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Synthesis and Antibacterial *in Vitro* Activity of Novel Analogues of Nematophin

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Abstract : The synthesis and *in vitro* antibacterial activity of new derivatives and analogues of nematophin are described. It was shown that the unsubstituted amide NH-group is essential for bioactivity. Alkyl- or aryl-substitution at the 1-position results in a distinct increase of antibacterial activity. Addition of protein (blood or serum) to the culture media reduces the inhibitory activity on bacteria.

Recently the novel antibiotic named nematophin **1** and its antibacterial activity were described.[1,2] The authors showed that **1** has a strong activity against *Staphylococcus spp.* They synthesized analogues with different side chains of the acid part (like compound **2**) and described the significant effects on the bioactivity. Other compounds with absence of the α-carbonyl acyl group like **3** or **4** showed no bioactivity, demonstrating the essential role of this structural element. Replacement of the α-keto amide function with an α-keto ester group gave compound **5** with remarkably lower antibacterial activity.[2]

Continuously increasing numbers of multidrug resistant bacteria[3,4,5] underline the urgent need for new classes of antibacterials, providing new modes of action. Nematophin represents the prototype of such a new class. Synthesis of new derivatives of nematophin and structural analogues could yield new drugs with an improved antibacterial profile. In this paper we report the synthesis of several new compounds with structural changes at the amide group or the indole ring, their antibacterial activity in comparison to nematophin, and some new structure-activity relationships.

Nematophin **1** and the isomer **2** were synthesized as the racemic compounds from tryptamine and 3-methyl-2-oxo-pentanoic acid chloride or 4-methyl-2-oxo-pentanoic acid according to the literature procedures.[2,6] The α-methyl and the N-ω-methyl derivatives **6** and **7** were synthesized by the same method. 1-Methyl-tryptamine was prepared starting with commercially available 1-methylindole-3-carbaldehyde[7] and reacted with 3-methyl- or 4-methyl-2-oxo-pentanoic acid yielding the 1-methyl analogue of nematophin **8**, and **9**. The synthesis of the other 1-substituted compounds **10** - **14** started with indole-3-carbaldehyde, which was reacted with 2-bromopropane,[8] benzyl chloride[8] or phenyl bromide,[9] respectively, to prepare the 1-substituted indole-3-carbaldehydes. The condensation of these aldehydes with nitromethane in the presence of ammonium acetate afforded the nitrovinyl derivatives,[8] which were reduced with lithium aluminium hydride to the 1-substituted tryptamines.[8,10]

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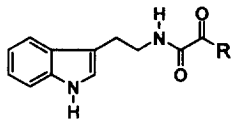
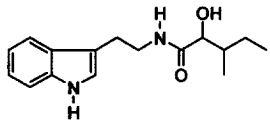


R = $CH(CH_3)CH_2CH_3$: **1** (racemic nematophin)
R = $CH_2CH(CH_3)CH_3$: **2**



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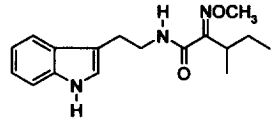

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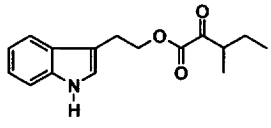

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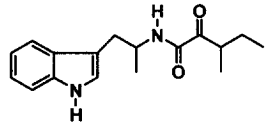
Figure 1: Structures of nematophin **1**, the isomer **2**, the ester analogue **5** and compounds lacking the α-carbonyl acyl group **3**, **4**.[2]



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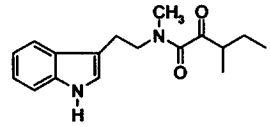

