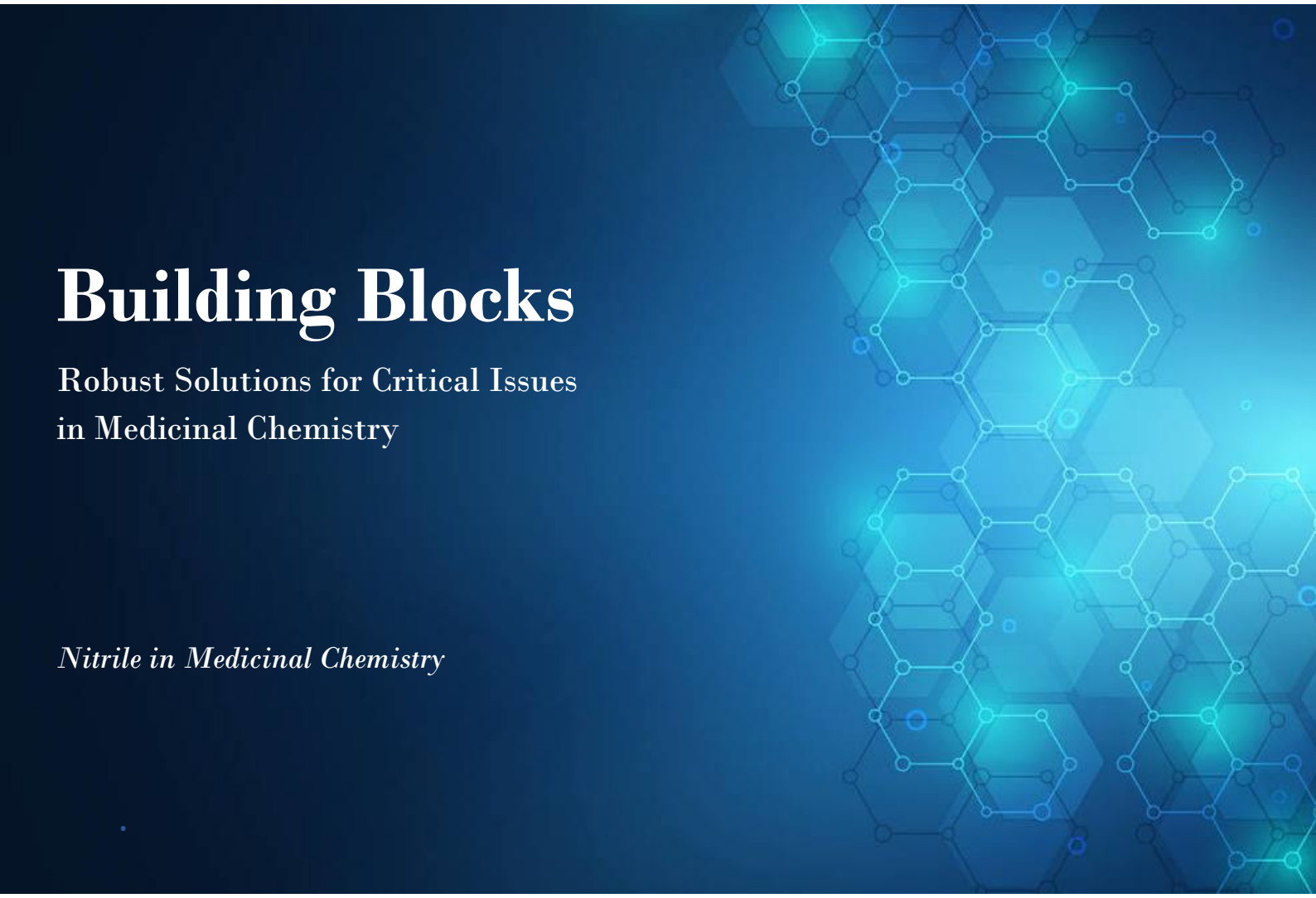

Building Blocks

Robust Solutions for Critical Issues in Medicinal Chemistry

Nitrile in Medicinal Chemistry

Nitrile in Medicinal Chemistry

The nitrile group has played an increasingly important role in medicinal chemistry, with more than 60 small-molecule approved drugs and a tremendous number of clinical candidates containing nitrile (**Figure 138**). [1-3] Since 2010, the FDA has approved at least one nitrile-containing drug every year, and reached the maximum number of five drugs in 2020. The marketed drugs with a nitrile moiety target a wide range of clinical disorders, including heart failure, hypertension, chronic myeloid leukemia, breast cancer, virus infection, etc.

