 (d, 6H, J = 7.2 Hz), 4.85 (hept, 1H, J = 7.2 Hz), 5.60 (s, 2H), 7.32–7.41 (m, 3H), 7.52 (d, 2H, J = 6.8 Hz), 8.82 (s, 1H, 8-H).

(2R)-2-[(6-Benzyloxy-9-isopropyl-purin-2-yl) amino]butan-1-ol (Aftin-3) (5)

A mixture of 5b (1.45 g, 4.7 mmol) and (R)-2-aminobutanol (3.58 mL, 0.4 mmol) was heated 6 h at 140°C. After cooling, the mixture was extracted with CH_2Cl_2 washed with



Scheme 3. Reagents and conditions. a) N-methylbenzylamine, N $(Et)_3$, in n-butanol, 85°C; b) *iso*-propylbromide, K_2CO_3, DMSO 16–18°C; c) (R)-2-aminobutanol, 160°C.



Scheme 4. Reagents and conditions. a) N-methylaniline, N $(Et)_3$, in n-butanol, 95°C; b) isopropylbromide, K_2CO_3, DMSO 16–18°C; c) (R)-2-aminobutanol, 160°C.

water (2×20 mL). Derivative 5 crystallized upon trituration with AcOEt. ^{1}H- NMR ($CDCl_3$) δ ppm: 0.99 (t, 3 H, J = 7.2 Hz), 1.53–1.61 (m, 1 H), 1.63 (d, 6 H, J = 7.2 Hz), 1.74–1.80 (m, 1 H), 3.59–3.69 (m, 2 H), 4.00–4.08 (m, 1 H), 4.85 (septet, 1H, J = 7.2 Hz), 5.60 (s, 2 H), 7.32–7.41 (m, 3 H), 7.52 (d, 2 H, J = 6.8 Hz), 8.82 (s, 1 H).

Preparation of Aftin-4 (6)

The preparation of Aftin-4 (6) is depicted in Scheme 3.

The amination of position 6 was first performed upon heating 2,6-dichloropurine with N-methylbenzylamine in *n*-BuOH. Alkylation of 6a was achieved using an alkylhalide in a dipolar aprotic solvent such as DMSO. In the last step, the amination was achieved upon heating the chloro derivatives 6b with an amine (Scheme 3).

N-Benzyl-2-chloro-N-methyl-9H-purin-6-amine (6a)

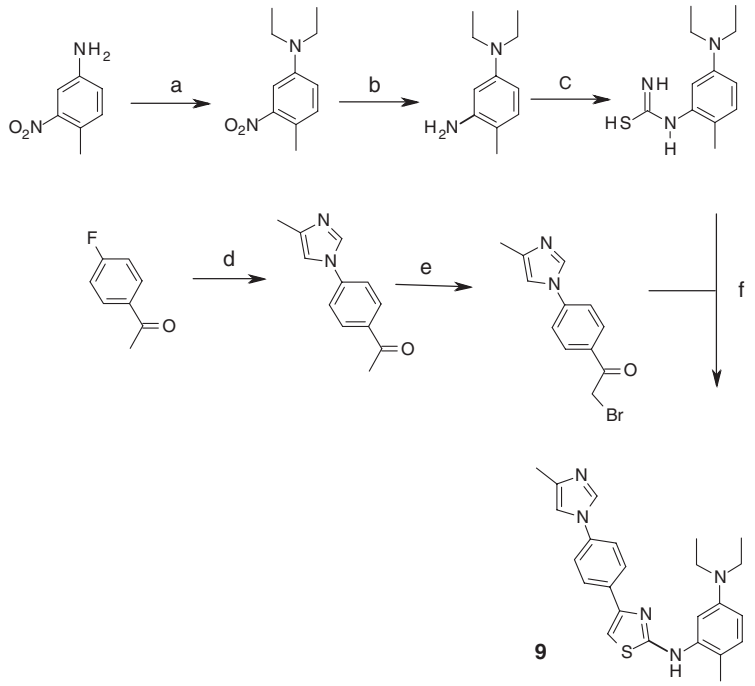
N-Methylbenzylamine (10.5 mL, 79.36 mmol) and triethylamine (29 mL, 211.63 mmol) were added to a solution of 2,6-dichloropurine (10.0 g, 52.91 mmol) in n-butanol (80 mL). The mixture was heated at 100°C for 3 h. The reaction was cooled to 40°C, and the precipitate was filtered at this temperature, washed with 10 mL cold (15°C) H_2O, and dried overnight in vacuo.

