Pyrimidines in Drug Discovery

Key Points

- Majority of marketed pyrimidine-containing drugs as kinase inhibitors
- Isostere for aromatic and heteroaryl rings
- Two possible contact points with target proteins

Overview

Pyrimidine motif is a privileged fragment in drug discovery, boasting more than 19 marketed pyrimidine-containing drugs, with the majority of them as kinase inhibitors. In particular, amino- and diamino-pyrimidine fragments are especially prevalent.

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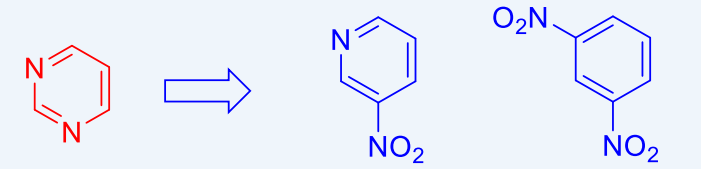
Electronically, pyrimidine is a deactivated arene comparable to 3-nitropyridine or dinitrobenzene. As far as aromaticity is concerned, pyrimidine's aromaticity is 67% relative to benzene (100%). As a privileged structure, pyrimidine has appeared in more than 19 drugs, with the majority of them as kinase inhibitors.



pyrimidine 3-nitropyridine dinitrobenzene

Pyrimidine-containing Drugs

More than 19 pyrimidine-containing drugs are currently on the market. One of the better known pyrimidine-containing drugs is AstraZeneca′s 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor rosuvastatin (Crestor, **1**) for lowering cholesterol. In place of the isopropyl group of many other statins, rosuvastatin (**1**)′s methylsulfonamide group renders the drug a non-substrate of CYP3A4 thus lowering potential for drug–drug interactions (DDIs) and bestowed the molecule with novel intellectual properties.

Both of Tibotec′s non-nucleoside reverse transcriptase inhibitors (NNRTI) inhibitors, etravirine (Intelence, **2**) and rilpivirine (Edurant, **3**) contain the diamino-pyrimidine core structures.

The majority of pyrimidine-containing drugs are protein kinase inhibitors. This has not come as a surprise because pyrimidine closely mimic the adenine fragment of adenosine triphosphate (ATP), which is critical to the phosphorylation process, the key function of kinases. The first kinase inhibitor on the market, Novartis′s Brc–abl inhibitor imatinib (Gleevec, **4**) contains a pyrimidine ring in addition to three additional aromatic rings. BMS's dual Bcr–abl and Src inhibitor dasatinib (Sprycel, **5**) has a diaminopyrimidine fragment. Novartis's followup Brc–abl inhibitor nilotinib (Tasigna, **6**) retained imatinib (**4**)′s pyridyl-pyrimidine motif. The difference between imatinib (**4**)′s and nilotinib (**6**)′s central methylphenyl structures is that the former has two amine groups thus higher chances of forming reactive metabolites via CYP oxidation.

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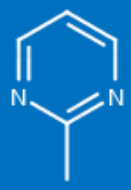

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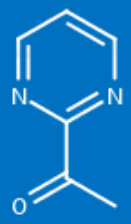

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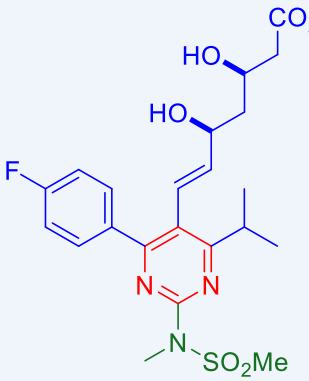
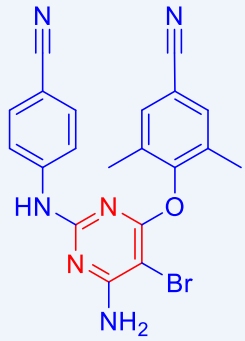
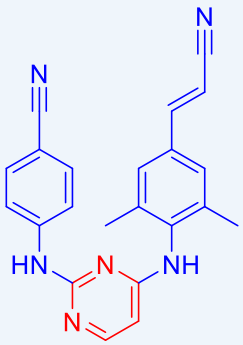


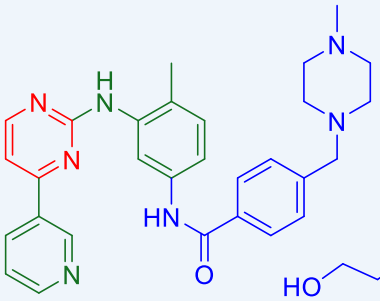
rosuvastatin (Crestor, **1**)
AstraZeneca, 2002
HMG-CoA reductase inhibitor



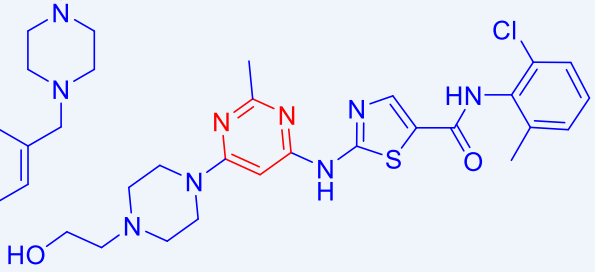
etravirine (Intelence, **2**)
Tibotec/J&J, 2008
NNRTI



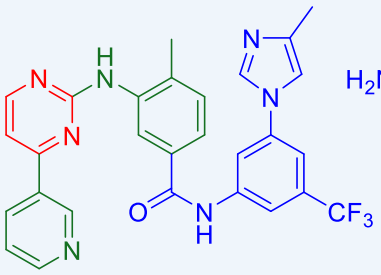
rilpivirine (Edurant, **3**)
Tibotec/J&J, 2011
NNRTI



