Sponsored by:

MDL Powering the Process of Invention™

Monitor Editor: Matthew Thorne
m.thorne@elsevier.com

monitor

MOLECULES

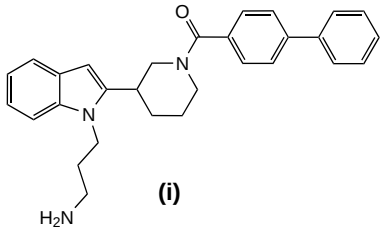
ZipA-FtsZ inhibitors

The prokaryotic tubulin analogue FtsZ is an essential protein involved in cell division in Gram-negative and Gram-positive bacteria. During this division process, cytoplasmic FtsZ is localized in the medial division site of the septal ring at an early stage in the division cycle, and remains associated with the leading edge of the ingrowing septum during cytokinesis. Impairment of septal ring assembly and resulting failure of the thermosensitive *fts*Z84 mutant of *Escherichia coli* to divide above the restrictive temperature indicates the importance of FtsZ in cell division.

FtsZ is present in nearly all Gram-negative and Gram-positive bacteria. ZipA was previously identified [1] as a protein that binds to FtsZ in *E. coli*, and this protein is localized at the site of cell division at a very early stage in this division process. This serves to tether the FtsZ protofilaments to the membrane during invagination of the septum. Binding of FtsZ protein to the membrane bound ZipA is required for septum formation of nonseptate filaments after overexpression or depletion of ZipA. However, proteins with significant homology to ZipA have not been found in Gram-positive bacteria and also appear to be absent in a number of Gram-negative bacteria. This indicates that ZipA is conserved in only a subset of Gram-negative genomes.

Illnesses caused by respiratory pathogens, for example, *Sreptococcus pnuemoniae*, are major global health problem. Pathogens such as this are the causative agents of a variety of life threatening infections and disease states such as chronic bronchitis. The appearance of strains of respiratory pathogens resistant to the antibiotics used to eradicate them is becoming more frequent, and gives us cause for concern. As ZipA is required for cell division is a number of Gram-negative bacilli, inhibition of the ZipA–FtsZ interaction represents a promising new target for antibacterial action. To this end, a programme dedicated to discovering small molecule inhibitors of the binding of FtsZ by identified bacterial ZipAs, while simultaneously searching for ZipA analogues in Gram-positive pathogens, has been reported [2]. Several libraries were synthesized in solution, giving a total of >100 compounds prepared as singletons. These compounds were evaluated for their effects on the binding of FtsZ peptide to $ZipA_{185\text{-}328}$ and an analogue of the C-terminal FtsZ peptide in a fluorescence polarization competitive assay. Additionally, to correlate *in vitro* and *in vivo* activities, selected compounds were evaluated as inhibitors of bacterial cell growth. Compounds were tested for activity against bacterial cell growth by examination using microscopy to see whether or not they caused cell elongation at sublethal concentrations in *Bacillus subtilis*, and in two strains of *E. Coli* 390 (inner membrane permeable mutant) and CH4 (a ZipA gene knock-out complemented with ZipA supplied on a thermosensitive plasmid).

Cytotoxic compounds that caused the formation of cell debris were distinguished from compounds causing cell elongation in this assay visually by both microscopy and histogram shape. One of the most potent ZipA analogues isolated was **(i)**, which possessed an IC_{50} of 296 μM. Results for this compound in the cell elongation assay is consistent with cell division inhibition. This work is important as it has demonstrated that small molecule inhibitors of ZipA–FtsZ could inhibit cell division in *E. coli* and more work is necessary to further our understanding in this area.



(i)

1 Hale, C.A. and de Boer, P.A.J. (1997) Direct binding of FtsZ to ZipA, an essential component of the septal ring structure that mediates call division in *E. coli*. *Cell* 88, 175–185

2 Jennings, L.D., *et al.* (2004) Combinatorial synthesis of substituted 3-(2-indolyl)piperidines and 2-phenyl indoles as inhibitors of ZipA-FtsZ interaction. *Bioorg. Med. Chem.* 12, 5115–5131

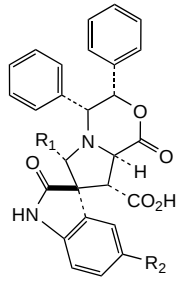
Actin polymerization inhibitors

One of the goals of chemical genetics is to find molecules that alter, in a highly specific manner, the individual functions of gene products, something that can also be accomplished by natural products. An unproven and intuitive hypothesis is that synthetic compounds embodying features characteristic of natural products might prove equally effective modulators of individual functions of gene products. This mimicry will arise, for example, from their rigidity, covalent bonding, hydrogen bonding capacity or stereochemistry when compared with the natural product.

