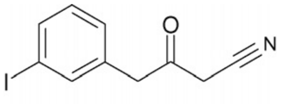
monitor

MOLECULES

Application of technologies and parallel chemistry for the generation of actives against biological targets

De novo design, synthesis and evaluation of inhibitors of reverse transcriptase of HIV

The reverse transcriptase (RT[a]) of HIV-1 is an essential enzyme in the lifecycle of the retrovirus. After entering the cell, RT copies the viral plus sense and single-stranded genomic RNA into double-stranded DNA. This process is catalyzed solely by RT and depends on two fundamental enzymatic activities: that of the DNA polymerase (which copies both RNA and DNA into DNA) and that of the RNaseH, which concomitantly cleaves the viral RNA strand in the RNA/DNA heteroduplex. The resulting double-stranded DNA is transported into the nucleus as part of a pre-integration complex and is subsequently incorporated into the DNA of the cell by the viral enzyme integrase [1]. An intense worldwide search for molecules that block RT activities is still in progress. To date, research efforts have led to several classes of inhibitors of HIV; one such class is the non-nucleoside RT inhibitors (NNRTIs). NNRTIs are a variety of noncompetitive inhibitors that bind specifically to a hydrophobic pocket in proximity to the DNA polymerase active site of the enzyme. The majority of inhibitors are highly specific against HIV-1 RT with minimal effects on the closely related HIV-2 RT [2]. This class of inhibitor is used in therapy against HIV-1 as part of the highly active anti-retroviral therapy (HAART). A significant obstacle for the use of NNRTIs is their very high specificity that reduces their efficacy against mutated variants of the virus [3]. Owing to the prevalence of resistance, major efforts have been directed in recent years to finding broad spectrum NNRTIs that inhibit both wild type and drug-resistant variants of RT. To date, discovering such inhibitors by screening of random libraries and optimizing active compounds by chemical modifications are time- and resource-consuming. To complement such efforts, faster and more efficient strategies that facilitate and shorten the discovery process would be beneficial. The *de novo* design of new molecules is a useful technique for new molecule discovery. The molecules thus designed are then synthesized and tested for their enzymatic inhibitory effects. Since the discovery of NNRTIs, crystal structures of wild type and drug-resistant variants of HIV-1 RT have been used in the design of novel NNRTIs. Recent work [4] has sought to elucidate a novel approach for *de novo* design of NNRTIs that inhibit wild type, as well as drug-resistant variants of HIV-1 RT. These researchers have used four different crystal structures of RT, one wild type and three mutated enzymes, which are resistant to several of the commonly used NNRTIs [5]. Using these four structures, small fragments of existing synthetic molecules were sought that could bind separately to each variant of the RT enzyme. The fragments that interacted with each RT structure were compared and only those that interacted with all four structures were linked together to construct final compounds. The resulting molecules were used as scaffolds for the synthesis of various chemically related molecules to be tested *in vitro* for inhibiting the activities of HIV-1 RT. For each of the 27 compounds synthesized in this way, each was tested against the DNA polymerase activity of HIV-1 RT. The IC_{50} values against the enzyme, were calculated for each compound from dose-response curves. From the library, several compounds were obtained that displayed activity. One of the most potent compounds tested was (i) which possessed an IC_{50} of 3.5 μM. This work is of interest because it has utilizes a parallel screen of small fragments against various structures of RT which successfully led to the identification of several inhibitors. Thus, filtering of molecules that could interact with the wild type and mutant variants is enabled. The computational approach used could serve as an alternative tool to other fragment-based methods, such as NMR or functional assay screening at high compound concentrations, both of which have gained increasing popularity recently. Further work in this area is warranted with a view to improve the drug-like properties of compounds within this series.