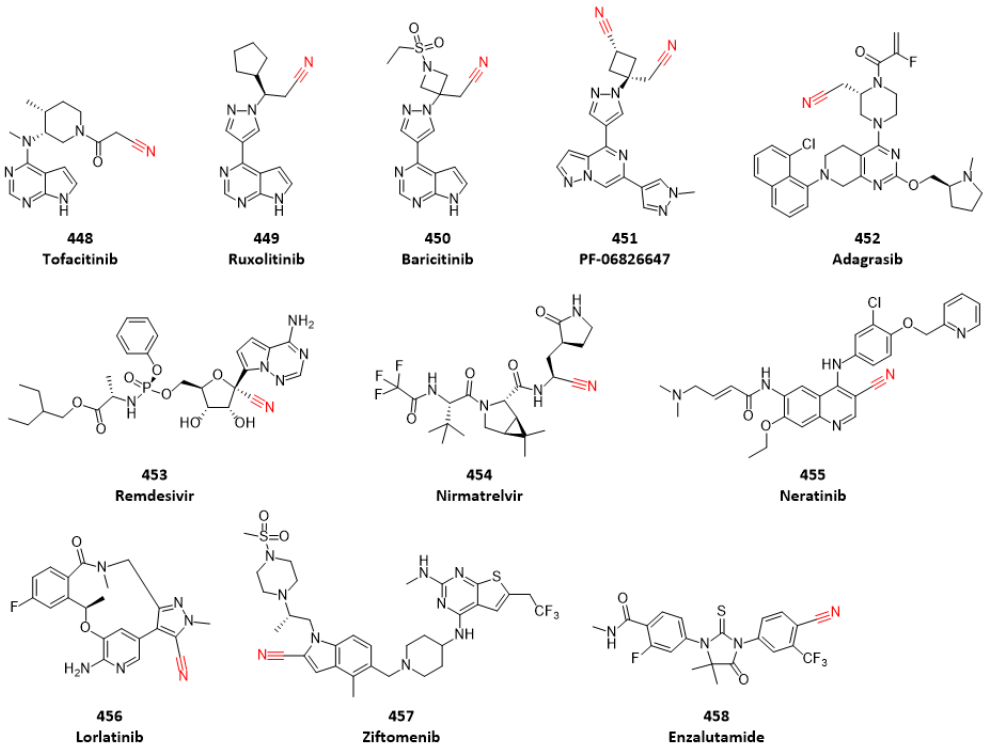

Figure 138. Approved drugs and clinical candidates containing nitrile group

Underpinning a wide range of applications of nitrile group in drug discovery are its unique features, including but not limited to the followings (**Figure 139**): [1-3]

a) Owing to its linear shape and low molecular volume, nitrile group can properly fit in the subsites of proteins and perform lipophilic interactions *via* the triple bond π system. The molecular volume of nitrile group is only one-eighth the size of methyl group, with 1.16 Å length of CN triple bond.

b) The carbon atom can act as an electrophile due to its electron deficiency, promoted by the high electronegativity of the nitrogen atom and high dipole moment in the triple bond; while the nitrogen atom can act as a hydrogen bond acceptor by its lone pair. So a nitrile group can simultaneously form two interactions with protein.

c) In addition to the non-bonded interactions, nitrile is a remarkable group that can for covalent adduct with proteins, mainly linked to a reactive cysteine or serine side chain. The most popular examples are DDP-4 inhibitors (**Vildagliptin** and **Saxagliptin**) and 3CL inhibitors (**Nirmatrelvir**).

d) Compounds containing nitrile group generally have a lower cLogP which indicates enhanced solubility. Compared to alkyne, nitrile decreases cLogP by 0.84 unit.

e) Nitrile group is considerably metabolically stable and non-toxic. It usually remains unchanged when it is eliminated from human body.