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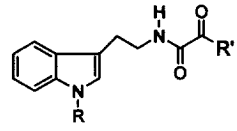
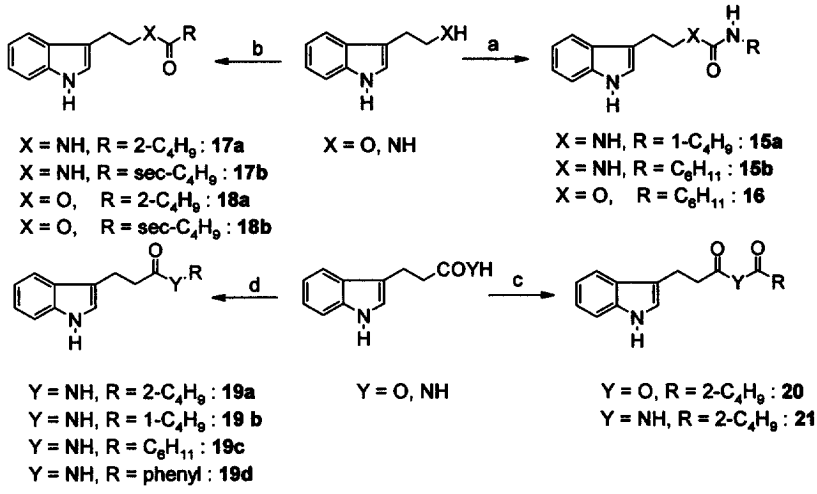


8 - 14

R = CH_3,	R' = $CH(CH_3)CH_2CH_3$	: **8**
R = CH_3,	R' = $CH_2CH(CH_3)CH_3$	: **9**
R = $CH(CH_3)_2$,	R' = $CH(CH_3)CH_2CH_3$	: **10**
R = $CH(CH_3)_2$,	R' = $CH_2CH(CH_3)CH_3$	: **11**
R = CH_2Ph,	R' = $CH(CH_3)CH_2CH_3$	: **12**
R = CH_2Ph,	R' = $CH_2CH(CH_3)CH_3$	: **13**
R = Ph,	R' = $CH_2CH(CH_3)CH_3$	: **14**

Figure 2: Structures of new derivatives of nematophin

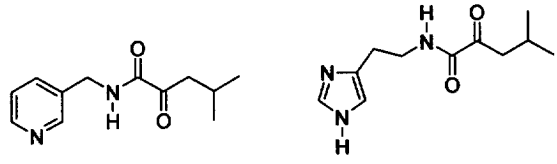
Reaction of tryptamine or tryptophol with alkyl isocyanates gave the ureas **15** and the carbamic acid ester **16**, respectively. In addition, the simple amides **17** and esters **18** were prepared starting with tryptamine and tryptophol, respectively. Reaction of 3-(3-indolyl)-propionic acid with amines or carboxylic acid chlorides gave the amides **19** and the anhydride **20**, respectively. The imide **21** was prepared from 3-(3-indolyl)-propionic acid amide and 2-methyl-butanoic acid chloride (Scheme 1).



a) R-NCO, Me_2CO, reflux; b) R-COCl, pyridine, room temperature; c) R-COCl, NEt_3 or pyridine, reflux; d) R-NH_2, NEt_3, THF, $NCP(O)(OEt)_2$, room temperature.

Scheme 1: Synthesis of new analogues of nematophin

Two new monocyclic compounds (replacement of the indole ring by a pyridine with omission of a methylene group resulting a pyridin-3-ylmethyl-amide **22** or by a imidazole ring to give **23**) were also synthesized by standard methods (Figure 3).



22 **23**

Figure 3: Monocyclic analogues of nematophin

Minimal inhibitory concentrations (MIC) of the synthesized compounds were determined by the agar dilution method according to NCCLS guidelines using iso sensitest agar.[11] As protein binding can influence the efficacy of antimicrobials MICs were also determined with supplementation of protein (sheep blood or

horse serum in amounts of 0.5% or 5% of the agar). Compounds **15**, **16**, **17**, **18**, **19**, **20** and **21** were completely inactive with MIC > 128 μg/ml.

