monitor

MOLECULES

The design and synthesis of libraries for the discovery of antibacterial and antifungal substances

Library synthesis of 2-(4,5-dihydroisoxazol-5-yl)-1,3,4-oxadiazoles as antifungal agents

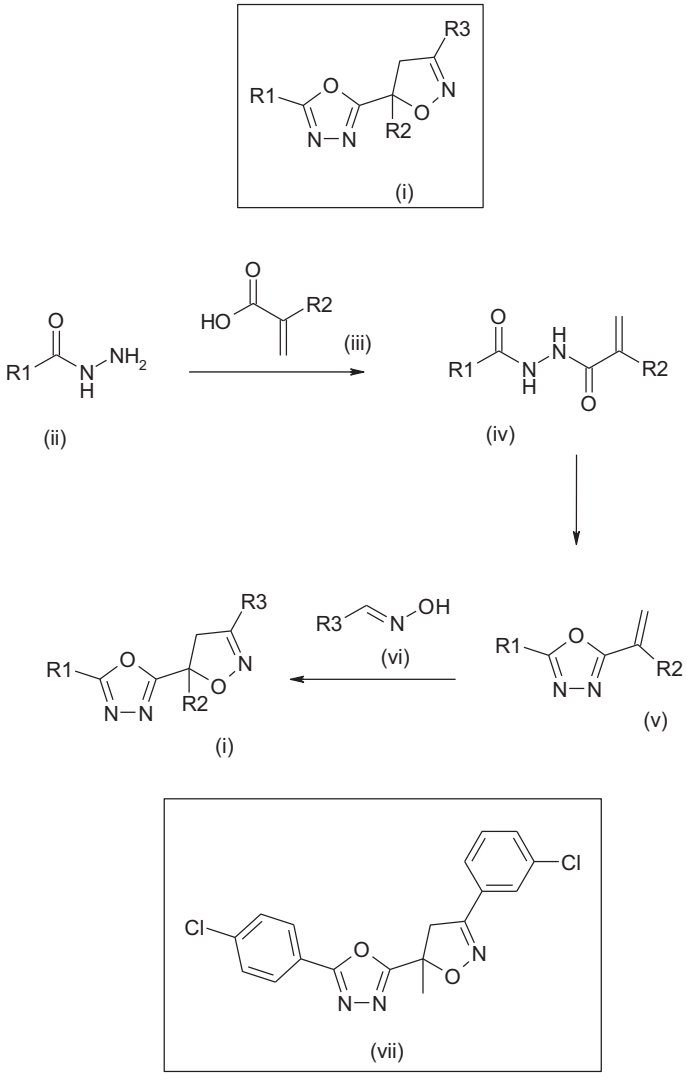
As a heterocyclic class of molecules, 1,3,4-oxadiazoles display a wide variety of biological activities, including antibacterial [1,2], antifungal [3,4] and anti-inflammatory activities [5], in addition to being considered a 'privileged' scaffold in medicinal chemistry [6]. Isoxazolines are also important heterocycles in medicinal chemistry and molecules containing this scaffold have been found to elicit antifungal [7] and antibacterial activities [8], among other biological activities seen with this scaffold.

It is common practice for pharmaceutical and agrochemical companies to use high-throughput screening for the discovery of new hit molecules. There are literature reports of combinatorial libraries being used as discovery tools in the agrochemical industry and numerous hits and leads have been produced in this way [9]. The success arising from library design and parallel synthesis being used to improve the biological activity of hits discovered from high throughput screens, coupled to the broad spectrum biological activity seen with both the oxadiazole and isoxazoline scaffolds, has provided a stimulus for recent work [10]. In this work, the synthesis of derivatives of heterocycle (i) has been undertaken.

It was reasoned that tethering of these two heterocycles and subsequent library diversification at three points on this newly created scaffold (i), would lead to new derivatives displaying interesting biological activities.

Thus, a library synthesis of 50 members was undertaken in solution by coupling the hydrazide derivatives (ii) with acrylic acids (iii) to provide intermediates (iv). Cyclization then ensued to deliver oxadiazole intermediates (v). These were reacted, through a 1,3-dipolar cycloaddition reaction, with a range of oximes (vi) to provide diheterocyclic products (i).

