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Synthesis and evaluation of antibacterial and trypanocidal activity of derivatives of monensin A

Marta Jędrzejczyk [a], Natalia Stępczyńska [a], Greta Klejborowska [a], Małgorzata Podsiad [b], Joanna Stefańska [c], Dietmar Steverding [d], Adam Huczyński [a,*]

[a] *Department of Medical Chemistry, Faculty of Chemistry, Adam Mickiewicz University, Uniwersytetu Poznańskiego 8, 61-614 Poznań, Poland*

[b] *Chair and Department of Biochemistry, Medical University of Warsaw, Banacha 1, 02-097 Warsaw, Poland*

[c] *Department of Pharmaceutical Microbiology, Centre for Preclinical Research, Medical University of Warsaw, Banacha 1b, 02-097, Warsaw, Poland*

[d] *Bob Champion Research and Education Centre, Norwich Medical School, University of East Anglia, Norwich, United Kingdom*

ABSTRACT

The synthesis and biological evaluation of eleven derivatives of the natural polyether ionophore monensin A (**MON**), modified at the C-26 position, is presented. Eight urethane and three ester derivatives were tested for their antimicrobial activity against different strains of *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli* and *Pseudomonas aeruginosa*. In addition, their antiparasitic activity was also evaluated with bloodstream forms of *Trypanosoma brucei*. The majority of the modified ionophores were active against a variety of Gram-positive bacterial strains, including methicillin-resistant *S. epidermidis*, and showed better antibacterial activity than the unmodified **MON**. The phenyl urethane derivative of **MON** exhibited the most promising antibacterial activity of all tested compounds, with minimal inhibitory concentration values of 0.25-0.50 μg/ml. In contrast, none of the **MON** derivatives displayed higher antitrypanosomal activity than the unmodified ionophore.

Keywords: antimicrobial activity; antiparasitic activity; natural products; polyether ionophores

*correspondence: adhucz@amu.edu.pl

Infectious diseases are one of the most common cause of human deaths worldwide. At present, the antimicrobial-resistance of bacteria is a major problem the health care has to face and new and effective antimicrobial agents have been in demand for decades. The number of deaths related to multidrug-resistant bacteria in the European Union (EU) amounts to 25,000 every year. Antimicrobial resistance has a huge impact on the healthcare systems and society as it generates enormous costs estimated to be approximately €1.5 billion in the EU alone.[1]

Among Gram-positive bacteria, the greatest threat to human health is posed by *Staphylococcus aureus* and *Staphylococcus epidermidis*.[2,3] *S. aureus* is responsible for malignant results of many different types of disorders including mild skin infection, endocarditis, fatal pneumonia, bacteraemia and sepsis.[4,5] The threat of *S. aureus* is due to its multidrug-resistance, which has been developed over the last few decades and MRSA (methicillin-resistant *S. aureus*) became the most common antimicrobial-resistant pathogen in almost every part of the world.[6] Like MRSA, methicillin-resistant *S. epidermidis* (MRSE) is a serious problem, which is responsible for bloodstream infections and nosocomial post-operative wound infections.[7]

Trypanosomiasis is parasitic infectious disease, caused by protozoan belonging to the genus *Trypanosoma*. *Trypanosoma brucei* is the causative agent of human and animal African trypanosomiasis (HAT and AAT) and is transmitted by the bite of infected tsetse flies. African trypanosomiasis has had a significant impact on the economic and cultural development of central Africa and has been a severe public health problem causing several epidemics .[8] Nowadays, the incidence of sleeping sickness is the lowest over the last 80 years, but the history of this disease shows that preventive measures of this disease should not be suspended the search for novel antitrypanosomal drugs to avoid potential future epidemics should be continued.[9] Furthermore, drug-resistant trypanosomes strains have

