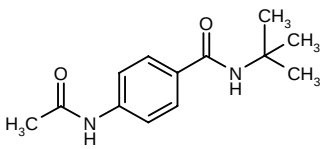
Drugs of the Future 2001, 26(7): 647-650

CCC: 0377-8282/2001

CPI-1189

Treatment of AIDS Dementia
Antiparkinsonian
Treatment of Alzheimer's Dementia

4-Acetamido-*N*-(*tert*-butyl)benzamide



$C_{13}H_{18}N_2O_2$ Mol wt: 234.2972

CAS: 183619-38-7

EN: 283852

Synthesis

Reaction of 4-nitrobenzoyl chloride (I) with *tert*-butylamine (II) in ethyl acetate gives the corresponding amide (III), which is reduced with H_2 over Pd/C in ethanol to yield 4-amino-*N*-*tert*-butylbenzamide (IV). Finally, this compound is acylated with acetyl chloride and TEA in ethyl acetate (1-3). Scheme 1.

Introduction

AIDS dementia is a progressive or relatively static condition that is characterized by a significant cognitive decline and motor dysfunction in individuals with HIV infection when other etiologies have been excluded. Cognitive decline manifests as mental slowing, memory loss and alterations in mood (*i.e.*, apathy, depression) and motor dysfunctions include difficulties in walking, clumsiness and tremor (4). AIDS dementia is estimated to occur in 5-15% of AIDS patients. Although the incidence of AIDS dementia appears to have been reduced by about 50% with the introduction of more effective antiretroviral therapies, it has been suggested that prevalence may actually increase as antiviral therapies prolong the survival of patients (5-7).

AIDS dementia and its associated brain damage is pathogenically characterized at the gross microscopic level as a chronic inflammatory reaction with cortical and subcortical atrophy, neuronal loss, reactive gliosis/astrocytosis, dendritic oversimplification resulting in a decrease in synaptic interactions and the presence of microglial nodules containing macrophage-derived multinucleated cells (8). It is thought that the cause of these microscopic changes which lead to dementia are the toxic products produced by activated HIV-infected monocyte-derived macrophages. Patients suffering from AIDS dementia display a significantly higher CD14/CD69 monocyte subset as compared to HIV-infected patients not presenting dementia (9, 10). Factors such as TNF-α, HIV glycoprotein 120 (gp120) and quinolinic acid have been implicated in AIDS dementia. For example, TNF-α has been demonstrated to be neurotoxic via induction of apoptosis and significantly more TNF-α mRNA has been detected in AIDS patients with dementia than those without (11-13). Results obtained *in vitro* suggest that gp120 from the HIV-1 envelope-induced DNA fragmentation (*i.e.*, apoptosis) in rat neurons and astrocytes appear to be a direct target for the glycoprotein, exhibiting increased glutamate release and a loss of glial fibrillary acidic protein following gp120 exposure (14-16). Quinolinic acid, a metabolite of L-tryptophan that is produced by activated macrophages, is neurotoxic and has been discovered to occur in increased levels in serum and cerebral spinal fluid of patients suffering from AIDS (17-19).