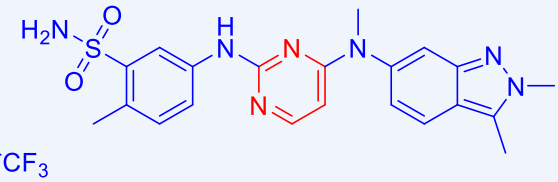
imatinib (Gleevec, **4**)
Novartis, 2001
Brc-abl inhibitor



dasatinib (Sprycel, **5**)
BMS, 2006
dual Bcr-abl and Src inhibitor



nilotinib (Tasigna, **6**)
Novartis, 2007
Bcr-Abl inhibitor



pazopanib (Votrient, **7**)
GSK, 2009
VEGFR, c-Kit inhibitor

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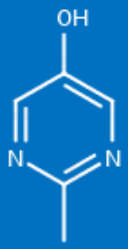

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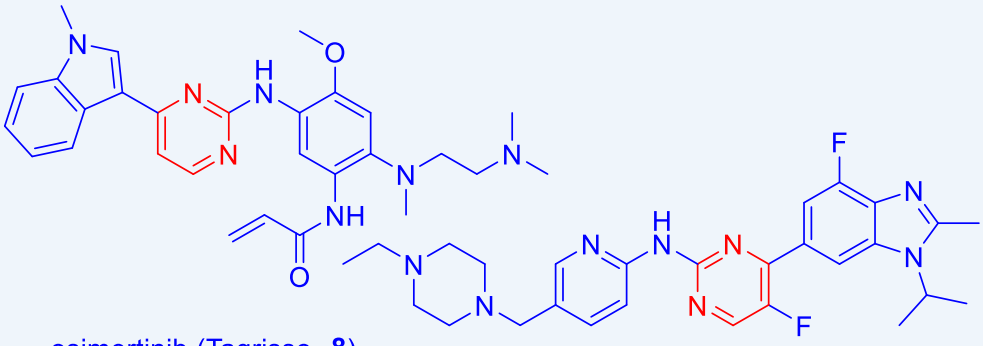
Apparently, diaminopyrimidine is a good pharmacophore for kinase inhibitors. GSK's dual vascular endothelial growth factor receptor (VEGFR) and c-Kit inhibitor pazopanib (Votrient, **7**) also has a diaminopyrimidine core structure.

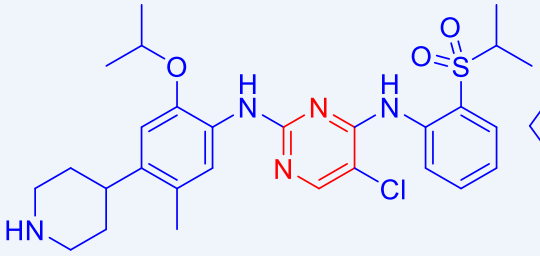
Epidermal growth factor receptor (EGFR) inhibitors are among the earliest kinase inhibitors on the market. But resistance invariably developed and covalent inhibitors have been invented to combat the L858R and T790M mutations by taking advantage of Cys-797 at EGFR′s active site. AstraZeneca's 3rd generation EGFR inhibitor osimertinib (Tagrisso, **8**) is a covalent inhibitor expressly designed to overcome the T790M mutation. Lilly′s aminopyrimidine-containing abemaciclib (Verzanio, **9**) is a cyclin-dependent kinase (CDK)4/6 inhibitor.

In addition, Novartis′s anaplastic lymphoma kinase (ALK) inhibitor ceritinib (Zykadia, **10**) contains a chloro-diaminopyrimidine core and Bayer's copanlisib (Aliqopa, **11**) is a pan-phospoinositide-3-kinase (PI3K) inhibitor with an aminopyrimidine fragment.

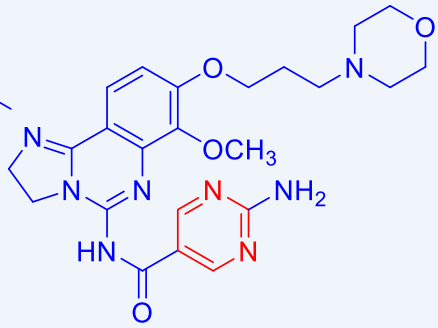


osimertinib (Tagrisso, **8**)
AZ, 2015
EGFR inhibitor (3rd generation)
C797S mutation

abemaciclib (Verzanio, **9**)
Lilly, 2017
CDK4/6 inhibitor



ceritinib (Zykadia, **10**)
Novartis, 2014
ALK inhibitor



copanlisib (Aliqopa, **11**)
Bayer, 2017
pan-PI3K inhibitor

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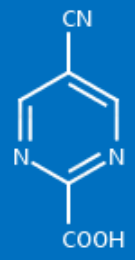

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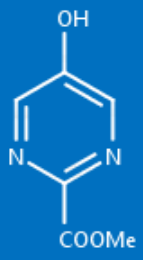

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The Ras–MAP kinase pathway has been implicated in tumor progression for a variety of human cancers. The Raf kinases, which are components of this cascade, are serine/threonine kinases that activate mitogen-activated protein kinase (MEK)1/2. Mutant B-Raf containing a V600E substitution (where B-Raf protein's 600th amino acid valine is replaced by glutamic acid) causes aberrant constitutive activation of this pathway and has high occurrence in several human cancers. Two of the three B-Raf kinase inhibitors approved by the FDA, dabrafenib (Tafinlar, **12**), and encorafenib (Braftovi, **13**), contain an aminopyrimidine moiety.

The chloro-diaminopyrimidine core seem to be a favored structure for ALK inhibitors. It appeared on Novartis′s ALK inhibitor ceritinib (**10**) and it also made an appearance on Ariad′s ALK and EGFR inhibitor brigatinib (Alunbrig, **14**).

