Pyrrolotriazines in Drug Discovery

Key Points

- Apply in nucleoside antiviral drugs and kinase inhibitors.
- Good bioisosteres of the adenine portion of ATP.
- Pyrrolotriazine-containing drugs contribute to the treatment of COVID-19.

Overview

Pyrrolotriazines and imidazolotriazines are bioisosteres for bases such as adenine, guanine, purine, etc. As a consequence, they have found utility in the fields of nucleoside antiviral drugs, kinase inhibitors, PDE, and HDAC inhibitors.

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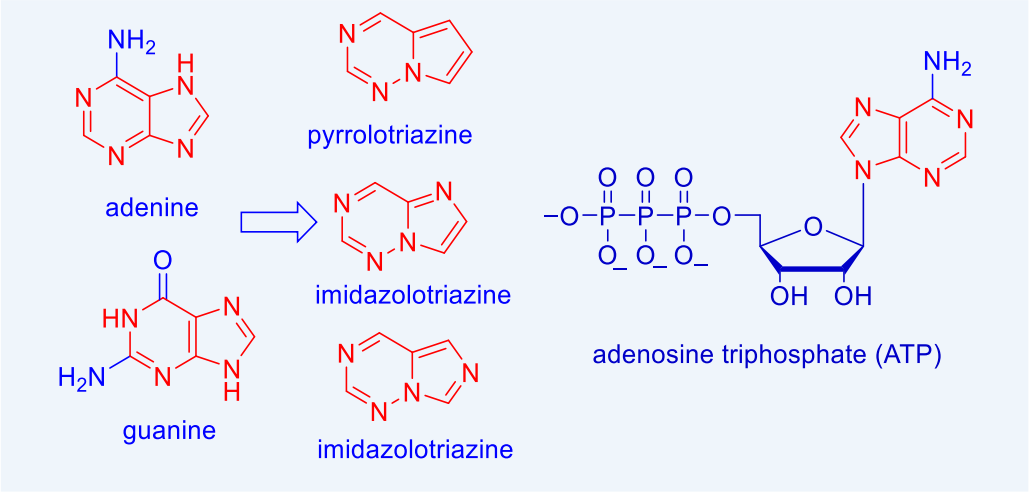
PharmaBlock designs and synthesizes over 520 Pyrrolotriazines and 59 Pyrrolotriazines products are in stock. CLICK HERE to find detailed product information on webpage.

Pyrrolotriazine-containing drugs have attracted intense attention recently since Gilead's nucleoside antiviral drug remdesivir (Veklury) has been catapulted to the limelight when, on May 1st, the FDA approved it for emergency use to treat COVID-19 patients.



Pyrrolotriazines and their aza-analogs imidazolotriazines have found widespread applications in nucleoside antiviral drugs and kinase inhibitors.

Bioisosterism is a powerful tool for drug discovery. Many old nucleoside antiviral drugs were discovered by mimicking the structures of DNA bases. There are two pairs of DNA bases: adenine, guanine, thymine, and cytosine. Another base, uracil, is only seen in RNAs. Pyrrolotriazines and their aza-analogs imidazolotriazines, non-natural bases, bear a striking resemblance to both adenine and guanine. Therefore, they are ideal bioisosteres for those two DNA bases. On the other hand, protein kinases phosphorylate amino acids such as tyrosine, serine, or threonine amino acids on target proteins by taking a phosphate from adenine triphosphate (ATP). Nearly all kinase inhibitors on the market are ATP competitive inhibitors by occupying the ATP-binding pockets of the kinases. Not surprisingly, pyrrolotriazines and imidazolotriazines are good bioisosteres of the adenine portion of ATP.

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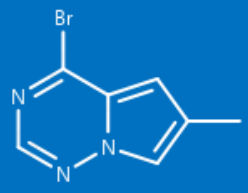

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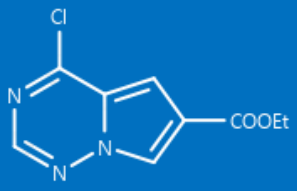

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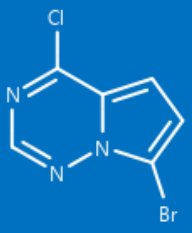

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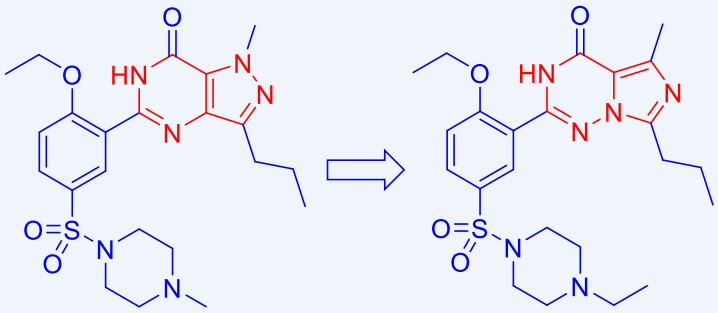
Pyrrolotriazine-containing Drugs

