Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters 15 (2005) 4666–4670
Bioorganic & Medicinal Chemistry Letters

The neuroprotective action of JNK3 inhibitors based on the 6,7-dihydro-5*H*-pyrrolo[1,2-*a*]imidazole scaffold

Piotr P. Graczyk,[a,*] Afzal Khan,[a] Gurpreet S. Bhatia,[a] Vanessa Palmer,[a] Darren Medland,[a] Hirotoshi Numata,[a] Hitoshi Oinuma,[a] Jacqueline Catchick,[a] Angela Dunne,[a] Moira Ellis,[a] Caroline Smales,[a] Jonathan Whitfield,[a] Stephen J. Neame,[a] Bina Shah,[a] Daniel Wilton,[a] Louise Morgan,[a] Toshal Patel,[a] Raymond Chung,[a] Howard Desmond,[a] James M. Staddon,[a] Nobuaki Sato[b] and Atsushi Inoue[b]

[a]*Eisai London Research Laboratories, University College London, Bernard Katz Building, Gower Street, London WC1E 6BT, UK*
[b]*Tsukuba Research Laboratories, Eisai Co., Ltd, Tsukuba-shi, Ibaraki 300-2635, Japan*

Received 9 June 2005; revised 26 July 2005; accepted 29 July 2005
Available online 8 September 2005

Abstract—Imidazole-based structures of p38 inhibitors served as a starting point for the design of JNK3 inhibitors. Construction of a 6,7-dihydro-5*H*-pyrrolo[1,2-*a*]imidazole scaffold led to the synthesis of the (*S*)-enantiomers, which exhibited p38/JNK3 IC_{50} ratio of up to 10 and were up to 20 times more potent inhibitors of JNK3 than the relevant (*R*)-enantiomers. The JNK3 inhibitory potency correlated well with inhibition of c-Jun phosphorylation and neuroprotective properties of the compounds in low K^+-induced cell death of rat cerebellar granule neurones.

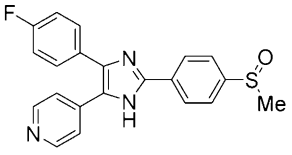
Contribution of apoptotic mechanisms to neurodegenerative diseases, such as Alzheimer's,[1] Huntington's,[2] and Parkinson's diseases,[3] as well as amyotrophic lateral sclerosis (ALS),[4] is becoming increasingly appreciated.[5] Therefore, the JNK pathway, which controls apoptosis in various cell death paradigms, has developed into an attractive drug target for neurodegenerative disorders.[6,7] Inhibitors of this pathway, such as SP600125[8] and TAT-fused peptide inhibitor of JNK substrate binding,[9] exhibited neuroprotective properties in preclinical studies, and CEP-1347, an inhibitor of the JNK pathway, had advanced to Phase III for Parkinson's disease.[10]

Although this pathway can be inhibited at multiple steps, we focused on c-Jun N-terminal kinase 3 (JNK3) due to its relatively selective expression in brain tissue[11] and an expected better drug safety profile. Validity of JNK3 as a target for neurodegenerative disorders has been supported by the resistance of hippocampal CA3 neurones of JNK3 knockout mice to kainic-acid-induced injury.[12] These mice are also protected against cerebral ischemia-hypoxia[13] and partially against toxicity due to MPTP, which serves as a model of Parkinson's disease.[14] Importantly, the JNK3 knockout animals exhibit normal phenotype.[12] Furthermore, mice in which the endogenous substrate of JNK, c-Jun, is replaced by a mutant Jun allele, which cannot be phosphorylated by JNK, are viable and fertile, and resistant to neuronal apoptosis induced by kainic acid.[15]

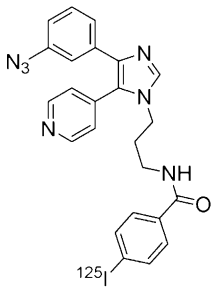
At the initial stages of the JNK3 program at Eisai, we decided to obtain a JNK3 inhibitor by modification of known structure acting in an ATP-competitive manner, similar to the approach adopted by others.[7] As the imidazole-based p38 inhibitor SB203580 had been recognized to inhibit JNK3α1 with a submicromolar IC_{50} value,[16] we decided to use a more structurally attractive p38 inhibitor, SB227931 (p38 IC_{50} 0.046 μM),[17] as a starting point for our initial drug design, hoping that it, too, would inhibit JNK3. To verify this assumption, we synthesized **1**, which showed inhibition of JNK3 with an IC_{50} value of 0.13 μM.

