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Studies on the Novel Anti-staphyloccal Compound Nematophin

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Abstract—A number of analogues of the recently described compound nematophin were prepared and studied for antibacterial activity. The 2-phenyl derivative was found to exhibit exceptional activity against methicillin resistant *Staphylococcus aureus* (MRSA) whereas the isosteric benzimidazole analogue was much less active. © 2000 Elsevier Science Ltd. All rights reserved.

Introduction

The alarming rise in the incidence of nosocomial acquired infections caused by antibacterial resistant organisms has become a cause for increasing concern over the last few years. It has been estimated that at least half of the approximately two million patients who acquire infections within the hospital environment are infected with bacteria resistant to most current antibiotic treatments.[1] Bacteraemia caused by the Gram positive organism *Staphylococcus aureus* is a significant cause of morbidity in intensive care units and there is a real need for the identification of both novel essential targets within bacteria, as well as new agents capable of combatting these pathogens.[2,3]

Nematophin, **1**, is a novel antibacterial compound isolated from *Xenorhabdus nematophilus*, a bacterial symbiont of a nematode (Fig. 1). The compound has only recently been described by a Canadian group[4,5] and some preliminary SAR work has been published by them and a group of researchers at the animal health division of Bayer AG.[6] The available published data indicate that this compound has potential as an anti-MRSA agent as it is very active against both wild type and methicillin resistant strains of *S. aureus* (MRSA), although the compound apparently loses antibacterial activity in the presence of serum in vitro. The mechanism of action of nematophin is not known.

The work of the Bayer group has established that substitution of the indole by a smaller heterocycle such as pyridine or imidazole leads to inactive compounds, however, no data is available for scaffolds which are known to be (bio)isosteric with indoles. They have also demonstrated the requirement for the α-ketoamide moiety within the molecule and that the indole hydrogen can be replaced by either an aryl, alkyl or benzyl group (e.g., **2**) with concomitant increase in in vitro anti-staphylococcal activity in the absence of serum.

Results and Discussion

The parent nematophin and the *N*-benzyl derivative **2** were prepared as standards according to the Bayer reference. The Suzuki cross-coupling reaction has recently been applied to *N*-phthalimide protected 2-bromotryptamine for the synthesis of biologically active molecules and was used here to introduce aryl groups as shown in Scheme 1.[7] Bromination of the indole nucleus with pyridinium tribromide required careful monitoring by HPLC to ensure a good yield of the monobromo compound and to avoid over-halogenation. The 2-(3-pyridyl) derivative **5b** was quaternized with both iodomethane and iodoacetamide to give the compounds **6a** and **6b** containing charged substituents.

The benzimidazole nucleus represents a readily accessible heterocyclic system which is isosteric with the indole ring. The direct alkylation of either benzimidazole or 2-phenylbenzimidazole with *N*-(2-bromoethyl) phthalimide using standard alkylation conditions (e.g., NaH/DMF or K_2CO_3/DMF) was not successful therefore

*This paper is dedicated to the memory of John Alexander Winders 9.12.59–12.4.99

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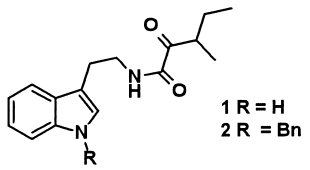
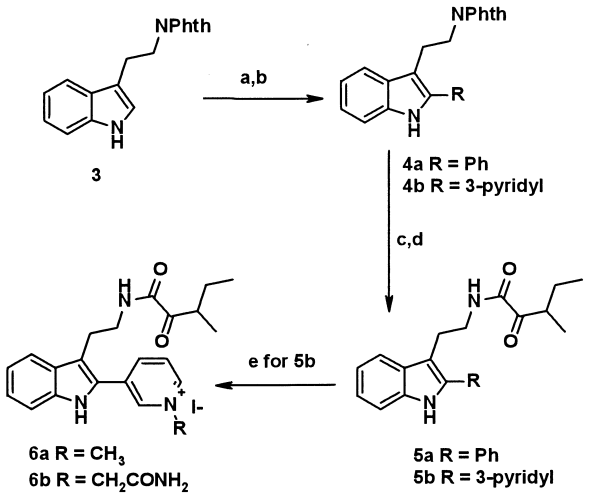


Figure 1. Nematophin (1) and its *N*-benzyl analogue (**2**).

