Exploring anti-leishmanial almiramide structure-activity relationships

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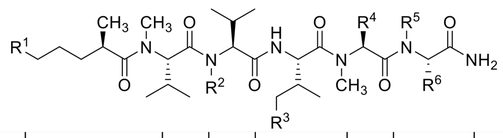
Abstract

Contemporary leishmaniasis therapy is unsatisfactory. Almiramide peptides exhibit anti-leishmanial activity with a relatively high therapeutic index.[1] In a structure-activity relationship study, the influences of *N*-methylation and conformation for almiramide peptide activity on parasite and host cells has been examined.

Leishmaniasis and almiramide

Leishmaniasis is a protozoan disease affecting between 12 to 15 million people worldwide.[1] With drug resistant strains on the rise and limitations in contemporary therapy, new anti-leishmanial therapy is urgently needed.[2] Almiramide peptides exhibit selective activity against parasites with low host cytotoxicity due likely to a novel mechanism of action.[3]

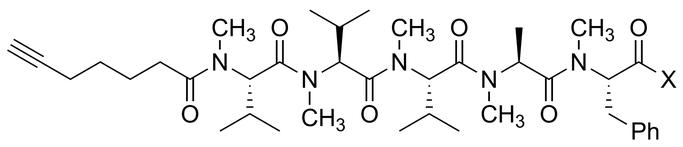
Natural almiramides A-D



Almiramide	R^1	R^2	R^3	R^4	R^5	R^6	Bioacitivity
A	-CH_2-CO-CH_3	Me	H	Me	Me	Bn	Antileishmanial activity Antitumor cytotoxicity
B	-CH_2-C≡CH	Me	H	Me	Me	Bn	
C	-CH_2-CH=CH_2	Me	H	Me	Me	Bn	
D	-CH_2-C≡CH	Me	Me	*sec*-butyl	H	Me	Antitumor cytotoxicity

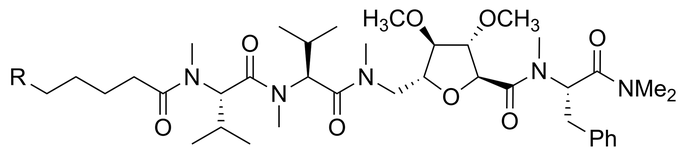
Background

The *C*-terminus of almiramide B was shown to be important for activity. Acid **1** and dimethyl amide **2** had μM IC_{50} values against *Leishmania (L.) donovani* and better therapeutic indices (CC_{50}/IC_{50}: **1** = **2** = 50.2) than almiramide B (17.4).[4]



X = **1**, OH; **2**, $N(CH_3)_2$

Almiramide-furan analogs **3** and **4** were observed by NMR spectroscopy to adopt active bent conformers, and exhibited activity against *L. donovani* with a selectivity index similar to standard of care miltefosine.[5]

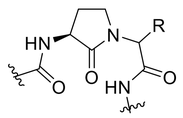


R = **3**, -$(CH_2)_3CH_3$; **4**, Ph

Goals

Employing peptide **1** as a lead which has relatively potent activity against *Leishmania*, we studied:

- The relevance of *N*-methylation by *N*-methyl scan
- The relevance of turn geometry by an α-amino γ-lactam (Agl) scan

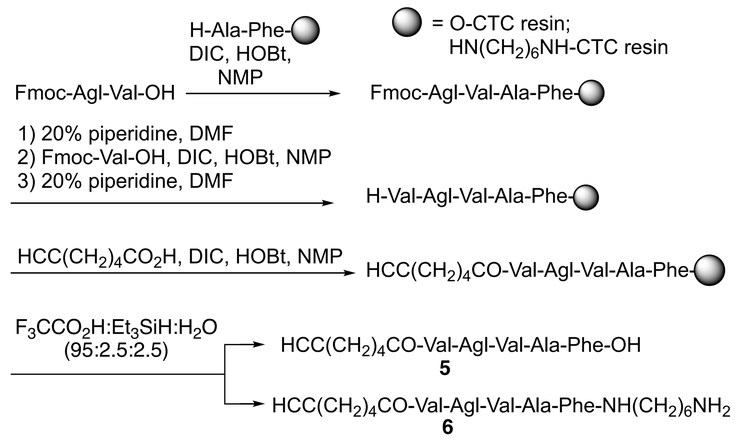


α-amino γ-lactam (Agl)

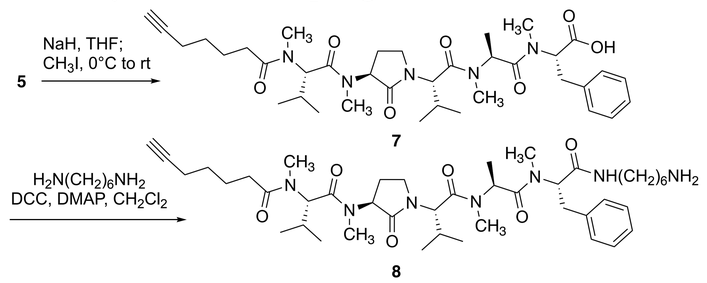
- Diamine amides (e.g., **1**, X = NH-$(CH_2)_6$-NH_2) for subsequent conjugate formation.

