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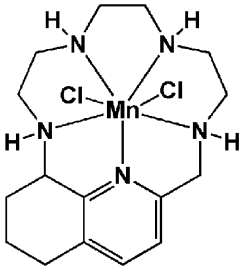
Macrocyclic Polyamines Targeting the Cellular HIV Co-receptors, CXCR4 and CCR5

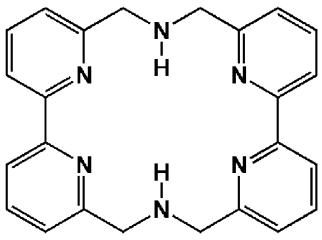
Sunil Hamal[1,*], Li Cui[1], Bell Thomas[1], Aquaro Stefano[2], Dana Huskens[3], Dominique Schols[3]

[1] *Department of Chemistry, University of Nevada, Reno, USA;* [2] *Department of Experimental Medicine and Biochemical Sciences, University of Rome 'Tor Vergata', Rome, Italy;* [3] *Rega Institute for Medical Research, Department of Microbiology and Immunology, Katholieke Universiteit Leuven, Leuven, Belgium*

A number of macrocyclic polyamines and/or their metal complexes are known to have anti-HIV activity. For example, CADA compounds are triazacyclododecanes that specifically down-modulate CD4, the principal cellular receptor for HIV. Bicyclams and their metal complexes act as entry inhibitors by a different mechanism, via specific binding to the cellular co-receptor CXCR4. Manganese(II) complexes of certain pentaazacyclopentadecanes are superoxide dismutase mimics and reduce oxidative stress in cells. One such compound, M40401, has been reported to decrease apoptosis in HIV-infected astrocytes [Mollace et al., 2002. J. Leukoc. Biol., 71, 65–72]. By synthesizing and screening various pyridine-fused macrocyclic polyamines, we have discovered two lead compounds that act as HIV entry inhibitors by binding to one or both cellular co-receptors, CXCR4 and CCR5. One of these new leads is SH06, the manganese(II) complex of a novel ring-fused pentaazacyclopentadecane. SH06 inhibits replication of HIV-1 IIIB and NL4.3 in MT-4 cell cultures with IC_{50} values of 0.2–0.4 μg/ml and with CC_{50} of 20 μg/ml. Remarkably, SH06 interacts with both HIV co-receptors CXCR4 and CCR5, according to specific chemokine-induced calcium-signaling assays. SH06 acts as an antagonist toward SDF-1-induced Ca-signaling in CXCR4-transfected cells (IC_{50}: 0.3 μg/ml), but acts as an agonist toward CCR5. In addition, the compound also has significant activity (IC_{50}: 1.6–3.4 μg/ml) against several R5 viruses in PBMCs and monocytes/macrophages. The other new lead is Cui3, a previously known ring-fused hexaazacyclooctadecane. Cui3 inhibits HIV-1 NL4.3 in MT-4 cell cultures (IC_{50}: 2.1 μg/ml; CC_{50} > 100 μg/ml), and it acts as a specific antagonist towards SDF-1-induced Ca-signaling in U87.CD4.CXCR4-transfected cells (IC_{50}: 1.7 μg/ml). Syntheses and anti-HIV-1 activities of these and some related ring-fused polyazamacrocycles will be presented.



SH06



Cui3

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Synthesis and Antiviral Activity of Substituted Uracils

Alexander Ivanov[1,*], Vera Tunitskaya[1], Olga Smirnova[1], Robert Buckheit[2], Katherine Seley-Radtke[3], Alexander Ozerov[4], Mikhail Novikov[4]

[1] *Engelhardt Institute of Molecular Biology, Moscow, Russia;* [2] *University of Maryland, Baltimore County, Baltimore, USA;* [3] *ImQuest BioSciences, Inc., Frederick, USA;* [4] *Volgograd State Medical University, Volgograd, Russia*

Hepatitis C virus (HCV), human immunodeficiency virus (HIV) and Epstein-Barr virus (EBV) belong to a group of infectious agents that are a global health threat due to their chronic nature. These viruses induce liver injury, mononucleosis and disrupt the immune system. They have been classified as group 1 carcinogens by the International Agency for Research in Cancer. Treatment of HCV and EBV infections is poorly developed, despite a large number of compounds that have shown potential as antiviral agents in cell culture systems. In contrast, several nucleoside and nonnucleoside inhibitors of HIV reproduction have been approved for AIDS therapy. Nevertheless, during extended treatment there is a high risk of developing resistance. Hence, the discovery of novel antiviral agents continues to be one of the major goals for modern medicinal chemistry.

