doi:10.2533/chimia.2022.435 Chimia 76 (2022) 435–447 

# Never Gonna Give You Up – Current Developments in Covalent Protein Kinase Inhibitors

Laura Hillebrand[a] and Matthias Gehringer*[ab]

*Abstract:* Covalent inhibitors have recently seen a revival in medicinal chemistry. Inhibitors addressing non-catalytic cysteine residues with weakly reactive electrophiles have been very successfully employed to target protein kinases, one of the major druggable protein families. Here we provide an overview of irreversible and reversible covalent protein kinase inhibitors in clinical development and beyond. We further spotlight recent advances in targeting amino acids other than cysteine and the reactive groups utilized in covalent protein kinase inhibitors.

**Keywords:** Covalent inhibitors · Cysteine targeting · Protein kinases

***Laura Hillebrand*** obtained her BSc in chemistry from TU Dresden and her MSc in the same field from RWTH Aachen. For her Master's thesis, she worked on photocontrollable arginine mimetics for MLL1 inhibition in Marburg. Since 2021, she is part of the Gehringer lab at the University of Tübingen as a PhD student, where she is working on covalent targeting of tyrosine side chains in protein kinases.

***Matthias Gehringer*** is an Assistant Professor for Medicinal Chemistry and Associate PI in the Cluster of Excellence 'Image-guided and Functionally Instructed Tumor Therapies' (iFIT) at the University of Tübingen. After studying chemistry in Karlsruhe, Montpellier, and Heidelberg, he received his PhD in Medicinal Chemistry from the University of Tübingen. As a postdoc, he worked with Prof. Karl-Heinz Altmann at the Swiss Federal Institute of Technology (ETH) in Zurich. His research focuses on protein kinase inhibitors and covalent targeting strategies.

## 1. Introduction

### 1.1 *Covalent Inhibitors*

Most small molecule drugs achieve their biological effect *via* reversible, non-covalent binding to a disease-relevant target. In contrast, medicinal chemists have largely avoided the design of covalent drugs which undergo a chemical reaction to form a covalent bond with their target protein.[1] This is due to concerns about their potentially indiscriminate reactivity, which was suspected to trigger off-target effects and idiosyncratic drug reactions. In fact, if drugs possessed a covalent mechanism, it has often been discovered serendipitously. This is illustrated by successful covalent drugs like acetylsalicylic acid (aspirin), β-lactam antibiotics, proton-pump inhibitors (*e.g.* omeprazole), or the platelet agglutination inhibitor clopidogrel.[2] More recently, however, deliberately designed covalent inhibitors such as the anticancer agents afatinib (**1**) or ibrutinib (**2**) have emerged.[3] As of the writing of this review, there are far more than 100 covalently-acting drugs approved by the FDA.[4] Regardless of their success, however, covalent inhibitors remain underrepresented among the approved therapeutics.

Despite the previous reluctance, covalent inhibitors have recently started to gain popularity in drug discovery,[5] particularly because of their prolonged drug–target residence times and their promise for targets where reversibly binding small molecules have been unsuccessful. As a result of this rekindled interest, a significant number of purposely designed covalent inhibitors have recently entered clinical trials.[1] Inhibitors addressing poorly conserved, non-catalytic residues are commonly referred to as targeted covalent inhibitors (TCIs).[2] The covalent inactivation takes place once the bond-forming functional group is positioned to react swiftly with a specific residue at the target site in the reversibly-bound complex (Fig. 1a).[5] Whereas most TCIs act in an irreversible manner, covalent bond formation can also be reversible. The durability of the covalent interaction depends on the chosen functional group, the so-called warhead, which typically is an electrophilic moiety designated to address an electron-rich residue (nucleophile) on the receptor.[3,6]

Reversible binding is an equilibrium process (Fig. 1b) with ligand affinity defined by the inhibition constant $K_i$, which reflects the ratio between the rate of ligand dissociation ($k_{off}$) and association ($k_{on}$). In contrast, irreversible covalent binding is a nonequilibrium process that can be described by two distinct steps. In the first step, a classical (reversible) binding event takes place. This step is described by the constant $K_I$ accounting for the ligand concentration necessary to attain a half-maximal rate of covalent modification. The second step is described by the first order rate constant $k_{inact}$, the maximal potential rate of covalent modification. The total efficiency of covalent binding is characterized by the second-order rate constant $k_{inact}/K_I$. Covalent inhibition is thus a time-dependent process, in which the apparent inhibitor potency increases with incubation time.[7]

A result of irreversible covalent binding is that target function can only be restored by *de novo* protein synthesis.[5] Moreover,

*Correspondence: Jun.-Prof. Dr. M. Gehringer, E-mail: matthias.gehringer@uni-tuebingen.de

[a]Department of Pharmaceutical & Medicinal Chemistry, Eberhard Karls University Tübingen, Auf der Morgenstelle 8, 72076 Tübingen, Germany; [b]Cluster of Excellence iFIT (EXC 2180) 'Image-Guided & Functionally Instructed Tumor Therapies', University of Tübingen, 72076 Tübingen, Germany.

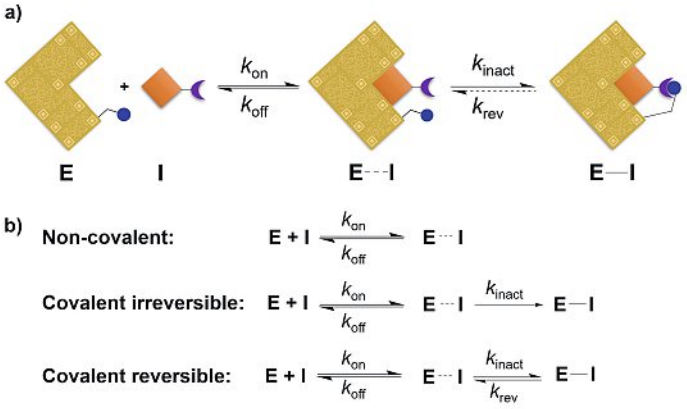



Fig. 1. a) Mechanism of irreversible and reversible targeted covalent inhibitors. After the reversible binding event in which the reactive warhead (purple) is positioned closely to the target amino acid (blue), the covalent trapping takes place. b) Comparison of the kinetic mechanisms of non-covalent and covalent binders.

Electrophilic warheads must fulfill several criteria to be useful for TCI design. Because the requirements differ between various targets, the reactivity of such functional groups should be tunable over a broad range. Beyond that, the warhead (and potential metabolites) should be non-toxic and adequately stable against metabolism and abundant nucleophiles such as glutathione (GSH).[11] Currently, the most prevalent CRGs in TCIs – including all FDA approved covalent kinase inhibitors – are acrylamides and related α,β-unsaturated amides (Scheme 1). Their popularity traces back to their relatively low intrinsic reactivity, their preference for 'soft' nucleophiles, and their synthetic accessibility.[7,12]


Scheme 1: Reaction of acrylamides with cysteine *via* thia-Michael addition. The reaction is commonly irreversible.

prolonged exposure can enable full and durable target occupancy, which can present a potential benefit in a clinical setting. Accordingly, a key advantage of covalent inhibition is the opportunity to increase the (apparent) on-target potency of a compound as a result of the non-equilibrium binding behavior.[1] A higher potency and extended duration of action allows for smaller and less frequent dosing regimens reducing the risk of idiosyncratic toxicities, which may improve both treatment outcome and patient compliance.[3] While prolonged duration of action can be desirable, especially for target proteins with slow turnover, it may also present a safety concern in some cases.[1] Generally, there is a fine line between risks and benefits associated with covalent inhibition in a therapeutic setting and the right balance largely depends on the projected therapeutic indication.

Beyond the merits and pitfalls of durable target modification, non-specific binding presents a major challenge for covalent inhibitors: if the intrinsic reactivity of a compound is too high, it can bind promiscuously to other proteins or DNA. On the other hand, covalent engagement of the target of interest may become too slow if reactivity is too low. Thus, designing inhibitors that selectively bind to, and rapidly inactivate a single target protein presents a challenge. This holds particularly true when the target is a part of a conserved protein class like protein kinases, a large superfamily of enzymes with very similar binding pockets.[1] The TCI concept has shown, however, that the covalent binding mechanism can be exploited to increase selectivity if a suitable, non-conserved target amino acid can be identified in the binding pocket and if the right degree of intrinsic warhead reactivity can be found.[5] To this end, selectivity can be improved through optimization of two orthogonal selectivity filters: target-specific covalent modification and non-covalent interactions to improve receptor recognition.[8]

There are several different strategies for the development of TCIs. Fragment-based covalent drug discovery has started to gain traction,[9] but most often TCIs are generated by structure-based design from optimized reversible ligands. The reversible ligands are then modified by attachment of the electrophilic covalent reactive group (CRG or warhead) to address a proximal amino acid – most frequently cysteine. This is because the nucleophilicity of the thiolate form of the cysteine side chain (pKa ca. 8.5) is the highest among the 20 canonical amino acids.[7] Besides the chemical nature of the nucleophile, its location and the surrounding protein microenvironment are important factors that not only influence its accessibility but also its reactivity. This results from alterations in the protonation state and spatial arrangement of the reacting groups, the geometries and energies of transition states and intermediates, and the stability of the reaction product.[10]

Beyond cysteine, it is also possible to address other amino acids, like lysine. As the most common alternative to cysteine, lysine is typically found on protein surfaces, on interfaces mediating protein–protein interactions, and in binding cavities. However, surface-exposed (unperturbed) lysine is almost entirely protonated at physiological pH ($pK_a$ *ca.* 10.5).[7] The three amino acids containing side chain hydroxyl groups (serine, threonine, and tyrosine) are a further group of possible targets for covalent inhibitors. Of these, the phenol moiety in tyrosine is the most acidic ($pK_a$ *ca.* 10) and its $pK_a$ is frequently perturbed to favor the highly nucleophilic phenolate form. Due to the increased 'hardness' of oxygen nucleophiles compared to sulfur, conjugate addition chemistry is less suitable to address such moieties. Harder electrophiles, such as sulfur(VI) fluorides are favored instead.[13] Moreover, nucleophilic aromatic substitution ($S_NAr$) chemistry has shown great promise in targeting cysteine, but also lysine and tyrosine residues.[7,14,15]

### 1.2 *Covalent Protein Kinase Inhibitors*

Post-translational modifications expand the functional diversity of the proteome and influence countless aspects of pathological and normal physiology. As one of the most important post-translational modifications, protein phosphorylation is executed by protein kinases which transfer the γ-phosphate of adenosine triphosphate (ATP) onto hydroxyl groups of serine, threonine and/or tyrosine. Acting as a reversible on/off switch for protein activity, this process plays a vital role in cellular signal transduction. However, events like gene amplifications, mutations, or chromosomal rearrangements can lead to abnormal regulation of protein kinases which is in turn linked to diseases, especially malignancies.[16,17] Consequently, large drug discovery efforts have been dedicated to the inhibition of protein kinases which are now among the most important drug targets. Since the groundbreaking approval of the first small molecule protein kinase inhibitor imatinib in 2001, over 70 inhibitors (including eight covalent ones) have been approved.[17] Nevertheless, and despite the impressive amount of research conducted, large parts of the human 'kinome' encoded by over 500 protein kinase genes have remained underexplored since most research continues to be centered around few well-validated targets.[18] While protein kinase inhibitors have been most successful in cancer treatment, these agents also hold great promise for other disorders where aberrant protein kinase activity is a driver including inflammation, neurodegeneration, infectious and metabolic diseases.[16]

Small-molecule inhibitors of protein kinases usually target the ATP binding pocket.[16] The degree of conservation of this binding

site in the human kinome adds an intrinsic challenge to the design of selective inhibitors. This challenge has partly been addressed, for example, by targeting less conserved regions of the ATP binding site or adjacent pockets exclusive to special kinase conformations. Still, the design of selective or narrow spectrum kinase inhibitors remains an ambitious endeavor. Furthermore, therapeutic targeting is complicated by high intracellular ATP concentrations competing with the ligand. Covalent inhibitors possess the potential to address these challenges because they combine limited ATP competitivity with the use of an additional, complementary selectivity filter, *i.e.* the requirement for a nucleophilic amino acid at a suitable position in the ATP binding site.[11] In 2009, a study by Gray and colleagues revealed that more than 200 kinases (about 40% of the kinome) possess an accessible cysteine residue that can potentially be targeted by covalent kinase inhibitors.[19] Together, these non-catalytic cysteines have been designated the protein kinases' cysteinome. Cysteine residues are scattered at numerous locations inside and around the ATP pocket presenting the opportunity to be addressed by TCIs.[11] A subsequent analysis by Chaikuad *et al.* concluded that there are at least 18 positions or subsites harboring cysteines with a high likelihood of being targetable by electrophilic inhibitors (Fig. 2).[12] In inactive conformations, even more cysteine positions become accessible.[20] Altogether, these cysteines provide the basis for the large majority of covalent protein kinases inhibitors developed so far.