* Corresponding author. Tel.: +44 2074131147; fax: +44 2074131121; e-mail: piotr_graczyk@eisai.net

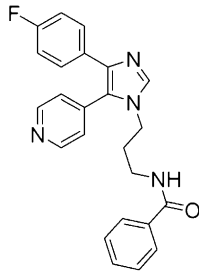
0960-894X/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2005.07.076



SB203580

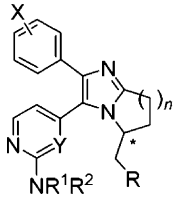


SB227931



1

To increase inhibitory potency, we decided to modify structure **1** by reducing conformational flexibility of the side chain. This could be achieved by introducing an additional ring ($n = 1, 2$) linking imidazole C(2) with the side chain, as shown in structure **2**. We also expected that formation of a chiral center in **2** would enable further control of selectivity, particularly against p38, as this kinase contributes to synaptic plasticity within the CNS.[18]

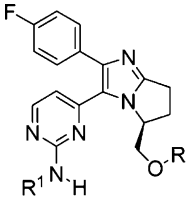


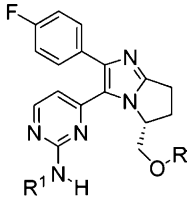
Y= CH, N

$n = 1, 2$

2

Furthermore, the target structure would possess several points for derivatization, which may be utilized to optimize potency and ADME properties of the compounds. Herein, we describe the synthesis of three enantiomeric pairs **3**–**5**, their biological properties and rationalization of the results based on an in silico approach.





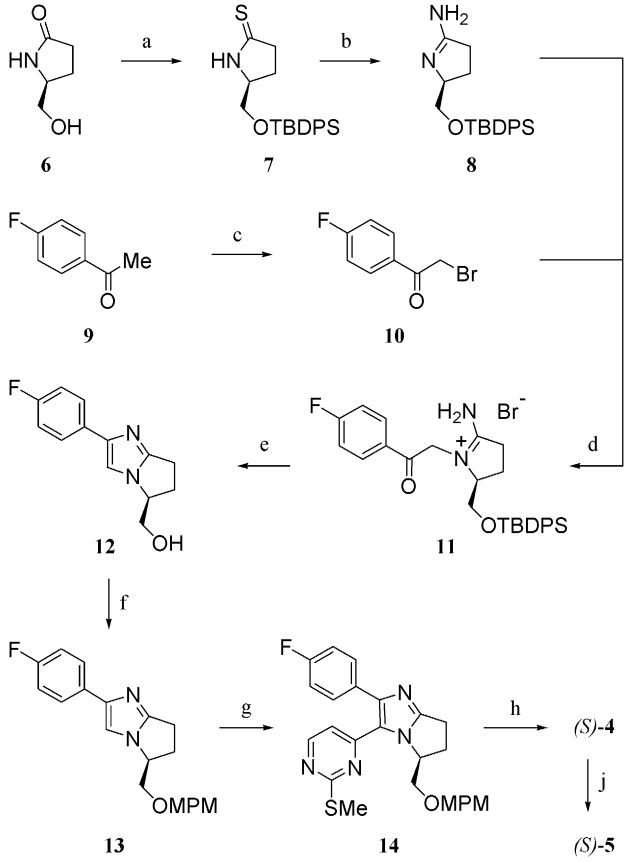
(S)-**3** $R^1 = H$, $R = p\text{-}MeOC_6H_4CH_2$ *(R)*-**3**

(S)-**4** $R^1 = n\text{-}Pr$, $R = p\text{-}MeOC_6H_4CH_2$ *(R)*-**4**

(S)-**5** $R^1 = n\text{-}Pr$, $R = H$ *(R)*-**5**

Preparation of (*S*)-**4** and (*S*)-**5**, which are representative examples of the series, is given in Scheme 1. Synthesis of amine **8**, from a commercially available pyrrolidinone **6**, was performed by modification of the methodology developed by Leutenegger et al.[19]

