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STRUCTURE-BASED SYNTHESIS - FROM NATURAL PRODUCTS TO DRUG PROTOTYPES*

STEPHEN HANESSIAN

Department of Chemistry, Universite de Montreal, C.P. 6128, Succ. Centre-ville, Montreal, QC, H3C 3J7, Canada
Department of Chemistry, University of California, Irvine, USA

Abstract: X-Ray crystallographic data available from complexes of natural and synthetic molecules with the enzyme thrombin has led to the design and synthesis of truncated and hybrid molecules exhibiting excellent inhibition *in vitro.*

Introduction

The vital importance of natural products for the well-being of man has been known for millennia. Their therapeutic benefits to alleviate pain or cure diseases continue to rank natural products among the primary sources of potential drugs [1]. Great advances have been made in the methods of isolation, identification, and structure elucidation of some of the most complex natural products in recent years. The advent of molecular biology and genetic mapping has also aided in our understanding of the intriguing biosynthetic pathways leading to various classes of therapeutically relevant antibiotic, anticancer, and related natural products. Synthetic chemistry has also been enriched with the discovery of diverse classes of natural products. Elegant and practical methodology has been developed leading to the total synthesis of virtually every class of medicinally important natural product [2]. In some cases, natural products or their chemically modified congeners have been manufactured by total synthesis on an industrial level which is a testament to the ingenuity of process chemists [3].

In spite of their potent activities in enzymatic or receptor-mediated assays, not all natural products are amenable to being developed as marketable drugs. In many instances unfavorable pharmacological effects cannot be overcome without drastic structural and functional modifications, which may also result in altered efficacy. Structure modification through truncation, functional group variations, isosteric replacements, and skeletal rigidifications aided by molecular modeling, X-ray crystallography of protein targets, or NMR data are valid objectives in the context of small molecule drug discovery starting with bioactive natural products [4]. A large proportion of these pertain to chemotherapeutic agents against cancer [5].

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From natural aeruginosins to unnatural analogues

The aeruginosins are a relatively new class of secondary metabolites isolated from cyanobacterial water blooms, sponges, and algae located in geographically and phytogenetically distinct bodies of water [6]. Twenty or more members of this class of linear peptides encompassing a 2-carboxy perhydroindole core motif have been isolated. The total syntheses of seven aeruginosins have been reported, with two of these involving revisions of the originally proposed structures [7]. The aeruginosins exhibit inhibitory activities against serine proteases such as thrombin and trypsin. Inhibition of thrombin in particular is highly relevant because it is the last enzyme in the cascade of events leading to blood coagulation [8]. In this regard our recent total synthesis, structure elucidation, and stereochemical assignment of chlorodysinosin A [9] is most noteworthy (Fig. 1). The simple replacement of the D-leucyl residue in dysinosin A [10] with a chloroleucyl residue brought about a dramatic improvement in the IC_{50} values for thrombin inhibition. Until this discovery, oscillarin [11] was the most potent in vitro thrombin inhibitor (Fig. 1). It is intriguing that dysinosin A, oscillarin and chlorodysinosin A, all share the novel 1-amidino Δ^3-pyrroline moiety as an arginine surrogate. The beneficial "chloro" effect has been rationalized on the basis of a hydrophobic effect in the P_3 active site of thrombin [9].

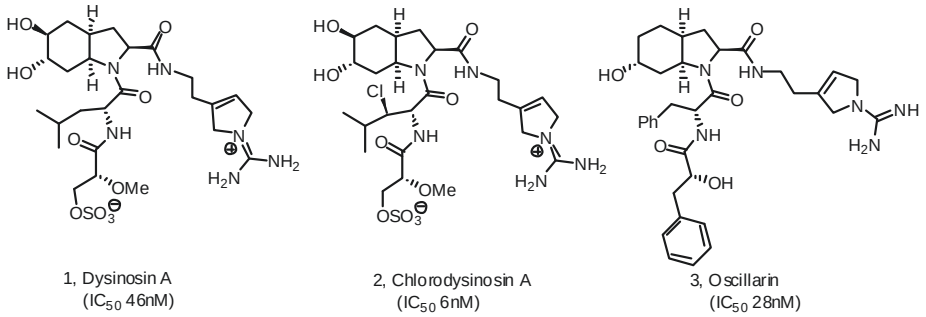

Fig. 1. Structure of aeruginosins isolated from distinct aquatic regions. In vitro inhibition activity against the enzyme thrombin (Factor IIA).

