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Pyrrolopyrimidine: A Versatile Scaffold for Construction of Targeted Anti-cancer Agents

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ABSTRACT

Pyrrolopyrimidine derivatives comprise a class of biologically active heterocyclic compounds that are well known to play a critical role in pharmaceutical drug design and health care. The pyrrolopyrimidines serve as promising scaffolds that were found in a number of biologically active compounds including antibiotics, anti-inflammatory, anti-viral and anticancer. Researchers were inspired to develop novel pyrrolopyrimidine derivatives for the treatment of Cancer. Currently several pyrrolopyrimidines are available commercially as anticancer drugs. The present review article offers a detailed account on the development of pyrrolopyrimidine derivatives with anticancer potential with emphasis on their activity as targeted kinase inhibitors.

Keywords: *Anti-cancer; Protein kinases; Pyrrolopyrimidine*

INTRODUCTION

Pyrrolopyrimidine derivatives occupy an important place among various heterocyclic systems because they include compounds with different types of biological activity. Furthermore, these bicyclic compounds represent structural analogues of biogenic purines and hence they can be regarded as potential antimetabolites in nucleic acid metabolism. These data have served and continue to serve as a basis for the synthesis of a wide variety of derivatives of pyrrolopyrimidines in order to discover physiologically active substances among them.[1]

Pyrrolopyrimidines have aroused recent attention from chemical and biological view points since they have useful properties as ant-inflammatory[2], antimicrobial[3,4], antiviral[5] and anti-cancer[6-11]. They have useful properties as antimetabolites in purine biochemical reactions. Several mechanisms are also involved in their cytotoxic activities as being antifolate inhibitors of dihydrofolate reductases[12].

The pyrrolopyrimidine scaffold is being extensively investigated and in the last few years many of such compounds resulted active as kinase inhibitors. The present review highlights the anticancer activity of pyrrolopyrimidine derivatives through inhibition of specific kinase enzymes.

Synthetic strategies

Pyrrolo[2,3-*d*]pyrimidine derivatives synthesis procedures are very abundant in the literature.[1] In general, it is favorable to synthesize the pyrrole ring first and to subsequently build up the pyrimidine ring, even though the opposite strategy has been applied as well[13].

Traditionally, There are two approaches toward the synthesis of pyrrolo[2,3-*d*]pyrimidines. One approach is that the pyrimidine ring is constructed on a substituted pyrrole, via two consecutive amide bonds using urea derivatives, esters, and formamides or their retro-synthetic analogues, e.g., isocyanates or ortho-esters. A second very common approach is the synthesis of 4- aminopyrimidines, containing a 2-oxo-ethyl (or alkyl) substituent at the 5-position. [14]These two substituents can then be condensed to form the desired fused pyrrole. However, the introduction of the 2-oxo-alkyl substituent is performed by reaction of 4- amino-

