REVIEW

Peptide and Peptidomimetic Libraries

Molecular Diversity and Drug Design

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Abstract

Various techniques for generation of peptide and peptidomimetic libraries are summarized in this article. Multipin, tea bag, and split-couple-mix techniques represent the major methods used to make peptides and peptidomimetics libraries. The synthesis of these libraries were made in either discrete or mixture format. Peptides and peptidomimetics combinatorial libraries were screened to discover leads against a variety of targets. These targets, including bacteria, fungus, virus, receptors, and enzymes were used in the screening of the libraries. Discovered leads can be further optimized by combinatorial approaches.

Index Entries: Combinatorial libraries; peptides; peptidomimetics; molecular diversity.

1. Introduction

Peptide and peptidomimetic research has significantly evolved over the past several decades as the result of new concepts and strategies in chemistry, biology, and related technologies. In recent years, the impact of automated technologies to accelerate peptide-based lead identification for receptors, peptidases, signal-transduction proteins, and other targets of therapeutic interest has resulted in the discovery of a plethora of novel compounds. In this review, we describe a few standard methodologies that have been developed and applied to the discovery (generation and/or optimization) of prototype lead compounds and drug candidates. Such studies exemplify the impact of peptide and peptidomimetic libraries on molecular diversity, drug design, and the so-called "paradigm shift" in drug discovery.

2. General Combinatorial Library Technologies

2.1. Multipin (MP) Method

This approach was originally introduced by Geysen and co-workers and is readily adapted to a 96-well format (1). The general strategy is summarized below (**Fig. 1A**). This technique is used for parallel synthesis of peptides on tips of polypropylene pins using classical solid phase proce-

dures. Multipin strategies also have been used to generate peptidomimetic libraries and optimization of organic synthetic methods for combinatorial chemistry development (2). The MP technique can be used to prepare ≤ 0.5 μmol of peptide per pin.

2.2. Tea Bag (TB) Method

This method was introduced by Houghten and co-workers (3) and is an extension of classical solid-phase peptide synthesis (4). The general strategy is described below (**Fig. 1B**). In this technique the solid support is partitioned into polypropylene "tea bags" and the resin can be immersed in a reaction mixture containing a single or multiple activated amino acids. The washing, deprotection, and cleavage steps to remove orthogonal protecting groups and release of final products from resin can be accomplished using several tea bags. The TB technique also can be used to make a several milligrams of peptides or peptidomimetics of interest.

2.3. Split-Couple-Mix (SCM) Method

Another version of multiple solid-phase synthesis of peptides is the split-couple-mix method as first described by Furka and co-workers (5,6). The one peptide (compound)-one bead method developed by Lam and co-workers (7) can be used

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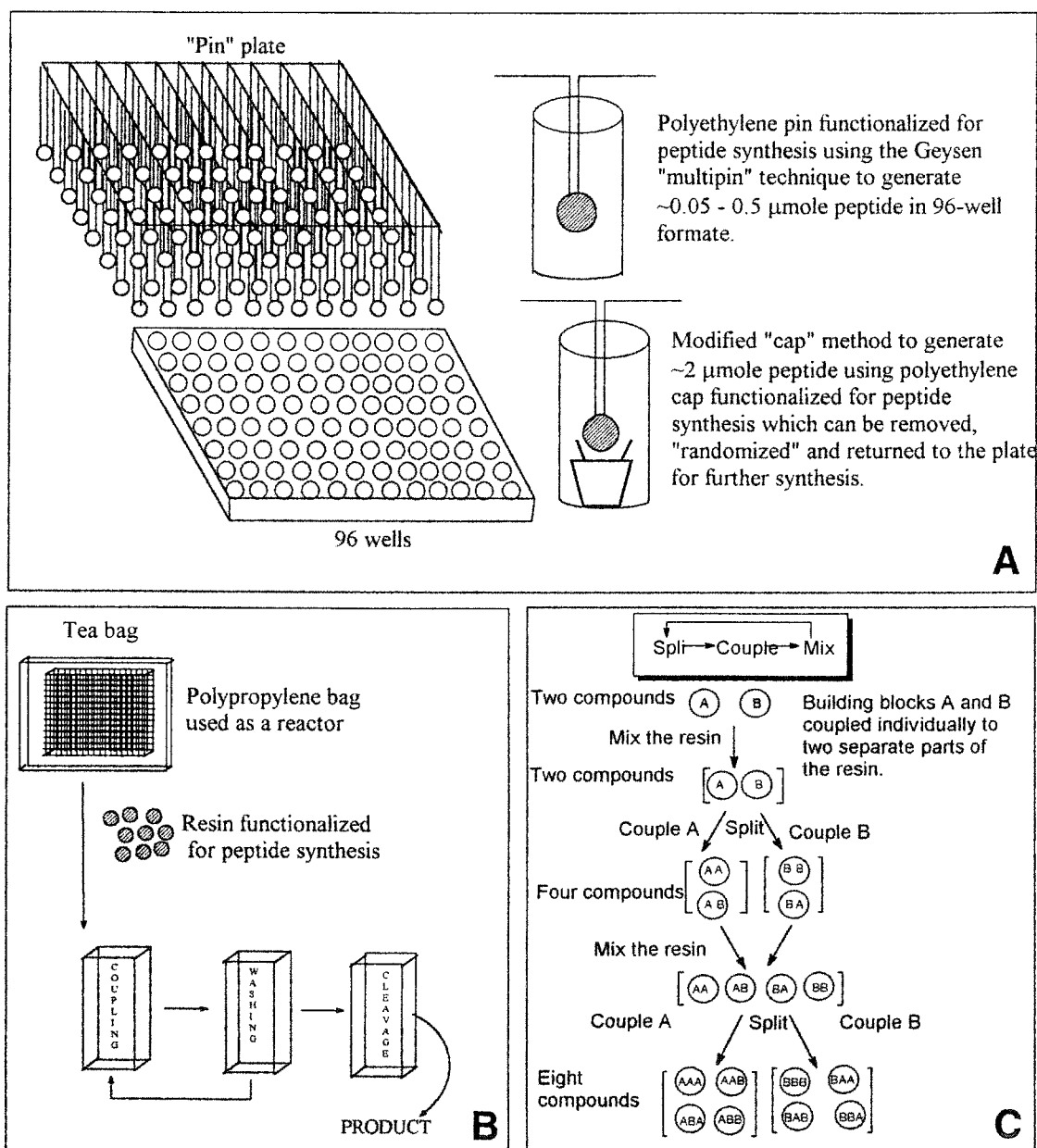
Fig. 1. The multipin (MP) method. (A) "Pin" plate, (B) tea bag (TB), (C) split-couple-mix (SCM).

for the generation of very large combinatorial libraries of peptides or peptidomimetics (>10^5–10^6 compounds). The SCM technique is illustrated in **Fig. 1C**.