N-Benzyl-2-chloro-9-isopropyl-N-methyl-purin-6-amine (6b)

2-Bromopropane (25.77 mL, 275.37 mmol) was added to a solution of 1 (10.76 g, 39.34 mol) and K_2CO_3 (21.74 g, 157.35 mmol) in 80 mL DMSO. After 12 h stirring, the mixture was diluted with 200 mL H_2O and extracted with AcOEt (3×20 mL). After concentration the remaining solid was triturated with 5 mL AcOEt filtered and dried overnight in vacuo. Yield 65%, mp 103–106°C. ^{1}H-NMR (DMSOd6) δ ppm: 1.47 (d, 6H, $(CH_3)_2CH$); 3.12 and 3.60 (2 brs, 3H, CH_3N); 4.75 (hept, 1H, J = Hz) 4.90 and 5.50 (2 brs, 2 H, $CH_2C_6H_5$); 75 (s, 1 H, 8-H). ^{13}C-NMR δ ppm: 22.7, 46.62, 119.07, 127.46, 128.64; 136.12; 137.31; 153.65.

(2R)-2-[Amino-[6-(benzyl(methyl)amino)-9-isopropyl-purin-2-yl]amino]butan-1-ol (Aftin-4) (6)

A mixture of the chloro derivative 2 (12.0 g, 37.99 mmol) (R)-2-aminobutanol (28.47 mL, 304 mmol) was heated 4 h at 160°C. After cooling, the mixture was extracted with CH_2Cl_2 washed with water



Scheme 5. Reagents and conditions. a) BrEt, K_2CO_3, DMF, 20°C; b) Fe, HCl, NH_4Cl, EtOH; c) 1. C_6H_5CONCO, 2. NaOH 80°C; d) K_2CO_3, DMSO, 85°C; e) Br_2, HBr, CH_3COOH; f) DMF 75°C.

(2×20 mL). Derivative 3 was crystallized upon trituration with AcOEt, followed by recrystallisation from 2-propanol. Yield, 65%, mp 114–117°C. ^{1}H-NMR ($DMSO_{d6}$) δ ppm:1.01 (t, 3H, CH_3CH_2); 1.49 (d, 6 H, $(CH_3)_2CH$); 1.51 (m, 2 H, CH_2CH_3); 3.26 (brs, 3 H, CH_3N) 3.77 and 3.81 (2 m, 2 H, CH_2O); 3.78 (m 1H, CHO); 4.55 (m, hept, 1 H), 4.70 (d, 1 H, NH); 5;21 (brs, $CH_2C_6H_5$);7.28 (m, 5 H, C_6H_5); 7.45 (s, 1 H, 8-H). ^{13}C-NMR δ ppm: 10.97; 22.52; 22, 56; 25.08; 46.16; 53.38 56.33; 68.76; 115.11; 127.12; 127.58; 128.52; 133.36; 138.19; 151.84, 155.08; 159.44.

Preparation of Aftin-5 (7)

The preparation of Aftin-5 (7) is depicted in Scheme 4. Briefly the amination of 2, 6-dichloropurine was first performed upon heating with N-methylaniline and NEt_3 in *n*BuOH. Alkylation was then performed followed by amination at the 2 position. This last step was achieved upon heating until completion with an 8 fold excess of (R)-2-aminobutanol at 160°C.

(2R)-2-[[9-isopropyl-6-(N-methylanilino)purin-2-yl]amino]butan-1-ol (Aftin-5) (7)

Yield 63%. ^{1}H-NMR ($CDCl_3$) δ ppm: 0.92 (t, 3 H, CH_3); 1.45 (d, 6 H, $(CH_3)_2CH$); 1.49 (m, 2 H, CH_2CH_3); 3.50 (m, 1 H, CHN); 3.69 (m, 2 H CH_2-O); 3.72 (s, 3 H, CH_3N); 4.54 (hept, 1 H, $(CH_3)_2CH$); 4.79 (brs, 2 H, NH_2); 7.24 and 7.34 (2 m, 5 H, C_6H_4) 7.39 (s, 1 H, 8-H). ^{13}C-NMR: 10.94 (CH_3); 22.48 and 22.54 (2 CH_3); 24.97 (CH_2); 40.21 (CH); 46.13 (CH); 56.28 (CH); 68.37 (CH_2); 115.3 (C); 126.43 (CH); 126.99 (CH); 129.04 (CH); 134.33 (CH) 14.28 (C); 154.13 (C); 155.14 (C); 159.04 (C).