emerged, which makes the treatment of trypanosomiasis more difficult.[10–13] In view of the above, the search for new, efficient and well-tolerated drugs to overcome the problem of drug-resistance is an important line of research work. Monensin A (**MON**, Figure 1), which belongs to the well-known group of polyether ionophore antibiotics, is a good candidate to consider in this respect, because it shows a wide spectrum of biological activities, including antimicrobial, antiproliferative, antiparasitic and antiviral actions.[14–17] In 2016, Steverding *et al.* showed that **MON** exhibits trypanocidal activity higher than suramin, the drug used in the treatment of sleeping sickness.[18] Furthermore, Stefańska *et al.* have investigated the effects of **MON** against methicillin-resistant *S. epidermidis* strains and confirmed the antibacterial activity of the ionophore antibiotic against MRSE strains, stronger than that of the reference antibacterial drug ciprofloxacin.[19,20]

Accordingly, the biological activities of **MON** derivatives such as urethanes and esters have been investigated.[21–25] For instance, *in vitro* studies of fourteen urethanes have shown that some of the derivatives exhibited a ten-fold higher activity against Gram-positive microorganisms, compared to that of the unmodified monensin.[21] The highest activities were displayed by phenyl urethanes, expressed by the MIC values lower than 0.1 μg/mL.[21] These findings have proved that modification of **MON** at the C-26 position is a promising direction of studies, which can lead to the discovery of new compounds with enhanced biological activity.

In this paper, both novel urethanes as well as the previously described phenyl urethanes and esters of **MON** were synthesized (Figure 1) and their antimicrobial and antiparasitic activities tested. We chose the three esters (**9-11**), which were examined in our previous study against four human cancer cell lines and exhibited the highest antiproliferative activity.[26] The aim of this study was to determine the structure-activity relationship (SAR) of monensin derivatives

found as promising in the search for efficient compounds, which could be developed into new treatments of bacterial and parasitic infections.

The sodium salt of monensin (MON-Na) was isolated from the premix - Coxidin® (a commercially available veterinary feed additive). **MON** was obtained from MON-Na by the extraction with H_2SO_4 solution (pH = 1.5) in CH_2Cl_2, according to the procedure described by Huczyński and co-workers.[27,28]

The urethane derivatives (**1-8**) were obtained in the reactions of **MON** with the respective isocyanates in anhydrous toluene (Figure 1)[21]. The esters (**9-11**) were synthesized according to Gaboyard's procedure in the reaction of MON-Na with the respective acyl chlorides in the presence of 4-(dimethylamino)pyridine (DMAP) in pyridine (Figure 1).[23]

All **MON** derivatives were readily purified chromatographically on a silica gel column using CombiFlash®Rf+ (hexane/ethyl acetate, increasing concentration gradient) with an integrated Evaporative Light Scattering Detector (ELSD), and their structures and purity were determined by ESI- MS, FT-IR, ^{1}H, and ^{13}C NMR methods.

^{1}H NMR and ^{13}C NMR spectra of the synthesized derivatives **1-11** can be found in the Supplementary Materials. In the ^{13}C NMR spectra of urethanes **1**–**8**, the chemical shifts of the C-27 atom of the urethane group were observed in the range of 152.4–157.9 ppm, depending on the substituent, and the signals of the C-1 atom in the carboxylic group were observed in the range of 177.3–179.7 ppm. In the spectrum of compound **8**, the chemical shift of the C-28 atom was observed at 172.1 ppm. On the other hand, the most characteristic signals of the C-27 atom of the ester group in compounds **9-11**, were observed in the range of 166.8–174.0 ppm, while the signals of the C-1 atom in the carboxylic group were observed in a narrower range of 177.0–178.0 ppm.

According to the ESI-MS spectra, all **MON** derivatives showed the ability to form complexes with sodium cations in the 1:1 stoichiometry (see Supplementary Materials). The ESI-MS studies confirmed that **MON** urethane and ester derivatives preserved the ionophoretic properties and were still able to complex metal cations.

