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THE INHIBITION OF C5a RECEPTOR BINDING BY ANALOGS OF L-156,602, A CYCLIC HEXADEPSIPEPTIDE ANTIBIOTIC

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Abstract - The cyclic hexadepsipeptide antibiotic L-156,602 (**1**) was found to be an inhibitor of anaphylatoxin C5a binding to its receptor (IC_{50}=1.7 μg/mL). This compound also caused nonspecific degranulation of neutrophils, but not through interactions with the C5a or other receptors. The effects of chemical modification of the structure of **1** on C5a receptor inhibition and nonspecific actions are reported.

The complement system is activated in response to numerous immunological and nonimmunological events and functions to focus and amplify the inflammatory response towards the offending agent(s).[1-11] An exquisite cascade of proteolytic and recombining processes serves to assemble the membrane attack complex (MAC) which forms broad channels in the membranes of suitably identified and labeled cells causing cell disruption. Numerous complement derived peptides are also released that interact with cellular receptors to further propagate and recruit inflammatory cells to the site of injury or inflammation. Among these is C5a, a highly cationic 74 amino acid protein released from the complement component C5 by C5-convertase. The biological actions of C5a include neutrophil chemotaxis and aggregation, stimulation of leukotriene and oxidative product release, cellular release of vasoactive amines and lysosomal enzymes, induction of interleukin-1 transcription by macrophages, enhanced antibody production, increased vascular permeability, and contraction of smooth muscle. The augmentation of the action of this inflammatory mediator by a selective antagonist may offer a potential treatment for a variety of diseases characterized by recruitment and activation of inflammatory cells.[12]

Our search for C5a antagonists with novel structures yielded the cyclic hexadepsipeptide L-156,602 (**1**), isolated from a strain of *Streptomyces*.[13] Its X-ray structure was determined[13] and was found to be similar to the antibiotics azinothricin [14], A83586C [15], variapeptin and citropeptin [16] and is apparently identical to PD124,966[17]. The members of this class of antibiotics are characterized by a cyclic depsipeptide portion containing several unusual amino acids with a highly functionalized tetrahydropyranyl side chain appended to the amino acid that forms the ester linkage.

Compound **1** inhibited the binding of radiolabeled C5a to neutrophil membranes with an IC_{50} = 1.7 μg/mL (~2.0 μM). Azinothricin was considerably less potent as an inhibitor of C5a binding (IC_{50} = 30 μg/mL).

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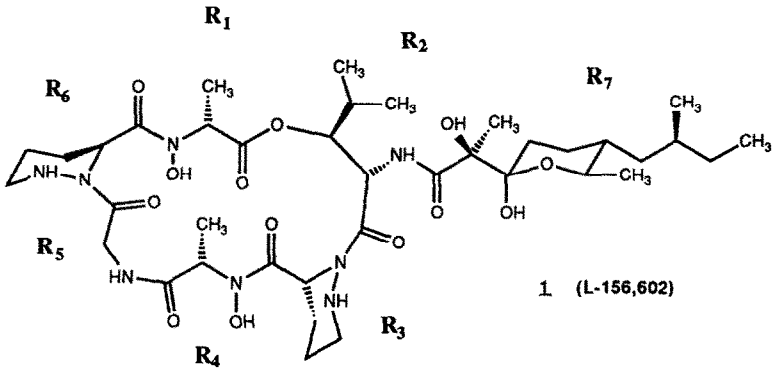