Preparation of the 'Torrey Pines' compound (9)

The synthesis of the 'Torrey Pines' compound 9 was achieved as depicted in Scheme 5.

N1, N1-diethyl-4-methyl-N3-[4-[4-(4-methylimidazol-1-yl)phenyl]thiazol-2-yl]benzene-1, 3-diamine (9)

^{1}H-NMR ($CDCl_3$) δ ppm: 1.05 (t, 6 H, $2CH_3CH_2$); 2.10 (s, 3 H, CH_3); 2.18 (s, 3 H, CH_3); 3.40 (m, 4 H, $2CH_2$); 6.45 (dd, J = 4 Hz, 2 Hz, 1 H); 6.98 (m, 2 H); 7.35 (d, 2 H, J = 7 Hz); 7.75 (s, 1 H, imidazol); 7.92 (d, 2 H, J = 7 Hz). ^{13}C-NMR (DEPT135): 12.67 (CH_3); 13.74 (CH_3); 16.57 (CH_3); 44.60 (CH_2); 102.13 (CH); 104.00 (CH); 108.32 (CH); 114.48 (CH); 121.00 (CH); 127.37 (CH); 131.67 (CH); 134.51 (CH).

SELECTIVITY STUDIES

Supplementary Table 1

Discoverx KinomeScan Selectivity Panel (442 kinases). Enzymes were prepared and interactions assays were run in the presence of 10 μM Aftin-5, as described in [2]. A semi-quantitative scoring of this primary screen was estimated. This score relates to a probability of a hit rather than strict affinity. Scores >10, between 1–10 and <1 indicate the probability of a being a false positive is <20%,<10%, <5 %, respectively

KINASE	SCORE
AAK1	98
ABL1 (E255K)-phosphorylated	89
ABL1 (F317I)-nonphosphorylated	72
ABL1 (F317I)-phosphorylated	100
ABL1 (F317L)-nonphosphorylated	100
ABL1 (F317L)-phosphorylated	74
ABL1 (H396P)-nonphosphorylated	100
ABL1 (H396P)-phosphorylated	70
ABL1 (M351T)-phosphorylated	50
ABL1 (Q252H)-nonphosphorylated	100
ABL1 (Q252H)-phosphorylated	96
ABL1 (T315I)-nonphosphorylated	100
ABL1 (T315I)-phosphorylated	52
ABL1 (Y253F)-phosphorylated	66
ABL1-nonphosphorylated	87
ABL1-phosphorylated	91
ABL2	100
ACVR1	100
ACVR1B	100
ACVR2A	100
ACVR2B	100
ACVRL1	100
ADCK3	100
ADCK4	63
AKT1	92
AKT2	100
AKT3	91
ALK	94
AMPK-alpha1	100
AMPK-alpha2	91
ANKK1	87
ARK5	100
ASK1	83
ASK2	93
AURKA	84
AURKB	89
AURKC	100
AXL	86
BIKE	100
BLK	80
BMPR1A	72
BMPR1B	70
BMPR2	86
BMX	93
BRAF	100
BRAF (V600E)	100
BRK	98
BRSK1	66
BRSK2	86
BTK	100
CAMK1	100

Supplementary Table 1
(*Continued*)

KINASE	SCORE
CAMK1D	86
CAMK1G	100
CAMK2A	94
CAMK2B	100
CAMK2D	100
CAMK2G	100
CAMK4	100
CAMKK1	100
CAMKK2	100
CASK	100
CDC2L1	67
CDC2L2	65
CDC2L5	62
CDK11	77
CDK2	80
CDK3	99
CDK4-cyclinD1	58
CDK4-cyclinD3	100
CDK5	96
CDK7	66
CDK8	89
CDK9	100
CDKL1	100
CDKL2	100
CDKL3	100
CDKL5	75
CHEK1	100
CHEK2	100
CIT	65
CLK1	98
CLK2	100
CLK3	100
CLK4	100
CSF1R	97
CSK	100
CSNK1A1	95
CSNK1A1L	100
CSNK1D	66
CSNK1E	96
CSNK1G1	96
CSNK1G2	92
CSNK1G3	93
CSNK2A1	76
CSNK2A2	100
CTK	68
DAPK1	95
DAPK2	100
DAPK3	82
DCAMKL1	100
DCAMKL2	100
DCAMKL3	100
DDR1	100
DDR2	88
DLK	100
DMPK	96
DMPK2	97
DRAK1	100
DRAK2	100
DYRK1A	73
DYRK1B	99