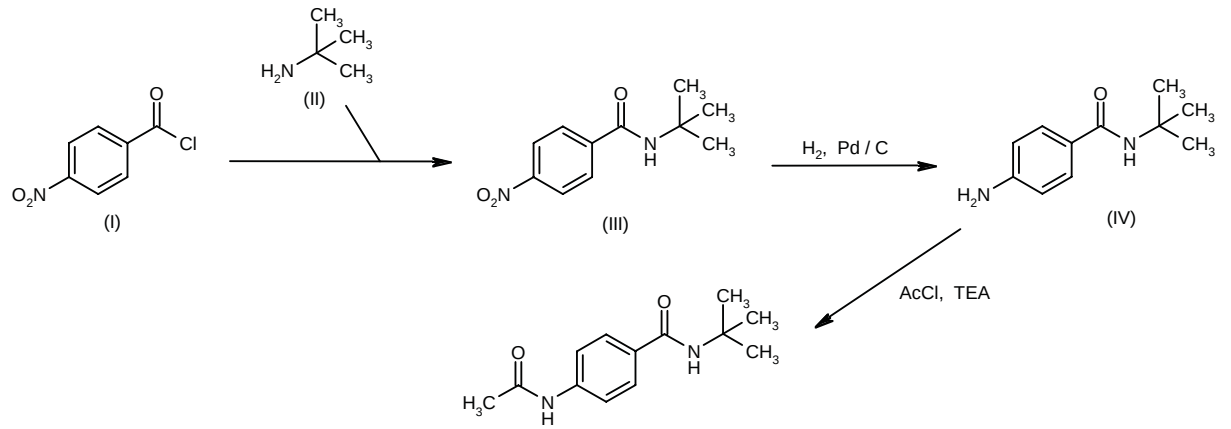
Despite intense research efforts, AIDS dementia remains elusive and this also applies to the progressive loss of cognitive ability and/or dementia associated with Parkinson's and Alzheimer's diseases (20, 21). CPI-1189, a highly orally bioavailable benzamide that has shown antiapoptotic activity *in vitro* and efficacy against cognitive disorders *in vivo*, has been selected for further development as a treatment for AIDS dementia as well as dementia associated with Parkinson's and Alzheimer's diseases.

Pharmacological Studies

Results from *in vitro* studies have shown that CPI-1189 protects against the neurotoxins implicated in AIDS dementia. A study using a human neuroblastoma cell line

L.A. Sorbera, P.A. Leeson, J. Castañer. Prous Science, P.O. Box 540, 08080 Barcelona Spain.

Scheme 1: Synthesis of CPI-1189



(SK-N-MC), differentiated into neurotropic cells following 5 days of incubation with retinoic acid (5 μM), showed that CPI-1189 (0.1-100 μM 1 h before TNF-α exposure) dose-dependently inhibited apoptosis resulting from exposure to increasing concentrations of TNF-α (0.03, 0.3 and 3 ng/ml). The increases in reactive oxygen species and reductions in bcl-2 protein seen 4 and 6 h after incubation with TNF-α treatment, respectively, were also attenuated by treatment with the agent (22).

Other *in vitro* studies using brain cell aggregate cultures prepared from second trimester elective abortion tissue reported similar effects. When brain cell aggregates were treated with monocyte/macrophage supernatant (20% v/v for 48 h) prepared from 4 of 5 HIV-positve patients with dementia, pretreatment with CPI-1189 (10 μM 1 h before) significantly reduced apoptosis by 50%; in experiments using brain cell aggregates treated with monocyte/macrophage supernatant prepared from the fifth demented HIV-positive patient, CPI-1189 reduced apoptosis by only 20%. Interestingly, no apoptosis was observed in brain cell aggregates treated with monocyte//macrophage supernatant prepared from nondemented HIV-positive subjects or HIV seronegative patients. Further experiments using this model were performed in an attempt to identify the mechanism of protection afforded by CPI-1189. Pretreatment of brain cell aggregates with CPI-1189 (0.03 μM and higher 1 h before) significantly protected against apoptosis induced by TNF-α (1 and 10 ng/ml) or recombinant gp120 (1 ng/ml). Moreover, pretreatment with the agent (3 μM 1 h before) significantly decreased apoptotic and necrotic cell death of brain cell aggregates occurring in response to 48-h prior exposure to quinolinic acid. CPI-1189 (10 μM) had no significant effects on TNF-α (0.3 ng/ml)-induced nuclear translocation of NFκB in brain cell aggregates. CPI-1189 also had no effect or only a slight decreasing effect on TNF-α-induced JNK (c-JUN-*N*-terminal kinase) and p38 MAPK (mitogen activated protein kinase) activity. However, while TNF-α treatment slightly activated ERK (extracellular signal-regulated kinase; 27% at 30 min), CPI-1189 + TNF-α treatment resulted in a 57% increase in ERK activity over control aggregates. Moreover, treatment of aggregates with TNF-α and PD-98059, a specific inhibitor of ERK's upstream MAPK kinase (MKK) 1 and MKK2, blocked the protective effects of CPI-1189 against TNF-α-induced neurotoxicity. Results suggest that the protective effects of CPI-1189 are associated with ERK activation (23).