Compound **1** did not inhibit the binding of other chemotactic peptides, such as fMLF, LTB4, and PAF. As corroboration that the compound **1** inhibits binding by interacting with the C5a receptor, we found that the molecule blocks C5a binding to detergent solubilized and purified receptor[18] with an IC_{50} = 5 ug/ml. However, compound **1** did inhibit binding of IL-8 to its receptor on neutrophils (IC_{50} = 5 μg/mL).[19] In a whole cell functional assay designed to determine degranulation of neutrophils as measured by myeloperoxidase (MPO) release, **1** caused degranulation with an EC_{50} = 0.28 μg/mL. Evidience suggested that this biological activity was not mediated by the C5a receptor. The C5a receptors on neutrophils were desensitized with C5a and washed. The addition of more C5a did not cause the release of MPO. However, the addition of **1** did cause degranulation. Furthermore, MPO release mediated by **1**, unlike that of C5a, was independent of cytocholasin B. Herein, we report the results of our efforts to increase the potency of **1** as an antagonist of the binding of C5a to its receptor while reducing or eliminating the unwanted side effect of nonspecific degranulation.

Chemistry

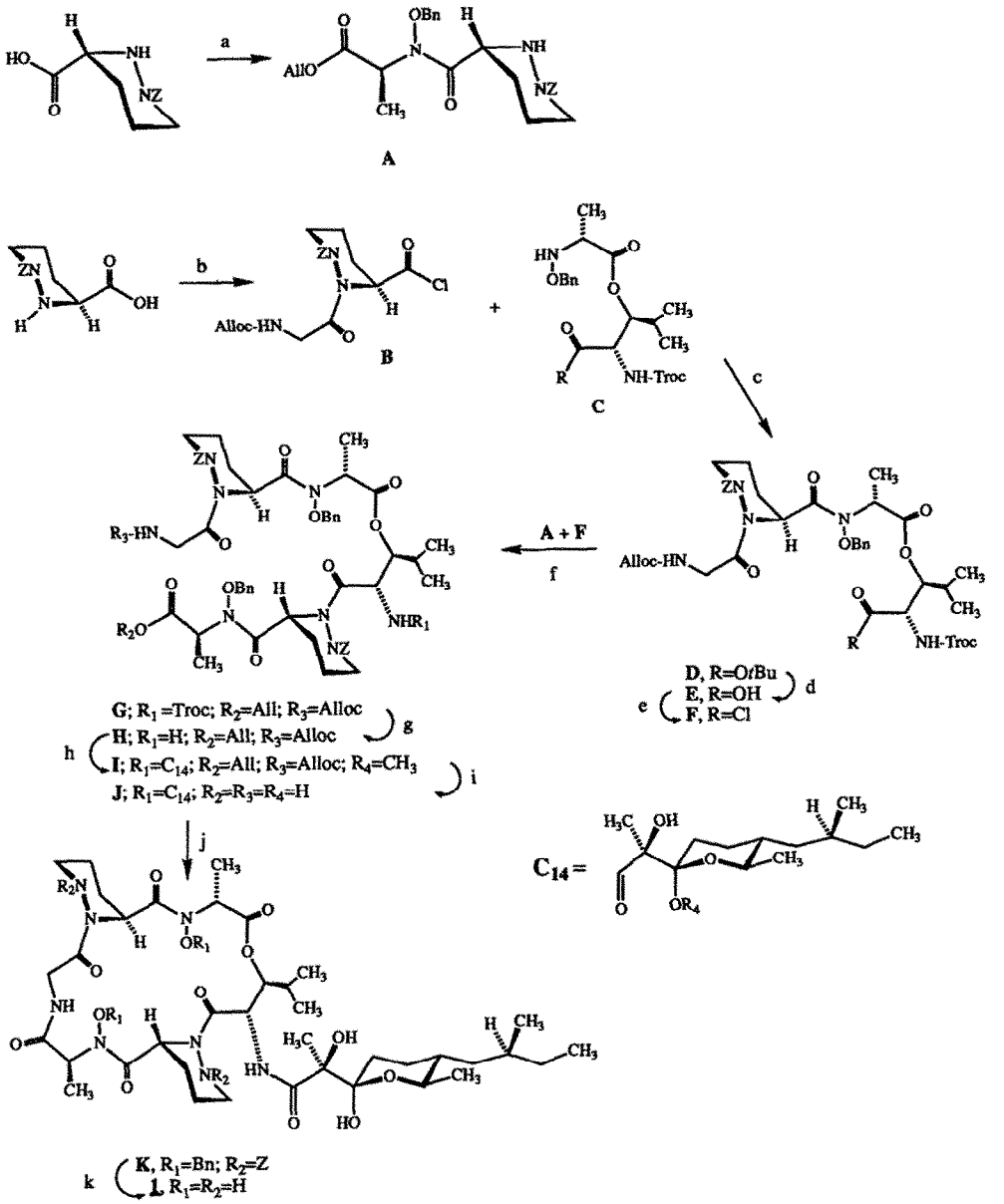
The total synthesis of **1** has been described and is outlined in Scheme 1.[20] The synthetic methodology required a variety of peptide bond forming techniques with appropriate protecting groups to form the cyclic depsipeptide. Two methods for preparing the fourteen carbon lipophilic side chain (C_{14}) have also been reported.[21] Related procedures were used to prepare the derivatives **3**, **5-9**, **11**, **12**, and **21-23**, substituting the individual amino acids as noted in Table 1. The preparation of the analogs **28-29** containing the simplified (R)- and (S)-twelve carbon side chains (C_{12}) followed the Seebach methodology described for the preparation of C_{14}.