Amine **8** was reacted with bromoketone **10**, which was readily available by bromination of ketone **9** with $CuBr_2$.[20] Formation of the imidazole ring occurred during reflux of an ethanolic solution of salt **11** in the presence of catalytic amount of hydrobromic acid. Treatment of the product with TBAF afforded alcohol **12**, which was then protected under phase-transfer conditions to form the MPM ether **13**. Stannylation of **13**, followed by Stille coupling with 4-iodo-2-methylthiopyrimidine, gave di(hetero)aryl-substituted imidazole **14**. Substitution of the methylthio group with the *n*-propylamino was achieved by oxidation of sulfur to the relevant sulfoxide and reacting it at room temperature with neat propylamine (liquid ammonia in a sealed tube in the case of (*S*)-**3** and (*R*)-**3**). The MPM group in (*S*)-**4** could be removed under acidic conditions to provide (*S*)-**5**.



Scheme 1. Reagents and conditions: (a) (i) TBDPSCl, imidazole, DMF, rt, 2 h; (ii) Lawesson's reagent, THF, rt, 2 h, 81%; (b) (i) MeI, CH_2Cl_2, rt, 5 h; (ii) NH_4Cl, MeOH, 50 °C, 3.5 h; (iii) NaOH, (87%); (c) $CuBr_2$, AcOEt, reflux, 5 h, 73%; (d) AcOEt, rt, 16 h; (e) (i) cat HBr/AcOH, 33% aq EtOH, reflux, 5.5 h, (ii) TBAF, THF, 0 °C—rt, 1 h, 62% (overall for steps d and e); (f) *p*-methoxybenzyl chloride, 50% aq NaOH, *n*-Bu_4NHSO_4 (cat), rt, 12 h, 99%; (g) (i) *n*-BuLi, THF, −78 °C, 30 min; (ii) Bu_3SnI (neat); (iii) 4-iodo-2-methylthiopyrimidine, DMF, $Pd(PPh_3)_4$ (cat), 80 °C, 4 days, 48%; (h) (i) H_2O_2, CH_3COOH, 48 h; (ii) *n*-$PrNH_2$ (neat), rt, 36%; (j) (i) 6 N $HCl_{(aq)}$, MeOH, 125 °C, 1 h; (ii) NaOH, 78%.

Table 1. Inhibitory activities (IC_{50}) of enantiomeric pairs **3**–**5** and the relevant p38/JNK3 selectivity ratios

Compound	JNK3 IC_{50}[a] (nM)	p38 IC_{50}[a] (nM)	p38/JNK3 IC_{50}
(*S*)-**3**	61.2 (± 12)	295 (± 27)	4.8
(*R*)-**3**	270 (± 47)	116 (± 17)	0.43
(*S*)-**4**	10.9 (± 3.6)	54 (± 4)	5.0
(*R*)-**4**	233 (± 46)	217 (± 26)	0.93
(*S*)-**5**	2.5 (± 0.1)	28 (± 2)	11
(*R*)-**5**	3.0 (± 0.1)	31 (± 2)	10

[a] Values are means of three experiments, standard error is given in parentheses. ATP concentration in both enzyme assays was 1.0 μM.

None of the synthetic stages was accompanied by racemization, which was verified by determination of the optical purity of final compounds and key intermediates.[21]

Inhibitory activities of compounds **3**–**5** against JNK3 and p38 are given in Table 1.

The (*S*)-enantiomers of MPM ethers **3** and **4** are more potent JNK3 inhibitors than their (*R*) counterparts. In particular, the JNK3 IC_{50} value for (*S*)-**4** is about 20 times lower than that for (*R*)-**4**. This observation can be rationalized by docking (*S*)-**4** and (*R*)-**4** within the JNK3 ATP-binding site using published coordinates for the protein.[22]

Compound (*S*)-**4** fits the ATP-binding site of JNK3 very well (Fig. 1A) and participates in several binding interactions identified by Scapin et al.[22] for analogous compounds. Furthermore, the methoxybenzyl group in (*S*)-**4** binds to the glycine-rich loop in the N-terminal domain of JNK3. In contrast, the methoxybenzyl group in (*R*)-**4** is shifted toward the C-terminal domain (Fig. 1B). Some of the hydrophobic interactions are lost and as a consequence (*R*)-**4** would exhibit a weaker JNK3 inhibition than (*S*)-**4**.

