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Reviews

Predicting the unpredictable: Recent structure–activity studies on peptide-based macrocycles

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ABSTRACT

Heterocycle-containing macrocycles are an emerging class of molecules that have therapeutic efficacy. Many biologically active natural products that have interesting biological properties fall into this class of molecules. The highly specific and selective biological activity is often attributed to the unique conformation of these macrocycles, which is affected by the elements of the macrocycles as well as its surroundings in biological systems. In this review, the structure–activity relationship studies of several recently developed biologically active heterocycle-containing macrocycles have been discussed in order to facilitate an understanding on how unpredictable structures can be controlled.

Contents

1. Introduction

Macrocycles are cyclic molecules composed of 12 or more atoms within their backbone. They are a class of molecules that are emerging as highly effective therapeutics [1,2]. Macrocycles are distinctive from other classes used as therapeutic leads and chemical biology tools because their cyclic nature forces the molecule to adopt specific conformation(s) likely lock the ring into a favourable binding conformation. Molecules lacking the rigid macrocyclic structure are flexible and contain rotatable bonds making them unoptimized inhibitors for entropic reasons. In contrast, macrocycles, if locked into the correct conformation will interact favourably with a receptor or biological target. Furthermore, the large surface of macrocycles interferes with protein–protein interactions more effectively than traditional small molecules, providing an opportunity for these medium sized rings

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to be better inhibitors than conventional drug leads [2–5]. The macrocycle's specific conformation is not only dictated by the components within the macrocyclic backbone such as the side chain orientations and size, but also by the external factors, such as polar and non-polar residues in the physiological system [6]. These structural benefits provide compounds that possess a broad spectrum of interesting biological properties, including anticancer, antibacterial, antifungal, antimalarial [7].

In addition the ring structure of the macrocycle, a higher degree of selectivity and specificity can be achieved via the incorporation of heterocyclic elements into the backbone and side-chains of the ring. Common elements seen in natural products include oxazoles, oxazolines, thiazoles, thiazolines and pyridines [8–11]. These heterocyclic elements are incorporated post-translationally into both linear and cyclic peptides, generating large rings. Due to their cyclic nature, heterocycles help to rigidify and increase the metabolic stability of the flexible peptide chains [8].

Pioneering the structure activity studies on heterocycle containing macrocyclic peptide, Wipf et al. worked on Lissoclinamide 7, a cyclopeptide metabolite with two thiazoline and one oxazoline within its macrocycle, and found that the macrocycle adopted a specific conformation dominated by both a type II β-turn and a β-loop due to the presence of rigid heterocycles [12]. Changes either on the composition of the heterocycles within the macrocycle and the stereochemistry of the amino acid influenced the cytotoxicity of the molecule [12]. Similarly, Doi et al. found that by altering the composition of the heterocycles within Ascidiacyclamide, a symmetrical cyclopeptide containing two thiazoles and two methyl oxazolines, the molecule adopted a flat squared conformation [13–15]. The changed conformation, however, was insignificant in improving the cytotoxicity of the molecule as compared to the one of the natural product.

One prominent structural change that is applied to macrocyclic peptides is the inclusion of *N*-Methyl amino acid [16]. Kessler et al. noted that *N*-methylation improved metabolic stability and the membrane permeability of a macrocycle, and modulated the overall conformation, which in turn, the selectivity of a macrocycle [16]. In addition, Lokey et al. demonstrated that, depending on the macrocyclic scaffold, side chain positioning was critical to the membrane permeability of the molecule [17]. Incorporation of D-Phe was also found to be significant in improving the cytotoxicity of the macrocycle [18–20].

In this review, macrocyclic peptides that underwent extensive structure–activity relationship analysis involving heterocycle and amino acid substitution over the past 7 years are discussed. Discussion of the compound and their analogues synthesis and how their structure correlates to their biological activity provides information on the most effective modifications to these macrocycles. Specifically we highlight examples that involve the following modifications to macrocycles in order to evaluate their biological activity: (a) changing a heterocycle (from Ox to Th or vice versa), (b) removing or adding a heterocycle, (c) exchanging amino acid structures (d) adding an *N*-methyl moiety, and (e) incorporating D-amino acids.

2. Macrocycles with antitumor activity

2.1. Sansalvamide A

Sansalvamide A (San A, Fig. 1), a macrocyclic depsipeptide isolated from the mycelium of a marine fungus *Fusarium* sp., was found to induce micromolar (μM) level of cytotoxicity on several cancer cell lines [21]. Investigations done by McAlpine and co-workers utilizing the peptide analog of San A (SM1, Fig. 1) showed that this San A derivative targeted heat shock protein 90 (Hsp90) [22]. Hsp90 is a major molecular chaperone that is involved in the progression and survival of cancer cells. Hsp90 regulates the trafficking and folding of over 400 misfolded/unfolded client proteins [23–25]. In depth structure–activity relationships lead to the McAlpine discovering SM145 (Fig. 1), which possessed better binding affinity to Hsp90 than SM1 (i.e., 3.6 versus 20 μM respectively). Classic Hsp90 inhibitors that are currently in various stages of clinical trials, have suffered set-backs related to a cytoprotective response that is triggered when they are used to inhibit Hsp90. This cytoprotective response is usually referred to as a heat shock response (HSR). Investigation of the SM145's mechanism showed that in contrast to the classic inhibitors, SM145 did not induce this HSR [26–28]. This successful discovery with SM145 drove the development of new molecules that were less hydrophobic and more potent than SM145.

Incorporation of the peptidomimetic moiety such as an oxazole can enhance the solubility of the macrocycle, while decreasing the peptidic nature when incorporated within the backbone of the ring [8,11]. When placed within the backbone of the ring, this moiety rigidifies the natural product and may facilitate the formation of a desirable conformation that is selective for the biological target. However, although the inclusion of an oxazole moiety into the SM145's scaffold (Fig. 2) reduced the rings flexibility, it did not have any cytotoxicity [29]. These data indicated that the backbone conformation was negatively impacted by rigid moieties such as the oxazole. Alternative heterocycles including triazoles and pseudoprolines had the same impact on the biological activity of the molecules demonstrating that the loss of potency was likely due to altering the macrocycles conformation [29]. Supporting these data is the loss or gain of activity when the stereochemistry of an amino acid in the backbone is altered from d to l or vice versa. If one stereoisomer is active, then switching the stereochemistry of a single amino acid produces an inactive molecule [30–33].

Given that heterocycles enhance potency and offer multiple binding modes to biological targets (pi-stacking, hydrogen donors, and hydrogen acceptors) McAlpine and co-workers evaluated how

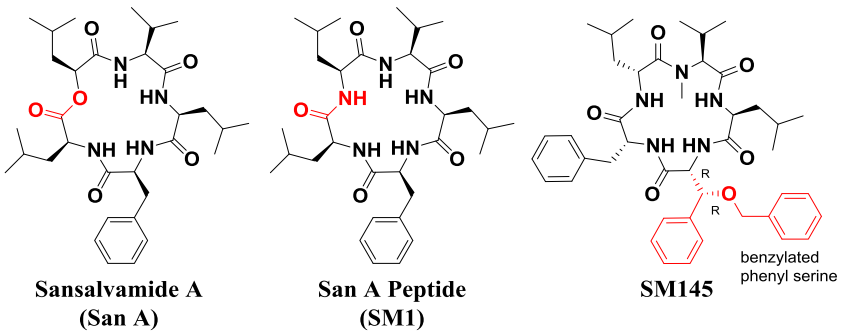

Fig. 1. Structures of Sansalvamide A (San A), San A peptide (SM1) and SM145.