proteins bind to help activate transcription) from a human protein called NF-κB p65.

The authors found that, of all their synthetic transcription factors, a DNA-binding domain from a liver-specific human protein called hepatocyte nuclear factor 1-alpha (HNF1α) activated gene transcription most potently in response to antigen binding. They therefore used this transcription factor as part of a fully human SNIPR for further assessment. The group demonstrated that the humanized SNIPR-to-CAR circuit has clinical potential in several mouse-tumour models – in each case, it enabled specific eradication of cells expressing both antigens required for circuit activation, and spared cells harbouring only one.

Zhu *et al.* have provided a comprehensive framework for the modular assembly of humanized SNIPRs that have the potential for clinical application in CAR-T-cell therapy. The concept could theoretically be extended to a broad range of cell types and diseases. However, the current study focused only on T cells, so the universality of the authors' design principles remains to be determined.

Notably, in certain designs, SNIPR-circuit activity was enhanced by activation of the T-cell receptor (TCR) – a surface protein responsible for T cells' normal role of triggering immune responses to foreign antigens. This property could be beneficial when the desirable output is the expression of a CAR, but could be unwanted when the local, titrated delivery of a potentially toxic therapeutic agent is necessary. In the latter case, the authors suggest that cells could be further edited to eliminate TCR expression.

Furthermore, there are still some concerns about the kinetics of CAR induction and decay – kinetics that define a CAR's ability to selectively target a tumour while protecting normal tissues. After T cells are injected systemically, they circulate in the body and localize in the tumour; induction of CAR expression needs to be fast enough to engage the tumour when T cells reach it, but the protein needs to decay before T cells begin to circulate again to other sites. The best-performing humanized SNIPR candidate induced CAR expression at levels similar to those achieved by the conventional synNotch, but significantly more slowly (over 72 hours, compared with 24 hours). As such, the SNIPR-carrying cells had lower and slower tumour-cell-killing capacity than do conventional CAR T cells.

Promisingly, no CAR expression was detected *in vivo* in tumours lacking SNIPR target antigens. However, Zhu *et al.* did not provide detailed evaluation of CAR decay data for SNIPRs compared with synNotch. In the synNotch system, decay of CAR expression was slow enough that the engineered cells activated immune responses against non-tumour cells that expressed the CAR target antigen if the non-tumour cells were in close proximity to the tumour[6]. Thus, careful antigen pairing is still required during SNIPR design, and the 'safe distance' that would protect healthy tissues from toxicity remains to be determined.

Overall, Zhu and colleagues' work demonstrates the tremendous potential of synthetic biology to control the behaviour of therapeutic cells. The SNIPR circuit has many improved features compared with its precursor. The use of building blocks of human origin reduced the protein's immunogenicity to levels comparable with a CAR currently in clinical use. This reduces the risk that the protein will be rejected by the immune system – although further assays are required to confirm this. Moreover, the humanized SNIPR's compact size compared with first-generation synNotch, its efficiency in low copy numbers, its high sensitivity to low levels of antigen and its potential for activation by a range of ligands further support its clinical potential. Together, these benefits mean that Zhu and colleagues' toolkit should help to expedite the clinical implementation of this type of synthetic circuit in cancer immunotherapy.

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Chemical biology

Synthesis provides insight into a traditional medicine

Nicholas P. R. Onuska & Joshua G. Pierce

A compound made by plants used in traditional medicine has been prepared by chemical synthesis, providing enough for biological testing. The unexpected finding that it acts at opioid receptors raises prospects for drug discovery. **See p.917**

The bark of *Galbulimima* plants is used in the traditional medicine and ritual practices of people in the Papua New Guinea region as a painkiller, fever remedy and hallucinogen[1]. The bark, sometimes together with leaves of the *Homalomena* plant, is consumed to induce visions and a dream-like state that lasts for about one hour, followed by a sense of calmness, euphoria and then drowsiness (see go.nature.com/3feq5fu). On page 917, Woo and Shenvi[2] describe a remarkably innovative and scalable approach for the preparation of GB18 – an alkaloid compound found in *Galbulimima* bark. By gaining access to this complex molecule through chemical synthesis, the authors demonstrate its previously unknown ability to act at opioid receptors. GB18 might therefore serve as a platform for the development of medicines that target these receptors.

Human medicine relies on the continual discovery and development of new types of biologically active organic molecule. The connectivity of the atoms and the corresponding 3D structure of such molecules determine pharmacological parameters such as potency, activity and selectivity for a biological target. Even small changes in the structure, such as the replacement of a single atom in a molecule, can lead to profound differences in biological activity and efficacy[3].

The field of drug discovery broadly encompasses the process of identifying and optimizing the structure of an organic molecule for use as a medicine. Synthetic organic chemistry is the technology that enables the construction of well-defined and structurally complex molecules for biological testing. Molecules isolated from living organisms – broadly known as natural products – have historically been a potent source of inspiration for organic chemists working in drug development. Between 1981 and 2019, 32% of approved small-molecule drugs had structures that were based on those found in bioactive natural products[4]. However, the typically low natural abundance of these compounds in the organisms from which they derive has necessitated the development of practical chemical syntheses to obtain sufficient quantities of complex natural products

for scientific studies[4,5].