In agreement with this explanation, enantiomeric alcohols (*S*)-**5** and (*R*)-**5**, in which the methoxybenzyl group is absent, display virtually the same inhibitory potencies against JNK3. A very low IC_{50} value for both enantiomers can be attributed to the possible hydrogen bond formation between the hydroxyl group of both enantiomers and enzyme Asn152 and/or Ser193. As these hydrogen bonds can also be formed with p38, there is virtually no difference in selectivity against p38 between isomeric alcohols (p38/JNK3 IC_{50} 11 vs. 10 for (*S*)-**5** and (*R*)-**5**, respectively).

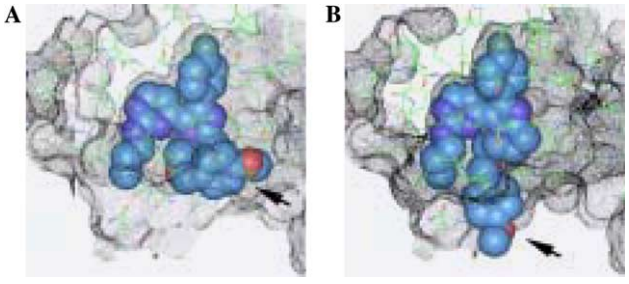


Figure 1. View of (A) (*S*)-**4** and (B) (*R*)-**4** within the JNK3 ATP-binding site (arrows point to the methoxybenzyl group in both molecules).

Docking simulations suggest that (*R*)-**3** and (*R*)-**4** fit the ATP-binding site of p38 better than the ATP-binding site of JNK3 because Val196 in JNK3 is replaced with a smaller Ala157 in p38. Furthermore, the (*R*)-enantiomers may participate in larger hydrophobic interactions with the C-terminal domain of p38. This may explain why (*R*)-**3** inhibits p38 more strongly than JNK3, although no such difference can be seen for (*R*)-**4**. Interestingly, the (*S*)-enantiomers inhibit p38 lesser than JNK3 because the methoxybenzyl group is located at the ribose binding site of p38 where it undergoes destabilizing interactions with Tyr35. The above differences are reflected in the selectivities, for example, p38/JNK3 IC_{50} 4.8 for (*S*)-**3** and p38/JNK3 IC_{50} 0.43 for (*R*)-**3**.

Due to similar cell membrane permeability, enantiomeric compounds with different selectivity profiles offer a very useful tool to study the importance of alternative signaling pathways for biological phenomena in cell systems. As the relative contribution of JNK- and p38-dependent pathways in different cell death mechanisms is still a matter of debate,[23] we examined the effect of the three enantiomeric pairs **3**–**5** on neuroprotection in cerebellar granule neurones (CGN). In this model, incubation of cells in the presence of 25 mM KCl prevents cell death. Reducing the KCl concentration to 5 mM induces c-Jun phosphorylation and apoptosis.[24]

The transcription factor c-Jun is suggested to be a JNK substrate involved in neuronal death.[25] Therefore, efficacy in c-Jun phosphorylation inhibition should follow the JNK3 inhibition profile.[26] As can be seen in Figure 2,

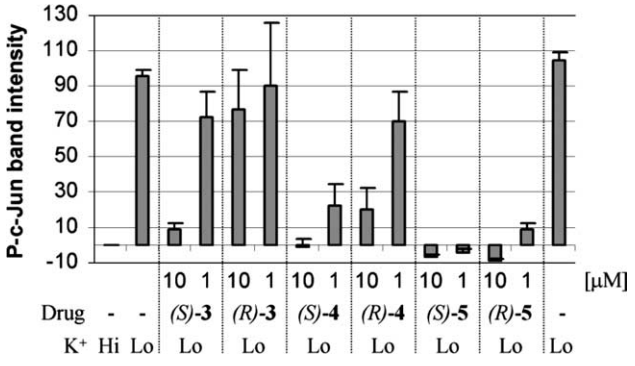


Figure 2. Inhibition of c-Jun phosphorylation by enantiomeric pairs **3**–**5** at concentrations of 10 and 1 μM. Phospho-c-Jun band intensity in immunoblot is expressed as a percentage of the signal at low K^+ in the absence of drug. Hi, 25 mM KCl; Lo, 5 mM KCl. The data are average of four experiments; error bars represent standard deviation.

