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A CASE STUDY OF COMBINATORIAL LIBRARIES: ENDOTHELIN RECEPTOR ANTAGONIST HEXAPEPTIDES

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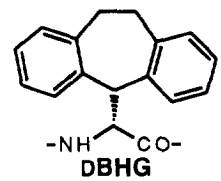
Abstract: Combinatorial libraries were constructed based on a hexapeptide endothelin receptor antagonist. Iterative optimization efficiently identified the most active member of the library and allowed preparation of a more potent antagonist.

Combinatorial libraries hold great potential for the discovery of leads for new drugs.[1] An efficient testing strategy employs the biological assay of mixtures of library components. "Deconvolution" of mixtures which possess biological activity allows identification of the active components. Iterative optimization is a powerful tool for this process.[2] We were particularly interested in employing this method using samples of isolated solids, since medicinal chemistry is usually practiced in this context. We set out to confirm the utility of iterative optimization by employing a known active substance, and to assess the feasiblity of identifying a more potent analog during this process. The active substance employed for this study was the hexapeptide **1**, reported by workers at Warner-Lambert as a potent antagonist of the endothelin receptor.[3,4]

Ac—**DBHG**—LEU—ASP—ILE—ILE—TRP—OH

1 ET_A IC_{50} = 8nM



We chose as our parent mixture the 19x19 combinatorial array shown in **2**, where the symbol ***EM*** represents an equimolar mixture of the 19 natural L-aminoacids, excluding cysteine. The parent mixture was prepared[5] on Sasrin resin[6] by the split synthesis method.[7] Standard Fmoc/t-butyl protection was employed in the synthesis.[8] The ET_A-antagonist assay employed a membrane preparation from porcine heart,[9] with ^{125}I-labelled ET-1 as the ligand. The IC_{50} value[10] for this 361-component mixture in the ET_A assay was 150nM.

1 2 3 4 5 6

Ac—DBHG—LEU—***EM***—***EM***—ILE—TRP—OH **2**

ET_A IC_{50} = 150nM

This mixture was deconvoluted at the 3-position by preparation of the 19 mixtures **3**, each comprised of 19 equimolar components,[11] where ***Aaa*** represents an individual L-amino-acid. Receptor antagonist activity is shown in Table 1. IC_{50} values were determined for the most potent members of this group, as shown in Table 2.[12] The most potent member (IC_{50} = 18nM) contains the Asp residue found in the known **1**. Next most potent is the Glu derivative, followed by the corresponding amides Asn and Gln, and finally by the hydroxy aminoacids Ser and Thr. Clear dependence on the polarity of the side-chain can be seen.

Ac—DBHG—LEU—$\overset{3}{\textbf{\textit{Aaa}}}$—$\overset{4}{\textbf{\textit{EM}}}$—ILE—TRP—OH **3**

Aaa	% Inh.	Aaa	% Inh.	Aaa	% Inh.
Ala	62	His	56	Ser	65
Asp	93	Ile	22	Thr	67
Asn	74	Leu	34	Trp	58
Arg	27	Lys	16	Tyr	45
Gln	66	Met	61	Val	46
Glu	88	Phe	42		
Gly	41	Pro	7	***EM***	66

Table 1. Percent inhibition of ET-1 binding by hexapeptides **3**, concentration 1000nM.

ACIDS		AMIDES		ALCOHOLS	
Aaa	**IC50 nM**	**Aaa**	**IC50 nM**	**Aaa**	**IC50 nM**
Asp	18	Asn	120	Thr	350
Glu	48	Gln	180	Ser	380

Table 2. IC50 values for most potent members of hexapeptides **3**.

Having identified Asp as the optimum residue in the 3-position, we incorporated this in the next stage of deconvolution, producing the 19 individual compounds **4**. While reliable purity determination for the multi-component mixtures **2** and **3** was not possible, we were gratified to observe HPLC purity of >95% for each crude product of structure **4**. Percent inhibition of ET-1 binding was determined for each compound (see Table 3), and IC50 values were obtained for the most active compounds, as shown in Table 4. The two most potent compounds **4** are the known Ile derivative **1** (IC50 = 8nM) and the Val analog (IC50 = 5nM). Next most potent are the Ala, Asn, and Met derivatives. Thus, deconvolution in the 4-position identified the known active, just as occurred for the 3-position. In the 4-position, good activity was seen for the closely related Val analog as well.