Supplementary Table 1
(*Continued*)

KINASE	SCORE
DYRK2	79
EGFR	100
EGFR (E746-A750del)	95
EGFR (G719C)	94
EGFR (G719S)	94
EGFR (L747-E749del, A750P)	86
EGFR (L747-S752del, P753S)	79
EGFR (L747-T751del, Sins)	94
EGFR (L858R)	93
EGFR (L858R, T790M)	100
EGFR (L861Q)	75
EGFR (S752-I759del)	79
EGFR (T790M)	100
EIF2AK1	98
EPHA1	95
EPHA2	90
EPHA3	86
EPHA4	91
EPHA5	100
EPHA6	100
EPHA7	100
EPHA8	90
EPHB1	100
EPHB2	100
EPHB3	100
EPHB4	100
EPHB6	65
ERBB2	92
ERBB3	100
ERBB4	96
ERK1	100
ERK2	100
ERK3	94
ERK4	72
ERK5	98
ERK8	100
ERN1	97
FAK	91
FER	100
FES	100
FGFR1	100
FGFR2	98
FGFR3	81
FGFR3 (G697C)	84
FGFR4	100
FGR	100
FLT1	100
FLT3	59
FLT3 (D835H)	99
FLT3 (D835Y)	98
FLT3 (ITD)	86
FLT3 (K663Q)	100
FLT3 (N841I)	84
FLT3 (R834Q)	84
FLT4	100
FRK	100
FYN	100
GAK	100
GCN2 (Kin.Dom.2, S808G)	100
GRK1	81

Supplementary Table 1
(*Continued*)

KINASE	SCORE
GRK4	98
GRK7	89
GSK3A	91
GSK3B	72
HCK	92
HIPK1	99
HIPK2	91
HIPK3	39
HIPK4	78
HPK1	84
HUNK	82
ICK	96
IGF1R	100
IKK-alpha	80
IKK-beta	100
IKK-epsilon	43
INSR	75
INSRR	95
IRAK1	59
IRAK3	60
IRAK4	54
ITK	100
JAK1 (JH1domain-catalytic)	82
JAK1 (JH2domain-pseudokinase)	99
JAK2 (JH1domain-catalytic)	95
JAK3 (JH1domain-catalytic)	100
JNK1	92
JNK2	100
JNK3	100
KIT	88
KIT (A829P)	100
KIT (D816H)	75
KIT (D816V)	91
KIT (L576P)	79
KIT (V559D)	86
KIT (V559D, T670I)	100
KIT (V559D, V654A)	100
LATS1	100
LATS2	84
LCK	100
LIMK1	100
LIMK2	100
LKB1	100
LOK	91
LRRK2	100
LRRK2 (G2019S)	100
LTK	100
LYN	100
LZK	100
MAK	95
MAP3K1	90
MAP3K15	100
MAP3K2	99
MAP3K3	100
MAP3K4	100
MAP4K2	90
MAP4K3	100
MAP4K4	100
MAP4K5	100
MAPKAPK2	100

Supplementary Table 1
(*Continued*)

KINASE	SCORE
MAPKAPK5	100
MARK1	98
MARK2	94
MARK3	92
MARK4	84
MAST1	100
MEK1	100
MEK2	100
MEK3	97
MEK4	100
MEK5	91
MEK6	100
MELK	100
MERTK	100
MET	89
MET (M1250T)	100
MET (Y1235D)	88
MINK	100
MKK7	100
MKNK1	89
MKNK2	92
MLCK	100
MLK1	93
MLK2	90
MLK3	93
MRCKA	98
MRCKB	100
MST1	100
MST1R	96
MST2	60
MST3	100
MST4	100
MTOR	100
MUSK	100
MYLK	73
MYLK2	100
MYLK4	100
MYO3A	100
MYO3B	100
NDR1	100
NDR2	97
NEK1	88
NEK11	98
NEK2	97
NEK3	100
NEK4	100
NEK5	86
NEK6	100
NEK7	94
NEK9	100
NIM1	88
NLK	90
OSR1	45
p38-alpha	100
p38-beta	100
p38-delta	77
p38-gamma	100
PAK1	73
PAK2	100
PAK3	100