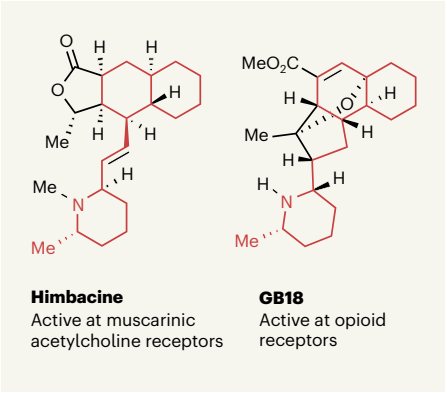
The compounds found in *Galbulimima* bark have long intrigued researchers, and 40 natural products unique to the *Galbulimima* genus have so far been isolated — the GB alkaloids. One of these, himbacine, shows promise as an antispasmodic drug (a compound that suppresses muscle spasms), and is a potent inhibitor of proteins known as muscarinic acetylcholine receptors[6]. GB18 is the only member of this alkaloid family that produces behavioural changes associated with cognition in mice, rather than changes in sensation or pain tolerance[1]. However, studies of GB18 activity have been limited by the low quantities of the compound in *Galbulimima* bark, and by the laborious process used to extract it.

The molecular structure of GB18 is similar to that of himbacine (the main 'scaffold' of the GB18 molecule can be thought of as a rearranged version of that of himbacine; Fig. 1), but there are marked differences in the biological activity of the compounds. The synthesis and biological testing of GB18 is therefore crucial to understanding the biological underpinnings of the GB alkaloids.

In pursuit of this goal, Woo and Shenvi sought a multistep organic synthesis that could gradually build up the complex, caged structure of the GB18 molecule from simple starting materials. A notable feature of their approach is the use of transition-metal compounds as catalysts to forge key bonds in the core of the molecule[7,8]. More specifically, the authors used a palladium-catalysed reaction with hydrogen (a hydrogenation reaction) as an early step in their synthetic route (see step 2 in Fig. 2 of ref. 2), and a new carbon–carbon bond-forming reaction (a cross-coupling reaction), catalysed by a mixture of manganese and nickel compounds, to introduce a nitrogen-containing group known as a pyridine ring (see the first step in Fig. 3a of ref. 2).

These catalytic reactions accomplish otherwise untenable chemical transformations at temperatures close to room temperature, and cleverly set up specific 3D orientations of crucial bonds within the molecule. Small quantities of the catalysts are used, relative to the amounts of the key reactants, decreasing the environmental impact and cost of the overall synthesis. Essential to the success of the cross-coupling reaction was the unprecedented use of an affordable and commercially available anti-inflammatory agent (praxadine) as an additive — in this context, praxadine stabilizes and attenuates the reactivity of the manganese and nickel atoms.

Woo and Shenvi then used another hydrogenation reaction to transform the pyridine ring into the corresponding piperidine ring — a process that involves the formation of five new carbon–hydrogen bonds. These bonds must form selectively on one face of the flat pyridine ring to match the 3D structure of GB18. Initial attempts at this reaction resulted in a one-to-one mixture of products arising from hydrogenation at both faces, but the determined researchers pressed on in pursuit of a solution.



Himbacine
Active at muscarinic acetylcholine receptors

GB18
Active at opioid receptors

Figure 1 | Molecular structures and biological activities of two *Galbulimima* alkaloids. The bark of *Galbulimima* trees is used in traditional medicine in Papua New Guinea. Two of its biologically active components are the alkaloid compounds himbacine and GB18. The main 'scaffold' of the GB18 molecule is a rearranged version of that of himbacine, although some parts of the two structures are the same (red). Himbacine acts at several muscarinic acetylcholine receptors, but the biological targets of GB18 were unknown because insufficient quantities of the alkaloid could be isolated for testing. Woo and Shenvi[2] report a synthesis of GB18 that allowed them to prepare gram-scale quantities for biological testing. They find that it acts at two opioid receptors, revealing that similar alkaloid scaffolds can have very different biological activities.

Just as the substitution of a single atom in a molecule can radically affect the molecule's biological activity, Woo and Shenvi found that the addition of a single oxygen atom to the nitrogen of the pyridine ring transformed the outcome of hydrogenation: using a rhodium catalyst, the hydrogenation occurred with the favoured selectivity (see step 9 in Fig. 3a of ref. 2). The resulting compound was then treated with metallic zinc to form the piperidine-containing molecule with excellent control of the 3D structure. Finally, the authors used a short series of chemical reactions to convert this molecule into GB18, which could be produced in gram-scale quantities.

The compound was prepared as a mixture of mirror-image isomers (enantiomers), which were separated to obtain the naturally occurring enantiomer. This approach was effective for the purposes of the current studies, but such separations can substantially increase the cost of syntheses carried out at multigram scales. Future research to develop a synthesis capable of producing a single enantiomer would therefore be valuable.

The authors screened GB18 against a panel of human receptors known to be associated with the activity of psychoactive drugs. This revealed that GB18 is a potent blocker of the κ- and μ-opioid receptors. The compound's activity at these receptors is comparable to that of opioid receptor blockers such as naltrexone, which is used to manage alcohol- and opioid-use disorders. Unlike himbacine, GB18 binds with low or statistically insignificant affinities to muscarinic acetylcholine receptors. The marked differences in the biological activity of GB18 and himbacine are remarkable given their structural similarities, and highlight the profound effect that scaffold rearrangements can have on the activity of GB alkaloids. The identification of GB18 as a blocker of the κ- and μ-opioid receptors is the first new assignment of biological targets to a GB alkaloid in more than 35 years[9].

This work emphasizes how research into bioactive natural products, synthesis and medicinal chemistry can advance understanding of traditional medicine. By using organic synthesis to produce multigram quantities of bioactive compounds, researchers can address biological questions, unconstrained by the natural abundance of such substances[10,11]. Woo and Shenvi's demonstration of problem-solving in chemistry also serves as an excellent example of determined scientific persistence in the face of the confounding and complex problems encountered during the synthesis of natural products, and sets the stage for future discoveries.

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