Here we present synthesis of a series of substituted pyrimidines and their evaluation as antiviral agents. For this purpose, two groups of compounds were synthesized: N^1-benzyl substituted-5-aminouracils and 2,5-disubstituted-2-thio-6-methyluracils. The compounds displayed only moderate inhibitory activity against HIV, however some of the 5-aminouracils showed notable anti-EBV activity. The highest activity observed was EC_{50} 2.3 μM for compound 11 that was not found to be toxic in the Akata cell line.

Several 2-thio-6-methyluracils inhibited HCV replication in cell culture. Their mechanism of action against HCV was dual: among the compounds active in replicon system only five inhibited viral RNA-dependent RNA polymerase. None of the compounds blocked helicase or NTPase activities of HCV NS3 protein, therefore, it is likely that 2-thiouracils may also alter some cellular process crucial for HCV replication.

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Synthesis and Biological Evaluation of Acyclic Nucleotide Analogues of Bicyclic Pyrimidine Bases

Zlatko Janeba[1,*], Antonín Holý[1], Robert Snoeck[2], Graciela Andrei[2], Eric De Clercq[2], Jan Balzarini[2]

[1] *Institute of Organic Chemistry and Biochemistry AS CR, Prague, Czech Republic;* [2] *Rega Institute for Medical Research, Leuven, Belgium*

The bicyclic furo[2,3-d]pyrimidine nucleoside analogues are potent and selective inhibitors of Varicella-Zoster virus (VZV). Antiviral compound Cf 1743 is one of the most potent antiviral agents that have ever been reported and is subject of phase-I clinical trials as its orally bioavailable 5′-valine prodrug derivative (McGuigan et al., 2007). Modifications on the sugar moiety of the furo[2,3-d]pyrimidine nucleosides led to compounds poorly active against VZV but with activity against human cytomegalovirus

(HCMV) (McGuigan et al., 2004). Replacement of the sugar at N3 with the (2-hydroxyethoxy)methyl group (present in the antiherpes drug acyclovir) afforded compounds with weak activity against both VZV and HCMV (Janeba et al., 2005). Phosphorylation of the furo[2,3-d]pyrimidine nucleoside analogues by the VZV TK is a prerequisite for their anti-VZV activity, but it is apparently not sufficient (Balzarini and McGuigan, 2002). To further elucidate the mechanism of antiviral action of this group of compounds, novel series of phosphoryl methoxy ethyl (PME) furo[2,3-d]pyrimidines were synthesized. The target compounds were prepared by the Sonogashira coupling of various alkynes with protected 5-iodo PMEU, followed by Cu(I)-promoted intramolecular cyclization, and removal of the iPr ester groups. The antiviral activity against HIV, HSV, VZV and HCMV of these compounds will be reported.

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HIV-1 Gag Matrix Protein Fragments and Polyacid Conjugates Designed for the HIV Inhibition

E. Karaseva*, A. Serbin, I. Rodionov, A. Vasiliev, V. Abramov, O. Alikhanova

Health RDF, Moscow, Russia

The HIV gag matrix protein (MA) plays an essential role in the HIV life cycle at earliest (viral uncoating, RNA delivery to nuclei) and latest (RNA re-transporting toward plasma membrane, virions assembly-maturation) steps. So, the MA, as promising anti-HIV therapeutic target, was included in priority of our anti-HIV inhibitors design strategy [Antivir. Res. 2003 57(3):50; 2006 70(1):85]. Here we report the recent experimental developments in frame of: (1) MA-derived peptides (MAP) design and synthesis; (2) a cooperation of the MA-interfere with an anti-RNA potency expected on macromolecular level of MAP grafted to specific polymers (NAM), mimicking furan- and acid-kind species alternation similar to polymeric backbone of nucleic acids. A number of MAP-imitators of MA helix 2–4 region fragments (responsible for MA–MA inter-self recognition-aggregation) were synthesized and modified to mono-amino group active reagents suitable for single-linked grafting to NAM, and the corresponding MAP-NAM conjugates were synthesized, purified and separated in soluble lyophilized forms too. The grafting link location within AA chain/N-terminus of MAP was regulated by regioselective variation of the active and protected –NH2 groups positions along the polypeptide chain. In parallel the fluorescent derivates of MAP and MAP-NAM were prepared. The newly synthesized candidates (Fig. 1) to therapeutic counter-intervention in HIV life cycle by MA-interfering and by cooperative RNA-antagonistic mechanisms are disposed to anti-HIV evaluation (particularly in A. Bukrinskaya Lab., Virol. Inst., Moscow), and current results will be discussed.