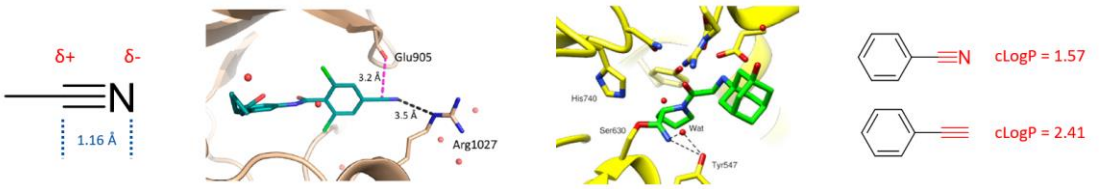

Figure 139. Unique features of nitrile group: an example where nitrile forms two interactions with protein (PDB code: 4GI1); [11] an example where nitrile forms a covalent bond with protein (PDB code: 6B1E); [2] compared to alkyne, nitrile decreases cLogP by 0.84 unit.

In the course of discovery of clinical candidate **Adagrasib** (compound **452**), in order to increase potency of compound **459**, X-ray crystal structure was obtained. Notable in this crystal structure is a bound water molecule complexed to Gly10 and Thr58 which is near the piperazine ring. This water molecule forms hydrogen bonds with the side chain hydroxyl of Thr58 and the carbonyl of Gly10. Analysis of the proximal hydrogen bonding network suggested that displacement of this water could lead to a large potency increase. An appropriately substituted piperazine ring would have the correct trajectory to displace this bound water. With this hypothesis in mind, a series of analogues were designed and synthesized. Among of them, compound **460** with *S*-CH_2CN was determined to be 440-fold more potent than compound **459** and 100-fold more potent than diastereomer **461**. In addition to binding, CH_2CN also impacted chemical reactivity of acrylamide. For example, compound **459** has a GSH $T_{1/2}$ = 17 h; on the hand, compound **460** and compound **461** have significantly decreased GSH $T_{1/2}$ = 4 h and 1 h respectively. Further optimization based on compound **460** led to identification of **Adagrasib** (compound **452**). X-ray crystal structure of **Adagrasib** (compound **452**) bound to KRAS G12C revealed that nitrile displaces the Gly10 bound water and forms a hydrogen bond to the backbone NH of Gly10 (**Figure 140**). [4]

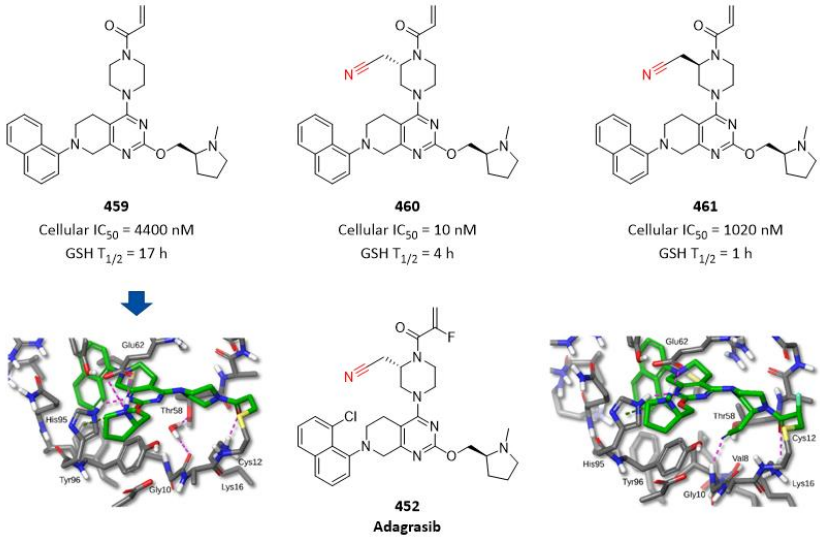

Figure 140. Nitrile displaces water molecule and increased potency. (PDB codes: 6USX, 6UT0)