Only one imidazolotriazine-containing drug is currently on the market, to the best of our knowledge. It is Bayer's vardenafil (Levitra, **2**),[1] a phosphodiesterase V (PDE5) inhibitor for the treatment of erectile dysfunction (ED). It is a "me-too" drug of Pfizer's blockbuster drug and social sensation, sildenafil (Viagra, **1**). Bayer discovered vardenafil (**2**) employing a classic "scaffold-hopping" strategy. Although vardenafil (**2**)'s ethyl group on piperazine bestowed the molecule with a superior PDE5/PDE1 selectivity over the prototype drug sildenafil (**1**), the minor change would not have created any novel intellectual properties (IP) for Bayer. What "brought home the bacon" was vardenafil (**2**)'s imidazolotriazinone fragment to replace sildenafil (**1**)'s pyrrolopyrimidinone core structure. This approach is also known as "analog-based drug discovery" strategy because imidazolotriazinone is an analog of pyrrolopyrimidinone.



sildenafil (Viagra, **1**)
Pfizer, 1998
PDE5 inhibitor

vardenafil (Levitra, **2**)
Bayer, 2003
PDE5 inhibitor

Several pyrrolotriazine- and imidazolotriazine-containing drugs have entered clinical trials, mostly as nucleoside antiviral drugs and kinase inhibitors. The most conspicuous of all is, of course, Gilead's nucleoside antiviral drug remdesivir (Veklury, **4**), initially discovered as a treatment of Ebola virus (EBOV) and entered phase III clinical trials.[2] It evolved from GS-6620 (**3**), the first *C-nucleoside* HCV polymerase inhibitor with demonstrated antiviral response in HCV infected patients.[3] Unlike normal

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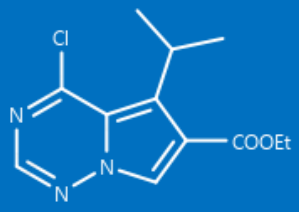

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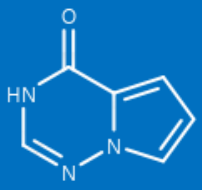

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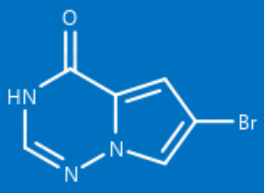

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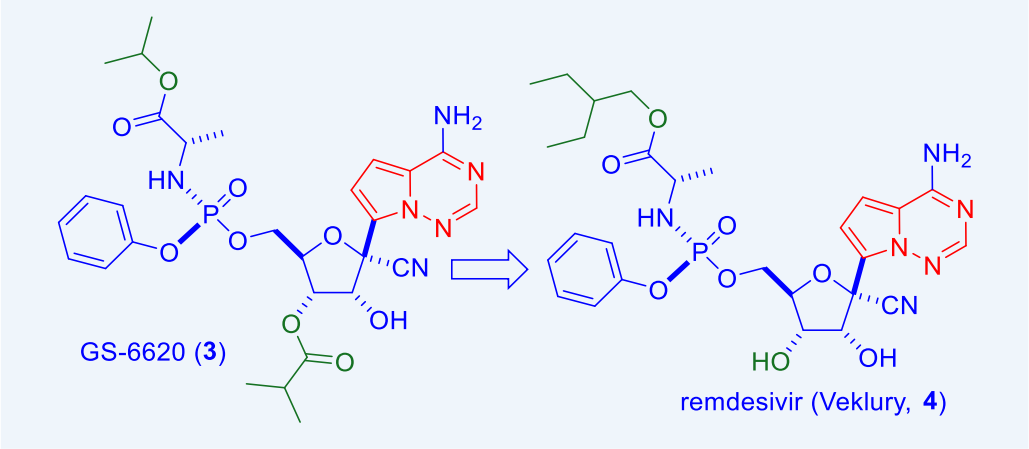
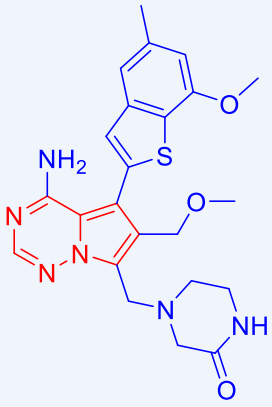
nucleosides where the base and the sugar are tied together by a N–C bond, the pyrrolotriazine fragment connects the sugar motif via a C–C bond for C-nucleosides. *C-Nucleosides have the potential for improved metabolism and pharmacokinetic properties over their N-nucleoside counterparts due to the presence of a strong carbon–carbon glycosidic bond and a non-natural heterocyclic base.*

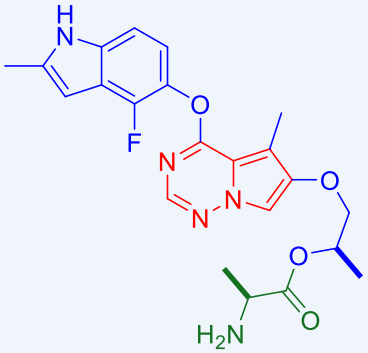


The field of kinase inhibitors is a fertile ground for flat heterocycles in general, and pyrrolotriazines in particular, because they mimic the adenine motif of the ATP molecule, the key player of the kinase functions. Bayer's rogaratinib (**5**) is a pan-fibroblast growth factor receptor (FGFR) inhibitor, inhibiting all four subtypes of this receptor: FGFR1–4. The drug has good drug metabolism and pharmacokinetic (DMPK) properties. Since rogaratinib (**5**) demonstrated tumor growth reduction in preclinical models bearing different FGFR-alterations in mono- and combination-therapy, it was brought to phase I clinical trials in 2018.[4]

