Pergamon

Bioorganic & Medicinal Chemistry Letters 8 (1998) 1035–1040

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

PF1022A - A NOVEL ANTHELMINTIC CYCLOOCTADEPSIPEPTIDE. MODIFICATION AND EXCHANGE OF THE N-METHYL LEUCINE RESIDUES

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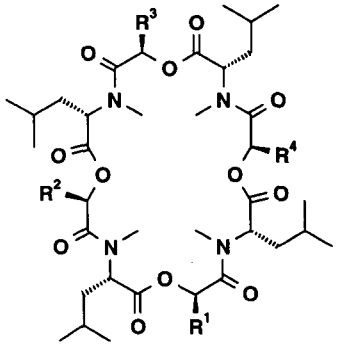
Received 17 December 1997; accepted 25 March 1998

Abstract: The first structure-activity relationships of the anthelmintic cyclooctadepsipeptide PF1022A have been established *via* a systematic exchange of the leucine residues by a series of related N-alkylated amino acids. The data presented strongly suggest that (L)-N-methyl-leucine is crucial for high *in vivo* activity. © 1998 Elsevier Science Ltd. All rights reserved.

Introduction

Plants, animals and humans are harmed to an immense extent by parasitic nematodes. It is estimated that about 1.300 million people are infected by *Ascaris lumbricoides* and nearly 1.000 million people by the hookworms *Ancylostoma duodenale* or *Necator americanus*.[1] Furthermore, nematodes cause enormous economic losses of crop plants and livestock.[1] A milestone in the chemotherapy of nematode infections, particularly in animals, was the discovery of the milbemycins[2] and the avermectins[3] during the 70's. Today these macrocyclic lactones are widely and intensively used in the control of gastrointestinal infections of livestock and companion animals.[4] The avermectins, however, are not without their problems, including increasing nematode resistance and slow degradation in the soil. Since the discovery of the highly active avermectins and milbemycins, reports of potent new classes of anthelmintics have been, to say the least, scarce.[5]

Figure 1.



1

	R^1	R^2	R^3	R^4
PF1022A (1)	Me	Bn	Me	Bn
PF1022B	Bn	Bn	Bn	Bn
PF1022C	Bn	Bn	Me	Bn
PF1022D	Me	Me	Me	Bn
PF1022E	Me	$CH_2C_6H_4OH$	Me	Bn
Bassianolide	^{i}Pr	^{i}Pr	^{i}Pr	^{i}Pr

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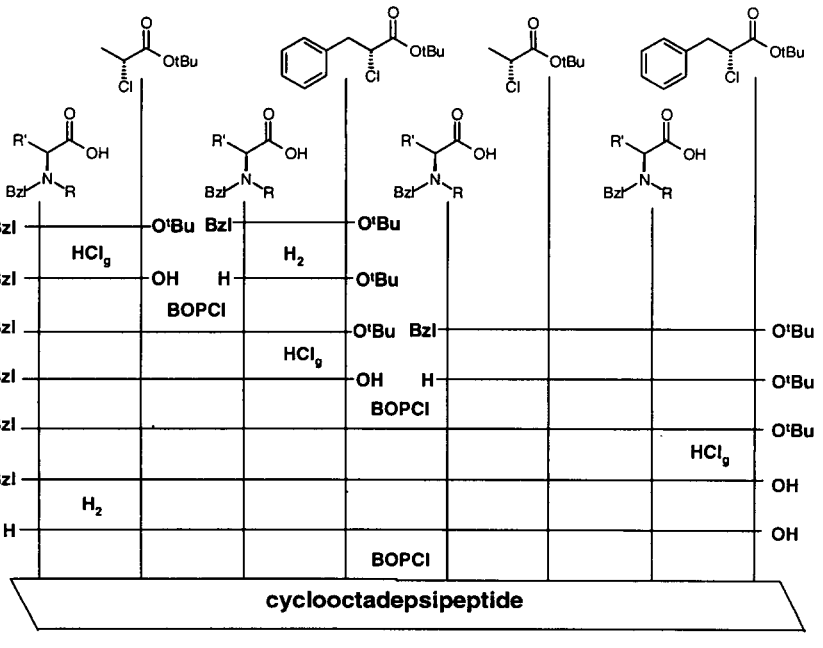
PII: S0960-894X(98)00156-5