2.4. Other Technologies

Other technologies for generating combinatorial libraries of peptides and peptidomimetics have been developed *(8)*. One non-chemical technology that has significantly impacted the generation of peptide (or protein) libraries for receptor- or enzyme-targeted drug discovery is phage display which utilizes recombinant DNA methods *(9)*. It has been frequently used for the discovery of novel peptide (or protein) ligands for receptors and enzymes. The phage display method will not be discussed here as this review will be

Table 1
Structures of Anti-Infective Leads from Peptide Libraries

No.	Structure	Target	IC_{50} $(\mu g/mL)^a$	Ref.
1	Ac-Arg-Arg-Trp-Trp-Cys-Arg-NH$_2$	*Staph. aureus*	3.4	*11*
2	Fmoc-Lys(NeTrp-D-Phe-Arg-NH$_2$)	*Staph. aureus*	2.0	
		Staph. sanguis	2.0	
3	Ac-Arg-Arg-Trp-Trp-Arg-Phe-NH$_2$	*E. coli*	8.0	
4	Ac-Arg-Arg-Trp-Trp-Arg-Arg-NH$_2$	*C. albicans*	28.0	
5	MePhe-MePhe-MeIle-MePhe-MePhe-MePhe	*Staph. aureus*	6.0	*12*
		Staph. aureus	7.0	
6	MePhe-MePhe-MePhe-MePhe-MePhe-MePhe	MRSA^b	7.0	
		Strep. sanguis	9.0	
7	MePhe-MePhe-MeIle-MePhe-MeHis-MePhe	*Staph. sanguis*	1.3	

^{a}Only compounds with highest activity (lowest IC_{50}) was selected.
^{b}MRSA is a mehthacillin resistant *Staph. aureus*.
Note: MePhe, MeIle, and MeHis refer to N^α-methylamino acids.

focused on peptide and peptidomimetic libraries prepared by chemical methods.

3. Examples of Peptide and Peptidomimetic Library Studies

3.1. Antibacterials and Antifungals

Using a combinatorial approach with peptide libraries, several potent antibacterial leads *(10–12)* have been discovered that were effective against gram-positive bacteria (*Staphylococcus aureus, Streptococcus sangis*), gram-negative bacteria (*Escherichia coli, Pseudomonas aeruginosa*) and fungi (*Candida albicans*). The chemical structure of the library and lead compounds are shown in **Table 1**. The synthesis of the library used a combination of the TB and SCM techniques. The library was a hexapeptide $O_1O_2X_1X_2X_3X_4$ with two positions fixed (O) and four positions randomized (X). In each X position, 19 proteinogenic amino acids (excluding Cys) were incorporated, and at each O position 20 amino acids were individually substituted. The sublibraries were modified by acylation, alkylation, reductive alkylation, and so forth. The final lyophilized powders of sublibraries bioassayed against selected targets of micro-organisms and the positive leads identified by iterative deconvolution synthesis. Several anti-infective leads were identified from both peptide and peptidomimetic libraries (**Table 1**). Significant differences in chemical structures of lead compounds

were determined using peptide libraries vs peptidom-imetic libraries (cf. compounds 1 and 6).

3.2. Vaccines and Epitope Mapping

Peptide vaccines have advantages over traditional vaccines, including easy preparation, safety and low liability, and high selectivity. Combinatorial libraries of peptides and peptidomimetics have been used for epitope mapping of several immunogens *(13–17)*. A few studies are detailed below, including the chemical structures of the libraries and/or lead compounds discovered.

3.2.1. Measles Virus (MV)

A heptamer combinatorial library was synthesized on polystyrene resin linked with stretches of polyethylene glycol *(18)*. Fmoc-chemistry and SCM methods were used to synthesize the library having 19 proteinogenic amino acids (excluding Cys) at each position. The deprotected peptide library was screened against monoclonal antibody (MAb) F7-2, which, in turn, was specific for the F protein of measles virus (MVF). Incubation of aliquots of the library beads with MAb using histochemical staining for visualization gave several positive beads. Sequencing of the active beads led to identification of several peptide sequences. Only one of the identified sequences (compound 1 in **Table 2**) was capable of inducing an antibody response with protection against fatal encephalitis caused by infection with MV. This result suggests that the mimeotope Asn-Ile-Ile-

Table 2
Structures of Epitope and Mimeotopes Leads from Peptide Libraries

No.	Target	Potency	Structures[a]	Ref.
1	MVF		Asn-Ile-Ile-Arg-Thr-Lys-Lys-Gln	*14*
2	Tab2 MAb[b]	$Kd = 8 \times 10^{-8} M$	Val-Ser-His-Phe-Asn-Asp	*16*
3	T-cell epitope		Lys-Gly-Tyr-Ala-Trp-Gln-Tyr-Thr	*17*
4	MHCII-hDRB1*0101	$IC_{50} = 70\ \mu M$	X_1-X_2-X_3-X_4-X_5-X_6-X_7-X_8-X_9-X_{10}-X_{11}	*18*
		$IC_{50} = 20\ \mu M$	X_1-**Phe**-X_3-X_4-X_5-X_6-X_7-X_8-X_9-X_{10}-X_{11}	
		$IC_{50} = 15\ \mu M$	X_1-X_2-X_3-X_4-**Met**-X_6-X_7-X_8-X_9-X_{10}-X_{11}	
		$IC_{50} = 1.2\ \mu M$	Ser-Phe-His-Thr-Met-Ser-Ala-Ala-Lys-Leu-Ile-NH_2	
5	N.03 MAb to JEV[c]	$IC_{50} = 9 \times 10^{-6} M$	Tyr-Gly-Gly-Ile-Tyr-Met-Asn-Gly	*20*
	N.08 MAb to JEV	$IC50 = 9 \times 10^{-5}$	Gln-Trp-Tyr-Asp-Asp-Arg	

[a]X refers to variable amino acids.
[b]Tab2 MAb antibody for transforming growth factor-alpha (TGFα).
[c]N.03 and N.08 MAbs against Japanese encephalitis virus (JEV).

Arg-Thr-Lys-Lys-Gln can adapt a conformation capable of inducing a polyclonal antibody (PAb) reaction with sufficient affinity and specificity to achieve virus neutralization and, thus, providing a potential synthetic peptide vaccine candidate.

3.2.2. Transforming Growth Factor-α Antibody

A hexamer peptide library $X_1O_1O_2O_3O_4X_2$ (immobilized on a cellulose membrane) was prepared to test against the Tab2 antibody for TGF-α as well as for comparison against sequences from phage-display libraries *(19)*. In the synthetic library, position X was randomized with 17 proteinogenic amino acids (excluding Cys, Trp, and Met) and position O represents specific amino acids that predetermined to be one of several known clusters (e.g., Arg/Pro/Gly, His/Lys/Arg, Phe/Tyr/Trp). The library was prepared by so-called spot synthesis on Whatman 540 paper using mixture coupling. The cellulose-bound libraries were incubated with the Tab2 antibody against (TGF-α), and the binding was visualized using an alkaline phosphatase-labeled secondary antibody. The sequences of the positive leads were determined and compared to the sequences derived from phage-display libraries. Both libraries gave similar results, although more variation in the structures of leads was observed for the chemical library (*see* Table 2).