In conjunction with our first total synthesis of dysinosin A [10], we were also intrigued by the potent inhibitory activity of analogues of D-Phe-L-Pro-L-Arg, particularly as a chloromethylketone analogue PPACK [12]. X-Ray crystallographic analysis of PPACK as a complex with thrombin revealed the crucial interactions within the Sj, S_2 and S_3 pockets, as well as the site of the catalytic triad involving a serine residue (Fig. 2). Based on this valuable information, we designed and synthesized a series of indolizidinone [13,14] and bicyclic sultam [15] analogues. The dependence of inhibitory activity against thrombin on the stereochemistry of the tertiary center bearing a benzyl and primary amino (or hydroxyl) group was evident [13,14]. X-Ray co-crystal structures revealed the expected interactions and corroborated the stereochemical dependence. As expected, the most potent analogue was the (5)-amino indolizidinone [14, 16].

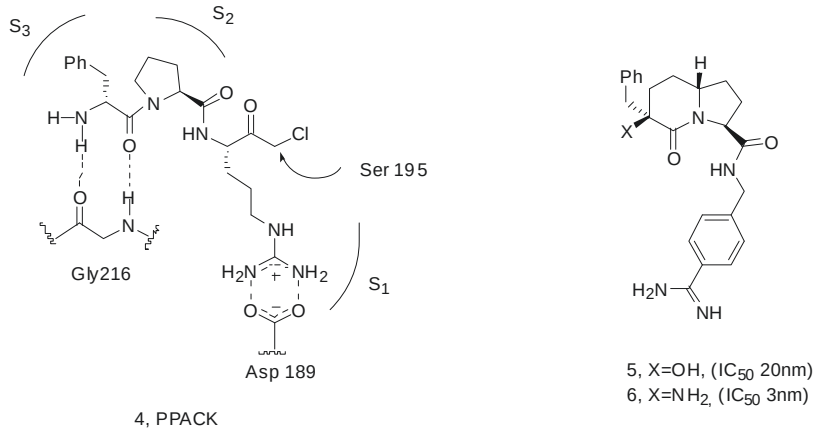

Fig. 2. Structure of PPACK(4). Constrained indolizidinone analogues (**5** and **6**) and their inhibitory activity against thrombin.

In an effort to maintain the hydroxy octahydroindole core in truncated analogues of oscillarin, we synthesized a series amides and sulfonamides that would mimic the three important pharmacophoric sites in the natural product [17]. Thus, the P_1 1-amidino Δ^3-pyrroline moiety was replaced by a 4-amidinobenzyl group, and the D-Phe-D-Pla residue was replaced by simpler surrogates such as **7-9** (Fig. 3). Unfortunately, only weak in vitro activity was found for three of the analogues. Thus, the P_3 replacements in these truncated analogues were not suitable, although the stereochemistry at the ring juncture was not crucial.

