



**Short communication**

## Search for drugs of the combined anti-inflammatory and anti-bacterial properties: 1-methyl-N'-(hydroxymethyl)nicotinamide

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**Abstract:**

It has already been reported that 1-methylnicotinamide (MNA<sup>+</sup>), a primary metabolite of nicotinamide (vitamin B<sub>3</sub>), possesses remarkable anti-inflammatory properties [3]. This communication shows that 1-methyl-N'-(hydroxymethyl)nicotinamide (MNAF<sup>+</sup>) can be regarded as MNA<sup>+</sup> precursor able to release simultaneously formaldehyde. Therefore, MNAF<sup>+</sup> can be viewed as a candidate for drug with the combined anti-inflammatory and anti-bacterial properties.

**Key words:**

1-methyl-N'-(hydroxymethyl)nicotinamide, anti-inflammatory, anti-bacterial, formaldehyde release

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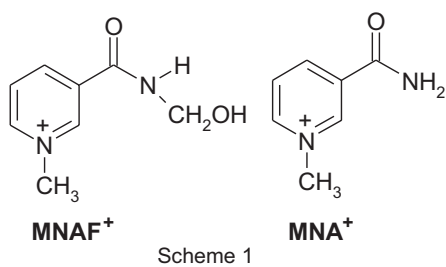
### Introduction

Over the past few decades the bacterial resistance to antibiotics has become one of the most important problems of infections treatment. Searching for new compounds, which would combine a non-specific activity against a broad spectrum of bacteria and low toxicity seems to be a promising way to overcome that problem.

Recently 1-methylnicotinamide (MNA<sup>+</sup>), a primary metabolite of nicotinamide, has been shown to act as a very efficient anti-inflammatory agent [3, 10] especially useful in the treatment of burns, wound healing and selected dermatologic diseases with inflammatory component. It has been found, however, that MNA<sup>+</sup> therapy is not sufficient in the cases with

accompanying bacterial infection as MNA<sup>+</sup> does not possess considerable antibacterial activity. To solve this problem a suitable modification of the structure of MNA<sup>+</sup> molecule should be offered. Additionally, this modification should not lead to any loss of remarkable anti-inflammatory properties of MNA<sup>+</sup>. We have accomplished this goal by applying successfully the strategy of “masked formaldehyde” compounds [5]. Formaldehyde is known as a powerful antibacterial agent, but its irritative properties and pungency make it clinically useless. The already known “masked formaldehyde” compounds, like taurolin or noxythiolin [1, 4] containing N-CH<sub>2</sub>-N or N-CH<sub>2</sub>-O groups in their structure, can slowly release formaldehyde, thus exerting an efficient antibacterial action without any undesirable side effects.

The appropriate modification of MNA<sup>+</sup> molecule was achieved by substitution of one of the amide hydrogen atoms by the hydroxymethyl group. Here, we present the preliminary results showing anti-bacterial properties of 1-methyl-N'-(hydroxymethyl)nicotinamide (MNAF<sup>+</sup>), which can be viewed as a candidate for non-toxic drug of the combined anti-inflammatory and anti-bacterial activities.



## Materials and Methods

### Materials

1-methyl-N'-(hydroxymethyl)nicotinamide chloride (MNAF<sup>+</sup>) was synthesized as follows: N'-(hydroxymethyl)nicotinamide (Sigma-Aldrich, 98%) was methylated with methyl iodide in methanol solution according to a known procedure [8]. The resulting iodide salt was converted to MNAF<sup>+</sup> by shaking its aqueous solution with freshly precipitated silver chloride. MNAF<sup>+</sup> was purified by repeated crystallization from water-acetone (1:14) solution, forming colorless crystals with the following characteristics: mp. 178°C (decomp.); <sup>1</sup>H NMR (Bruker, 250 MHz, D<sub>2</sub>O), δ ppm: 4.49 (s, 3H, CH<sub>3</sub>), 4.95 (s, 2H, CH<sub>2</sub>), 8.19 (m, 1H), 8.95 (dd, 2H), 9.28 (s, 1H); UV-VIS (Philips PU 8710, H<sub>2</sub>O): λ<sub>max</sub> 265 nm; ε<sub>max</sub> 4.1 × 10<sup>3</sup> l/mol cm.