Further *in vitro* studies using primary rat astrocyte cultures to investigate the mechanism of action responsible for the neuroprotective effects of CPI-1189 showed that the agent (10 nM or less) inhibited IL-1β-induced p38 MAPK phosphorylation by 70%; a maximum inhibition of 70-80% was observed with CPI-1189 at concentrations of 10-300 nM. Thus, antagonism of p38-MAPK may be involved in the neuroprotective effects of CPI-1189 (24).

Two *in vivo* studies using a rat model of AIDS dementia in which rats were subjected to chronic ventricular infusions of TNF-α (50 ng/μl i.c.v. bilaterally for 3 or 7 days) showed that concurrent treatment with CPI-1189 (20 mg/kg b.i.d. p.o.) significantly prevented TNF-α-induced weight loss, learning and memory impairment, enlargement of ventricles and increases in apoptosis. Both CPI-1189 and TNF-α reduced glial fibrillary acidic protein (GFAP) staining around the infusion site. However, only CPI-1189 treatment significantly increased the blood-brain arrier integrity at the infusion site. No effects were observed on oxidative stress parameters such as lipid peroxidation and hydroxyl free radical production. Results not only indicate the potential efficacy of the agent in the treatment of AIDS dementia but also that CP-1189 may be effective in diseases presenting high levels of brain TNF-α such as Alzheimer's and Parkinson's diseases (25, 26).

Preclinical studies have also demonstrated the potential efficacy of CPI-1189 as a treatment of inflammatory bowel disease. Two studies using a murine (3% dextran

sulfate sodium in the drinking water for 7 days) or rat (intrarectal enema of 50 mg/kg trinitrobenzene [TNBS]) model of experimental colitis reported that oral CPI-1189 (10-90 mg/kg p.o.) dose-dependently improved disease activity indices and mucosal damage. Moreover, when CPI-1189 was given 4, 8 and 24 h after TNBS administration in rats, a significant inhibition of TNBS-induced colonic inflammatory responses was observed, indicating possible efficacy of the agent in healing severe colitis (27, 28).

Pharmacokinetics and Toxicology

Pharmacokinetic studies in animals have shown that orally administered CPI-1189 was highly bioavailable. A $t_{1/2}$ of approximately 40 h was obtained with little accumulation of the agent observed. In monkeys, about 80% of the dose was shown to penetrate the brain as compared to blood concentrations. Results also indicated that there are no effects of cytochrome P-450 enzymes, suggesting that drug-drug interactions would be rare (29).

The toxicity of CPI-1189 was investigated in 6- and 12-month studies in animals. No unexpected systemic effects were observed with long-term CPI-1189 treatment. Moreover, single- or multiple-CPI-1189 dosing of approximately 200 human subjects showed no significant treatment-limiting adverse events (29).

Clinical Studies

Results from a randomized, double-blind, placebo-controlled phase II trial conducted in 64 subjects with mild to moderate HIV-associated cognitive impairment and receiving HIV therapy, failed to show the efficacy of CPI-1189 (50-100 mg/day for 10 week). No significant changes in Z-scores for 8 neuropsychological measures (NPZ8) or alterations in function parameters were observed with treatment. Treatment was well tolerated with only 2 CPI-1189-treated patients discontinuing due to rash; incidence of rash was similar in both treated and placebo groups. One patient developed a cataract while receiving CPI-1189. No clinically significant toxicities were observed, and no changes in viral load or CD4 counts were reported during treatment. It was concluded that a longer study is required to examine the neuroprotective efficacy of the agent (30).

