Pyrazines in Drug Discovery

Key Points

- May serves as a hydrogen bond acceptor to interact with an amino acid in the hinge region of the kinase protein

Overview

At least eight pyrazine-containing drugs have been approved by the FDA. In medicinal chemistry, pyrazine has been employed as bioisostere of benzene, pyridine, and pyrimidine. For pyrazine-containing kinase inhibitors, the pyrazine nitrogen atom frequently serves as a hydrogen bond acceptor to interact with an amino acid in the hinge region of the kinase protein.

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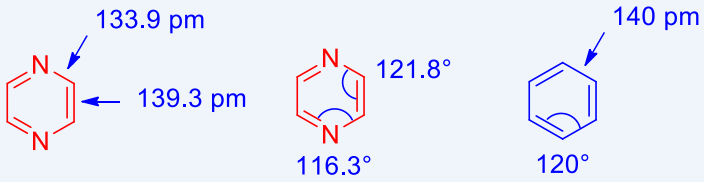
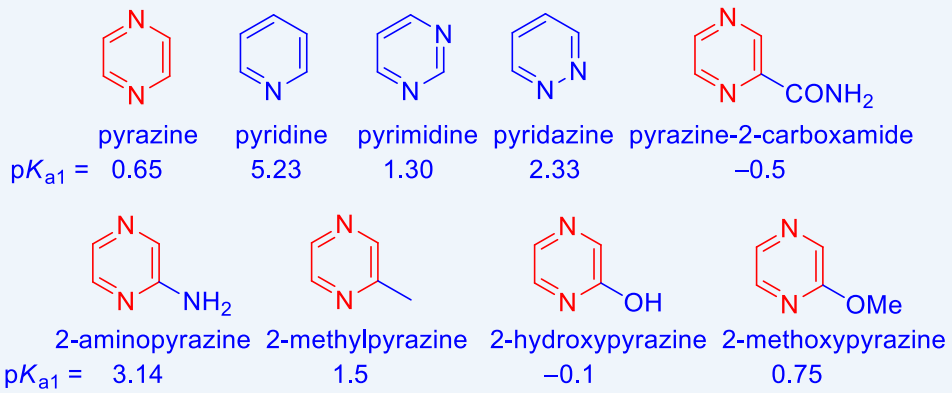
PharmaBlock designs and synthesizes over 1391 Pyrazines and 159 Pyrazine products are in stock. CLICK HERE to find detailed product information on webpage.

Structurally, pyrazine is a planar hexagon, similar to benzene, in both bond angles and lengths. Its C–N bonds are shorter and C–N–C bond angles are smaller than their phenyl counterparts.



Pyrazine's Bond Lengths and Angles

With 6 π-electrons, pyrazine is an electron-*deficient* (also known as electron-*poor*) aromatic heterocycle because of the increased electronegativity of the nitrogen atoms. Due to the presence of the electronegative nitrogen atoms, the electron density on the ring carbons is less than one. The lone-pair electrons do not take part in the delocalization for aromaticity so this molecule can act as a mild base. Its first pK_{a1} = 0.65 and second pK_{a2} = 5.78. Basic dissociation constants (pK_{a1}) of pyrazine, some pyrazine derivatives, and other diazines are listed below:[1]



Pyrazine-containing Drugs

There are approximately eight pyrazine-containing drugs on the market in the US. For instance, Pfizer's glipizide (Glucotrol, **1**) is an old sulfonylurea antidiabetic, which works by stimulating insulin secretion to metabolize carbohydrates. Its mechanism of action (MOA) is serving as a potassium channel blocker.

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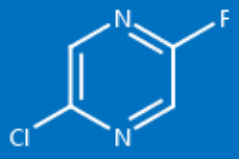

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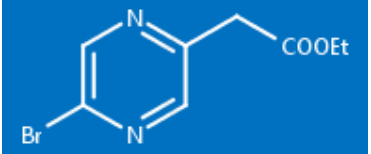
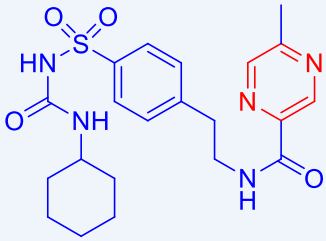


PBU3187

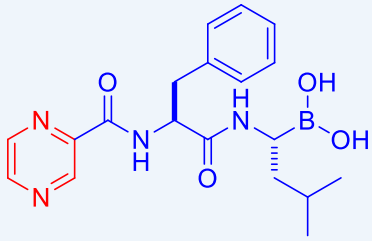
Vertex's bortezomib (Velcade, **2**) was the first proteasome inhibitor approved to treat relapsed multiple myeloma (MM) and mantle cell lymphoma. Mechanistically, the boron atom binds, with high affinity and specificity, to the catalytic site of 26S proteasome, which maintains the immortal type of myeloma.

Rhone–Poulenc Rorer's older sleeping pill, zopiclone (Imovane, **3**), functions as a $GABA_A$ modulator. It is no longer available in US because of its dubious benefit/risk profile. Having realized that the *S*-enantiomer is significantly more active and less toxic than the *R*-enantiomer, Separacor separated them and arrived at eszopiclone (Lunesta, **4**), the *S*-isomer.

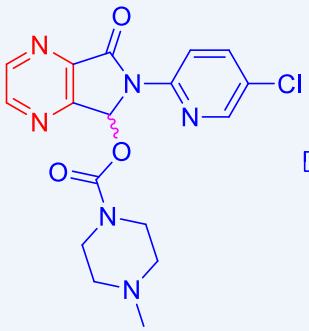
Also in the CNS arena, Pfizer's varenicline (Chantix, **5**) works as a partial agonist of α4β2 nicotinic acetylcholine receptor (nAChR) for helping smoke cessation.