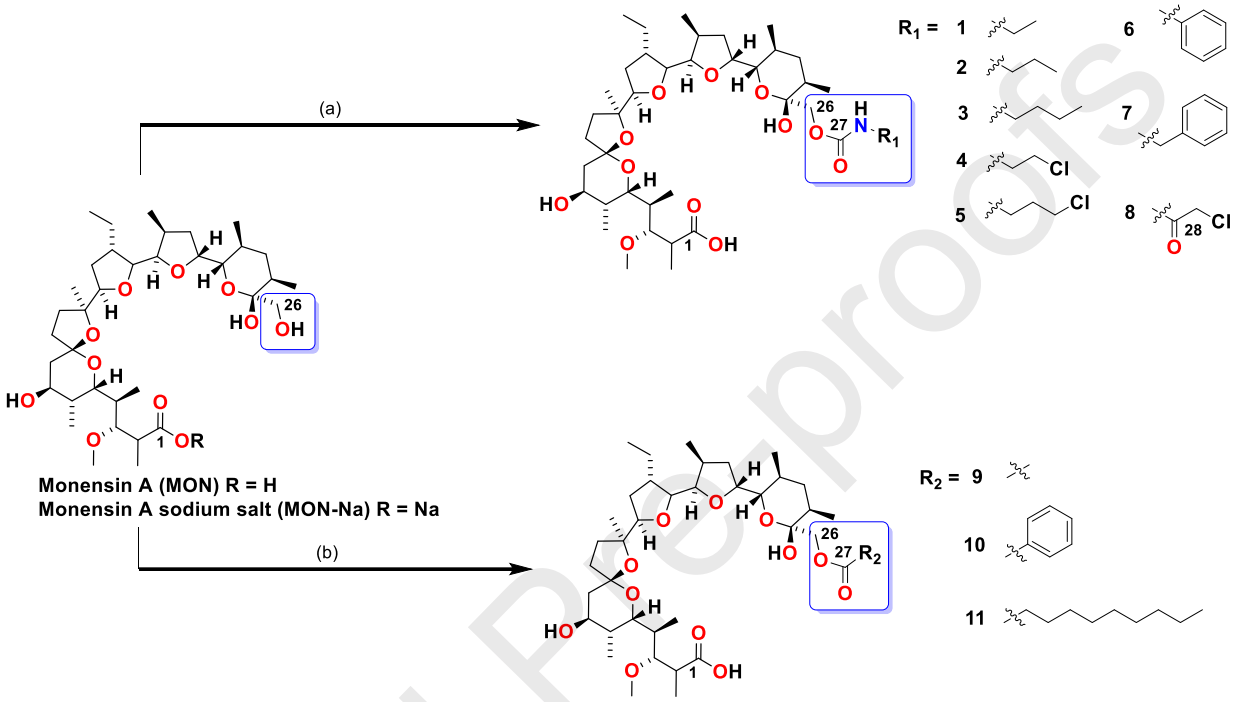

Figure 1. Reactions and conditions: (a) MON-H (1 eq), respective isocyanate (0.95 eq), anhydrous toluene, rt, 14 days; (b) MON-Na (1 eq), DMAP (catalytic amount), acyl chloride (3 eq), pyridine, rt, 48 hr. For yields of isolated and purified products see SupplementaryMaterials.[21,23]

In the present study, we tested the *in vitro* antibacterial activity of **MON** and its derivatives (**1–11**) against several strains of the Gram-positive bacteria *S. aureus* and *S. epidermidis*, and of the Gram-negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa*, respectively. The results are presented as minimal inhibitory concentration (MIC) in **Table 1**, based on the protocol described in the Supplementary Materials.

Table 1. Minimal inhibitory concentration (MIC) of **MON** and its derivatives on different strains of Gram-positive and Gram-negative bacteria.