3.2.3. T-Cell Epitope

In an approach to T-cell epitope determination *(20)*, an octapeptide library $X_1O_1X_2O_2X_3O_3X_4O_4$-NH_2 was synthesized by the SCM technique. Mix-

ture coupling at randomized positions X utilized 19 proteinogenic amino acids (excluding Cys) and single amino acids were coupled at each defined position O. The positionally randomized and defined amino acids were alternated within the sequence of the peptide library. Thus, eight sublibraries were synthesized with 19 amino acids in each defined position, and screening was performed using a whole-cell bioassay. It was found that the identified synthetic epitopes were unique relative to the natural epi-topes (*see* Table 2), and application to future vaccine development was described.

3.2.4. Human Leukocyte Antigen MHC II

Both randomized combinatorial libraries *(21)* and positional scanning peptide libraries *(22)* have been designed, synthesized and tested against a human leukocyte antigen class II (MHC II) molecule DRB1*0101. Each randomized position (X) was coupled with a mixture of 20 proteinogenic amino acids (except for Cys, which was replaced by α-aminobutyric acid). Thus, the totally randomized library had a complexity of 20^{11} different peptides. A total of 220 sublibraries (20^{10} different peptides) for defined positions (O) were prepared using a Rink resin, and the peptide mixtures screened in solution by competition with fluorescently labeled peptide binding to purified hMHC-DRB1*0101 molecule (HLA-DR1). The IC_{50} values of the tested sublibraries were determined and several leads were identified (*see* Table 2).

Library Structure:
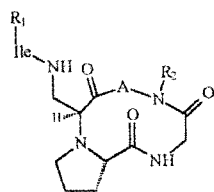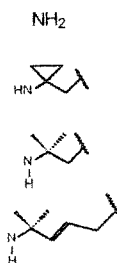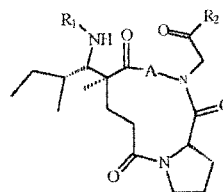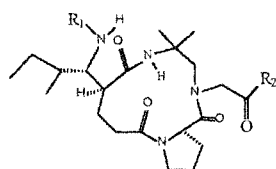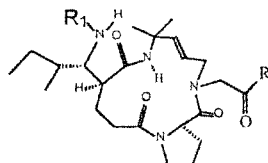
Sublibrary I   A   Sublibrary II

R1 = Gly-Pro-Lys-Glu-Pro-Phe-Arg-Asp-Tyr-Val-Asp-Arg-Phe-Tyr-Lys-Asn-Lys-Arg
-Lys-Arg-Ile-His
R2 = Arg-Ala-Phe

Leads:

Compound A   Compound B

Fig. 2. Conformationally modified peptide library for immunogen against HIV V3 loop.

3.2.5. Japanese Encephalitis Virus (JEV)

A totally randomized pentamer peptide library $(X_1X_2X_3X_4X_5)$ was prepared by the SCM technique and tested against two clones of MAbs N.03 and N.08 directed against JEV (23). Each randomized position (X) had 20 proteinogenic amino acids to give 20^5 possible different peptide components. The library was screened on beads and the first generated leads identified were subsequently optimized by secondary libraries to give highly active epitopes against both antibodies N.03 and N.08 (*see* **Table 2**). The identification of these mimeotopes was described to be useful in the development of a novel type of peptide vaccine against this virus.

3.2.6. Human Immunodeficiency Virus (HIV) V3 Loop

HIV infection of cells follows binding of the viral envelope glycoprotein gp120 to the cell-surface receptor CD4, and this event is initiated by thrombin or Factor Xa cleavage of the V3 loop of

gp120 at Arg315-Ala316. From analysis of the possible mechanisms of cleavage of the V3 loop by thrombin, a combinatorial peptide library was designed that contained peptidomimetic replacements of the cleavage-site sequence Ile-Gly-Pro-Gly-Arg as based on a parent linear immunogen peptide (24) The library was screened against gp120 binding to the V3 loop using a MAb 50.1, directed to V3. Two compounds were identified as leads (cf. compounds A and B in **Fig. 2**), and one was selected for further evaluation as a potential vaccine.

3.3. Receptor Antagonists and Agonists

3.3.1. Bradykinin B₂ Receptor

Using structure-based design, the synthesis of peptidomimetic libraries to discover novel bradykinin B_2 receptor antagonists has been reported (25). The synthesis of 10 libraries was achieved by using the SCM method, and the pool size was adjusted to give 25 components in each directed library (**Fig. 3**). The sublibraries were bioassayed for

Library Structure:

$\underline{D}$-Arg-Arg-X$_1$-X$_2$-Arg

X$_1$ and X$_2$ = Various combination of different building blocks (see below).

Examples:

Lead:

Fig. 3. Nonpeptide library for bradykinin B$_2$ receptor antagonists.

Library Structure:

$\boxed{X_1X_2X3O\text{-}\underline{D}\text{-}Asp}$

O = Pro,Val,Leu,Trp,Arg,Glu
X = Pro,Val,Leu,Trp,Arg,Glu

Leads :

Pro-$\underline{D}$-Pro-Leu-$\underline{D}$-Trp-$\underline{D}$-Asp

I

Pro-$\underline{D}$-Pro-Trp-$\underline{D}$-Trp-$\underline{D}$-Asp

II

Pro-$\underline{D}$-Val-Leu-$\underline{D}$-Trp-$\underline{D}$-Asp

III

Pro-$\underline{D}$-Val-Trp-$\underline{D}$-Trp-$\underline{D}$-Asp

IV

Fig. 4. Cyclic peptide library for endothelin receptor antagonists.

binding to human B$_2$ receptor (vs [^{3}H]bradykinin). Each sublibrary yielded positive leads, and upon deconvolution a potent compound (K$_i$ = 80 n*M*) was identified.

3.3.2. Endothelin Receptor

A cyclic pentapeptide library was designed to give 48 mixtures (sublibraries) containing 82,944 possible head-to-tail cyclized peptides *(26)*. The peptides were cleaved from a hydroxymethyl resin and partially purified by C$_{18}$ reversed-phase-high-pressure liquid chromatography (RP-HPLC).

Solution screening of the cyclic peptide sublibraries was conducted using a radioligand binding assay. Four lead compounds were identified and their structures confirmed by re-synthesis (**Fig. 4**). A previously reported ET antagonist BQ-123 *(27)* was included among the most potent leads.

3.3.3. Fibrinogen Receptor (GPIIb/IIIa)

A peptidomimetic library incorporating a ß-turn scaffold was designed, synthesized, and tested for the identification of novel gpIIb/IIIa receptor antagonists *(28)*. More than 200 analogs

Library structure :

n = 0, 1, 2 or3

B =CH$_2$CH$_2$CO, CH$_2$OCO, CH$_2$NHCO,
CH$_2$CH$_2$SO$_2$

A = CH or N

R = 12 different side chains

Lead:

RWJ-50042

Ki (dog) = 0.41 uM (R-enantiomer)

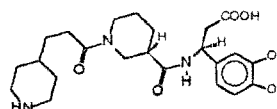*Optimized Lead:*

Ki (dog) = 0.015 uM

half-life (dog) > 180 min.
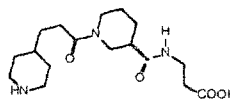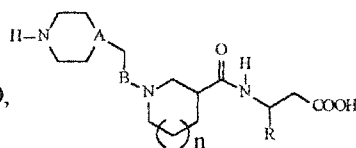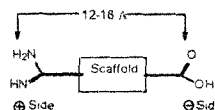
Fig. 5. Nonpeptide library for gpIIb/IIIa receptor antagonists.