Phosphodiesterase 5 (PDE5) inhibitors include well-known names such as sildenafil (Viagra), vardenafil (Levitra), and tadalafil (Cialis) for the treatment of erectile dysfunction (ED). Vivus′s PDE5 inhibitor avanafil (Stendra, **15**) possesses two pyrimidine rings. An intramolecular hydrogen bond forms a pseudo ring and renders the core pyrimidine "behave" almost like a bicycle. Triaminopyrimidine riociguat (Adempas, **16**) by Bayer is a stimulator of soluble guanylate cyclase (sGC) approved for the treatment of adults with persistent/recurrent chronic thromboembolic pulmonary hypertension (CTEPH).

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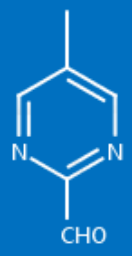

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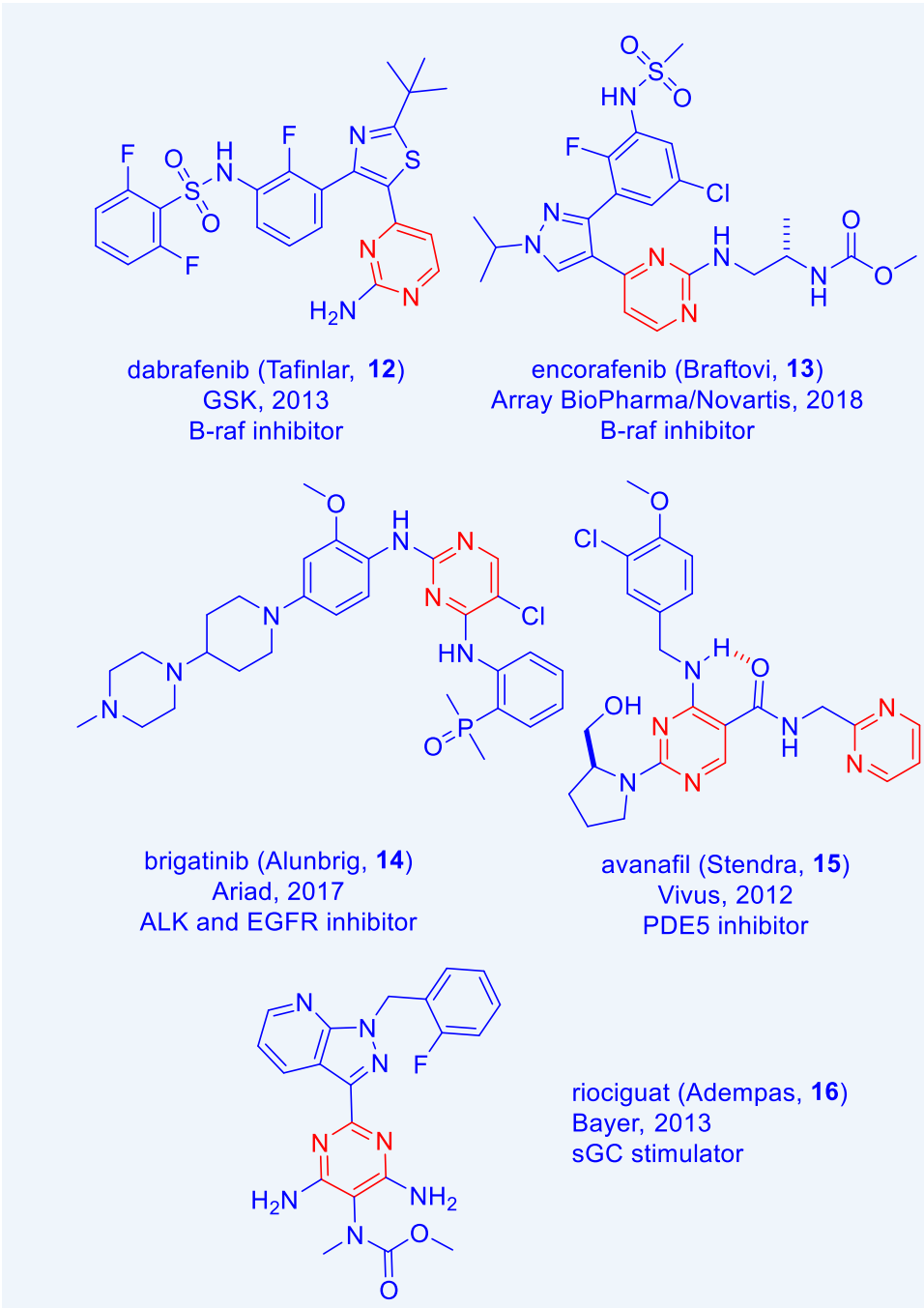

Three pyrimidine-containing drugs were approved in 2019. Karyopharm's selinexor (Xpovio, **17**) is a first-in-class selective inhibitor of nuclear export (SINE) XPO1 antagonist for the treatment of patients adult patients with relapsed or refractory multiple myeloma (RRMM). Eisai's lemborexant (Dayvigo, **18**) is a dual antagonist of the orexin OX1 and OX2 receptors approved for the treatment of insomnia. Impact's fedratinib (Inrebic, **19**) is a Janus kinase (JAK)-2-selective inhibitor for treating high risk myelofibrosis.