In the course of discovery of clinical candidate **PF-06826647** (compound **451**) as a selective TYK2 inhibitor, compound **462** was identified as a promising lead compound. X-ray crystal structure of compound **462** bound to TYK2 revealed that the positioning of the alkyl cyano group was shown to be toward the C-terminal lobe in the active site lower lipophilic pocket. The trifluoromethyl azetidine group provided the desired TYK2 potency through engagement of the P-loop, but the overall moderate LipE reflected the high overall lipophilicity of the molecule. In order to improve the modest metabolic stability as judged by human liver microsome turnover, the team attempted to replace trifluoroethylamino group. In addition, replacement of the trifluoroethylamino group

was preferred due to association with testicular toxicity liability for potential metabolites. Unique within this series, the cyanomethyl group of compound **463** recapitulated the potency of compound **462**, and also lowered HLM clearance. Further optimization based on compound **463** led to identification of **PF-06826647** (compound **451**). X-ray crystal structure of **PF-06826647** (compound **451**) bound to TYK2 revealed that the *cis*-cyclobutanecarbonitrile moiety was positioned below and toward the tip of the P-loop. The end of the P-loop contains mainly hydrophobic residues and a generally satisfied hydrogen bond network which may contribute to the calculated lability of this modeled water in the apo structure. This predicted high energy water is displaced by the cyclobutanecarbonitrile group of **PF-06826647** (compound **451**). The electrostatic mapping of this pocket also showed a positive electrostatic potential which could provide a favorable interaction with the cyano group (**Figure 141**). [5]

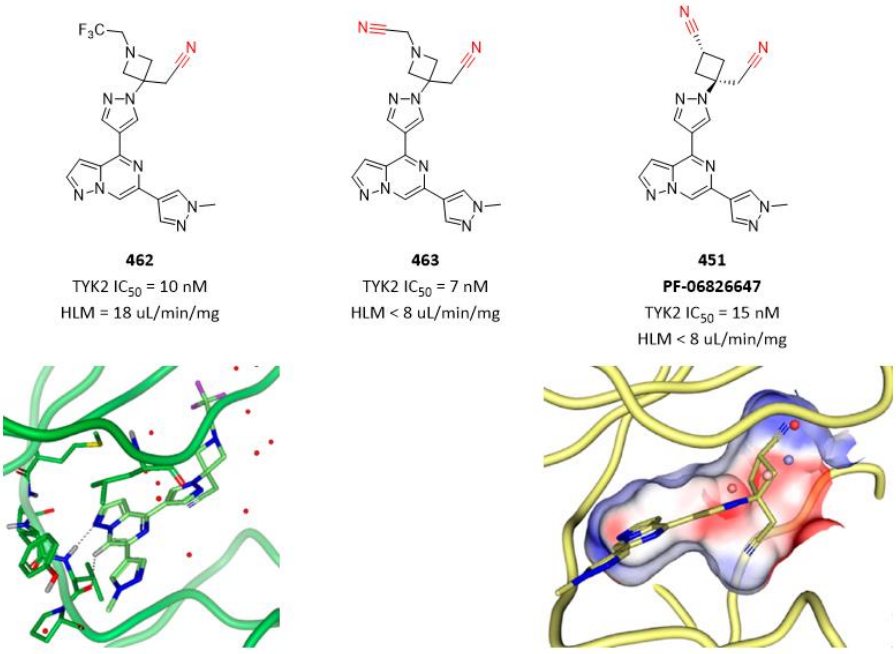

Figure 141. Nitrile replace water molecule and increased metabolic stability. (PDB codes: 6X8F, 6X8G)

Nirmatrelvir inhibits the SARS-CoV-2 main protease, also known as 3C-like protease ($3CL^{pro}$), which plays a crucial role in cleaving the coronavirus polyprotein to form smaller essential proteins required for virus replication and pathogenesis. Nitrile in **Nirmatrelvir** forms a covalent bond with Cys145 of $3CL^{pro}$, which is a critical interaction for high potency. Removing nitrile in compound **464** lost potency completely, while transforming nitrile to amide in compound **465** also lost potency completely, suggesting that an electrophilic warhead is essential for inhibiting 3CL (**Figure 142**). [6] X-ray crystal structure of **Nirmatrelvir** bound to $3CL^{pro}$ revealed that there is a covalent bond between nitrile and Cys145.