One of the most outstanding anthelmintics, is the recently reported[6] cyclooctadepsipeptide PF1022A, the most active member of a new class of anthelmintic agents.[7] PF1022A was isolated from a mycelia cake of *Mycelia sterilia* belonging to the order Agonomycetales. *In vitro* motility of the intestinal nematode *Angiostrongylus cantonensis* was completely inhibited 2 hrs post treatment with 10^{-7} g/ml PF1022A.[8] Oral application of the compound in dogs infected with *Toxocara canis* or *Ancylostoma caninum* at a dosage of 1.0 mg/kg was completely efficacious, and worms were expelled very quickly from the host.

The potency and rapid action,[9] in combination with low toxicity, prompted us to further investigate the structure-activity relationships of PF1022A analogues. PF1022A consists of four *N*-methyl-(S)-leucine (MeLeu) residues, two 3-phenyl-(R)-lactate (PheLac) units and two (R)-lactate moieties linked together in a pattern giving the molecule a C_2-axis of symmetry. Initially, we focused our attention on the four N-methyl-leucine residues which, together with the phenyllactic acid units, impart on the molecule a unusually high lipophilicity. We considered the following points to be worthy of further investigation: i) the significance of the lipophilicity of PF1022A, ii) the specificity of the leucine residues, and iii) the significance of the C_2-symmetry as requirements for anthelmintic activity.

Thus, two series of compounds were synthesized. Firstly, the N-alkyl substituents on leucine were varied, affording increasingly more lipophilic derivatives. Here we planned to assess the effect of increasing lipophilicity on anthelmintic activity without necessarily adversely affecting specific structural features, i.e. the amino acid side chains. Secondly, the N-methyl substituent was kept constant and the leucine residues were successively exchanged for related lipophilic amino acids. We hoped that these two studies would provide us with clues as to the reasons behind the differing *in vitro* and *in vivo* results, reported in the literature.[10,11d]

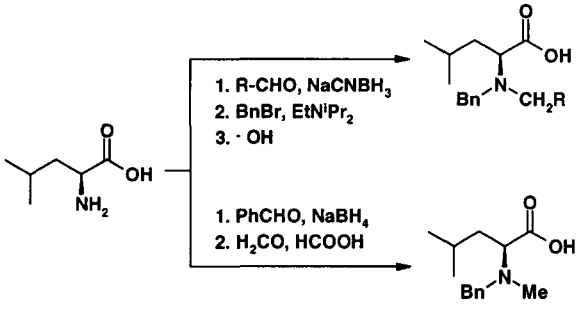
Scheme 1.



Chemistry

To date, several total syntheses of PF1022A have been reported.[11] The key feature of the most efficient synthesis[11a] is the highly efficient BOP-Cl (N,N'-bis(2-Oxo-3-oxazolidinyl)phosphinic chloride) mediated macrocyclisation to afford PF1022A in an excellent yield of 87%. Our syntheses were performed according to scheme 1,[11a] using *tert*-butyl esters and N-benzyl groups for our orthogonal protecting group strategy. Thus the cyclooctadepsipeptides were synthesized by a series of fragment condensations starting from the known (S)-2-chloropropanoic acid and (S)-2-chloro-3-phenylpropanoic acid.[12] The N-benzyl-N-methyl amino acids were prepared by standard literature procedures.[13] The N-alkyl-N-benzyl-leucines were synthesized from (L)-leucine by reductive alkylation with the corresponding aldehyde or ketone followed by alkylation with benzylbromide and subsequent hydrolysis of the benzyl esters (scheme 2). Noteworthy are the generally good-to-excellent yields of the BOP-Cl mediated lactamization reaction of the linear precursors leading to the 24 membered macrocycles. The ease of this peptide bond forming macrocyclisation may be attributed to the *cis*-amide rotamers in the linear precursors, resulting in an U-type conformation with the terminal amino- and carboxyl groups spatially close together. Even in the case of the very sterically hindered N-isopropyl analogue, the cyclisation could be accomplished in a 26% yield by refluxing the linear precursor in dioxane for 24 h.[14]

Scheme 2.