Other chemicals and solvents were purchased from Sigma-Aldrich or Merck.

### Bacterial strains

The following bacterial strains were used: *Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus* MR3, *Escherichia coli* ATCC 25922, *Escherichia coli* 35218, *Pseudomonas aeruginosa* ATCC 27853, *Enterococcus faecalis* ATCC 29212. All the bacteria were obtained from the State Institute of Hygiene, Warsaw, Poland.

### Determination of formaldehyde (CH<sub>2</sub>O) content in aqueous solutions of MNAF<sup>+</sup>

The content of CH<sub>2</sub>O in aqueous solutions was determined according to Barker-Summerson's method [9] which relies on the reaction of CH<sub>2</sub>O with CuSO<sub>4</sub>-(4-phenyl)phenol complex to form a highly colored product. As MNAF<sup>+</sup> interferes with this method, the content of CH<sub>2</sub>O in MNAF<sup>+</sup> solutions cannot be determined directly.

Samples (0.5 ml) of MNAF<sup>+</sup> solutions in deionized water were lyophilized under the pressure of 0.01 mmHg. The evaporated samples of water were collected in traps cooled with liquid nitrogen and stored at -20°C. Before analysis for CH<sub>2</sub>O content the samples of water were heated to room temperature.

Samples of water evaporated from MNAF<sup>+</sup> solutions (0.2 ml) were placed in 10 ml flasks containing 3.5 ml of 96% H<sub>2</sub>SO<sub>4</sub> (suprapur, Merck). The resulting solutions were stirred for 1 min and left for 10 min at room temperature. Then 50 μl of CuSO<sub>4</sub> solution (2.6%, in doubly-distilled water) and 100 μl of 4-phenylphenol solution (1.5%, in 96% ethanol) were added. The solutions were stirred for 2 min and then left for 1 h at room temperature. The absorbance of the colored product was measured at 608.8 nm. The calibration curve correlating the absorbance and the concentration of CH<sub>2</sub>O was generated using known concentrations of CH<sub>2</sub>O and used to calculate its concentration in water evaporated from MNAF<sup>+</sup> solutions. The detection limit was close to 35 μmol/l.

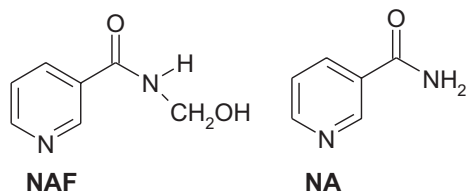
### Determination of minimal inhibitory concentration (MIC)

Serial two-fold dilutions of MNAF<sup>+</sup> were prepared over the range from 4.10 to 0.13 mg/ml in Mueller-Hinton liquid nutrient broth. The solutions were inoculated with the test bacteria (10 μl). Checking for growth was performed after 48 h incubation at 37°C. MIC was defined as the lowest MNAF<sup>+</sup> concentration with no visible growth.

## Results and Discussion

MNAF<sup>+</sup> is the methylated derivative of N'-hydroxymethylnicotinamide (NAF) [7], that is used as active substance of the old drug to treat biliary disorder, sold under commercial name of Bilamid (Cilag). NAF

can also be considered as “masked formaldehyde” compound derived from nicotinamide (NA), however, the anti-bacterial activity of NAF (MIC: 10 mg/ml, determined against *Staphylococcus aureus* and *Escherichia coli*) is much lower than that of MNAF<sup>+</sup>.