The laboratories of Schreiber have been actively engaged in determining whether diversity-orientated synthesis (DOS) might be useful in testing this hypothesis [3]. DOS aims

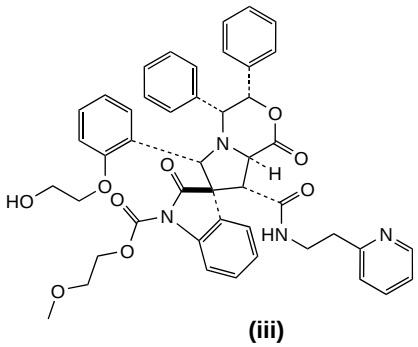
1359-6446/05/$ – see front matter ©2005 Elsevier Ltd. All rights reserved. PII: S1359-6446(05)03506-3

to synthesize compounds whose diversity results from variations in skeletons and stereochemistry. Additionally, products having functionalities that enable follow-up chemistry that can be performed effectively and systematically are highly valued. The selection of reactions to be incorporated in DOS pathways is critical to the value of the resultant library as a tool to investigate the biological effects of the compounds synthesized. Complexity-generating reactions are appealing because molecules embodying the features of natural products can be assembled from simple building blocks. A three-component coupling reaction was used to generate a library of over 3500 single-skeleton spirooxindoles [for this generic structure, see **(ii)**] on solid phase using a split-pool approach to their synthesis. In this library, diversity arose from use of alternating dipolarophiles and by the removal of an auxiliary that yielded an amino acid used for subsequent skeleton-determining reactions.



(ii)

Although the molecular weight and lipophilicity (as determined by cLogP) of the library members was high, to demonstrate the value of these compounds as effective probes a chemical genetic modifier screen was used to search for bioactive compounds. This type of screen identifies compounds that enhance or suppress cellular phenotypes, for example those induced by a small molecule with a known mechanism of action. An assay was developed to identify enhancers of the growth arrest induced by latrunculin B, a natural product that sequesters monomeric actin and prevents the formation of actin microfilaments. Latrunculin B has been a valuable tool in elucidating the roles of the actin cytoskeleton in mammalian cells [4]. On of the most potent compounds isolated from this approach was **(iii)** which possessed an EC_{50} of 550 nM for enhancing the inhibitory effect of latrunculin B. This work is of interest as the molecules synthesized have been shown to act as novel probes for cell circuitry, specifically for the actin regulatory network, by the discovery of enhancers of latrunculin B, an actin polymerisation inhibitor. Further work in this area is merited.



(iii)

3 Lo, M.M.-C. *et al.* (2004) A library of spirooxindoles based on a stereoselective three-component coupling reaction. *J. Am. Chem. Soc.* 126, 16077–16086

4 Peterson, J.R. and Mitchinson, T.J. (2002) Small molecules, big impact: A history of chemical inhibitors and the cytoskeleton. *Chem. Biol.* 9, 1275–1285

Paul Edwards
paul.edwards@santhera.com

NEUROSCIENCE

Alzheimer's disease: BACE the Ace

BACE, the β-Amyloid Converting Enzyme cleaves amyloid precursor proteins (APP) and the resulting products are further processed by γ-secretase into amyloidogenic peptides. β-amyloid, which results from these peptides, is the major component of senile plaques found in the brain of Alzheimer patients. When BACE is inhibited or its level is reduced, less β-amyloid is produced. BACE1 knockout mice are healthy and have almost no β-amyloid production. It has therefore been of interest to find a possibility to influence the activity of BACE1, via drugs or other regulatory proteins. Reticulons (RTNs) are small proteins, of which RTN4 has been implicated in the inhibition of neuronal growth. RTN4 and RTN3 are expressed in mouse brain.

In their recent study, He *et al.* showed that BACE1 binds to RTN3 and RTN4 when expressed in a cell line and in human brain cortex extracts [1]. Colocalization of RTN3 with BACE1 was shown in neurons in mouse brain slices with confocal microscopy.

Overexpression of RTN3 in a cell line that stably overexpresses APP, led to a decrease of Aβ1-40 and Aβ1-42 secretion in these cells. This effect could be shown for RTN1,2,3 and 4; it indicates, that RTNs are inhibiting BACE1 and thereby the (pathological) β-amyloid production. Decreasing RTN3 by RNAi in an APP expressing cell line led to a further increase in APP secretion, strengthening the idea of RTN3 as a negative modulator of BACE1 in these cells.

Using a pulldown essay, the author show that the protease-inactive BACE1 interacts with APP. Coexpression of increasing amounts of RTN3 in these cells led to a decrease in coimmunoprecipitation of BACE1 with APP, but increased coimmunoprecipitation of BACE1 with RTN3. This indicates that RTN3 blocks the access of BACE1 to APP.

Interfering with BACE1 seems to be ideal for the therapeutic block of β-amyloid production. As this enzyme is located intracellularly it is hard to reach for drugs and a specific modulator is difficult to find. He *et al.* describe a cellular modulator that might be an alternative target for drugs aiming to treat or prevent Alzheimer's disease.

1 He, W. *et al.* (2004) Reticulon family members modulate bace1 activity and amyloid-beta peptide generation. *Nat. Med.* 10, 959–965

Angelika Lampert
angelika.lampert@yale.edu

Cocaine and brain

Drug addiction is a relatively common, and potentially devastating phenomenon, incurring both economic and personal costs. Understanding the neurobiological basis of addiction is a pre-requisite to developing effective therapeutic strategies. In the case of cocaine, there is some evidence suggesting that addiction results from drug-induced perturbations of the dopamine system, and the circuits it regulates.

Volkow and colleagues used positron emission tomography (PET) scanning (an index of brain metabolic function), to compare responses to the cocaine-analogue methylphenidate (MP) in cocaine-addicted and control subjects [2]. By co-administering MP with the dopamine D2 receptor antagonist raclopride, the authors were able to correlate metabolic differences between the two experimental groups with dopaminergic effects.

MP administration resulted in metabolic increases in the cerebellum and the occipital cortex, and decreases in the caudate and the

1359-6446/05/$ – see front matter ©2005 Elsevier Ltd. All rights reserved. PII: S1359-6446(05)03496-3