DISCOVERY REPORT

An ACS member exclusive

ACS Chemistry for Life®

The future of covalent drugs

Latching molecules to proteins may yield new treatments

Published by c&en

$39.99

The future of covalent drugs

Chemistry is all about making bonds, particularly covalent ones in which atoms share electrons. And some of the world's longest-known pharmaceuticals—including the anti-inflammatory aspirin and the antibiotic penicillin V—act by forming covalent bonds with their biological targets.

But drugmakers have generally avoided developing such drugs, fearing that the molecules would do more harm than good by binding to nontarget proteins or triggering immune responses. Instead, pharmaceutical scientists have focused on molecules that bind reversibly through noncovalent interactions.

The recent success of several covalent anticancer drugs has demonstrated that the fears around covalent drugs are, if not unfounded, perhaps overblown.

In this report, you'll discover what chemists have learned about covalent drugs that has turned them into a mainstay of drug development. You'll learn the chemistry behind targeting proteins previously considered to be undruggable. You'll meet the scientists developing new tools to demonstrate the selectivity of drug candidates. And you'll encounter the start-ups taking the molecules into the clinics.

Contributing editor Brian Owens, an independent journalist who covers health and the environment, edited this report with Jyllian Kemsley, C&EN's executive editor for policy and content partnerships. It includes a reading list of papers and patents curated by our sources, as well as by information scientists at the CAS division of the American Chemical Society.

As an ACS member, you get exclusive access to Discovery Reports, quarterly publications that bring you cutting-edge research defining the chemical sciences and our industry. Look for the next one in the fourth quarter of 2022.

Bibiana Campos Seijo
Editor in chief, C&EN
@BibianaCampos

INSIDE

Published by
c&en

Editors: Jyllian Kemsley, Brian Owens

Contributing writers: Bethany Halford, Shi En Kim

Creative: Robin Braverman, Rob Bryson, Chris Gash

Copyeditor: Michele Arboit

Production: Arminda Downey-Mavromatis, Marsha-Ann Watson

Comments or questions? Email cenfeedback@acs.org

ACS Chemistry for Life®

CHEAT SHEET

5 questions and answers about covalent drugs

Q. Can we move beyond cysteine?

» **The vast majority of covalent drugs,** both approved and in development, use an acrylamide to bind with a cysteine amino acid on their target protein.

» **But that limits the proteins** that can be targeted, as cysteine is uncommon in binding sites and is usually accessible only in intracellular proteins.

» **New functional groups** that can bind to other amino acids, such as lysine, would expand the field of druggable proteins.

Q. Can covalent drugs be useful outside oncology?

» **The first targeted covalent inhibitors**—drugs deliberately designed to use covalent bonds, rather than those discovered serendipitously, like penicillin—were all anticancer drugs, because the risk of toxicity is considered more acceptable when the stakes are life and death.

» **The oncology drugs have proved to be well tolerated** and have not triggered broad toxicities, giving researchers and companies the leeway to expand into other areas.

» **Inflammatory diseases have emerged** as a rich target for new covalent drugs.

Q. How many 'undruggable' proteins could covalent drugs reach?

» **Of the roughly 20,000 proteins** in the human proteome, only about 3,000 are thought to be druggable with conventional drugs, and just 700 actually have drugs that target them.

» **Drugs that bind to nonfunctional parts of a protein,** to cause conformational changes or serve as anchors to allow interactions elsewhere, could help greatly expand the number of druggable targets.

» **Efforts to drug the entire proteome** are likely to rely heavily on covalent drugs.

Q. Have the risks of toxicity from covalent drugs been dealt with adequately?

» **The biggest hurdle to developing** covalent drugs has always been the risk of off-target effects that trigger an immune response and toxicity.

» **Stabilizing the protein-drug complex** is one of several techniques that scientists use to reduce the risk of side effects and make covalent drugs more specific.

» **In the case of kinase inhibitors,** covalent drugs have a lower chance of causing liver toxicity than conventional reversible drugs.

Q. Can biologic drugs make use of covalent bonds?

» **Small-molecule drugs** have dominated covalent drug development to date.

» **Proteins and other biologics** are generally not able to form covalent bonds with their targets, but techniques such as incorporating synthetic amino acids could give them that ability.

6 experts identify the challenges and opportunities for covalent drugs

Elena De Vita

» **Marie Skłodowska-Curie fellow, Imperial College London**

Perhaps the biggest advantage of covalent drugs is their potential to attack targets once thought to be "undruggable," Elena De Vita says. They are part of a suite of approaches, including things like next-generation sequencing, that are helping researchers expand the number of druggable proteins.

Of the roughly 20,000 proteins in the human proteome, about 3,000 are considered druggable with conventional drugs, yet only 700 have drugs that target them, De Vita says. The Target 2035 initiative is a global effort to create libraries of small molecules, chemical probes, and functional antibodies for the entire proteome—along with covalent drugs to target as many proteins as possible.

In her own work, De Vita focuses on Rab27, a small enzyme that hydrolyzes guanosine triphosphate to guanosine diphosphate and is involved in cancer metastasis. The family of GTPases was long considered undruggable, because the enzymes have only one binding pocket, which is highly conserved across all versions of the protein. The similarity of the binding sites makes it difficult to target a specific protein. In addition, there is a lot of GTP in a cell, so it is hard to outcompete the substrate with a drug. But De Vita and others working on GTPases found that a protein fragment that binds covalently elsewhere on the protein causes a conformational change that opens up a new pocket near the GTP-binding site to create a new target for a drug. Researchers can then build on that fragment to develop a novel drug that can take advantage of the new site.

> **If we can find something with even a weak affinity for the protein, that's a starting point we can use to develop a more potent chemical.**

"If we can find something with even a weak affinity for the protein, that's a starting point we can use to develop a more potent chemical," De Vita says. "This opens up a lot of possibilities that we wouldn't even be able to consider without covalent inhibitors.

Jeffery Kelly

» **Professor of chemistry, Scripps Research-California**

In drug discovery, researchers normally isolate a specific biological target, then throw hundreds or thousands of novel chemicals at it to see what sticks and what might make a useful drug. Jeffery Kelly does the opposite.

Kelly's research group uses a concept he calls inverse drug discovery. He and his colleagues start with a few chemicals with underexplored "Goldilocks" functional groups—ones they know have the potential to covalently bind with one or a few proteins but aren't so reactive that they'll disappear in the biological environment. They screen those molecules against all the proteins in a living cell to see which proteins the molecules bind to. They can then decide which, if any, look worthwhile to pursue further. "It's a really good strategy for making covalent ligands for the human proteome," Kelly says. "If we did it for a few years, we would eventually find ligands for the majority" of proteins.

The technique is better suited to academic drug discovery and big pharmaceutical companies than to small biotechs focused on a particular disease, Kelly says, because "we have no idea what we're going to

CREDIT: COURTESY OF INSTITUTIONS

find." His group has already identified some promising leads linked to cancer and is now concentrating on proteins involved in lysosomal degradation, which plays a role in some metabolic disorders.

Inverse drug discovery works best for finding covalent inhibitors, because the workflow involves using affinity chromatography to remove the bound proteins from the mixture. That separation technique requires extremely tight bonds. Kelly says his interest in covalent drugs stems from the fact that they're underused in drug discovery because of concerns about off-target effects. But there are now many approaches to delineate how selective covalent drugs are for their targets, he adds, and that makes them a viable option for future development.

György Keserű

» Director of medicinal chemistry, Hungarian Academy of Sciences

Many covalent drugs are created by taking an existing small-molecule drug and adding a covalent "warhead" to increase the drug's binding affinity for the target protein. György Keserű prefers to start with the covalent ligand and build the rest of the drug around it, a process known as fragment-based drug discovery.

"We can explore different subpockets [of the protein], then connect or grow new parts of the molecule," Keserű says. "Rather than trying to fit an existing house into a plot of land, you build it out of smaller pieces, like bricks, into a shape that fits the space available."

While fragment-based drug discovery does not require covalent ligands, there are several benefits to combining the two. First, because the starting point is a small part of a molecule, it can be difficult to determine when it has attached to a target without a covalent bond. Covalent ligands are much easier to detect. Second, as researchers add new moieties to an initial fragment, there's a risk that the binding mode will change, affecting the drug's efficacy. That is much less likely with covalent bonds, which makes covalent drugs easier to optimize. Finally, it can help identify new targets by screening covalent fragments against protein libraries, as in the inverse drug discovery method Jeffrey Kelly uses.

Building a molecule to fit the available space can also help reduce off-target effects. Assembling the molecule bit by bit allows researchers to add pieces that bind to other, nearby elements of the target, to both stabilize the bonds and make the drug more specific. "The specificity comes from those noncovalent parts," Keserű says.

> **"There are a whole host of drug targets out there that are not being targeted because they don't have opportune cysteines that you can go after."**

Juswinder Singh

» Chief scientific officer, Ankaa Therapeutics

Every covalent drug used today is essentially an acrylamide, or a slight variation on one. "That's good," Juswinder Singh says. "It shows companies are getting comfortable with the idea of targeting proteins with an acrylamide."

But that comfort also causes restrictions, Singh says, as it means covalent drugs are essentially limited to targeting cysteine residues. And those targeted cysteines are primarily on intracellular proteins. In contrast, cysteines on extracellular proteins are generally sequestered into disulfide bonds. "There are a whole host of drug targets out there that are not being targeted because they don't have opportune cysteines that you can go after," he says.