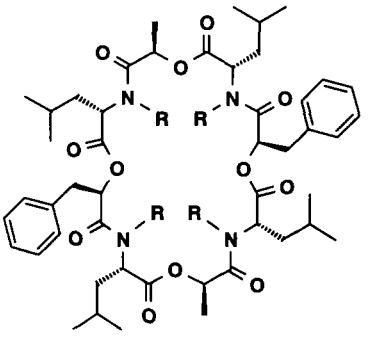
Biological Testing

Sheep (Merino or Schwarzkopf breed, 25-35 kg body weight) were infected experimentally with 5000 *Haemonchus contortus* L3 larvae and treated with the test substance after the end of the pre-patency period of the parasite. The test compounds were administered orally in gelatine capsules or intravenously, as recently described.[15] Anthelmintic effects of the test substances against *H. contortus* adults were measured as a function of the reduction in the faecal egg count. For the purpose of counting eggs, freshly obtained faeces from experimental animals were prepared using the McMaster method as modified by Wetzel and the egg count was calculated per gram of faeces.[16] The egg counts were determined at regular intervals before and after treatment. The anthelmintic evaluation is expressed as a function of the egg reduction as follows: 3 ≥ 90%, 2 = 75-90%, 1 = 50-75% and 0 ≤ 50% egg reduction.

Results and Discussion

The anthelmintic activities of the compounds **2** - **4**, in which the four N-methyl groups of PF1022A were successively exchanged for N-ethyl, N-propyl, and N-isopropyl groups, showed a significant dependance on their lipophilicities. The small change from the N-methyl to the N-ethyl group increased the log P value from 5.9 to 7.2. The anthelmintic activity of the N-ethyl derivative **2** was comparable to PF1022A at a dosage of 0.5 mg/kg, while the highly lipophilic N-propyl derivative **3** was somewhat less active, and compound **4** was virtually inactive even at a dosage of 1 mg/kg. A plausible explanation for this low anthelmintic activity is decreased bioavailability.

Table 1. Anthelmintic activities and lipophilicities of N-alkyl leucine PF1022A analogues



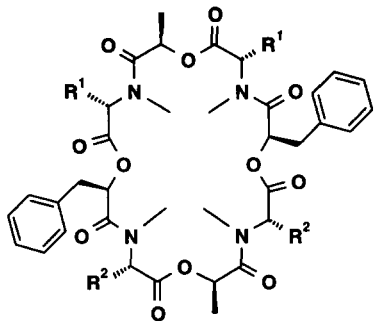
	R	cyclization yield[a] (%)	activity p.o. (mg/kg)	log P[c]
1	methyl	87	0.1 / 3[b]	5.9
2	ethyl	57	0.25 / 2-3	7.2
3	propyl	63	0.25 / 1	8.4
4	^{i}propyl	26	1 / 2	8.6

a) cyclisation conditions for the compounds 2, 3, and 5-11: CH_2Cl_2 (0.1%), 40°C, 6-8 h. Compound 4: Dioxane (0.1%), 100°C, 24 h. b) 3 ≥ 90%, 2 = 75-90%, 1 = 50-75%, 0 ≤ 50% reduction in faecal egg count. c) log P: partition coefficient n-octanol / water; determined by reversed phase HPLC (pH 7)

In the second series of analogues, the N-methyl-leucine residues were completely (entries **5** to **9**), or partially (entries **10** and **11**) exchanged for a series of related N-methylated amino acids, e.g. isoleucine (Ile), valine (Val), norvaline (Nva), alanine (Ala), and phenylalanine (Phe).[14] The log P value of the compounds varied in the range from 3.1 to 6.6. With the exception of the isoleucine and phenylalanine derivatives, the others in the series showed a reduced lipophilicity compared to PF1022A. Again, all the derivatives have been tested against the nematode *Haemonchus contortus* in sheep.

The exchange of leucine for isoleucine (**5**) resulted in a nearly complete loss of anthelmintic activity following oral administration and led to a decrease in activity by at least a factor of ten, when applied intravenously. The reasons for this effect remain somewhat unclear. The increased lipophilicity of **5** and / or a specific effect caused by reduced receptor binding of isoleucine might contribute to the attenuation of anthelmintic activity. On the other hand the valine (**6**) and norvaline (**7**) analogues with log P values lower than PF1022A also exhibited a remarkably reduced activity, and the phenylalanine analogue (**9**) with a log P value identical to that of PF1022A was inactive, even at a dosage of 5 mg/kg. These results identify the (L)-N-methyl-leucines of PF1022A as an important pharmacophore for *in vivo* anthelmintic activity.

