LEAD DISCOVERY

Build me up, buttercup

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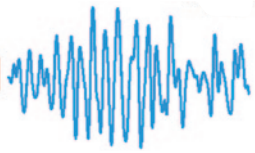
Fragment-based lead discovery relies on identifying low-molecular-weight molecules that bind weakly to a protein target, with the hope that those fragments could be enlarged or linked up to make a drug-like molecule that binds with higher affinity and selectivity. Unfortunately, practitioners of fragment-based lead discovery can spend a great deal of time screening libraries and studying hits that seem promising, only to find out that they are false positives. In an attempt to streamline this process, Silvestre *et al.* screened a library of 1,250 fragments against *Mycobacterium tuberculosis* pantothenate synthetase using fluorescence-based thermal shift assays. They identified 39 potential hits and used NMR spectroscopy to show that 17 of them were bona fide binders of pantothenate synthetase. Isothermal titration calorimetry was then used to determine the thermodynamic binding parameters of all 17 compounds, and X-ray crystal structures of eight of those compounds bound to pantothenate synthetase were determined. Four of the compounds bound the adenine-binding region of the protein, three bound the pantoate-binding region of the protein, and one bound very deeply in the pantoate-binding pocket, noncompetitively with ATP. The application of multiple biophysical techniques resulted in an approach that optimized the use of faster, less precise methods to rapidly focus on a smaller collection of fragments, which could then be subjected to a time-intensive, robust analysis to determine exactly how those fragments bound the target protein. *JMF*

NEUROSCIENCE

A positive for GABA

Neuron **78**, 1063–1074 (2013)

JOSEPH LYNCH

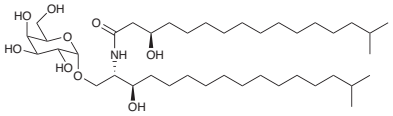


GABA is an inhibitory neurotransmitter that acts in various pathways, including the modulation of the susceptibility and duration of absence seizures, common brief lapses of consciousness. Benzodiazepines are psychoactive synthetic compounds that target $GABA_A$ receptors and can act as either positive (PAM) or negative (NAM) allosteric modulators. On the basis of the presence of an endogenous set of inhibitors comprising DBI and related proteins, which bind the allosteric benzodiazepine site on $GABA_A$ receptors to act as NAMs, Christian *et al.* suspected that an endogenous PAM might also exist. In their search for a PAM, the authors unexpectedly found a clue that linked back to DBI: mice with either a point mutation that abolished benzodiazepine binding in $GABA_A$ receptors or a chromosomal deletion that included the *Dbi* gene showed a decrease in the duration of $GABA_A$ receptor–mediated spontaneous inhibitory postsynaptic currents (IPSCs). Experiments with a benzodiazepine site antagonist provided *in vitro* validation that the benzodiazepine site is targeted by the PAM in modulating the IPSC duration, and electroencephalogram recordings of the mutant mice found that *Dbi* gene products suppressed seizure activity. Further experiments using caged GABA and GABA transporter antagonists served to define the specific brain regions, relevant to the antiseizure activity, where DBI products behave as PAMs. These results suggest that endogenous benzodiazepine-mimicking PAM effects exist and that they are mediated by DBI proteins. *MB*

SPHINGOLIPIDS

Microbes talk to T cells

PLoS Biol. **11**, e1001610 (2013)



Sphingolipids are signaling molecules that are prevalent in eukaryotes but infrequent in bacteria. One exception is the prevalent gut microbe, *Bacteroides fragilis*, in which sphingolipids are abundant in the membrane. To learn more about these sphingolipids and their potential roles in host biology, Brown *et al.* first searched for enzymes involved in sphingolipid biosynthesis. Knockout of BF2461, anticipated to serve as the first committed step in sphingolipid biosynthesis, resulted in the loss of three sphingolipids as compared to the wild-type strain, one of which was an unknown species. MS and NMR characterization, as well as comparison with a synthetic standard, defined the new species as an α-galactosylceramide named α-$GalCel_{Bf}$. The only other known naturally occurring α-galactosylceramides are the sponge-derived agelasphins, with the synthetic natural killer T (NKT) cell agonist KRN7000 derived from agelasphin-9b. Given this structural similarity, the authors tested whether α-$GalCel_{Bf}$ might similarly have a role in immune signaling. α-$GalCel_{Bf}$ was shown to bind the host immune receptor CD1d and to stimulate mouse and human NKT cells. NKT cells isolated from mice immunized with purified α-$GalCel_{Bf}$ also showed signs of activation. Tests of germ-free and specific pathogen–free mice colonized with wild-type and DBF2461 *B. fragilis* were inconclusive given variations in NKT cell numbers, but the high prevalence of both *B. fragilis* and its sphingolipid components in the human gut suggest that α-$GalCel_{Bf}$ could serve as the endogenous complement to KRN7000. *CG*

MICROBIOLOGY

Fighting to stay awake

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The tropical disease African sleeping sickness or African trypanosomiasis, caused by *Trypanosoma brucei* infection, results in symptoms such as sleep disturbances and, if left untreated, death. The evolving drug resistance of the parasite and high prevalence of side effects have stymied treatment for this disease, with researchers focusing on the use of protein kinase inhibitors as a therapeutic alternative. Nishino *et al.* found that administration of a fungus-derived compound, hypothemycin, a covalent inhibitor of the CDXG family of protein kinases, promoted the death of cultured *T. brucei* and increased survival of infected mice compared to untreated controls. However, high doses of hypothemycin resulted in side effects owing to nonspecific inhibition of protein kinases. To identify the CDXG protein kinases that are targeted at low hypothemycin concentrations and thus may serve as potential therapeutic targets, the authors synthesized an analog of hypothemycin by replacing the methyl ether with an equipotent propargyl ether, enabling pulldown of hypothemycin-labeled kinases in proteomic assays. RNAi knockdown of labeled kinases revealed that *Tb*GSK3short and a previously uncharacterized kinase, *Tb*CLK1, were required for *T. brucei* viability. Finally, *in vitro* hypothemycin preferentially inhibited *Tb*CLK1 activity. As *Tb*CLK1 has little sequence homology with its human counterpart, the development of drugs that specifically target *Tb*CLK1 activity may be a promising new avenue to combat African sleeping sickness. *GM*

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