Despite its apparent complexity and base sensitivity, **1** was selectively modified by a variety of chemical procedures.[22] Selective reductions of the N-hydroxy-alanines gave **2**, **17**, and **19**. The regioselective reductions were attributed to a strong internal hydrogen bond that was suggested by the X-ray structure between the N-hydroxyl group of (R)-N-OH-alanine (R_1) and the α-amide carbonyl of the appended C_{14} side chain on β-hydroxyleucine . The secondary nitrogens of both piperazic acids were methylated by reductive amination to give **10**. Treatment of **1** with diazomethane gave the (R)-O-methylated alanine **13** and the (R)- and (S)-bis-O-methylated alanine derivative **18**. Treatment of **3** under the same conditions with diazomethane gave **20**.

Receptor binding and degranulation assays

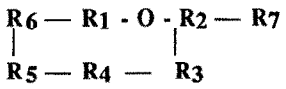
Compounds were tested for their ability to prevent the binding of radiolabeled C5a to intact membrane-bound receptors prepared from human neutrophils. These assays were carried out as described previoulsy except

SCHEME I.



(a) (i) TMS-Cl,then Fmoc-Cl; (ii) $(COCl)_2$, cat. DMF; (iii) N-OBn-(S)-Ala-O-allyl, 10% $NaHCO_3$, CH_2Cl_2; (iv) Et_2NH;
(b) (i) $(CH_3)_2C{=}CH_2$, H_2SO_4; (ii) N-Alloc-GlyCl, 10% $NaHCO_3$, CH_2Cl_2; (iii) TFA; (iv) $(COCl)_2$; (c) AgCN,
toluene, 90°; (d) TFA; (e) $(COCl)_2$; (f) 10% $NaHCO_3$, CH_2Cl_2; (g) Zn, AcOH; (h) HOBt-C_{14}, DMF;
(i) Bu_3SnH, $(Ph_3P)_2PdCl_2$, CH_2Cl_2, H_2O; (j) $[nPrP(O)O]_3$, DMAP, CH_2Cl_2, 10^{-4}M; (k) H_2, MeOH, 10% Pd(C)

Table 1. Inhibition of C5a Receptor Binding and Nonspecific MPO Release by Cyclic Hexadepsipeptides.