Synthesis of almiramide derivatives

Representative peptide SPPS synthesis



Permethylation and coupling to 1,6-diaminohexane

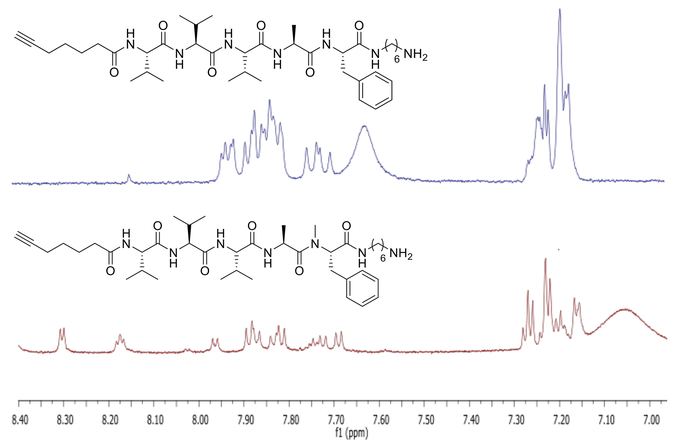


N-methyl scan and activity against *L. infatum*

Examining activity against wild type *L. infantum* (Ldi WT) and cytotoxicity (CC_{50}) in murine LM-1 macrophages, the 1,6-diaminohexane amides were respectively more potent than the acid counterparts. In the *N*-methyl series, peptides with no methyl groups and only *N*-methyl-Phe were most active and exhibited a good selectivity index (SI).

Sequence: R = -$(CH_2)_4$CCH	Ldi WT (μM)	CC_{50} (μM)	SI
RCO-Val(Me)-Val(Me)-Val(Me)-Ala(Me)-Phe(Me)-OH	68.20	281	4.1
RCO-Val(Me)-Val(Me)-Val(Me)-Ala(Me)-Phe(Me)-$NH(CH_2)_6NH_2$	48.11	338	7.1
RCO-Val-Val-Val-Ala-Phe-OH	44.90	316	7.0
RCO-Val-Val-Val-Ala-Phe-$NH(CH_2)_6NH_2$	23.25	446	19.2
RCO-Val(Me)-Val-Val-Ala-Phe-$NH(CH_2)_6NH_2$	36.44	177	4.9
RCO-Val-Val(Me)-Val-Ala-Phe-$NH(CH_2)_6NH_2$	81.56	316	3.8
RCO-Val-Val-Val(Me)-Ala-Phe-$NH(CH_2)_6NH_2$	54.28	281	6.9
RCO-Val-Val-Val-Ala(Me)-Phe-$NH(CH_2)_6NH_2$	75.31	1440	19.1
RCO-Val-Val-Val-Ala-Phe(Me)-$NH(CH_2)_6NH_2$	29.18	446	15.3

The ^{1}H NMR spectrum of *N*-(Me)Phe in DMSO-d_6 exhibited downfield shifting and isomeric pairs relative to nonmethylated counterpart indicating conformational change.



Agl scan and activity against *L. infatum*

Except the Agl[2] analog, Agl restriction resulted in lower activity than *N*-methyl and nonmethyl counterparts.

Sequence: R = -$(CH_2)_4$CCH	Ldi WT (μM)	CC_{50} (μM)	SI
RCO-Agl-Val-Val-Ala-Phe-$NH(CH_2)_6NH_2$	75.33	416	5.5
RCO-Val-Agl-Val-Ala-Phe-$NH(CH_2)_6NH_2$	47.63	407	8.5
RCO-Val-Val-Agl-Ala-Phe-$NH(CH_2)_6NH_2$	65.40	389	5.9
RCO-Val-Val-Val-Agl-Phe-$NH(CH_2)_6NH_2$	91.82	389	4.2
RCO-Val-Val-Val-Agl-D-Phe-$NH(CH_2)_6NH_2$	92.85	338	3.6
RCO-Agl(Me)-Val-Val(Me)-Ala(Me)-Phe(Me)-$NH(CH_2)_6NH_2$	120.35	407	3.4
RCO-Val(Me)-Agl(Me)-Val-Ala(Me)-Phe(Me)-$NH(CH_2)_6NH_2$	33.80	398	11.8
RCO-Val(Me)-Val(Me)-Agl(Me)-Ala-Phe(Me)-$NH(CH_2)_6NH_2$	78.90	371	4.7

Conclusions

- Seventeen almiramide analogs were synthesized featuring *N*-methyl and Agl substituents.
- 6-Aminohexamide analogs were more active than acid counterparts
- Peptide with no or mono-methylation at Val[1] and Phe[5] exhibited best activities.
- Replacement of the Val[2] residue by Agl[2] in a per-methylated peptide increased activity.
- Conformers extended at the central residues and mobile at the extremities of the peptide may favor almiramide activity.

References

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