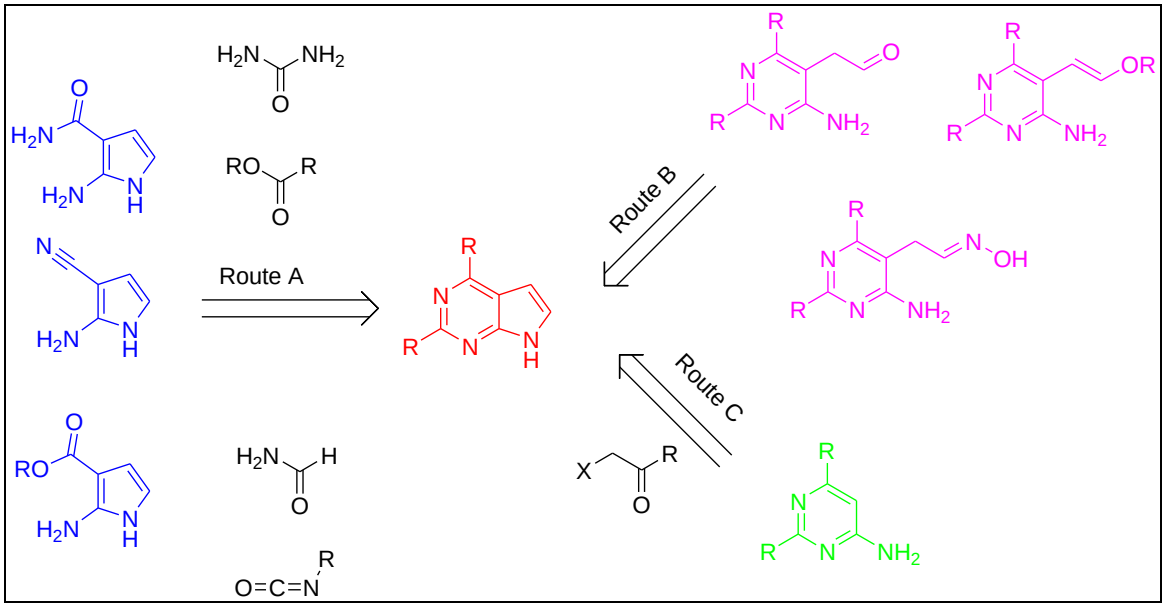


Figure 1. Classical Approaches to Pyrrolo[2,3-*d*]pyrimidines.

pyrimidines with α- halogenated ketones or aldehydes and is combined with the subsequent pyrrole condensation in a one-pot procedure **(Figure 1)**.[15]

N1- Substituted-2-amino-3-cyanopyrroles are essential precursors for the preparation of pyrrolo[2,3-*d*]pyrimidines bearing reactive functionalities such as amino or oxo group at C4. N1- substituted pyrrole with alkyl and benzyl groups was utilized and described in literature as precursors to prepare the N7-unsubstituted pyrrolo[2,3-*d*]pyrimidine.[16,17]A dealkylation reaction in polyphosphoric acid, followed by ammonium hydroxide neutralization was descriped for elimination of N1 alkyl substitution. The Aryl protecting group was removed with $AlCl_3$ in toluene to give the final unsubstituted derivatives.[16,17]

2-amino-3-cyanopyrrole could be refluxed in 85% formic acid or formamide to afford the corresponding pyrrolo[2,3-*d*]pyrimidine-4-one or pyrrolo[2,3-*d*] pyrimidine-4-amine respectively.[1,16]

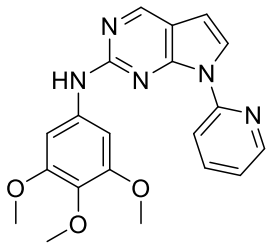
Pyrrolopyrimidines as kinase inhibitors and potential anticancer agents

Protein Kinase inhibitors

Protein kinases play an important role in signal transduction pathways that regulate numerous cellular functions, including proliferation, differentiation, migration, apoptosis, and angiogenesis. The up regulation of the signal transduction pathways is observed in tumor cells, this makes inhibitors of protein kinases that target the up-regulated pathways attractive candidates for cancer therapy.[13,14] Kinase inhibitors have played an increasingly important role in the treatment of cancer. A detailed study on the role of pyrrolopyrimidine derivatives in inhibition of protein kinases is demonstrated below.

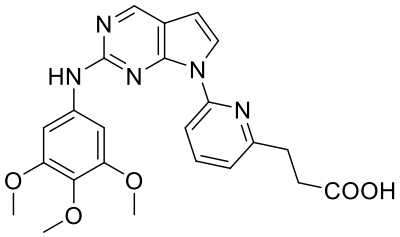
FAK

Focal adhesion kinase (FAK) is a tyrosine kinase that plays an important role in cellular movement and survival pathways. FAK is a potential target for the treatment of both primary cancers and the prevention of tumor metastasis.[15-17] **(Compound 1)** showed submicromolar inhibitory activity against FAK, and a co-crystal structure of this with FAK was successfully obtained (pdb code 2ETM).[18]



Compound 1

Later on, A series of 2-amino-9-aryl-7H-pyrrolo[2,3-d]pyrimidines were designed and synthesized as focal adhesion kinase (FAK) inhibitors using molecular modeling in conjunction with a co-crystal structure. This study resulted in the identification of potent FAK inhibitors. (**Compound 2**) possessed single-digit nanomolar IC_{50} and represents one of the most potent FAK inhibitors discovered.[19]