	Minimal inhibitory concentration [µg/ml]												
	MON	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**Cipro**
***S. aureus* NCTC 4163**	8	8	2	1	2	2	0.25	1	2	2	1	2	0.25
***S. aureus* ATCC 25923**	2	4	2	1	2	2	0.25	1	1	1	1	1	0.5
***S. aureus* ATCC 6538**	4	8	2	1	4	2	0.25	1	2	2	1	2	0.125
***S. aureus* ATCC 29213**	4	8	4	2	2	4	0.25	2	2	2	1	2	0.125
***S. epidermidis* ATCC 12228**	4	8	4	1	2	4	0.25	1	2	1	1	2	0.5
***S. epidermidis* ATCC 35984**	8	16	8	1	8	4	0.50	2	2	2	1	2	0.25
***E. coli* NCTC 10538**	>256	>256	>256	>256	>256	>256	>256	>256	>256	>256	>256	>256	0.125
***E. coli* ATCC 25922**	>256	>256	>256	>256	>256	>256	>256	>256	>256	>256	>256	>256	0.125
***P. aeruginosa* ATCC 15442**	>256	>256	>256	>256	>256	>256	>256	>256	>256	>256	>256	>256	<0.125
***P. aeruginosa* NCTC 27853**	>256	>256	>256	>256	>256	>256	>256	>256	>256	>256	>256	>256	<0.125

Table 2. Minimal inhibitory concentration (MIC) of **MON** and its derivatives in comparison to that of the reference antibiotic ciprofloxacin on planktonic cells of various methicillin-resistant *S. epidermidis* clinical strains.

	Minimal inhibitory concentration [µg/ml]												
	MON	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**Cipro**
***S. epidermidis* 825/19**	0.25	8	2	1	2	2	0.25	1	8	0.25	0.50	1	4
***S. epidermidis* 829/19**	2	8	4	1	4	4	0.25	2	16	1	2	2	≤0.125
***S. epidermidis* 830/19**	1	8	4	1	4	4	0.25	2	16	1	1	2	64
***S. epidermidis* 834/19**	1	4	4	2	4	4	0.50	2	8	1	1	2	≤0.125
***S. epidermidis* 840/19**	4	8	4	2	4	8	0.50	2	16	2	2	2	≤0.125
***S. epidermidis* 845/19**	4	8	4	2	4	4	0.50	2	32	2	2	2	16
***S. epidermidis* 848/19**	2	16	4	2	4	8	0.50	2	16	1	2	2	≤0.125
***S. epidermidis* 851/19**	2	16	4	2	4	4	0.50	2	16	2	1	2	≤0.125

The majority of the tested compounds showed higher or similar antimicrobial activity against Gram-positive strains compared to **MON**. Only one derivative (**1**) showed lower activity in comparison to that of the unmodified **MON**. It is worth noticing that one of the urethanes, (**6**) exhibited a significant activity against all the strains of *S. aureus* and *S. epidermidis* , characterized by the MIC values of 0.25 – 0.50 µg/ml, which were up to 32-times lower, when compared to that of the unmodified parent compound. For the other compounds, the MIC values ranged between 1 and 8 µg/ml.

Against Gram-negative bacteria, **MON** and its derivatives appeared to be inactive (**Table 1**), which is most likely due to the fact that their cell walls are not permeable to large and hydrophobic molecules such as those of ionophore antibiotics.[29]

Furthermore, **MON** and the newly synthesized compounds were also tested against eight different methicillin-resistant clinical strains of *S. epidermidis* (MRSE) (**Table 2**).

Ciprofloxacin (Cipro), used for treatment of a variety of bacterial infections, was assumed as a reference. As for the non-methicillin-resistant strains, the phenyl urethane **6** was the most active compound. Its activity was described by MIC values lower than that of **MON** for most strains, and for some strains (825/19, 830/19 and 845/19) it was also up to 256-times more effective than ciprofloxacin. The other derivatives showed the activities equal to or lower than that of the unmodified **MON**. The least active **MON** derivatives were compounds **1** and **8**.