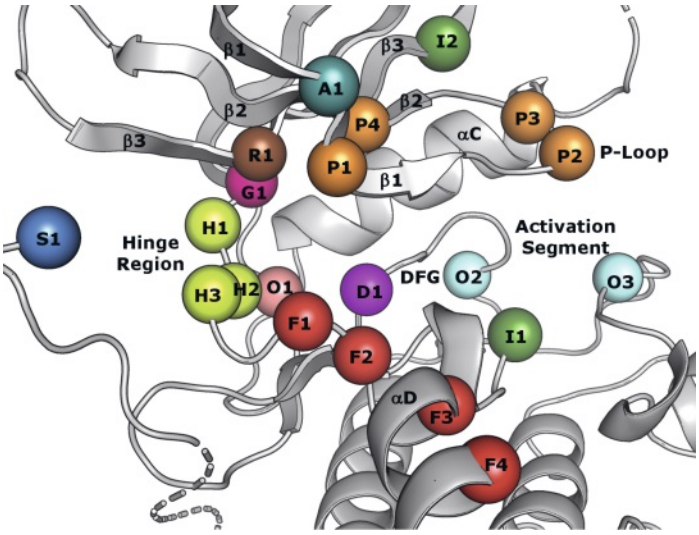



Fig. 2. Cysteines possibly amenable to covalent targeting according to Chaikuad *et al*.[12] Colors were designated corresponding to the kinase regions where the cysteines are located. Orange: P-loop; pink: gatekeeper residue; light green: hinge region; red: front region; magenta: pre-DFG motif; salmon: backpocket; brown: roof region; cyan blue: activation segment; blue: outside the ATP pocket; dark green: additional positions. Positions are labeled according to nomenclature used by Chaikuad *et al*.[12] Position A1 has been supplemented according to Yen-Pon *et al*.[21] Cysteines are unevenly distributed at these positions. A list assigning kinases to the respective cysteine positions can be found in ref. [11] and [12].

## 2. Approved Covalent Protein Kinase Inhibitors

Currently, eight covalent protein kinase inhibitors targeting two different kinase families are approved by the US Food and Drug Administration (FDA; see Fig. 3):[21] afatinib (2013, **1**), dacomitinib (2018, **3**), osimertinib (2015, **4**), mobocertinib, (2021, **5**) neratinib (2017, **6**) as well as ibrutinib (2013, **2**), acalabrutinib (2017, **7**) and zanubrutinib (2019, **8**).[22,23]

Of these, afatinib (**1**) was the first one to gain FDA approval in 2013. It is a gefitinib-derived second-generation EGFR/HER (ErbB) family kinase inhibitor that was developed by Boehringer Ingelheim and is being used in the therapy of metastatic non-small-cell lung cancer (NSCLC) driven by activating epidemical growth factor receptor(EGFR)-mutations. Similarly, dacomitinib (**3**), a structural analog of afatinib (**1**) developed by Pfizer is also utilized for this purpose as well as metastatic NSCLC with exon 19 deletions. Osimertinib (**4**), meanwhile, is a third-generation mutant-selective EGFR inhibitor approved for the treatment of metastatic NSCLC harboring the EGRF-T790M resistance mutation of the so-called gatekeeper residue.[11] The most recently approved covalent kinase inhibitor is mobocertinib (**5**), another third-generation EGFR inhibitor which received FDA approval in September 2021 as a therapy for EGFR exon 20 insertion-positive NSCLC.[23] All of the mentioned inhibitors covalently bind to a front-pocket cysteine (F2 position, compared to Fig. 2) of the EGFR kinase domain (Cys797) that is also present in HER2 and HER4.[11,24] Neratinib (**6**), which is a quinoline-derived pan-HER-inhibitor for treatment of HER2-positive breast cancer, also irreversibly binds to this particular cysteine.[22]

Also approved in 2013, ibrutinib (**2**) is an inhibitor of Bruton's tyrosine kinase (BTK) targeting an F2-positioned cysteine (Cys481) as well.[22] Ibrutinib (**2**) was developed by Pharmacyclics/AbbVie from a reversible screening hit bearing a cyclopentyl residue instead of the piperidine acrylamide. Through molecular modeling the distance of the cyclopentyl group to Cys481 was estimated at 3.8 Å, a suitable distance for attachment of a warhead. In consequence, numerous different linkers and warhead moieties were tested. Out of these experiments, ibrutinib (**2**) emerged as the lead candidate.[25] While at first, it was only approved for treating mantle cell lymphoma (MCL), the authorization was later expanded to other illnesses such as chronic and small lymphocytic leukemia (CLL, SLL) as well as graft-versus-host disease which renders ibrutinib (**2**) one of the few small molecule kinase inhibitors utilized for the treatment of immune disorders.[11,26] Since then, two second-generation covalent BTK inhibitors have been developed. Acalabrutinib (**7**), notable for its alkynamide warhead, and zanubrutinib (**8**) were both designed by exchanging the pyrazolopyrimidine core of ibrutinib (**2**) with comparably substituted, bioisosteric hinge binding scaffolds and attaching the respective warhead.[22] Both are being used in the therapy for MLL while acalabrutinib (**7**) is also utilized for treating CLL and SLL.[26]

## 3. Covalent Protein Kinase Inhibitors in (Clinical) Development

While the approval of eight covalent kinase inhibitors since 2013 proves the tremendous success of TCIs, many more of these agents are currently in clinical development. In the following chapter, we discuss the most important protein kinases for which inhibitors have advanced to clinical trials and highlight the corresponding inhibitors as well as selected examples of how such compounds have been developed.

In the field of EGFR inhibitors, more third-generation, mutant-selective irreversible inhibitors are being developed. Nazartinib/EGF816 (**9**, Fig. 4), which was designed by Novartis based on a reversible screening hit, is currently in phase II trials for metastatic NSCLC and bears an alternative scaffold to osimertinib's 2-aminopyrimidine core.[27] BLU-451/LNG-451, whose structure has not been disclosed, will begin clinical trials for advanced cancers with EGFR exon 20 insertion mutations soon and is especially interesting due to its good CNS penetration as determined in pre-clinical models, which may make it predominantly beneficial for patients with brain metastases.[28] Pyrotinib (**10**, Fig. 4), which is structurally very similar to neratinib (**6**) is already approved in

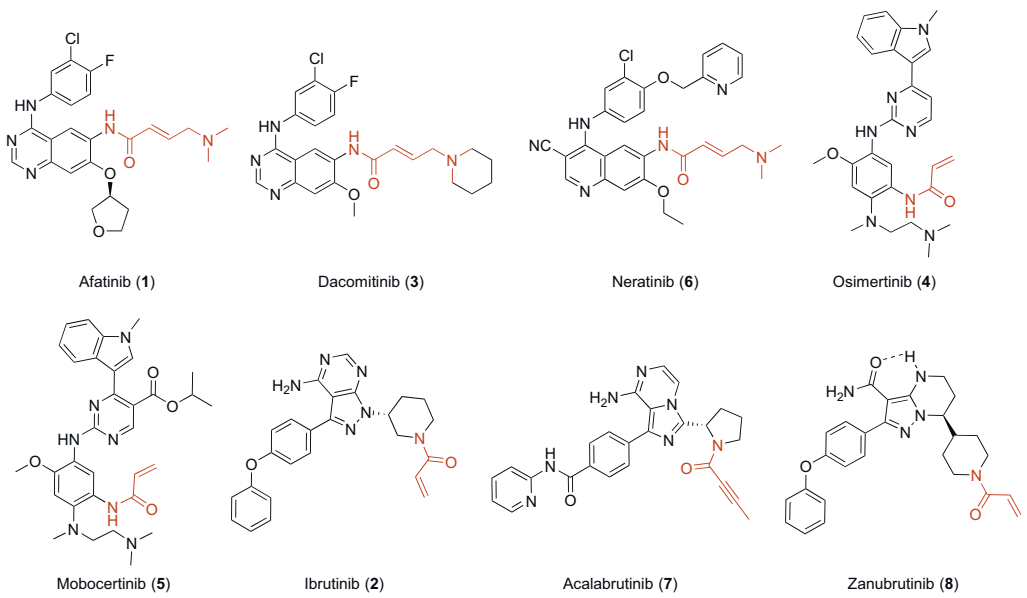



Fig. 3. Currently FDA-approved covalent protein kinase inhibitors. The respective warhead is marked in red.

China and still undergoing clinical trials for HER2-positive solid tumors[29] while poziotinib/HM781-36 (**11**, Fig. 4), which bears the same quinazoline core as the second-generation EGFR inhibitors, is being investigated for a multitude of oncological diseases with EGFR or HER2 exon 20 insertions.[30,31] Another interesting clinical candidate targeting HER2 is TAS0728 (**12**, Fig. 4) which is currently under investigation for advanced solid tumors with abnormalities in HER2 or HER3, the pseudokinase member of the HER family. This molecule bears an aminopyrazolopyrimidine scaffold like ibrutinib (**2**) and is therefore markedly different to the quinazoline core of afatinib (**1**) and the quinoline-3-carbonitrile of neratinib (**6**) which also hit HER2. TAS0728 (**12**) binds covalently to Cys805 and is selective for HER2.[32,33] Only very recently, Xia and colleagues from Shanghai Pharmaceuticals published the discovery program of SPH5030 (**13**, Fig. 4), another selective HER2 inhibitor. They used a molecular hybridization strategy, analyzing tucatinib (**14**, a reversible HER2 inhibitor), pyrotinib (**10**) and neratinib (**6**) for their different characteristics and the underlying interactions between their molecular features and HER2.

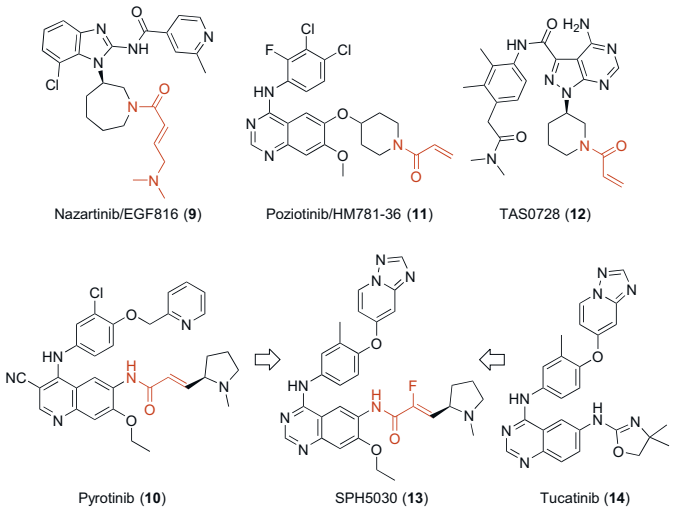



Fig. 4. Covalent EGFR/HER2 inhibitors currently in clinical trials. Tucatinib (**14**), a non-covalent HER2-selective inhibitor, is depicted as a structural template for the molecular hybridization design strategy that led to SPH5030 (**13**).

Examining the differences between the three molecules, they concluded that the 4-aminoquinazoline core of tucatinib (**14**) as well as its triazolopyrimidine side chain reaching into a hydrophobic back pocket near the α-C helix (compare to Fig. 2) were responsible for the HER2 selectivity. Meanwhile the Michael acceptor on pyrotinib (**10**) bearing a methylpyrrolidine as the substituent in the β-position demonstrated the best potency. The pyrotinib quinazoline 7-ethoxy group was adopted for enhanced PK properties and the fluoro substituent on the warhead's α-position not only ameliorated physicochemical properties but also increased HER2 selectivity further. Taken together, the inhibitor shows much better potency than neratinib (**6**) and pyrotinib (**10**) and is also selective for wildtype HER2 as well as several mutants. Phase I clinical trials of SPH5030 (**13**) are expected to start soon in China.[34]

An inhibitor that targets mutant forms of both EGFR and BTK and that is also being investigated beyond the realm of oncology is avitinib/AC0010, also known as abivertinib/STI-5656 (**15**, Fig. 5a).[35,36] It is structurally related to third-generation irreversible EGFR inhibitors, most of which are based on screening hit WZ4002 (**16**). The latter compound binds to the ATP binding site of EGFR mutant forms in a U-shaped conformation with two hydrogen bonds being formed between the central 2-aminopyrimidine and the hinge region (Fig. 5b).[37] However, in compound **15**, the common 2-aminopyrimidine core was replaced by 2-amino-7*H*-pyrrolo[2,3-*d*]pyrimidine capable of forming a third hydrogen bond towards the hinge backbone. Avitinib (**15**) is currently in advanced clinical trials for metastatic NSCLC and B-cell lymphoma but has also recently been investigated for the treatment of Covid-19. Due to the irreversible binding to BTK, the compound showed potent immunomodulatory activity *in vitro* by inhibiting the production of pro-inflammatory cytokines associated with cytokine storm and poor outcomes in Covid-19 patients with acute respiratory distress syndrome (ARDS).[38] Of note, other BTK inhibitors, like zanubrutinib (**8**) and acalabrutinib (**7**) have also been investigated for their use against Covid-19.[39]

Due to the important role of BTK in the inflammatory machinery, BTK inhibitors are also expected to have a favorable impact on autoimmune diseases caused by autoreactive B-cells and immune complex-driven inflammation.[39] Remibrutinib/LOU064 (**17**, Fig. 6a), a BTK inhibitor developed for the treatment of autoimmune disorders, is an interesting example because it binds to BTK in an inactive conformation which is most likely the cause for its selectivity over related kinases, including BTK's close relatives from the TEC family of nonreceptor protein-tyrosine