Ac—DBHG—LEU—$\overset{3}{\text{ASP}}$—$\overset{4}{\textbf{\textit{Aaa}}}$—ILE—TRP—OH **4**

Aaa	% Inh.	Aaa	% Inh.	Aaa	% Inh.
Ala	47	His	12	Ser	0
Asp	0	**Ile**	**62**	Thr	12
Asn	41	Leu	9	Trp	0
Arg	0	Lys	0	Tyr	9
Gln	12	Met	26	**Val**	**70**
Glu	0	Phe	12		
Gly	41	Pro	0		

Table 3. Percent inhibition of ET-1 binding by hexapeptides **4**, concentration 10nM.

Aaa	IC50, nM	Aaa	IC50, nM	Aaa	IC50, nM
Val	5	Ala	30	Met	40
Ile	8	Asn	40		

Table 4. IC50 values for the most potent members of hexapeptides **4**.

Having now fixed the residues in the 3- and 4-positions, we decided to examine the influence of the residue in the 2-position, and we prepared the 19 individual hexapeptides **5**. Here, too, the individual crude products showed a high degree of purity according to HPLC.[13] IC50 values were obtained for these compounds, as shown in Table 5. In general, all side chains are reasonably well tolerated in this region of the molecule. Interestingly, both the amide side chains Asn and Gln, and the basic side chains Arg and Lys, led to a modest improvement in potency (Gln IC50=2nM) relative to the lipophilic Leu in lead compound **1**.

Ac—DBHG—^{2}***Aaa***—^{3}ASP—^{4}ILE—ILE—TRP—OH **5**

Aaa	ETA IC50, nM	Aaa	ETA IC50, nM	Aaa	ETA IC50, nM
Ala	6	His	50	Ser	6
Asp	11	Ile	21	Thr	4
Asn	3	Leu	8	Trp	11
Arg	3	Lys	4	Tyr	11
Gln	2	Met	8	Val	12
Glu	11	Phe	24		
Gly	19	Pro	6		

Table 5. ETA antagonist IC50 values for hexapeptides **5**.

With this information in hand, we undertook a limited optimization of the hexapeptide structure. A set of compounds **6** was produced, where Asn and Gln were employed in addition to Leu at the 2-position. At the 4-position, three other branched aminoacids were examined in addition to Leu and Val. IC50 values are shown in Table 6. While BHG[14] and Chg (L-cyclohexylglycine) are apparently too large to be accomodated in the binding site of the receptor, tLeu is well tolerated. In combination with Gln at the 2-position, Val produced the greatest activity, with IC50=1.7nM (cf. lead compound **1**, IC50=8nM).[15] Quite interestingly, the -Gln-Asp-Val-Ile-Trp pentapeptide moiety is identical to the C-terminal fragment of the homolgous vasoactive peptides sarafatoxins 6a, 6b, and 6c.[16]

Ac—DBHG—^{2}***Aaa***—ASP—^{4}***Bbb***—ILE—TRP—OH **6**

Aaa	Bbb	IC50, nM	Aaa	Bbb	IC50, nM
Gln	Val	1.7	Gln	t-Leu	5.0
Asn	Val	4.0	Leu	t-Leu	5.0
Gln	Chg	49%*	Leu	BHG (A)†	45%*
Leu	Chg	47%*	Leu	BHG (B)†	0%*

Table 6. ETA antagonist IC50 values for double combinatorial hexapeptides **6**.
* % inhibition of ET binding at 100 nM † separated diastereomers enantiomeric at BHG center

These results demonstrate clearly the reliability of the iterative optimization technique, particularly employing isolated solid samples. Furthermore, a limited combinatorial lead optimization effort, when coupled with the SAR generated during the iterative process, led to an unanticipated more potent analog of the lead structure.[17] This result is nonetheless compatible with reported SAR.[3,4] The success of this case study provided a reliable basis for our successful exploration of novel combinatorial libraries, to be reported shortly.