Centaur Pharmaceuticals has reported the efficacy of CPI-1189 in significantly improving cognitive function in psychomotor tests according to results of two different double-blind, placebo-controlled phase IIa trials conducted in patients with AIDS dementia (3-month daily dosing) and dementia related to Parkinson's disease (5-month daily dosing). CPI-1189 continues to undergo phase II testing as a treatment for dementia associated with AIDS and Parkinson's disease (31).

Manufacturer

Centaur Pharmaceuticals, Inc. (US).

References

1. Flitter, W.D., Garland, W.A., Wilcox, A.L., Paylor, R.E. (Centaur Pharmaceuticals, Inc.). *Benzamides for neurodegenerative disorder treatment.* US 5955506, WO 9631462.

2. Garland, W. (Centaur Pharmaceuticals, Inc.). *Benzamide treatment of dementia, associated with AIDS virus (HIV-1) infection.* WO 9738684.

3. Flitter, W.D., Garland, W.A., Irwin, I. (Centaur Pharmaceuticals, Inc.). *Benzamide therapeutics for the treatment of inflammatory bowel disease.* US 6194465, WO 9959569.

4. Brew, B.J. *AIDS dementia complex.* Neurol Clin 1999, 17: 861-81.

5. McArthur, J.C., Hoover, D.R., Bacellar, H. et al. *Dementia in AIDS patients: Incidence and risk factors.* Neurology 1993, 43: 2245-52.

6. Qureshi, A.I., Hanson, D.L., Jones, J.L., Janssen, R.S. *Estimation of the temporal probability of human immunodeficiency virus (HIV) dementia after risk stratification for HIV-infected persons.* Neurology 1998, 50: 392-7.

7. Clifford, D.B. *Human immunodeficiency virus-associated dementia.* Arch Neurol 2000, 57: 321-4.

8. De Girolami, U., Anthony, D.C., Frosch, M.P. *The central nervous system.* In: Robbins Pathological Basis for Disease, R.S. Cotran, V. Kumar and T. Collins (Eds.), W.B. Saunders, Philadelphia, 1999, 1320-1.

9. Pulliam, L., Gascon, R., Stubblebine, M., McGuire, D., McGrath, M.S. *Unique monocyte subset in patients with AIDS dementia.* Lancet 1997, 349: 692-5.

10. Pulliam, L., Herndier, B.G., Tang, N.M., McGrath, M.S. *Human immunodeficiency virus infected macrophages produce soluble factors that cause histological and neurochemical alterations in cultured human brains.* J Clin Invest 1991, 87: 503-12.

11. Wesselingh, S.L., Power, C., Glass, J.D., Tyor, W.R., McArthur, J.C., Farber, J.M., Griffin, J.W., Griffin, D.E. *Intracerebral cytokine messenger RNA expression in acquired immunodeficiency syndrome dementia.* Ann Neurol 1993, 33: 576-82.

12. Pulliam, L., Zhou, M., Stubblebine, M., Bitler, C.M. *Differential modulation of cell death proteins in human brain cells by tumor necrosis factor alpha and platelet activating factor.* J Neurosci Res 1998, 54: 530-8.

13. Shi, B., Raina, J., Lorenzo, A., Busciglio, J., Gabuzda, D. *Neuronal apoptosis induced by HIV-1 Tat protein and TNF-alpha: Potentiation of neurotoxicity mediated by oxidative stress and implications for HIV-1 dementia.* J Neurovirol 1998, 4: 281-90.

14. Muller, W.E., Schroder, H.C., Ushijima, H., Dapper, J., Bormann, J. *gp120 of HIV-1 induces apoptosis in rat cortical cell cultures: Prevention by memantine.* Eur J Pharmacol 1992, 226: 209-14.

15. Benos, D.J., Hahn, B.H., Shaw, G.M., Bubien, J.K., Benveniste, E.N. *gp120 mediated alterations in astrocyte ion transport.* Adv Neuroimmunol 1994, 4: 175-9.