From this collection, 16 compounds were screened broadly for herbicidal, fungicidal and insecticidal activities against, for example, *Helianthus annuus* (sunflower) and *Digitaria*



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sanguinalis (crabgrass) as a measure of herbicidal activity, and *Pyricularia oryzae* (rice blast) and *Septoria tritici* (wheat leaf blotch) for fungicidal activity. None of the compounds tested displayed herbicidal activity. However, several compounds displayed modest insecticidal disease control, albeit below the activity of the fungicide standard used, azoxystrobin. One of the most potent compounds isolated was (vii), which displayed a 50% mortality rate on *Spodoptera exigua*.

This work is of interest because it has led to the rapid development and synthesis of a library of diheterocycles. Future efforts could include increasing insecticidal activity through expansion of the library's structural diversity and encompassing other heterocycles. Further work in this area is therefore warranted.

Discovery and optimization of antibacterial AccC inhibitors

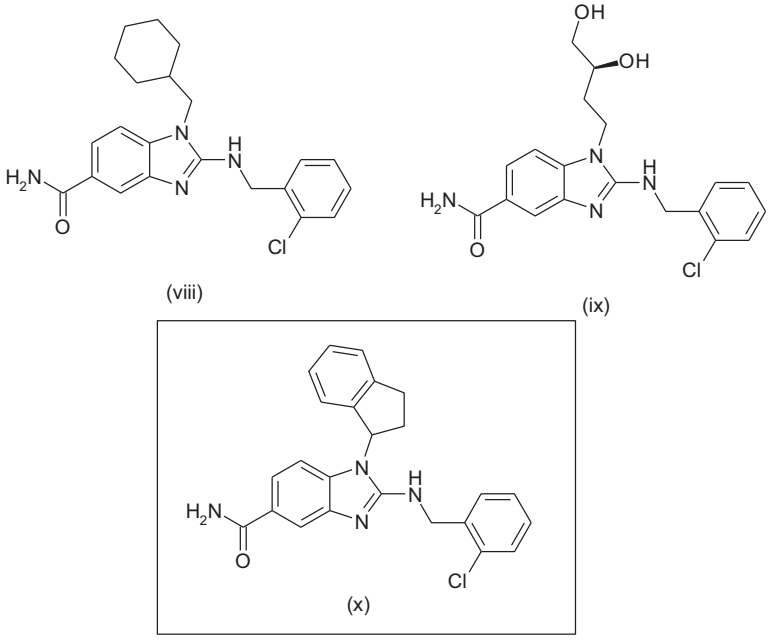
Antibiotic resistance is a major health problem and the pharmaceutical industry continues to develop broad spectrum antibiotics to meet this challenge. One way to tackle antibiotic resistance could be through targeting enzyme components of the bacterial acetyl coenzyme-A carboxylase (ACCase) with suitable inhibitors [11,12]. ACCase catalyzes the first committed step in fatty acid biosynthesis and is essential for bacterial viability [13]. ACCase is a multi-component system made up of two enzymes (AccC and AccAD) and the biotin carrier protein (BCCP or AccB) [14]. The catalytic mechanism itself comprises the ATP-dependent carboxylation of biotin, which is catalyzed by AccC (biotin carboxylase) followed by AccA/D (carboxyltransferase), where the carboxy group is transferred from biotin to acetyl-CoA to form the final product: malonyl-CoA.

The amino acids of the ACCase components are highly conserved across all bacteria, making ACCase a good target for seeking broad spectrum antibacterials.

Previous work [15] has identified compound (viii) from screening a mixture-based combinatorial library. Recent work [16] has sought to further optimize this series of inhibitors.

Using X-ray crystallography of co-crystals of (viii) bound to the AccC protein, to inform combinatorial library design, around 300 mass-encoded, mixture-based compounds were synthesized on solid-phase (Rink resin) using the split/pool technique. From this first library, 16 compounds were found to be of interest and these were synthesized as single, purified, entities. One of the most potent ACCase ATP-competitive inhibitors isolated from this library was (ix), which displayed an IC_{50} of 0.4 μM against ACCase ATP. Further optimization, based on project SAR obtained, led to the synthesis and biological testing of compound (x), which displayed an IC_{50} of 20 nM. Compound (x) was also found to be active in a cell-based assay, using a sensitive strain of *Escherichia coli* (*E. coli* HS294) in which the major drug efflux pumps have been deleted and cell permeability is enhanced by a reduction in LPS synthesis. Compound (x) displayed an MIC of 0.8 μg/mL in this assay.

This work is of interest because it describes the synthesis of a library of AccC ATP-competitive inhibitors which, as a class, provide a novel mode of action that could tackle antibiotic resistance. They also provide broad spectrum enzyme inhibitors. Further work in this area is warranted, to improve the activity and pharmaceutical properties of members of this series of inhibitors.



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