compound (*S*)-**3** almost completely suppressed the phosphorylation of c-Jun at a concentration of 10 μM, while its enantiomer, (*R*)-**3**, a weaker JNK3 inhibitor (IC_{50} 270 nM), was ineffective at this dose. A much more potent JNK3 inhibitor, (*S*)-**4** (IC_{50} 10.9 nM), showed clear effect, even at 1 μM concentration (Fig. 2), while its enantiomer was less active. Alcohols (*S*)-**5** and (*R*)-**5**, both very potent JNK3 inhibitors (IC_{50} of about 3 nM), exhibited a virtually complete suppression of c-Jun phosphorylation in the range of studied concentrations (Fig. 2). Therefore, the degree of inhibition of c-Jun phosphorylation in this system reflects the JNK3 inhibitory potency. Furthermore, the effects on c-Jun phosphorylation for (*S*)-**3** and (*R*)-**3** are opposite to what could be anticipated based on p38 inhibition (IC_{50} 295 and 116 nM, respectively).

To determine whether inhibition of JNK3 and c-Jun phosphorylation correlate with neuroprotection, we followed the survival of cerebellar granule neurones by MTT[27] or lactate dehydrogenase (LDH)[28] colorimetric assays. The results for enantiomeric pairs **3**–**5** are given in Figure 3 as a percentage of survival at three drug concentrations: 0.1, 1, and 10 μM.

Compounds (*S*)-**3** and (*R*)-**3** had no effect on survival up to a concentration of 1 μM, regardless of the assay used (Fig. 3A). At a concentration of 10 μM, the neuroprotective properties of the (*S*)-enantiomer were significantly stronger than those of (*R*)-**3**, as expected based on a lower JNK3 IC_{50} value for (*S*)-**3** (61.2 nM vs. 270 nM, Table 1) and in agreement with a stronger inhibition of c-Jun phosphorylation by (*S*)-**3** (Fig. 2). Since (*S*)-**3** is a weaker p38 inhibitor than (*R*)-**3** (IC_{50} 295 and 116 nM, respectively), the survival effects indicate that p38-dependent pathway is unimportant in this cell death paradigm, in agreement with Cao et al.[23]

Both enantiomers of **4** exhibited clear dose-dependent effects on survival. Neuroprotective properties of (*S*)-**4** and (*R*)-**4** correlate very well with their JNK3 IC_{50} values. Just as inhibitory potency of (*S*)-**4** is stronger than that of (*S*)-**3** (10.9 nM vs. 61.2 nM, respectively), so a clear protective effect of (*S*)-**4** could already be seen at a concentration of 1 μM (Fig. 3B). The effects of (*R*)-**4** were weaker (JNK3 IC_{50} 233 nM).

Alcohols (*S*)-**5** and (*R*)-**5**, which do not differ in terms of JNK3 inhibitory activity (IC_{50} values of 2.5 and 3.0 nM, respectively), showed survival profiles that were very similar. Due to their high inhibitory potency against JNK3, the compounds showed an almost complete neuroprotection at a concentration of 1 μM.

In conclusion, starting from the structures of known p38 inhibitors we designed a series of compounds based on the 6,7-dihydro-5*H*-pyrrolo[1,2-*a*]imidazole scaffold. Decoration of the scaffold enabled us to increase the JNK3 inhibitory potency and achieve IC_{50} values in the very low nanomolar range and up to 10-fold selectivity for JNK3 over p38. At submicromolar concentrations, these compounds exhibited strong neuroprotective properties, which were attributable to the JNK pathway inhibition. The results of additional SAR studies on this template will be reported in due course.