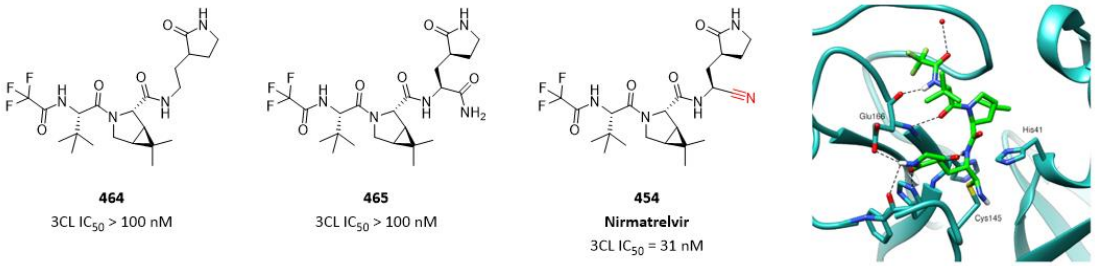

Figure 142. Nitrile forms a covalent bond with Cys145. (PDB code: 7RFW)

Vildagliptin (compound **466**) and **Saxagliptin** (compound **467**), which are DDP-4 inhibitors, can form a reversible covalent bond with Ser630. Their binding modes were characterized by the crystal structure of these inhibitors bound to the enzyme in the covalent state (**Figure 143**). [2]

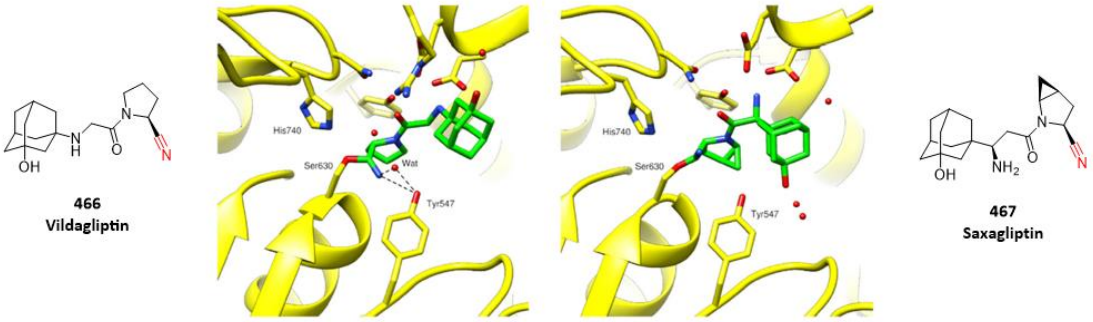

Figure 143. Nitrile in DDP-4 inhibitors can form covalent bond with Ser630. (PDB codes: 6B1E, 3BJM)

As described in above examples, aliphatic building blocks containing nitrile are of great value for discovery of covalent inhibitors (**Figure 144**).

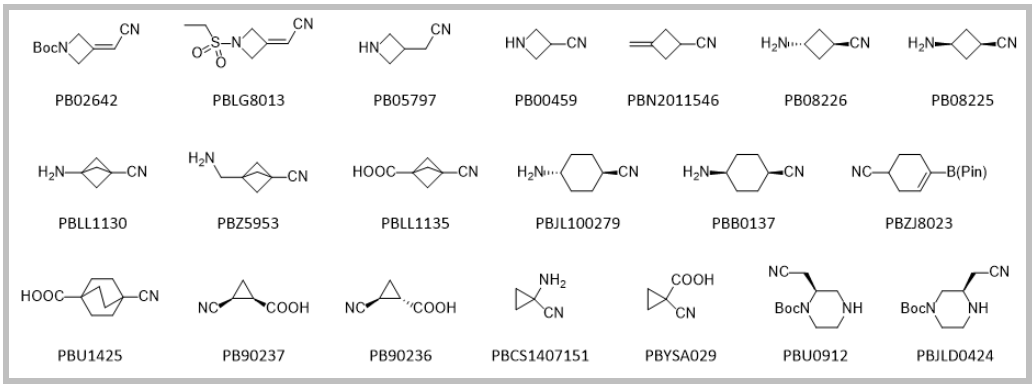

Figure 144. Aliphatic building blocks containing nitrile

It was found that 4-anilinoquinoline-3-carbonitrile **468** is an effective inhibitor of EGFR with activity comparable to 4-anilinoquinazoline **469**. A new homology model of EGFR was constructed based on the X-ray structures of Hck and FGF receptor-1 kinase. The model suggests that with the quinazoline-based inhibitor, the N3 atom is hydrogen bonded to a water molecule which, in turn, interacts with Thr830. It is proposed that the quinolone-3-carbonitrile bind in a similar manner where the water molecule is displaced by the cyano group which interacts with the same Thr830 residue (**Figure 145**). [7]

