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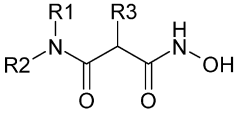
MONITOR

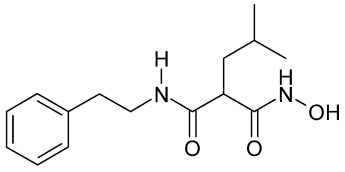
Syntheses of novel compounds targeting metalloprotease and antibacterial targets

A library approach to the synthesis of novel hydroxamic acids targeting the metalloprotease family

Proteases have been seen as important targets for the pharmaceutical industry for some time now. Potent inhibitors for the four classes of proteases (cysteine-, aspartyl-, serine- and metallo-) have been discovered. For example, inhibitors of ACE (angiotensin-converting enzyme) have been developed as antihypertensive agents [1], and inhibitors of the aspartyl-protease of HIV have also been discovered [2]. More recently, effort has focused on metalloproteases and closely related metallohydrolases for the treatment of cancer or arthritis, with inhibitors of TACE (TNF-α-converting enzyme), HDAC (histone desacetylase) and MMPs (matrix metalloproteases) being recently discovered. Thus, recent work [3] has sought to clarify the functions of metalloproteases by design and synthesis of specific inhibitors containing a hydroxamate group. This group is a recognized and well-characterized Zn^{2+} binding group [4], and diversely substituted hydroxamates should therefore be useful in clarifying functions of metalloproteases. Furthermore, they could represent potential starting points for the development of novel therapeutic agents, as exemplified by current clinical trials [5]. The work described here [3] centers on the preparation and first screening results of a library of malonylhydroxamic acids of general structure (i). Their synthesis is based on a simple convergent solution phase synthesis using diverse amines and *O-tert*-butyl hydroxamic acids bearing a free carboxylic acid function. As a proof of concept, the authors screened their library against APN (an enkephalin-processing enzyme also known as APN/CD13), the prototypal metalloprotease of the M1 family. In this way, a 217 membered library of singletons was synthesized in solution using amines and malonic acid derivatives as synthetic substrates. The library of 217 compounds was screened against mammalian neutral aminopeptidase (microsomal), in order to prove that the library could deliver hits on a given zinc metallo-enzyme and be a useful tool for the study of other metallo-enzymes. Screening was performed at a concentration of 10 μM. Enzyme activity was assessed at 405 nm by the release of the chromogenic *p*-nitro-aniline resulting from the cleavage of the substrate Leu-*p*-nitro-anilide. A significant number of hits against APN were obtained. Library members displaying a percent inhibition above 80% were re-synthesized for IC_{50} determination. One of the most potent hits obtained in this way was (ii) that displayed an IC_{50} of 82 nM for inhibition of APN. This work is important because it provides proof of concept for inhibition of APN, a prototypal aminopeptidase of the M1 family. Not only can the new inhibitors be used as tools for further optimization, but also the library itself may be a useful tool in attempts to elucidate the function of other, recently identified metalloproteases of the M1 family. Furthermore, the diversity of the library suggests that it could be useful for the study of other classes of Zn metalloproteases. Further work in this area is therefore warranted.



(i)



(ii)

Solid phase synthesis of new inhibitors of the essential cell division FtsZ enzyme as a new potential class of antibacterials

The widespread (mis)use of antibiotics has enabled and facilitated a great pressure for selecting bacteria resistant to all known classes of antibiotics. This problem is of the utmost importance to human health, and so antibiotic resistance among bacterial pathogens worldwide necessitates the development of structurally new antibacterials. One avenue for exploration is designing novel antibiotics against targets essential for growth, whose inhibition leads to a lethal phenotype [6]. This bacterial cell division process encodes essential proteins forming the divisome, and as such are perceived as representing some of the best antibacterial targets available. These proteins are extremely sensitive to inhibition since the cell division depends on recruitment of specific proteins in an essential cascade for forming the divisome [7]. Among those proteins, FtsZ can be considered as a specific target because this is the most important and conserved protein of the cell division machinery [8]. Recent work has exploited FtsZ as a target in the design of inhibitors of cell division [9]. Work here has focused efforts on the FtsZ protein of *Pseudomonas aeruginosa*, one of the major

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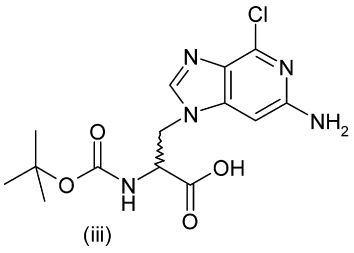
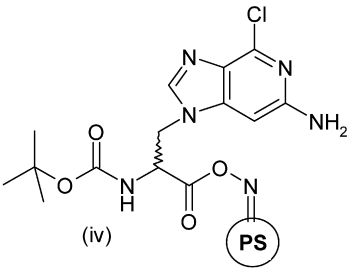
opportunistic pathogens causing severe nosocomial infections [10]. These researchers [9] synthesized a small number of molecules using combinatorial chemistry methodology on solid phase, preparing a set of highly diversified GTP analogues as potential FtsZ inhibitors. The GTP binding and hydrolytic activities of the purified *P. aeruginosa* FtsZ protein were characterized. The inhibitory activity of each was synthesized.

GTP analogue was then evaluated individually *in vitro* against the FtsZ GTPase activity and *in vivo* on whole bacterial cells. The core structure (iii) was utilized for parallel synthesis and oxime resin was chosen as the solid support, capturing the core via its acid functionality to give (iv). After removal of the Boc group from (iv) the nitrogen was derivatized and the final compounds cleaved from resin as the amide, following addition of a set of nucleophilic amines, giving (v). Synthesized compounds were analyzed for their capability to inhibit GTPase activity of FtsZ *in vitro*. The FtsZ conversion of GTP into GDP was used to assess the inhibitory properties of the synthesized compounds. All the GTP analogues inhibited the GTPase activity of FtsZ with significant IC_{50} values between 450 μM and 2.6 mM. One of the most potent analogues was (vi) with an IC_{50} of 450 μM for inhibition of the GTPase activity of FtsZ. Additionally, these compounds possessed antimicrobial activity of 10 mM, inhibiting the growth of *S. aureus* cells. This work is of interest as it represents the first report describing GTP analogues having promising inhibitory properties of the GTPase activity of FtsZ, and validated antimicrobial properties against whole bacterial cells. Further work in this area is warranted to investigate the structure activity relationships for the design of efficient and specific inhibitors of bacterial cell division.

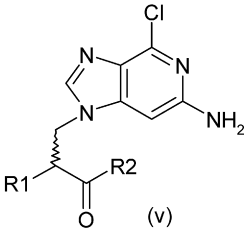


(iii)

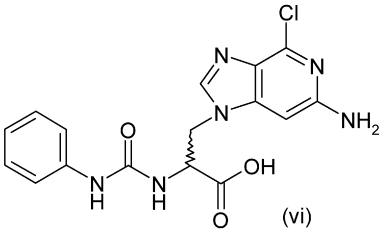


(iv)

PS = Polystryene Oxime resin



(v)



(vi)

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