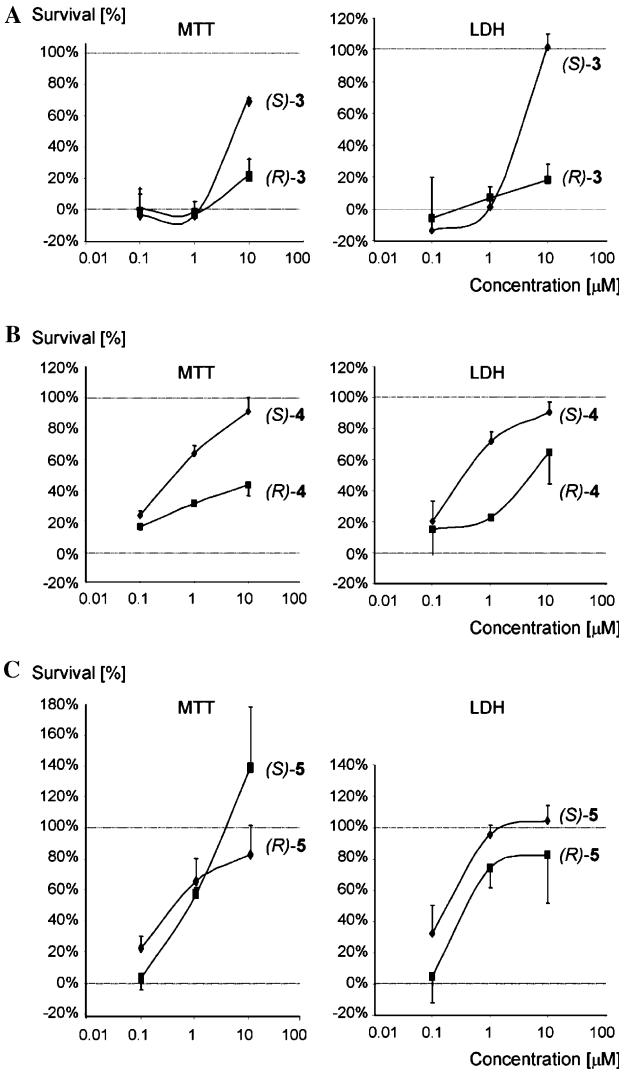


Figure 3. The effect of enantiomeric pairs **3**–**5** on the survival of cerebellar granule neurons, as measured by MTT and LDH assays (in triplicate, error bars represent standard deviation). Death was induced by withdrawal of K^+. Complete survival (100% of mean values) corresponds to the assay reading at high K^+ concentration, while 0% survival (cell death) is the reading at low K^+. (A) (*S*)-**3** and (*R*)-**3**; (B) (*S*)-**4** and (*R*)-**4**; (C) (*S*)-**5** and (*R*)-**5**.

References and notes

1. (a) Mattson, M. P.; Partin, J.; Begley, J. G. *Brain Res.* **1998**, *807*, 167; (b) Barinaga, M. *Science* **1998**, *281*, 1303; (c) McPhie, D. L.; Coopersmith, R.; Hines-Peralta, A.; Chen, Y.; Ivins, K. J.; Manly, S. P.; Kozlowski, M. R.; Neve, K. A.; Neve, R. L. *J. Neurosci.* **2003**, *23*, 6914; (d) Katayama, T.; Imaizumi, K.; Manabe, T.; Hitomi, J.; Kudo, T.; Tohyama, M. *J. Chem. Neuroanat.* **2004**, *28*, 67.
2. (a) Gutekunst, C. A.; Norflus, F.; Hersch, S. M. *Curr. Opin. Neurol.* **2000**, *13*, 445; (b) Hickey, M. A.; Chesselet, M.-F. *Prog. Neuro-psychoph.* **2003**, *27*, 255.
3. (a) Hartley, A.; Stone, J. M.; Heron, C.; Cooper, J. M.; Schapira, A. H. *J. Neurochem.* **1994**, *63*, 1987; (b) Ziv, I.;

Melamed, E.; Nardi, N.; Luria, D.; Achiron, A.; Offen, D.; Barzilai, A. *Neurosci. Lett.* **1994**, *170*, 136; (c) Vermes, I.; Steur, E. N.; Jirikowski, G. F.; Haanen, C. *Movement Disord.* **2004**, *19*, 1252.