Singh says the next big opportunity in covalent drug discovery is to expand the field of drug targets to include ones that make use of residues other than cysteine. "People are putting a lot of focus on lysine," he says. "Several companies have recently been financed to do this" (see page 17). Lysine is more prevalent than cysteine in protein-binding sites and is not limited to intracellular proteins. But because it is less nucleophilic than cysteine, in most cases it makes for a more challenging target for covalent drugs.

Singh himself has been working on this problem with his team at Ankaa for the past several years. They have been looking at chemicals other than acrylamide that can target cysteine and at ways to target other residues like lysine. If their search proves successful, it would greatly expand the possibilities of covalent drugs. "There's a whole landscape out there to be discovered," Singh says.

Jack Taunton

» Professor of cellular molecular pharmacology, University of California, San Francisco

The most distinctive feature of covalent drugs is the tight, almost unbreakable bonds they form with their target proteins. But Jack Taunton is going in another direction, designing covalent inhibitors that are completely reversible.

The discovery of such reversible covalent inhibitors was a surprise. Michael Cohen, one of Taunton's graduate students, started exploring electrophiles made from cyanoacrylates—the same type of chemical moiety used in adhesives such as

CREDIT: COURTESY OF INSTITUTIONS

Super Glue. Taunton was skeptical, thinking the compounds would be so reactive that they would bind to every cysteine in the cell. But when they looked at what should have been a cyanoacrylate compound bound to the ribosomal S6 kinase (RSK) protein, mass spectrometry showed no covalent modification, even though the molecule was the most potent RSK inhibitor they had yet seen. Further investigation showed that cyanoacrylates—and cyanoacrylamides—are indeed highly selective and completely reversible covalent inhibitors.

Reversible inhibitors rely on cooperative interactions like hydrogen bonds and Van der Waals interactions to stabilize them. Without those interactions, the covalent bond quickly reverses, reducing off-target effects. The same thing happens when the target protein is denatured or otherwise disrupted.

"Against a folded target the binding is, if not completely irreversible, at least long lasting. But as soon as you unfold or denature the protein, the covalent bond reverses as fast as you can make the measurement," Taunton says. Sanofi currently has a drug based on this kind of reversible covalent bond, rilzabrutinib, in Phase 3 trials for immune thrombocytopenia.

Reversible binding lets researchers design or optimize compounds on the basis of their distinct off rates by constructing drugs that take advantage of the specific stabilizing interactions available on their target. "Fully reversible interactions, even when covalent, give you more opportunities for off-rate-driven selectivity, which is completely unavailable to irreversible covalent approaches," Taunton says.

> **When proteins have the same covalent-binding ability, they should have the same benefits as with small molecules,"**

Lei Wang

» Professor of chemical biology, University of California, San Francisco

The benefits of covalent drugs are obvious: The tighter bond means they are more potent and stay in the body longer, so dosages can be lower. And they allow pharmaceuticals to attack targets previously thought to be undruggable. But so far, almost all the covalent drugs created have been small molecules. There are no covalent protein drugs, because proteins usually can't form covalent bonds with other proteins. Lei Wang wants to change that, bringing the advantages of covalency to biologic drugs.

"When proteins have the same covalent-binding ability, they should have the same benefits as with small molecules," Wang says. And they may also have greater specificity than small molecules, a characteristic that reduces concerns about off-target effects.

To create covalent proteins, Wang and his colleagues turned to the well-established method of genetic code expansion, replacing one of a protein's natural amino acids with a synthetic one that can form covalent bonds with its target. For example, they added a new amino acid to human programmed cell death protein 1 (PD-1), allowing the protein to bind irreversibly to its target, PD-L1. The result was an antitumor therapeutic efficacy equivalent to or better than that of an anti-PD-L1 antibody in mice.

While Wang has demonstrated the potential of covalent protein drugs as a treatment for cancer, he says the genetic code expansion method could be used to convert almost any protein drug—or other biologic drug—into a covalent one. "This is a platform technology that can be applied to a broad range of diseases that protein drugs can treat," Wang says. ■

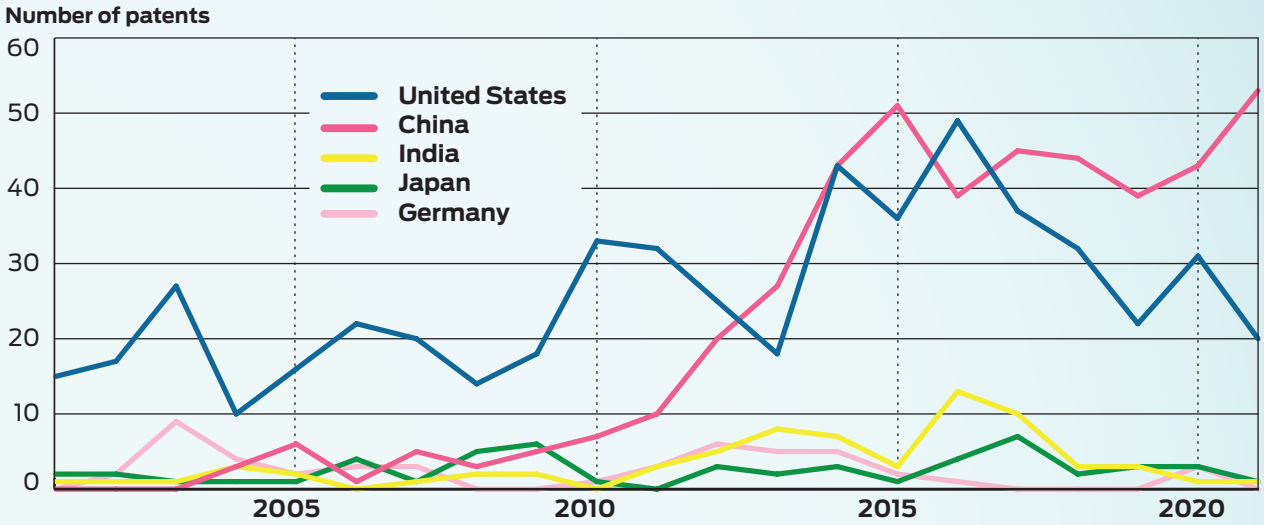
CREDIT: COURTESY OF INSTITUTIONS

BY THE NUMBERS

Discover trends in covalent drugs

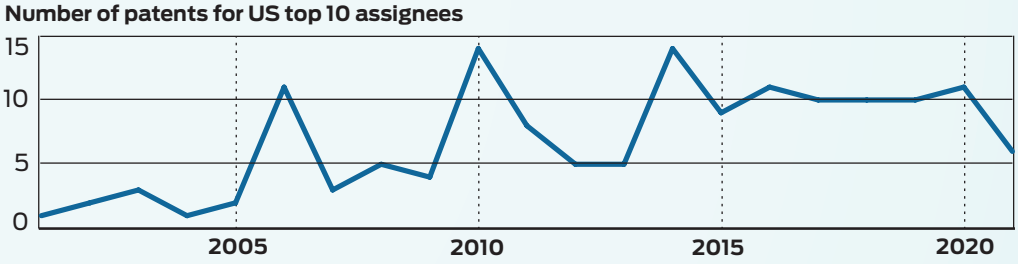
Where in the world

As in most fields, China and the US lead worldwide patenting for covalent drugs, with China overtaking the US around 2016.



Why did the US drop?

The drop in all US covalent drug patents around 2016 is not reflected in the numbers for the top 10 assignees, suggesting that major players stayed the course while others shifted their attention.



Cancer concentration

Although people working in the field of covalent drugs say it is expanding beyond cancer treatments, the top patenting concepts remain focused on oncology.

2007–11	2012–16	2017–21
Antitumor agents	Antitumor agents	Antitumor agents
Homo sapiens	Homo sapiens	Homo sapiens
Neoplasm	Neoplasm	Neoplasm
Drug delivery sustems	Pharmaceutical carriers	Pharmaceutical carriers
Anti-inflammatory agents	Anti-inflammatory agents	Mammary gland neoplasm
Mammary gland neoplasm	Mammary gland neoplasm	Lung neoplasm
Inflammation	Inflammation	Pharmaceutical excipients
Pharmaceutical excipients	Pharmaceutical excipients	Anti-inflammatory agents
Combination chemotherapy	Combination chemotherapy	Ovary neoplasm
Peptides	Autoimmune disease	Pancreatic neoplasm

Who's who

Four of the top six patent assignees for 2000–2021 were based in the US.