Table 2. Anthelmintic activities and lipophilicities of N-methyl amino acid analogues of PF1022A



	R^1	R^2	cyclization yield (%)	activity (mg/kg) i.v.	activity (mg/kg) p.o.	log P[c]
1	^{i}Bu	^{i}Bu	87	0.1/3[b]	0.1/3[b]	5.9
5	^{s}Bu	^{s}Bu	94	1.0/3	5.0/0	6.6
6	^{i}Pr	^{i}Pr	93	1.0/3	0.25/1	5.5
7	^{n}Pr	^{n}Pr	72	0.5/0	n.d.	5.1
8	Me	Me	14[a]	0.5/0	n.d.	3.1
9	Bn	Bn	92	5.0/0	n.d.	5.9
10	^{i}Bu	Me	60	0.5/0	n.d.	4.6
11	^{i}Bu	^{n}Pr	72	0.5/3	0.1/0	5.5

a) losses during chromatographic separation.b) 3 ≥ 90%, 2 = 75-90%, 1 = 50-75%, 0 ≤ 50% reduction in faecal egg count. c) log P: partition coefficient n-octanol / water; determined by reversed phase HPLC (pH 7)

Considering the C_2-axis of symmetry of PF1022A, it might be assumed that only one half of the molecule is responsible for biological activity while the other half determines the conformation required for receptor binding. According to this hypothesis the replacement of the leucines in only one half of the molecule should not result in loss of the anthelmintic activity, as long as the global conformation remains unchanged. Based on this consideration the mixed PF1022 analogs **10** and **11**, were synthesized.[14] Surprisingly, both compounds exhibited significantly diminished activity, being complete inactive at a dosage of 0.1 mg/kg, respectively. ^{1}H NMR spectroscopic analysis in solution indicated only minor conformational differences of the synthetic analogues when compared to PF1022A (^{1}H NMR - ROESY experiments in $CDCl_3$ and CD_3CN / water). Molecular modeling studies did not reveal a correlation between conformation and biological activity.

In summary the first data with regard to structure-activity relationships of the anthelmintic cyclooctadepsipeptide PF1022A have been reported, based on a systematic exchange of the (L)-NMe-leucines by a series of related amino acids. A significant increase in lipophilicity, via the introduction of homologous N-alkyl groups is critical with respect to the bioavailability of the corresponding cyclooctadepsipeptides. On the other hand the anthelmintic activities of the compounds with similar lipophilicities are strongly dependent on the nature of the N-methyl amino acid. The data presented here strongly suggests the (L)-N-methyl leucine residues to be a critical part of the pharmacophore, essential for *in vivo* anthelmintic activity. Despite the twofold axis of symmetry all four leucines are necessary for high biological activity. The synthetic utility of BOP-Cl mediated macrolactamizations has been expanded to include N-alkyl groups other than methyl, allowing cyclisations of sterically hindered depsipeptides.

Acknowledgements: We thank Meiji Seika Kaisha Ltd. for providing us with an authentic sample of PF1022A and Dr.Daniel Gondol for his help in interpreting the NMR spectra.

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14. All compounds gave satisfactory spectral and/or accurate mass data. Characteristical mass data (FAB-MS: m/z (%)) of the synthetic cyclooctadepsipeptides are given below. **2**: 1027 (100, $(M+Na)^+$, 1005 (38, $(M+H)^+$), **3**: 1083 (100, $(M+Na)^+$), 1061 (65, $(M+H)^+$), **4**: 1083 (22, $(M+Na)^+$, 1061 (40, $(M+H)^+$), **5**: 972 (16, $(M+Na)^+$, 949 (84, $(M+H)^+$), **6**: 915 (100, $(M+H)^+$, 893 (43, $(M+H)^+$), **9**: 1107 (100, $(M+Na)^+$), **8**: 803 (57, $(M+Na)^+$, 781 $(M+H)^+$), **7**: 915 (16, $(M+Na)^+$, 893 (100, $(M+H)^+$), **10**: 887 (40, $(M+Na)^+$, 865 $(M+H^+)$, **11**: 943 (38, $(M+Na)^+$, 921 (100, $(M+H)^+$).
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