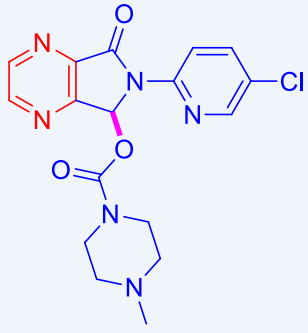
glipizide (Glucotrol, **1**)
Pfizer, 1984
sulfonylurea antidiabetic
(potassium channel blocker)



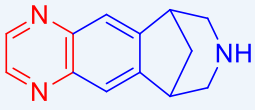
bortezomib (Velcade, **2**)
Vertex, 2003
Malignancy of bone marrow



zopiclone (Imovane, **3**)
not available in US
$GABA_A$ modulators for insomnia



eszopiclone (Lunesta, **4**)
Separacor, 2004
$GABA_A$ modulators for insomnia



varenicline (Chantix, **5**)
Pfizer, 2006
partial agonist of α4β2
nicotinic acetylcholine receptor (nACh)

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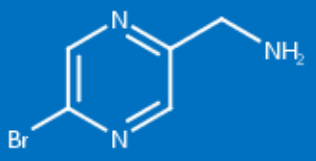

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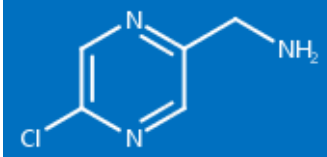
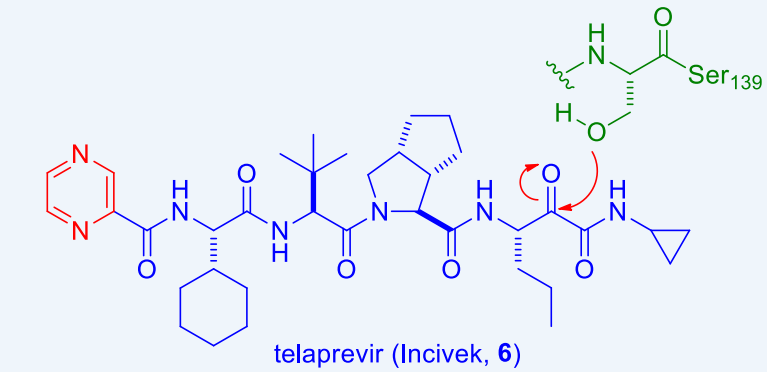


PB08068

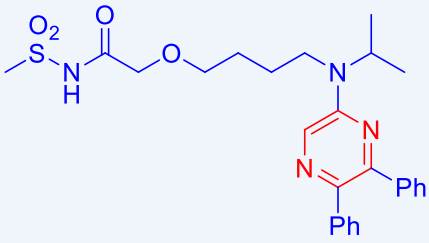
Vertex's telaprevir (Incivek, **6**) is an HCV NS3/4A serine protease inhibitor. It is a reversible covalent inhibitor—the protease's serine$_{139}$ forms an acetal tetrahedral intermediate with the ketone functional group of the terminal keto-amide "warhead."

Nippon Shinyaku licensed its PGI-2 receptor agonist selexipag (Uptravi, **7**) to Actelion. The drug is an oral treatment of pulmonary arterial hypertension (PAH). Interestingly, the *N*-methylsulfonamide is hydrolyzed to the corresponding carboxylic acid *in vivo* to provide a slow-release pharmacological effect.

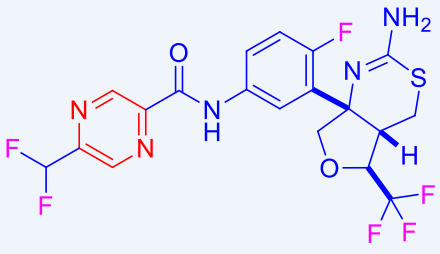
The quest for a new treatment of Alzheimer's disease (AD) has met with failures too numerous to count. Eisai/Biogen's elenbecestat (**8**) is a β-secretase-1 (BACE1, also known as beta-site amyloid precursor protein cleaving enzyme) inhibitor that failed phase III clinical trials for lack of efficacy.



telaprevir (Incivek, **6**)
Vertex, 2011
HCV NS3/4A serine protease inhibitor



selexipag (Uptravi, **7**)
Nippon Shinyaki/Actelion, 2015
PGI-2 receptor agonist



elenbecestat (**8**)
Eisai/Biogen
BACE1 inhibitor

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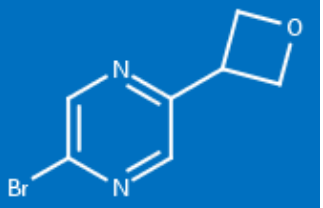

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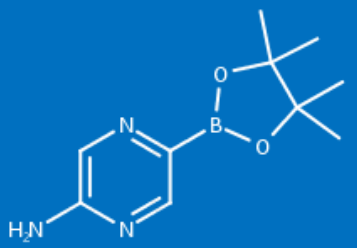
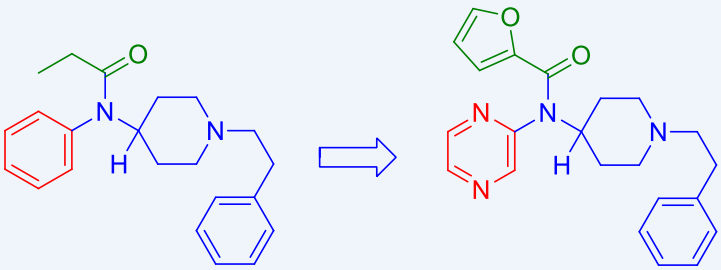


PB93487

Pyrazines in Drug Discovery

In drug discovery, pyrazine has served as a bioisostere for phenyl and heteroaryl moieties. For instance, mirfentanil (**10**), a μ opioid agonist, is an analog of fentanyl (Duragesic, **9**). Here, pyrazine on mirfentanil (**10**) replaced fentanyl (**9**)'s phenyl group.[1] One of the factors that contributed to today's opioid epidemic is the fact that so many fentanyl analogs exist, which makes even their detection a challenge.