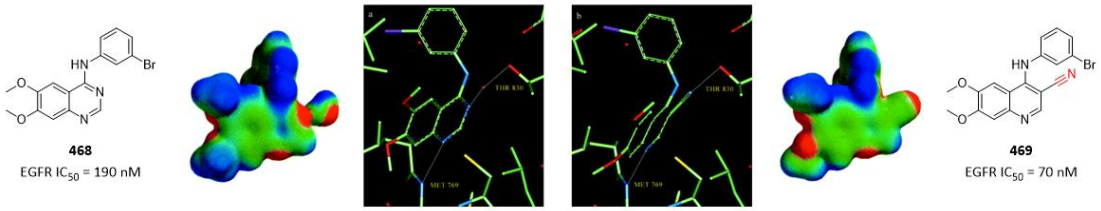

Figure 145. Nitrile displace water molecule, and forms hydrogen bond directly with Thr830.

Based on above findings, several EGFR inhibitors were discovered and approved. One of them is **Neratinib**, a covalent EGFR inhibitor with nitrile forming a hydrogen bond with protein directly (**Figure 146**). [8]

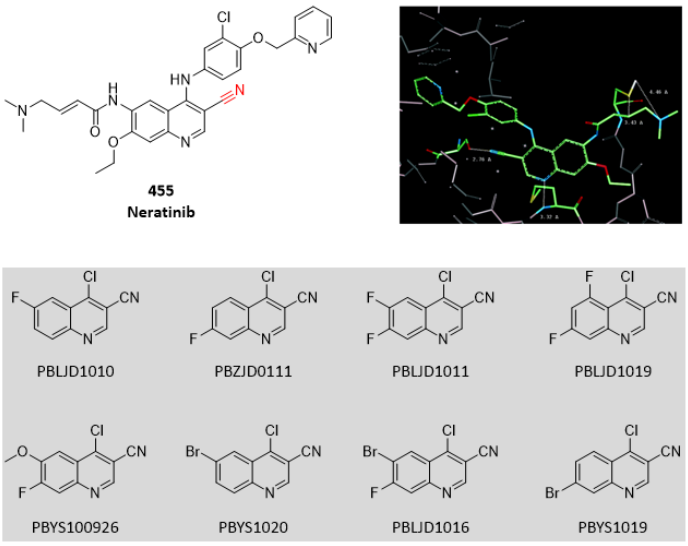

Figure 146. Nitrile in **Neratinib** forms a hydrogen bond with protein directly.

Replication of SARS-CoV-2 depends on the viral RNA-dependent RNA polymerase (RdRp), which is the target of the **Remdesivir** (compound **453**), a pro-drug of compound **473**. **Remdesivir** shows broad-spectrum antiviral activity against RNA viruses, and previous studies with RdRps from Ebola virus and Middle East respiratory syndrome coronavirus (MERS-CoV) have revealed that delayed chain termination is **Remdesivir**'s mechanism of action. [9] Comparing compound **470**, compound **471**, compound **472** and compound **473**, these data established that 1'-CN group was optimal among several 1'-modifications on the adenine C-nucleoside for RSV potency. [10] Structural studies demonstrated that 1'-CN of **Remdesivir** sits in a pocket formed by residues Thr-687 and Ala-688 at the beginning of chain prolongation. At i+4, 1'-CN moiety encounters a steric clash with Ser-861, and prevents RdRp from advancing into i+4, resulting in chain termination (**Figure 147**).

