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Mini Review

Drugs from Slugs. Part II – Conopeptide bioengineering

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ABSTRACT

The biological transformation of toxins as research probes, or as pharmaceutical drug leads, is an onerous and drawn out process. Issues regarding changes to pharmacological specificity, desired potency, and bioavailability are compounded naturally by their inherent toxicity. These often scuttle their progress as they move up the narrowing drug development pipeline. Yet one class of peptide toxins, from the genus *Conus*, has in many ways spearheaded the expansion of new peptide bioengineering techniques to aid peptide toxin pharmaceutical development. What has now emerged is the sequential bioengineering of new research probes and drug leads that owe their lineage to these highly potent and isoform specific peptides. Here we discuss the progressive bioengineering steps that many conopeptides have transitioned through, and specifically illustrate some of the biochemical approaches that have been established to maximize their biological research potential and pharmaceutical worth.

Contents

Abbreviations: Ab, antibody; Abu, α-amino butyric acid; ACE, angiotensin-converting enzyme; Agl, L-allylglycine; ACh, acetylcholine; AChBP, acetylcholine binding protein; AChR, acetylcholine receptor; εAHX, 6-aminohexanoic acid; αα, amino acid(s); Aopn, 5-amino-3-oxapentanoic acid; Aph, 4-aminophenylalanine; Atda, (D,L)-2-aminotetradecanoic acid; AuNPs, gold nanoparticles; BOC, *tert*-butyloxycarbonyl; BODIPY, boron-dipyrromethene; BSA, bovine serum albumin; CapBi, caproylbiotin; CD, circular dichroism; Ctt, cytsathionine thioether; Da, dalton; dicarba-Iml, (2,8)-dicarba-(3,12)-cystino R α-conotoxin Iml; DMSO, dimethyl sulfoxide; Dmt, 2,6-dimethyltyrosine; DGR, dorsal root ganglia; DTT, dithiothreitol; EDC, 1-ethyl-3,3′-dimethyl-amino-propyl-carbodiimide; EDTA, ethylenediaminetetraacetic acid; ELISA, enzyme linked immunosorbant assay; FA, fatty acid; FDA, food and drug administration; FMOC, 9-fluoroenyloxycarbonyl; GI, gastrointestinal; Gla, γ-carboxyglutamic acid; ^{3}H-NEM, *N*-[ethyl,1-2-^{3}H]maleimide; hNET, human norepinephrine transporter; Hyp, *4-trans*-hydroxyproline; i.c., intracerebroventricularly; I_{Na}, sodium currents; i.v., intravenous; Laa, 2-amino-D,L-dodecanoic acid; LHR, luteinizing hormone-releasing hormone; MD, molecular dynamic; Mtt, 4-methyltrityl; nAChR, nicotinic acetylcholine receptor; Na_v, sodium voltage gated channel; NCL, native chemical ligation; NHS, *N*-hydroxysuccinimidyl ester; Nle, norleucine; NMDA, *N*-methyl-D-aspartate; NMR, nuclear magnetic resonance; Nva, norvaline; OPG, orthogonal protection groups; N-VGCC, N-type voltage gated calcium channel; PEG, polyethylene glycol; PET, positron emission tomography; PTM, post-translational modification; RP-HPLC, reverse phase high performance liquid chromatography; SARs, structure–activity relationships; SN, sciatic nerve; SPPS, solid-phase peptide synthesis; TAMRA, tetramethylrhodamine; TFA, trifluoroacetic acid; TMR, tetramethylrhodamine; TRH, thyrotropin-releasing hormone; TTX, tetrodotoxin; TTXs, TTX-sensitive.

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1. Introduction

Native conopeptides extracted from the predatory marine snails (cone snails) are small highly post-translationally modified (PTM) peptides (~1000–5000 Da), which have proven their pharmaceutical worth [1]. Presently 9 conopeptides are in clinical phase development, with one receiving United States Food and Drug Administration (FDA) approval, as discussed in our previous review paper entitled "Drugs from Slugs – past, present and future perspectives of ω-conotoxin research" [2].

Peptide toxins are often not considered as direct therapeutic agents due to hindering issues of therapeutic ratio/index, bioavailability and circulatory stability [3]. However their unsurpassed ability for sub-type ion channel isoform selectivity makes it worth the costly transition from peptide toxin to therapeutic drug [4–6]. This process takes many routes; from a single isolated natural compound, to a combinatorial library of thousands of chemical derivatives, all in an effort generate lead compounds possessing efficacious features of therapeutic necessity and commercial value. During this pipeline process most lose their original structural features, reflecting efforts of pharmacological refinement. Yet the majority of candidates often become ineffective at their original pharmacological target, stressing the need to account for structure–activity relationships (SARs) of individual and combined ligand binding regions in the toxin bioengineering stratagem.

Most clinical drugs have small molecular masses (<1000 Da) and are preferentially delivered via oral route, making difficult work in designing drugs based on hydrolytic peptides [7,8]. Yet successful use of bioactive peptides as lead compounds are illustrated in the development of angiotensin-converting enzyme (ACE) inhibitors, these being effective treatment for hypertension, post myocardial infarction and congestive heart failure [9]. Thirty-seven years ago, Bristol-Myers-Squibb developed the drug Captopril (Capoten®) from Teprotide, a 9 amino acid (αα) venom peptide, isolated from the Brazilian viper *Bothrops jararca* [10–12]. This ACE inhibitor represents amongst one of the earliest successful transitions of a natural product via a structure-based drug design approach. Even at this early stage the potential pharmaceutical value of small bioactive peptide templates was recognized [13].

Around this same time it was discovered that the bioactivity in *Conus* venom was peptidic in nature [14]. This finding, and later total chemical synthesis of the first conopeptide, α-conotoxin GI in 1978 [15], laid the foundation of a 34-year chapter that has undoubtedly advanced small peptide toxins as therapeutics agents. This advancement parallels the chemical refinement in peptide synthesis, the expansion of bioengineering techniques and the availability of derivatizing agents. This provides the present platform in the development of new synthetic bioengineered molecules, including novel conopeptide research probes and drugs.

In our second review in the 'Drugs from Slugs' series, we examine some of the key chemical bioengineering developments and uses of conopeptide derivatives as research tools and probes. We consider the potential to maximize existing families of conopeptide sequences as parent based templates in an endeavor to design new non-native conopeptide-like sequences, then illustrate the integration of peptide bioengineering to examine modifications within parent peptide-toxin scaffolds, and provide insight into the gradual development of 'cono-(peptido)mimetics' based on the present studies of ω-conotoxin MVIIA/Prialt® and χ-conotoxin MrIA ('CMrVIB' [16]). These examples illustrate the extensive diversification of biological functionality and pharmaceutical