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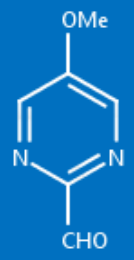

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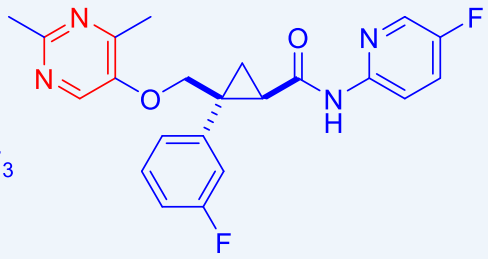
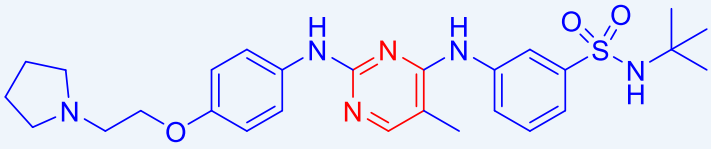
selinexor (Xpovio, **17**)
Karyopharm, 2019
XPO1 antagonist



lemborexant (Dayvigo, **18**)
Eisai, 2019
dual OX1 and OX2 antagonist

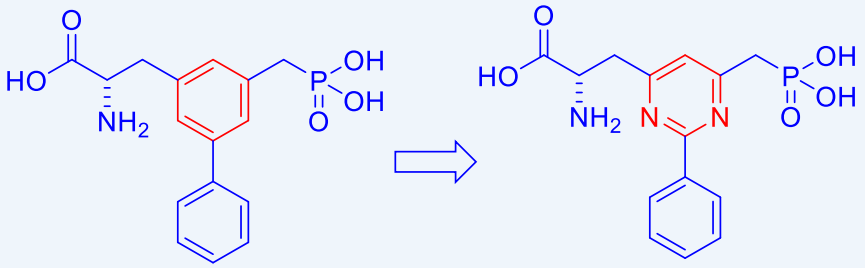


fedratinib
(Inrebic, **19**)
Impact, 2019
JAK2 inhibitor

Pyrimidines in Drug Discovery

1. Pyrimidine as a bioisostere

In theory, pyrimidine may serve as an isostere for all aromatic and heteroaryl rings. Historically, pyrimidine as a bioisostere had mixed results. Case in point was the pyrimidine isostere **21** for SDZ EAB 515 (**20**), a potent *N*-methyl-D-aspartate (NMDA) receptor antagonist. To overcome **20**'s high polarity, Sandoz scientists in 1994 prepared its pyrimidine analog **21**, which displayed a pK_i of 4.3 and a pA_2 value of 4.6 was measured in the cortical wedge test for functional activity. For the original drug **20**, these values were 6.7 and 6.94, respectively. Thus, the pyrimidine isostere **21** was more than two orders of magnitude less active than the potent NMDA antagonist **20**. The results are quite astonishing in light of the seemingly minor structural differences.[1]



SDZ EAB 515 (**20**)
pK_i = 6.7, pA_2 = 6.94

pyrimidine analog **21**
pK_i = 4.3, pA_2 = 4.6

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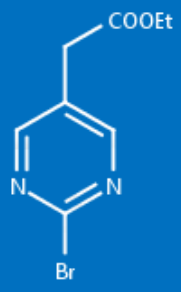

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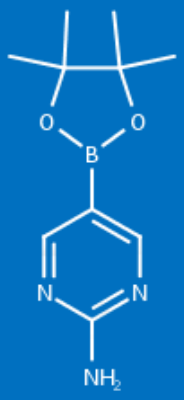

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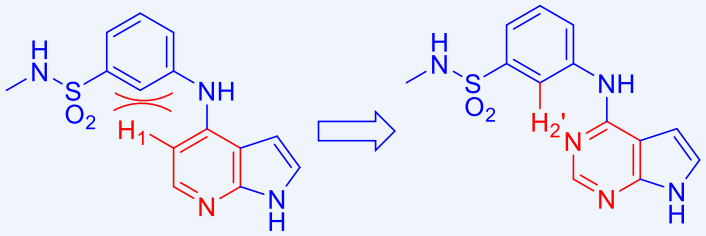
The next example of isostere had a more positive outcome. Pyrrolopyridine **22** as a type I inhibitor of troponin I-interacting kinase (TNNI3K) is not very potent with an IC_{50} value of 8,000 nM. Switching the pyridine moiety to the pyrimidine isostere gave rise to pyrrolopyrimidine **23**, which is 100-fold more potent in binding affinity with an IC_{50} value of 80 nM. Scrutiny of the X-ray crystal structure of a similar TNNI3K inhibitor bound to the ATP binding site of the kinase revealed that the steric clash of H1 and H2′ in the coplanar aryl orientation of **22** prevented it from adopting the correct (more flat) conformation required for effective binding. On the other hand, pyrrolopyrimidine **23** with an extra strategically placed nitrogen atom allows free rotation to adopt a more planar conformation therefore more effective binding.[2]



pyrrolopyridine **22**
TNNI3K IC_{50} = 8,000 nM

pyrrolopyrimidine **23**
TNNI3K IC_{50} = 80 nM

Antagonists of retinol-binding protein 4 (RBP4) have potential as treatment of atrophic age-related macular degeneration (AMD) and Stargardt disease. A RBP4 antagonist **24** with the anthranilic acid appendage had a reasonable *in vitro* and *in vivo* PK and PD profile already. To further enhance the *in vitro* and *in vivo* potency, 6-methyl-pyrimidine-4-carboxylic acid **25** was prepared because it could provide a suitable isostere for the amide of **24** while still presenting the acid group as a favorable interaction. Pyrimidine here reduces *the number of rotatable bonds* and was expected to lead improved RBP4 binding affinity. Pyrimidine-acid **25** was tested indeed more potent in an *in vitro* binding assays (RBP4 SPA IC_{50} = 12.8 nM, SPA = scintillation proximity assay) in comparison to the parent compound **24** (IC_{50} = 72.7 nM), a more than 5-fold improvement. Meanwhile, pyrimidine-acid **25** showed boosted functional RBP4–TTR (TTR = transthyretin) interaction antagonist (HTRF) activity (RBP4 HTRF IC_{50} = 43.6 nM, HTRF = homogeneous time resolved fluorescence), a nearly 7-fold enhancement against the parent compound **24** (IC_{50} = 294 nM).[3]