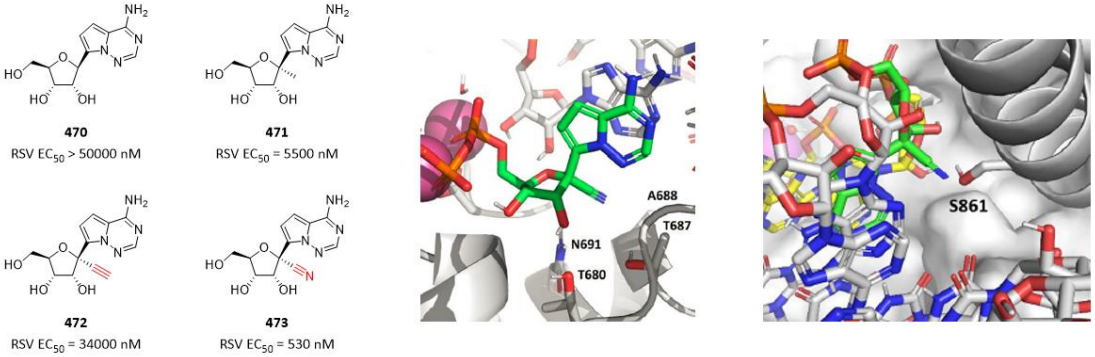

Figure 147. Critical role of 1'-CN and mechanism of action of Remdesivir

Based on compound **474**, addition of a Br in compound **475** increased biochemical potency, but did not increased cellular potency proportionally. It was surprisingly that addition of a cyano in compound **476** increased both biochemical potency and cellular potency significantly. Docking model revealed that the nitrogen atom of nitrile of compound **476** forms a hydrogen bond with Lys590 of protein (**Figure 148**). [12]

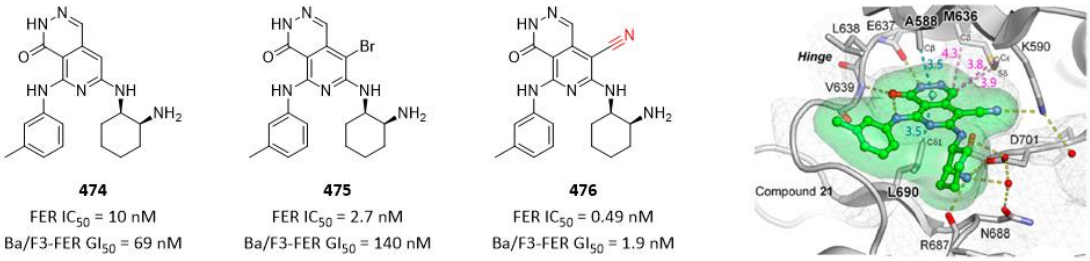

Figure 148. Nitrile increased potency by forming a hydrogen bond with protein.

In the course of discovery **Lorlatinib** (compound **456**) as a selective ALK inhibitor, to increase selectivity of compound **477** against TrkB and other kinases, the team employed the residue difference between ALK (Leu1198) and TrkB (Tyr635). The pyrazole cyano moiety in **Lorlatinib** (compound **456**) was efficient for obtaining selectivity because the cyano contains only one more heavy atom than the unselective methyl analogue **477** and gains at least 35-fold selectivity. It is hypothesized that the nitrile makes an unfavorable contact with the CE atom of the Tyr635 in TrkB, closer to the midpoint of the side chain rather than at the terminus. Unfavorable desolvation penalties or electrostatics due to the proximity of the electron-rich nitrile nitrogen atom and tyrosine may further enhance selectivity (**Figure 149**). [13]

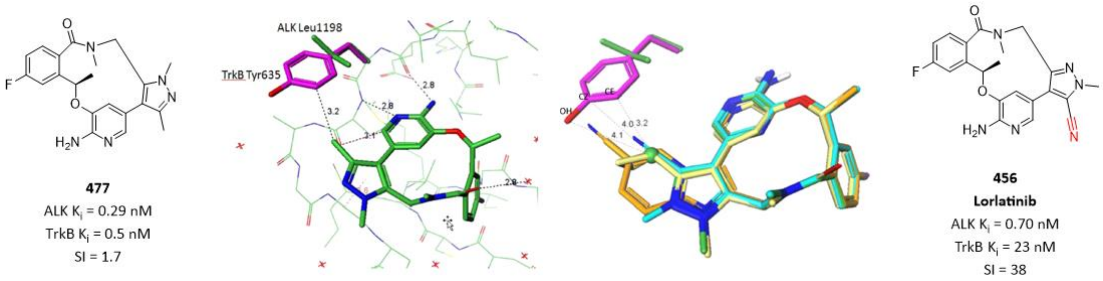

Figure 149. Nitrile increased selectivity. (PBD codes: 4CMU, 4AT3, 4CLI)