Similarly, BMS's pyrrolotriazine-containing drug brivanib alaninate (**6**) is a dual inhibitor of FGFR-1 and vascular endothelial growth factor-2 (VEGFR2). It is a prod-rug of BMS-540215 in an attempt to boost the solubility and bioavailability. Brivanib alaninate (**6**) has completed phase III clinical trials as a treatment of malignant tumors, is probably in the process of seeking governmental approval.[5]

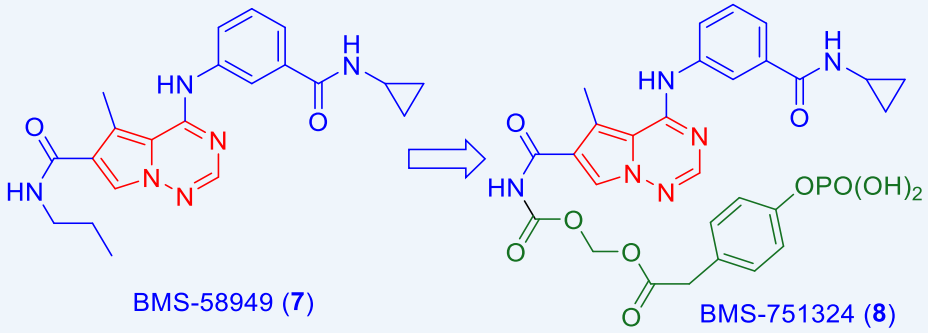


rogaratinib (**5**)
Bayer
pan-FGFR inhibitor



brivanib alaninate (**6**)
BMS, dual FGFR-1
and VEGFR-2 inhibitor

BMS also employed the prod-rug tactic to arrive at BMS-751324 (**8**), a clinical p38α mitogen-activating protein (MAP) kinase inhibitor. It is a carbamoylmethylene-linked prod-rug of the parent drug, BMS-582949 (**7**), which was advanced to phase II clinical trials for the treatment of rheumatoid arthritis (RA), but suffered low solubility and low exposure at higher pH. The unique pro-drug BMS-751324 (**8**) is not only stable but also water soluble under both acidic and neutral conditions. It is effectively biotransformed into parent drug BMS-582949 (**7**) *in vivo* by alkaline phosphatase and esterase in a stepwise manner.[6]



BMS-58949 (**7**)

BMS-751324 (**8**)

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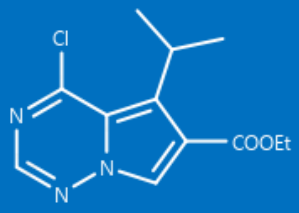

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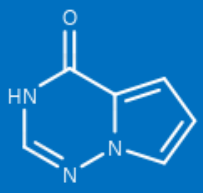

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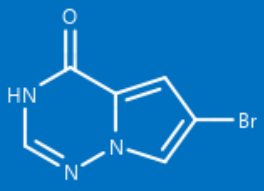

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Pyrrolotriazines in Drug Discovery

How does remdesivir (**4**) work as a nucleoside antiviral drug? Well, it is a long story……

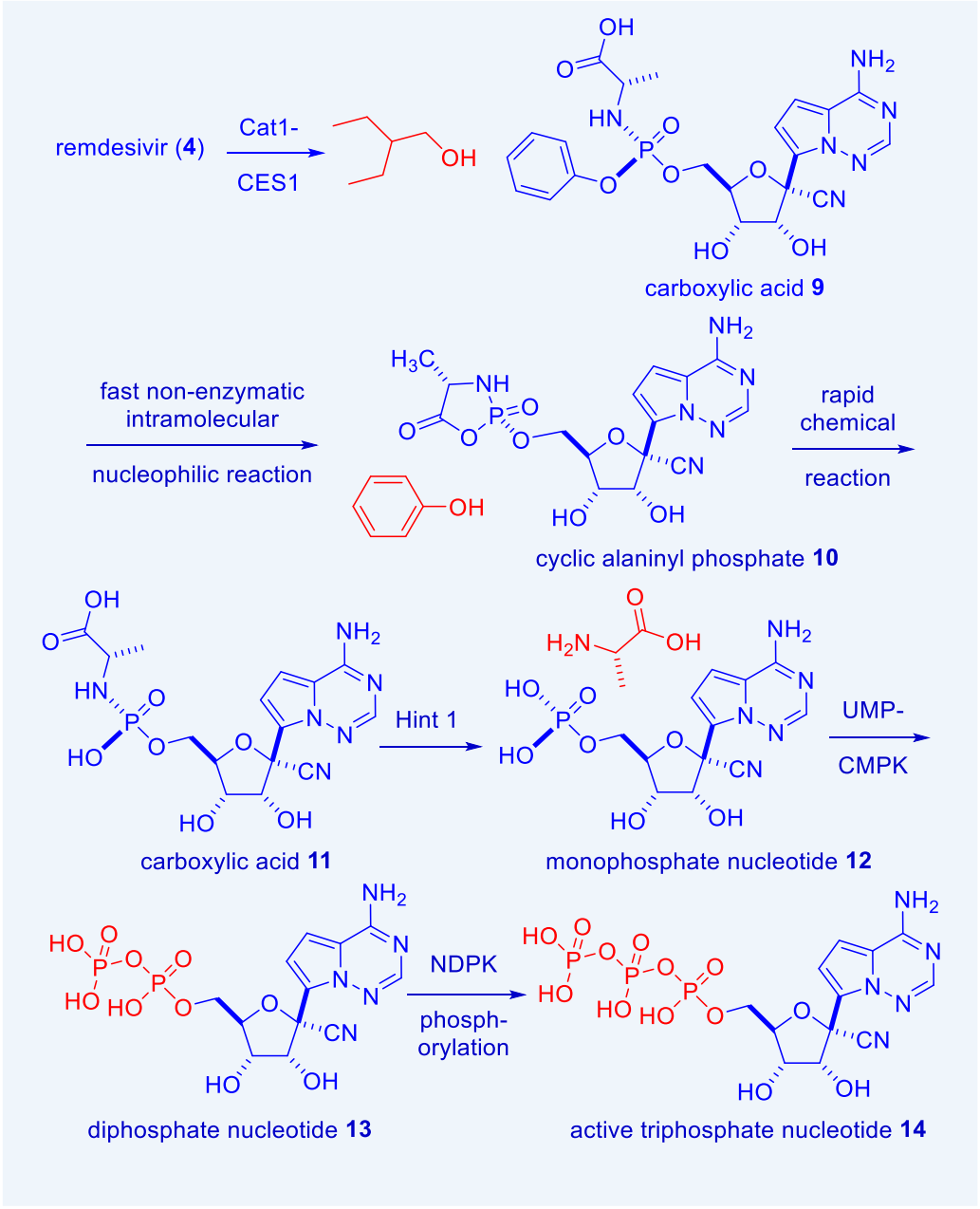
Nucleosides have long been recognized as direct-acting antivirals (DAAs). In terms of mechanism of action (MoA), remdesivir (**4**) belongs to a class of nucleoside antiviral drugs known as antimetabolites. By definition, antimetabolites are drugs that interfere with normal cellular function, particularly the synthesis of DNA that is required for replication. In another word, remdesivir (**4**) "pretends" to be a nucleoside building block and participates the viral DNA synthesis. But since it is not a *bona fide* DNA building block, DNA replication is interrupted and the virus is killed.