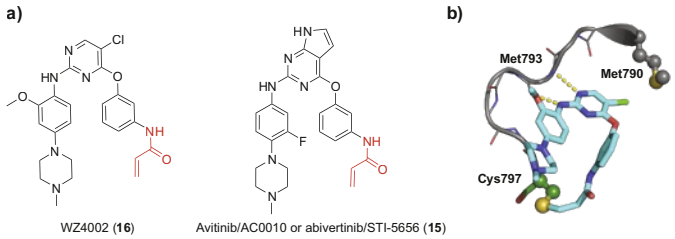
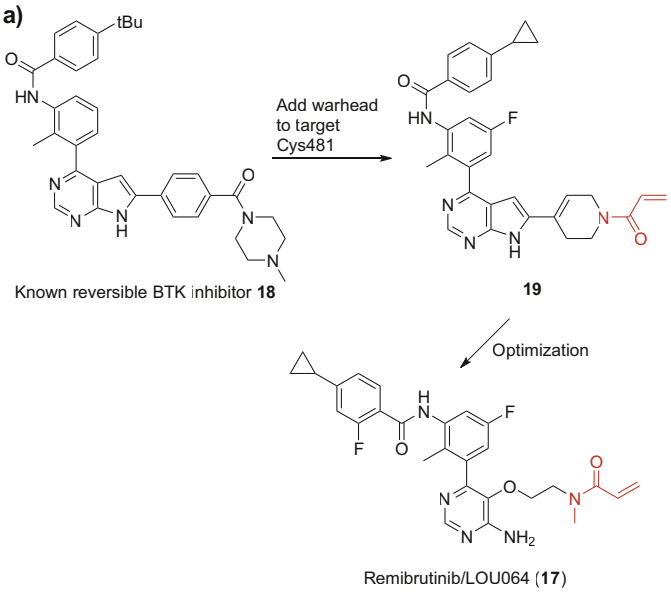



Fig. 5. a) Structures of avitinib (**15**) and structural prototype WZ4002 (**16**). b) X-ray crystal structure of WZ4002 (**16**) bound to EGFR T790M showing the classic U-shaped conformation of many third-generation irreversible EGFR inhibitors (PDB: 3IKA). The dashed lines indicate hydrogen bonds of the molecule to the backbone of the hinge region (Met793). The gatekeeper residue (Met790) and the targeted cysteine (Cys797) are depicted in spheres.

kinases (namely ITK, BMX, TXK, and TEC). The design was based on the known reversible BTK inhibitor **18** whose carboxamide-substituted phenyl ring showed close proximity to Cys481 providing a suitable starting point for attachment of electrophilic warheads. This led to irreversible BTK inhibitor **19** which already demonstrated very high potency towards BTK ($IC_{50}$ = 0.9 nM) but showed unfavorable physicochemical properties. Further optimization improving the ADME profile finally led to remibrutinib (**17**) which is currently in clinical trials for autoimmune diseases like Sjögren's syndrome (SS) and chronic spontaneous urticaria (CSU). An X-ray crystal structure of the inhibitor bound to BTK



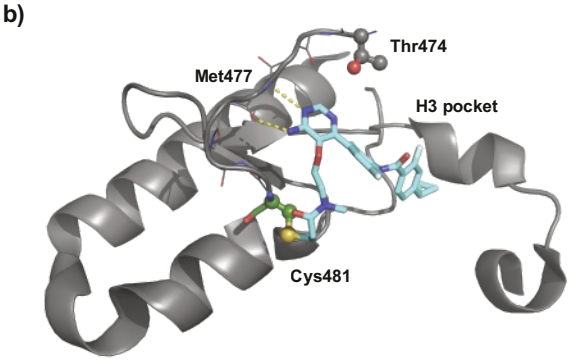
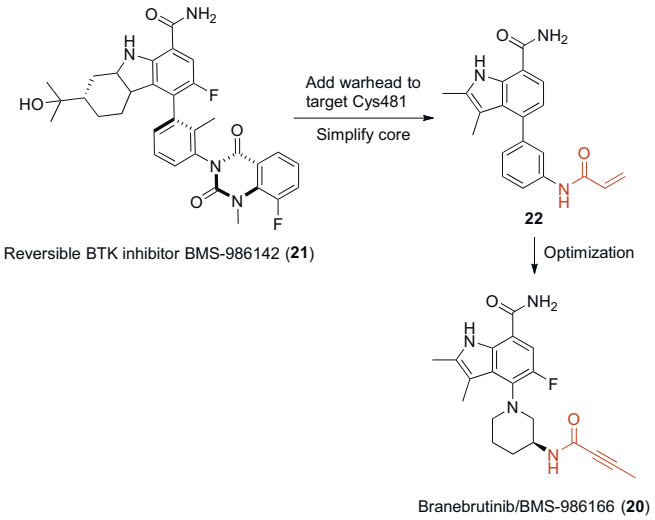




Fig. 6. a) Discovery of remibrutinib (**17**) starting from the reversible BTK inhibitor **18**. b) X-ray crystal structure of remibrutinib (**17**) in complex with BTK (PDB: 6TFP).

(Fig. 6b) confirmed the covalent engagement of Cys481 and demonstrated that the cyclopropyl-substituted fluorophenyl moiety inhabits the so-called 'H3 pocket' stabilizing BTK in its inactive conformation.[39,40]

Branebrutinib/BMS-986166 (**20**, Scheme 2) was also designed from a reversible BTK inhibitor, BMS-986142 (**21**). The crystal structure of **21** bound to BTK (PDB: 5T18) showed the quinazoline dione group pointing towards the desired cysteine. Attaching the warhead in the determined position and simplifying the scaffold resulted in irreversible inhibitor **22**. However, this compound exhibited poor plasma PK *in vivo* which was ascribed to the high intrinsic reactivity of the *N*-aryl acrylamide. Reactivity was reduced by replacement with a 3-aminopiperidine-linked but-2-ynamide, ultimately leading to branebrutinib (**20**) which only showed some off-target activity against the other TEC family members that possess a similar cysteine.[41] It is now in phase II for rheumatoid arthritis (RA), SS and lupus.[39]




Scheme 2. Discovery of branebrutinib (**20**) from non-covalent BTK inhibitor BMS-986142 (**21**).

There are several other BTK inhibitors (Fig. 7) in different stages of clinical development for autoimmune diseases as well as oncological illnesses. These compounds bear a striking resemblance to ibrutinib (**2**) with slight changes around the pyrazolopyrimidine hinge binding motif. For example, in evobrutinib (**23**), developed by Merck, this motif was replaced by a diaminopyrimidine. All of them, except for spebrutinib/CC-292 (**24**), carry the phenoxyphenyl moiety that is positioned in the hydrophobic back pocket behind the gatekeeper residue. Tirabrutinib/GS-4059 (**25**) and orelabrutinib (**26**) are already approved for treatment of lymphoma in Japan and China, respectively.[42,43] Tolebrutinib (**27**) is in phase III studies for multiple sclerosis (MS) and may be the first disease-modifying therapy to address the source of MS damage in the brain.[39]

Interleukin-2-inducible T-cell kinase (ITK) is the other member of the TEC family to which significant research efforts have been devoted. Up to now, however, there has been no ITK inhibitor in clinical trials. CPI-818 is the first ITK inhibitor to enter the clinical development stage for the treatment of T-cell lymphoma. Its structure is so far undisclosed.[44,45] Other TEC family members have been far less pursued. Notably, recent efforts from our own group and others aimed towards the development of selective covalent inhibitors of BTK's closest relative, the bone marrow tyrosine kinase on chromosome X (BMX).[46–48] Compounds developed so far, however, have significant off-target activity on BTK or other TEC family members.

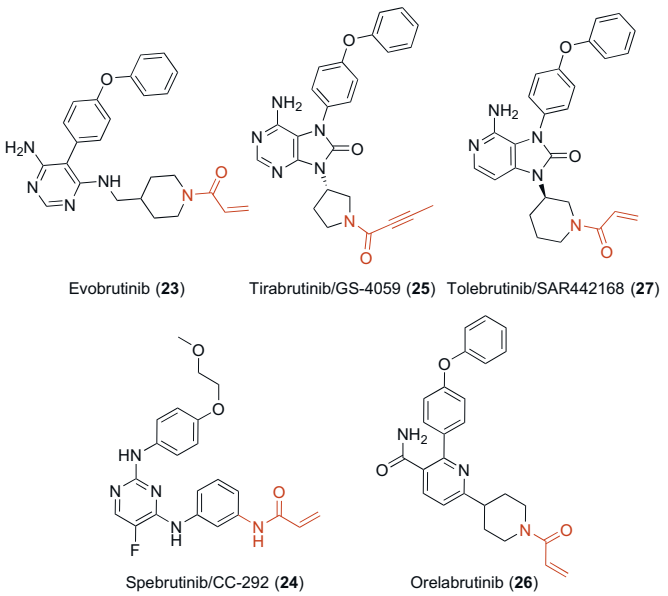



Fig. 7. Covalent BTK inhibitors with high resemblance to ibrutinib (**2**) currently in clinical trials.

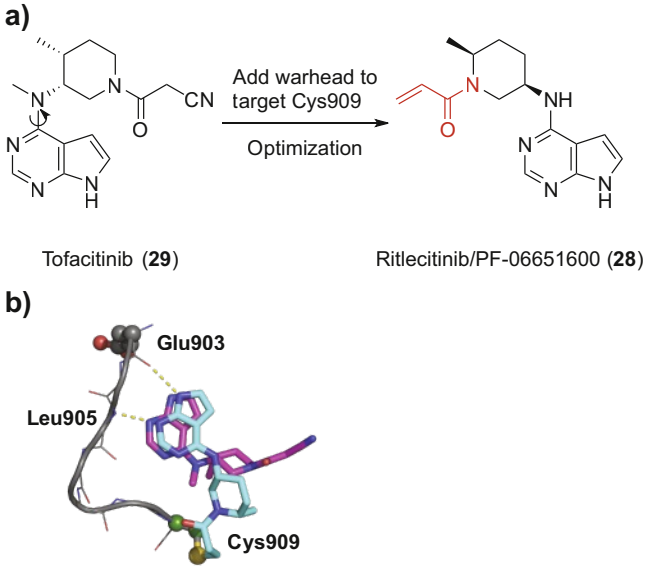



Fig. 8. a) Development of ritlecitinib (**28**) from the approved reversible pan-JAK inhibitor tofacitinib (**29**). b) Overlay of X-ray crystal structures of tofacitinib (**29**), magenta, PDB: 3LXK) and of ritlecitinib (**28**, cyan, PDB: 5TOZ) in complex with JAK3, highlighting the required 180° rotation of the 3-piperidyl substituent.

Beyond the realm of the TEC and EGFR families, covalent targeting of an equivalently positioned (F2) cysteine has been very successfully applied to the Janus kinase JAK3. The Janus kinase (JAK) family is comprised of the four members JAK1, JAK2, JAK3, and TYK2, which are validated targets for autoimmune-related diseases and myeloproliferative neoplasms.[49] JAK3 stands out among the JAKs since it exclusively mediates signaling *via* cytokine receptors featuring the comon γ-chain, and it has been suggested as a target for downregulating immune response with few side effects. Most of the currently approved (non-covalent) JAK inhibitors display poor selectivity within the JAK family. The cysteine in the F2 position of JAK3 (Cys909), however, is a serine in the other three family members, providing an opportunity to achieve isoform selectivity through covalent targeting.[50] Ritlecitinib/PF-0665100 (**28**, Fig. 8), the most advanced covalent JAK3 inhibitor, was designed by Pfizer using their reversible pan-JAK inhibitor tofacitinib (**29**, Fig. 8) as a starting point. Tofacitinib (**29**) binds the JAKs with the amino-linked 3-piperidyl moiety pointing to the same side as the pyrrole ring of the 7*H*-pyrrolo[2,3-*d*]pyrimidine core and thus towards the inside of the ATP pocket. The development of covalent inhibitors was based on the assumption that a 180° rotation of the 3-piperidyl moiety around the exocyclic $C_{Aryl}$–N bond would place its amide substituent in close proximity to Cys909.[51] The required conformational preference was achieved by removing tofacitinib's *N*-methyl and its piperidine 4-methyl group, the latter being re-introduced in the piperidine's 6-position to optimize reactivity and PK properties. An X-ray crystal structure of ritlecitinib (**28**) bound to JAK3 (Fig. 8b) validated the predicted binding mode with the acrylamide forming a covalent bond to Cys909.[52] Ritlecitinib (**28**) is currently in phase III studies for alopecia areata and in phase II for vitiligo, Crohn's disease and ulcerative colitis.[22]

Beyond cysteines in the front region, there are several kinase families that possess cysteines in other areas of the ATP binding site (compare to Fig. 2). One of these is the FGFR family that is comprised of the four members FGFR1–4, which all contain a cysteine in the P-loop (Cys488 in FGFR1; Position P2). FGFRs are deemed to be promising targets for anticancer drug development, but reversible inhibitors often suffer from dose-limiting toxicities or rapid clearance providing an encouraging rationale for covalent inhibitors.[53] Among covalent pan-FGFR inhibitors, most advanced in clinical trials is futibatinib/TAS-120 (**30**, Fig. 9) which is currently in phase II studies for advanced solid tumors harboring FGFR aberrations.[54] The hinge binding motif looks similar to ibrutinib (**2**) and the alkyne-linked dimethoxyphenyl ring binds to the hydrophobic behind-gatekeeper pocket in a similar manner as observed in other FGFR-selective inhibitors. Crystal structures (Fig. 9b) revealed that the pyrrolidine ring rotates upwards in order to meet the P-loop and form the covalent bond to Cys488.[55]