Central Constraint Concept :

Structure of the Library:
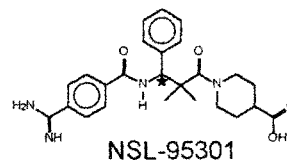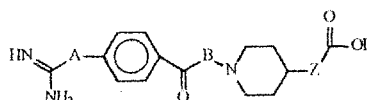
A = O, NH, CH$_2$NH

B = 21 variants

Z = O, CH$_2$, OCH$_2$

Leads:

(+) isomer IC$_{50}$ =0.092 mM

(-) isomer IC$_{50}$ = 31 mM

NSL-95301

Fig. 6. Nonpeptide library for gpIIa/IIIb receptor antagonist.

were prepared using solid-phase parallel synthesis, and several compounds were identified for further testing as preclinical leads (**Fig. 5**).

In another approach, a peptidomimetic library was designed using a central constraint concept to identify, and used to optimize novel gpIIb/IIIa receptor antagonists *(29)*. Synthesis of the library was performed by Fmoc-chemistry using solid phase methods, and HPLC purified compounds were screened. This led to the discovery of the lead compound NSL-95301 which was potent and selective for the gpIIb/IIIa receptor (**Fig. 6**).

3.3.4. μ-Opioid Receptor

A so-called "peptoid" library containing N-substituted Gly building blocks was screened for

binding to the μ-opioid receptor *(30)*. A structurally novel lead compound was discovered having 6 nM affinity to the μ-opioid receptor (**Fig. 7**). Of particular significance was that these compounds provided metabolically-stable analogs, and the N-substituent could be varied to include either amino acid-like sidechain moieties or quite different chemical groups.

3.4. Proteases and Metabolic Enzyme Inhibitors

Potent and selective enzyme inhibitors have long been recognized as important biochemical tools and/or therapeutic agents. Several discoveries of enzyme inhibitors or substrates have been accomplished recently using combinatorial peptide or peptidomimetic libraries as exemplified below.
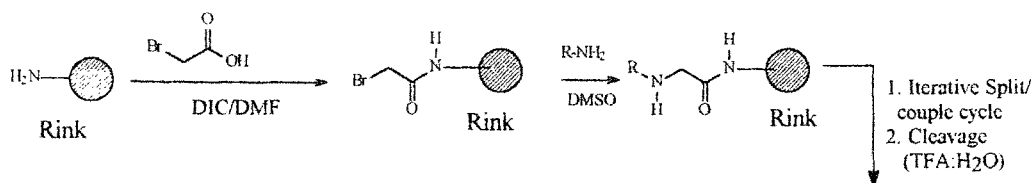
Library Structure
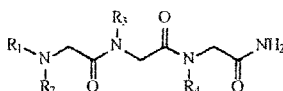
R_1 = 3 building blocks

R_2 = R_3 = R_4 = 21 possible alkyl, hydroxyalkyl, aryl or heteroaryl groups

Synthesis : SCM method on Rink-polystyrene resin.

Complexity of the library: 500 compounds per mixtures.
(18 sublibraries)
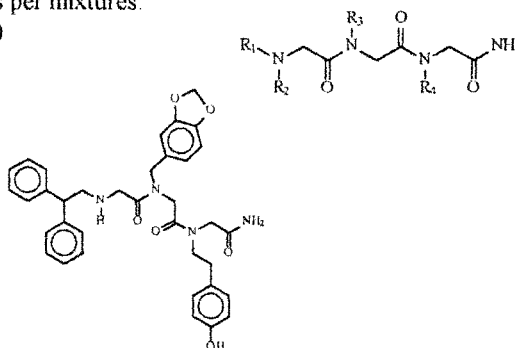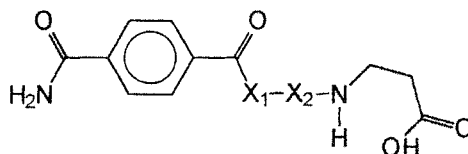
Lead:

CHIR4531
K_i = 6 nM

μ-opiate receptor

Fig. 7. NSG peptoid library for G-protein-coupled receptor agonists and antagonists.

Sublibrary I: X_1 and X_2 = 17 L-Amino Acids = 289 compounds
(excluding Cys,Met,Trp)

Sublibrary II: X_1 and X_2 = 16 D-Amino Acids = 256 compounds
(excluding Cys,Met,Trp,Ile)

Fig. 8. Dipeptide library for carbonic anhydrase inhibitors.

3.4.1. Carbonic Anhydrase

A dipeptide library containing 17 protein-ogenic amino acids (excluding Cys, Met, and Trp) at positions AA_1 and AA_2, and a second dipeptide library containing 16 D-amino acids (excluding Cys, Met, Trp, and Ile) were designed and synthesized *(31)*. Both libraries were screened in solution using electrospray ionization-mass spectrometry (ESI-MS). A tight binding inhibitor con-

taining Leu in both positions AA_1 and AA_2 was identified (**Fig. 8**).

3.4.2. Neurolysin

Discovery of selective and potent inhibitors of the zinc endopeptidase neurolysin was achieved by using combinatorial chemistry of peptido-mimetics incorporating phosphinic acid "transition state" replacements *(32)*. A lead compound (**Fig. 9**) was found having high affinity (K_i = 4 n*M*)
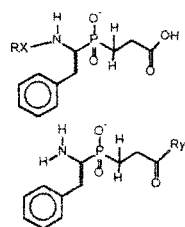
Rx = 20 different amino acids (Cys replaced by Nle)

Ry = 20 different amino acids (Cys replaced by Nle)

Rx-D/L-PheΨ(PO_2CH_2)Gly-Ry

Tripeptide = 40 different peptides per mixture
Tetrapeptide = 40 different peptides per mixture
Lead: Pro-PheΨ(PO_2CH_2)Gly-Pro
K_i = 4 nM against Neurolysin
K_i = 8100 nM against Thimet Oligopeptidase

Fig. 9. Peptidomimetic library for zinc endopeptidase neurolysin inhibitors.

Leads:

Cbz-(R,S)-PheΨ[P(O)O(OMe)]Leu-Ala-OH K_i = 122 nM

Cbz-(R,S)-PheΨ[P(O)O(OMe)]Leu-Ala-NH_2 K_i = 49 nM

Cbz-(R,S)-PheΨ[P(O)O(OMe)]Leu-Arg-NH2 K_i = 64 nM

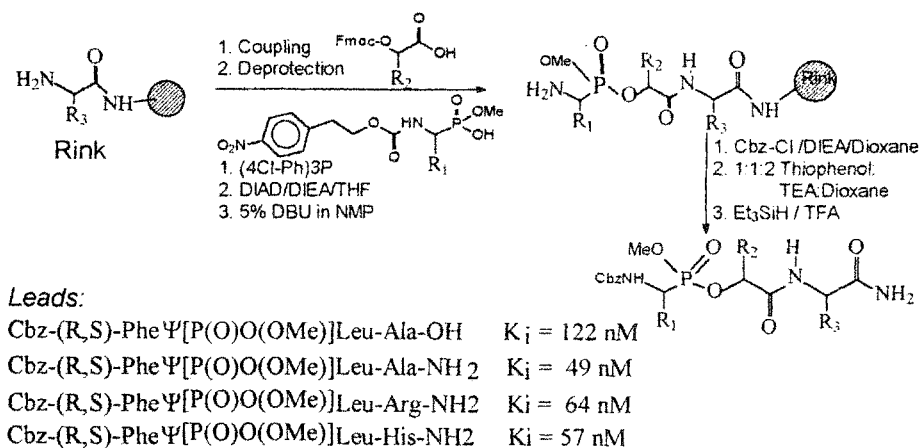Cbz-(R,S)-PheΨ[P(O)O(OMe)]Leu-His-NH2 K_i = 57 nM

Fig. 10. Peptidomimetic library for thermolysis inhibitors.

and marked selectivity (>2000-fold vs other zinc endopeptidases).