4. (a) Rabizadeh, S.; Gralla, E. B.; Borchelt, D. R.; Gwinn, R.; Valentine, J. S.; Sisodia, S.; Wong, P.; Lee, M.; Hahn, H.; Bredesen, D. E. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 3024; (b) Ferri, A.; Nencini, M.; Casciati, A.; Cozzolino, M.; Angelini, D. F.; Longone, P.; Spalloni, A.; Rotilio, G.; Carri, M. T. *FASEB J.* **2004**, *18*, 1261.
5. (a) Graczyk, P. *Prog. Med. Chem.* **2002**, *39*, 1; (b) Vila, M.; Przedborski, S. *Nat. Rev. Neurosci.* **2003**, *4*, 1; (c) Waldmeier, P. C.; Tatton, W. G. *Drug Discovery Today* **2004**, *9*, 210.
6. Lund, S.; Porzgen, P.; Mortensen, A. L.; Hasseldam, H.; Bozyczko-Coyne, D.; Morath, S.; Hartung, T.; Bianchi, M.; Ghezzi, P.; Bsibsi, M.; Dijkstra, S.; Leist, M. *J. Neurochem.* **2005**, *92*, 1439.
7. (a) Bozyczko-Coyne, D.; Saporito, M. S.; Hudkins, R. L. *Curr. Drug Targets: CNS Neurol. Disord.* **2002**, *1*, 31; (b) Manning, A. M.; Davis, R. J. *Nat. Rev. Drug Disc.* **2003**, *2*, 554.
8. (a) Gao, Y.; Signore, A. P.; Yin, W.; Cao, G.; Yin, X. M.; Sun, F.; Luo, Y.; Graham, S. H.; Chen, J. *J. Cerebr. Blood F. Met.* **2005**, 1; (b) Guan, Q. H.; Pei, D. S.; Zhang, Q. G.; Hao, Z. B.; Xu, T. L.; Zhang, G. Y. *Brain Res.* **2005**, *1035*, 51.
9. Borsello, T.; Clarke, P. G.; Hirt, L.; Vercelli, A.; Repici, M.; Schorderet, D. F.; Bogousslavsky, J.; Bonny, C. *Nat. Med.* **2003**, *9*, 1180.
10. Clinical trial has been discontinued (Cephalon press release on 11 May, 2005).
11. Mohit, A. A.; Martin, J. H.; Miller, C. A. *Neuron* **1995**, *14*, 67.
12. Yang, D. D.; Kuan, C.-Y.; Whitmarsh, A. J.; Rincon, M.; Zheng, T. S.; Davis, R. J.; Rakic, P.; Flavell, R. A. *Nature* **1997**, *389*, 865.
13. Kuan, C.-Y.; Whitmarsh, A. J.; Yang, D. D.; Liao, G.; Schloemer, A. J.; Dong, C.; Bao, J.; Banasiak, K. J.; Haddad, G. G.; Flavell, R. A.; Davis, R. J.; Rakic, P. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 15184.
14. Hunot, S.; Vila, M.; Teismann, P.; Davis, R. J.; Hirsch, E. C.; Przedborski, S.; Rakic, P.; Flavell, R. A. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 665.
15. Behrens, A.; Sibilia, M.; Wagner, E. F. *Nat. Genet.* **1999**, *21*, 326.
16. Lisnock, J. M.; Griffin, P.; Calaycay, J.; Frantz, B.; Parsons, J.; O'Keefe, S. J.; LoGrasso, P. *Biochemistry* **2000**, *39*, 3141.
17. Young, P. R.; McLaughlin, M. M.; Kumar, S.; Kassis, S.; Doyle, M. L.; McNulty, D.; Gallagher, T. F.; Fisher, S.; McDonnell, P. C.; Carr, S. A.; Huddleston, M. J.; Seibel, G.; Porter, T. G.; Livi, G. P.; Adams, J. L.; Lee, J. C. *J. Biol. Chem.* **1997**, *272*, 12116.
18. (a) Bolshakov, V. Y.; Carboni, L.; Cobb, M. H.; Siegelbaum, S. A.; Belardetti, F. *Nat. Neurosci.* **2000**, *3*, 1107; (b) Guan, Z.; Kim, J. H.; Lomvardas, S.; Holick, K.; Xu, S.; Kandel, E. R.; Schwartz, J. H. *J. Neurosci.* **2003**, *23*, 7317.