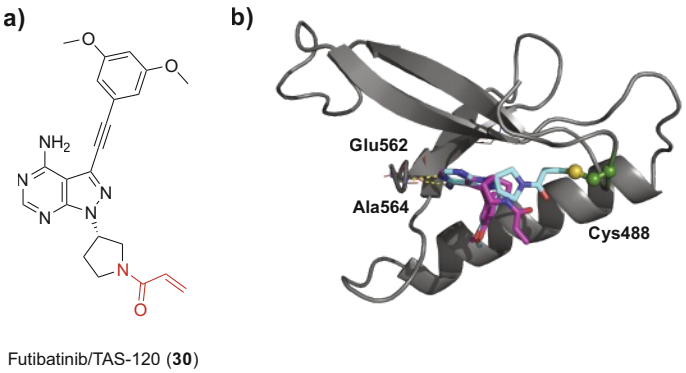
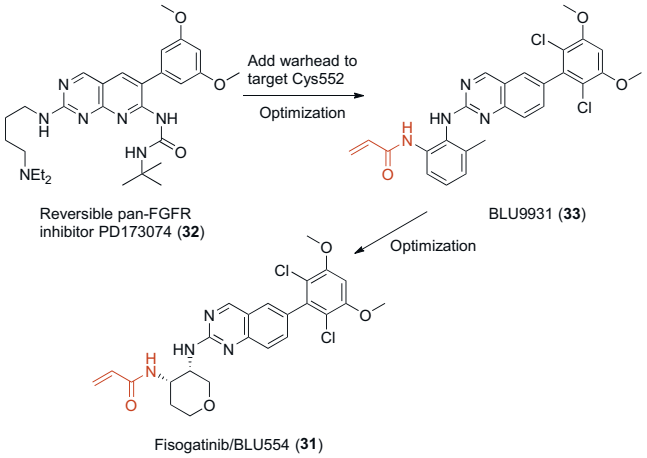



Fig. 9. a) Structure of covalent pan-FGFR inhibitor futibatinib (**30**). b) X-ray crystal structure of futibatinib (**30**) in complex with FGFR1 in the non-covalently bound state (magenta, C488A mutant, PDB: 6MZQ) and the irreversible complex (cyan, PDB: 6MZW) in which the pyrrolidine ring rotates upwards to direct the acrylamide warhead towards the P-loop.

Besides the conserved cysteine located in the P-loop, FGFR4 is the only family member that contains a second cysteine in the hinge region (Cys552, H1 position, compare to Fig. 2) which is present in only four other protein kinases (TTK/MPS1, MAPKAPK2, MAPKAPK3 and p70S6Kβ/S6K2). In FGFR1–3 this position is occupied by a tyrosine, providing a feature that facilitates the design of isoform-selective FGFR4 inhibitors.[11]

One FGFR4-selective inhibitor currently in clinical trials for the treatment of hepatocellular carcinoma (HCC), is fisogatinib/BLU554 (**31**, Scheme 3).[56] This covalent binder is based on the reversible pan-FGFR inhibitor PD173074 (**32**). Installation of an *ortho*-phenylene diamine linker equipped with an acrylamide in the quinazoline C2 position was key to reach Cys552. A methyl group in the other *ortho*-position further favored the required out-of-plane twist of the two aryl rings. Along with other modifications, this design strategy gave rise to prototype covalent FGFR4 inhibitor BLU9931 (**33**).[57] Further optimization of this molecule finally led to the clinical candidate fisogatinib/BLU554 (**31**).[58] Notably, we recently used an analogous design strategy to obtain a first-in-class covalent inhibitor of the Monopolar spindle kinase 1 (MPS1 or TTK).[59]



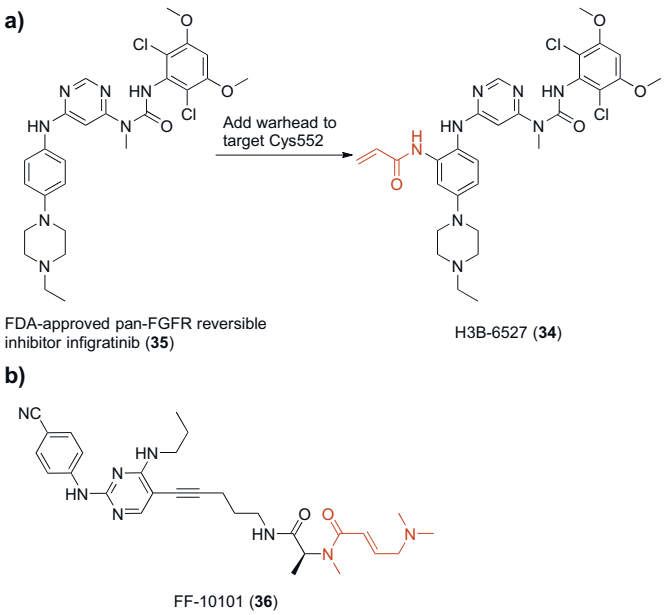

Scheme 3. Discovery of fisogatinib (**31**) starting from the reversible pan-FGFR inhibitor PD173974 (**32**) *via* covalent prototype BLU9931 (**33**).

Another promising FGFR4-selective inhibitor is H3B-6527 (**34**, Scheme 4a) which is also currently in clinical trials for HCC and is based on the FDA-approved reversible inhibitor infigratinib (**35**). As for the aforementioned compounds, the acrylamide not only is able to bind to Cys552 of FGFR4 but also induces a steric clash with the tyrosine present in the other FGFR isoforms leading to close to 1000-fold selectivity over FGFR1–3.[60]

Finally, another kinase bearing a cysteine in the hinge region is FLT3, a promising target for the treatment of acute myeloid leukemia (AML). Compared to FGFR4, the cysteine (Cys695) is located two positions further C-terminal (H3).[11] There are reversible FLT3 inhibitors that have shown efficacy in clinical studies but acquired resistance to these leads to poor outcomes in the treatment of AML. FF-10101 (**36**, Scheme 4b) is an irreversible inhibitor that not only demonstrated potent activity against wildtype FLT3 but also against the D835Y mutation (a mutation sometimes acquired at relapse).[61] It is currently in clinical trials for the treatment of refractory or relapsed AML patients.[62]

## 4. Alternative Warhead Chemistries for Cysteine-Targeted Irreversible Protein Kinase Inhibitors

While there are many recent developments with respect to novel kinase targets and inhibitors that have entered clinical testing, the warhead chemistry of these compounds is limited to Michael acceptors – mostly acryl amide and its derivatives or rarely alkynamides. In order to diversify the types of warheads available to medicinal chemists, a variety of alternative chemistries have been investigated. Here, we highlight selected examples. A broader discussion of




Scheme 4. a) Discovery of isoform-selective FGFR4 inhibitor H3B-6527 (**34**) from the FDA-approved pan-FGFR reversible inhibitor infigratinib (**35**). b) Structure of the FLT3 inhibitor FF-10101 (**36**).

warheads suitable to address non-catalytic cysteines is, however, beyond the scope of this review and can be found elsewhere.[7]

Nucleophilic aromatic substitution reactions have recently been leveraged for covalent protein kinase inhibition. In 2017, researchers from Novartis identified dipyridylamine **37** (Fig. 10) in a high-throughput screening campaign for selective FGFR4 inhibitors. Due to the molecule's relatively small size and its high level of FGFR4 potency and selectivity, the authors suspected a covalent mode of action. Binding to Cys552, the hinge cysteine discussed before in the context of covalent FGFR4 inhibitors, was demonstrated by testing the inhibitory activity against an FGFR4 C552A mutant and protein mass spectrometry. An X-ray crystal structure confirmed the reaction of Cys552 with the chloronitropyridine moiety *via* $S_NAr$ displacement of the chloride.[63] However, no further development of this compound has been disclosed.

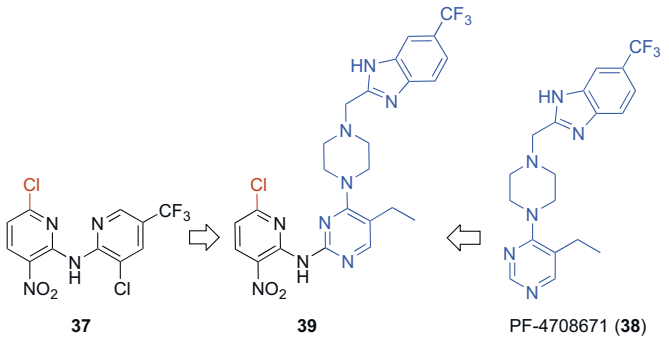



Fig. 10. Merging of screening hit **37** with S6K1 inhibitor PF-4708671 (**38**) to develop $S_NAr$-based S6K2 inhibitor **39**.

Our group deliberately used the aforementioned approach to selectively address the kinase S6K2, an understudied member of the p70 ribosomal protein S6 kinase (S6K) family.[64] In order to engage Cys150 in the ATP binding site of S6K2 (p70S6Kβ), which is not present in its closely related and much more studied homolog S6K1 (p70S6K), we combined the known S6K1-selective inhibitor PF-4708671 (**38**, Fig. 10) with the abovemen-

tioned chloronitropyridine warhead from FGFR4 inhibitor **37**. A favorable positioning of hybrid compound **39** (Fig. 10) in the binding pocket was predicted in docking studies. Biological profiling confirmed high S6K2 inhibitory potency ($IC_{50}$ = 22 nM), excellent selectivity against S6K1 ($IC_{50}$ > 5 μM) and a clean profile in a kinase panel. The covalent binding mode was corroborated by synthesis of an unreactive analog devoid of the chloride showing negligible potency towards S6K2.[65] Even though they have yet to be incorporated into clinical candidates, $S_NAr$ warheads show great potential for medicinal chemistry applications due to their defined rigid structure, synthetic accessibility and, in particular, their high tunability. Furthermore, these reactive groups may be useful to address amino acids beyond cysteine.[7]

A very recent and innovative approach to covalently address kinases employs carbocyclic strain-release electrophiles such as bicyclo[1.1.0]butane (BCB) sulfones or amides.[66,67] Tokunaga *et al.* streamlined several synthetic routes to prepare a variety of BCB amides which they then used to develop BTK-targeting covalent ligands. Using ibrutinib (**2**) as their starting point, they obtained a probe (**40**, Fig. 11) that showed ameliorated selectivity for BTK in human cells. In a chemical proteomic study, they also demonstrated that different warheads on the same BTK probe exhibit distinct off-target profiles, indicating that the incorporation of a BCB-amide as an electrophile may expand the ability to synthesize covalent inhibitors with the desired selectivity profile.[67]

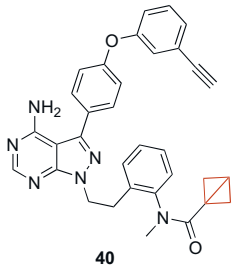
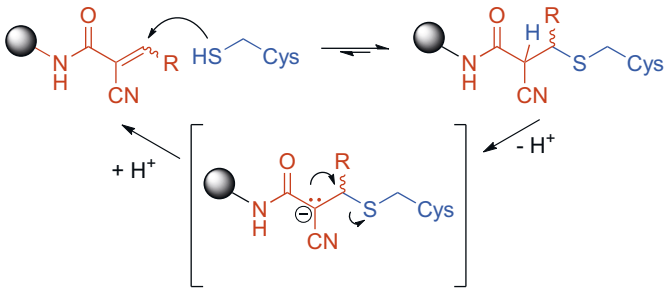



Fig. 11. Ibrutinib-based probe bearing a BCB warhead developed by Tokunaga *et al.*

## 5. Reversible Covalent Protein Kinase Inhibitors

Despite the resurgence of covalent drugs in the last decade, some safety concerns relating to the irreversible modification of proteins through covalent agents remain. Consequently, reversible covalent inhibitors have gained popularity in recent years. Such a strategy avoids permanent protein modification yet can significantly extend residence time which often leads to superior efficacy.[68] In 2012, Serafimova *et al*. reported the first reversible covalent kinase inhibitor for RSK2 designed by modulating the reactivity of the warhead of an irreversible covalent kinase inhibitor. They found that adding a nitrile group to a Michael acceptor such as an acrylamide will render the warhead hyperreactive but reversible and therefore reduces the chance of toxicities arising from permanent protein modification.[69] Reversibility can be attributed to the increased CH-acidity at the adduct's α-carbon atom, which facilitates proton abstraction triggering the reverse reaction (Scheme 5). The intrinsic reactivity of α-cyanoacrylamide warheads and the dissociation rates of the corresponding reversible covalent inhibitors can be modified by adding steric bulk to the β-position or by replacement of the amide group with an electron-withdrawing heteroarene making this warhead class highly tunable.[68,70,71]

Meanwhile, α-cyanoacrylamides have been used as warheads for multiple kinase targets. Forster *et al.* designed reversible co-




