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A SMALL LIBRARY OF PEPTIDOMIMETICS TO SYSTEMATICALLY VARY AND TEST THE EFFECTS OF X^1 CONSTRAINTS

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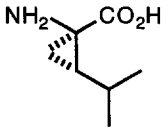
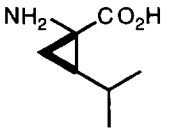
Abstract: A library of 47 peptidomimetics of YGGFLRFa was prepared wherein 32 of these contained 2,3-methanoleucine. This library was tested for binding the neuropeptide FF receptor where neuropeptide FF is FLFQPQRFa, an anti-opiate peptide. This study illustrates how conformational constraints can be systematically varied in a library format to determine the best conformational restrictions for the desired activity.

Small biased libraries have become important for the development of biologically active synthetic substances such as pharmaceuticals, herbicides, and pesticides. Libraries of this kind are most useful after a target structure is identified. For example, modifications of an active compound selected from a large combinatorial library[1-3] might be highly desirable, and small biased libraries are ideal for this. They facilitate systematic and rapid accumulation of structure/activity data to accelerate the evolution of molecular candidates for more advanced biological trials, thereby reserving more expensive and time consuming testing for compounds showing evidence of promising activities.

Approaches to the design of small biased libraries vary. Diversity, a much discussed facet of large combinatorial libraries,[4,5] is a less important issue in this case than *focus*. For instance, small libraries may be designed to probe steric effects, electrostatic factors, and/or lypophilicity. In this paper we describe a library designed to hone in on a very specific question: what conformational constraints can be introduced using 2,3-methanomethionine[6] without significantly diminishing the biological potency? As far as we are aware, this is the first library designed to probe effects of χ^1 restrictions in a series of peptidomimetics.



2*R*,3*S*-cyclo-Leu



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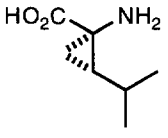

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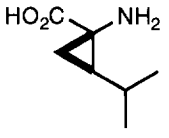

2*R*,3*R*-cyclo-Leu

47 peptidomimetics of YGGFLRFa wherein 32 contain a cyclo-Leu stereoisomer

Geysen's pins[7] from Chiron Mimetopes were used for the synthesis of the library. The pins used in this experiment were functionalized with Rink's amide handle[8] to facilitate cleavage of the peptidomimetics from the resin under relatively mild acidic conditions. This approach also ensured that the products after cleavage had the desired (*vide infra*) carboxamide *C*-terminus. Couplings were performed using FMOC-protected amino acids, BOP/HOBt mediated couplings, and piperidine for deprotection of the FMOC protecting groups, and the final cleavage was performed using TFA with ethanedithiol as a scavenger. The latter step also removed the ^{t}Bu and Mtr[9] side chain protecting groups used for the Tyr and Arg residues, respectively. All constituents of the library were analyzed by HPLC and MALDI-MS.

Table 1 indicates the format of the library. All the peptidomimetics are based on the sequence YGGFLRFa which was prepared in the top left well as depicted in Table 1. Each row in this table represents one particular amino acid at residue-5 (Leu or one of the cyclo-Leu stereoisomers). The columns in Table 1 are indicative of any other changes that were made in the rest of the sequence (none in the left-most column, D-Ala for Gly at residue 2 in the second, *etc*).

Table 1. The Library of YGGFLRFa Peptidomimetics, and Their Percent Inhibition of Radiolabeled neuropeptide FF Binding to neuropeptide FF Receptors in Rat Spinal Cord.[a]

deviations from the YGGFLRFa sequence[b]	-	D-Ala^2	D-Ala^3	D-Ala^2 D-Ala^3	D-Phe^4	D-Ala^2 D-Phe^4	D-Ala^3 D-Phe^4	D-Ala^2 D-Ala^3 D-Phe^4
-	84	76	75	65	98	60	81	58
D-Leu^5	31	35	28	63	48	40	48	69
2R,3S-cyclo-Leu^5	21	-7	22	35	30	20	34	32
2S,3R-cyclo-Leu^5	10	9	13	15	19	19	17	30
2S,3S-cyclo-Leu^5	**57**	14	27	27	**55**	37	**43**	32
2R,3R-cyclo-Leu^5	35	22	29	13	42	38	37	34

[a] Average of two experiments using 90 nM of peptidomimetic in competition with 0.28 nM of radiolabeled neuropeptide FF (which has a K_i of 0.44 ± 0.06 nM in this assay). [b] The difference between various rows represents changes from the Leu^5 in YGGFLRFa, and the variation between columns is indicative of other changes.