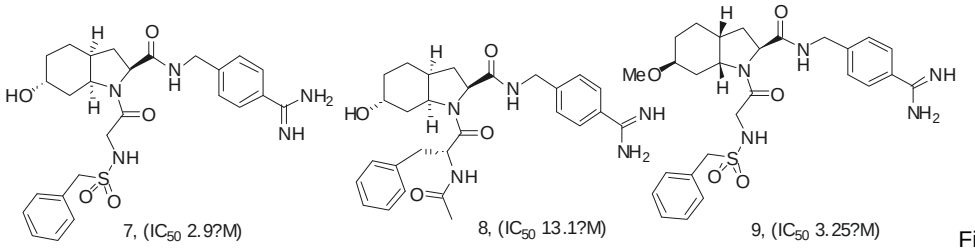

Fig. 3. Truncated octahydroindole analogues **7-9.**

The primordial importance of the nature of the amide appendage harboring the P_3 moiety was shown in a series of highly potent analogues in which we initially incorporated the chloroleucyl moiety found in chlorodysinosin A, while substituting the 1-amidino Δ^3-pyrroline unit with the 4-amidino benzyl group and removing the 6-hydroxyl group as in **10** [18] (Fig. 4). Clearly the chlorine group has a profound effect on the thrombin inhibitory activity compared to the des-chloro analogue **11.** Relying on the X-ray co-crystal structure of chlorodysinosin A [9] and dysinosin A [10] with thrombin, we concluded that the (*R*)-configured chlorine atom offered a better hydrophobic interaction in the S_3 site, possibly excluding water and giving an entropic gain. Indeed, molecular dynamics calculations revealed a more restricted rotation around the χ^1 angle in the D-Leu moiety bearing the chlorine substituent in chlorodysinosin A compared to dysinosin A [9]. The obvious conclusion was that other more hydrophobic and spatially compatible substituents on the P_3 amino acid residue between the D-Pla and hydroindole core might also be active inhibitors of the enzyme.

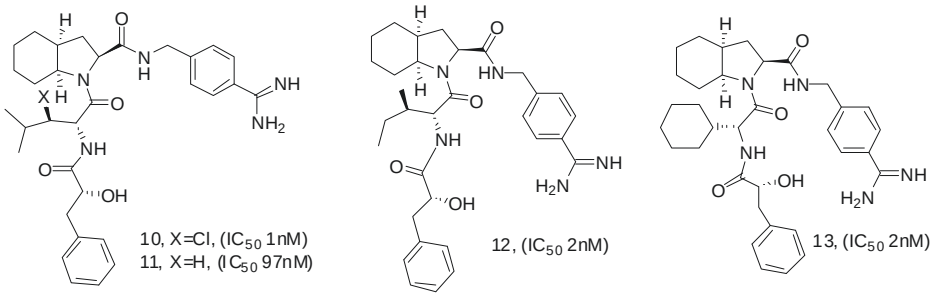

Fig. 4. Octahydroindole analogues incorporating a D-leucyl C-3 substituent and their inhibitory activity against thrombin

Indeed the D-isoleucyl and 3-cyclohexylleucyl analogues **12** and **13** respectively, were highly potent inhibitors. There remains to see if there is a stereochemical preference for the C-3 substituent on the P_3 D-leucyl moiety.

Thus, isosteric and functional replacements of the P_1, P_2 and P_3 subunits of chlorodysinosin *A*, led to even more potent thrombin inhibitors as truncated analogues.

From natural aeruginosins to achiral drug prototypes

Extensive modeling studies in collaboration with the AstraZeneca group in Molndal, Sweden allowed the conception and design of inhibitor prototypes that were devoid of stereogenic centers and structurally far removed from the quasi exotic aeruginosins in comparison. The conceptual basis of these prototypical classes is illustrated in Fig. 5. In the first class, the crucial antiparallel H-bonded bridge with Gly216 in PPACK (as well as the aeruginosins), would be simulated by an o-aminophenol.

A small set of 22 analogues consisting of the o-aminophenol core was synthesized by segment coupling [19]. Variations in the nature of the aromatic substituents in the P_3 region, and relying mostly on a sulfonamide type linkage with the o-aminophenol, revealed the naphthalene sulfonamide **14** to be the most potent, with a good selectivity for thrombin over trypsin (IC_{50} ratio of 67). A co-crystal structure of the sulfonamide **14** with thrombin revealed the expected interactions.[19] The 4-amidinobenzyl group occupies the S_1 pocket with a salt bridge between the amidino moiety and Asp 189. Similarly, the H-bond from the P_1-P_2 amide linkage to the C=O of Ser214 is conserved. The A-ring of the P_3-naphthyl group interacts partially with the lipophilic distal S_3 pockets but is also exposed to bulk water. Essential H-bonds to Gly216, Ser214 and the ionic interaction with Asp 189 could be clearly seen. The naphthalene moiety occupied the hydrophobic distal pocket.