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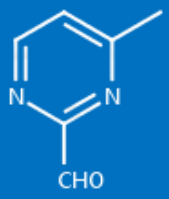

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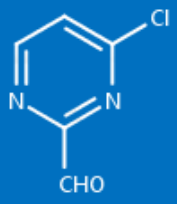

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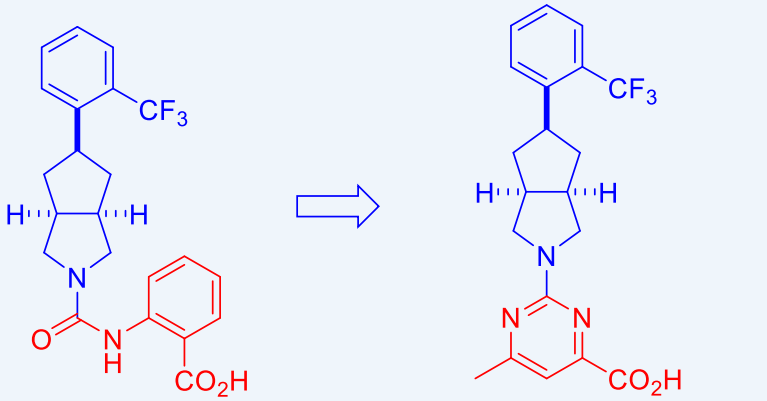
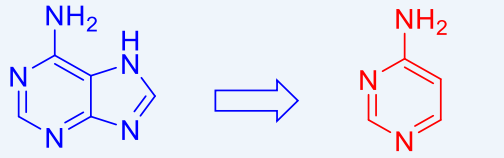


anthranilic acid **24**
RBP4 SPA IC_{50} = 72.7 nM
RBP4 HTRF IC_{50} = 294 nM

pyrimidine-4-carboxylic acid **25**
RBP4 SPA IC_{50} = 12.8 nM
RBP4 HTRF IC_{50} = 43.6 nM

2. Interactions with target proteins

There are two nitrogen atoms on pyrimidine, providing two possible contact points with target proteins in the form of hydrogen bond acceptors. A quick glance of pyrimidine-containing kinase inhibitors revealed that aminopyrimidines are prevalent. As shown below, since aminopyrimidine closely mimics the left portion of adenine, an important fragment of ATP, which is critical to the phosphorylation process, the key function of kinases. The amino group on aminopyrimidines not only offers its electron-donating properties to the electron-deficient pyrimidine ring, but also provides interacting points with target kinase proteins as a hydrogen bond donor.



adenine aminopyrimidine

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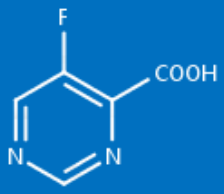

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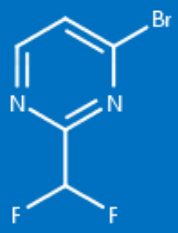
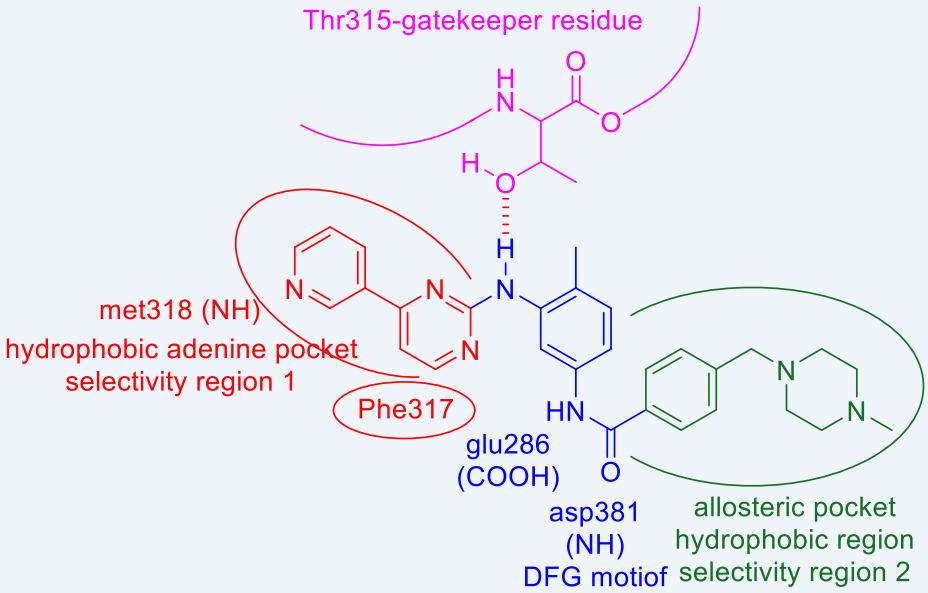


PBU0849

The first marketed kinase inhibitor, Brc–abl inhibitor imatinib (**4**) contains a pyridyl-aminopyrimidine motif, as does Novartis' follow-up Brc–abl inhibitor nilotinib (**6**). Crystal structure of imatinib (**4**)–abelson (Abl) tyrosine kinase complex indicated that the amine sandwiched between the pyrimidine and phenyl rings serves as a hydrogen bond donor to interact with the side chain hydroxyl group of Thr315 of the target protein. The pyridyl nitrogen accepts a hydrogen bond from the amide of Met318, which is normally hydrogen-bonded to the nitrogen N1 in ATP. These two interactions contribute to imatinib (**4**)'s selectivity. One of the two nitrogen atoms on the pyrimidine ring serves as a hydrogen bond acceptor with a water molecule nearby.[4]



interactions between imatinib (**4**) and Brc-abl enzyme

Protein–ligand interactions between ceritinib (**10**) and its target protein, wild-type ALK, are shown below. Ceritinib (**10**) forms two hydrogen bonds at the hinge area via the pyrimidine and amino nitrogen atoms with the backbone nitrogen and oxygen of M1199. The central pyrimidine ring of the ceritinib (**10**) is sandwiched between residues A1148 and L1256, probably interacting via π-stacking. The chlorine substituent on the pyrimidine ring is involved in a hydrophobic interaction with the gatekeeper residue L1196. The piperidine ring extends to the solvent and the isopropyl group adjacent to the sulfonyl bends down into the cavity lined by residues L1256 and D1270.[5]