Organization: Araxes Pharma — 31 (Number of patents)

Schering Corp. — 20

Dana-Farber Cancer Institute — 15

Shanghai Institute of Materia Medica — 15

Avila Therapeutics — 14

Pharmacyclics — 14

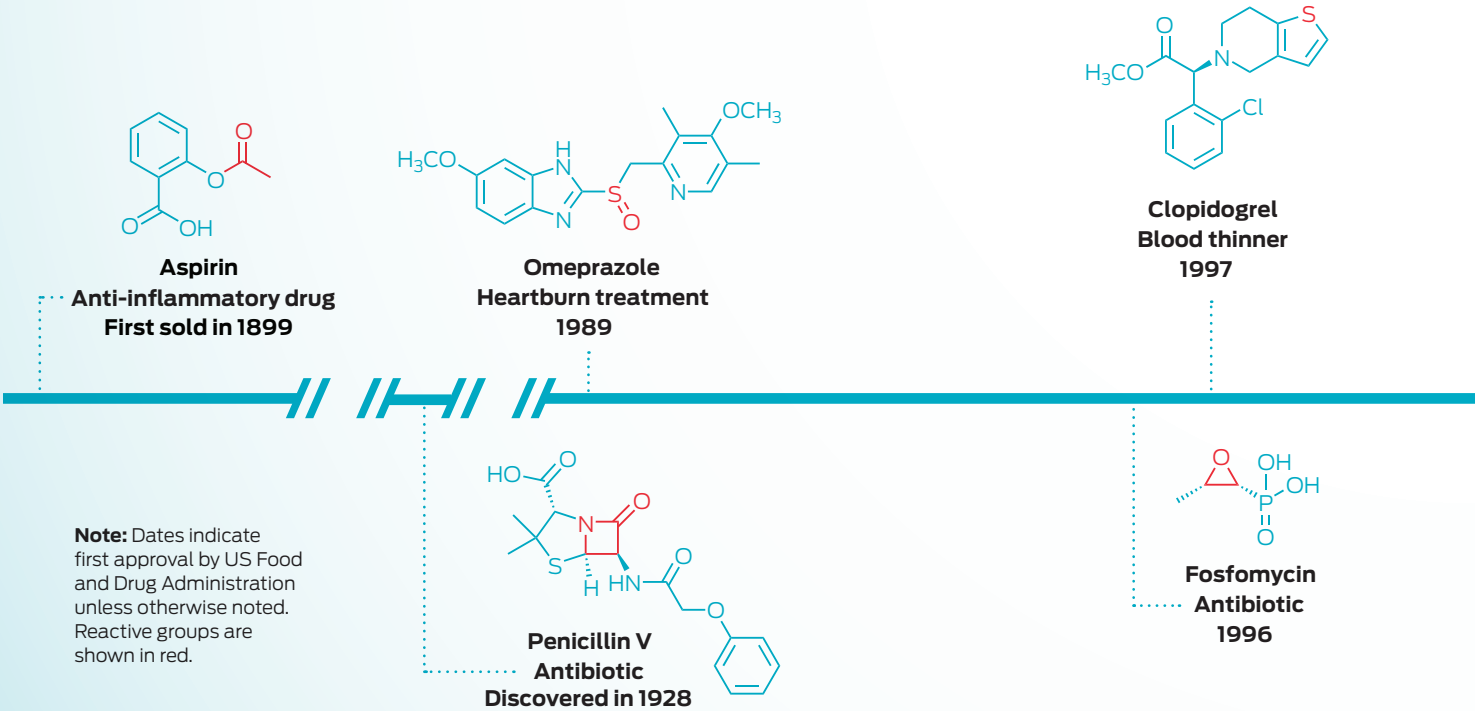
Source: CAS Content Collection.
Notes: CAS information scientists searched patents and publications containing the concept of covalent drugs for the years indicated. Patents may mention more than one type of technology. Figures for Germany may include patents that were filed in the former East Germany and published after a long delay.

ANALYSIS

Covalent drugs go from fringe field to fashionable endeavor

BETHANY HALFORD, C&EN STAFF

Enticing atoms to share their electrons—to make a covalent bond, in other words—is at chemistry's core. But when chemists make drug candidates, they've historically shied away from molecules that might engage in this type of bond making with biological targets, preferring instead to use molecules that drift in and out of their protein targets. After all, what's to guarantee that a reactive compound will make a bond only with an amino acid on its intended target and not one on some other protein in the human body's complex biochemical soup?



Note: Dates indicate first approval by US Food and Drug Administration unless otherwise noted. Reactive groups are shown in red.

Over the past decade, however, researchers have shifted their thinking about drug candidates that make covalent bonds. After the success of several covalent anticancer drugs, many medicinal chemists are now designing drug candidates that form bonds with their targets. These scientists are finding footholds on proteins that were once considered undruggable; they do this by using analytical techniques that help them zero in on specific amino acids and by developing novel reactive groups that expand the types of amino acids they can target.

Drugs that make covalent bonds to their targets certainly aren't new. Aspirin's reactive acetyl group gets transferred onto serines in certain enzymes, preventing the enzymes from making molecules that lead to inflammation and clotting. Of course, no one knew that in 1899, when Bayer started selling the drug. Even widely used contemporary drugs like the heartburn pill Prilosec (omeprazole) and the blood thinner Plavix (clopidogrel) act by a covalent mechanism that wasn't discovered until after they were in development. It wasn't engineered into the molecules.

"If you look back over 100 years, some of the most important medicines advanced have a covalent mechanism of action. I think that was underappreciated until the early 2000s," says Juswinder Singh, founder and chief scientific officer at Ankaa Therapeutics. "People didn't realize the importance of the bond that was being formed," he says. "The power of covalency is that you've got a small molecule that essentially silences the drug target."

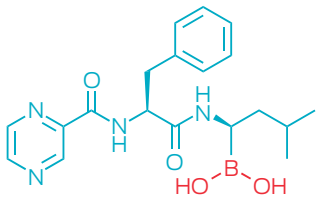
Taboo to trendy

A noncovalent, or reversible, drug slips in and out of its target, a disease-linked protein. But a covalent drug bonds to the protein target, shutting it down. That protein won't be active again until the body resynthesizes it—a process that can take days. That means that doctors don't have to give the drug as often and can give it in lower doses.

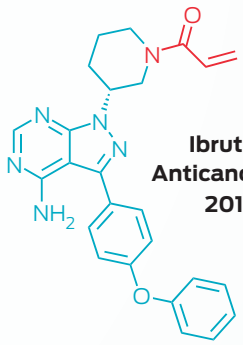
Although those qualities sound appealing, designing covalent drug candidates was considered a fringe idea as recently as 2006. That's when Singh cofounded Avila Therapeutics, a company dedicated to making covalent anticancer drugs. At the time, drugmakers worried about creating compounds that, in theory, could form bonds with other proteins in the body and cause dangerous, off-target effects. They also thought immune cells might see the drug-bound proteins as foreign and potentially trigger an immune response.

Margaret Chu-Moyer, vice president of research and head of chemistry, characterization, and technology at Amgen, says drugmakers saw how a class of covalent drugs called DNA-alkylating agents, which includes the cancer therapy cisplatin, reacted widely. "They were not only toxic to tumor cells, which is what you wanted; they were like a bomb going off throughout the body," because of their toxic reactions in other cells too, she says. With that context, it was tough to think about designing covalent drugs that would also be safe, she says.

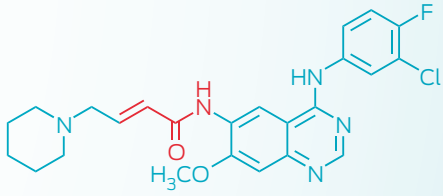
In fact, Chu-Moyer says, when she started as a medicinal chemist in the 1990s, she avoided mak-



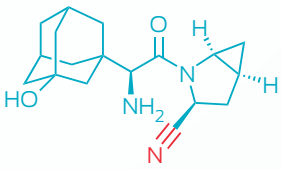
Bortezomib
Anticancer drug
2003



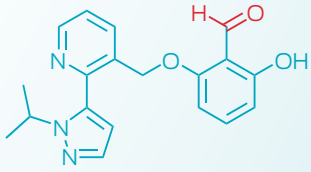
Ibrutinib
Anticancer drug
2013



Dacomitinib
Anticancer drug
2018



Saxagliptin
Antidiabetic drug
2009



Voxelotor
Sickle cell disease treatment
2019

"That experience really solidified for me that covalent drugs are the way. Not only are they a tool in the toolbox; they are a paradigm unto themselves that really offers a lot of advantages."

ing molecules with reactive handles or molecules that could be metabolized into compounds with reactive handles. "It was verboten, almost, to think of these in a prospective manner," she says.

Singh adds that many drugmakers thought covalent drugs simply weren't necessary. With rational drug design, he says, medicinal chemists thought they could solve every problem with a reversible drug. "I think it's still the case that a lot of people believe that," he says. But now there are plenty of data showing the problems covalent drugs can solve.

"The drug that really put covalent inhibitors on the map is ibrutinib," says Dan Erlanson, vice president of chemistry at Frontier Medicines, a company that's taking a covalent approach to drug discovery.

Ibrutinib, which is marketed as Imbruvica, covalently inhibits Bruton's tyrosine kinase (BTK), an enzyme that is active in certain cancer cells. The drug is one of several covalent BTK inhibitors that researchers began pursuing in the first decade of the 2000s—Avila's BTK inhibitor was bought by Celgene in 2012. Along with EGFR inhibitors, they were among the first drug candidates to be designed to act covalently.

Ibrutinib was originally designed at Celera Genomics in 2005 as a tool for studying the biology of BTK. Celera sold the compound in 2006 as part of a package deal to Pharmacyclics, which took the molecule through clinical development.

"Part of the reason ibrutinib caused people to take note is because it had been dismissed by a lot of people in pharma," Erlanson says. Many looked at the structure and thought it wouldn't be selective. But the US Food and Drug Administration approved the compound to treat mantle cell lymphoma in 2013, and it is now used to treat five other types of cancer too. "That caused people to reassess their assumptions," Erlanson says.

Another consideration: ibrutinib brings in a lot of money. Its success prompted AbbVie to pay $21 billion for Pharmacyclics in 2015.

Roman Fleck, CEO of Janpix, a biotech firm working on covalent inhibitors, says he and his colleagues see ibrutinib as a benchmark against which they judge their drug candidates. "It was the first molecule that validated in a big way that covalent inhibition is worthwhile—at least in oncology," he says.

Designing from scratch

Since 1990, the FDA has approved 55 drugs with a covalent mechanism of action, according to a 2022 analysis by Singh (*J. Med. Chem.*, DOI: 10.1021/acs.jmedchem.1c02134). Many approved in the last decade had shapes and scaffolds that had not appeared in any previous FDA-approved drugs. The novelty of the approved covalent drugs in the 2010s is a spike compared with the 2 drugs with novel shapes and scaffolds approved by the FDA in the first decade of the 2000s and none in the 1990s.