But the reality is not that simple. Because triphosphate nucleotide **14** (once a nucleoside is attached to a phosphate, it becomes a nucleotide) as the active antimetabolite has a low solubility and low bioavailability and could even be toxic. To overcome these shortcomings, the pro-drug tactic is resorted here, but with a twist. To achieve optimal pharmacokinetic and pharmacodynamic outcome, several forms of pro-drugs are installed onto triphosphate nucleotide **14**. Therefore, remdesivir (**4**) may be considered as a pro-drug of a pro-drug of a pro-drug of triphosphate nucleotide **14**.

As shown in the scheme, once remdesivir (**4**) is ingested orally, the key enzymes initially involved in the metabolism of remdesivir (**4**) are human cathepsin A 1 (CatA1) and carboxylesterase 1 (CES1), which are responsible for the hydrolysis of the carboxyl ester between the alaninyl moiety and the isopropyl alcohol. This stereospecific reaction gives rise to the corresponding carboxylic acid **9**. A nonenzymatic intramolecular nucleophilic attack then results in the formation of an alaninyl phosphate intermediate **10**, which undergoes a rapid chemical reaction to hydrolyze the cyclic phosphate to a linear phosphate as carboxylic acid **11**. The next step is speculated to involve the histidine triad nucleotide-binding protein 1 (Hint 1) enzyme in which the alaninyl phosphate intermediate is

deaminated to form a monophosphate nucleotide **12**. The final two steps involve consecutive phosphorylation reactions mediated by cellular kinases, uridine monophosphate–cytidine monophosphate kinase (UMP–CMPK) and nucleoside diphosphate kinase (NDPK), producing the diphosphate nucleotide **13** and subsequently the active triphosphate nucleotide **14**.[7] Triphosphate nucleotide **14** is then incorporated to the virus DNA to stop its replication. We eagerly look forward to seeing how remdesivir (**4**) fare in phase III clinical trials for treating COVID-19.

In addition to remdesivir (**4**), several additional series of pyrrolotriazine-containing drugs in the areas of nucleotide antiviral drugs and protein kinase inhibitors as anti-cancer drugs.



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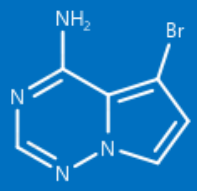

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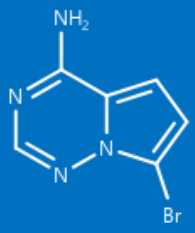

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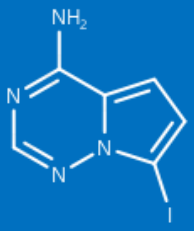