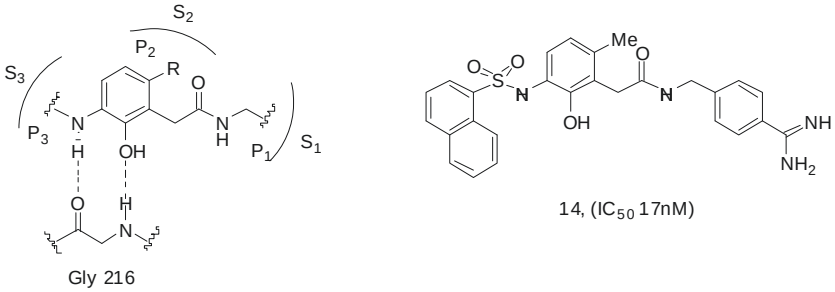

Fig. 5. Left: Model for binding of a P_2/P_3 phenolic core. Right: Thrombin inhibitory activity of an a chiral naphthylsulfonamide phenol analogue (**14**).

The second and third classes were represented by JV-amino-pyrididin-2-one and JV-amino-dihydropyridin-2-one core motifs. These analogues were prepared in chronological order, revealing a truly remarkable validation of molecular modeling in this series.

The *N*-amino-pyridin-2-one series [20] consisted of arylsulfonamides and arylethyl analogues having a variety of aromatic substituents including a naphthalene moiety following the lead of the phenol series. Variations in the P, basic residue produced 29 compounds. The most active analogue **15** showed a thrombin to trypsin ratio of 42, with a modest IC_{50} against thrombin (Fig. 6). The 4-amidino benzyl group was preferred to an 6-amino-3-fluoro-4-methyl-2-pyridylmethyl group as a P_1 surrogate.

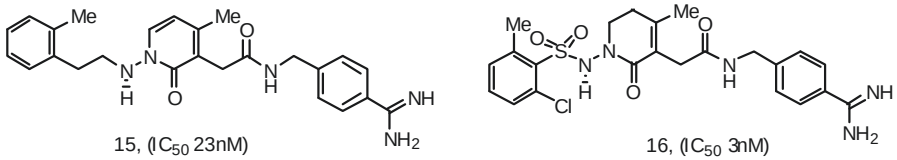

Fig. 6. *N*-Amino-pyridin-2-one, jV-amino-dihydropyridin-2-one core motifs (**15** and **16**) and their thrombin inhibitory activity.

Surprisingly, the *N*-amino-arylsulfonamido dihydropyridin-2-one analogue **16** was exceptionally potent with superb selectivity [21]. Several other derivatives in this series also exhibited single and double digit nanomolar IC_{50} activities against thrombin with excellent selectivity. Perhaps the more basic dihydropyridin-2-one core subunit confers a favorable interaction with the enzyme although definitive proof must await further studies.

Synopsis

Total syntheses of dysinosin A, oscillarin, and chlorodysinosin A revealed progressively improved inhibitory activity against the enzyme thrombin.

Inspired by the mode of binding of our synthetic aeruginosins as evidenced from X-ray co-crystal structures, it was possible to prepare truncated and functionally modified analogues. Highly active thrombin inhibitors in which the three principal P_1, P_2 and P_3 binding units were chemically modified or replaced with mimetic groups were identified. X-ray crystallography was also instrumental in the design and synthesis of indolizidinones as thrombin inhibitors, delineating the favored spacial disposition and stereochemistry of the P_3 substituent.

Structure-based synthesis in this series has culminated with the design and synthesis of achiral inhibitors centered around phenolic, *N*-amino-pyridin-2-one, and *N* -amino-dihydropyridin-2-one core structures. Of these, the dihydropyridin-2-ones have shown to be the most active and selective inhibitors of thrombin.

Our studies in this field have taken us from the conception of new methods for the elaboration of enantiopure 2-carboxy-hydroxyoctahydroindoles (as in dysinosin A, oscillarin, and chlorodysinosin A), to asymmetric functionalization of indolizidinones, to totally achiral molecules. Interestingly, in spite of the structural dissimilarity of the natural aeruginosins and the achiral inhibitors, they share the same binding site in thrombin with the added advantage of higher selectivity for the non-natural synthetic variants.

The prevention of stroke by inhibiting thrombin among other enzymes remains as an unmet medical need. It is hoped that an effective therapeutic agent will emerge in the near future. In this regard, fruitful collaborations between academia and pharmaceutical or biotech companies could continue to play an important role [22].

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