The boost in structural novelty points to another shift in the area of covalent drugs. Chemists designing covalent kinase inhibitors from 2000 to the early 2010s took molecules that bound reversibly to their targets and outfitted them with a reactive group, like an acrylamide or a chloroacetamide, that could latch on to an amino acid in the target.

While medicinal chemists still use that strategy, scientists are now able to find covalent inhibitors without starting from a molecule that already binds to the target reversibly. Instead, they screen their targets with libraries of small reactive fragments to find ones that make covalent bonds. Those become the starting point for designing covalent inhibitors that can latch on to targets that don't have deep pockets for a molecule to bind within—targets once considered intractable, like KRAS G12C.

KRAS is a key protein involved in the signaling processes that make cells divide and proliferate. But KRAS and its family members, mutants of which are found in 30% of cancers, are smooth as cue balls. Drug developers spent decades trying—and failing—to find a toehold on the proteins.

In 2013, a team led by Kevan Shokat of the University of California, San Francisco, found a possible way to covalently inhibit one of those mutants, KRAS G12C, in which the glycine at the peptide's 12th amino acid has been swapped out for a cysteine. Shokat discovered that the sulfur in KRAS G12C's mutant cysteine could act as a nucleophile and covalently latch on to a small-molecule electrophile. And because that mutation is present only in cancer cells, regular KRAS would be unaffected.

"When you're doing reversible binding, you can take advantage of van der Waals interactions and salt interactions and water," Shokat says. "But when you've got a nucleophile and an electrophile, it's a reaction, so it's got a much steeper transition state. You've got to get everything right."

In the case of KRAS G12C, Shokat says, the key was that the acrylamide electrophile the researchers used was perfectly poised to react with the cysteine, with an assistive tug from a nearby lysine. When they tried a similar strategy with KRAS G13C, a mutant in which a glycine just one position further along the protein chain is modified to a cysteine, they couldn't get anything to work. "It's probably because it's too far from the lysine," Shokat says.

That work inspired a number of companies to make compounds that latch on to that cysteine. "I

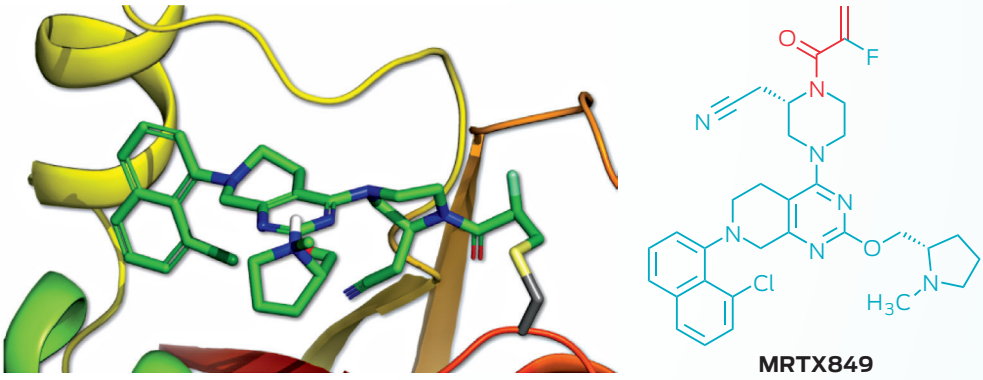
think KRAS represents a real change in the way that people looked at covalent inhibitors," says Victor Cee, vice president of chemistry at Oncovalent Therapeutics. Before joining Oncovalent, Cee worked at Amgen on the first KRAS G12C inhibitor to enter clinical trials, sotorasib. Cee says the KRAS G12C inhibitors that have gone into clinical trials came from screening for and then iteratively optimizing molecules that could react with cysteine.

Chemists didn't understand if they were optimizing the molecules' binding affinity or their rate of reaction, Cee says, and successful KRAS G12C inhibitors are "oddball molecules" compared with the kinase inhibitors that had been retrofitted with a reactive handle. The drugs that eventually went into the clinic generally don't have a strong affinity for KRAS G12C, but when they do bind, they react quickly with that cysteine on the protein. So chemists don't need to get a lot of their covalent inhibitors onto the target as long as they react quickly during a chance encounter.

"This opens up a whole new world of targets for covalent inhibition," Cee says. A huge family of proteins is considered undruggable because it's impossible to get a high enough concentration of drugs onto a target to effectively silence it, he says. The KRAS work shows there's a different way.

Larry Burgess, head of chemistry at Vividion Therapeutics, remembers reading the work from Shokat's team when it came out. At the time, Burgess was executive director of drug discovery at Array BioPharma, and he and the company's chief scientific officer decided that day that they had to go after KRAS G12C. They'd already gotten comfortable with the idea of covalent drugs, he says, through earlier work to modify reversible kinase inhibitors. After seeing Shokat's strategy, they wanted to see if they could create a drug using a de novo approach. The company ultimately teamed up with Mirati Therapeutics to develop MRTX849, another KRAS G12C covalent inhibitor that is in clinical trials.

"That experience really solidified for me that covalent drugs are the way. Not only are they a tool in the toolbox; they are a paradigm unto themselves that really offers a lot of advantages," Burgess says.



MRTX849

MRTX849's acrylamide (red in the structure, right) forms a covalent bond to a cysteine's sulfur (yellow) in KRAS G12C (shown in the crystal structure on the left).

CREDIT: *J. MED. CHEM.*

Progress with proteomics

So drugmakers had figured out how to design covalent inhibitors. They'd shown that they could be effective drugs. And they'd used them to go after some challenging targets.

But there still remained the question of selectivity.

Would you develop a covalent drug only to find out late in development that it has an off-target effect you didn't know about and can't work around? "In every project, that is the biggest fear," Cee says.

Those fears have largely subsided, thanks to proteomic screening, according to Burgess and Cee. This technique allows drug developers to look at all the proteins expressed by a cell or an organism and see if their candidate compound will, for example, latch on to only a specific cysteine or if it will make bonds to cysteines in other proteins as well. It is essentially a competition experiment in which scientists expose a proteome—all the proteins in a biological system—to a small molecule that covalently binds to a specific amino acid on a protein of interest. They then throw in a probe that would modify that amino acid indiscriminately and use mass spectrometry to see which proteins have made covalent bonds to the small molecule instead of the probe.

"Before the rise of these high-throughput proteomic techniques, it was kind of a shot in the dark if I said, 'I'm going to make a covalent inhibitor,'" Cee says.

Proteomic screening "gives people confidence that they're not going in blind," says Benjamin F. Cravatt of Scripps Research in California, who pioneered the technique and cofounded Vividion in 2014 to use the method for drug discovery. "Our goal all along was to try to demystify the process of covalent ligands in drug discovery, to try to make it more of a science with data that can drive decision-making," Cravatt says.

In the late 1990s, his lab started looking in the proteome for amino acid residues that are good nucleophiles and can latch on to small electrophiles—precisely what someone developing a covalent drug would want to know. The technique is mostly used to look for nucleophilic cysteines and serines, but it can be applied to other residues, too, like lysines and tyrosines. As high-throughput mass spectrometry–based methods became more advanced, Cravatt says, "it was clear that you can actually make a pretty rigorous science out of covalent-ligand drug discovery, probably a more rigorous science than you can make out of reversible-ligand discovery."

That's because the mass spectrometry can directly detect when a bond has been made, but it can't do the same for reversible interactions.

Cravatt says he encountered a lot of skepticism when he first suggested the technique to pharmaceutical companies a decade ago. But since then, drugmakers have used it to go back and determine whether marketed covalent drugs and clinical candidates are selective for their protein targets. And they've been using proteomic screening as a tool in current campaigns. Consequently, Cravatt says, "a lot of the drugs that are now being developed are way more selective than the ones that have already been approved."

John Tallarico, who heads chemical biology and therapeutics at the Novartis Institutes for BioMedical Research, agrees. "Over and over, I'm surprised with how clean these molecules are. I'm not saying they only interact with one target, but it's not hundreds; it's tens of targets sometimes, which is fantastic."

Finding footholds

Using proteomics and reactive fragment screening to go after targets that were once considered undruggable is an area that's hotly pursued by many companies, including Novartis, Vividion, and Frontier Medicines, a start-up cofounded by Daniel Nomura, a professor at the University of California, Berkeley, who studied with Cravatt as a postdoctoral fellow.

Over 90% of proteins in humans are considered undruggable, Nomura says. "I would argue that's one of the biggest bottlenecks in modern drug discovery." But, he says, combining proteomic screening and approaches for discovering covalent small molecules, like using libraries of small reactive fragments to find reactive residues, has "enabled us to tackle areas of the proteome, particularly the undruggable proteome, in ways that we couldn't access before."

In collaboration with Tallarico and others at Novartis, Nomura's lab recently reported it was able to find a small molecule that covalently binds to MYC, a transcription factor, or gene-reading protein, that promotes cell growth and proliferation. Scientists have long considered MYC to be a key driver of cancer, but, like most transcription factors, much of the protein is disordered. "There's no obvious pocket on this for us to stick a small molecule in, even as a tool," Tallarico says.

Using proteomic screening, Nomura and coworkers found a cysteine within a disordered region of MYC that they realized they could use to grasp a molecule. They then screened a library of small molecules and found one that targets this intrinsically disordered region, destabilizes MYC, and leads to its destruction.

Tallarico says the small molecule is an interesting tool, but he doesn't think it will become a drug. Nomura says his team is trying to repurpose the molecule as a starting point for a protein degrader, a bifunctional molecule that binds to both a protein of interest and an enzyme that helps tag the protein for breakdown.