fentanyl (Duragesic, **9**)
Janssen, 1968
μ opioid agonist

mirfentanil (**10**)
μ opioid agonist

Bayer's sorafenib (Nexavar, **11**) is a dual platelet-derived growth factor receptor (PDGF) and vascular endothelial growth factor receptor (VEGF) inhibitor. An exercise of replacing the pyridine ring on sorafenib (**11**) with pyrazine led to a series of analogs. Their biochemical activities suggested that the substituents on urea is essential for interaction with c-Raf. One of the pyrazine derivatives **12** exerted cytostatic activities that surpassed sorafenib (**11**) in inhibitory effects on proliferation of cancer cell lines including Hela, A549, and HepG2.[2]

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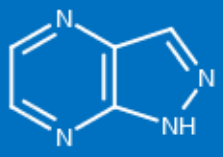

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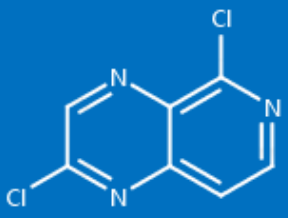

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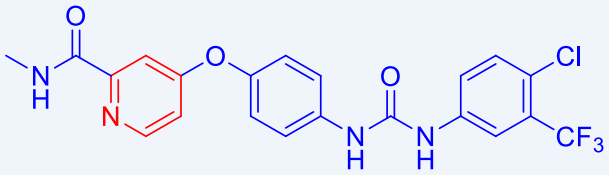
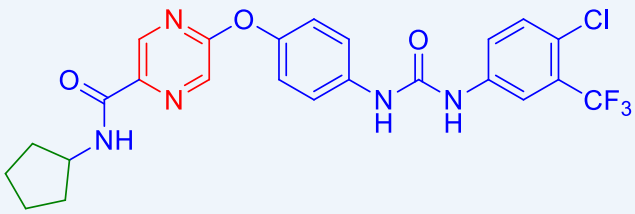


sorafenib (Nexavar, **11**)
Bayer/Onyx, 2007
PDGF and VEGF inhibitor
HeLa cell , IC_{50} = 7.1 nM
A549 cell , IC_{50} = 6.1 nM
HepG2 cell , IC_{50} = 6.2 nM



pyrazine analog **12**
HeLa cell , IC_{50} = 0.6 nM
A549 cell , IC_{50} = 0.8 nM
HepG2 cell , IC_{50} = 0.7 nM

Point mutations in isocitrate dehydrogenase (IDH) 1 and 2 are found in multiple tumors, including glioma, cholangiocarcinoma, chondro-sarcoma, and acute myeloid leukemia (AML). Agios's ivosidenib (Tibsovo) is a potent, selective, and, more importantly, metabolically stable, IDH1 inhibitor that was approved by the FDA for the treatment of IDH1-mutant cancers.

Novartis reported a novel class of mutant IDH1 (mIDH1) inhibitors with a 3-pyrimidin-4-yl-oxalidin-2-one motif. Among them, NI-1 (**13**) inhibited both biochemical and cellular production of oncometabolite D-2-hydroxyglutatrate (D2HG) with an IC_{50} value of 94 nM (reduced D2HG level by 24.7% at 50 μM) for IDH1 R132H. Mutant R132H is the predominant mutant of the IDH1 enzyme where Arg_{132} is substituted by His. Employing pyrazine as a bioisostere for NI-1 (**13**)'s pyridine gave rise to 3-pyrazine-2-yl-oxazolidin-2-one (**14**). It too effectively suppressed the D2HG production level in cells transfected with IDH1-R132H mutation. Pyrazine analog **14** also showed a good ability to penetrate the blood-brain barrier (BBB) using a parallel artificial membrane permeability assay (PAMPA).[3]

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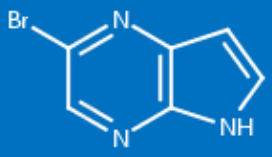

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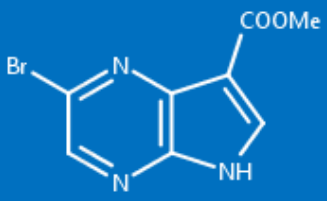

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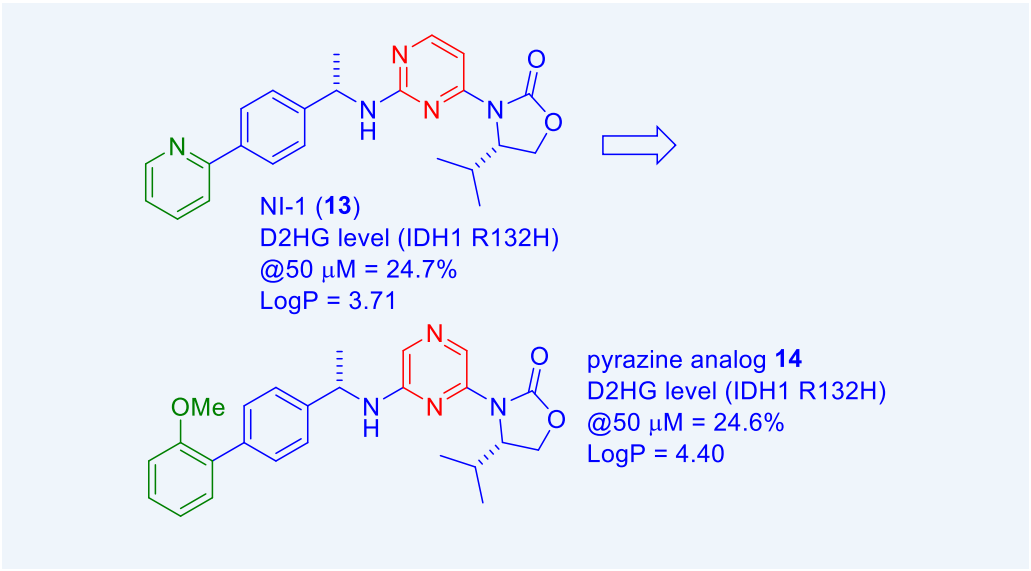
Docking analysis of both NI-1 (**13**) and pyrazine **14** revealed that the carbonyl of their oxazolidinone establishes a hydrogen bond with the amine of Leu_{120}. While pyrazine **14**'s C2-amine group forms a donor–acceptor polar interaction (a salt bridge) with Ile_{128}, its 4-N atom of its pyrazine cannot interact with Ile_{128} although NI-1 (**13**) does. This may be the reason why pyrazine **14** showed decreased inhibitory activity compared to NI-1 (**13**).[3]



