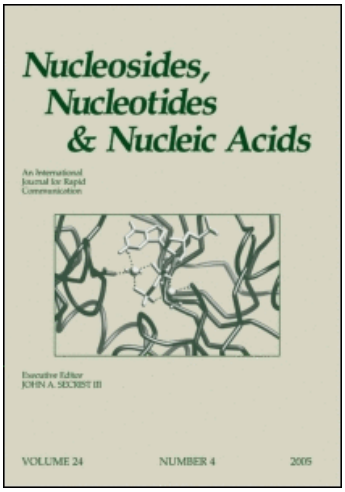

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

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To cite this Article Franchetti, Palmarisa , Petrelli, R. , Cappellacci, L. , Pasqualini, M. , Vita, P. , Sorci, L. , Mazzola, F. , Raffaelli, N. and Magni, Giulio(2005) 'Synthesis and Biological Evaluation of NAD Analogs as Human Pyridine Nucleotide Adenylyltransferase Inhibitors', Nucleosides, Nucleotides and Nucleic Acids, 24: 5, 477 — 479
To link to this Article: DOI: 10.1081/NCN-200060013
URL: http://dx.doi.org/10.1081/NCN-200060013

Nucleosides, Nucleotides, and Nucleic Acids, 24 (5–7):477–479, (2005)

ISSN: 1525-7770 print/ 1532-2335 online
DOI: 10.1081/NCN-200060013

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SYNTHESIS AND BIOLOGICAL EVALUATION OF NAD ANALOGS AS HUMAN PYRIDINE NUCLEOTIDE ADENYLYLTRANSFERASE INHIBITORS

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▫ *NAD analogs modified at the ribose adenylyl moiety, named N-2′-MeAD and Na-2′-MeAD, were synthesized as ligands of pyridine nucleotide (NMN/NaMN) adenylyltransferase (NMNAT). Both dinucleotides resulted selective inhibitors against human NMNAT-3 isoenzyme.*

Keywords NAD Analogs, Human NMN Adenylyltransferase (NMNAT), NMNAT Inhibitors

INTRODUCTION

The central role of nicotinamide adenine dinucleotide (NAD) or its deamidated form NaAD in cellular metabolic processes justifies a great focus of investigation toward the biosynthesis of the dinucleotides. NMN-adenylyltransferase (NMNAT, referred also as pyridine nucleotide adenylyltransferase)[1–3] is an indispensable enzyme in the NAD biosynthetic pathways.

Three distinct human NMNAT isoenzymes, named hNMNAT-1, hNMNAT-2, and hNMNAT-3, have been identified and characterized.[1–3] The tissue distribution pattern of hNMNATs is different, suggesting that the expression of these isoforms is differentially controlled at transcriptional level. Thus, each isoenzyme may have a distinct cellular and physiological role.

The aim of our study was to obtain hNMNAT inhibitors to better understand the biological function of the enzyme isoforms and to define their suitability as

This work was supported by grants of Italian MIUR (PRIN 2002). We thank Dr. G. Rafaiani, F. Lupidi, and M. Brandi for technical assistance.

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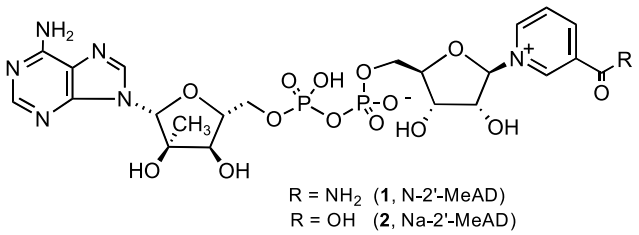

FIGURE 1

specific targets for drug development. On the basis of the knowledge that the structure of a nucleotide analog and the conformation about the glycosidic bond are determinant factors in the drug–enzyme interaction, we designed a NAD analog and its deamido derivative in which the adenylyl part was modified at the *C*2′ position of the furanose ring by introduction of a methyl group. The dinucleotides N-2′-MeAD and Na-2′-MeAD were synthesized and assayed to evaluate their activity against the hNMNAT-1, hNMNAT-2, and hNMNAT-3 isoforms (Figure 1).

CHEMISTRY

The synthesis of dinucleotides N-2′-MeAD and Na-2′-MeAD was carried out by coupling of nicotinamide or nicotinic acid riboside 5′-monophosphates (NMN/NaMN) as imidazolide derivatives with the *n*-tributylammonium salt of 2′-*C*-methyl-adenosine 5′-monophosphate (2′-*C*-MeAMP). Structural assignment for dinucleotides **1** and **2** was based on spectroscopic data (^{1}H-NMR, ^{31}P-NMR spectroscopy, and mass spectrometry API-ES). Information concerning the predominant solution conformation of both NAD analogs was obtained by proton nuclear overhauser enhancement (n.O.e.) effects in D_2O. The complete lack of H-8 enhancement in the purine ring when the anomeric proton H-1′ of the 2′-*C*-methyl-adenosine of **1** or **2** was irradiated, supports a spatial arrangement where H-8 of adenine and H-1′ are not proximate, as would be the case in the *anti* conformer. Because of the overlapping of the protons signals in positions 3 and 4 of the ribose moiety, it was impossible to determine the sugar puckering of these dinucleotides by ^{1}H-NMR experiments. However, we previously found that 2′-*C*-methyl-adenosine has a marked preference for the North ($^{3}T_2$) conformation in solution.[4] Thus, it is likely that the preference for this conformation is maintained in N-2′-MeAD and in the deamidated analog Na-2′-MeAD.

BIOLOGICAL RESULTS AND DISCUSSION

The effect of dinucleotides N-2′-MeAD and Na-2′-MeNAD was tested on hNMNATs activity (Table 1). Enzymatic assay was carried out at 37°C in an

TABLE 1 Inhibitory Effect of N-2′-MeAD and Na-2′-MeAD on hNMNATs Activity[a]

	Inhibition (%)		
compd	hNMNAT-1	hNMNAT-2	hNMNAT-3
Na-2′-MeAD	28	33	81
N-2′-MeAD	9	9	65

[a]hNMNATs activity was assayed in the presence of 1 mM dinucleotide and compared to the activity measured in the absence of the compound. Values are reported as percentage of inhibition of the enzymatic activity. NMN and ATP were used at 1 mM concentrations.

incubation mixture of 100 μL, containing 35 mM Tris buffer (pH 7.5), 20 mM $MgCl_2$, different concentrations of ATP, NMN, and dinucleotides and a suitable aliquot of recombinant enzymes.

Both dinucleotides showed an inhibitory activity against hNMNATs with a significant selectivity for the type-3 isoform. Hence, the inhibitory concentration of the compounds was determined toward hNMNAT-3 activity. Surprisinghly, IC_{50} value for N-2′-MeAD (0.19 mM) resulted significantly lower with respect to that of deamidate analog Na-2′-MeAD (1.12 mM). Moreover, Na-2′-MeAD showed *K*i values towards NMN and ATP of 0.25 and 0.32 mM, respectively. Inhibition analysis was performed according to Dixon.[5]

The identification of N-2′-MeAD and Na-2′-MeAD as good selective inhibitors of hNMNAT-3 might be helpful for the rational design of ligand molecules potentially useful as new chemotherapeutic agents.

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