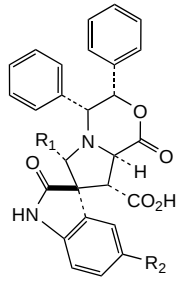
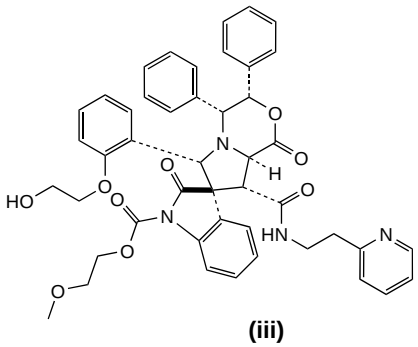
to synthesize compounds whose diversity results from variations in skeletons and stereochemistry. Additionally, products having functionalities that enable follow-up chemistry that can be performed effectively and systematically are highly valued. The selection of reactions to be incorporated in DOS 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

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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

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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

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temporal insula in both groups. In contrast, opposite metabolic effects in the two groups were seen in the right medial orbital prefrontal cortex (OMPFC), a structure implicated in salience attribution, inhibitory control and compulsive behaviours. Specifically, metabolism was increased in addicted subjects, but decreased in control subjects. In all subjects, these metabolic changes were associated with an enhanced desire for methylphenidate, and in addicts with a cocaine craving.

The strong correlation between MP-induced metabolism in one area of the OMPFC [Brodmann's area (BA) 25] and yearning for either methylphenidate or cocaine suggests that the abnormal activation of this region underlies the intense desire to take the drug. At the psychological level, the authors suggest that BA25 could be involved in processing the emotional reactivity to the drug, whereas a second region of the OMPFC, BA11, might be involved with 'processing the saliency value of the drug to the subject and the motivation to procure it'. The raclopride experiment indicated that MP-induced increases in metabolism in the OMPFC were associated with an increase in dopamine in the thalamus, but not in the striatum. This result indicates that the mesothalamic dopaminergic projection regulates the responses of the OMPFC to MP, or even vice versa, given the reciprocal nature of the connections between the two structures.

In summary, the results strengthen the argument for a role of the OMPFC in cocaine addiction, and propose a mediatory role for mesothalamic dopaminergic projections. Abnormalities of the former region have also been observed in subjects addicted to a variety of other drugs (including heroin, methamphetamine, marijuana and alcohol) suggesting a common neurobiological basis to addiction amenable to therapeutic intervention.

2 Volkow, N.D. *et al.* (2005) Activation of orbital and medial prefrontal cortex by methylphenidate in cocaine-addicted subjects but not in controls: relevance to addiction. *J. Neurosci.* 25, 3932–3939

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MOLECULAR BIOLOGY

ATM is activated by the Mre11–Rad50–Nbs1 complex

DNA double-strand breaks (DSBs) must be repaired to maintain genomic stability. The kinase ATM is activated by DSBs and phosphorylates targets such as p53 and Chk2 to initiate cell cycle arrest and DNA repair. However it is not clear how ATM itself is activated. Lee *et al.* show that ATM is activated and recruited to DNA ends by the Mre11–Rad50–Nbs1 (MRN) complex [3].

ATM forms an inactive multimer *in vivo* and becomes monomeric when it is activated. Therefore the authors purified inactive multimeric ATM and showed using glycerol gradients that it was a dimer. This dimer was activated slightly by the MRE complex alone, but activity increased two orders of magnitude if linear DNA was also present. If closed-circular DNA was used no stimulation was seen, showing that DNA ends are required for activity. A complex lacking the Nbs1 subunit (MR) was not sufficient for the stimulation of activity.

The authors also examined DNA binding. Using biotinylated DNA they showed that the MRN complex bound DNA and that ATM only bound when MRN was also present. The MR complex also recruited ATM to DNA, whereas Mre11 alone did not. This shows that recruitment to the DNA requires Rad50 and that DNA binding is not sufficient to activate ATM, because activation requires Nbs1.

The MRN complex unwinds DNA and this activity requires Nbs1 and ATP. The authors used a mutant of Rad50 that inhibits the ATPase activity and showed that this prevented activation of ATM. A DNA substrate with closed hairpins on the ends did not stimulate ATM, whereas DNA with a non-complementary end did. These data show that ATP-dependent unwinding of the DNA ends is required for the activation of ATM.

3 Lee, J.-H. and Paull, T.T. (2005) ATM activation by DNA double-strand breaks through the Mre11–Rad50–Nbs1 complex. *Science* 308, 551–554

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Bioavailability, divide and conquer

Drugs differ from non-drugs, and humans have a preference for simple categories. Taken together this sparked Lipinski's 'rule of five' some years ago: You are likely to encounter good absorption and permeation if you have fewer than five hydrogen bond donors, fewer than ten hydrogen bond acceptors, a molecular weight of less than 500 and a logP smaller than five. This rule generalizes across all classes of drugs, however, there are exceptions to it such as antibiotics and antifungals, but overall it provides a simple guideline for what's absorbed and what's not, from which to deviate one needs at least a good reason.

Recently, Martin, realizing that commercial ADME tools were unable to predict bioavailability reliably, discovered a new twist to this tale: the influence of charge [1]. Based on Caco-2 permeability and rat bioavailability data the 'rule of five' was not able to form a satisfactory predictor. Not on its own, that is: it failed just for anions, although giving acceptable results for neutral and positively charged compounds. For anions a different property emerged as being important, the polar surface area (PSA) of the molecule. Whereas small anions (defined as PSA $\leq 75 Å^2$) are very well absorbed, this already changes for medium-sized structures ($75 Å^2 \leq$ PSA $< 150 Å^2$), whereas large, negatively charged molecules (PSA $\geq 150 Å^2$) are very unlikely to show favourable behaviour. Martin has thus identified a hidden variable relevant for bioavailability, charge, whose particular effect on absorption and permeation is as yet unknown.

Together with the 'rule of five', the 'rule of three' (for lead-like compounds) and similar approaches the score developed here is another useful concept to cut losses early in drug development. It is overall predictive for bioavailability, yet simple enough to be understood. As always, remember, there are exceptions to every rule.

1 Martin, Y.C. (2005) A bioavailability score. *J. Med. Chem.* 48, 3164–3170

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