3.4.3. Thermolysin

Another zinc endopeptidase, thermolysin, was targeted by a library of peptidomimetics containing phosphonate "transition state" replace-ments *(33)*. A total of 540 compounds were synthesized using the SCM method. The library was designed to target the P_1-P_1'-P_2' sites as related to the substrate sequence. Six pools of peptidylphosphonate sequences with 90 possible compounds per mixture were generated, and several potent lead compounds (K_i ~ 50 nM range) were identified (**Fig. 10**).

3.4.4. Factor Xa (FXa)

FXa is a serine protease that plays a central role in vascular hemostasis *(34)*, and it has been identified recently as a promising target for drug discovery *(35)*. A synthetic resin-bound octapeptide library was screened against FXa (**Fig. 11**), and several lead compounds were identified by Edman sequencing of the positive beads *(36)*. Each of the peptide leads were found to contain a tripeptide motif of Tyr-Ile-Arg. The key lead compounds were highly selective against FXa as they were essentially inactive against other serine proteases involved in blood coagulation.

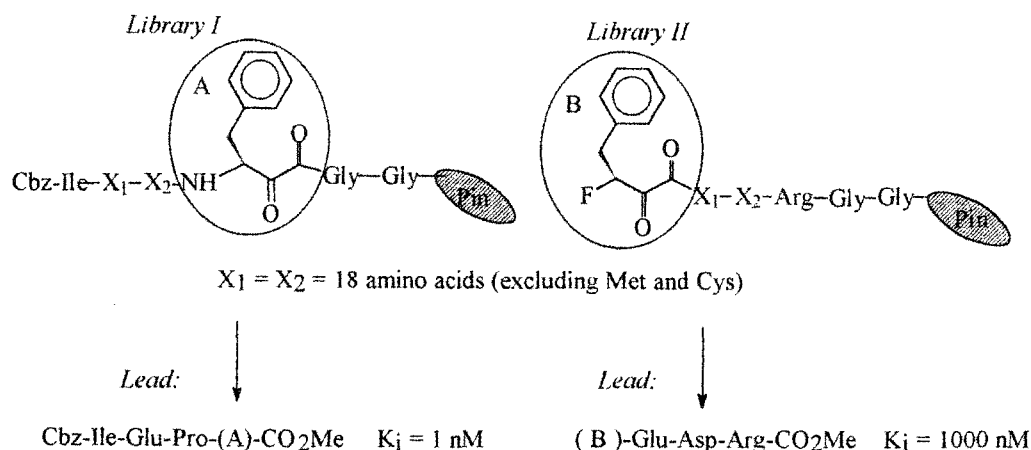Fig. 11. Peptide library for factor Xa inhibitors.

Fig. 12. Peptidomimetic libraries for chymase inhibitors.

3.4.5. Human Heart Chymase

Human-heart chymase is a chymotrypsin-like enzyme that converts angiotensin I to angiotensin II. Two peptide libraries containing either 3-fluorobenzylpyruvamide or α-keto-amide type Phe mimetics were prepared (37) by the MP method (**Fig. 11**). The library containing 3-fluorobenzylpyruvamide was modified at the P₁, P₁', and P₂' positions relative to the substrate sequence, whereas a second library containing a Phe α-keto-amide was modified at the P₁, P₂, and P₃ positions Several lead compounds were identified having inhibitory activities in the 1 nM–1 µM range (**Fig. 12**).

3.4.6. Chymotrypsin

Using the anti-tryptic loop region of the Bowman-like inhibitor, D4 from *Macrotyloma axillare*, as a template a cyclic peptide library was synthesized (38). The on-bead cyclic peptide library was screened by incubation with biotinylated α-chymotrypsin, and positive beads were sequenced to determine the structure of the lead compounds. Lead compounds were confirmed by re-synthesis, and several highly potent inhibitors of α-chymotrypsin were dis-

covered (**Fig. 13**). Thus, a naturally-occurring protein inhibitor of α-chymotrypsin was rapidly converted to a series of simplified cyclic peptide "second generation" leads.

3.4.7. Glycosomal Phosphoglycerate Kinase (gPGK) and Fructose-1,6-Bisphosphate Aldolase

Trypanosoma bruci, the causative factor of sleeping sickness, is very susceptible to compounds interfering with glycolysis owing to the high glucose consumption and the lack of other sources of metabolic energy. In one approach, a peptide library was designed and screened (39) to provide a prototype inhibitor (IC₅₀ = 80 µM) against gPGK (**Fig. 14**). Another pentapeptide library containing D-amino acids was screened against fructose-1,6-biphosphate aldolase (40), and several positive hits were identified (**Fig. 14**).

3.4.8. HIV Protease

A tetrapeptide library was prepared (41) to identify lead compounds having high affinity against the HIV protease. The synthetic library Ac-X₁X₂-Sta-X₃-NH₂ was prepared using the SCM method and a potent HIV protease inhibitor
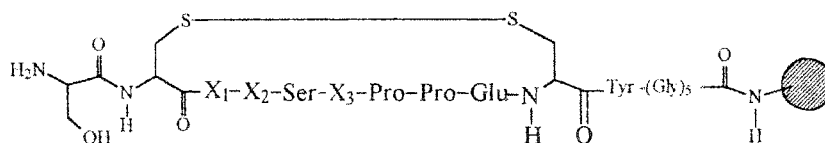
X$_1$ = X$_2$ = X$_3$ = 20 amino acids (Cys replaced by Nle)
Library Synthesis: Fmoc-chemistry using SMC technique.
Complexity : 8000 peptides.

Leads:	Ki (nM)
Ser-Cys-**Thr**-**Phe**-Ser-**Ile**-Pro-Prp-Glu-Cys-Tyr-(Gly) 5-NH$_2$	19
Ser-Cys-**Thr**-**Phe**-Ser-**Ieu**-Pro-Prp-Glu-Cys-Tyr-(Gly) 5-NH$_2$	20
Ser-Cys-**Thr**-**Phe**-Ser-**Nle**-Pro-Prp-Glu-Cys-Tyr-(Gly) 5-NH$_2$	19
Ser-Cys-**Thr**-**Tyr**-Ser-**Ile**-Pro-Prp-Glu-Cys-Tyr-(Gly) 5-NH$_2$	17

Fig. 13. Peptide library for chymotrypsin inhibitors.