In both tests, the highest antibacterial activity was observed for phenyl urethane **6**. It is probably due to the aromatic substituent, increasing the solubility of this monensin urethane in the bacterial cell membrane, which in turn facilitates the ion transport into the intracellular environment.

Table 3. GI_{50} and MIC values and ratios of monensin derivatives for *T. brucei* and HL-60 cells.

	T. brucei		***HL-60 cells***		***Selectivity***	
	MIC (μM)	GI_{50} (μM)	MIC (μM)	GI_{50} (μM)	MIC (μM) ratio	GI_{50} (μM) ratio
MON	0.01	0.0027	10	1.78	1000	659
1	10	3.05	100	85.8	10	28.1
2	10	2.94	100	24.5	10	8.3
3	10	0.47	100	11.4	19	24.3
4	10	3.48	100	22.4	10	6.4
5	10	3.05	100	18.1	10	5.9
6	1	0.27	100	8.94	100	33.1
7	1	0.31	100	19.5	100	62.9
8	0.1	0.0047	10	3.51	100	746
9	1	0.29	10	5.51	10	19.0
10	10	2.62	100	18.3	10	7.0
11	1	0.038	100	10.0	100	263
suramin	1	0.05	>100	>100	>100	>2000

It should also be pointed out that the cytotoxicity of phenyl urethane **6** is 10 times lower / albo weaker/ than that of **MON**. When determining the cytotoxicity using human HL-60 cells, **MON** was characterized by a MIC value of 10 μM (6.7 μg/ml) while the MIC value

obtained for **6** was 100 μM (77.6 μg/ml) (**Table 3**). This shows that it is possible to increase the antibacterial activity with simultaneous reduction of the general cytotoxicity of **MON** by chemical modification.

The trypanocidal activities of the **MON** derivatives were tested *in vitro* with bloodstream forms of *T. brucei* using the resazurin cell viability assay described previously.[31] Although all **MON** derivatives displayed trypanocidal activity, they were less potent than the unmodified **MON** (**Table 3**). The aliphatic urethanes **1-5** were between 100 to 1000-fold less active, while the aromatic urethanes **6** and **7** were about 100-fold less potent than **MON**, which 50% growth inhibition (GI_{50}) value and MIC value was 2.7 nM and 10 nM, respectively (**Table 3**). Only urethane **8** exhibited potent trypanocidal activity characterized by a GI_{50} value (4.7 nM) similar to that of **MON** and a MIC value of 100 nM (**Table 3**). The three esters **9-11** were found to be 10 to 1000-fold less trypanocidal than the unmodified **MON**. The reason why derivatization of **MON** did not result in compounds with increased antitrypanosomal activity lies probably in the fact that this ionophore already displays strong trypanocidal potency surpassing that of the commercial drug suramin (**Table 3**) and which might be difficult to improve further by chemical modification. A similar observation has been previously made for C1 **MON** esters.[22] Although the general cytotoxicity of all derivatives was lower than the general cytotoxicity of **MON**, the selectivity indices of the derivatives were not improve compared to **MON** (**Table 3**). Only the urethane **8** had a marginal better GI_{50} ratio than **MON** (**Table 3**).

To summarize, we synthesized eleven derivatives of **MON** modified at the C-26 position - **MON** urethanes **1-8** and **MON** esters **9-11**. All of the compounds were tested against typical Gram-positive and Gram-negative bacteria and against the protozoan parasite *T. brucei*. All of the **MON** derivatives exhibited average to good *in vitro* antibacterial activity against Gram-

positive species. This study has shown that a phenyl moiety, at the C-26 position in particular, increases the antibacterial activity. However, modification at the C-26 position did not result in derivatives with enhanced antitrypanosomal activity compared to that of the unmodified parent compound **MON**.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary Materials

Supplementary Materials to this article can be found online at…

1. Antimicrobial resistance | European Medicines Agency. https://www.ema.europa.eu/en/human-regulatory/overview/public-health-threats/antimicrobial-resistance

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