Checkpoint kinase 1 (CHK1) is an intracellular, serine/threonine kinase that plays a central role in the DNA damage response pathway. CHK1 inhibitors, meanwhile, are of current interest as potential antitumor agents although many advanced CHK1 inhibitors are not orally bioavailable. From hybridization of two lead scaffolds derived from fragment-based drug design (FBDD) and subsequent optimization, CCT244747 (**15**) was obtained using a cellular-based assay cascade. Although the compound showed high biochemical kinase potency and selectivity for CHK1-dependent mechanism of action (MOA) in human cancer cells, its micromolar human ether-a-go-go-related gene (hERG) inhibition was a source of concern.[4a]

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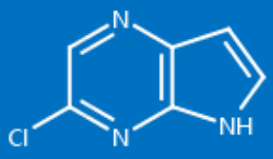

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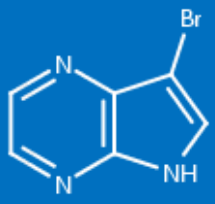

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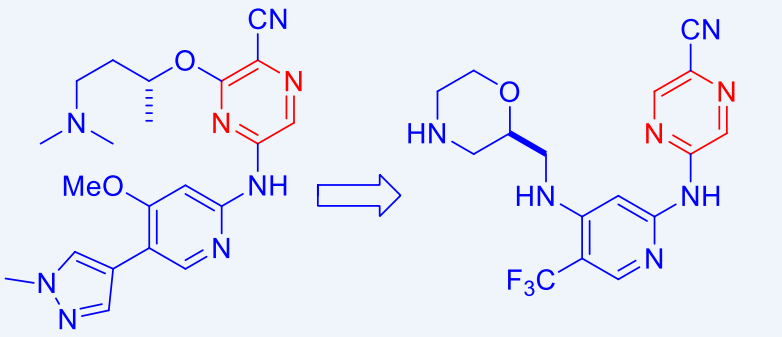
Generally speaking, hERG inhibition is dependent on lipophilicity and basicity of the compounds. During further optimization of **15**, its 5-aminopyrazine-2-carbonitrile group was retained because it was shown, through extensive structural biology, to optimally interact with unique *protein-bound water molecule* in CHK1, conferring high selectivity over other kinases. Eventually, extensive SAR produced CCT245737 (**16**) as a potent and selective CHK1 inhibitor. It also has low predicted doses and exposure in humans which mitigated the residual weak *in vitro* hERG inhibition. It is now a clinical candidate as an oral treatment of RAS-mutant non-small cell lung cancer (NSCLC) and Eμ-MYC driven B-cell lymphoma.[4b]

The crystal structure of CCT245737 (16) bound to CHK1 revealed that the cyanopyrazine was positioned close to the side-chain of Lys38. Importantly, both the nitrile and N-4 of the pyrazine ring were positioned to interact with protein-bound water at the entrance to the pocket beyond the gatekeeper residue, one of a network of conserved water molecules resulting from the presence of the unique polar residue Asn59 in this pocket in CHK1 instead of the more common lipophilic side chains. Interactions with these bound waters are a CHK1 selectivity determinant for this and other series of CHK1 inhibitors including other pyrazines.[4b]



CCT244747 (**15**)
CHK1 IC_{50} = 7.7 nM
hERG IC_{50} = 5.0 μM

CCT245737 (**16**)
CHK1 IC_{50} = 1.3 nM
CHK2 IC_{50} = 2,440 nM

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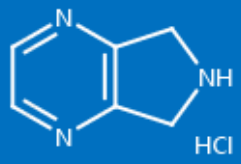

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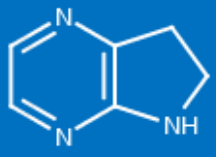

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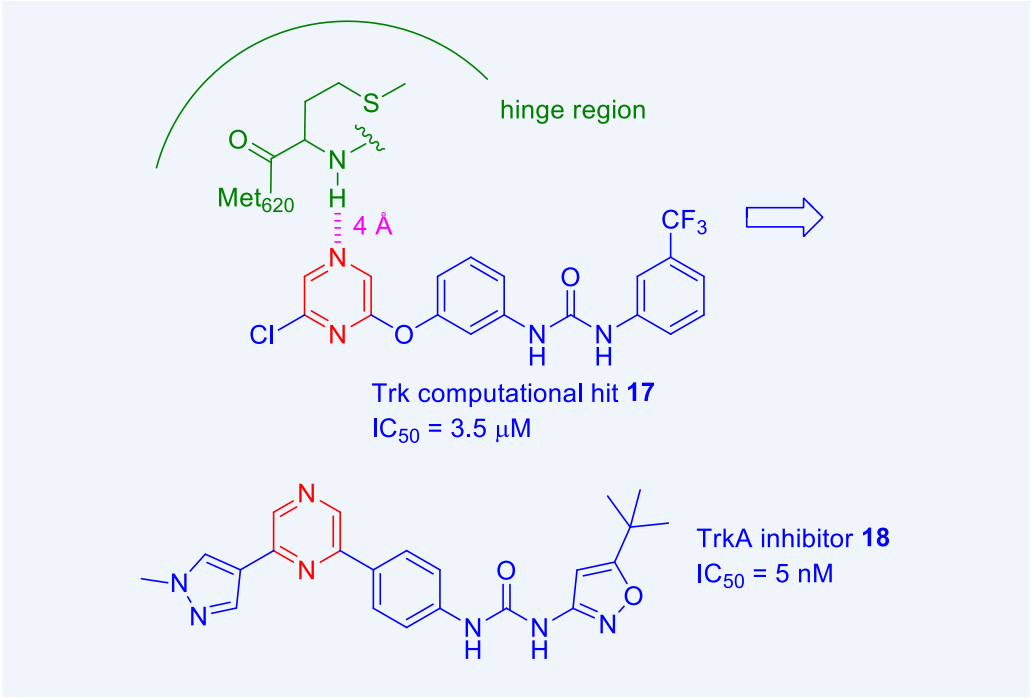
Even though electron-deficient, pyrazine is still an aromatic ring. Like many aromatic rings, pyrazine has made frequent appearance as a fragment of receptor tyrosine kinase (RTK) inhibitors. One of the two nitrogen atoms on pyrazine may serve as a hydrogen bond acceptor (HBA) to form a hydrogen bond with target kinase protein.