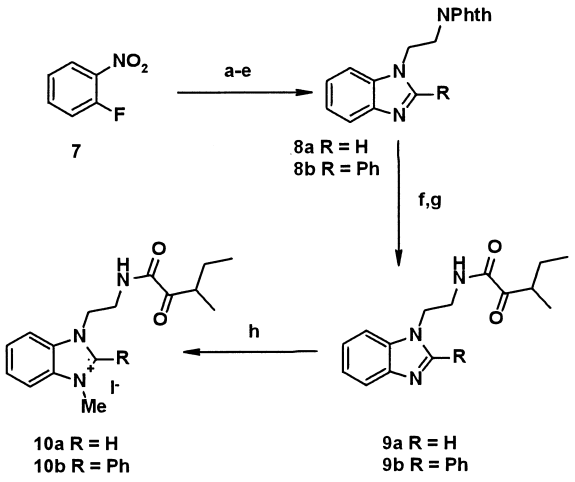


Scheme 1. Reagents: (a) pyridinium tribromide/THF/0 °C; (b) $Pd(PPh_3)_4$/LiCl/2 M Na_2CO_3/phenylboronic acid or 3-pyridylboronic acid 1,3-propanediol cyclic ester/EtOH/toluene; (c) 2 M Me_2NH in MeOH; (d) (*rac*)-3-methyl-2-oxovaleric acid/HOBT/EDCI/DMF/ overnight; (e) **6a**: MeI/CH_2Cl_2/rt; **6b**: ICH_2CONH_2/CH_2Cl_2/rt.

these analogues were prepared by the less direct route shown in Scheme 2.

The reaction of mono-Boc-protected ethylenediamine with 2-fluoronitrobenzene is known[8] and was followed by switching of the protecting group to phthalimide. The nitro compound was easily reduced using tin (II) chloride in DMF, although the reaction was somewhat sluggish.[9] The intermediate diamine was cyclised through reaction with trimethylorthoformate or triethylorthobenzoate in the presence of acid to form the benzimidazoles **8a** and **8b**, respectively.[10] In the latter case, the product recrystallised directly from the reaction medium. The phthalimide protecting group was removed and the amines coupled with 3-methyl-2-oxovaleric acid to give the benzimidazole analogues **9a** and **9b**. Methylation of these gave the charged benzimidazolium species **10a** and **10b**, respectively.

The antibacterial activities of the compounds prepared along with those of the two standards **1** and **2** are collected in Table 1. The minimum inhibitory concentrations (MIC) of the compounds were measured against the methicillin sensitive organism *S. aureus* 853 and the methicillin-resistant organism *S. aureus* COL,[11] as well as *Bacillus subtilis* to determine if the activity observed extended to non-staphylococcal bacteria. It is noteworthy that the MIC values of **1** and **2** against the MRSA organism *S. aureus* COL are much higher than



Scheme 2. Reagents: (a) *N*-Boc ethylenediamine/Et_3N/toluene/reflux/8 h; (b) TFA/CH_2Cl_2/ reflux (c) *N*-carboethoxyphthalimide/Et_3N/THF/ reflux/24 h; (d) $SnCl_2 2H_2O$/DMF/60 °C/12 h; (e) **8a**: triethylorthoformate/H^+/toluene/100 °C/1 h; for **8b** triethyl orthobenzoate/H^+/toluene/100 °C/1 h; (f) Me_2NH 2.0 M in MeOH; (g) (*rac*)-3-methyl-2-oxovaleric acid/HOBT/EDCI/DMF/ overnight; (h) MeI/CH_2Cl_2.

Table 1. Miniminum inhibitory concentrations (μg ml^{-1}) against Gram positive bacteria for compounds described in the text[a]

Compound[b]	*Staphylococcus aureus* 853	*Staphylococcus aureus* COL	*Bacillus subtilis*
1	2	16	>32
2	2	>32	>32
5a	<0.03	0.06	8
5b	0.5	4	>32
6a	>32	>32	>32
6b	>32	>32	>32
9a	>32	>32	>32
9b	8	16	>32
10a	>32	>32	>32
10b	>32	>32	>32

[a]Measured according to the standard method described in ref 13.
[b]Spectroscopic data for compounds **2**–**10b** is provided in note[17].

those against the sensitive strain and no activity was observed for either compound against *B. subtilis*. This observation contrasts with the reported literature values and deserves comment. First of all, the previously described values were measured in clinical isolates of MRSA which exhibit different phenotypes to the well-characterised strain used routinely in this work and secondly, the MIC measurements were taken using the serial broth dilution method, whereas previous workers report MICs obtained using the agar method.