(i)

High-throughput synthesis and screening of cyclic peptide antibiotics

Cyclic peptides and depsipeptides are a class of privileged molecular structures. With reference to linear peptides, cyclic peptides are more stable against proteolytic degradation owing to their lack of free N- or C-termini, and also possess reduced conformational freedom. The entropic advantages associated with the increased rigidity also make cyclic peptides tighter-binding and potentially more specific ligands of macromolecular receptors. Additionally, cyclic peptides are more highly bioavailable (than linear peptides) owing to the absence of N- and C-terminal charges and their ability to form intramolecular hydrogen bonds as they traverse the lipid bilayer [6]. Cyclic peptides and depsipeptides are widely distributed in nature and possess a broad range of biological activities [7]. Several cyclic peptides, such as the immunosuppressant cyclosporin A, [8] are clinically used therapeutic agents. Given

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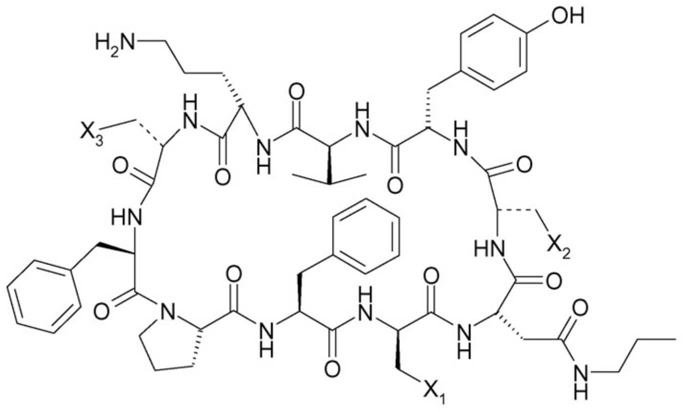
their potential as drugs, drug leads and molecular tools in biomedical research, there has been interest in the generation of cyclic peptide natural product analogues, either chemically or enzymatically to improve further their native activities, or to endow new biological activities. In principle, large cyclic peptide libraries (up to 10^7 variants) should be readily accessible via combinatorial synthesis by the split-and-pool method [9]. However, combinatorial synthesis necessitates post-screening sequence determination of 'hit' peptides and, until very recently, sequence determination of cyclic peptides had been a challenging problem. To address this challenge, recent work [10] has been directed toward the development of a methodology for combinatorial synthesis and high-throughput screening of cyclic peptides and to demonstrate its validity by the identification of a biologically active tyrocidine analogue from a 400-member library. This work has recently been extended [11] to generate a library of 1716 tyrocidine A analogues. From this library, several analogues were obtained with an improved therapeutic index. The tyrocidine analogue library was synthesized on TentaGel macrobeads (280–320 μm, 0.27 mmol/g, and 3.5 nmol/bead). To facilitate sequence determination of hit peptides, a 'one-bead two-compound' strategy was adopted [12]. Here, each resin bead was rendered to contain two peptide molecules; approximately 90% of the peptide molecules were cyclic and attached to the TentaGel resin by a labile ester linkage, whereas the other 10% of the molecules were the corresponding linear peptides, linked to the resin via a stable amide bond. After library synthesis, approximately 2400 beads were randomly selected from the tyrocidine analogue library and placed in 96-well microtiter plates (one bead/well). The cyclic peptide on each bead was cleaved from the solid support by aminolysis of the ester linkage with neat propylamine. The linear encoding peptide, which was linked to the resin via an amide linkage, was unaffected. The excess propylamine was evaporated, and the resulting peptides subjected to antibacterial assays (using *Bacillus subtilis* cultures). Out of the 2400 samples screened, 22 (approximately 1%) showed visible growth inhibition of *B. subtilis*. The 22 beads were retrieved from the microtiter plates, and the identity of the cyclic peptides determined by sequencing the remaining linear encoding peptides by partial Edman degradation/mass spectrometry (PED/MS). Several active cyclic peptides were obtained upon screening of this library. One of the most potent was (ii) which possessed an MIC value of 2 μg/mL. This work is of interest because a general methodology for high-throughput synthesis and screening of cyclic peptide libraries has been developed allowing the generation of libraries of up to 10^7 different compounds. Several active tyrocidine A derivatives have been synthesized, but with improved therapeutic indices. Further work in this area is warranted to improve the properties of this series of inhibitors and potentially lead to clinically-useful antibiotics.



(ii): X1 = D-Arg; X2 = L-Ser ; X3 = L-Fpa

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