Library Structure: X$_1$X$_2$X$_3$X$_4$X$_5$- resin
X = 19 proteinogenic amino acids (excluding Cys).

Library Synthesis: SCM method
Screening: On bead screening using FITC or Biotin labelled gPGK.
Lead: Asn-Trp-Met-Met-Phe IC$_{50}$ = 80 mM

Fig. 14. Peptide library for glycosomal phosphoglycerate kinase (gPGK) inhibitors.

was discovered having the sequence Ac-Trp-Val-Sta-D-Leu-NH$_2$ (**Fig. 15**).

3.4.9. Matrix Metalloproteinase

The inhibition of matrix metalloproteinases MMP-3 and MMP-8 using a N-carboxyalkyl-modified peptide library was conducted to identify leads and integrate structure-based drug design to further explore the binding of selected inhibitors using molecular modeling and an X-ray structure of the target enzyme *(42)*. A potent inhibitor of both MMP-3 and MMP-8 (Ki's of 148 n*M* and 1.9 n*M*, respectively) was designed with a lipophilic aromatic group replacement for a methyl group (**Fig. 16**).

3.5. Signal Transduction Protein Antagonists, Substrates, and Inhibitors

3.5.1. Src Homology-2 (SH2) Domains

A combinatorial peptide library was synthesized by the SCM technique using small diameter resin beads (9.7 μ) to permit screening by flow

cytometric processing *(43)*. This process has an advantage of a high sample throughput (>1000 beads/s). The peptide library was screened against target SH2 domains using (FITC)-labeled antibodies recognizing an SH2-glutathione-S-transferase (GST) fusion construct protein. The structures of peptides recognized by the SH2 domain were determined by Edman sequencing. A pool sequencing approach was used owing to the low concentration of peptide (~8 fmol/bead), and subsequent Edman sequencing provided structural identification following alignments with phosphotyrosine and deconvolution. Phospho-peptides specific for Grb2 and Syk SH2 were discovered and showed p-Tyr-Glu-Asn-ASP and pTyr-Glu-Glu-Leu, respectively, as preferred motifs (**Fig. 17** and **Table 3**).

Randomized peptide libraries were designed and synthesized to study the specificity of Lim2 and Lim3 domains of cytoplasmic Enigma protein and their interactions with receptor tyrosine kinases *(44)*. Screening using radioac-

$X_1-X_2-Sta-X_3-NH2$

$X_1 = X_2 = X_3 = 22$ amino acids : Asp, Phe, His, Ile, Lys, Met, Pro, Gln, Arg, Ser, Trp, Tyr;
D- Ala, D-Asn, D-Glu, D-Phe, D-Lys, D-Leu, D-Pro, D-Val, D-Trp, D-Tyr

Sta
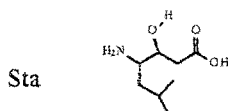
Library Synthesis: SCM technique.

Complexity: 10648 different tetrapeptides amides per each of the 22 sublibraries.
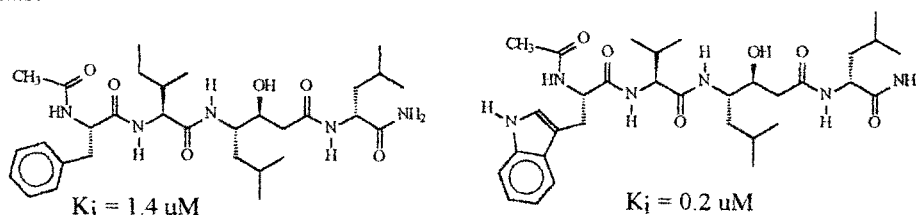
Leads:

$K_i = 1.4$ uM $K_i = 0.2$ uM

Fig. 15. Peptidomimetic library for HIV-1 Protease inhibitors.

Library synthesis:

library

1. Pyruvic acid /HBTU

2. HCl.H₂NCH(R₁)CO₂Fm
NaCNBH₃/DIEA/HOAc/DMF
3. 20% piperidine

1. PFP-OH/DIC

2. R₂-NH2

$R_2NH_2 > 100$ amines

1.screening
2.resynthesis
3.docking

Lead:

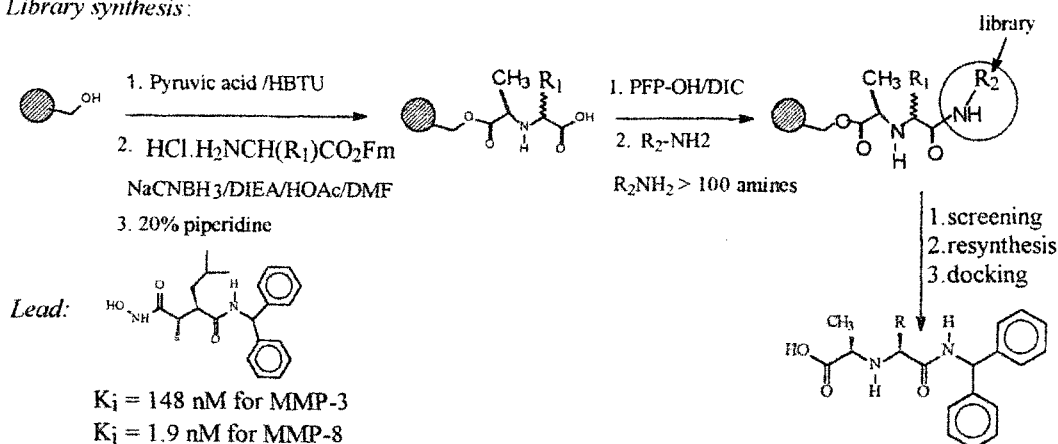$K_i = 148$ nM for MMP-3
$K_i = 1.9$ nM for MMP-8

Fig. 16. Peptidomimetic library for MMP inhibitors.

tive aden-osine triphosphate (ATP) as source of phosphate in combination with Lims domains resulted in the discovery of the phosphorylated peptide Glu-Glu-Gly-Pro-pTyr-Gly-Pro-Val-Phe, which was determined to be an effective ligand for the Lim2 and Lim3 SH2 domains (**Table 3**). Finally, the phosphopeptide libraries have been screened to identify specific ligands for the SH2 domains of Shc, Crk and Nck as summarized in **Table 3** *(45).*

3.5.2. Transforming Growth Factor-β (TGFβ) Receptor Ser/Thr Kinases

Type I and II receptors for the TGF-β are transmembrane Ser/Thr protein kinases that are essential for TGF-β signaling. A combinatorial peptide library was designed to determined the specific substrates for the TGF-β receptor kinases *(46).* Synthesis of the library was performed on a hydrophilic carrier synthesized from porous polyethylene discs (**Fig. 18**), functionalized with a

Library Structure:

X_1 = 6 amino acids (Glu, Phe, His, Leu, Gln, Ser)
X_2 = X_4 = 19 amino acids (excluding Cys)
X_3 = X_5 = 7 amino acids (Asp, Gly, His, Pro, Arg, Thr, Trp)
X_6 = 7 amino acids (Glu, Leu, Asn, Gln, Arg, Ser, Tyr)
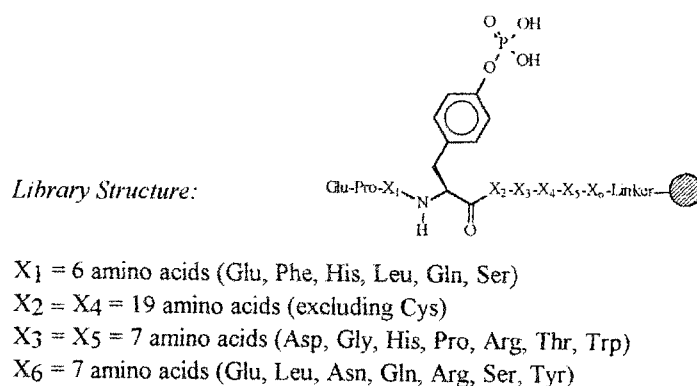
Library Synthesis: SMC technique
Complexity = 636,804 different peptides
Leads: see Table III

Fig. 17. Phosphopeptide library for SH2 domains.