Table 1: Minimum Inhibitory Concentrations (MIC) of nematophin **1** and derivatives on different strains of *Staphylococcus spp.*[12] in iso sensitest agar

	Staph. aureus			*Staph. hyicus*		*Staph. intermedius*	
Compound	ATCC 6538	ATCC 25923	ATCC 29213	9621	9622	ATCC 29663	9503
1	0,25	0,125	0,125	1	0,5	8	2
2	0,125	0,25	0,25	0,25	0,03	2	1
6	1	nt[a]	nt	1	0,5	nt	0,5
8	0,015	0,015	0,015	0,06	0,06	2	0,5
9	0,03	0,03	0,03	0,03	0,03	2	0,5
10	0,03	0,03	0,03	1	0,5	4	2
11	0,125	0,06	0,25	0,06	0,25	2	1
12	0,125	0,125	0,125	1	1	8	2
13	1	2	2	1	2	>64	2
14	0,03	0,06	0,06	0,06	0,015	1	0,125

a) nt = not tested

Table 2 : Minimum Inhibitory Concentrations (MIC) of nematophin **1** and derivatives on different strains of *Staphylococcus spp.* in iso sensitest agar with addition of blood or serum

	Staph. aureus			*Staph. hyicus*		*Staph. intermedius*	
Compound	ATCC 6538	ATCC 25923	ATCC 29213	9621	9622	ATCC 29663	9503
1							
0,5% blood	>128	nt[a]	nt	0,25	0,125	nt	1
5% blood	>128	nt	nt	>128	>128	nt	>128
0,5% serum	>128	nt	nt	>128	1	nt	8
5% serum	>64	4	4	>64	>64	16	16
8							
0,5% blood	0,06	nt	nt	0,03	0,03	nt	0,06
5% blood	>128	nt	nt	>128	>128	nt	>128
0,5% serum	0,25	nt	nt	0,25	0,125	nt	2
5% serum	>32	16	16	>32	>32	32	>32
9							
5% serum	>64	16	16	>64	>64	16	32
10							
5% serum	>32	16	8	>32	>32	>32	>32
11							
5% serum	>64	32	32	>64	>64	32	64
12							
5% serum	>64	16	16	>64	>64	>64	>64
13							
5% serum	>64	>64	>64	>64	>64	>64	>64
14							
5% serum	>64	>64	>64	>64	>64	>64	>64

a) nt = not tested

These results confirm the essential role of the α-keto amide group for antibacterial activity: replacement of the keto moiety by CHMe or CH_2 in **17** or by NH in **15** results in total loss of bioactivity. The imide **21**, representing the retro-amide analogue of nematophin (CONHCO instead of NHCOCO), is also devoid of activity. The monocyclic compounds **22** and **23** are also completely inactive.

The MICs of the compounds **6** - **14** in comparison with **1** and **2** are of special interest. Whereas the α-methyl substitution in **6** (mixture of stereoisomers) has almost no effect on the MIC, the N-methyl substitution in **7** results in a complete loss of activity (MIC > 128 μg/ml). This indicates that in addition to the α-keto functionality, the secondary amide group (NH) is essential for antibacterial activity. The N-substitution in the indole ring gives the compounds **8** - **14** with a substantially increased activity. The compound with the best activity is **8** - the 1-methyl derivative of nematophin (MICs up to fourfold lower than those of **1**). The 1-isopropyl derivatives **10** and **11** show a little bit weaker activity, but are still better in comparison to nematophin, whereas the antibacterial activity of the compounds **12** and **13** with a benzyl group at the 1-position is in the range of **1**. The 1-phenyl compound **14** on the other hand shows a much better efficacy. The results are summarized in Table 1.

We found a strong dependence of MICs on the medium used for the determination of inhibitory effects. The supplementation of protein resulted in a strong decrease of activity at an amount of 5% with all compounds tested (see Table 2). The reasons for this behavior remain unclear at the present time. Possible explanations could be strong protein binding, or a cleavage at the α-keto amide moiety.

In conclusion, the results of our study confirmed the importance of the α-keto amide functionality for antistaphylococcal activity. In addition, we found that substitution of the amide NH-group results in total loss of activity. This gives rise to the hypothesis that a hydrogen bond involving this NH-group is important for binding the compound in the active site. Alternatively the NH-group could be essential for penetration into the bacterial cell. N-Alkylation or arylation at the indole ring gives derivatives with much better antibacterial activity. Addition of blood or serum to the culture media resulted in strong decrease or even loss of in vitro antibacterial activity. Studies regarding the consequences of these findings are in progress.

References and Notes

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