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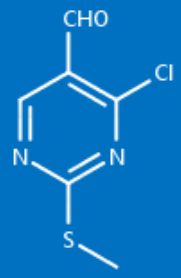

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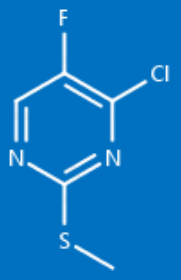

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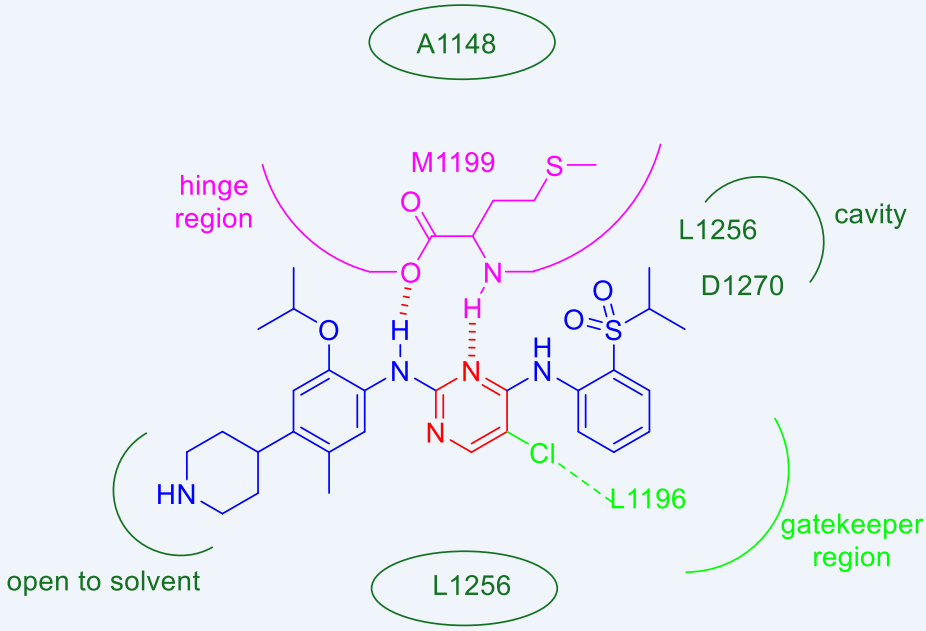
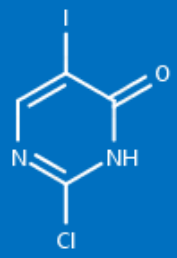


interactions between ceritinib (**10**) and wild-type ALK enzyme

Synthesis of Some Pyrimidine-containing Drugs

The 12-step synthesis of imatinib mesylate (**4**) in the manufacturing process was accomplished by Novartis in an astonishingly short time. The synthesis began with a condensation between 3-acetylpyridine (**26**) and ethyl formate. Deprotonation of the methyl group on **26** using freshly prepared sodium methoxide afforded an enolate. Condensation of the enolate with ethyl formate was followed by exchange with dimethylamine to produce 3-dimethylamino-1-(3-pyridyl)-2-propen-1-one (**27**). Alternatively, **27** could be prepared from the condensation of **26** and *N,N*-dimethylformamide dimethylacetal [$(MeO)_2CHN(Me)_2$]. Meanwhile, nitration of 2-amino-toluene (**28**) gave 2-amino-4-nitrotoluene nitrate, with the nitro group serving as a masked amine group. Refluxing 2-amino-4-nitrotoluene nitrate with cyamide furnished 2-amino-5-nitrophenylguanidine (**29**). Subsequently, condensation of 3-dimethylamino-enone **27** and guanidine **29** was achieved in the presence of NaOH in refluxing isopropanol to assemble pyrimidine **30**. Palladium-catalyzed hydrogenation of nitrophenylpyrimidine **30** unmasked the nitro group to provide aminophenylpyrimidine. Amide formation was accomplished by treatment of the aminophenyl-pyrimidine with 4-(4-methylpiperazinomethyl)benzoyl chloride to deliver **4**. Finally, the mesylate of **4** was readily accessed by the addition of one equivalent of methanesulfonic acid.[6]

PharmaBlock Products



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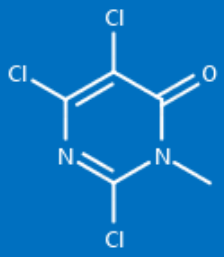