Tropomyosin receptor kinase (Trk) was first discovered as an oncogene that is activated through chromosomal rearrangement in human colon carcinoma. A computational Trk hit **17** was generated from a kinase-directed virtual library screen. Crystal structure of compound **17** and TrkA indicated that it forms a hydrogen bond at the kinase hinge through a weak hydrogen bond (~4 Å) with Met_{620}. Extensive SAR investigations identified compound **18** as a potent and selective TrkA inhibitor (there are three Trk isoforms: TrkA, TrkB, and TrkC).[5]



Spleen tyrosine kinase (Syk) is a cytosolic non-receptor protein tyrosine kinase that plays an essential role in immune-receptor signaling, mainly in B cell receptors. X-Ray structure of a Syk inhibitor **19** bound to Syk indicated that its aminopyrazine motif forms that key hydrogen bond actions with Glu_{449} and Ala_{451} in the hinge region.[6]

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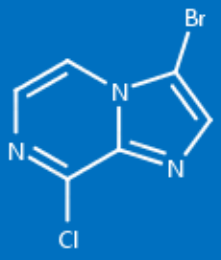

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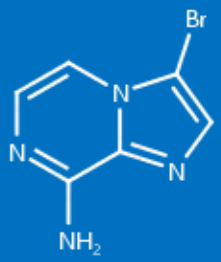

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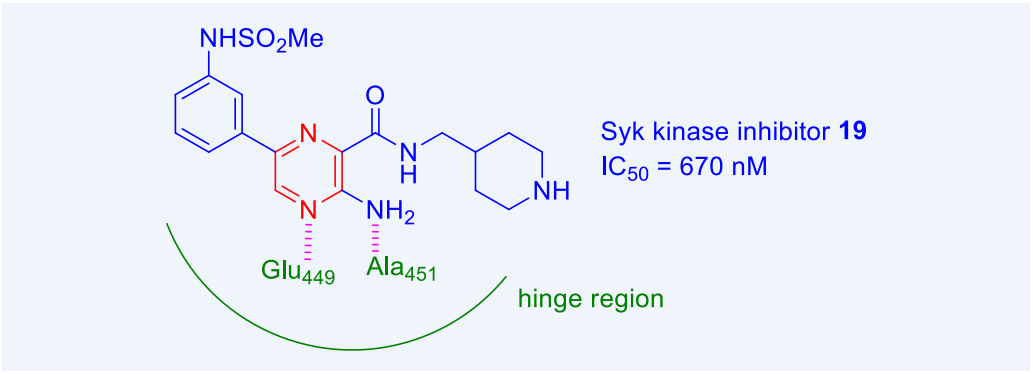
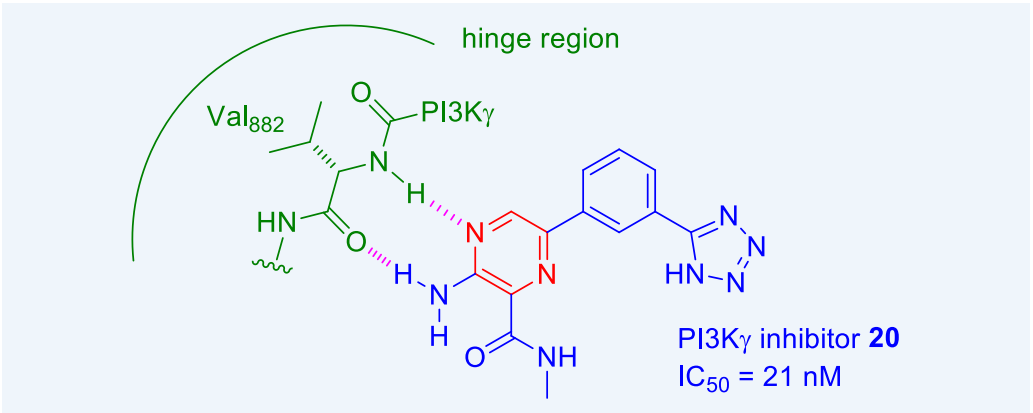


Deregulation of the well-known phospoinositide-3-kinase (PI3K) pathway has been implicated in numerous pathologies such as cancer, diabetes, thrombosis, rheumatoid arthritis (RA), and asthma. Two PI3K inhibitors have been approved by the FDA. One is Gilead's idelalisib (Zydelig), which is a PI3Kδ selective inhibitor. And the other is Bayer's copanlisib (Aliqopa) that is a pan-PI3K inhibitor.