References and notes:

1. The most recent and comprehensive reviews are:
   (a) Gallop, M. A.; Barrett, R. W.; Dower, W. J.; Fodor, S. P. A.; Gordon, E. M. *J. Med. Chem.* **1994,** *37,* 1233.
   (b) Gordon, E. M.; Barrett, R. W.; Dower, W. J.; Fodor, S. P. A.; Gallop, M. A. *J. Med. Chem.* **1994,** *37,* 1385.
   (c) Desai, M. J.; Zuckermann, R. N.; Moos, W. H. *Drug Devel. Res.* **1994**, *33*, 174.
   In addition, informative symposia-in-print are:
   (d) *BioorgMed. Chem. Lett.* **1993**, *3*(3).
   (e) *Drug Devel. Res.* **1994**, *33*(2).
2. Houghten, R. A.; Pinilla, C.; Blondelle, S. E.; Appel, J. R.; Dooley, C. T.; Cuervo, J. H. *Nature* **1991,** *354*, ,84.
3. (a) Dogherty, A. M.; Cody, W. L.; DePue, P. L.; He, J. X.; Waite, L. A.; Leonard, D. M.; Leitz, N. L.; Dudley, T. D.; Rapundalo, S. T.; Hingorani, G. P.; Haleen, S. J.; LaDouceur, D. M.; Hill, K. E.; Flynn, M. A.; Reynolds, E. E. *J. Med. Chem.* **1993**, *36*, 2585.
   (b) Beylin, V. G.; Chen, H. G.; Dunbar, J.; Goel, O. P.; Harter, W.; Marlatt, M.; Topliss, J. G. *Tetrahedron Lett.* **1993**, *34*, 953.
   (c) Cody, W. L.; Dogherty, A. M.; He, J. X.; DePue, P. L.; Waite, L. A.; Topliss, J. G.; Haleen, S. J.; LaDouceur, D. M.; Flynn, M. A.; Hill, K. E.; Reynolds, E. E. *Med. Chem. Res.* **1993**, *3*, 154.
4. A combinatorial approach to hexapeptide endothelin anatagonists based on Ac-Dtyr-Leu-Asp-Ile-Ile-Trp-OH is described by Spellmeyer, D. C.; Brown, S.; Stauber, G. B.; Geysen, H. M.; Valerio, R. *BioorgMed. Chem. Lett.* **1993**, *3*, 519. In contrast to the iterative optimization of mixtures employed in our work, that study utilized single materials from systematic variation (46 aminoacids) at each position in the peptide.
5. Couplings were performed manually, with each cleaved and deprotected product isolated as a precipitated solid.
6. Fmoc-Trp-Sasrin resin was obtained from BACHEM Biosciences, King of Prussia, PA.
7. (a) Furka, A.; Sebestyen, F.; Asgedom, M.; Dibo, G. *Int. J. Pept. Prot. Res.* **1991**, *37*, 487.
   (b) Lam, K.; Salmon, S.; Hersh, E.; Hruby, V.; Kazmierski, W.; Knapp, R. *Nature* **1991**, *354*, 82.
8. H-DBHG-OH was prepared according to the detailed procedure of Patent Application WO 93/21176. Fmoc-DBHG-OH was prepared from the aminoacid hydrochloride, Fmoc-Cl, and Na_2CO_3 in aqueous dioxane. The white powder showed mp 257-8 °C and $[\alpha]_D^{25} = -54.2$ ° (MeOH, c=1).
9. The ETA assay described in reference 3(c) employed rabbit renal artery as the membrane source and determined IC_{50}=4nM for peptide **1**, close to the value found in our assay.
10. IC_{50} values were determined from a plot of inhibition at five concentrations with half-log intervals. Each point was determined in duplicate. As a known standard, the Banyu cyclic pentapeptide BQ-123 was synthesized and shown to have IC_{50}=14nM in this assay.
11. While we have inexact HPLC evidence of the equimolar character of this mixture, deconvolution of the Asp example gave comparable yields of each of its 19 components.
12. Replicate IC_{50} values were determined for some of these mixtures. In all cases, values were reproduced within 25%. This consistency is a reflection of the 10-point method employed for IC_{50} values and use of weighed samples in the assay.
13. HPLC purity >97% (except for His) obviated purification. Analytical HPLC employed a Vydac 218TP54 column and 25-80% MeCN-H2O (each 0.1% TFA) gradient over 30 min.
14. Racemic H-BHG-OH was prepared according to reference 3b and converted to Fmoc-BHG-OH.
15. This magnitude increase in potency is significant and reproducible.
16. We thank a referree for pointing out the sarafotoxin sequence (*Br. J. Pharmacol.* **1990**, *101*, 232).
17. A similar combinatorial study has been based upon the Fujisawa endothelin antagonist FR-139,317, a modified pentapeptide. Terrett, N. K.; Bojanic, D.; Brown, D.; Bungay, P. J.; Gardner, M.; Gordon, D. W.; Mayers, C. J.; Steel, J. *BioorgMed. Chem. Lett.* **1995**, *5*, 917.

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