

Synthesis and characterization of (4-amino-phenyl)-(1-aza-bicyclo[2.2.2]oct-4-yl)-methanone, a quinuclidine derivative with anti-retroviral properties



Laurent Lecanu^{1,2}, Wenguo Yao^{1,2}, Alexander Piechot⁴, Janet Greeson³, Dimitrios Tzalis⁴ and Vassilios Papadopoulos^{1,2}

¹ Department of Biochemistry and Molecular Biology, Georgetown University Medical Center, Washington DC, 20057, USA

² Samaritan Research Laboratories, Georgetown University Medical Center, Washington DC, 20057, USA; ³ Samaritan Pharmaceuticals, Las Vegas NV, 89109, USA; ⁴ Taros Custom Chemicals, Marburg, 35043, Germany

The global HIV/AIDS epidemic killed more than 3 million people in 2003, and an estimated 5 million acquired the human immunodeficiency virus (HIV) – bringing to 40 million the number of people living with the virus around the world, urging for new treatments. We report herein the synthesis and the characterization of the antiretroviral properties of a new quinuclidine derivative, (4-amino-phenyl)-(1-aza-bicyclo[2.2.2]oct-4-yl)-methanone (SP-03), as the lead compound of a new series of antiretroviral small molecules. SP-03 displayed a highest efficacy than AZT and ddI in inhibiting HIV-1IIB. Without a pre-medication protocol, SP-03 inhibited the HIV-1 IIB viral replication with a higher efficiency than the classical antiviral agent ddI when given at concentrations up to 1 μ M. The maximum effect was observed for the concentration 10 nM with a 50% inhibitory effect on the HIV-1 IIB viral replication. With 6 hours pre-medication, SP-03 displayed a highest efficacy at low concentrations up to 10 nM than AZT in inhibiting the viral replication. 12 hours pre-medication resulted in an equivalent effect of SP-03 and AZT on the viral replication with an inhibition of 70% and 78% and an IC₅₀ of 3.11 μ M and 3.83 μ M respectively. 48 hours pretreatment with SP-03 inhibited by 50% HIV replication with an IC₅₀ of 73.9 pM compared to AZT (IC₅₀=40.8 nM). In addition SP-03 was able to significantly reduce the replication of the multidrug resistant viral strain MDR769 with a maximum effect of 60% and an IC₅₀ of 1 nM. In addition SP-03 was able to protect neuronal cells against gp120 neurotoxicity and displayed a very low *in vitro* toxicity compared to the reference compounds AZT and ddI, as studied on HeLa cells. These results present therefore SP-03 as a very interesting drug candidate to treat AIDS and HIV-related dementia, and to overcome anti-retroviral resistance

Introduction

The global HIV/AIDS epidemic killed more than 3 million people in 2003, and an estimated 5 million acquired the human immunodeficiency virus (HIV) – bringing to 40 million the number of people living with the virus around the world. Despite progress in developing anti-viral regimens, there is not a fully effective therapy for AIDS. Emerging resistances due to virus genotype mutations and serious side-effects are strong limitations to the treatment efficacy. Currently, there is a need for effective anti-retroviral agents. We report herein the antiretroviral properties of a new quinuclidine derivative, (4-amino-phenyl)-(1-aza-bicyclo[2.2.2]oct-4-yl)-methanone (SP003). SP003 displayed a highest efficacy than AZT and ddI in inhibiting HIV-1IIB and the multi-drug resistant strain MDR-769 infectivity as well as in reducing gp120 toxicity in neuronal cells.

Figure 1: Chemical synthesis

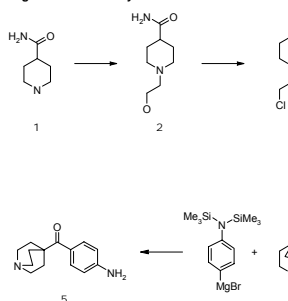


Figure 3: SP-03 antiretroviral effect on HIV-1 MDR769

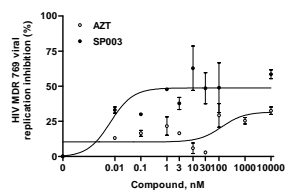


Figure 2: SP-03 antiretroviral effect on HIV-1 IIB

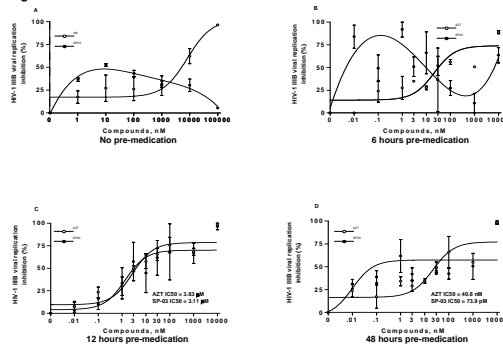


Figure 4: SP-03 protects neuronal cells against gp120 toxicity

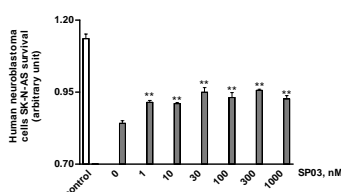
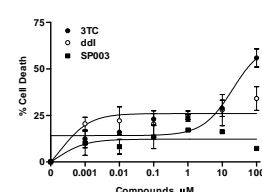