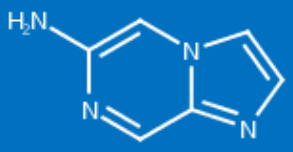
A novel series of potent, selective (against PI3Kα, PI3Kβ, and PI3Kδ), and orally bioavailable PI3Kγ inhibitors, as represented by inhibitor **20**, have been reported. Cocrystal structure of **20** bound to PI3Kγ indicated that its aminopyrazine moiety forms key hydrogen bonding with kinase hinge residue Val_{882}.[7]



Synthesis of Some Pyrazine-containing Drugs

For preparation of Vertex's proteasome inhibitor bortezomib (**2**), its pyrazine motif was installed at the end of the synthesis. Amide formation between pyrazine-carboxylic acid (**21**) and pinanediol-boronate intermediate **22** was facilitated by 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethylaminium tetrafluoroborate (TBTU) as the coupling agent. Conversion of pinanediol-boronate to the corresponding boronic acid was carried out using 1 N HCl and isobutylboronic acid to deliver bortezomib (**2**).[8]

PharmaBlock Products



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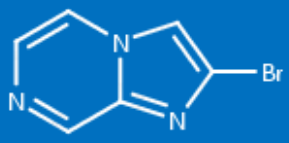
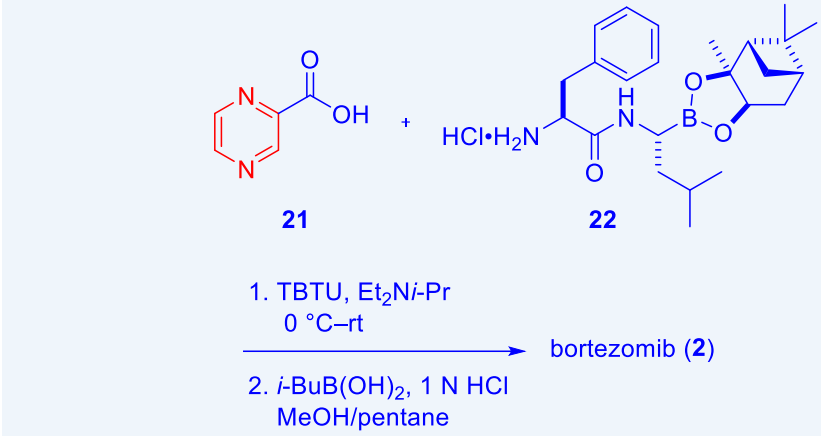
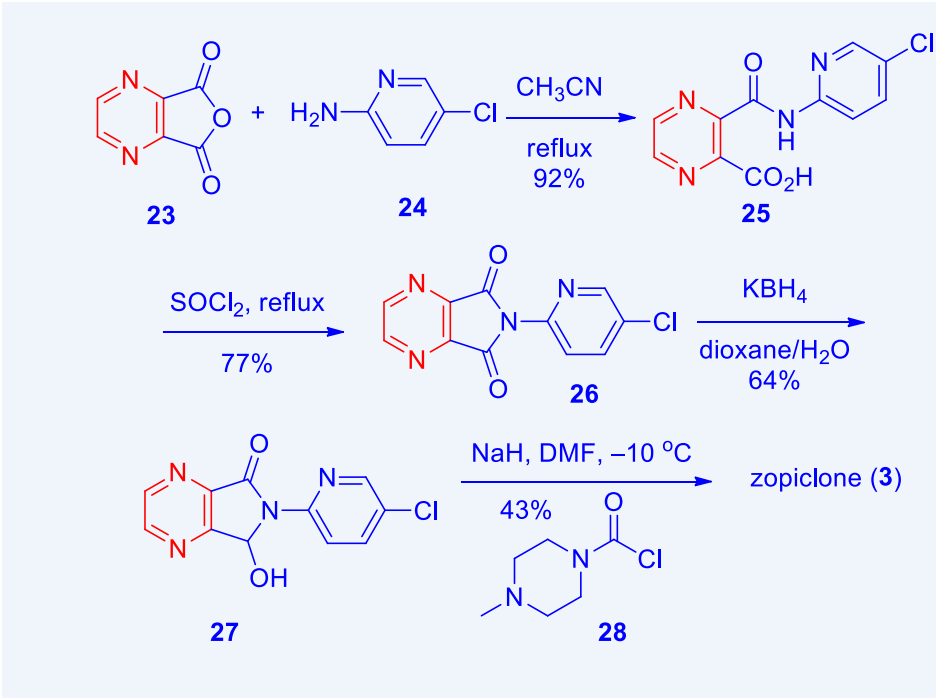


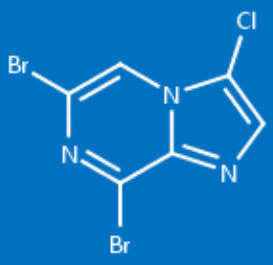
PBZ3482



Rhone–Poulenc's synthesis of racemic zopiclone (**3**) commenced with the treatment of pyrazine anhydride (**23**) with 2-amino-5-chloropyridine (**24**) to give amide **25** in good yield. Refluxing amide **25** with thionyl chloride produced imide **26**. Mono-reduction of the imide with KBH_4 afforded alcohol **27**. Acylation of alcohol **27** with 4-methylpiperazine-1-carbonyl chloride (**28**) using NaH in DMF then produced racemic zopiclone (**3**).[9]