Introduction of a phenyl substituent in the 2-position of the indole (**5a**) afforded considerable gains in activity with respect to the parent nematophin. This compound displayed an MIC at the lower limit of detection against *S. aureus* 853 and 8-dilutions lower than the parent against *S. aureus* COL. This gain in activity was reflected in the 3-pyridyl compound **5b** although not to quite the same levels. Interestingly, the 2-phenyl analogue was the only compound prepared that also displayed activity,

albeit modest, against the Gram positive organism *B. subtilis*. The incompatibility of charge with maintenance of activity is most clearly demonstrated in this series: the methylpyridinium compound **6a** being devoid of activity against both *S. aureus* and *B. subtilis*. Neither **5a** nor **5b** showed signs of cytotoxicity at 10 μg ml^{-1} (data not shown).[12]

The benzimidazole analogue of nematophin **9a** proved to be completely inactive showing that the benzimidazole ring cannot be considered to be *bio*-isosteric with an indole in this system and that factors more subtle than simple shape are at play here. Interestingly, the 2-phenyl analogue **9b** displayed some activity against both *S. aureus* 853 and *S. aureus* COL, although the MIC values are not comparable with those obtained for the corresponding 2-phenyl indole derivative **5a**. This shows that the presence of an aryl ring in this position is sufficient to overcome some of the negative characteristics of the benzimidazole nucleus. As with the pyridinium species **6a** described above, the presence of charge in the compounds **10a** and **10b** was not compatible with antibacterial activity.

The parent compound is known to lose activity in the presence of serum and therefore the MIC values of the compounds were also measured in the presence of 5% bovine serum (data not shown). None of the compounds prepared, including the charged pyridinium and the benzimidazolium derivatives were active in the presence of serum, despite several well-documented examples of the use of charged groups as modifiers of excessive serum protein binding.[14–16] This result indicates that the loss of activity in the presence of serum is a general property of the class.

Conclusions

The biological data obtained show that this class is probably not suited to systemic administration but that such agents could be considered for topical use. The restricted spectrum of activity of these compounds which are apparently only active against some strains of *S. aureus* (with some weak sign of activity against other Gram positive bacteria) is a limiting factor for the class, even though such narrow spectrum agents may become more important in the medium-term with the advent of rapid and accurate diagnostics.

The surprising level of activity seen with the 2-phenyl derivative **5a**, as well as the apparent ability of the phenyl group in the 2-position to allow a recovery of activity in the otherwise inactive benzimidazole series, points to quite specific substituent effects possibly related to enhanced interaction with the target. This could warrant further exploration. It would appear that these compounds are optimally active against a target (or targets) which are present in *Staphylococcus* sp., although there are considerable differences between strains which could reflect structural differences at the level of the target or strain dependent expression of the target.

Acknowledgements

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17. Selected compound data. Note: all compounds containing chiral centres were prepared as racemates.

Compound 2: waxy solid; IR (film) cm^{-1} 3410, 1714, 1685, 1517, 1464, 1451; ^1H NMR (400 MHz, $CDCl_3$) δ 0.89 (3H, t), 1.09 (3H, d), 1.39 (1H, m), 1.79 (1H, m), 3.03 (2H, t), 3.49 (1H, m), 3.64 (2H, m), 5.29 (2H, s), 6.97, (1H, s), 7.06 (1H, s), 7.13 (3H, m), 7.21, (1H, m), 7.26–7.34 (2H, m), 7.62 (1H, bd) ES–MS *m*/*z* 363 $(MH)^+$ 234 $(M\text{-}C_6H_{10}NO_2)^+$.

Compound 5a (±)-***N*-[2-(2-phenyl-1*H*-indol-3-yl)ethyl]-3-methyl-2-oxo-pentanamide**: colourless oil; IR (film) cm^{-1} 3393–3326, 1714–1671, 1457; ^1H NMR (400 MHz, $CDCl_3$) δ 0.88 (3H, t), 1.05 (3H, d), 1.37 (1H, m), 1.69 (1H, m), 3.18 (2H, t), 3.44 (1H, m), 3.64 (2H, t), 7.03 (1H, bt), 7.18–7.26 (2H, m), 7.40 (1H, t), 7.42 (1H, d), 7.49 (2H, t), 7.57 (2H, d), 7.67 (1H, d), 8.13 (1H, bs), ES–MS *m*/*z* 220 $(M-NHCOCOCH(CH_3)C_2H_5)^+$.

Compound 5b (±)-***N*-[2-(2-(3-pyridyl)-1*H*-indol-3-yl) ethyl]-3-methyl-2-oxo-pentanamide**; pale yellow gum; IR (nujol) cm^{-1} 1720, 1660; ^1H NMR (400 MHz, $CDCl_3$) δ 0.86 (3H, t), 1.05 (3H, d), 1.38 (1H, m), 1.69 (1H, m), 3.14 (2H, t), 3.44 (1H, m), 3.64 (2H, t), 7.19 (1H, m), 7.26 (1H, t) (+solvent), 7.44 (2H, m), 7.7 (1H, m), 7.94 (1H, m), 8.44 (1H, bs), 8.59 (1H, dd), 8.87 (1H, d); ES–MS *m*/*z* 220 $(M-NHCOCOCH(CH_3)C_2H_5)^+$ 100%.