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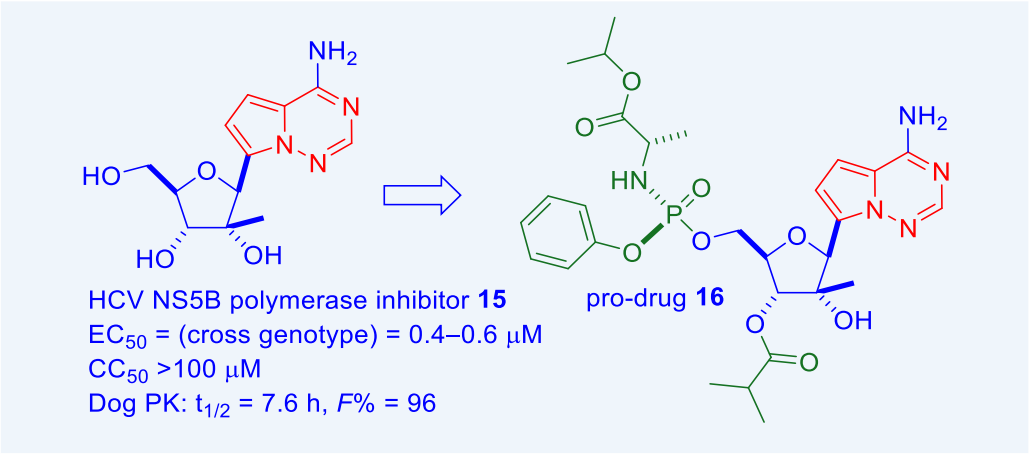
Biota and Boehringer Ingelheim collaborated to discover a series of pyrrolotriazine-containing C-nucleosides as potential novel anti-HCV agents. The adenosine analog **15** was found to inhibit HCV NS5B polymerase (its triphosphate to be exact) and have excellent pharmacokinetic properties. *In vitro*, C-nucleoside **15** was tested efficacious cross all genotypes with minimal cytotoxicity (CC_{50} >100μM). Unfortunately, it was not pursued as a drug candidate (DC) because adverse effects were observed in its rat safety studies due to cytotoxicities.[8] Prodrugs of pyrrolotriazine C-nucleoside **15** were prepared to overcome the toxicity issues. Very similar to Gilead's GS-6620 (**3**) structurally, pro-drug **16** was found to increase anti-HCV activity and enhance nucleotide triphosphate concentrations *in vitro*.[9]

In the realm of kinase inhibitors, both pyrrolotriazines and imidazolotriazines have been used as they are bioisosteres of adenine moiety of ATP, a key player of kinase functions.



Interleukin-1 receptor-associated kinase 4 (IRAK4) is an enzyme important in innate immunity, and its inhibition is predicted to be beneficial in treating inflammatory diseases. The fact that PF-06650833, a selective IRAK4 inhibitor, was advanced to phase I clinical trials for the treatment of RA and inflammatory bowel disorder (IBD). This has boosted the confidence in rationale (CIR) for this drug target.

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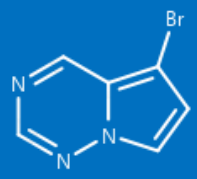

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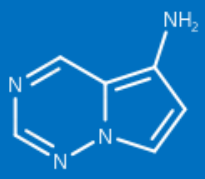

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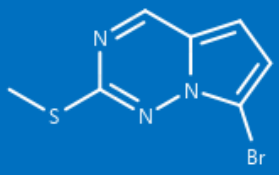

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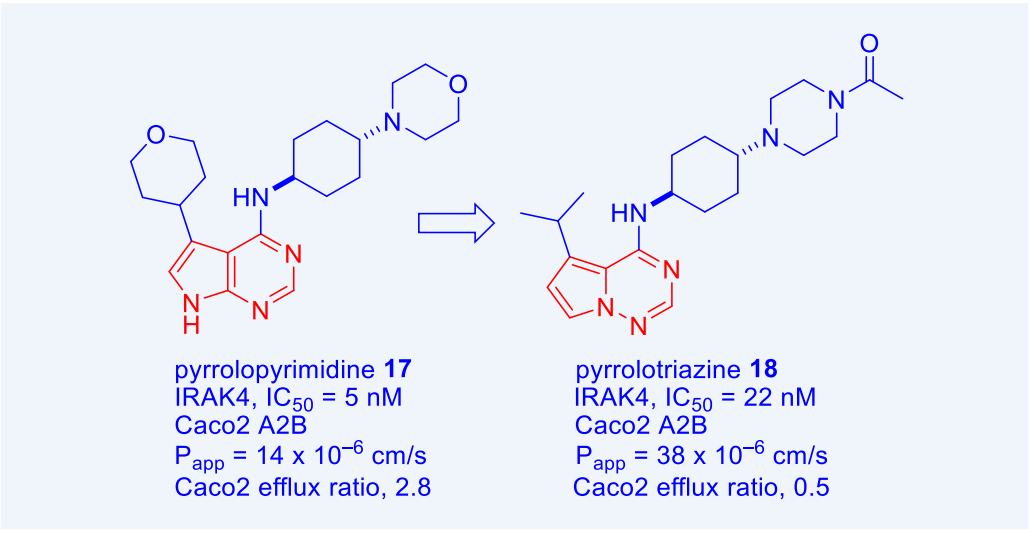
AstraZeneca initially obtained a series of pyrrolopyrimidines as represented by **17** as IRAK4 inhibitors. While the pyrrolopyrimidines were potent in both enzymatic and cellular assays, they suffered from low permeability and high efflux. A scaffold-hopping strategy led to the discovery of a series of pyrrolotriazines as analogous IRAK4 inhibitors. *In comparison to the pyrrolopyrimidines, the pyrrolotriazines contain one fewer formal hydrogen bond donor and are intrinsically more lipophilic.* Cell permeability increases as the number of hydrogen bond donors decreases. Optimization of the series culminated the discovery of pyrrolotriazine **18** with higher permeability and lower efflux in Caco2 assays in comparison to the prototype **17**. Pyrrolotriazine **18** was a promising *in vivo* probe to assess the potential of IRAK4 inhibition in cancer treatment. It demonstrated tumor regression in combination with ibrutinib, a covalent Bruton's tyrosine kinase (Btk) inhibitor.[10]



Just like pyrrolotriazines, imidazolotriazines are also used as fragments in nucleoside antivirals and kinase inhibitors. Furthermore, imidazolotriazines also found applications in the fields of PDE and histone deacetylase (HDAC) inhibitors.