Supplementary Table 1
(*Continued*)

KINASE	SCORE
PAK4	90
PAK6	100
PAK7	86
PCTK1	82
PCTK2	82
PCTK3	100
PDGFRA	100
PDGFRB	100
PDPK1	95
PFCDPK1 (*P. falciparum*)	100
PFPK5 (*P. falciparum*)	84
PFTAIRE2	100
PFTK1	100
PHKG1	96
PHKG2	100
PIK3C2B	100
PIK3C2G	100
PIK3CA	100
PIK3CA (C420R)	100
PIK3CA (E542K)	97
PIK3CA (E545A)	100
PIK3CA (E545K)	100
PIK3CA (H1047L)	100
PIK3CA (H1047Y)	100
PIK3CA (I800L)	100
PIK3CA (M1043I)	100
PIK3CA (Q546K)	86
PIK3CB	100
PIK3CD	88
PIK3CG	100
PIK4CB	63
PIM1	94
PIM2	94
PIM3	98
PIP5K1A	99
PIP5K1C	95
PIP5K2B	100
PIP5K2C	100
PKAC-alpha	84
PKAC-beta	100
PKMYT1	100
PKN1	100
PKN2	89
PKNB (*M. tuberculosis*)	100
PLK1	100
PLK2	87
PLK3	100
PLK4	83
PRKCD	100
PRKCE	100
PRKCH	100
PRKCI	100
PRKCQ	100
PRKD1	81
PRKD2	87
PRKD3	84
PRKG1	92
PRKG2	99
PRKR	90
PRKX	100

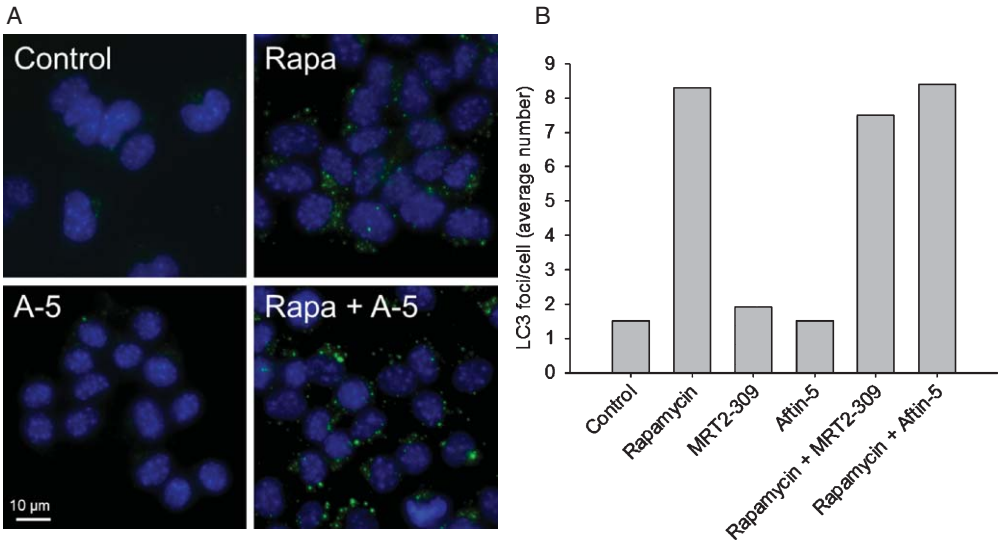
Supplementary Table 1
(*Continued*)

KINASE	SCORE
PRP4	88
PYK2	100
QSK	98
RAF1	100
RET	99
RET (M918T)	89
RET (V804L)	95
RET (V804M)	94
RIOK1	96
RIOK2	100
RIOK3	87
RIPK1	89
RIPK2	84
RIPK4	100
RIPK5	77
ROCK1	92
ROCK2	100
ROS1	100
RPS6KA4 (Kin.Dom.1-N-terminal)	93
RPS6KA4 (Kin.Dom.2-C-terminal)	80
RPS6KA5 (Kin.Dom.1-N-terminal)	100
RPS6KA5 (Kin.Dom.2-C-terminal)	100
RSK1 (Kin.Dom.1-N-terminal)	86
RSK1 (Kin.Dom.2-C-terminal)	100
RSK2 (Kin.Dom.1-N-terminal)	83
RSK3 (Kin.Dom.1-N-terminal)	100
RSK3 (Kin.Dom.2-C-terminal)	93
RSK4 (Kin.Dom.1-N-terminal)	98
RSK4 (Kin.Dom.2-C-terminal)	88
S6K1	100
SBK1	100
SgK110	100
SGK3	100
SIK	88
SIK2	89
SLK	100
SNARK	100
SNRK	100
SRC	96
SRMS	100
SRPK1	100
SRPK2	66
SRPK3	76
STK16	100
STK33	90
STK35	100