Researchers at Janpix have also had some success using covalent inhibitors to block transcription factors, in this case two proteins related to blood cancers, STAT3 and STAT5. The company has been working with University of Toronto Mississauga medicinal chemist Patrick Gunning on a family of small molecules targeting STAT3 and STAT5. Not only do the molecules covalently bind to these targets, but in the case of STAT5, the inhibitors also unravel the protein so that it gets degraded, Janpix's Fleck says. The company, which became part of Centessa in 2021, is looking for third-party financing to pay for preclinical development of one of its candidates, Fleck says.

Ties that bind

The Janpix molecules also stand out because they have an unusually reactive handle. Ibrutinib, sotorasib, MRTX849, and many other covalent drugs and drug candidates use an acrylamide as their electrophilic handle, which gets attacked by cysteine nucleophiles.

"Acrylamides are getting to be really popular because people have figured out a lot of the rules for their reactivity and how to modulate that reactivity effectively," Oncovalent's Cee says. "They're the Goldilocks electrophile." Acrylamides also have a history of success, and they're not difficult to add to a molecule later in a synthesis. "If you can use an acrylamide, you would," Cee says.

In contrast, the reactive handle on Janpix's molecules is a pentafluorobenzene sulfonamide. Cysteines go after this handle too, latching on to the para position of the pentafluorobenzene and displacing the fluoride there. The molecules are outfitted with other groups that encase the reactive pentafluorobenzene and spring open only when they encounter STAT3 and STAT5, Fleck explains. This reactive electrophile is new in covalent drugs, "so people

Combining proteomic screening and approaches for discovering covalent small molecules, like using libraries of small reactive fragments to find reactive residues, has "enabled us to tackle areas of the proteome, particularly the undruggable proteome, in ways that we couldn't access before."

are unfamiliar with it, and people may have reservations about it," he says. "But an acrylamide could not be shielded in the same way."

There are already a few approved drugs that feature reactive handles and aren't based on acrylamides—for example, the anticancer drug bortezomib uses a boronic acid to bind to a threonine, and the antibiotic fosfomycin uses an epoxide to target a cysteine. But they are exceptions rather than general classes that medicinal chemists can use.

Medicinal chemists who want to target amino acids other than cysteine are going to have to look for reactive groups beyond acrylamides, says Matthias Gehringer, a medicinal chemist at the University of Tübingen who is studying new reactive groups for covalent inhibitors. The conjugate addition chemistry that works so well to link cysteine's sulfur to the acrylamide isn't suited to other amino acids, he says.

Determining how to create reactive small molecules that selectively latch on to amino acids like lysine, tyrosine, and aspartate is going to take creative chemistry, UCSF's Shokat says. "Whenever I go to give talks at chemistry departments and have lunch with the students, I always tell them, 'We need reactions that work in water and attack aspartate,'" he says. That's because the KRAS that drives pancreatic cancer has a mutant aspartate. Finding a molecule that could selectively lock on to that amino acid, Shokat says, "would be fantastic."

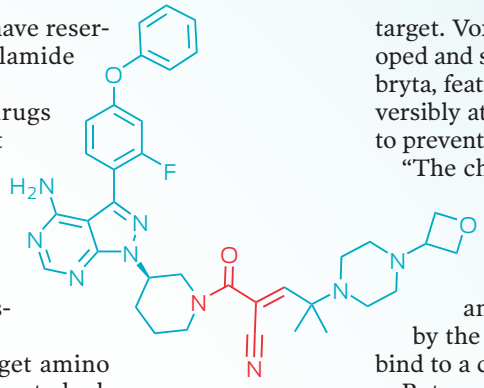
Several academic chemists are developing new reactive groups for amino acids beyond cysteine. UC Berkeley chemists Christopher J. Chang and F. Dean Toste have used oxaziridines that latch on to methionines in proteins, for example.

The sulfur(VI) fluoride exchange, a type of click chemistry developed by K. Barry Sharpless at Scripps Research in California, offers one way medicinal chemists can tack small molecules on to other amino acids, including tyrosines, lysines, serines, histidines, and threonines. Chemical intuition suggests that the sulfonyl fluorides and sulfuramidimidoyl fluorides developed in Sharpless's lab wouldn't be very selective, but these substituents have proved otherwise. They hook up with an amino acid only when the protein environment is just right.

This feature prompted the lab to nickname these groups "sleeping beauties." Only when the molecules encounter the right amino acid prince will they awaken for the key bond-making event. Sharpless says they are still trying to establish what makes the perfect protein environment for the sulfur(VI) fluoride exchange to occur. He suspects there must be a positively charged amino acid nearby that pulls the fluoride away so that the sulfonyl or sulfuramidimidoyl makes a covalent bond to the amino acid.

As they develop new reactive electrophilic handles, chemists have also created molecules that form covalent bonds reversibly—that is, they can be broken and remade at various sites on a protein target. Voxelotor, a sickle cell disease drug developed and sold by Global Blood Therapeutics as Oxbryta, features an aldehyde reactive handle that reversibly attaches to the N-terminus of hemoglobin to prevent it from polymerizing in red blood cells.

"The chemistry is as old as organic chemistry," UCSF's Jack Taunton says of reversible covalent inhibitors. "And applying reversible covalent approaches to drug discovery is also very old." The antidiabetic drug saxagliptin, approved by the FDA in 2009, uses a nitrile to reversibly bind to a catalytic serine on a key protease.



Rilzabrutinib

But reversibly binding to noncatalytic residues is relatively unexplored, Taunton says. His lab has made reversible covalent inhibitors using cyanoacrylamides as electrophiles. He notes that a cyanoacrylamide is the key electrophile in rilzabrutinib, a BTK inhibitor that was developed by Principia Biopharma and is currently in late-stage clinical trials for immune-mediated diseases. The reversibility of the covalent bond lets the compound attach and detach from cysteines in various kinases at different rates, Taunton says. Although the details of rilzabrutinib's selectivity haven't been disclosed, the compound seems to spend more time on BTK than it does on other kinases.

While much of the innovation in designing covalent drugs has been in oncology, medicinal chemists are using covalent inhibition for other diseases too. The coronavirus pandemic brought an explosion of work to develop covalent inhibitors of the main protease of SARS-CoV-2. This protease's active site has a cysteine that's essential for its activity and therefore a good nucleophilic target for either reversible or irreversible covalent molecules.

> "This opens up a whole new world of targets for covalent inhibition"

Part of the COVID Moonshot project is devoted to screening small fragments that make covalent bonds to that key cysteine with the goal of using that affinity to create drugs. Similarly, Novartis's Tallarico says the company is working on covalent inhibitors of SARS-CoV-2. And Pfizer's antiviral Paxlovid contains nirmatrelvir, which incorporates a nitrile as an electrophile. Pfizer announced on June 30 that it had submitted a new drug application for full FDA approval to treat COVID-19.

As the boom in covalent drugs continues, researchers say there's still plenty of room for innovative chemistry. Whether it's making new reactive groups, expanding the libraries that are used to screen possible targets, or establishing footholds on proteins previously thought to be undruggable, chemists have a valuable role to play. "I think we're at a stage now where covalent ligands in drug discovery are here to stay," Scripps's Cravatt says. "I think many companies and many academic labs would prefer a covalent ligand over a noncovalent ligand, which is amazing to say, because 10 years ago that would have been blasphemy." ■

This article is reprinted with permission from C&EN. A version of this article was published on Nov. 9, 2020 on cen.acs.org and in digital and print issues on page 28.

We choose 16 promising companies pursuing new covalent drugs

Acerta Pharma

» **Acerta Pharma**
» acerta-pharma.com
» **Based:** Oss, the Netherlands
» **Launched:** 2013
» **Money raised in start-up funding rounds:** $60 million
» **Publicly traded:** No
» **Key partnerships:** AstraZeneca
» **Strategy:** Acerta Pharma focuses on the development of covalent binding technology solutions to create therapies for cancer.
» **Why watch:** AstraZeneca acquired a majority stake in the company in 2016. AstraZeneca's interest was driven by acalabrutinib, Acerta's lead inhibitor for Bruton's tyrosine kinase (BTK). The US Food and Drug Administration approved the drug in 2017.

Ankaa Tx

» **Ankaa Therapeutics**
» ankaatx.com
» **Based:** Southborough, Massachusetts
» **Launched:** 2014
» **Money raised in start-up funding rounds:** Not disclosed
» **Publicly traded:** No
» **Key partnerships:** Not disclosed
» **Strategy:** Ankaa Therapeutics develops targeted small-molecule drugs and therapies, including covalent drugs, intended to address drug resistance and cancer treatments.
» **Why watch:** The company's president and chief scientific officer, Juswinder Singh (page 5), is a pioneer in the discovery and development of targeted covalent drugs.

Atomwise

» **Atomwise**
» atomwise.com
» **Based:** San Francisco
» **Launched:** 2012
» **Money raised in start-up funding rounds:** $176.6 million
» **Publicly traded:** No
» **Key partnerships:** Sanofi
» **Strategy:** Atomwise uses artificial intelligence for structure-based drug discovery, focusing on targets previously thought to be undruggable.
» **Why watch:** The company aims to train its deep learning algorithm to identify and characterize covalent inhibitors.