Compd. No.	R_1	R_2	R_3	R_4	R_5	R_6	R_7	C5a binding[1] ± SD μg/mL (n)[5]	MPO release[2] ± SD μg/mL (n)[5]
1	(R)-N-OH-Ala	(2S,3S)-3-OH-Leu	(R)-Piz	(S)-N-OH-Ala	Gly	(S)-Piz	C_{14} (X=OH) [3]	1.8 ± 0.6 (27)	0.27 ± 0.14 (14)
2	(R)-Ala							21 ± 3	7 ± 3
3	(R)-N-CH_3-Ala							5 ± 2	4 ± 2
4	(R)-N-OCH_3-Ala							2 ± 2	2 ± 1
5		(S)-Ser						>100	26 ± 2 (2)
6		(2S,3S)-Thr						2 ± 1	1.0 ± 0.5
7			(R)-Pip					75 ± 25 (3)	20 ± 3
8			(R)-Orn					60 ± 10	>200
9			(R)-Nγ-Ac-Orn					>100	>50
10			(R)-N-CH_3-Piz			(S)-N-CH_3-Piz		11 ± 3	10 ± 1
11				(S)-Ala				>100	ND
12				(S)-N-CH_3-Ala				5 ± 2	2 ± 1
13				(S)-N-OCH_3-Ala				5 ± 1.5	0.3 ± 0.1
14				(S)-N-OCH_2Ph-Ala				4 ± 1	0.6 ± 0.3
15				(S)-N-Ac-Ala				4 ± 2	0.3 ± 0.2
16				(S)-N-t-BOC-Ala				~ 100	>50
17	(R)-Ala			(S)-Ala				40 ± 10	>50
18	(R)-N-OCH_3-Ala			(S)-N-OCH_3-Ala				6 ± 3	3 ± 2
19	(R)-Ala			(S)-N-OCH_2Ph-Ala				>100	18 ± 5
20	(R)-N-CH_3-Ala			(S)-N-OCH_3-Ala				7 ± 1	20 ± 10
21					β-Ala			>100	>50
22					(S)-Ala			60 ± 30	ND
23					(R)-Ala			>100	>100
24							$COCOCH_3$	>100	>13
25							$COCH(OH)CH_3$	23 ± 2 (2)	23 ± 2 (2)
26							$COCH(OH)CH_3$	>100	>20
27							C_{14} (X=H)	3.6 ± 1.5	0.3 ± 0.2
28							(R)-C_{12} [4]	~ 100	12 ± 4
29							(S)-C_{12}	>100	9 ± 3

[1] Inhibition of radiolabeled C5a binding to its membrane bound receptor.

[2] Nonspecific degranulation of PMN's as measured by MPO release.

[3] C_{14} (X=OH) is (2S)-2-[(2R, 5R, 6R)-2-hydroxy-6-methyl-5-[(2S)-2-methylbutyl]tetrahydro-2*H*-pyran-2-yl]propanoic acid (Reference 10).

[4] C_{12} (X=OH) is 2(R or S)-hydroxy-2-methyl-5-phenyl-pentanoic acid.

[5] n = number of determinations if more than one.

that the compounds were incubated with the membranes for 60 min prior to addition of ^{125}I-C5a.[23-25] These data are reported as IC_{50}'s in Table 1. To determine whether the compounds could also cause neutrophil degranulation, their ability to induce the nonspecific release of the azurophilic granule constituent MPO from intact neutrophils was measured and the data are reported as EC_{50}'s in Table 1. The upper limits of test concentrations were determined by the solubility of the individual compounds. The assay conditions are as previously reported[26] except that the cells were incubated with the compounds for 20 min prior to the addition of C5a.

Results and Discussion

It is clear from the data in Table 1 that chemical modifications of the structure of **1** did not significantly improve the activity profile, defined as the ratio of IC_{50} to EC_{50}, of the lead compound. Replacement of the N-hydroxyl group of (R)-N-OH-Ala (R_1) with N-methyl (**3**) or N-methoxyl (**4**) groups slightly reduced potency. Substitution with (R)-Ala (**2**) at that position significantly reduced activity. The (S)-N-OH-Ala position (R_4) also appeared tolerant of substitution. While the (S)-Ala analog (**11**) was much less potent, N-methyl (**12**) and N-O-alkyl (**13-14**) and N-O-acetyl (**15**) derivatives retained the activity of **1**. The N-O-t-BOC derivative (**16**) was much less active. The bis-NH-Ala replacements at R_1 and R_4 (**17**) still retained significant activity, while the bis-N-methoxy derivative **18** was comparable to **1**. The (R)-N-CH_3-Ala; (S)-N-OCH_3-Ala (**20**) derivative was only slightly less active than **1** in both assays.