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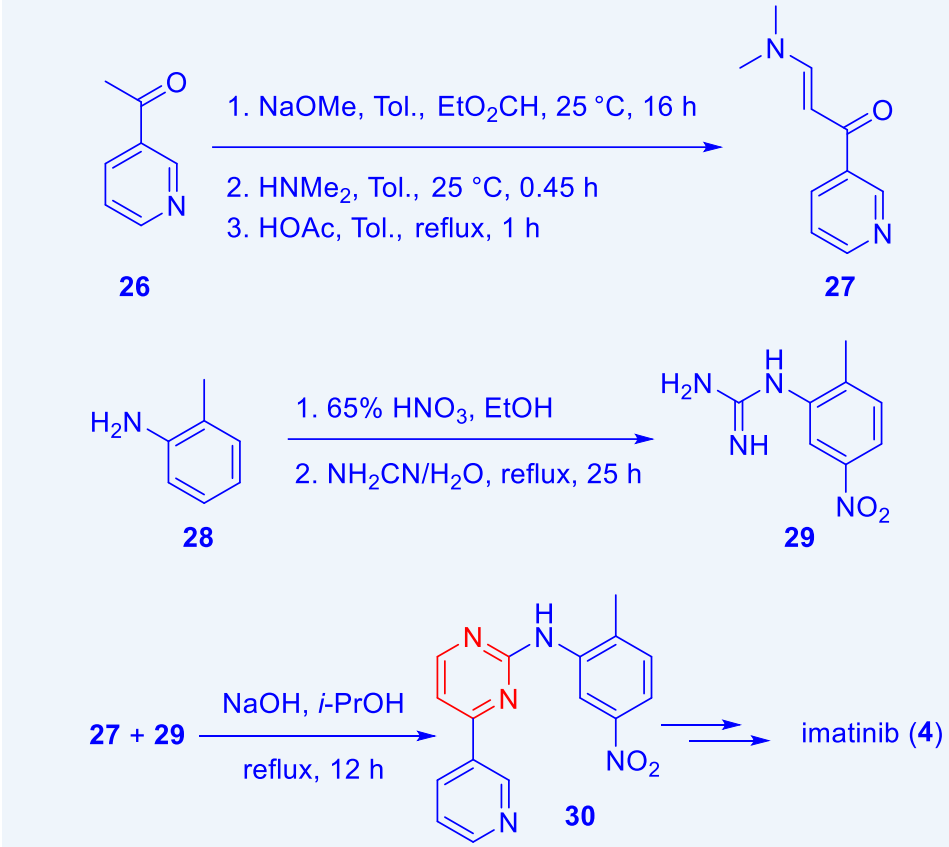
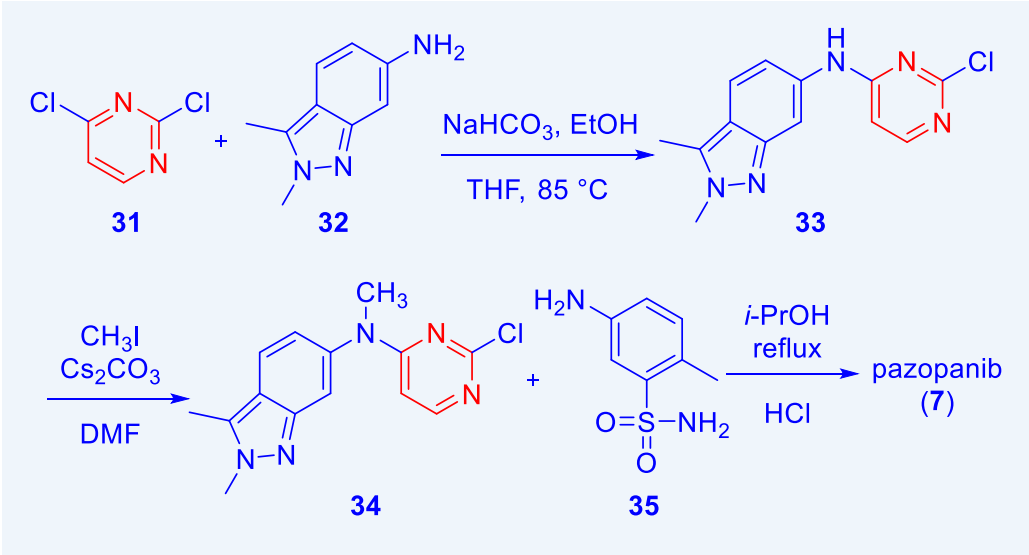


The synthesis of pazopanib (**7**) involves amination of 2,4-dichloropyrimidine (**31**) with 6-amino-2,3-dimethylindazole (**32**) in the presence of sodium bicarbonate in ethanol/THF to produce **33**. Subsequent *N*-methylation of **33** with iodomethane and cesium carbonate produced **34**. The 2-chloro group of pyrimidine on **34** was then allowed to react with 5-amino-2-methylbenzene-sulfonamide (**35**) in catalytic HCl/isopropanol at reflux to deliver pazopanib hydrochloride (**7**) in good yield.[7]



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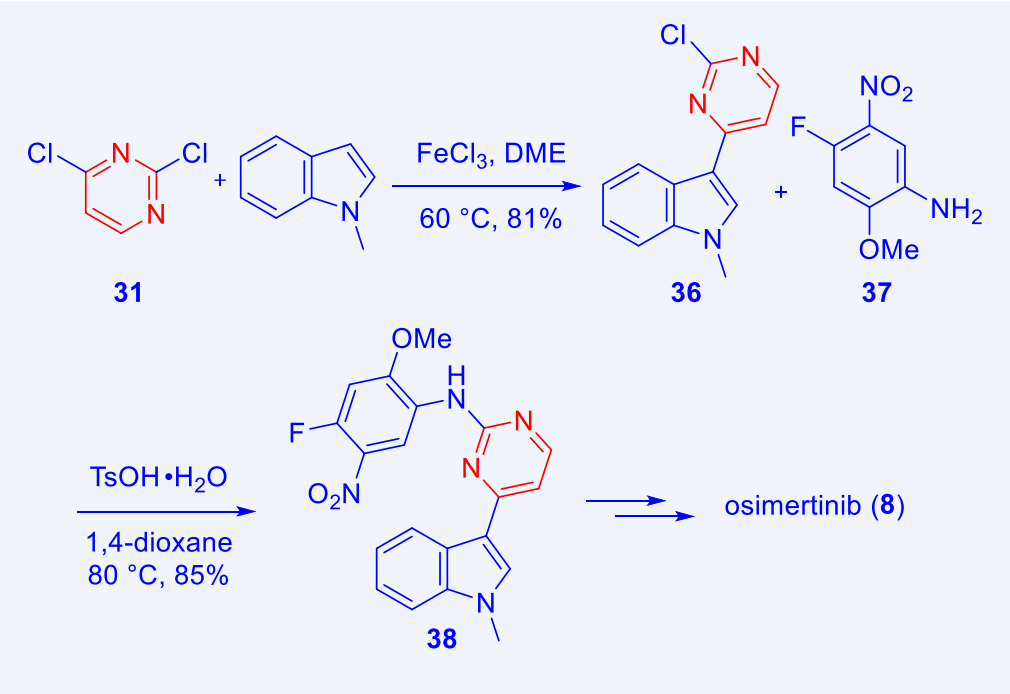
AstraZeneca's synthesis of osimertinib (**8**) also employed 2,4-dichloro-pyrimidine (**31**) as its starting material. Its coupling with dimethyl-indole assembled adduct **36** with the help of $FeCl_3$. Further coupling of **36** with aniline **37** afforded 2-phenylamino-3-indolylpyrimidine **38**, which was converted to the desired osimertinib (**8**) in three additional steps.[8]