This library was designed to be tested for anti-opiate activities, and the rationale for the selection of these particular compounds is as follows. All the peptidomimetics have -RFa *C*-termini because there is substantial evidence that this structural feature imparts anti-opiate properties.[10] Peptides of this kind tend to accumulate in mammalian brain tissue as a response to endogenous opiates or morphine treatment. They can precipitate tolerance and abstinence syndrome in rats; tolerance apparently arises when the concentration of -RFa peptides increases in response to morphine treatment, and withdrawal effects can be attributed to an abundance of -RFa peptides once the treatment is discontinued or the dose is reduced.[10-14]

In the rat, an important naturally occurring -RFa peptide is FLFQPQRFa (neuropeptide FF). Assays to test for binding to neuropeptide FF receptors in rat brain and spinal cord have been established. Structure-

activity relationships have shown that the RFa *C*-terminus is critical for binding to neuropeptide FF receptors.[15] No appreciably active compounds have been identified in which these residues were modified. There is, however, considerable scope for making changes on the *N*-terminal side of the -RFa fragment.

2-Substituted-2,3-methanoamino acids[16] impart various conformational constraints to peptidomimetics. They lock the side chain χ^1 rotamer at values of approximately 0° and ± 150°, depending on the particular stereoisomer chosen.[17] This rigid constraint has more subtle effects on the stereochemistry of the peptide around the 2,3-methanoamino acid, leading to, for instance, γ-turn structures.[17,18] They also give peptidomimetics that are more proteolytically stable than the parent peptide sequence.[19-22] In this research, the sequence chosen for elaboration, YGGFLRFa, has obvious homologies between leucine enkephalin and two other peptides in the -RFa series, ie YGGFMRF-NH_2 and LPLRF-NH_2. Most of the peptidomimetics contained cyclo-Leu stereoisomers substituted for the Leu^5 residue to explore which χ^1 constraints are tolerated/favored with respect to receptor binding. The position of the 2,3-methanoamino acid was chosen to favor maximum influence on the -RFa fragment conformation, without directly altering this critical structure. The cyclopropylamino acid at this position would also partially protect the RFa terminus from proteolytic cleavage, a phenomenon that could be investigated and explored in later studies.

Binding inhibition data for the library are shown in Table 1; compounds that bound strongly have high percent binding inhibitions. Some trends are immediately apparent for the set of 32 compounds containing cyclo-Leu, *notably the three peptidomimetics in this class that bound most strongly were all in the 2S,3S-cyclo-Leu series* (data for these three compounds is shown in bold face). *2R,3R*-Cyclo-Leu tended to give the second most strongly bound compounds out of the other three cyclo-Leu stereoisomers. *2S,3S*- and *2R,3R*-cyclo-Leu are enantiomers, and both compounds have a *cis*-orientation of the *iso*-propyl side-chain relative to the amine functionality. *For both these 2,3-methanoleucine stereoisomers, χ^1 is approximately 0°*. The other trends in this data relate to the peripheral amino acids. Replacement of Gly^2 or Gly^3 with D-Ala residues is generally detrimental to the binding. Conversely, substitution of the L-Phe^4 residue with D-Phe^4 is generally advantageous; in fact, these data indicate the most potent compound in the library was YGG(D-F)LRFa. Overall, the most active compound in the series did not contain 2,3-methanoamino acids, but it is important to remember that the loss of binding affinity is likely to be offset by increased proteolytic stability of the "cyclopropylogs" in pharmacological studies.

The three 2,3-methanoamino acid derived peptidomimetics that were most active in the preliminary screen (Table 1) were tested under more stringent conditions. The following K_i values were obtained (nM) (data from three experiments, errors quoted represent standard error of the mean, SEM): YGGF(*2S,3S*-cyclo-L)RFa, 6.28 ± 0.11; YGG(D-F)(*2S,3S*-cyclo-L)RFa, 16.21 ± 0.15; YG(D-A)(D-F)(*2S,3S*-cyclo-L)RFa, 47.18 ± 0.05. The trend in these three activities follows that indicated in Table 1, increasing confidence in the reliability of the original screen. Neuropeptide FF has a K_i of 0.44 ± 0.06 nM under similar conditions. Consequently, all three peptidomimetics have reasonably strong affinities to the neuropeptide FF receptor.