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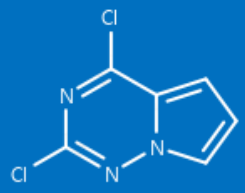

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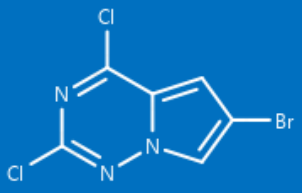

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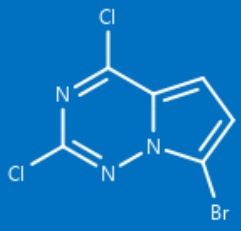

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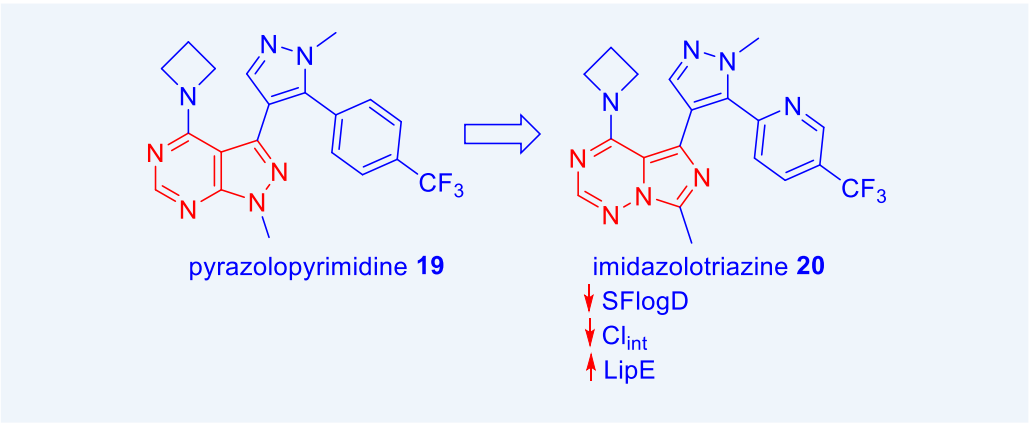
(*R*)-Roscovitine (seliciclib) is a cyclin-dependent kinase (CDK) inhibitor in phase II clinical trials. In a scaffold-hopping exercise, the purine core structure on (*R*)-roscovitine was replaced with its imidazolotriazine isostere, giving rise to an imidazolotriazine analog as a potent CDK inhibitor.[11]

PDE 2A inhibitors may have potential as central nervous system (CNS) drugs. Employing a late-stage microsomal oxidation of an existing PDE 2A inhibitor, Pfizer identified PF-06815189, an imidazolotriazinone derivative, with reduced clearance by cytochrome 450 (CYP450) enzymes, minimized the risk of drug–drug interactions (DDIs), and improved physiochemical properties.[12]

Pfizer identified pyrazolopyrimidine **19** as a potent, selective, and brain penetrant PDE 2A inhibitor. Its estimated human dose, 108 mg/day, is high. A design strategy to reduce its clearance in human liver microsomes (HLM C_{lint}) by reducing drugs′ lipophilicity led to the discovery of imidazolotriazine **20**. It is a potent, highly selective, and brain penetrant PDE 2A inhibitor clinical candidate with a significantly lower human dose, 30 mg/day, qd. It is likely that the core structure change boosted hydrogen bonding strength, thus reducing lipophilicity and clearance while elevating its lipophilic ligand efficiency (LipE).[13]



Synthesis of Some Pyrrolotriazine-containing Drugs

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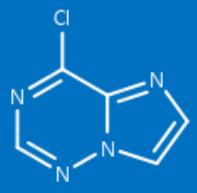

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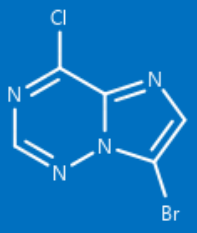

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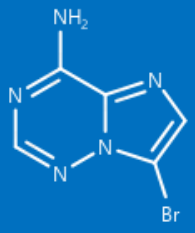