For the process synthesis of riociguat (**16**), condensation of amidine **39** with malononitrile **40** assembled pyrimidine **41**. The phenyldiazine group on **41** was cleaved via palladium-catalyzed hydrogenation to afford tri-aminopyrimidine **42**. Treatment of **42** with dimethyl dicarbonate selectively protected the amine at the 4'-position in the middle to offer carbamate **43** in 95% yield. Eventually, carbamate **43** was methylated and the resulting compound was recrystallized with DMSO and EtOAc sequentially to deliver the desired active pharmaceutical ingredient (API) riociguat (**16**).[9]

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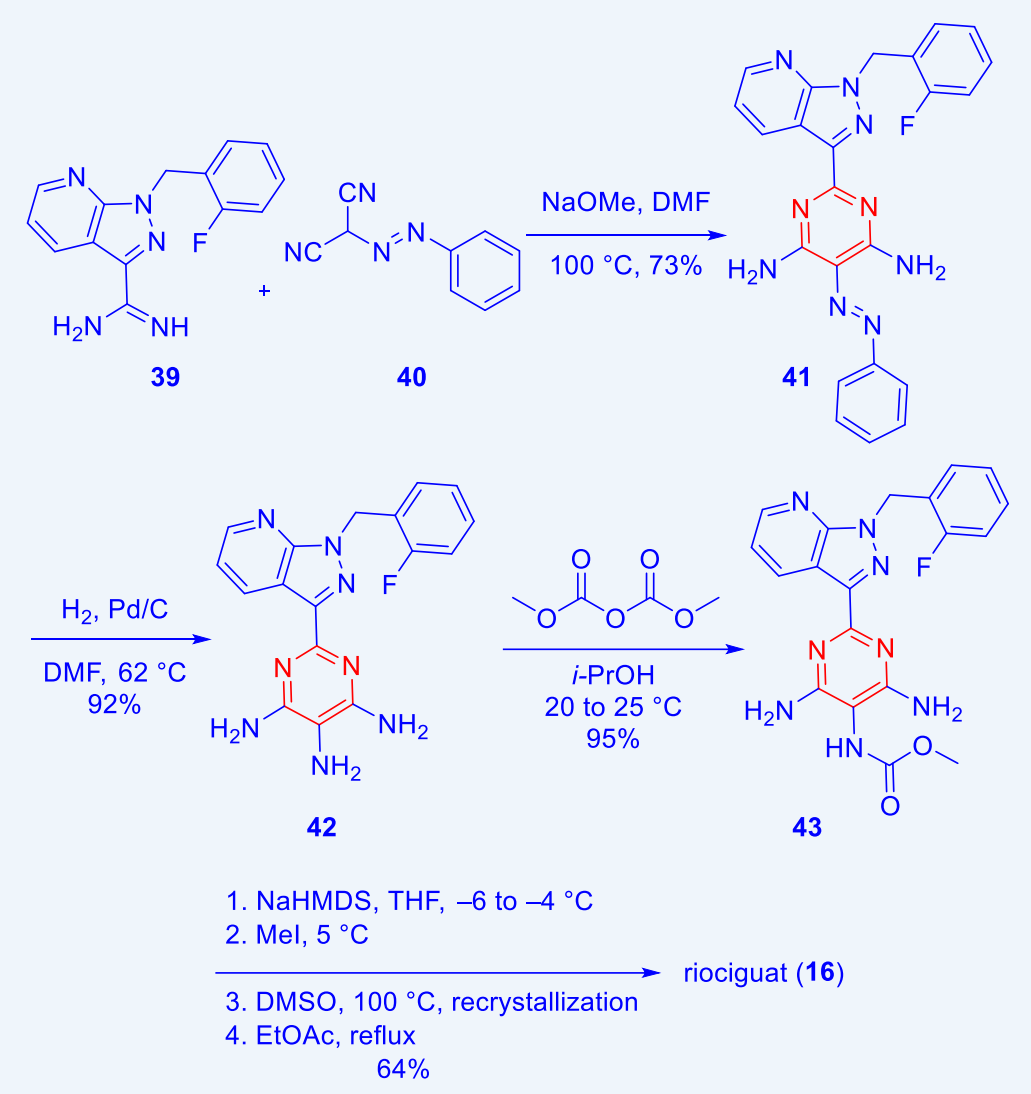

In summary, pyrimidine motif is a privileged fragment in drug discovery, boasting more than 19 marketed pyrimidine-containing drugs, with the majority of them as kinase inhibitors. In particular, amino- and diamino-pyrimidine fragments are especially prevalent.

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