Supplementary Table 1
(*Continued*)

KINASE	SCORE
STK36	100
STK39	100
SYK	100
TAK1	91
TAOK1	97
TAOK2	100
TAOK3	88
TBK1	74
TEC	96
TESK1	100
TGFBR1	99
TGFBR2	100
TIE1	94
TIE2	100
TLK1	100
TLK2	100
TNIK	92
TNK1	100
TNK2	100
TNNI3K	100
TRKA	100
TRKB	82
TRKC	100
TRPM6	100
TSSK1B	100
TTK	70
TXK	100
TYK2 (JH1domain-catalytic)	97
TYK2 (JH2domain-pseudokinase)	84
TYRO3	100
ULK1	71
ULK2	96
ULK3	100
VEGFR2	100
VRK2	33
WEE1	100
WEE2	88
YANK1	100
YANK2	100
YANK3	94
YES	100
YSK1	75
YSK4	100
ZAK	100
ZAP70	59

AUTOPHAGY STUDIES



Supplementary Figure 1. Aftin-5 is not involved in autophagy. A) Immunofluorescence of N2A-AβPP695 cells treated with the indicated drug for 18 h (A-5: Aftin-5; Rapa: Rapamycin). Cells were stained for LC3 (green), DNA is visualized with DAPI (blue). Bar: 10 µm. B) Quantification of LC3 foci present per cell following treatment with 0.6 µM Rapamycin, 100 µM Aftin-5 or 100 µM MRT2-309 (2), an inactive analogue of Aftin-5, or DMSO vehicle (control). When cells were treated with two drugs, rapamycin was added two hours after Aftin-5 or MRT2-309.

MITOCHONDRIAL STUDIES

Material and methods - Results

Assessment of mitochondrial swelling in cultured cells

N2a-AβPP695 cells were treated with Aftin-5 (25, 50, and 100 µM) in presence or absence of cyclosporine A (10 µM; PTP inhibitor) for 6 and 24 h. Then cells were fixed with 2% glutaraldehyde in 0.1 M Na-cacodylate buffer, pH 7.2 for 3 h at 4°C. After 2 washes with 0.2 M sucrose in 0.1 M Na-cacodylate buffer, pH 7.2, the specimens were post-fixed with 1% osmium tetroxide containing 1.5% potassium cyanoferrate, dehydrated in gradual ethanol (30–100%) and embedded in Epon. 70 nm thin sections were collected onto 200 mesh cupper grids, counterstained with uranyl acetate and lead citrate before examination with a Zeiss EM 902 transmission electron microscope at 80 Kvolt (MIMA2, Electron Microscopy Platform-GPL, Jouy-en-Josas).

Assessment of $\Delta\Psi_m$ loss in cultured cells

N2a-AβPP695 cells were treated with Aftin-5 for 6 and 24 h before measure of mitochondrial transmembrane potential ($\Delta\Psi_m$) which was monitored by flow cytometry (FACSCalibur; BD Biosciences) using 10 nM Dioc-6 dye. N2a-AβPP695 cells treated with 1 µM staurosporine were used as a positive control for 100% $\Delta\Psi_m$ loss.

Determination of mitochondrial cytochrome c release in cultured cells

N2a-AβPP695 cells were treated with Aftin-5 for 6 and 24 h, then permeabilized by digitonin, fixed and labeled with anti-cytochrome *c* antibody (BD Pharmingen) and secondary antibody (Alexa Fluor 488) for detection of cytochrome *c* by flow cytometry (FACSCalibur; BD Biosciences). Cells treated with 1 µM staurosporine were used as a positive control for 100% cytochrome *c* release.

Measurement of oxygen consumption in cultured cells

N2a-AβPP695 cells were treated with Aftin-5 for 6 and 24 h before measure of cellular respiration. Cells were incubated in presence of the oxygen-sensitive fluorescent dye MitoXpress (Luxcel, Cork, Ireland) in 96-well plates. Oxygen consumption was measured in real-time by spectrofluorimetry (Tecan Infinite 200;

$\lambda_{Excitation}$ 380 nm; $\lambda_{Emission}$ 650 nm). Treatment with rotenone 2 μM was used as positive control for 100% inhibition of oxygen consumption.