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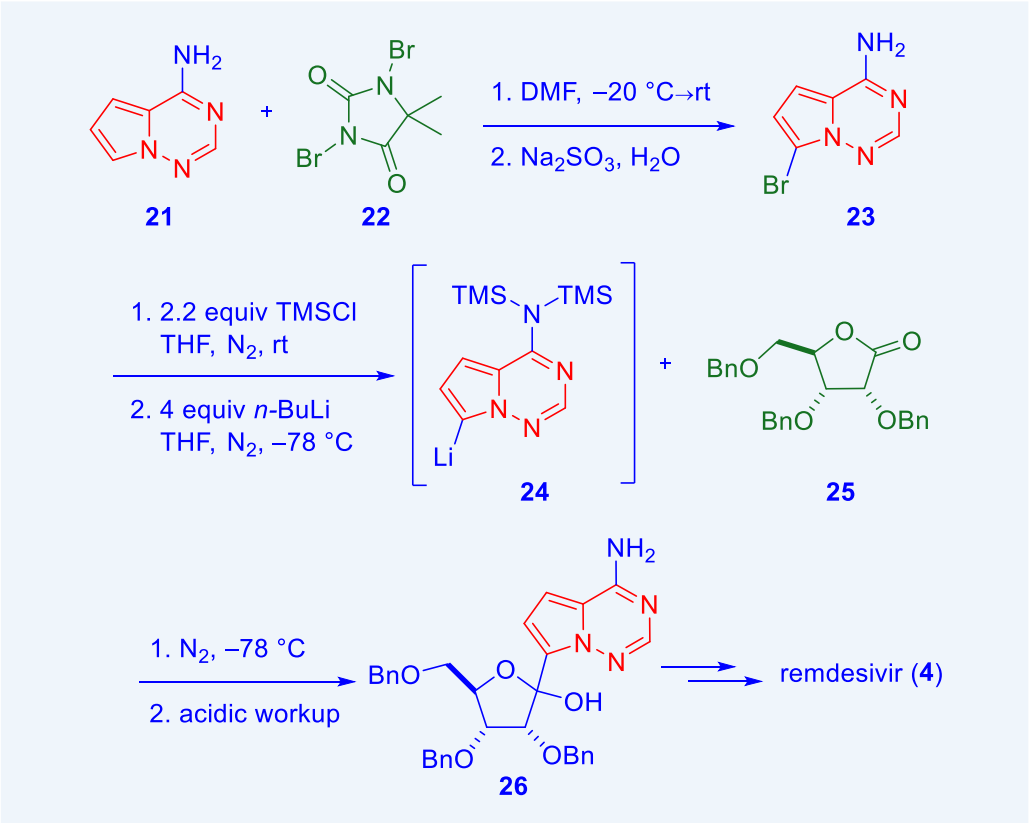
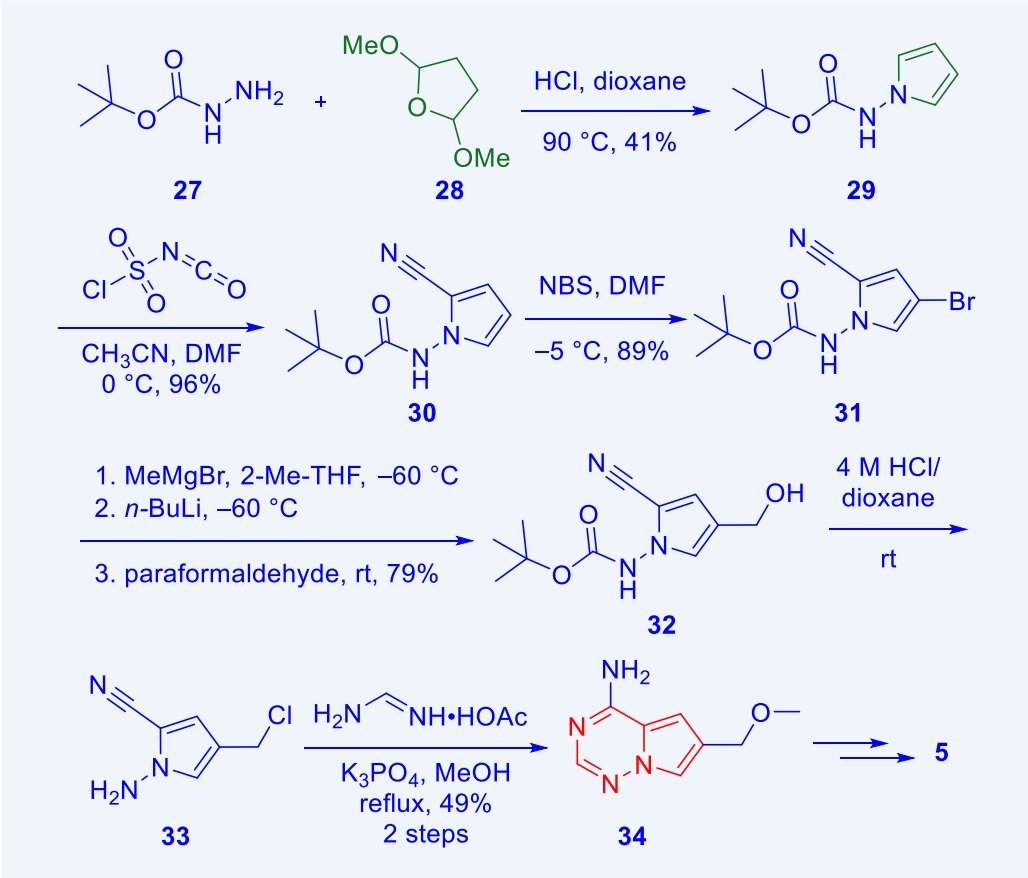
In Gilead's process-scale synthesis of remdesivir (**4**), aminopyrrolotriazine **21** was brominated with 1,3-dibromo-5,5-dimethylhydantoin (DBDMH, **22**) to prepare bromide **23**. After protecting the aniline with two TMS groups, halogen–metal exchange prepared the lithium intermediate **24**, which was added to a cold solution of lactone **25** to assemble lactol **26** as a 1:1 mixture of two anomers after quenching the reaction with a weakly acidic aqueous solution. Separation of the anomers and installation of the pro-drug motif then delivered remdesivir (**4**) after a few minor functional group manipulations.[4]