Scheme 5. Reversible reaction of α-cyanoacrylamides with cysteine *via* thia-Michael addition. The reverse β-elimination is enabled by increased acidity of the Cα-proton.

valent JAK3 inhibitors based on a tricyclic scaffold, which led to the chemical probe FM-381 (**41**, Fig. 12) and the even more selective inhibitor FM-409 (**42**, Fig. 12), both featuring sub-nanomolar potency and excellent selectivity against other JAK family members.[72] Interestingly, a crystal structure of JAK3 in complex with inhibitor FM-409 (**42**) demonstrated the coexistence of both the unreacted as well as the covalently bound form which may be a result of the reversible covalent interaction.[73] Rauh and colleagues harnessed the reversible covalent strategy to generate α-cyanoacrylamide-based EGFR inhibitors (*e.g.* **43**, Fig. 12).[74]

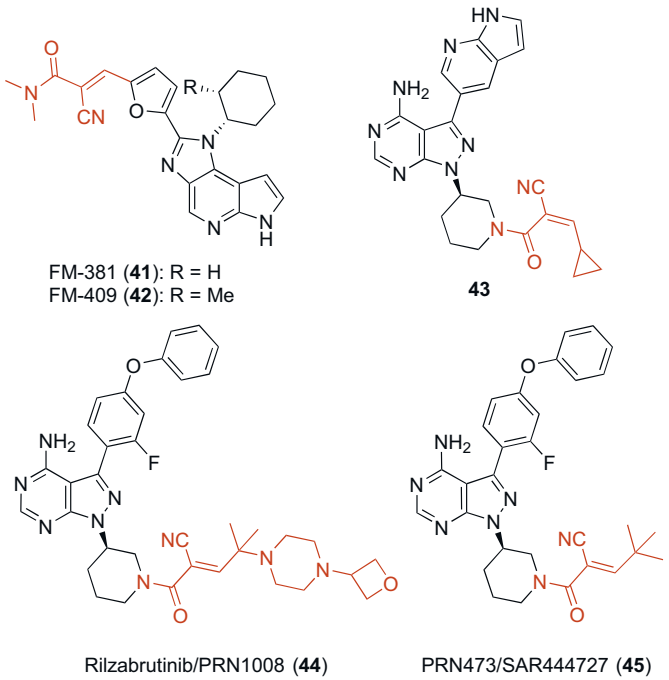
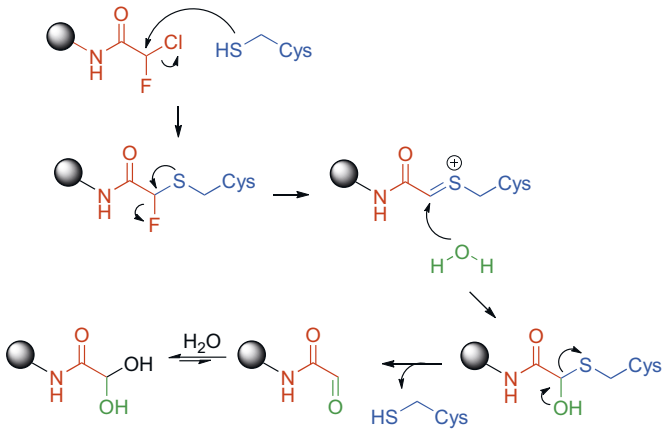



Fig. 12. Examples of reversible covalent protein kinase inhibitors bearing α-cyanoacrylamide warheads.

The largest progress in this field, however, has been achieved with inhibitors of BTK. Here, rilzabrutinib/PRN1008 (**44**, Fig. 12) as well as PRN473/SAR444727 (**45**, Fig. 12) have both progressed into phase II studies for atopic dermatitis and the former is also being investigated for immunoglobulin G4 (IgG4)-related disease (phase II) as well as immune thrombocytopenia (phase III).[22,75] Besides reversible covalent protein kinase inhibitors, there have also been some recent reports of incorporation of α-cyanoacrylamides (as well as other warheads) into proteolysis-targeting chimeras (PROTACs).[76,77]

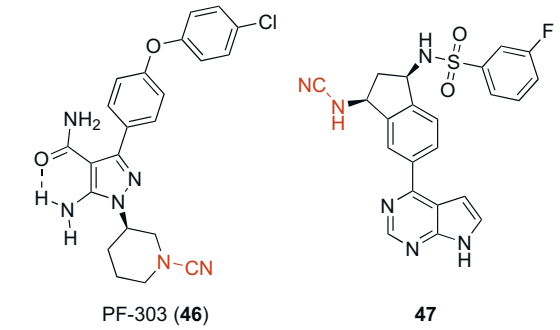
Apart from α-cyanoacrylamides, several other reversible covalent warheads have been discovered and utilized in protein kinase inhibitors in the last decade. In 2019, Shindo *et al.* introduced α-chlorofluoroacetamide (CFA) as a new type of reversible covalent electrophilic group reacting with cysteine *via* nucleophilic displacement of the chloride. By screening a series of α-haloacetamides they determined CFAs to possess the most suitable reactivity range for application in TCIs and developed several BTK and EGFR inhibitors bearing this warhead. In contrast to α-cyanoacrylamides, where the unmodified inhibitor is recovered *via* the reverse reaction, the covalent bond of the CFA-thiol reaction product is cleaved by hydrolysis to give hydrated glyoxamides (Scheme 6). Interestingly, while *N*-acetyl cysteine-adducts were hydrolyzed within a few hours, the covalent complex with Cys797 of EGFR was found to be stable for over 72 h which was attributed to the covalent linkage being solvent-sequestered within the ATP binding pocket. This phenomenon may lead to reduced off-target effects since unwanted adducts with cysteines in solvent-exposed binding pockets will be hydrolyzed rapidly while the desired inhibitor-cysteine bond is very stable.[78]



Scheme 6. Reaction of cysteine with CFA and ensuing hydrolysis mechanism.

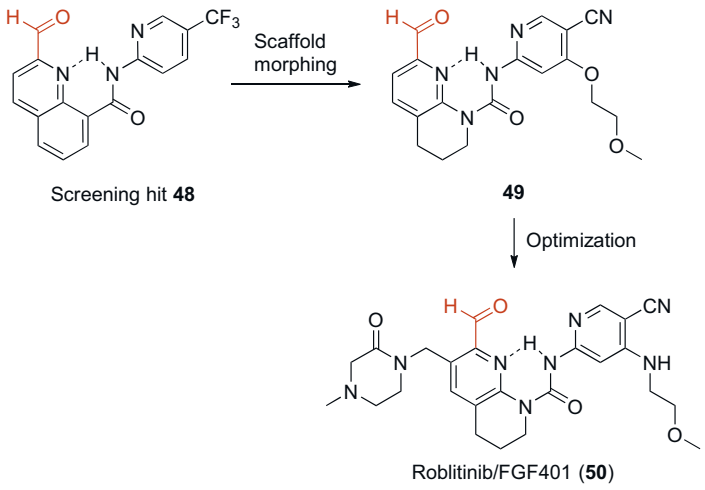
Beyond the realm of kinases, FDA-approved drugs with reversible covalent warheads already exist. For example, the serine protease inhibitor Saxagliptin functions by forming a covalent bond *via* a nitrile as the electrophile.[79] Nitriles are rather inert functional groups, so for covalent adduct formation highly reactive active site nucleophiles are usually required and the electrophilic carbon atom should be precisely situated. Nevertheless, the electrophilicity of the nitrile moiety can be increased by attachment of electron-withdrawing groups. Cyanamides, which reversibly form isothioureas in a reaction with cysteine,[80] for example, exhibit similar reaction kinetics with GSH as acrylamides.[81] Applying this concept, researchers from Pfizer developed the cyanamide-based reversible covalent inhibitor PF-303 (**46**, Fig. 13) that was utilized as a probe to study the phenotype of BTK inhibition in mice. The inhibitor was based on ibrutinib (**2**) and by switching aminopyrazolopyrimidine hinge binding motif to an aminopyrazole carboxamide and utilizing a cyanamide warhead instead of the irreversible acrylamide, the best combination of BTK potency and EGFR selectivity was reached.[82] Cyanamides have also been successfully employed to address JAK3 (*e.g.* **47**, Fig. 13).[83]

Furthermore, reversible covalent kinase targeting has been achieved with carbonyl warheads (*e.g.* aldehydes or trifluoromethyl ketones) forming hemithioacetal adducts with the respective cysteine. Aldehyde-based kinase inhibitors have resulted from the same screening campaign that identified $S_NAr$-based inhibitor **37**. Here, Fairhurst and colleagues discovered that 2-formylquinoline amide **48** (Scheme 7) and its analogues act as isoform-selective, reversible covalent FGFR4 inhibitors.[63] Despite initial skepticism about the druglikeness of the aldehyde group, these compounds were developed further. Structure–activity relationship (SAR) studies showed the quinoline nitrogen atom and the carboxamide proton to be required for positioning the aldehyde by stabilizing a pseudotricyclic arrangement *via* intramolecular hydrogen bonding. To increase solubility, a 2-formylpyridine urea scaffold was introduced as a bioisosteric replacement of the 2-formylquinoline amide leading to inhibitor **49**. Further optimizations led to clinical candidate roblitinib/FGF401 (**50**) which has been in phase II studies for FGFR4 and β-klotho positive solid tumors as well as for HCC.[84] For this target, a reversible covalent approach was deemed particularly promising because of the very rapid resynthesis rate of FGFR4 (<2 h) in HCC cell lines negating the benefits of irreversible covalent binders.[85.86]



PF-303 (**46**) **47**

Fig. 13. Reversible covalent inhibitors bearing cyanamide warheads.



Screening hit **48** **49** Roblitinib/FGF401 (**50**)

Scheme 7. Development of reversible covalent FGFR4 inhibitor (**50**) from screening hit **48**.

## 6. Developments Beyond Cysteine

As mentioned in the introduction, besides cysteine, mainly lysine and tyrosine side chains in kinases have been targeted with covalent modifiers. Compared to cysteine, lysine is more abundant in the proteome (5.8% vs. 1.9%)[87] but with a p$K_a$-value of approx. 10.5, surface-exposed lysines are almost exclusively protonated at physiological pH. Depending on the protein environment, however, lysine side chains can undergo a p$K_a$ shift of up to five units.[88] Almost 30 years ago, it was discovered that the natural product wortmannin reacts covalently with a lysine side chain in the phosphoinositide 3-kinase γ (PI3Kγ) by aza-Michael addition.[89] However, many aza-Michael additions with lysines

have been found serendipitously rather than by design and Michael acceptors are typically more reactive towards the 'softer' cysteine thiols which may lead to cysteine-mediated off-targetactivity. Intriguingly, for α,β-unsaturated sulfonamides the opposite trend has been observed.[90] Anscombe *et al.* used this property to develop the first irreversible inhibitor of cyclin-dependent kinase (CDK) 2. Their starting point was a non-covalently binding sulfonamide. By replacing this moiety with a vinyl sulfone, a covalent inhibitor was obtained as confirmed by X-ray crystallography. The probe, however, displayed fairly slow inactivation kinetics and only moderate reversible binding affinity ($K_i$ = 1.31 μM).[91]

Beyond Michael acceptors, other covalent reactive groups have proven to be more suitable for targeting lysine. For example, several research groups used sulfonyl fluoride-based probes to address lysine side chains. In comparison to sulfonyl chlorides, sulfonyl fluorides are thermodynamically more stable and less susceptible to hydrolysis and reduction. Furthermore, they exclusively react at the sulfur atom.[92] Zhao *et al.* designed pyrimidinyl 3-aminopyrazole XO44 (**51**, Fig. 14) as a clickable broad-spectrum kinase ligand to facilitate chemoproteomic selectivity profiling. Not only did this probe label the model kinases c-SRC and EGFR, but XO44 (**51**) also hit more than 50% of the 375 protein kinases in the panel. Furthermore, the probe bears the advantage of being cell-penetrant.[93] Mukherjee and colleagues used 5'-fluorosulfonylbenzoyladenosine (FSBA) analogues bearing a sulfonyl fluoride warhead (**52**, Fig. 14) to target Lys514 in FGFR1.[94] Using a different type of warhead, namely an activated ester (**53**, Fig. 14), Dalton *et al.* were able to form a covalent bond with Lys779 of PI3Kδ.[95] Finally, Quach *et al.* recently reported an iminoboronate-based BCR-ABL inhibitor (**54**, Fig. 14). In this case, the boronic acid adjoining an aldehyde moiety serves two functions: it accelerates imine formation and stabilizes the final Schiff base.[96] It is worth noting that all of the lysine-targeted inhibitors depicted in Fig. 14 address a conserved lysine at the end of the β3-sheet in the N-lobe (compare to Fig. 2) which is required for nucleotide binding and sometimes referred to as the catalytic lysine.