Purification of N2a-AβPP695 mitochondria

Mitochondria were isolated from N2a-AβPP695 cell line as previously described [3]. To ensure quality of mitochondrial preparations, samples were subjected to various assays for integrity and functionality as described in Lecoeur et al. [4] and Buron et al. [3].

Assessment of swelling and $\Delta\Psi_m$ loss in isolated mitochondria

Mitochondrial swelling and $\Delta\Psi_m$ were evaluated as described previously [5] in the presence of succinate and rotenone. Calcium ($CaCl_2$; 50 μM) and mCICCP (50 μM) were used as the 100% baseline for swelling and loss of $\Delta\Psi_m$, respectively.

Determination of cytochrome c release in isolated mitochondria

Cytochrome *c* release was evaluated as previously described [5] using an ELISA kit (R&D Systems, France). Treatment with 20 μg/mL Alamethicin was used as the 100% baseline.

Measurement of oxygen consumption in isolated mitochondria

Oxygen consumption was monitored as previously described [5] using the oxygen-sensitive phosphorescent dye MitoXpress (Luxcel, Cork, Ireland). Treatment with rotenone 2 μM and Oligomycin A 1 μM were used as positive control for 100% inhibition of oxygen consumption by complex I and complex II respectively. Untreated mitochondria were the 0% activation/inhibition of oxygen consumption. Results are presented in Supplementary Table 2.

RESULTS

Supplementary Table 2

Effects of Aftin-5 on mitochondria from N2a-AβPP695 cells. Aftin-5 was tested for its ability to induce swelling, loss of $\Delta\Psi_m$, cytochrome c release, and inhibition of mitochondrial respiration. Assays were performed in both whole cells (6 and 24 h-treatment; concentration range from 1.56 to 100 μM) and isolated mitochondria (concentration range from 3.13 to 200 μM). The results shown are for 100 μM on cultured cells (24 h treatment) and for 200 μM on isolated mitochondria and are means of 3 independent experiments

	Mitochondrial effects of Aftin-5				
	Swelling	$\Delta\Psi_m$ loss	Cyto c	O_2 consumption	
Cultured cells (24 h-100 μM	No effect	No effect	No effect	34% inhibition	
Isolated mitochondria (200 μM)	No effect	No effect	No effect	31% activation Complex I	No effect Complex II

REFERENCES

[1] Oumata N, Bettayeb K, Ferandin Y, Demange L, Lopez-Giral A, Goddard M-L, Myrianthopoulos V, Mikros E, Flajolet M, Greengard P, Meijer L, Galons H (2008) Roscovitine-derived, dual-specificity inhibitors of cyclin-dependent kinases (CDKs) and casein kinase 1 (CK1). *J Med Chem* **51**, 5229-5242.

[2] Karaman MW, Herrgard S, Treiber DK, Gallant P, Atteridge CE, Campbell BT, Chan KW, Ciceri P, Davis MI, Edeen PT, Faraoni R, Floyd M, Hunt JP, Lockhart DJ, Milanov ZV, Morrison MJ, Pallares G, Patel HK, Pritchard S, Wodicka LM, Zarrinkar PP (2008) A quantitative analysis of kinase inhibitor selectivity. *Nat Biotechnol* **26**, 127-132.

[3] Buron N, Porceddu M, Brabant M, Desgue D, Racoeur C, Lassalle M, Pechoux C, Rustin P, Jacotot E, Borgne-Sanchez A (2010) Use of human cancer cell lines mitochondria to explore the mechanisms of BH3 peptides and ABT-737-induced mitochondrial membrane permeabilization. *PLoS ONE* **5**, e9924.

[4] Lecoeur H, Langonné A, Baux L, Rebouillat D, Rustin P, Prèvost MC, Brenner C, Edelman L, Jacotot E (2004) Real-time flow cytometry analysis of permeability transition in isolated mitochondria. *Exp Cell Res* **294**, 106-117.

[5] Porceddu M, Buron N, Roussel C, Labbe G, Fromenty B, Borgne-Sanchez A (2012) Prediction of liver injury induced by chemicals in human with a multiparametric assay on isolated mouse liver mitochondria. *Toxicol Sci* **129**, 332-345.