A HSQC-based screening of 13,000 fragments revealed 20 fragments hits that displayed chemical shift perturbation patterns of KRAS G12V different than those observed with compounds that bind to switch I/II. Among these compounds was a cluster that included both aminocyanothiophenes and aminothiazoles. NMR titration of the hits using the modified KRAS construct revealed that fragment hit **478** bound to the modified protein with an affinity of 116 uM. Aminothiazole fragment hit **479** showed reduced affinity compared to fragment hit **478**. Using a combination of targeted commercial purchasing and discrete compound synthesis, the cyano group is shown to be important based on complete loss of affinity observed for compound **480**. X-ray crystal structure of fragment hit **478** bound to GDP-KRAS G12V revealed that nitrile interacts with both the amide NH of Glu63 as well as nearby bound water. This network of interactions readily explains the rigid requirement for nitrile for the best affinity (**Figure 150**). [14]

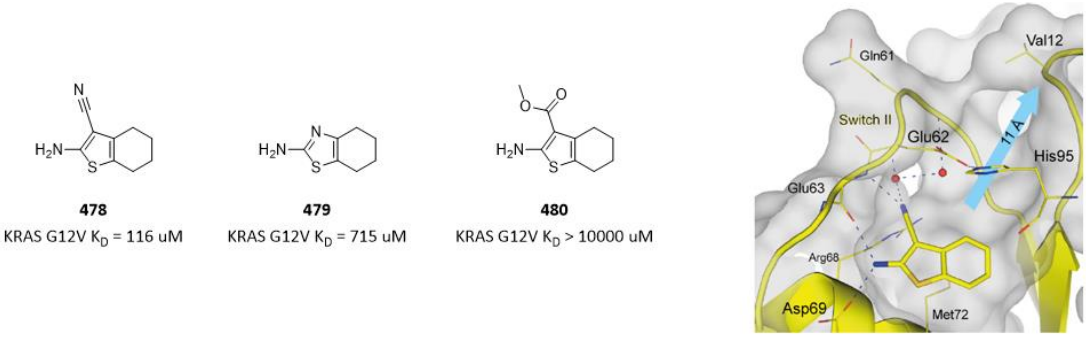

Figure 150. Nitrile interacts with both NH of backbone and nearby bound water. (PDB code: 7U8H)

The above described cases displayed great value of aromatic nitrile building blocks for quick exploration of SAR and SRP (**Figure 151**).

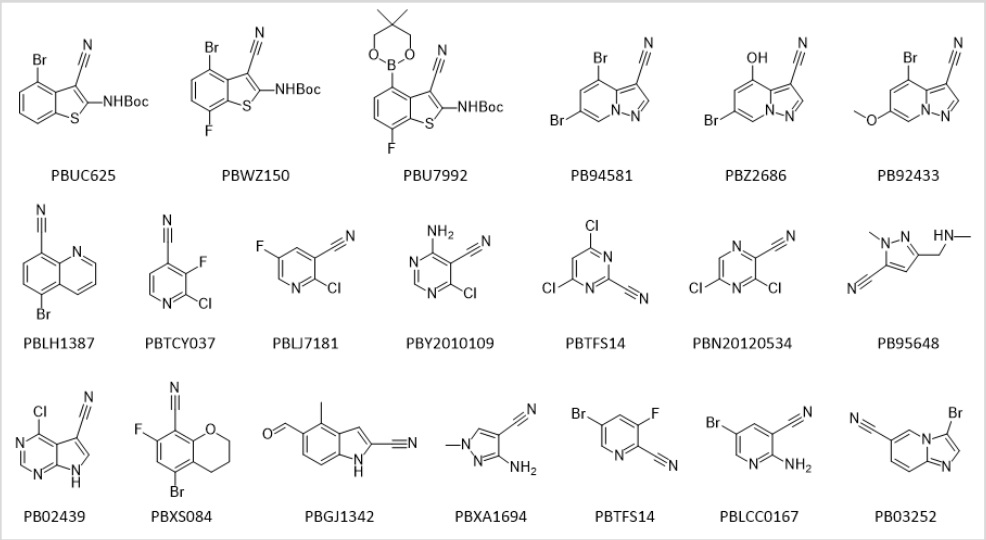

Figure 151. Aromatic nitrile building blocks

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