Only very limited efforts towards kinase inhibitors targeting tyrosine moieties have been disclosed so far and all of these studies relied on sulfur (VI) chemistry. Hatcher *et al.* designed an inhibitor based on the approved reversible anaplastic lymphoma kinase (ALK) inhibitor alectinib (**55**, Fig. 14). By introducing a *m*-phenylsulfonyl fluoride (SRPKIN-1, **56**, Fig. 14) they were able to covalently engage a unique tyrosine (Tyr227) in the SR-protein kinase SRPK1 as suggested by washout experiments and MS analysis.[97] Finally, the group of Hsu recently discovered that sulfonyl triazoles can be optimized for high tyrosine chemoselectivity. Compared to the aforementioned sulfur-fluoride exchange (SuFEx) chemistry, sulfur-triazole exchange (SuTEx) showed a more favorable reactivity at protein sites.[98] The authors used this chemistry in chemical proteomic studies and identified a wealth of tyrosine (and lysine) labeling sites in kinases and further ATP-/NAD-binding proteins.[99,100]

## 7. Conclusions and Outlook

Despite the historical reluctance of medicinal chemists to develop covalent drugs, eight approvals of covalent protein kinase inhibitors by the FDA since 2013 and more than 15 compounds currently under clinical review corroborate the potential of this approach to improve potency, selectivity, and clinical efficacy, overcome acquired resistances, and address targets for which no specific inhibitors exist. While many of the compounds in clinical development target kinases for which several drugs are already approved (*i.e.* EGFR/the HER family and BTK), there are increasing efforts to address other parts of the kinome with covalent inhibitors.

As of now, warhead chemistry of irreversible inhibitors in clinical studies has been limited to α,β-unsaturated amides targeting cysteine. Advances for other covalent reactive groups, such as $S_NAr$ or BCB warheads, exist but they are still in early development stages. It remains to be seen when such non-canonical electrophiles will be incorporated into clinical candidates.

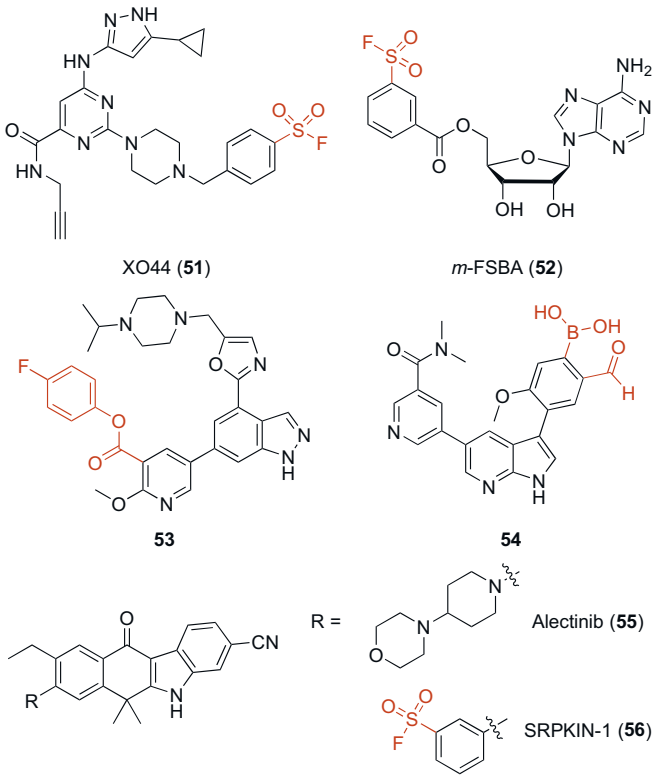



Fig. 14. Lysine- and tyrosine-targeted covalent inhibitors.

Meanwhile, reversible covalent kinase inhibitors relying on α-cyanoacrylamide or carbonyl electrophiles have progressed into clinical trials. The tunable characteristics of the covalent reversible mode of action will help to mitigate risks associated with permanent protein modification including idiosyncratic drug reactions as well as target or off-target mediated side effects. Considering the progress of reversible covalent kinase inhibitors in clinical trials, it is likely that we will see the first approval soon.

Developments in targeting amino acids beyond cysteine are still in their early infancy and few kinases have been specifically addressed *via* lysine or tyrosine side chains. Despite intrinsic challenges linked to the lower nucleophilicity and protonation state of latter amino acids, and the urgent need for novel warhead chemistries to address these residues, this approach holds the promise to target the parts of the kinome that do not possess a suitable cysteine. Considering the fact that only a small fraction of human kinases has been therapeutically explored and that the functions of many kinases remain unclear, there is an obvious need to expand the scope of covalent targeting approaches to help illuminating the 'dark' parts of the kinome.

### *Acknowledgements*

The authors thank the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) under Germany's Excellence Strategy-EXC 2180-390900677, the Fonds der Chemischen Industrie (SK 206/10 and SK 207/25), and the Postdoctoral Fellowship Program of the Baden-Württemberg Stiftung (1.16101.18) for financial support. Laura Hillebrand thanks the Studienstiftung des deutschen Volkes and the Evonik Stiftung for their support. Dr. Raimund Nieß and Stefan Gerstenecker are acknowledged for technical support and discussion.

Received: April 13, 2022

[1] R. Lonsdale, R. A. Ward, *Chem. Soc. Rev.* **2018**, *47*, 3816, https://doi.org/10.1039/c7cs00220c.

[2] J. Singh, R. C. Petter, T. A. Baillie, A. Whitty, *Nat. Rev. Drug Discov.* **2011**, *10*, 307, https://doi.org/10.1038/nrd3410.

[3] A. Abdeldayem, Y. S. Raouf, S. N. Constantinescu, R. Moriggl, P. T. Gunning, *Chem. Soc. Rev.* **2020**, *49*, 2617, https://doi.org/10.1039/c9cs00720b.

[4] Self-Collected Data.

[5] M. Gehringer, *Future Med. Chem.* **2020**, *12*, 1363, https://doi.org/10.4155/fmc-2020-0118.

[6] F. Sutanto, M. Konstantinidou, A. Dömling, *RSC Med. Chem.* **2020**, *11*, 876, https://doi.org/10.1039/d0md00154f.

[7] M. Gehringer, S. A. Laufer, *J. Med. Chem.* **2019**, *62*, 5673, https://doi.org/10.1021/acs.jmedchem.8b01153.

[8] R. Mah, J. R. Thomas, C. M. Shafer, *Bioorganic Med. Chem. Lett.* **2014**, *24*, 33, https://doi.org/10.1016/j.bmcl.2013.10.003.

[9] J. M. Ostrem, U. Peters, M. L. Sos, J. A. Wells, K. M. Shokat, *Nature* **2013**, *503*, 548, https://doi.org/10.1038/nature12796.

[10] L. Petri, A. Egyed, D. Bajusz, T. Imre, A. Hetényi, T. Martinek, P. Ábrányi-Balogh, G. M. Keseru, *Eur. J. Med. Chem.* **2020**, *207*, 112836, https://doi.org/10.1016/j.ejmech.2020.112836.

[11] M. Gehringer, in 'Proteinkinase Inhibitors', 1 ed., Springer, Cham, Switzerland, **2020**, pp. 43, https://doi.org/10.1007/7355_2020_103.

[12] A. Chaikuad, P. Koch, S. A. Laufer, S. Knapp, *Angew. Chemie - Int. Ed.* **2018**, *57*, 4372, https://doi.org/10.1002/anie.201707875.

[13] S. Ray, A. S. Murkin, *Biochemistry* **2019**, *58*, 5234, https://doi.org/10.1021/acs.biochem.9b00293.

[14] L. A. Crawford, E. Weerapana, *Mol. Biosyst.* **2016**, *12*, 1768, https://doi.org/10.1039/c6mb00250a.

[15] D. A. Shannon, R. Banerjee, E. R. Webster, D. W. Bak, C. Wang, E. Weerapana, *J. Am. Chem. Soc.* **2014**, *136*, 3330, https://doi.org/10.1021/ja4116204.

[16] M. M. Attwood, D. Fabbro, A. V. Sokolov, S. Knapp, H. B. Schiöth, *Nat. Rev. Drug Discov.* **2021**, *20*, 839, https://doi.org/10.1038/s41573-021-00252-y.

[17] P. Cohen, D. Cross, P. A. Jänne, *Nat. Rev. Drug Discov.* **2021**, *20*, 551, https://doi.org/10.1038/s41573-021-00195-4.

[18] R. A. M. Serafim, J. M. Elkins, W. J. Zuercher, S. A. Laufer, M. Gehringer, *J. Med. Chem.* **2021**, https://doi.org/10.1021/acs.jmedchem.1c00980.

[19] J. Zhang, P. L. Yang, N. S. Gray, *Nat. Rev. Cancer* **2009**, *9*, 28, https://doi.org/10.1038/nrc2559.

[20] E. Leproult, S. Barluenga, D. Moras, J. M. Wurtz, N. Winssinger, *J. Med. Chem.* **2011**, *54*, 1347, https://doi.org/10.1021/jm101396q.

[21] S. Laufer, J. Bajorath, *J. Med. Chem.* **2022**, *65*, 891, https://doi.org/10.1021/acs.jmedchem.1c02126.

[22] X. Lu, J. B. Smaill, A. V. Patterson, K. Ding, *J. Med. Chem.* **2022**, *65*, 58, https://doi.org/10.1021/acs.jmedchem.1c01719.

[23] A. Markham, *Drugs* **2021**, *81*, 2069, https://doi.org/10.1007/s40265-021-01632-9.

[24] F. Gonzalvez, S. Vincent, T. E. Baker, A. E. Gould, S. Li, S. D. Wardwell, S. Nadworny, Y. Ning, S. Zhang, W. S. Huang, Y. Hu, F. Li, M. T. Greenfield, S. G. Zech, B. Das, N. I. Narasimhan, T. Clackson, D. Dalgarno, W. C. Shakespeare, M. Fitzgerald, J. Chouitar, R. J. Griffin, S. Liu, K. K. Wong, X. Zhu, V. M. Rivera, *Cancer Discov.* **2021**, *11*, 1672, https://doi.org/10.1158/2159-8290.CD-20-1683.

[25] Z. Pan, H. Scheerens, S. J. Li, B. E. Schultz, P. A. Sprengeler, L. C. Burrill, R. V. Mendonca, M. D. Sweeney, K. C. K. Scott, P. G. Grothaus, D. A. Jeffery, J. M. Spoerke, L. A. Honigberg, P. R. Young, S. A. Dalrymple, J. T. Palmer, *ChemMedChem* **2007**, *2*, 58, https://doi.org/10.1002/cmdc.200600221.

[26] R. Roskoski, *Pharmacol. Res.* **2021**, *165*, 105422, https://doi.org/10.1016/j.phrs.2021.105422.

[27] G. Lelais, R. Epple, T. H. Marsilje, Y. O. Long, M. McNeill, B. Chen, W. Lu, J. Anumolu, S. Badiger, B. Bursulaya, M. DiDonato, R. Fong, J. Juarez, J. Li, M. Manuia, D. E. Mason, P. Gordon, T. Groessl, K. Johnson, Y. Jia, S. Kasibhatla, C. Li, J. Isbell, G. Spraggon, S. Bender, P. Y. Michellys, *J. Med. Chem.* **2016**, *59*, 6671, https://doi.org/10.1021/acs.jmedchem.5b01985.

[28] P. Timmins, *Ther. Deliv.* **2021**, *12*, 427, https://doi.org/10.4155/tde-2021-0031.

[29] H. A. Blair, *Drugs* **2018**, *78*, 1751, https://doi.org/10.1007/s40265-018-0997-0.

[30] Y. Y. Elamin, J. P. Robichaux, B. W. Carter, M. Altan, D. L. Gibbons, F. V. Fossella, V. K. Lam, A. B. Patel, M. V. Negrao, X. Le, F. E. Mott, J. Zhang, L. Feng, G. Blumenschein, A. S. Tsao, J. V. Heymach, *J. Clin. Oncol.* **2022**, *40*, 702, https://doi.org/10.1200/jco.21.01113.

[31] W. Ji, J. Shen, B. Wang, F. Chen, D. Meng, S. Wang, D. Dai, Y. Zhou, C. Wang, Q. Zhou, *Pharm. Biol.* **2021**, *59*, 457, https://doi.org/10.1080/13880209.2021.1914114.

[32] H. Irie, K. Ito, Y. Fujioka, K. Oguchi, A. Fujioka, A. Hashimoto, H. Ohsawa, K. Tanaka, K. Funabashi, H. Araki, Y. Kawai, T. Shimamura, R. Wadhwa, S. Ohkubo, K. Matsuo, *Mol. Cancer Ther.* **2019**, *18*, 733, https://doi.org/10.1158/1535-7163.MCT-18-1085.

[33] S. A. Piha-Paul, A. Azaro, H. T. Arkenau, D. Y. Oh, M. D. Galsky, S. K. Pal, K. Hamada, Y. He, I. Yamamiya, K. A. Benhadji, A. Hollebecque, *Invest. New Drugs* **2021**, *39*, 1324, https://doi.org/10.1007/s10637-021-01104-7.

[34] D. Li, Y. Tu, K. Jin, L. Duan, Y. Hong, J. Xu, N. Chen, Z. Zhang, H. Zuo, W. Gong, J. Zhang, Q. Wang, H. Qian, X. Wang, Y. Ke, G. Xia, *J. Med. Chem.* **2022**, https://doi.org/10.1021/acs.jmedchem.1c00710.