Inspection of the X-ray structure of **1**, suggested that the N-hydroxyl groups at R_1 and R_4 arranged as they are in a cyclic structure might tightly bind metal ions. This may impart "ionophoric" properties to **1** and might explain the nonreceptor mediated degranulation of neutrophils that was observed. The retention of both receptor binding activity and MPO release by analogs which replaced or modified those N-hydroxyls (**3, 4, 12, 13, 14, 15, 18,** and **20**) makes this proposed metal binding site unlikely. Each of these substitutents would be expected to modify the affinity for metal binding and would thus have an effect on neutrophil degranulation. However, ionophoric mechanisms involving the other amino acids of **1** cannot be ruled out.

Replacement of the β-hydroxyleucine at R_2 with (2S, 3S)-threonine gave **6** which was essentially equipotent with **1**. Elimination of the side chain residue at this position ((S)-serine) to give **5** eliminated activity. This loss of activity may be associated with the elimination of a specific interaction with the receptor or the formation of an unfavorable conformation.

The replacement of the (R)-piperazic acid at R3 with (R)-pipecolic acid **7** (2^o-NH→CH_2) resulted in a loss of inhibition even though such a substitution might be expected to have minimal effects on the overall conformation of the molecule. The (R)-ornithine analog **8**, which is the equivalent of cleaving the N-N bond of the (R)-piperazic acid of **1**,was also marginally active but is one of the few analogs to abolish MPO release. N_δ-Acetylation of this ornithine in **9** abolished receptor binding. Bis-N-methylation of the secondary nitrogens of both piperazic acids in **10** by reductive amination resulted in 10-20 fold loss of activity in both assays with respect to **1**. The basic residues at this position may be interacting with acidic amino acids proposed to be located on the extracellular domain of the receptor.[26,27]

The glycine at R_5 appears crucial. Replacement of this glycine with β-alanine in **21** resulting in a larger ring eliminated activity. Addition of a methyl group in **22** ((S)-Ala) or **23** ((R)-Ala) gave compounds with greatly reduced activity.

Finally, modification of the fourteen carbon side chain also attenuated the receptor binding inhibition as well as neutrophil degranulation. The pyruvamide **24** was inactive. One of the diastereomeric lactamides **25**

from reduction of **24** retained significant activity while the other isomer **26** was inactive. It was thought that the stereochemistry of the α-hydroxyl group of the side chain might also be crucial to binding. However, replacement of the fourteen carbon side chain with a twelve carbon mimic C_{12} gave gave **28** which placed the α-hydroxyl group in the opposite configuration of **1**. This compound retained significant activity in the C5a inhibition assay but had limited solubility in the MPO assay. The other C_{12} stereoisomer **29** which had the same configuration at that stereocenter was inactive. Selective reduction of **1**, which removed the anomeric hydroxyl group, gave **27**, a compound that retained most of the activities of **1**. It is difficult to draw any conclusions with respect to the important binding interactions in this part of the molecule.

The extensive structure-activity relationship around **1** revealed a complex interplay of this compound with the C5a receptor and an uncharacterized nonspecific interaction. It does reenforce the strategy that analysis of natural products along with selective screening[26] and substrate modification[28] will lead to novel structures that can elucidate both structural and functional features of a biological target.