19. Leutenegger, U.; Umbricht, G.; Fahrni, C.; von Matt, P.; Pfaltz, A. *Tetrahedron* **1992**, *48*, 2143.
20. King, L. C.; Ostrum, G. K. *J. Org. Chem.* **1964**, *29*, 3459.
21. Optical purity was determined using Chiralpak AD and propan-2-ol/hexane = 3:7 (v/v; compounds **3** and **5**) and propan-2-ol/hexane = 1:9 (v/v; compounds **4**) as mobile phase: (*S*)-**3**: ee > 99%; $[\alpha]_D^{20}$ −271.3 (*c* 0.91, MeOH); (*R*)-**3**: ee > 99%; $[\alpha]_D^{20}$ +269.9 (*c* 0.41, MeOH); (*S*)-**4**: ee > 99%; $[\alpha]_D^{20}$ −237.6 (*c* 0.87, MeOH); *(R)*-**4**: ee 99%; $[\alpha]_D^{20}$ +247.6 (*c* 0.54, MeOH); (*S*)-**5**: ee > 99%; $[\alpha]_D^{20}$ −193.9 (*c* 1.02, MeOH); (*R*)-**5**: ee > 99%; $[\alpha]_D^{20}$ +195.7 (*c* 0.89, MeOH). (*S*)-**4**: ^{1}H NMR (400 MHz, $CDCl_3$) δ [ppm] 0.98 (t, *J* = 7.4 Hz, 3H), 1.62 (sextet, *J* = 7.3 Hz, 2H), 2.55–2.61 (m, 1H), 2.71–2.89 (m, 2H), 2.97–3.07 (m, 1H), 3.35 (q, *J* = 6.6 Hz, 2H), 3.51 (dd, *J* = 9.5, 5.2 Hz, 1H), 3.66 (dd, *J* = 9.5, 2.9 Hz, 1H), 3.75 (s, 3H), 4.22 (d, *J* = 11.7 Hz, 1H), 4.32 (d, *J* = 11.7 Hz, 1H), 5.11 (bs, 2H), 6.36 (d, *J* = 5.3 Hz, 1H), 6.79 (d, *J* = 8.7 Hz, 2H), 7.01–7.09 (m, 4H), 7.54 (dd, *J* = 8.7, 5.5 Hz, 2H), 7.98 (d, *J* = 5.2 Hz, 1H); HRMS (CI) calcd for $C_{28}H_{30}FN_5O_2$, 488.2462 (M+1); found: 488.2467. (*S*)-**5**: ^{1}H NMR (400 MHz, $CDCl_3$) δ [ppm] 0.98 (t, *J* = 7.4 Hz, 3H), 1.62 (sextet, *J* = 7.3 Hz, 2H), 2.34–2.46 (m, 1H), 2.67–2.86 (m, 2H), 2.99–3.09 (m, 1H), 3.33 (q, *J* = 6.6 Hz, 2H), 3.72 (dd, *J* = 11.4, 6.7 Hz, 1H), 3.87 (dd, *J* = 11.4, 3.6 Hz, 1H), 4.88 (bs, 1H), 5.19 (bs, 1H), 6.34 (d, *J* = 5.3 Hz, 1H), 7.01(t, *J* = 8.7 Hz, 2H), 7.47 (dd, *J* = 8.7, 5.5 Hz, 2H), 7.99 (d, *J* = 4.4 Hz, 1H); HRMS (CI) calcd for $C_{20}H_{22}FN_5O$, 368.1887 (M+1); found: 368.1874.
22. Scapin, G.; Patel, S.; Lisnock, J.; Becker, J.; LoGrasso, P. *Chem. Biol.* **2003**, *10*, 705, Structure: 1PMN.
23. Cao, J.; Semenova, M. M.; Solovyan, V. T.; Han, J.; Coffey, E. T.; Courtney, M. J. *J. Biol. Chem.* **2004**, *279*, 35903.
24. CGNs were isolated from 7 day Sprague–Dawley rat pups.[23] Neurons were cultured for 7 days in basal modified Eagle's medium containing 2 mM L-glutamine, 25 mM KCl, 35 mM glucose, and 10% foetal bovine serum.
25. Eilers, A.; Whitfield, J.; Babij, C.; Rubin, L. L.; Ham, J. *J. Neurosci.* **1998**, *18*, 1713.
26. Due to very high homology between JNK isoforms inhibitors **3**–**5**, were expected to inhibit c-Jun phosphorylation, regardless of the isoform.
27. Mosmann, T. *J. Immunol. Methods* **1983**, *65*, 55.
28. da Costa, A. O.; de Assis, M. C.; Marques Ede, A.; Plotkowski, M. C. *Biocell* **1999**, *23*, 65.