Researchers modify traditional poison used by Asian fishermen for potential neurological drugs

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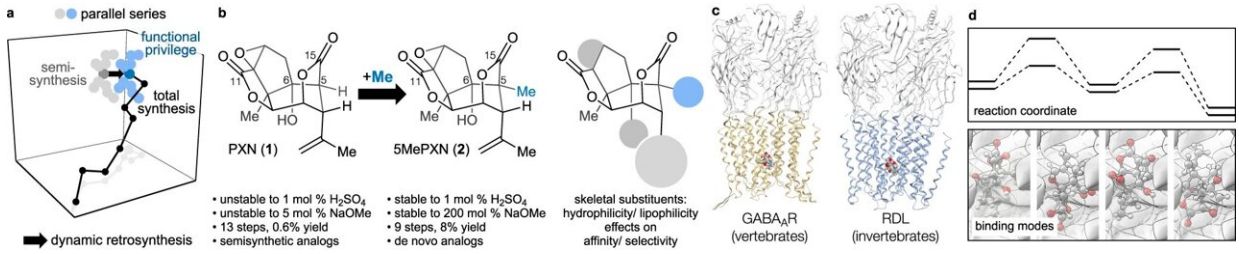

Overview: target modification to explore functionally privileged chemical space. **a** Chemical space plot of parallel series to explore the effects of a scaffold uniquely accessible through total synthesis; (**b**) C5 methylation increases stability to base and acid, increases yield, decreases required steps and increases receptor selectivity; (**c**) Assay against $GABA_A$ and RDL receptors, representative of vertebrate (e.g. human) and invertebrate (e.g. insect) ligand-gated ion channels (LGICs), respectively. Left: rat $GABA_A$ homology model from PDB 6×40 template with sequence from *R. norvegicus*, gold. Right: fly RDL homology model from PDB 6×40 template with sequence from *D. melanogaster*, blue. **d** Computational analyses provide models for increased stability and selectivity of the 5MePXN series. PXN picrotoxinin, 5MePXN 5-methylpicrotoxinin. Credit: *Nature Communications* (2023). DOI: 10.1038/s41467-023-44030-3

Picrotoxinin, a plant-derived toxin that Asian fishermen traditionally have used to paralyze and catch fish, has long been seen as a possible starting point for new human therapeutics and other neuroactive

products.

Yet little progress has been made due to picrotoxinin's chemical instability and toxicity and the difficulty of making and modifying its complex structure. However, chemists at Scripps Research have found a relatively easy way to make versions of picrotoxinin with improved properties.

In a study published in *Nature Communications*, the researchers showed that close chemical variants of picrotoxinin that contain a single small modification have better chemical stability, are much easier to make and modify, and are safer for humans. This opens the door to developing new neurological drugs, safer pesticides, and even anti-parasite treatments.

"Just a small alteration to the natural product gives it properties that have been elusive for decades," says study senior author Ryan Shenvi, Ph.D., a professor in the Department of Chemistry at Scripps Research.

The first author was Guanghu Tong, Ph.D., a postdoctoral research associate in the Shenvi Lab during the study.

Picrotoxinin comes from the seeds—often called "fishberry" seeds because of their use by fishermen—of Anamirta cocculus, a plant found in parts of Southeast Asia and India. The toxin potently blocks the activity of neuronal receptors found in most higher organisms.

In mammals, these are called $GABA_A$ receptors, and they exist throughout the brain, largely to prevent other neurons from becoming overactive. Even at small doses, picrotoxinin's blocking of these receptors can cause seizures and fatally disrupt the nerve signals that control breathing.

It might seem contradictory that chemists would turn to poisons for making new medicines, but many plant toxins, in addition to hitting desirable targets, already have good drug-like properties such as getting to their targets via oral dosing.

In the case of picrotoxinin, chemists would like to modify it to develop drugs for psychiatric and neurological disorders, safe and effective pesticides and anti-parasite drugs, and laboratory tools to manipulate GABA precisely $_{A}$ receptors. The problem has been that picrotoxinin's other chemical properties, such as its synthetic difficulty and tendency to react with ordinary solvents, have made it extraordinarily hard to tame.

Shenvi's lab uses organic chemistry techniques to overcome such challenges and find ways to improve natural products. For years, he and his team have been focusing on molecules that target $GABA_A$ receptors, and in 2020, they reported the shortest-ever organic synthesis of picrotoxinin.

In that study, they found they could much more easily synthesize a compound that was almost the same as picrotoxinin. 5Me-picrotoxinin, as they called it, could still bind to $GABA_A$ receptors and only differed from its chemical cousin by the addition of a cluster of atoms—called a methyl group—at a key position on the molecule. Given this one structural change, Shenvi's team investigated 5Me-picrotoxinin's novel properties for the new study.

The team synthesized two parallel sets of picrotoxinin and 5Me-picrotoxinin variants, determining how the absence or presence of the methyl group changes the molecule's stability and receptor binding selectivity.

They discovered that the methylated version is chemically much more stable, with a bloodstream half-life that seems to be nearly triple that of

ordinary picrotoxinin. They also found that 5Me-picrotoxinin is much less prone to reactions with common solvents, including alcohols and acids. Co-authors Shuming Chen, Ph.D., assistant professor of chemistry at Oberlin College, and her lab member Anna Crowell explained this using computational modeling.

Another surprise was that the methylated version has lower potency against mammalian $GABA_A$ receptors while retaining high potency against insect versions of the receptor—just what one would want for a safe insect-killing compound.

"The fact that picrotoxinin targets a family of receptors including $GABA_A$ receptors has been known for several decades, but this is the first time we've been able to change its selectivity for those receptors," Tong says.

The experiments with picrotoxinin variants and insect receptors were conducted by collaborating researchers at Corteva Agriscience, developers of pest-control products. Models built for the study by Corteva computational chemist Avery Sader, Ph.D., suggest further ways to modify 5Me-picrotoxinin to make it more selective for insect pests and thus safer for humans.

The researchers plan to continue synthesizing and investigating new variants of 5Me-picrotoxinin for their potential to be developed into new medicines and other products.

More information: Guanghu Tong et al, C5 methylation confers accessibility, stability and selectivity to picrotoxinin, *Nature Communications* (2023). DOI: 10.1038/s41467-023-44030-3

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