References and Notes

1. Kinoshita, T. *Immunol.Today* **1991**, *12*, 291.
2. Farries, T.C.; Atkinson, J.P. *Immunol.Today* **1991**, *12*, 295.
3. Morgan, B. P.; Walport, M. J. *Immunol.Today* **1991**, *12*, 301.
4. Sim, R.B.; Reid, K.B.M. *Immunol.Today* **1991**, *12*, 307.
5. Lachmann, P.J. *Immunol.Today* **1991**, *12*, 312.
6. Esser, A.F. *Immunol.Today* **1991**, *12*, 316.
7. Bhakdi, S.; Tranum-Jensen, J. *Immunol.Today* **1991**, *12*, 318.
8. Frank, M.M.; Fries, L.F. *Immunol.Today* **1991**, *12*, 322.
9. Cooper, N.R. *Immunol.Today* **1991**, *12*, 327.
10. Erdei, A.; Fust; G.; Gergely, J. *Immunol.Today* **1991**, *12*, 332.
11. Sims; P. J.; Wiedmer, T. *Immunol.Today* **1991**, *12*, 338.
12. Morgan, B. P. *Complement: Clinical Aspects and Relevance to Disease*; Academic Press: New York 1990; pp. 112-192.
13. Hensens, O. D.; Borris, R. P.; Koupal, L. R.; Caldwell, C. G.; Currie, S. A.; Haidri, A. A.; Homnick, C. F.; Honeycutt, S. S.; Lindenmayer, S. M.; Schwartz, C. D.; Weissberger, B. A.; Woodruff, H. B.; Zink, D. L.; Zitano, L.; Fieldhouse, J. M; Rollins, T.; Springer, M. S.; Springer, J. P. *J. Antibiotics* **1991**, *44*, 249.
14. Maehr, H.; Liu, C.-M.; Palleroni, N. J.; Smallheer, J.; Todaro, L.; Williams, T. H.; Blount, J. F. *J. Antibiotics* **1986**, *39*, 17.
15. Smitka, T. A.; Deeter, J. B.; Hunt, A. H.; Mertz, F. P.; Ellis, R. M.; Boeck, L. D.; Yao, R. C. *J. Antibiotics* **1988**, *41*, 726.
16. Nagawa, M.; Hayakawa, K.; Furihata, K.; Seto, H. *J. Antibiotics* **1990**, *43*, 477.
17. Hurley, T. R.; Bunge, R. H.; Willmer, N. E.; Hokanson, G. C.; French, J. C. *J. Antibiotics* **1986**, *39*, 1651.
18. Rollins, T.E., Sicliano, S., Kobayashi, S., Cianciarulo, D. N., Bonilla-Argudo, V., Collier, K., Springer, M.S. *Proc. Nat. Acad. Sci. USA* **1991**, *88*, 971.
19. Springer, M. S., unpublished results.
20. Durette, P. L.; Baker, F.; Barker, P. L.; Boger, J.; Bondy, S. S.; Hammond, M. L.; Lanza, T. J.; Pessolano, A. A.; Caldwell, C. G. *Tet. Lett.* **1990**, *31*, 1237.
21. Caldwell, C. G.; Rupprecht, K. M.; Bondy, S. S.; Davis, A. A. *J. Org. Chem.* **1990**, *55*, 2355.
22. Kopka, I. E. *Tet. Lett.* **1990**, *33*, 4711.
23. Rollins, T. E.; Springer, M. S. *J. Biol. Chem.* **1985**, *260*, 7157.
24. Rollins, T. E.; Siciliano, S.; Springer, M. S. *J. Biol. Chem.* **1988**, *264*, 520.
25. English, D.; Andersen, B. R. *J. Immunolog. Methods* **1974**, *5*, 249.
26. Lanza, T. J.; Durette, P. L.; Rollins, T.; Siciliano, S.; Cianciarulo, D. N.; Kobayashi, S. V., Caldwell, C. G.; Springer, M. S.; Hagmann, W. K. *J. Med. Chem.* **1992**, *35*, 252.
27. Gerard, N. P.; Gerard, C. *Nature* **1991**, *349*, 614.
28. Or, Y.-S.; Clark, R. F.; Mollison, K. W.; Carter, G. W.; Luly, J. R. *J. Med. Chem.* **1992**, *35*, 402.