Table 3
Structures of Ligands for SH2 Domains from Phosphopeptide Libraries

No.	Domain	Structures[a]	Ref.
1	Shc-SH2	Glu-Glu-Tyr-**pTyr**-Glu-X-Leu	*45*
2	Crk SH2	Glu-Asp-Tyr-**pTyr**-Asp-X-Pro	
3	Nck SH2	X-His-Tyr-**pTyr**-Asp-Asp-Val	
4	Shc PTB	Asn-Pro-X-**pTyr**-Phe-X-Arg	
5	Grb2 SH2	Glu-Pro-Phe-**pTyr**-Glu-Asn-Asp-Pro	*43*
6	Syk (C) SH2	Glu-Pro-Glu-**pTyr**-Glu-Glu-Leu-Asp	
7	Lim2 and Lim3 SH2	Glu-Glu-Gly-Pro-**pTyr**-Gly-Pro-Val-Phe	*44*

[a]The bold letter amino acid is the target amino acid, which is phosphorylated by the enzyme. X refers to variable amino acids.

Library Structure: X_1-O_1-O_2-Lys-O_3-O_4-Ser/Thr-Leu-Gly-Linker —

X = mixture of 19 amino acids (excluding Cys)
O = defined position containing one of 17 amino acids (excluding Ser,Thr,Cys)

Ser/Thr = mixture of Ser and Thr.
Library Synthesis: Fmoc-chemistry, SMC and mixture coupling in positions X.
The randomized position X was moved over the library with alternation with the
O position.
Complexity: 6612 compounds.
Leads: See Table IV

Fig. 18. Peptide library for Ser/Thr kinase substrates.

proper linker containing hydrophilic stretches of carboxymethyl-dextran. Ten peptide sublibraries were synthesized by permutation of randomized positions (X) and positions containing mixtures of amino acids (O). Screening was done by incubating the library (on discs) in kinase buffer containing 10 µCi of [γ-^{32}P]-ATP, and the ^{32}P-labeled discs were subsequently sequenced to identify the structure of the phosphorylated products of the substrate peptides. From these studies a decapeptide, Lys-(Lys)$_5$-Ser-$X_1X_2X_3$, was discovered that showed preferen-

Table 4
Structures of Protein Kinase Substrates from Peptide Libraries

No.	Protein kinase (PK)	Type of PK	Structures[a]	Ref.
1	Cam kinase II	Ser	Lys-Arg-Gln-Gln-**Ser**-Phe-Asp-Leu-Phe	*47*
2	Phosphorylase kinase	Ser	Phe-Arg-Met-Met-**Ser**-Phe-Phe-Leu-Phe	
3	CKI	Ser	Glu-Phe-Asp-Thr-Gly-**Ser**-Ile-Ile-Ile-Phe-Phe	
4	CKII	Ser	Glu-Asp-Glu-Glu-**Ser**-Glu-Asp-Glu-Glu	
5	P35/CDK4	Ser	Lys-His-His-Lys-**Ser**-Pro-Lys-His-Arg	
6	Erk1	Ser	Thr-Gly-Pro-Leu-**Ser**-Pro-Gly-Pro-Phe	
7	NIMA	Ser	Arg-Phe-Arg-Arg-**Ser**-Arg-Arg-Met-Ile	
8	TβRII	Ser/Thr	Lys-Lys-Lys-Lys-Lys-Lys-(**Ser/Thr**)-X-X-X	*46*
9	TβRI	Ser/Thr	Lys-Lys-Lys-Lys-Lys-Lys-(**Ser/Thr**)-X-X-X	
10	C-Fps/Fes (human	Tyr	Glu-Glu-Glu-Ile-**Tyr**-Glu-Glu-Ile-GLu	*49*
11	MT/c-Src (chicken)	Tyr	Glu-Glu-Glu-Ile-**Tyr**-Gly-Glu-Phe-Phe	
12	v-Src (chicken)	Tyr	Glu-Glu-Glu-Ile-**Tyr**-Gly-Phe-Phe-Asp	
13	Lck	Tyr	X-Glu-X-Ile-**Tyr**-Gly-Val-Leu-Phe	
14	c-Abl	Tyr	Ala-Glu-Val-Ile-**Tyr**-Phe-Phe-Leu-Phe	
15	EGF receptor	Tyr	Glu-Glu-Glu-Glu-**Tyr**-Phe-Glu-Leu-Val	
16	PDGF receptor	Tyr	Glu-Glu-Glu-Glu-**Tyr**-Val-Phe-Ile-Glu	
17	FGF receptor	Tyr	Ala-Glu-Glu-Glu-**Tyr**-Phe-Phe-Leu-Phe	
18	Insulin receptor	Tyr	X-Glu-Glu-Glu-**Tyr**-Met-Met-Met-Met	
19	PDGF receptor	Tyr	Ala-Ala-(Asn/Leu)-(Ile/Arg)-**Tyr**-Ala-Ala-Arg-Arg-Gly	*48*

[a]The bold letter amino acid is the target amino acid, which is phosphorylated by the protein kinase. X refers to a variable amino acid.

Library Structure:

X = 20 amino acids.

Ala-Ala-X₁-X₂-X₃—N   Ala-Ala-Arg-Arg-Gly-OH

Library Synthesis: SMC technique
Complexity = 8000 different peptides
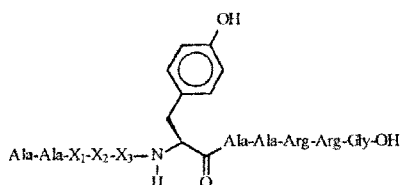*Leads*::see Table IV:

Fig. 19. Peptide library for PDGF receptor kinase substrates.

tial phosphorylation by both the TGFβR-I and TGFβR-II kinases (**Table 4**). At the X position, amino acid variability existed, but hydrophobic amino acids were predominant. Negatively charged amino acids at the X position resulted in inactive substrates for both TGFβR-I and TGFβR-II kinases.

3.5.3. Casein Kinases and Other Ser/Thr Kinases

The structural basis for the substrate specificities of casein kinase I and II, as well as several other Ser/Thr kinases, have been determined using peptide libraries *(47)* as summarized in **Table 4**.