PharmaBlock Products



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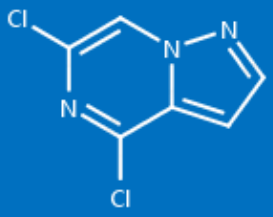
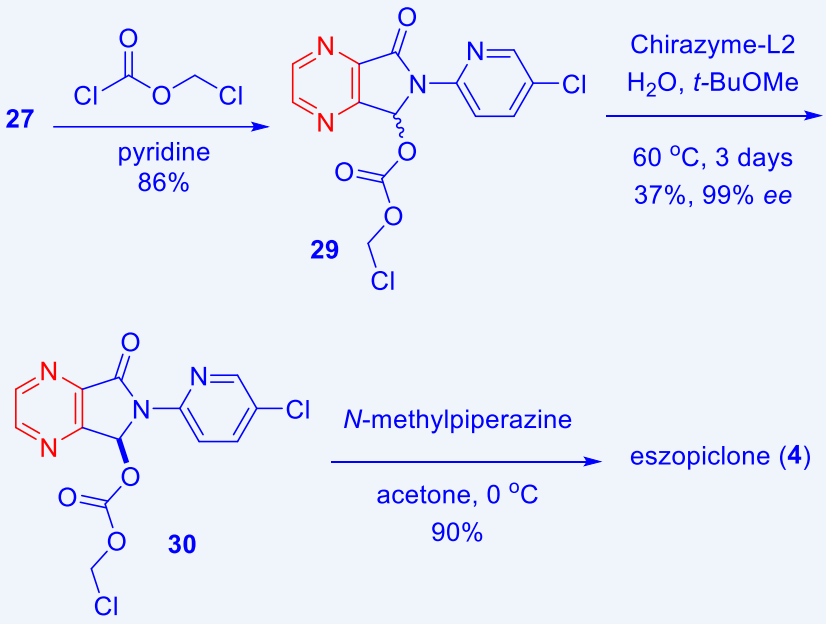


PBLJD1172

Several methods have been described for the preparation of the eszopiclone (**4**). Chiral resolution of **3** by diastereomeric salt formation and recrystallization has been described with both malic acid and O,O′-dibenzoyltartaric acid. Another conceptually different approach involving an enzymatic resolution of carbonate intermediates has also been described.[10] An immobilized form of lipase B from *Candida Antarctica* (Chirazyme-L2) catalyzed the hydrolysis of carbonate **29** to give optically active carbonate **30** with the correct absolute (*S*)-stereochemistry required for the synthesis of eszopiclone (**4**) and racemic **27**. The enzyme preparation can be recycled ten times without any loss to the activity of the catalyst or enantioselectivity of the reaction. Furthermore, racemic **27** can be recycled to improve the efficiency of the asymmetric synthesis.[10]



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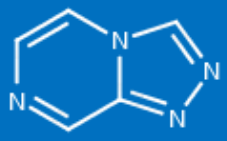
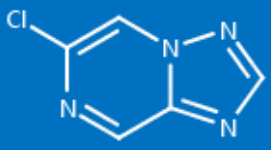


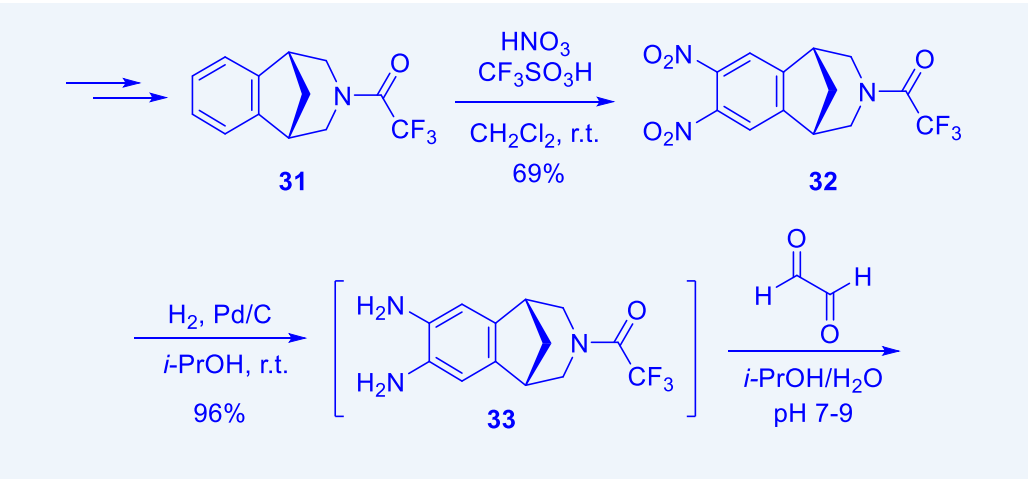
PBZ0157

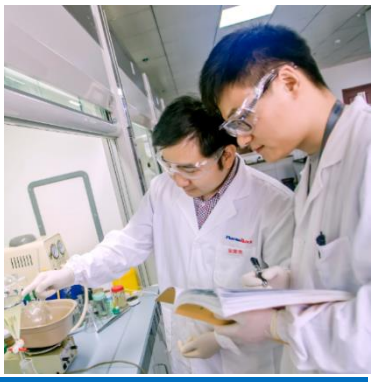


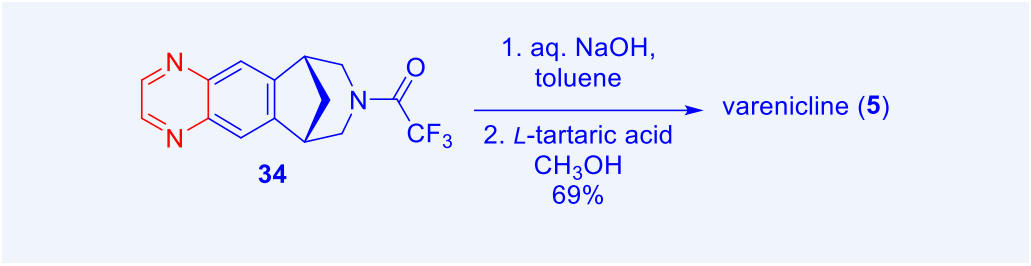
PBU1590

Many synthetic routes have been published for the preparation of varenicline (**5**). In one of Pfizer's process routes, bicyclic intermediate [3,2,1]-benzazepine (**31**) was assembled before installation of the pyrazine ring. Trifluoroacetamide protection was specifically introduced to insulate the nitrogen by removing electron density to avoid the formation of doubly charged cationic intermediates. This protection allowed nitration to proceed. With an excess of 2 equivalents of nitronium triflate, dinitrated product **32** was obtained in >75% yield. Regioselectivity for this conversion likely derives from steric and electronic factors driven by the bicyclic core, leading to 7:1–11:1 preference for the desired *ortho*-dinitro over the *meta*-dinitro regioisomer.[11]