BeiGene

» **BeiGene**
» beigene.com
» **Based:** Beijing
» **Launched:** 2010
» **Money raised in start-up funding rounds:** $172 million
» **Publicly traded:** Yes
» **Key partnerships:** Amgen, Bristol Myers Squibb, Novartis
» **Strategy:** BeiGene is a clinical stage biopharma company developing targeted and immuno-oncology drugs that address unmet medical needs in a variety of cancer indications.
» **Why watch:** BeiGene's covalent BTK inhibitor zanubrutinib has been approved for the treatment of mantle cell lymphoma, Waldenström's macroglobulinemia, marginal zone lymphoma, and chronic lymphocytic leukemia. BeiGene is also investigating zanubrutinib, both as a monotherapy and in combination with other drugs, to treat a variety of additional B-cell malignancies.

bridgebio therapeutics

» **Bridge Biotherapeutics**
» bridgebiorx.com
» **Based:** Seongnam, South Korea
» **Launched:** 2015
» **Money raised in start-up funding rounds:** Not disclosed
» **Publicly traded:** Yes
» **Key partnerships:** Scripps Research
» **Strategy:** Bridge Biotherapeutics is a clinical stage biotech company focusing on unmet medical needs in areas such as ulcerative colitis, fibrotic diseases, and cancers.
» **Why watch:** In February 2022, the firm launched a collaboration with Scripps Research to discover and characterize novel reactive groups that covalently target noncysteine amino acids. This makes it possible to access new druggable sites in targets of high therapeutic value.

- **BridGene Biosciences**
- **bridgenebio.com**
- **Based:** San Jose, California
- **Launched:** 2018
- **Money raised in start-up funding rounds:** $50.5 million
- **Publicly traded:** No
- **Key partnerships:** Takeda Pharmaceutical
- **Strategy:** BridGene Biosciences uses chemoproteomic technology to perform proteome-wide screening of small molecules in live cells. The approach identifies highly selective small molecules that bind traditionally undruggable targets.
- **Why watch:** The company has used its platform to pinpoint several potential oncology targets. Takeda is now using the platform, and BridGene may be eligible for more than $500 million in up-front and milestone payments from that partnership.

- **Centessa Pharmaceuticals**
- **centessa.com**
- **Based:** Cambridge, Massachusetts
- **Launched:** 2021
- **Money raised in start-up funding rounds:** $250 million
- **Publicly traded:** Yes
- **Key partnerships:** None
- **Strategy:** Centessa Pharmaceuticals was formed by the merger of 10 biotech companies, each with its own portfolio of highly validated programs. Centessa provides oversight and manufacturing, regulatory, and operational support for the programs under its umbrella.
- **Why watch:** One of the companies that went into Centessa was Janpix, which is developing a novel class of small-molecule covalent drugs for the treatment of hematological malignancies, including leukemias and lymphomas.

- **Enlaza Therapeutics**
- **Based:** San Diego
- **Launched:** 2021
- **Money raised in start-up funding rounds:** $4 million
- **Publicly traded:** No
- **Key partnerships:** None
- **Strategy:** Enlaza aims to transform the field of biologic therapeutics by applying the concept of covalency to protein biologic drugs.
- **Why watch:** Enlaza is about to emerge from stealth mode, and it is the first company to work on covalent protein drugs.

- **Frontier Medicines**
- **frontiermeds.com**
- **Based:** South San Francisco
- **Launched:** 2018
- **Money raised in start-up funding rounds:** $155.5 million
- **Publicly traded:** No
- **Key partnerships:** AbbVie
- **Strategy:** Frontier Medicines uses chemoproteomics, covalent drug discovery, and machine learning to develop medicines. It is beginning with anticancer therapies against disease-causing proteins previously considered undruggable.
- **Why watch:** Its collaboration with AbbVie will use Frontier's chemoproteomics platform to identify small molecules for oncology and immunology targets.

- **Global Blood Therapeutics**
- **gbt.com**
- **Based:** South San Francisco
- **Launched:** 2011
- **Money raised in start-up funding rounds:** $88.7 million
- **Publicly traded:** Yes
- **Key partnerships:** None
- **Strategy:** Global Blood Therapeutics is a clinical stage biopharmaceutical firm that focuses on developing treatments for sickle cell disease.
- **Why watch:** GBT's first therapy for sickle cell disease, voxelotor, received FDA approval in December 2021, and the company has several other potential treatments under investigation.

- **Pardes Biosciences**
- **pardesbio.com**
- **Based:** Carlsbad, California
- **Launched:** 2020
- **Money raised in start-up funding rounds:** $51.6 million
- **Publicly traded:** Yes
- **Key partnerships:** None
- **Strategy:** Pardes Biosciences uses structure-based drug design and a reversible covalent chemistry platform to develop antivirals for SARS-CoV-2.
- **Why watch:** Founded by former executives from Assembly Bio, Gilead, and other pharmaceutical companies, Pardes is developing PBI-0451 as an oral antiviral drug designed to inhibit the SARS-CoV-2 main protease, which has a sequence that is highly conserved in proteases across all coronaviruses.

- **Scorpion Therapeutics**
- **scorpiontx.com**
- **Based:** Boston
- **Launched:** 2020
- **Money raised in start-up funding rounds:** $270 million
- **Publicly traded:** No
- **Key partnerships:** AstraZeneca
- **Strategy:** Scorpion Therapeutics aims to broaden the reach of precision medicine in oncology, by developing drugs for targets previously considered undruggable.
- **Why watch:** In partnership with AstraZeneca, Scorpion is using covalent chemistry to find ways to target transcription factors and other difficult-to-drug proteins.

- **Terremoto Biosciences**
- **terremotobio.com**
- **Based:** South San Francisco
- **Launched:** 2021
- **Money raised in start-up funding rounds:** $75 million
- **Publicly traded:** No
- **Key partnerships:** None
- **Strategy:** Terremoto Biosciences aims to expand the alphabet of amino acids

available to covalent drugs by developing molecules that latch onto the amino acid lysine rather than cysteine.
» **Why watch:** Moving beyond cysteine to target other amino acids is one of the next major areas of study in covalent drug discovery.

» **Totus Medicines**
» totusmedicines.com
» **Based:** Emeryville, California
» **Launched:** 2020
» **Money raised in start-up funding rounds:** $40 million
» **Publicly traded:** No
» **Key partnerships:** None
» **Strategy:** The Totus Medicines platform uses covalent chemistry and artificial intelligence to create and screen molecules across every protein-coding gene in the human genome to find drugs for undruggable targets.
» **Why watch:** The company's lead compound, TOS-358, is the first highly specific, potent inhibitor of the protein made by the common oncogene PI3K. The drug should enter Phase 1 trials soon.

» **Vividion Therapeutics**
» Vividion.com
» **Based:** San Diego
» **Launched:** 2013
» **Money raised in start-up rounds:** $371.5 million
» **Publicly traded:** No
» **Key partnerships:** Bayer
» **Strategy:** Vividion Therapeutics uses its chemical proteomics platform to discover previously unknown functional pockets on the surface of proteins and identify small molecules that selectively bind to those targets.
» **Why watch:** The company, which was acquired by Bayer in August 2021, is optimizing leads for several oncology and immunology drugs.

» **X-Chem**
» x-chemrx.com
» **Based:** Waltham, Massachusetts
» **Launched:** 2009
» **Money raised in start-up rounds:** Not disclosed
» **Publicly traded:** No
» **Key partnerships:** None
» **Strategy:** X-Chem provides biotechnological services, including using artificial intelligence for drug discovery and medicinal chemistry.
» **Why watch:** The company recently completed a proof-of-concept study that used its chemical library to generate large numbers of potential covalent inhibitors.

Note: Companies were included because of the novelty and potential of their methods, amount of capital raised, number of partnerships, and number and identity of investors.

Sources: Crunchbase (accessed August 2022), company websites, news reports.

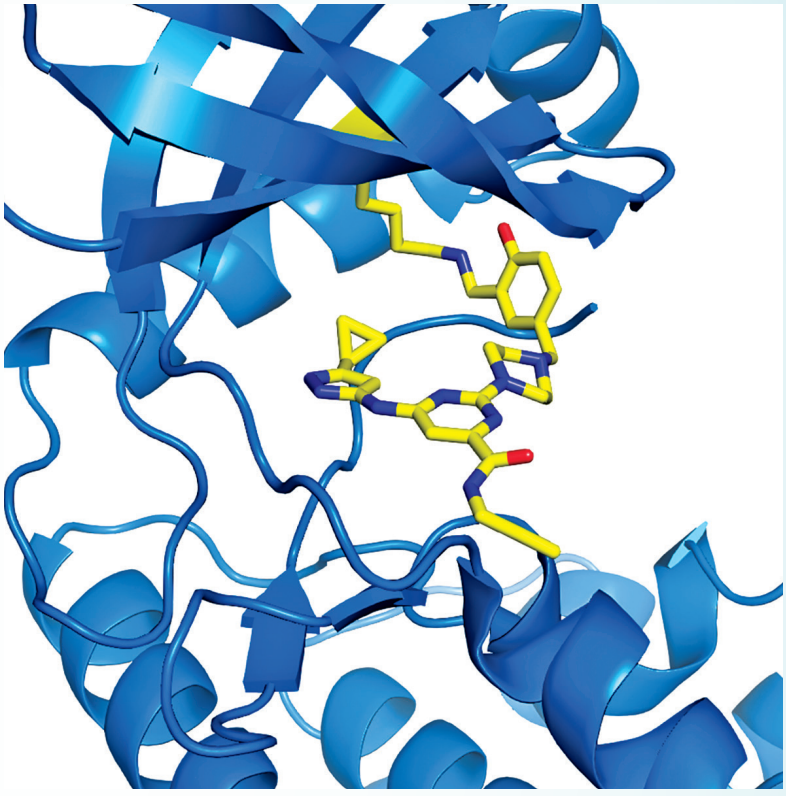
Looking to lysine

SHI EN KIM, C&EN STAFF

Misbehaving proteins are behind many diseases. One way drugmakers incapacitate these bad actors is to deploy molecules that bind to them. But finding solid footholds on the proteins to block their action isn't always easy.