3.5.4. Platelet-Derived Growth Factor Receptor (PDGF) Tyr Kinase

Recently, Chan et al. *(48)* described the use of a combinatorial peptide library with three degenerate positions at the site N-terminal to tyrosine for discovery of a specific substrate for PDGF receptor kinase. In this study, screening for phosphorylated substrate peptides was achieved using immunoaffinity chromatography to an immobilized monoclonal antiphosphotyrosine antibody. Several optimal substrates for PDGF receptor kinase were identified by HPLC purification and sequencing (**Fig. 19**).

Library Structure:

OH

Glu-Pro-X₁—N—X₂-X₃-X₄-X₅-X₆—Linker—
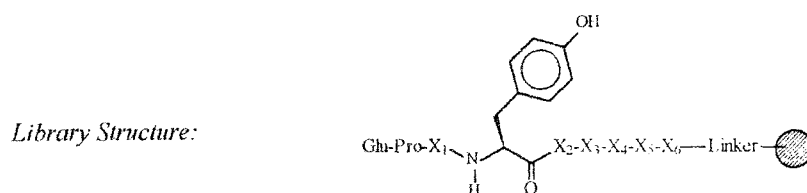
X₁ = 6 amino acids (Glu, Phe, His, Leu, Gln, Ser)
X₂ and X₄ = 19 amino acids (excluding Cys)
X₃ and X₅ = 7 amino acids (Asp, Gly, His, Pro, Arg, Thr, Trp)
X₆ = 7 amino acids (Glu, Leu, Asn, Gln, Arg, Ser, Tyr)

Library Synthesis: SMC technique
Complexity = 636,804 different peptides
Leads: see Table IV.

Fig. 20. Peptide library for insulin receptor kinase substrate.

3.5.5. Insulin-Receptor Tyr Kinases

Peptide libraries were prepared having randomized N- and C-terminal sequences relative to a fixed hydroxylated amino acid (Tyr, Ser, or Thr; **Fig. 20**) to examine the substrate specificity of nine protein kinases *(49)*. The libraries were prepared by the SCM technique, cleaved from the solid support, and subjected to screening by incubation with a protein kinase and [γ-^{32}P]-ATP. The phosphopeptides were isolated using diethylaminoethyl (DEAE)-Sephacel and ferric chelation chromatography, and their structure deduced by sequencing. Nine PTKs were tested by this method and their specific substrate sequences were identified (**Table 4**). As a specific example, the peptide Glu-Glu-Glu-Tyr-Met-Met-Met-Met was identified as a substrate for the insulin-receptor kinase.

3.5.6. Epidermal Growth Factor (EGF) Receptor Tyr Kinase

A combinatorial peptide library of the structure Glu-Val-Pro-Glu-Tyr-X₁X₂X₃X₄-Ser-Pro-Leu-Leu-Leu was synthesized for screening against both native and a mutant form (M857T) of the EGF receptor kinase *(50)*. Each randomized position (X) incorporated 18 proteinogenic amino acids (excluding Cys and Trp). The synthesis of the library was achieved by coupling a mixture of amino acids, cleaved from the solid support, and screened. Several lead compounds were identified for both mutant and wild-type

receptors. Substrate sequences included P_{+1} Glu (or Asp), P_{+2} Val, and P_{+3} Phe (Leu or Ile) at the C-terminal positions relative to the Tyr.

3.5.7. Src Tyr Kinase

A heptamer combinatorial peptide library was synthesized on polyethylene glycol grafted polystyrene beads by the SCM technique using Fmoc-chemistry *(51)*. Each random position incorporated 19 proteinogenic amino acids (Cys excluded), and the completed library was depro-tected and screened on beads against human pp60^{c-src} (Src) using auto-radiography technique to visualize ^{32}P-labeled beads. The labeled beads were sequenced, and several lead compounds were identified. The hepta-peptide Tyr-Ile-Tyr-Gly-Ser- Phe-Lys was identified as the most effective substrate (Km = 55 μM).

3.5.8. Ras Farnesyl Transferase

A tetrapeptide combinatorial library was screened against farnesyl protein transferase (FPT) and structurally novel inhibitors were discovered *(52)*. The design of the library was biased against the substrate peptide analogs in that both Cys and a C-terminal carboxylic acid were excluded. As shown in **Fig. 21**, the most potent FPT inhibitor discovered was D-Trp-D-Met-D-Phe(p-Cl)-Gla-NH₂ (Gla refers to γ-carboxyglutamic acid) which was >10,000-fold selective to inhibit FPT (Kᵢ = 2 nM) vs geranyl-geranyl-protein transferase type I (Kᵢ > 50 μM).

220   *Al-Obeidi, Hruby, and Sawyer*

Library Structure : X_1-X_2-X_3-X_4-NH_2

X_1 = X_2 = X_3 = X_4 = 68 amino acids (L- and D natural and unnatural; exceluding (Cys).
O = defined residue.
Library Synthesis : SCM using positional scanning approach.

O-X_2-X_3-X_4-NH_2
X_1-O-X_3-X_4-NH_2
X_1-X_2-O-X_4-NH_2
X_1-X_2-X_3-O-NH_2

Leads: D-Trp-D-Met-D-Phe(p-Cl)-Gla-NH_2 IC_{50} = 1.3 nM
          D-Trp-D-Met-D-Phe-Gla-NH_2 IC_{50} = 2.1 nM
          D-Trp-D-Trp-D-Phe(p-Cl)-Gla-NH_2 IC_{50} = 12.9 nM

          Gla = γ-carboxyglutamic acid.

Fig. 21. Peptide library for farnesyl-protein transferase inhibitors.

4. Summary and Future Developments

As exemplified in this review, peptide and peptidomimetic libraries have made a significant impact on both basic research and drug discovery. A variety of therapeutic targets have been examined using such libraries to identify receptor agonists or antagonists, peptidase inhibitors, and signal transduction protein antagonists or inhibitors In several cases, the application of peptide or peptidomimetic library methods have resulted in novel lead compounds for further drug design studies. The future of this area of research is extraordinary with respect to the use of novel building blocks (e.g., N-substituted Gly derivatives, β-amino acids, novel amino acids, aminobenzoic acids, and pseudodipeptides) to expand molecular diversity beyond naturally occurring amino acids (53). Similarly, secondary structural features of peptides (e.g., α-helix, β-turn, and β-sheet), as well as topographical features (54) may be explored by the use of peptide or peptidomimetic libraries for a variety of applications. In such cases, either linear or macrocyclic libraries may be tested. Solid-phase synthetic methods development, robotics and computational technologies are rapidly emerging to further advance peptide and peptidomimetic chemical diversity and drug design. Furthermore, the interface of peptide and peptidomimetic libraries with key biophysical technologies such as X-ray crystallography, nuclear magnetic resonance spectroscopy, mass spectrometry, and affinity capillary electrophoresis are noteworthy (55–58). In conclusion, the impact of peptide and peptidomimetic libraries to molecular diversity, drug design, and the so-called paradigm shift in drug discovery has been significant. Future applications to identify leads for an increasing number of therapeutic targets as projected from human genome studies (59) remains extremely promising, as well as applications to various aspects of drug development such as bioavailability, metabolism, and delivery of peptide or peptidomimetic therapeutic agents.

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