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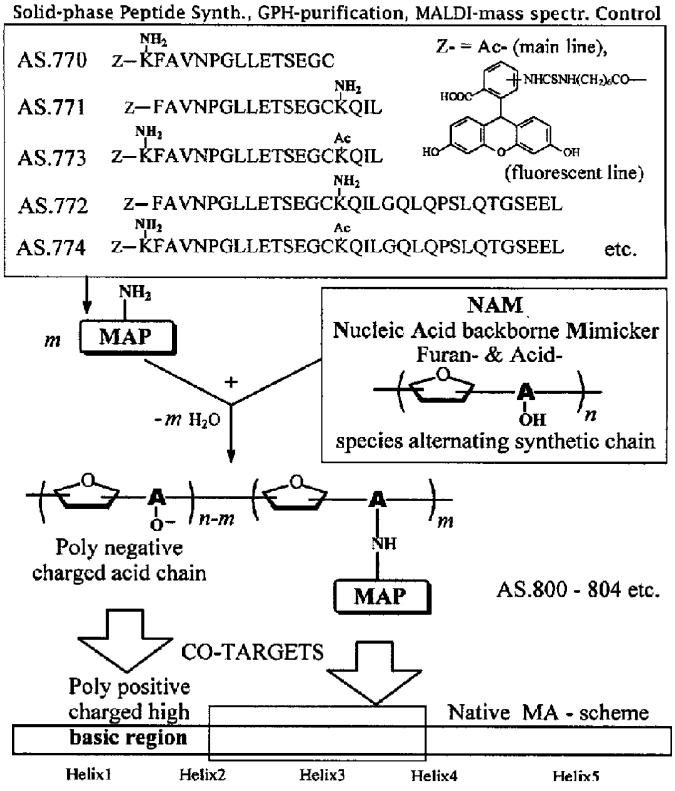

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CXCR4 Antagonists: A New Generation of Configurationally Restricted Bis-azamacrocyclic Compounds

Abid Khan[1,*], Gary Nicholson[1], Graeme McRobbie[1], John Greenman[4], Christophe Pannecouque[2], Dirk Daelemans[2], Dominique Schols[2], Erik De CLercq[2], Timothy J. Hubin[3], Stephen J. Archibald[1]

[1] *Department of Chemistry, University of Hull, Hull, United Kingdom;* [2] *Rega Institute for Medical Research, Katholieke Universiteit Leuven, Leuven, Belgium;* [3] *Department of Chemistry and Physics, Southwestern Oklahoma State University, Weatherford, USA;* [4] *Centre for Medical Research, University of Hull, Hull, United Kingdom*

AMD3100 is a bis-azamacrocyclic compound that has been demonstrated to be a highly effective antagonist of the CXCR4 chemokine receptor. The two azamacrocyclic rings have been shown to interact with aspartate residues on the receptor via hydrogen bonding and electrostatic interactions. However, it is proposed that AMD3100 may bind metal ions in vivo and the active form may be a metal containing drug compound. This presents an issue as the metal complexes of AMD3100 exist in a configurational equilibrium some of which are better at binding to the receptor than others. The aim of this research is to design, synthesise and characterise new azamacrocyclic metal complexes with fixed configurations to provide optimised interactions with the CXCR4 receptor, increasing the potency and residence times of the new drugs relative to AMD3100. Informed design of a metal containing drug requires a comprehensive knowledge of coordination chemistry principles and must incorporate high kinetic stability of the complex to pre-