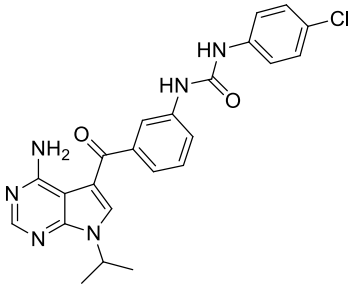
Compound 2 (IC_{50}=0.004 µM)

Tie-2

Tie-2 is a receptor tyrosine kinase (RTK) that is essential for the formation of the embryonic vasculature and is implicated in tumor angiogenesis.[6,25,26] Pyrrolopyrimidines have been reported to bind to the Tie-2 kinase domain (**Figure 2**)[27-29]

However, no specific details were available with regard to Tie-2 kinase/cellular potency, selectivity, SAR, ADMET and in vivo attributes. Later on there have been several reports on non-pyrrolopyrimidine based Tie-2 inhibitors [30,31] but few showed kinase selectivity at the time.

In 2013, novel pyrrolopyrimidines were evaluated as potent Tie-2 inhibitors with good selectivity against other key angiogenesis kinases and adequate ADMET properties.[32] The ketophenyl urea derivative (**Compound 3**) was identified as reversible and ATP competitive Tie-2 inhibitor with potent activity in whole-cell assays.

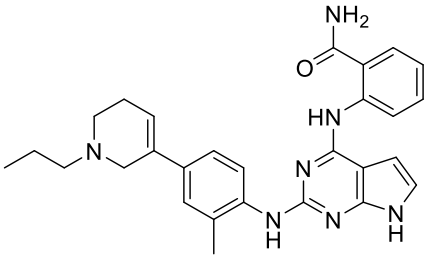


Compound 3

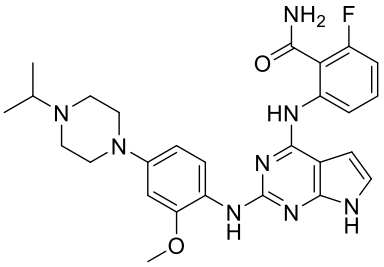
IGF-1R

The IGF-1R signaling pathway is activated in a wide range of human cancers including prostate[33], breast [34], colon and pancreas [35] by the overexpression of IGF-1R or its ligands IGF-1 and 2 and/or by the decrease of levels of IGF binding proteins. Inhibition of IGF-1R through many approaches resulted in decreased proliferation and survival of tumor cells in vitro and in vivo.[36]

From this point a number of potent small-molecule inhibitors of IGF-1R were developed. A series of 4,6-bis-anilino-1H-pyrrolo[2,3-d]pyrimidines, were investigated as potent inhibitors of the IGF-1R receptor. Pyrrolopyrimidine derivative **(Compound 4)** was identified from a focused library of small molecules with known kinase inhibitory motifs. A remarkable kinase selectivity profile was observed. A docking model of this complex with IGF-1R kinase domain was constructed to understand the bases of structural optimization. **(Compound 5)** was found to possess excellent potency against IGF-1R with an IC_{50} of 2 nm. The binding mode for **Compound 5** was similar to that modeled for **Compound 4** in complex with IGF-1R (PDB:3EKN).[37]



Compound 4



Compound 5

Later on various IGF-1R inhibitors have been developed based on the fact that the link to cancer was through targeting the kinase activity by binding within the adenosine triphosphate (ATP) binding site[38,39]. Of these, linsitinib is the most advanced and it is curently in phase II studies, in a wide range of cancers, in combination with other treatments[40,41]. linsitinib workers at Novartis had described the identification of the structurally related 5-phenyl-4-aminopyrrolopyrimidine derivative **NVP-AEW541**; **(Compound 6)** which has been used extensively as an IGF-1R inhibitor tool in a various preclinical investigation [42-44] Optimization of this target resulted in the identification of **(Compound 7)**. Compared with **NVP-AEW541**, **Compound 7** exhibited increased IGF-1R potency with an IC_{50} of 13 nM[45].

m-TOR

The mammalian target of rapamycin mTOR is a serine/therionine-specific protein kinase that belongs to the family of phosphatidylinositol-3-kinase (PI3K)