Drugs that form covalent bonds with proteins latch irreversibly onto their targets. This means they have a longer action time and are effective in smaller doses than noncovalent drugs, which can come and go from the proteins they attach to. In recent years, covalent molecules have become increasingly popular among drug developers for their potency and their ability, if designed properly, to bind selectively to their intended targets and shut them down for good.

Covalent drugs usually zero in on reactive amino acid side chains that jut from the surface or nooks of a disease-causing protein. For the past few decades, efforts to develop covalent drugs have targeted cysteine. But now another amino acid promises to vastly expand the space of druggable targets: lysine.



A small molecule carrying an aldehyde group covalently binds to a lysine (yellow segment on the blue ribbon) on a kinase protein.

CREDIT: JACK TAUNTON

An alternative to cysteine

Cysteine as a covalent binding site is a double-edged sword. On the one hand, it offers some degree of selectivity: the fraction of cysteine-containing proteins in the human body is small, so drug molecules have fewer opportunities to bind to the wrong place. On the other hand, many proteins lack a cysteine, which puts them out of contention as potential covalent drug targets.

Lysine promises to make many more human proteins available to target with covalent drugs. This amino acid residue is nearly three times as abundant as cysteine in the body: an average of 32 lysines dot every protein.

Research on cysteine-targeting covalent drugs has also found that some cancer-related proteins, such as the epidermal growth factor receptor, can mutate to swap out their cysteine residue for another amino acid, rendering the once-druggable protein impervious to attack. Homing in on lysine promises a lower probability of proteins picking up mutations that lead to drug resistance. That's because, for certain large classes of proteins such as in kinases, the lysine is critical to the protein's function and can't be substituted for another amino acid. As a drug target, lysine isn't just versatile—it's also reliable.

Lysine has already drawn interest from the pharmaceutical industry. Several companies developing covalent drugs are looking to this amino acid after focusing on the well-established cysteine.

"There is tremendous utility in being able to expand the covalent alphabet to lysine," says Peter Thompson, CEO and cofounder of Terremoto Biosciences, a San Francisco–based company that recently emerged to develop lysine-based covalent drugs. The company, which has not disclosed details about diseases it is targeting, expects competition in the future: "There will probably be many companies that will pursue this," Thompson says.

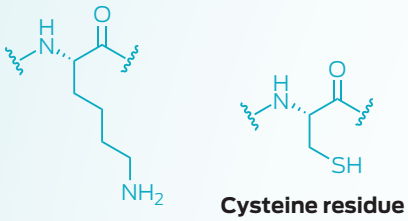
Experts figure that lysine is more than a decade behind cysteine as a covalent drug target. There are no lysine-targeting drugs available yet, while cysteine drug developers boast several game-changing medicines, such as the anticancer drugs ibrutinib and osimertinib. But the field of lysine covalency is "being developed as we speak,"

Thompson says. “We’re moving very rapidly—faster than I thought.”

Lysine’s potential

Among all the amino acids, cysteine is drugmakers’ top choice for a covalent target because it’s the most nucleophilic and hence reactive. That means it’s the most eager among the amino acids to share its electrons with an electrophile to make a bond. Lysine is slightly less nucleophilic, so it’s a logical next covalent target.

Despite their prevalence, not all lysines are options as drug-docking sites. When exposed to an acidic or even mildly basic environment, such as at the physiological pH of 7.4, lysine can become



Lysine residue Cysteine residue

protonated and will no longer make a covalent bond. So drug discovery efforts typically go after lysines nestled within the inner folds of a protein, where they are protected from protonation.

To explore the extent of lysine’s potential, researchers recently charted the landscape of lysines in human proteins to look for druggable sites. Their effort showed plenty of opportunities in this relatively unexplored space. Chemist Mikail Abbasov of Cornell University and colleagues combed through the proteins in human immune cells and cancer cells for lysines that could be targeted given their protected location in the protein (*Nat. Chem.* 2021, DOI: 10.1038/s41557-021-00765-4). The researchers mapped more than 14,000 lysines; at least 3,000 were potentially druggable with a covalent molecule.

The group also performed experiments to evaluate various reactive groups’ efficiency at binding to the lysines at different sites. Understanding their binding behavior could provide a starting point for covalent drug design. “This was the first work that not only assessed the reactivity of a large variety of small molecules,” Abbasov says, “but also screened in cancer and immune systems” for lysines that small molecules could bind to. The landscape is vast for covalent drug candidates that seek out lysine. “There are a lot of things to explore,” he says.

“There’s been a lot of work in the lysine-targeting field, especially in the last 5 years,” says Katya Vinogradova, a chemical biologist who studies immunology and proteomics at the Rockefeller University. “The [lysine covalent] platform is very powerful.”

“I see it as having a compound with the best of the two worlds—the covalent and noncovalent.”

To bind or not to bind

Lysine’s lower nucleophilicity compared with cysteine requires that researchers use more reactive electrophiles as drug candidates. But that reactivity may make the electrophiles less stable, causing them to potentially react with water, enzymes, or other compounds in the bloodstream. Drugmakers need to balance reactivity with stability in electrophile design.

Scientists led by Maurizio Pellecchia of the University of California, Riverside, have approached this balance from both directions: in one case they started with a highly reactive sulfonyl fluoride and reined it in by tacking on the right electron-donating groups (*J. Med. Chem.* 2021, DOI: 10.1021/acs.jmedchem.1c01459). In another case, the researchers designed a workaround to coax reluctant fluorosulfates to react. They made a molecular scaffold to hold the fluorosulfate group across from the lysine of interest long enough for the two to form a covalent bond (*J. Med. Chem.* 2019, DOI: 10.1021/acs.jmedchem.9b01108).

Pellecchia is marching these custom-made fluorosulfate molecules toward commercialization. He is the president and founder of Armida Labs, which is seeking seed funding as it moves to test its best candidate, called CovaLys, in mouse models to treat cancer.

One of the key components of a covalent drug molecule is, ironically, its noncovalent portion: the ligand that’s attached to the electrophile. While the electrophile forms the covalent bond to the amino acid, the ligand strikes up noncovalent interactions with the protein frame surrounding the correct amino acid target, thereby guiding the drug to the right spot. The ligand reinforces the attraction to the site of interest, locking the electrophile in place so that a covalent bond with the right amino acid can form.

Cysteine-selective drugs already use this trick, but it’s even more critical when abundant lysine is the target.

Scientists at Kyoto University, for example, employed a ligand to guide highly reactive sulfonamide groups onto a lysine on Hsp90, a protein typically found in cancer cells (*Nat. Commun.* 2018, DOI: 10.1038/s41467-018-04343-0). Their molecule was able to tamp down the expression of the protein and suppress cancer cell growth.

Without the bulky ligand, the antsy sulfonamide would stray to other lysines and even water molecules in the protein environment, says Itaru Hamachi, the chemical biologist who helmed the research. “The ligand [gave] us binding and selectivity.”

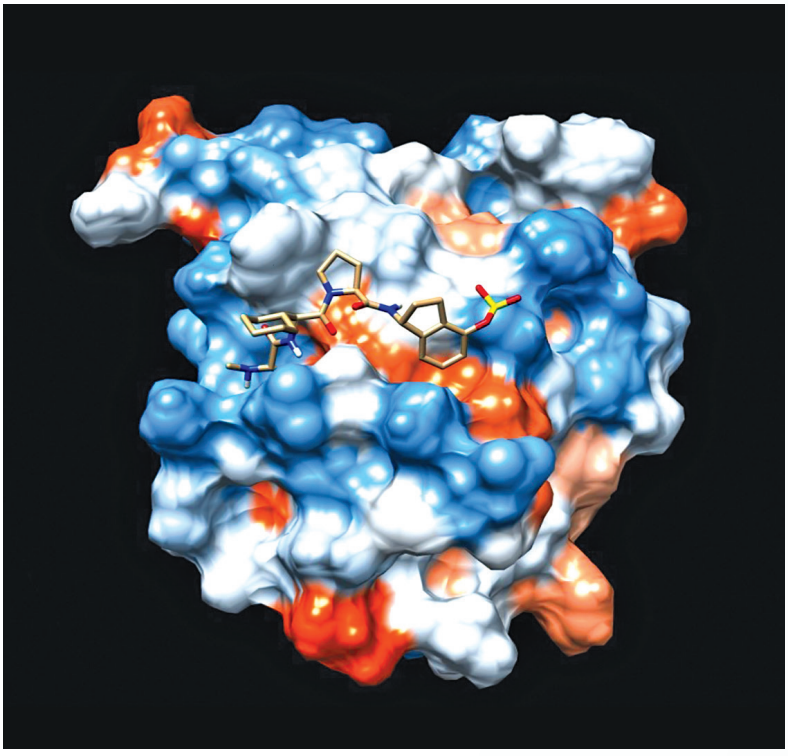
Making and breaking covalent bonds

Another emerging strategy to achieve selectivity is counterintuitive: some researchers are mov-

ing from fully covalent drugs to explore molecules that bind to proteins covalently but reversibly. In this case, the irreversible bond that first sold scientists on covalent drugs is now deliberately engineered to be breakable.

"I see it as having a compound with the best of the two worlds—the covalent and noncovalent," Abbasov says.