[35] S. Huang, J. Pan, J. Jin, C. Li, X. Li, J. Huang, X. Huang, X. Yan, F. Li, M. Yu, C. Hu, J. Jin, Y. Xu, Q. Ling, W. Ye, Y. Wang, J. Jin, *Cancer Lett.* **2019**, *461*, 132, https://doi.org/10.1016/j.canlet.2019.07.008.

[36] H. Wang, R. Pan, X. Zhang, X. Si, M. Wang, L. Zhang, *Thorac. Cancer* **2020**, *11*, 594, https://doi.org/10.1111/1759-7714.13302.

[37] W. Zhou, D. Ercan, L. Chen, C. H. Yun, D. Li, M. Capelletti, A. B. Cortot, L. Chirieac, R. E. Iacob, R. Padera, J. R. Engen, K. K. Wong, M. J. Eck, N. S. Gray, P. A. Jänne, *Nature* **2009**, *462*, 1070, https://doi.org/10.1038/nature08622.

[38] N. Alizadehmohajer, A. Behmardi, S. Najafgholian, S. Moradi, F. Mohammadi, R. Nedaeinia, S. Haghjooy Javanmard, E. Sohrabi, R. Salehi, G. A. Ferns, A. Emami Nejad, M. Manian, *Netw. Model. Anal. Heal. Informatics Bioinforma.* **2022**, *11*, https://doi.org/10.1007/s13721-021-00341-3.

[39] Z. Xie, X. Yang, Y. Duan, J. Han, C. Liao, *J. Med. Chem.* **2021**, *64*, 1283, https://doi.org/10.1021/acs.jmedchem.0c01511.

[40] D. Angst, F. Gessier, P. Janser, A. Vulpetti, R. Wälchli, C. Beerli, A. Littlewood-Evans, J. Dawson, B. Nuesslein-Hildesheim, G. Wieczorek, S. Gutmann, C. Scheufler, A. Hinniger, A. Zimmerlin, E. G. Funhoff, R. Pulz, B. Cenni, *J. Med. Chem.* **2020**, *63*, 5102, https://doi.org/10.1021/acs.jmedchem.9b01916.

[41] S. H. Watterson, Q. Liu, M. Beaudoin Bertrand, D. G. Batt, L. Li, M. A. Pattoli, S. Skala, L. Cheng, M. T. Obermeier, R. Moore, Z. Yang, R. Vickery, P. A. Elzinga, L. Discenza, C. D'Arienzo, K. M. Gillooly, T. L. Taylor, C. Pulicicchio, Y. Zhang, E. Heimrich, K. W. McIntyre, Q. Ruan, R. A. Westhouse, I. M. Catlett, N. Zheng, C. Chaudhry, J. Dai, M. A. Galella, A. J. Tebben, M. Pokross, J. Li, R. Zhao, D. Smith, R. Rampulla, A. Allentoff, M. A. Wallace, A. Mathur, L. Salter-Cid, J. E. Macor, P. H. Carter, A. Fura, J. R. Burke, J. A. Tino, *J. Med. Chem.* **2019**, *62*, 3228, https://doi.org/10.1021/acs.jmedchem.9b00167.

[42] S. Dhillon, *Drugs* **2021**, *81*, 503, https://doi.org/10.1007/s40265-021-01482-5.

[43] S. Dhillon, *Drugs* **2020**, *80*, 835, https://doi.org/10.1007/s40265-020-01318-8.

[44] S. Lindner, N. Lee, G. K. Armijo, M. D. da Silva, P. S. Herrera, C. L. Nguyen, E. A. Dwomoh, R. Ghale, M. Smith, M.-A. Perales, K. A. Markey, J. J. Buggy, J. W. Janc, M. van den Brink, *Blood* **2021**, *138*, 3814, https://doi.org/10.1182/blood-2021-153360.

[45] M. S. Khodadoust, T. A. Feldman, D. H. Yoon, C. K. Yannakou, D. Radeski, Y. H. Kim, N. Mehta-Shah, A. Khot, R. A. Wilcox, W. S. Kim, S. M. Horwitz, J. J. Buggy, A. Hotson, C. M. Hill, B. Munneke, S. Mahabhashyam, R. A. Miller, J. W. Janc, M. Mobasher, *Blood* **2020**, *136*, 19, https://doi.org/10.1182/blood-2020-137782.

[46] M. Forster, X. J. Liang, M. Schröder, S. Gerstenecker, A. Chaikuad, S. Knapp, S. Laufer, M. Gehringer, *Int. J. Mol. Sci.* **2020**, *21*, 1, https://doi.org/10.3390/ijms21239269.

[47] J. D. Seixas, B. B. Sousa, M. C. Marques, A. Guerreiro, R. Traquete, T. Rodrigues, I. S. Albuquerque, M. F. Q. Sousa, A. R. Lemos, P. M. F. Sousa, T. M. Bandeiras, D. Wu, S. K. Doyle, C. V. Robinson, A. N. Koehler, F. Corzana, P. M. Matias, G. J. L. Bernardes, *RSC Chem. Biol.* **2020**, *1*, 251, https://doi.org/10.1039/d0cb00033g.

[48] F. Liu, X. Zhang, E. Weisberg, S. Chen, W. Hur, H. Wu, Z. Zhao, W. Wang, M. Mao, C. Cai, N. I. Simon, T. Sanda, J. Wang, A. T. Look, J. D. Griffin, S. P. Balk, Q. Liu, N. S. Gray, *ACS Chem. Biol.* **2013**, *8*, 1423, https://doi.org/10.1021/cb4000629.

[49] J. D. Clark, M. E. Flanagan, J. B. Telliez, *J. Med. Chem.* **2014**, *57*, 5023, https://doi.org/10.1021/jm401490p.

[50] M. Forster, M. Gehringer, S. A. Laufer, *Bioorganic Med. Chem. Lett.* **2017**, *27*, 4229, https://doi.org/10.1016/j.bmcl.2017.07.079.

[51] A. Thorarensen, M. E. Dowty, M. E. Banker, B. Juba, J. Jussif, T. Lin, F. Vincent, R. M. Czerwinski, A. Casimiro-Garcia, R. Unwalla, J. I. Trujillo, S. Liang, P. Balbo, Y. Che, A. M. Gilbert, M. F. Brown, M. Hayward, J. Montgomery, L. Leung, X. Yang, S. Soucy, M. Hegen, J. Coe, J. Langille, F. Vajdos, J. Chrencik, J. B. Telliez, *J. Med. Chem.* **2017**, *60*, 1971, https://doi.org/10.1021/acs.jmedchem.6b01694.

[52] J. B. Telliez, M. E. Dowty, L. Wang, J. Jussif, T. Lin, L. Li, E. Moy, P. Balbo, W. Li, Y. Zhao, K. Crouse, C. Dickinson, P. Symanowicz, M. Hegen, M. E. Banker, F. Vincent, R. Unwalla, S. Liang, A. M. Gilbert, M. F. Brown, M. Hayward, J. Montgomery, X. Yang, J. Bauman, J. I. Trujillo, A. Casimiro-Garcia, F. F. Vajdos, L. Leung, K. F. Geoghegan, A. Quazi, D. Xuan, L. Jones, E. Hett, K. Wright, J. D. Clark, A. Thorarensen, *ACS Chem. Biol.* **2016**, *11*, 3442, https://doi.org/10.1021/acschembio.6b00677.

[53] M. Katoh, *Nat. Rev. Clin. Oncol.* **2019**, *16*, 105, https://doi.org/10.1038/s41571-018-0115-y.

[54] A. Hollebecque, T. Doi, O. Saavedra, O. Takahashi, H. He, K. A. Benhadji, K. Shitara, *J. Clin. Oncol.* **2020**, *38*, TPS470, https://doi.org/10.1200/JCO.2020.38.4_suppl.TPS470.

[55] M. Kalyukina, Y. Yosaatmadja, M. J. Middleditch, A. V. Patterson, J. B. Smaill, C. J. Squire, *ChemMedChem* **2019**, *14*, 494, https://doi.org/10.1002/cmdc.201800719.

[56] R. D. Kim, D. Sarker, T. Meyer, T. Yau, T. Macarulla, J. W. Park, S. P. Choo, A. Hollebecque, M. W. Sung, H. Y. Lim, V. Mazzaferro, J. Trojan, A. X. Zhu, J. H. Yoon, S. Sharma, Z. Z. Lin, S. L. Chan, S. Faivre, L. G. Feun, C. J. Yen, J. F. Dufour, D. H. Palmer, J. M. Llovet, M. Manoogian, M. Tugnait, N. Stransky, M. Hagel, N. E. Kohl, C. Lengauer, C. A. Sherwin, O. Schmidt-Kittler, K. P. Hoeflich, H. Shi, B. B. Wolf, Y. K. Kang, *Cancer Discov.* **2019**, *9*, 1696, https://doi.org/10.1158/2159-8290.CD-19-0555.

[57] M. Hagel, C. Miduturu, M. Sheets, N. Rubin, W. Weng, N. Stransky, N. Bifulco, J. L. Kim, B. Hodous, N. Brooijmans, A. Shutes, C. Winter, C. Lengauer, N. E. Kohl, T. Guzi, *Cancer Discov.* **2015**, *5*, 424, https://doi.org/10.1158/2159-8290.CD-14-1029.

[58] X. Lin, Y. Yosaatmadja, M. Kalyukina, M. J. Middleditch, Z. Zhang, X. Lu, K. Ding, A. V. Patterson, J. B. Smaill, C. J. Squire, *ACS Med. Chem. Lett.* **2019**, *10*, 1180, https://doi.org/10.1021/acsmedchemlett.9b00196.

[59] R. A. M. Serafim, A. da Silva Santiago, M. P. Schwalm, Z. Hu, C. V. dos Reis, J. E. Takarada, P. Mezzomo, K. B. Massirer, M. Kudolo, S. Gerstenecker, A. Chaikuad, L. Zender, S. Knapp, S. Laufer, R. M. Couñago, M. Gehringer, *J. Med. Chem.* **2022**, *65*, 3173, https://doi.org/10.1021/acs.jmedchem.1c01165.

[60] J. J. Joshi, H. Coffey, E. Corcoran, J. Tsai, C. L. Huang, K. Ichikawa, S. Prajapati, M. H. Hao, S. Bailey, J. Wu, V. Rimkunas, C. Karr, V. Subramanian, P. Kumar, C. MacKenzie, R. Hurley, T. Satoh, K. Yu, E. Park, N. Rioux, A. Kim, W. G. Lai, L. Yu, P. Zhu, S. Buonamici, N. Larsen, P. Fekkes, J. Wang, M. Warmuth, D. J. Reynolds, P. G. Smith, A. Selvaraj, *Cancer Res.* **2017**, *77*, 6999, https://doi.org/10.1158/0008-5472.CAN-17-1865.

[61] T. Yamaura, T. Nakatani, K. Uda, H. Ogura, W. Shin, N. Kurokawa, K. Saito, N. Fujikawa, T. Date, M. Takasaki, D. Terada, A. Hirai, A. Akashi, F. Chen, Y. Adachi, Y. Ishikawa, F. Hayakawa, S. Hagiwara, T. Naoe, H. Kiyoi, *Blood* **2018**, *131*, 426, https://doi.org/10.1182/blood-2017-05-786657.

[62] M. J. Levis, C. C. Smith, A. E. Perl, G. J. Schiller, A. T. Fathi, G. J. Roboz, E. S. Wang, J. K. Altman, M. Ando, T. Suzuki, R. A. Subach, G. Maier, T. Madden, M. Johansen, K. Cheung, M. R. Kurman, *J. Clin. Oncol.* **2021**, *39*, 7008, https://doi.org/10.1200/JCO.2021.39.15_suppl.7008.

[63] R. A. Fairhurst, T. Knoepfel, C. Leblanc, N. Buschmann, C. Gaul, J. Blank, I. Galuba, J. Trappe, C. Zou, J. Voshol, C. Genick, P. Brunet-Lefeuvre, F. Bitsch, D. Graus-Porta, P. Furet, *Med. Chem. Commun.* **2017**, *8*, 1604, https://doi.org/10.1039/c7md00213k.

[64] O. E. Pardo, M. J. Seckl, *Front. Oncol.* **2013**, *3 JUL*, 1, https://doi.org/10.3389/fonc.2013.00191.

[65] S. Gerstenecker, L. Haarer, M. Schröder, M. Kudolo, M. P. Schwalm, V. Wydra, R. A. M. Serafim, A. Chaikuad, S. Knapp, S. Laufer, M. Gehringer, *Cancers (Basel).* **2021**, *13*, 1, https://doi.org/10.3390/cancers13205133.

[66] J. M. Lopchuk, K. Fjelbye, Y. Kawamata, L. R. Malins, C. M. Pan, R. Gianatassio, J. Wang, L. Prieto, J. Bradow, T. A. Brandt, M. R. Collins, J. Elleraas, J. Ewanicki, W. Farrell, O. O. Fadeyi, G. M. Gallego, J. J. Mousseau, R. Oliver, N. W. Sach, J. K. Smith, J. E. Spangler, H. Zhu, J. Zhu, P. S. Baran, *J. Am. Chem. Soc.* **2017**, *139*, 3209, https://doi.org/10.1021/jacs.6b13229.