Compound 6a (±)-***N*-[2-(2-(3-*N*-methylpyridinium)-1*H*-indol-3-yl)ethyl]-3-methyl-2-oxo-pentanamide iodide**. Yellow solid (mp 103–104 °C softens); IR (nujol) cm^{-1} 3383, 3178, 1668; ^1H NMR (400 MHz, $CDCl_3$) δ 0.89 (3H, t), 1.09 (3H, d), 1.37 (1H, m), 1.69 (1H, m), 3.13 (2H, t), 3.44 (1H, m), 3.54 (2H, t), 4.60 (3H, s), 7.15 (1H, bt), 7.27 (2H, m), 7.57 (1H, d), 7.69 (1H,

d), 7.91 (1H, m), 8.32 (1H, d), 8.97 (1H, d), 9.87 (1H, s), 11.18 (1H, s); ES–MS m/z 364 $(M-I)^+$ 100%.

Compound 6b (±)-***N*-[2-(2-(3-*N*-(2-acetamido) pyridinium)-1*H*-indol-3-yl)ethyl]-3-methyl-2-oxo pentanamide iodide**. Yellow solid (mp 210–211 °C); IR (nujol) cm^{-1} 3320, 3120, 1690, 1670; ^1H NMR (400 MHz, DMSO) δ 0.79 (3H, t), 0.96 (3H, d), 1.29 (1H, m), 1.57 (1H, m), 3.10 (t, 2H,), 3.30 (m, 1H,), 3.39 (2H, t), 5.52 (2H, s), 7.11 (1H, t), 7.24 (1H, d), 7.45 (1H, d), 7.68 (1H, d), 7.74 (1H, s), 8.07 (1H, s), 8.30 (1H, d), 8.83 (1H, d), 8.85 (1H, t), 8.92 (1H, d), 9.26 (1H, s), 11.71 (1H, s); ES–MS m/z 407 $(M-I)^+$ 100%.

Compound 9a (±)-*N*-[2-(1-benzimidazolyl)ethyl]-3-methyl-2-oxo-pentanamide. White solid (mp 101–102 °C); IR (nujol) cm^{-1} 3417, 1717, 1689; ^1H NMR (400 MHz, $CDCl_3$) δ 0.88 (3H, t), 1.08 (3H, d), 1.39 (1H, m), 1.69 (1H, m), 3.44 (1H, m), 3.75 (2H, t), 4.41 (2H, t), 7.16 (1H, m), 7.32 (2H, m), 7.44 (1H, dd), 7.81 (1H, dd), 7.92 (1H, s); ES–MS m/z 274 $(MH)^+$.

Compound 9b (±)-***N*-[2-(2-phenyl-1-benzimidazolyl) ethyl]-3-methyl-2-oxo-pentanamide**. White solid (mp 115–116 °C); IR (nujol) cm^{-1} 3411, 1716, 1690; ^1H NMR (400MHz, $CDCl_3$) δ 0.45 (3H, t), 1.01 (3H, d), 1.31 (1H, m), 1.62 (1H, m), 3.31 (1H, m), 3.62 (2H, q), 4.48 (2H, t), 6.95 (1H, bt), 7.34 (2H, m), 7.51 (4H, m), 7.69 (2H, m), 7.84 (1H, m); ES–MS m/z 350 $(MH)^+$ 100%.

Compound 10a (±)-*N*-[2-(3-methyl-1*H*-benzimidazolium)ethyl]-3-methyl-2-oxo-pentanamide iodide. Pale yellow powder (mp 148–149 °C, softens); IR (nujol) cm^{-1} 3330, 1708, 1666; ^1H NMR (400 MHz, DMSO) δ) 0.70 (3H, t), 0.85 (3H, d), 1.17 (1H, m), 1.43 (1H, m), 3.11 (1H, m), 3.64 (2H, q), 4.07 (3H, s), 4.60 (2H, t), 7.67 (2H, m), 8.00 (1H, m), 8.82 (1H, t), 9.96 (1H, s); ES–MS m/z 288 $(M-I)^+$ 100%.

Compound 10b (±)-***N*-[2-(3-methyl-2-phenyl-1*H*-benzimidazolium)ethyl]-3-methyl-2-oxo-pentanamide iodide**. Off-white powder (softens ca. 105 °C); IR (nujol) cm^{-1} 3415, 1717, 1667; ^1H NMR (400 MHz, DMSO) δ 0.70 (3H, t), 0.82 (3H, d), 1.14 (1H, m), 1.40 (1H, m), 3.04 (1H, m), 3.42 (2H, q), 3.84 (3H, s), 4.45 (2H, t), 7.70–7.83 (7H, m), 8.06 (1H, m), 8.13 (1H, m), 8.71 (1H, t); ES–MS m/z 364 $(M-I)^+$ 100%.