16. Pulliam, L., West, D., Haigwood, N., Swanson, R.A. *HIV-1 envelope gp120 alters astrocytes in human brain cultures.* AIDS Res Hum Retroviruses 1993, 9: 439-44.

17. Nottet, H.S., Flanagan, E.M., Flanagan, C.R., Gelbard, H.A., Gendelman, H.E., Reinhard, J.F. Jr. *The regulation of quinolinic acid in human immunodeficiency virus-infected monocytes.* J Neurovirol 1996, 2: 111-7.

18. Achim, C.L., Heyes, M.P., Wiley, C.A. *Quantitation of human immunodeficiency virus, immune activation factors, and quinolinic acid in AIDS brains.* J Clin Invest 1993, 91: 2769-75.

19. Heyes, M.P., Brew, B.J., Martin, A. et al. *Quinolinic acid in cerebrospinal fluid and serum in HIV-1 infection: Relationship to clinical and neurological status.* Ann Neurol 1991, 29: 202-9.

20. Prous Science Drug R&D Backgrounders: *Parkinson's disease (online publication).* Updated June 26, 2001.

21. Prous Science Drug R&D Backgrounders: *Alzheimer's disease (online publication).* Updated July 2, 2001.

22. Irwin, I., MacGowan, E., Flitter, W.D., Garland, W.A. *CPI-1189 attenuates the toxic effects of TNFalpha in SK-N-MC cells.* Soc Neurosci Abst 1998, 24(Part 2): Abst 596.8.

23. Pulliam, L., Irwin, I., Kusdra, L., Rempel, H., Flitter, W.D., Garland, W.A. *CPI-1189 attenuates effects of suspected neurotoxins associated with AIDS dementia: A possible role for ERK activation.* Brain Res 2001, 893: 95-103.

24. Hensley, K., Robinson, K.A., Pye, Q.N., Floyd, R.A., Cheng, I., Garland, W.A., Irwin, I. *CPI-1189 inhibits interleukin 1beta-induced p38-mitogen activated protein kinase phosphorylation: An explanation for its neuroprotective properties?* Neurosci Lett 2000, 281: 179-82.

25. Bjugstad, K.B., Flitter, W.D., Garland, W.A., Su, G.C., Arendash, G.W. *Preventive actions of a synthetic antioxidant in a novel animal model of AIDS dementia.* Brain Res 1998, 795: 349-57.

26. Bjugstad, K.B., Flitter, W.D., Garland, W.A., Philpot, R.M., Kirstein, C.L., Arendash, G.W. *CPI-1189 prevents apoptosis and reduces glial fibrillary acidic protein immunostaining in a TNF-alpha infusion model for AIDS dementia complex.* J Neurovirol 2000, 6: 478-91.

27. Greenwood-Van Maerveld, B., Tyler, K.R., Johnson, A.C., Adesina, A.M., Dunn, S.T., Irwin, I., Garland, W. *CPI-1189 protects against dextran sulfate sodium-induced colitis in mice.* Dig Dis Week (May 16-19, Orlando) 1999, Abst 4029.

28. Greenwood-Van Meerveld, B., Tyler, K.R., Peters, L.J., Flitter, W., Garland, W. *Anti-inflammatory effects of a novel antioxidant in rats.* Dig Dis Week (May 17-20, New Orleans) 1998, Abst 3070.

29. McGuire D, Marder K. *Pharmacological frontiers in the treatment of AIDS dementia.* J Psychopharmacol 2000, 14: 251-7.

30. Clifford, D., McArthur, J.C., Schifitto, G., Cohen, B., Ellis, R., Marder, K., Marra, C., Kieburtz, K., McDermott, M., Flitter, W. *Phase II trial of CPI-1189 for HIV-associated motor cognitive impairment.* 8th Conf Retroviruses Opportunistic Infect (Feb 4-8, Chicago) 2001, Abst 600.

31. *Centaur's CPI-1189 shows efficacy in phase IIa trials in patients with dementia.* DailyDrugNews.com (Daily Essentials) May 16, 2001.