[67] N. Shindo, A. Ojida, K. Tokunaga, M. Sato, K. Kuwata, C. Miura, H. Fuchida, N. Matsunaga, S. Koyanagi, S. Ohdo, *J. Am. Chem. Soc.* **2020**, *142*, 18522, https://doi.org/10.1021/jacs.0c07490.

[68] J. M. Bradshaw, J. M. McFarland, V. O. Paavilainen, A. Bisconte, D. Tam, V. T. Phan, S. Romanov, D. Finkle, J. Shu, V. Patel, T. Ton, X. Li, D. G. Loughhead, P. A. Nunn, D. E. Karr, M. E. Gerritsen, J. O. Funk, T. D. Owens, E. Verner, K. A. Brameld, R. J. Hill, D. M. Goldstein, J. Taunton, *Nat. Chem. Biol.* **2015**, *11*, 525, https://doi.org/10.1038/nchembio.1817.

[69] I. M. Serafimova, M. A. Pufall, S. Krishnan, K. Duda, M. S. Cohen, R. L. Maglathlin, J. M. McFarland, R. M. Miller, M. Frödin, J. Taunton, *Nat. Chem. Biol.* **2012**, *8*, 471, https://doi.org/10.1038/nchembio.925.

[70] S. Krishnan, R. M. Miller, B. Tian, R. D. Mullins, M. P. Jacobson, J. Taunton, *J. Am. Chem. Soc.* **2014**, *136*, 12624, https://doi.org/10.1021/ja505194w.

[71] E. H. Krenske, R. C. Petter, K. N. Houk, *J. Org. Chem.* **2016**, *81*, 11726, https://doi.org/10.1021/acs.joc.6b02188.

[72] M. Forster, A. Chaikuad, T. Dimitrov, E. Döring, J. Holstein, B. T. Berger, M. Gehringer, K. Ghoreschi, S. Müller, S. Knapp, S. A. Laufer, *J. Med. Chem.* **2018**, *61*, 5350, https://doi.org/10.1021/acs.jmedchem.8b00571.

[73] M. Forster, A. Chaikuad, S. M. Bauer, J. Holstein, M. B. Robers, C. R. Corona, M. Gehringer, E. Pfaffenrot, K. Ghoreschi, S. Knapp, S. A. Laufer, *Cell Chem. Biol.* **2016**, *23*, 1335, https://doi.org/10.1016/j.chembiol.2016.10.008.

[74] S. Smith, M. Keul, J. Engel, D. Basu, S. Eppmann, D. Rauh, *ACS Omega* **2017**, *2*, 1563, https://doi.org/10.1021/acsomega.7b00157.

[75] Y. Xing, K. A. Chu, J. Wadhwa, W. Chen, J. Zhu, J. M. Bradshaw, J. Shu, M. C. Foulke, N. Loewenstein, P. Nunn, K. By, P. Phiasivongsa, D. M. Goldstein, C. L. Langrish, *ImmunoHorizons* **2021**, *5*, 581, https://doi.org/10.4049/immunohorizons.2100063.

[76] R. Gabizon, A. Shraga, P. Gehrtz, E. Livnah, Y. Shorer, N. Gurwicz, L. Avram, T. Unger, H. Aharoni, S. Albeck, A. Brandis, Z. Shulman, B. Z. Katz, Y. Herishanu, N. London, *J. Am. Chem. Soc.* **2020**, *142*, 11734, https://doi.org/10.1021/jacs.9b13907.

[77] W. H. Guo, X. Qi, X. Yu, Y. Liu, C. I. Chung, F. Bai, X. Lin, D. Lu, L. Wang, J. Chen, L. H. Su, K. J. Nomie, F. Li, M. C. Wang, X. Shu, J. N. Onuchic, J. A. Woyach, M. L. Wang, J. Wang, *Nat. Commun.* **2020**, *11*, 1, https://doi.org/10.1038/s41467-020-17997-6.

[78] N. Shindo, H. Fuchida, M. Sato, K. Watari, T. Shibata, K. Kuwata, C. Miura, K. Okamoto, Y. Hatsuyama, K. Tokunaga, S. Sakamoto, S. Morimoto, Y. Abe, M. Shiroishi, J. M. M. Caaveiro, T. Ueda, T. Tamura, N. Matsunaga, T. Nakao, S. Koyanagi, S. Ohdo, Y. Yamaguchi, I. Hamachi, M. Ono, A. Ojida, *Nat. Chem. Biol.* **2019**, *15*, 250, https://doi.org/10.1038/s41589-018-0204-3.

[79] D. J. Augeri, J. A. Robl, D. A. Betebenner, D. R. Magnin, A. Khanna, J. G. Robertson, A. Wang, L. M. Simpkins, P. Taunk, Q. Huang, S. P. Han, B. Abboa-Offei, M. Cap, L. Xin, L. Tao, E. Tozzo, G. E. Welzel, D. M. Egan, J. Marcinkeviciene, S. Y. Chang, S. A. Biller, M. S. Kirby, R. A. Parker, L. G. Hamann, *J. Med. Chem.* **2005**, *48*, 5025, https://doi.org/10.1021/jm050261p.

[80] D. N. Deaton, A. M. Hassell, R. B. McFadyen, A. B. Miller, L. R. Miller, L. M. Shewchuk, F. X. Tavares, D. H. Willard, L. L. Wright, *Bioorganic Med. Chem. Lett.* **2005**, *15*, 1815, https://doi.org/10.1016/j.bmcl.2005.02.033.

[81] M. E. Flanagan, J. A. Abramite, D. P. Anderson, A. Aulabaugh, U. P. Dahal, A. M. Gilbert, C. Li, J. Montgomery, S. R. Oppenheimer, T. Ryder, B. P. Schuff, D. P. Uccello, G. S. Walker, Y. Wu, M. F. Brown, J. M. Chen, M. M. Hayward, M. C. Noe, R. S. Obach, L. Philippe, V. Shanmugasundaram, M. J. Shapiro, J. Starr, J. Stroh, Y. Che, *J. Med. Chem.* **2014**, *57*, 10072, https://doi.org/10.1021/jm501412a.

[82] M. E. Schnute, S. E. Benoit, I. P. Buchler, N. Caspers, M. L. Grapperhaus, S. Han, R. Hotchandani, N. Huang, R. O. Hughes, B. M. Juba, K. H. Kim, E. Liu, E. McCarthy, D. Messing, J. S. Miyashiro, S. Mohan, T. N. O'Connell, J. F. Ohren, M. D. Parikh, M. Schmidt, S. R. Selness, J. R. Springer, V. Thanabal, J. I. Trujillo, D. P. Walker, Z. K. Wan, J. M. Withka, A. J. Wittwer, N. L. Wood, L. Xing, C. W. Zapf, J. Douhan, *ACS Med. Chem. Lett.* **2019**, *10*, 80, https://doi.org/10.1021/acsmedchemlett.8b00461.

[83] A. Casimiro-Garcia, J. I. Trujillo, F. Vajdos, B. Juba, M. E. Banker, A. Aulabaugh, P. Balbo, J. Bauman, J. Chrencik, J. W. Coe, R. Czerwinski, M. Dowty, J. D. Knafels, S. Kwon, L. Leung, S. Liang, R. P. Robinson, J. B. Telliez, R. Unwalla, X. Yang, A. Thorarensen, *J. Med. Chem.* **2018**, *61*, 10665, https://doi.org/10.1021/acs.jmedchem.8b01308.

[84] R. A. Fairhurst, T. Knoepfel, N. Buschmann, C. Leblanc, R. Mah, M. Todorov, P. Nimsgern, S. Ripoche, M. Niklaus, N. Warin, V. H. Luu, M. Madoerin, J. Wirth, D. Graus-Porta, A. Weiss, M. Kiffe, M. Wartmann, J. Kinyamu-Akunda, D. Sterker, C. Stamm, F. Adler, A. Buhles, H. Schadt, P. Couttet, J. Blank, I. Galuba, J. Trappe, J. Voshol, N. Ostermann, C. Zou, J. Berghausen, A. Del Rio Espinola, W. Jahnke, P. Furet, *J. Med. Chem.* **2020**, *63*, 12542, https://doi.org/10.1021/acs.jmedchem.0c01019.

[85] S. L. Chan, C.-J. Yen, M. Schuler, C.-C. Lin, S. P. Choo, K.-H. Weiss, A. Geier, T. Okusaka, H. Y. Lim, T. Macarulla, A. X. Zhu, T. Kakizume, Y. (Gary) Gu, D. G. Porta, A. P. Myers, J.-P. Delord, *Cancer Res.* **2017**, *77*, CT106, https://doi.org/10.1158/1538-7445.AM2017-CT106.

[86] T. Knoepfel, P. Furet, R. Mah, N. Buschmann, C. Leblanc, S. Ripoche, D. Graus-Porta, M. Wartmann, I. Galuba, R. A. Fairhurst, *ACS Med. Chem. Lett.* **2018**, *9*, 215, https://doi.org/10.1021/acsmedchemlett.7b00485.

[87] A. Jones, X. Zhang, X. Lei, *Cell Chem. Biol.* **2017**, *24*, 537, https://doi.org/10.1016/j.chembiol.2017.05.003.

[88] D. G. Isom, C. A. Castañed, B. R. Cannon, B. E. García-Moreno, *Proc. Natl. Acad. Sci. U. S. A.* **2011**, *108*, 5260, https://doi.org/10.1073/pnas.1010750108.

[89] M. P. Wymann, G. Bulgarelli-Leva, M. J. Zvelebil, L. Pirola, B. Vanhaesebroeck, M. D. Waterfield, G. Panayotou, *Mol. Cell. Biol.* **1996**, *16*, 1722, https://doi.org/10.1128/mcb.16.4.1722.

[90] U. P. Dahal, A. M. Gilbert, R. S. Obach, M. E. Flanagan, J. M. Chen, C. Garcia-Irizarry, J. T. Starr, B. Schuff, D. P. Uccello, J. A. Young, *Medchemcomm* **2016**, *7*, 864, https://doi.org/10.1039/c6md00017g.

[91] E. Anscombe, E. Meschini, R. Mora-Vidal, M. P. Martin, D. Staunton, M. Geitmann, U. H. Danielson, W. A. Stanley, L. Z. Wang, T. Reuillon, B. T. Golding, C. Cano, D. R. Newell, M. E. M. Noble, S. R. Wedge, J. A. Endicott, R. J. Griffin, *Chem. Biol.* **2015**, *22*, 1159, https://doi.org/10.1016/j.chembiol.2015.07.018.

[92] J. Dong, L. Krasnova, M. G. Finn, K. Barry Sharpless, *Angew. Chemie - Int. Ed.* **2014**, *53*, 9430, https://doi.org/10.1002/anie.201309399.

[93] Q. Zhao, X. Ouyang, X. Wan, K. S. Gajiwala, J. C. Kath, L. H. Jones, A. L. Burlingame, J. Taunton, *J. Am. Chem. Soc.* **2017**, *139*, 680, https://doi.org/10.1021/jacs.6b08536.

[94] H. Mukherjee, J. Debreczeni, J. Breed, S. Tentarelli, B. Aquila, J. E. Dowling, A. Whitty, N. P. Grimster, *Org. Biomol. Chem.* **2017**, *15*, 9685, https://doi.org/10.1039/c7ob02028g.
[95] S. E. Dalton, L. Dittus, D. A. Thomas, M. A. Convery, J. Nunes, J. T. Bush, J. P. Evans, T. Werner, M. Bantscheff, J. A. Murphy, S. Campos, *J. Am. Chem. Soc.* **2018**, *140*, 932, https://doi.org/10.1021/jacs.7b08979.
[96] D. Quach, G. Tang, J. Anantharajan, N. Baburajendran, A. Poulsen, J. L. K. Wee, P. Retna, R. Li, B. Liu, D. H. Y. Tee, P. Z. Kwek, J. K. Joy, W. Q. Yang, C. J. Zhang, K. Foo, T. H. Keller, S. Q. Yao, *Angew. Chemie - Int. Ed.* **2021**, *60*, 17131, https://doi.org/10.1002/anie.202105383.
[97] T. Zhang, J. M. Hatcher, M. Teng, N. S. Gray, M. Kostic, *Cell Chem. Biol.* **2019**, *26*, 1486, https://doi.org/10.1016/j.chembiol.2019.09.012.
[98] H. S. Hahm, E. K. Toroitich, A. L. Borne, J. W. Brulet, A. H. Libby, K. Yuan, T. B. Ware, R. L. McCloud, A. M. Ciancone, K. L. Hsu, *Nat. Chem. Biol.* **2020**, *16*, 150, https://doi.org/10.1038/s41589-019-0404-5.
[99] R. L. McCloud, K. Yuan, K. E. Mahoney, D. L. Bai, J. Shabanowitz, M. M. Ross, D. F. Hunt, K. L. Hsu, *Anal. Chem.* **2021**, *93*, 11946, https://doi.org/10.1021/acs.analchem.1c01591.
[100] T. Huang, S. Hosseinibarkooie, A. L. Borne, M. E. Granade, J. W. Brulet, T. E. Harris, H. A. Ferris, K. L. Hsu, *Chem. Sci.* **2021**, *12*, 3295, https://doi.org/10.1039/d0sc06623k.

### *License and Terms*



The definitive version of this article is the electronic one that can be found at https://doi.org/10.2533/chimia.2022.435