The syntheses described in this paper were performed in such a way that one half of the 96 pins of a standard apparatus were used to generate the library shown in Table 1, while the other half were used to simultaneously prepare a library of YGGFLa peptidomimetics for comparison. Details of the binding assays for both sets of peptidomimetics will be reported in a full paper describing this complete study.[22]

Acknowledgments

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References and Notes

1. Pinilla, C.; Appel, J.; Blondelle, S.; Dooley, C.; Dorner, B.; Eichler, J.; Ostresh, J.; Houghten, R. A. *Biopolymers* **1995**, *37*, 221.
2. Gallop, M. A.; Barrett, R. W.; Dower, W. J.; Fodor, S. P. A.; Gordon, E. M. *J. Med. Chem.* **1994**, *37*, 1233.
3. Gordon, E. M.; Barrett, R. W.; Dower, W. J.; Fodor, S. P. A.; Gallop, M. A. *J. Med. Chem.* **1994**, *37*, 1385.
4. Martin, E. J.; Blaney, J. M.; Siani, M. A.; Spellmeyer, D. C.; Wong, A. K.; Moos, W. H. *J. Med. Chem.* **1995**, *38*, 1431.
5. Sadowski, J.; Wagener, M.; Gasteiger, J. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 2674.
6. Burgess, K.; Li, W. *Tetrahedron Lett.* **1995**, *36*, 2725. There were errors R and S nomencalture assigned to the products in our original communication of this work, but those shown in this paper are correct.
7. Maeji, N. J.; Bray, A. M.; Valerio, R. M.; Seldom, M. A.; Wang, J.-X.; Geysen, H. M. *Pep. Res.* **1991**, *4*, 142.
8. Rink, H. *Tetrahedron Lett.* **1987**, *28*, 3787.
9. Wakimasu, M.; Kitada, C.; Fujino, M. *Chem. Pharm. Bull* **1982**, *30*, 2766.
10. Rothman, R. B.; Xu, H.; Yang, H.-Y. T.; Long, J. B.*Anti-opioid peptides in morphine tolerance and dependence: focus on NPFF*; CRC Press: New York. 1992.
11. Yang, H.-Y. T.; Tang, J.; Iadorola, M.; Panula, P.; Costa, E. *Endocoids* **1985**, 313.
12. Yang, H.-Y. T.; Fratta, W.; Majane, E. A.; Costa, E. *Proc. Natl. Acad. Sci. USA* **1985**, *82*, 7757.
13. Malin, D. H.; Lane, J. R.; Fowler, D. E.; Hammond, M. V.; Brown, S. L.; Leyva, J. E.; Prasco, P. E.; Dougherty, T. M. *Peptides* **1990**, *11*, 277.
14. Malin, D. H.; Lake, J. R.; Hammond, M. V.; Fowler, D. E.; Rogillio, R. B.; Brown, S. L.; Sims, J. L.; Leecraft, B. M.; Yang, H.-Y. T. *Peptides* **1990**, *11*, 969.
15. Payza, K.; Akar, C. A.; Yang, H.-Y. T. *J. Pharmacol. Expt. Therap.* **1993**, *267*, 88.
16. Burgess, K.; Ho, K.-K.; Moye-Sherman, D. *SYNLETT* **1994**, *8*, 575.
17. Burgess, K.; Ho, K.-K.; Pal, B. *J. Am. Chem. Soc.* **1995**, *117*, 3808.
18. Burgess, K.; Ke, C.-Y. *J. Org. Chem.* **1996**, submitted.
19. Kimura, H.; Stammer, C. H.; Ren-Lin, C.; Stewart, J. *Biochem. Biophys. Res. Commun.* **1983**, *115*, 112.
20. Ogawa, T.; Shimohigashi, Y.; Yoshitomi, H.; Sakamoto, H.; Kodama, H.; Waki, M.; Stammer, C. H. *Pept. Chem.* **1988**, 25.
21. Ogawa, T.; Shimohigashi, Y.; Shiota, M.; Waki, M.; Stammer, C. H.; Ohno, M. *Pept. Chem.* **1989**, 43.
22. Ogawa, T.; Yoshitomi, H.; Kodama, H.; Waki, M.; Stammer, C. H.; Shimohigashi, Y. *Febs Lett* **1989**, *250*, 227.

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