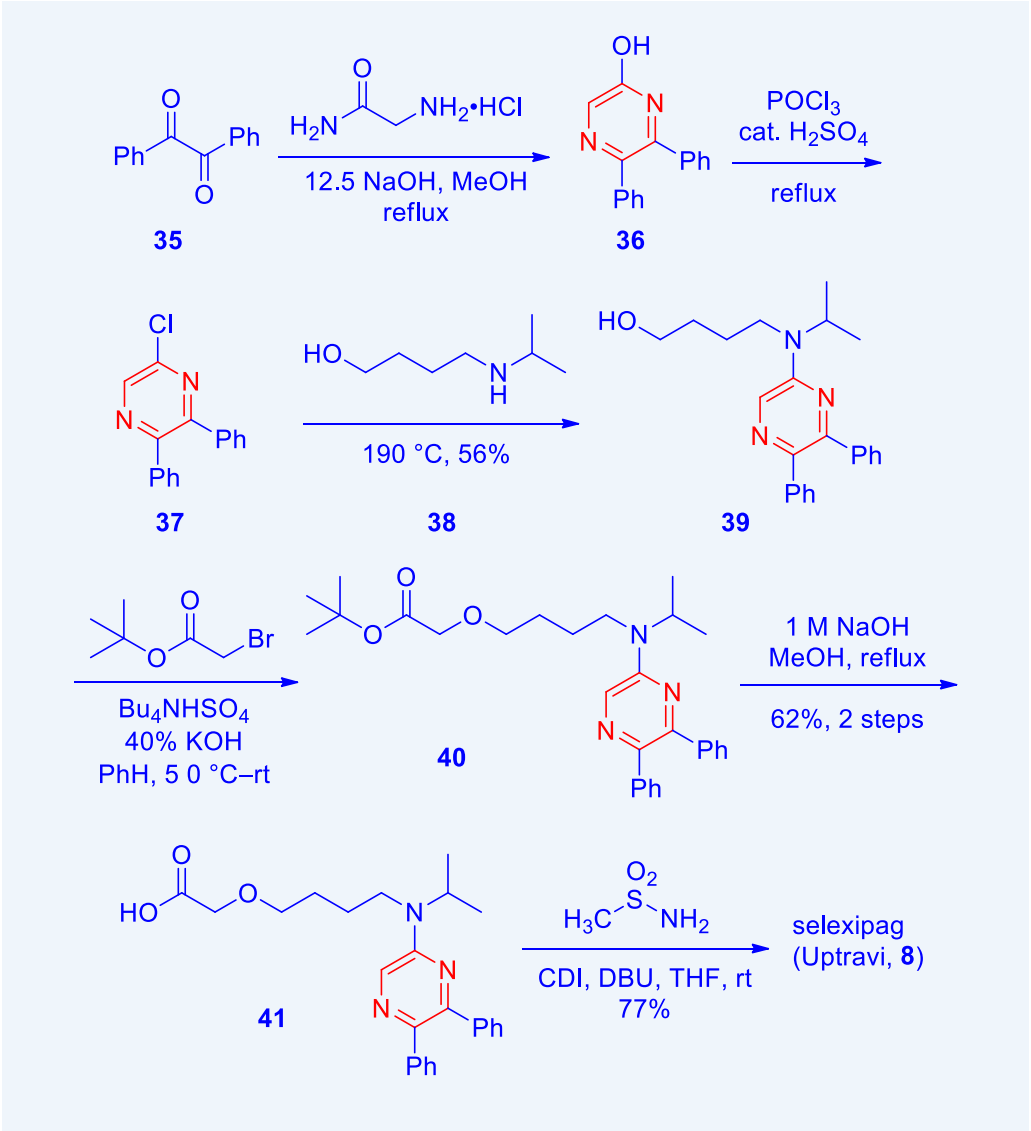
Dinitro intermediate **32** is readily reduced to dianiline **33** via palladium-catalyzed hydrogenation. Although dianiline **33** is stable in solid form, it decomposes after prolonged standing in solution, thus processes were set up without isolation of **33** using direct addition of aqueous glyoxal to form the desired quinoxaline **3**4. Added bicarbonate controlled the pH to avoid unwanted side products in this step. The process was completed with rapid and quantitative trifluoroacetamide hydrolysis via treatment with sodium hydroxide, thus combining steps via non-isolated intermediates and telescoping directly into the formation of the L-tartrate salt in methanol to deliver varenicline (**5**)-tartarate. The reaction control of the tartrate salt polymorph to the desired form B has been described by Rose and co-workers.[12]







A synthetic route of PGI-2 receptor agonist selexipag (**7**) began with condensation of benzil (**35**) with glycinamide to assemble hydroxypyrazine **36**. Chlorination of **36** in refluxing $POCl_3$ was facilitated by a catalytic amount of H_2SO_4 to afford chloride **37**. S_NAr displacement of the chloride by amino-alcohol **38** gave rise to adduct **39**. A phase-transfer catalyzed S_N2 reaction between **39** and *tert*-butyl bromoacetate provided ether **40**. After basic hydrolysis of **40** to make acid **41**, it was converted to selexipag (**7**) by coupling acid **41** with methanesulfonamide.[13]



In summary, at least eight pyrazine-containing drugs have been approved by the FDA. In medicinal chemistry, pyrazine has been employed as bioisostere of benzene, pyridine, and pyrimidine. For pyrazine-containing kinase inhibitors, the pyrazine nitrogen atom frequently serves as a hydrogen bond acceptor to interact with an amino acid in the hinge region of the kinase protein.

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