Preparation of the aminopyrrolotriazine fragment **30** of Bayer's pan-FGFR inhibitor rogaratinib (**5**) is accomplished in six linear steps. At first, an acid-catalyzed condensation of *tert*-butyl carbazate (**27**) and 2,5-dimethoxytetrahydro-furan (**28**) afforded the protected aminopyrrole **29** in 41% yield after crystallization. Subsequent C1 electrophilic substitution

was achieved by treating **29** with chlorosulfonylisocyanate followed by an intramolecular elimination to install cyanopyrrole **30**. Bromination of **30** at the C4 position was carried out at low temperature to boost the desired regioselectivity and minimize the overreaction (to dibromopyrrole). The resulting bromide **31** then underwent a halogen–metal exchange reaction and was quenched by paraformaldehyde to afford the hydroxymethyl-pyrrole **32**. Treating **32** with 4 M HCl not only removed the Boc protective group, but also converted the alcohol to chloride to produce **33**. The very reactive chloromethyl **33** was directly converted to methoxymethyl-pyrrolotriazine **34** in a one-pot, two-step reaction sequence by treating **33** with methanol, followed by formamidine acetate and potassium phosphate at reflux. The key intermediate **34** was employed as a building block to construct rogaratinib (**5**).[4]



For synthesis of imidazolotriazine-containing drugs, Pfizer's PDE 2A inhibitor clinical candidate imidazolotriazine **20** is showcased here as an example. As shown underneath, 4-bromo-1-methylpyrazole (**35**) was

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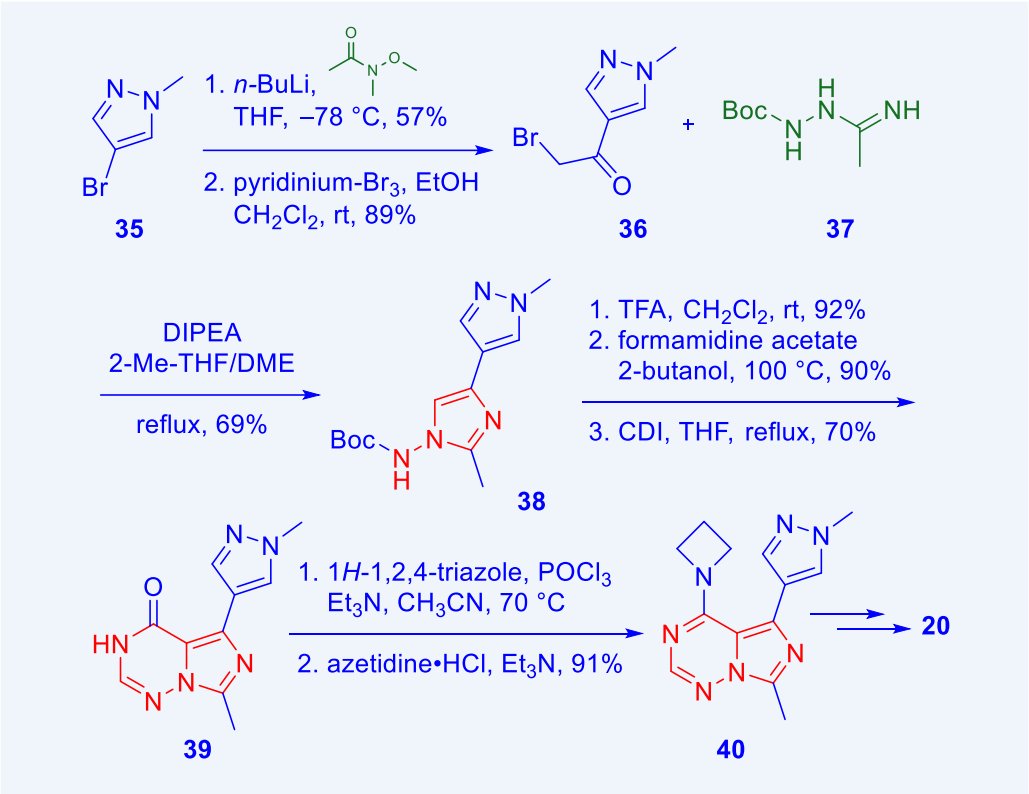
lithiated and trapped with N-methoxy-N-methylacetamide to give the methyl ketone, which was selectively brominated to afford α-bromoketone **36**. Condensation of **36** with N-aminoamidine **37** led to imidazole **38**. Treatment of **38** with TFA was followed by reaction with formamidine, and the resulting intermediate underwent an intramolecular cyclization with the aid of CDI to produce imidazolotriazinone **39**. Chlorination of **39** was followed by an S_NAr reaction with azetidine to assemble the key intermediate **40**, which was transformed to imidazolotriazine **20** in several additional steps.[13]



In summary, pyrrolotriazines and imidazolotriazines are bioisosteres for bases such as adenine, guanine, purine, etc. As a consequence, they have found utility in the fields of nucleoside antiviral drugs, kinase inhibitors, PDE, and HDAC inhibitors.

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