Figure 5: Cytotoxicity of SP-03 assessed on HeLa cells



Experimental section

Synthesis of the SP-03 compound, (4-Amino-phenyl)-(1-aza-bicyclo[2.2.2]oct-4-yl)-methanone (Fig 1)

Compound 4 was synthesized by the method of Kanai²¹. N,N-Bis-(trimethylsilyl)-4-bromoaniline was purchased from Sigma-Aldrich. Solvents were purified by standard methods. Under an argon atmosphere, N,N-Bis-(trimethylsilyl)-4-bromoaniline (2.5 ml, 8.86 mmol) was added dropwise to a suspension of Mg turnings (250 mg, 10.3 mmol) in dry THF (2 ml) at such a rate that consumption of Mg was observed. An aliquot of 1.1 ml of this Grignard reagent was added dropwise to 4 (200 mg, 1.47 mmol) dissolved in dry THF (2 ml). After stirring the solution under reflux for 2 h, ice-cold aqueous HCl (20%, 4 ml) was added. After stirring overnight at ambient temperature the mixture was neutralized with saturated aqueous NaHCO₃ and extracted with dichloromethane. The organic layer was separated, dried over MgSO₄ and concentrated *in vacuo*. The residue was purified by PTLC on silica gel to afford 5 (44 mg, 0.19 mmol, 13%).

HeLa cells culture and treatment

In order to study the viral replication *in vitro*, the GenPhar AV-Finder™-HIV Drug Discovery Assay was used, a novel technology that consists of two components: (1) a cloned, continuous-passage HeLa cell line containing an HIV-1 tat-activated molecular switch and a Green Fluorescent Protein reporter gene and (2) a recombinant adenovirus (rAd) vector containing the genes for all three of the HIV-1 receptor/co-receptors (CD4, CXCR4, and CCR5) to transduce into HeLa cells and convert them into highly susceptible HIV-1 indicator cells for use in the assay.

Detector plates are set up at day 1 by adding HeLa cells (3000/well) to the adenovirus AD-3R in DMEM containing Cosmic Calf Serum (Calf serum supplemented in iron and growth factor) (Hyclone, Logan UT) in 96-well plates and incubated at 37°C under 95% humidity and 5% CO₂ for 2 days. To study the effect of SP-03 on HIV-1 IIB infectivity, SP-03 was assessed using a protocol with or without pre-medication. Without pre-medication, at day 3, HIV-1 IIB (200IP/well) and increasing concentrations of SP-03 or reference compounds (AZT) were added and incubated overnight. At day 4, the medium was replaced by fresh medium containing the corresponding concentration of the compounds of interest. The infectivity was assessed by measuring the fluorescence on each well at day 7 (λ_{em}=485 nm; λ_{exc}=520 nm). With 6, 12 or 48 hours pre-medication, increasing concentrations of SP-03 or reference compounds (AZT) were added at day 3 and incubated the corresponding period of time. Thereafter, HIV-1 IIB (200IP/well) and increasing concentrations of SP-03 or reference compounds (AZT) were added and incubated overnight. Then, the medium was replaced by fresh medium containing the corresponding concentration of compounds of interest and the infectivity was assessed by measuring the fluorescence on each well 3 days later. Results are expressed as percentage of inhibition of the viral replication. The effect of SP-03 on the multi-drugs resistant strain MDR-769 has been assessed without any pre-medication.

Assessment of SP-03 neuroprotective properties against gp120 toxicity

Human neuroblastoma cells SK-N-AS (ATCC, Manassas, VA) were seeded in 96-well plates (4.104 cells/well) and incubated overnight in DMEM, 10% FBS at 37°C and 5% CO₂. SP-03 was then added at increasing concentrations and cells were incubated for 2 days. After this period of time, the medium was replaced by a fresh culture medium containing gp120 20 nM and the corresponding concentration of SP-03. The cell viability was assessed 24 hours later by measuring the level of the cellular 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) reduction.

Assessment of SP-03 toxicity

Following the above described cell treatment protocol, the cytotoxicity of SP-03 was assessed by measuring the levels of cellular 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt (MTS) reduction into a formazan dye (reading at 490 nm).

In conclusion, the data herein demonstrate the anti-retroviral properties of the (4-amino-phenyl)-(1-aza-bicyclo[2.2.2]oct-4-yl)-methanone (SP03). SP-03 inhibited in a dose-dependent manner the infectivity of the HIV-1 IIB in engineered HeLa cells. The necessity to pre-incubate the HeLa cells with SP03 to obtain a strong anti-proliferative effect seems to indicate that this compound acts most likely on the cells by increasing their resistance to the virus entry rather than acting directly on the virus itself. Although the mechanism of action is not fully understood, acting on the host cells, rather than on the virus, is expected to lower the rate of emergence of resistant strains and therefore to increase the efficacy of anti-retroviral therapies. Moreover, the reduction of MDR769 viral replication induced by SP03 treatment associated with a neuroprotective effect against gp120 deleterious properties present this quinuclidine derivative as a potentially interesting anti-retroviral agent. In addition, SP03 displayed a very low *in vitro* toxicity. Taken together, these results suggest that SP03 as a new therapeutic strategy to treat AIDS and HIV-related dementia, and to overcome antiretroviral resistance, in association or not with HAART and mega-HAART regimens.