Engineering reversibility into the bond contributes to the drug candidate's selectivity by giving it room for self-correction. The electrophiles are still reactive enough to form strong covalent bonds, but they will detach from off targets until they find the lysine that they have the most affinity with, which is, by design, on the target protein.



The drug candidate CovaLys binds covalently to a lysine in the cancer-linked protein XIAP.

CREDIT: UDOMPHOLKUL P., ET AL., PEGAN S., AND PELLECCHIA M. OF UC RIVERSIDE. THE IMAGE WAS PRODUCED USING CHIMERA FROM THE COMPUTER GRAPHICS LABORATORY, UCSF (SUPPORTED BY NIH P41 RR-01081).

Irreversible electrophiles' selectivity depends only on one rate constant—a factor called the on rate, which is the rate the molecule attaches to the right lysine site, according to Jack Taunton, a chemist at the University of California, San Francisco. "With reversible electrophiles, you also have an off rate, and so it just gives you an extra dimension of selectivity."

By tinkering with this off rate of covalent bond formation, Taunton's team of researchers demonstrated that its aldehyde-based covalent inhibitors eventually landed on the desired proteins and didn't stay put on the wrong targets, despite the high on rate. The team showed that a collection of these molecules could selectively seek over 200 different protein targets in human cell lines and mice (*Nat. Chem. Biol.* 2022, DOI: 10.1038/s41589-022-01019-1).

Taunton has another reason to bet on aldehydes: there's a precedent for drugs that rely on these functional groups, suggesting they can be safe in the human body. In 2019, the US Food and Drug Administration approved Global Blood Therapeutics' voxelotor to treat sickle cell disease. The compound relies on an aldehyde to bind to an *N*-terminal amine on hemoglobin.

And although Novartis decided for business reasons to abandon it, the company took the reversible covalent inhibitor roblitinib, a cysteine-seeking aldehyde, through Phase 2 clinical trials as a potential therapy for liver cancer. Neither of these molecules targets lysine, but Taunton thinks a lysine-based covalent drug with an aldehyde handle has good odds of being safe.

Taunton hopes engineering delicately balanced, reversible agents will eventually allow him to hit a loftier goal: molecules that anchor on surface lysines just outside the protective inner pockets of proteins. These targets are swarmed with water molecules that weaken the lysine's nucleophilicity, so the optimal trade-off between the reactivity of the electrophile and the stability of the reversible interactions is tricky to achieve.

"It's going to require really paying close attention to the chemistry," Taunton says.

Lots to do for lysine

Fashioning effective covalent drugs requires putting all the pieces together. "Everything matters—the scaffold, the [electrophile] reactivity, and the local environment surrounding the lysine residue," Abbasov says. There is no one electrophile candidate that stands above the rest; the more researchers can devise, the more options will be available to tailor a drug to a specific lysine on a candidate protein.

Despite its abundance, lysine isn't meant to replace cysteine as the covalent target of choice. Instead, researchers hope that cysteine and lysine will complement each other to widen the overall druggable space. Researchers are already exploring ways to covalently snare other amino acids, such as tyrosine and serine. Each brings its own challenges but has the potential to expand drug developers' disease-fighting repertoire.

A breadth of options will be a win beyond drug development: researchers can use the same chemistry to tag amino acids with covalent molecular probes that can discover a protein's secrets. Having more sites available to bind to allows scientists to poke, prod, and modify a protein to better understand its structure and function.

"There's a lot to do in this field," Abbasov says. "This is just the beginning of what we can envision for the future."

This article is reprinted with permission from C&EN. A version of this article was published on Aug. 29, 2022, on cen.acs.org and in digital and print issues on page 15.

READING LIST

Our picks of the patent and journal literature on covalent drugs

2022

» Singh, Juswinder. **"The Ascension of Targeted Covalent Inhibitors."** *J. Med. Chem.* 65, no. 8 (April 28, 2022): 5886–901. https://doi.org/10.1021/acs.jmedchem.1c02134.

2021

» Dubiella, Christian, Benika J. Pinch, Kazuhiro Koikawa, Daniel Zaidman, Evon Poon, Theresa D. Manz, Behnam Nabet, et al. **"Sulfopin Is a Covalent Inhibitor of Pin1 that Blocks Myc-Driven Tumors in Vivo."** *Nat. Chem. Biol.* 17, no. 9 (Sept. 2021): 954–63. https://doi.org/10.1038/s41589-021-00786-7.

» Amgen. **"KRAS G12C Inhibitors and Methods of Using the Same."** US Patent 11,090,304, filed May 3, 2019, and issued Aug. 17, 2021.

» Su, Haixia, Sheng Yao, Wenfeng Zhao, Yumin Zhang, Jia Liu, Qiang Shao, Qingxing Wang, et al. **"Identification of Pyrogallol as a Warhead in Design of Covalent Inhibitors for the SARS-CoV-2 3CL Protease."** *Nat. Commun.* 12 (June 15, 2021): 3623. https://doi.org/10.1038/s41467-021-23751-3.

» Kuljanin, Miljan, Dylan C. Mitchell, Devin K. Schweppe, Ajami S. Gikandi, David P. Nusinow, Nathan J. Bulloch, Ekaterina V. Vinogradova, et al. **"Reimagining High-Throughput Profiling of Reactive Cysteines for Cell-Based Screening of Large Electrophile Libraries."** *Nat. Biotechnol.* 39, no. 5 (May 2021): 630–41. https://doi.org/10.1038/s41587-020-00778-3.

» Roskoski Jr., Robert. **"Orally Effective FDA-Approved Protein Kinase Targeted Covalent Inhibitors (TCIs)."** *Pharmacol. Res.* 165 (March 2021): 105422. https://doi.org/10.1016/j.phrs.2021.105422.

2020

» Syros Pharmaceuticals. **"Inhibitors of Cyclin-Dependent Kinase 7 (CDK7)."** US Patent 10,738,067, filed Feb. 11, 2020, and issued Aug. 11, 2020.

» Fell, Jay B., John P. Fischer, Brian R. Baer, James F. Blake, Karyn Bouhana, David M. Briere, Karin D. Brown, et al. **"Identification of the Clinical Development Candidate MRTX849, a Covalent KRASG12C Inhibitor for the Treatment of Cancer."** *J. Med. Chem.* 63, no. 13 (July 9, 2020): 6679–93. https://doi.org/10.1021/acs.jmedchem.9b02052.

» Li, Qingke, Qu Chen, Paul C. Klauser, Mengyuan Li, Feng Zheng, Nanxi Wang, Xiaoying Li, et al. **"Developing Covalent Protein Drugs via Proximity-Enabled Reactive Therapeutics."** *Cell* 182, no. 1 (July 9, 2020): 85–97. https://doi.org/10.1016/j.cell.2020.05.028.

» Chen, Shiyu, Scott Lovell, Sumin Lee, Matthias Fellner, Peter D. Mace, and Matthew Bogyo. **"Identification of Highly Selective Covalent Inhibitors by Phage Display."** *Nat. Biotechnol.* 39, no. 4 (April 2021): 490–498). https://doi.org/10.1038/s41587-020-0733-7.

» Wan, Xiaobo, Tangpo Yang, Adolfo Cuesta, Xiaming Pang, Trent E. Balius, John J. Irwin, Brian K. Shoichet, and Jack Taunton. **"Discovery of Lysine-Targeted eIF4E Inhibitors through Covalent Docking."** *J. Am. Chem. Soc.* 142, no. 11 (March 18, 2020): 4960–64. https://doi.org/10.1021/jacs.9b10377.

» Hamrick, Jodie C., Jean-Denis Docquier, Tsuyoshi Uehara, Cullen L. Myers, David A. Six, Cassandra L. Chatwin, Kaitlyn J. John, et al. **"VNRX-5133 (Taniborbactam), a Broad-Spectrum Inhibitor of Serine- and Metallo-β-Lactamases, Restores Activity of Cefepime in Enterobacterales and Pseudomonas aeruginosa."** *Antimicrob. Agents Chemother.* 64, no. 3 (Feb. 21, 2020): e01963–19. https://doi.org/10.1128/AAC.01963-19.

2019

» Shindo, Naoya, Hirokazu Fuchida, Mami Sato, Kosuke Watari, Tomohiro Shibata, Keiko Kuwata, Chizuru Miura, et al. **"Selective and Reversible Modification of Kinase Cysteines with Chlorofluoroacetamides."** *Nat. Chem. Biol.* 15, no. 3 (March 2019): 250–58. https://doi.org/10.1038/s41589-018-0204-3.

» Mons, Elma, Ineke D. C. Jansen, Jure Loboda, Bjorn R. van Doodewaerd, Jill Hermans, Martijn Verdoes, Constant A. A. van Boeckel, et al. **"The Alkyne Moiety as a Latent Electrophile in Irreversible Covalent Small Molecule Inhibitors of Cathepsin K."** *J. Am. Chem. Soc.* 141, no. 8 (Feb. 27, 2019): 3507–14. https://doi.org/10.1021/jacs.8b11027.

» Johansson, Henrik, Yi-Chun Isabella Tsai, Ken Fantom, Chun-Wa Chung, Sandra Kümper, Luigi Martino, Daniel A. Thomas, et al. **"Fragment-Based Covalent Ligand Screening Enables Rapid Discovery of Inhibitors for the RBR E3 Ubiquitin Ligase HOIP."** *J. Am. Chem. Soc.* 141, no. 6 (Feb. 13, 2019): 2703–12. https://doi.org/10.1021/jacs.8b13193

Note: This list was chosen by experts who work in the field, CAS information scientists, and C&EN editorial staff.

Download a copy of this and all Discovery Reports at www.acs.org/discoveryreports