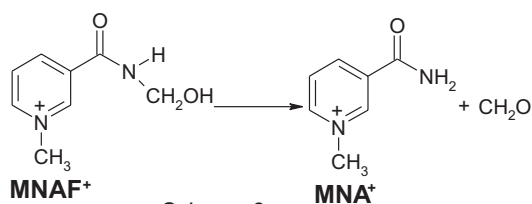
Scheme 2

The results of our preliminary study have shown that MNAF<sup>+</sup> possesses remarkable anti-bacterial activity against all the bacteria tested (Tab. 1). Among them *Enterococcus faecalis* was the most susceptible (MIC: 0.51 mg/ml), whereas *Pseudomonas aeruginosa* was much more resistant (MIC: 2.0 mg/ml). This is not surprising as *Pseudomonas aeruginosa* is also intrinsically resistant to many antibiotics [2]. These

Tab. 1. Minimal inhibitory concentration (MIC) of MNAF<sup>+</sup> against various bacterial strains

No.	Bacterial strain	MIC [mg/ml]
1	<i>Enterococcus faecalis</i> 29212	0.51
2	<i>Staphylococcus aureus</i> 25923	1.02
3	<i>Staphylococcus aureus</i> MR3	1.02
4	<i>Escherichia coli</i> 25922	1.02
5	<i>Escherichia coli</i> 35218	2.05
6	<i>Pseudomonas aeruginosa</i> 6749	2.05

characteristics can be attributed to low permeability of outer membrane of this bacterial cell. The anti-bacterial activity of MNAF<sup>+</sup> is about 25 times greater than that of MNA<sup>+</sup> (MIC: 50 mg/ml against *Pseudomonas aeruginosa*). It is reasonable to assume that the considerable anti-bacterial activity of MNAF<sup>+</sup> can be attributed to formaldehyde (CH<sub>2</sub>O) released upon MNAF<sup>+</sup> decomposition that can proceed according to the reaction:



Scheme 3

Tab. 2. Detected concentration of CH<sub>2</sub>O released in MNAF<sup>+</sup> solution (1%) containing various numbers of bacterial cells.

Bacterial strain	Number of bacterial cells in 1 ml <sup>a</sup>	CH <sub>2</sub> O concentration [μmol/l] <sup>b</sup>
<i>Escherichia coli</i>	10 <sup>6</sup>	360
	10 <sup>7</sup>	535
<i>Staphylococcus aureus</i>	10 <sup>6</sup>	450
	10 <sup>7</sup>	515
none	0	190

<sup>a</sup> Bacteria were added to the solution 24 h before the measurements.

<sup>b</sup> CH<sub>2</sub>O detection limit: 35 μmol/l

The second product of the above reaction is 1-methylnicotinamide (MNA<sup>+</sup>), which would exert an efficient anti-inflammatory action.

The concentrations of CH<sub>2</sub>O determined in water removed by lyophilization from 1% MNAF<sup>+</sup> solutions containing the bacteria are shown in Table 2. It is evident from these data that the MNAF<sup>+</sup> decomposition is faster in the presence of bacteria. Additionally we have found that the time required for bactericidal action of MNAF<sup>+</sup> is close to 6 h and does not depend strongly on MNAF<sup>+</sup> concentration (Tab. 3). The much shorter time, about 60 min, was determined for bactericidal action of free CH<sub>2</sub>O (0.8% solution) [6].

The presence of bacteria in MNAF<sup>+</sup> solution can increase the amount of CH<sub>2</sub>O released, most likely *via* participation of enzymes present in bacterial outer

Tab. 3. Bactericidal action of MNAF<sup>+</sup> as a function of contact time and number of bacterial cells present in the solution

MNAF <sup>+</sup> concentration [%]	Time [h]	<i>Staphylococcus aureus</i> <sup>a</sup>			<i>Pseudomonas aeruginosa</i> <sup>a</sup>		
		10 <sup>6</sup> /ml	10 <sup>7</sup> /ml	10 <sup>8</sup> /ml	10 <sup>6</sup> /ml	10 <sup>7</sup> /ml	10 <sup>8</sup> /ml
0.1	1	+	+	+	+	+	+
	6	-	-	+	-	+	+
	24	-	-	-	-	-	+
0.5	1	+	+	+	+	+	+
	6	-	-	+	-	+	+
	24	-	-	-	-	-	+
1.0	1	+	+	+	+	+	+
	6	-	-	+	-	+	+
	24	-	-	-	-	-	-

<sup>a</sup> growth observed (+); no growth (-)

membranes. However, at present it is impossible to differentiate quantitatively two effects responsible for CH<sub>2</sub>O release, namely, the thermal decomposition of MNAF<sup>+</sup> or decomposition assisted by bacterial enzymes.

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