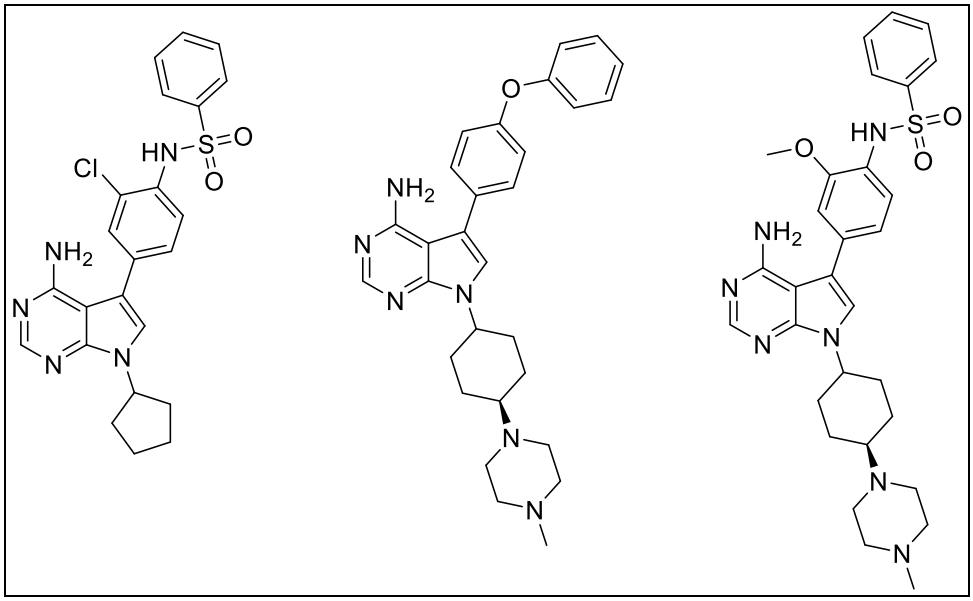
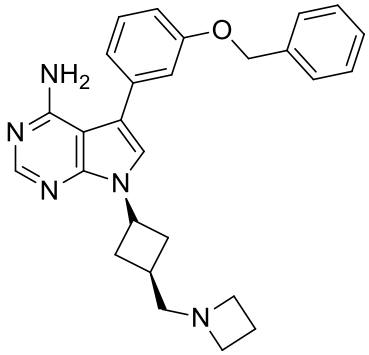


Figure 2. Active Tie-2 Pyrrolopyrimidines

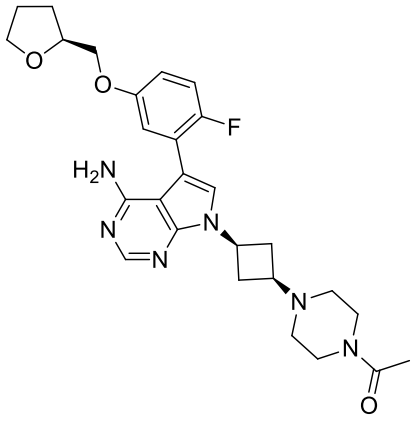
and plays a central role as a regulator of cell growth, metabolism, and proliferation.[46]

Inhibition of mTOR signaling can be used for the treatment of solid tumors with mutations or deletion of the tumor suppressor phosphatase and tensin homolog (PTEN) gene.[47] Guided by structure-based design, a series of mTOR selective N-7 substituted imidazolopyrimidines were evaluated and synthesized, the SAR was expanded to include N-Me-pyrrolopyrimidine and N-Me-pyrazolopyrimidine scaffolds and these similar ring systems have been reported as potent PI3K and/or mTOR inhibitors[48-52].

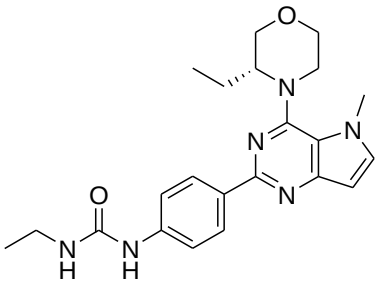
In 2013, W. Lee *et al*. were able to evaluate a variety of pyrrolopyrimidine derivatives as mTOR inhibitors with selectivity against PI3K **(Compound 8)**[53].



NVP-AEW541;(Compound 6)



Compound 7



Compound 8 [mTor (Ki 6 nM)]

BTK

Bruton's tyrosine kinase (BTK) is a non-receptor tyrosine kinase that is expressed in most hematopoietic cells such as B cells, mast cells, and macrophages. [54-56]

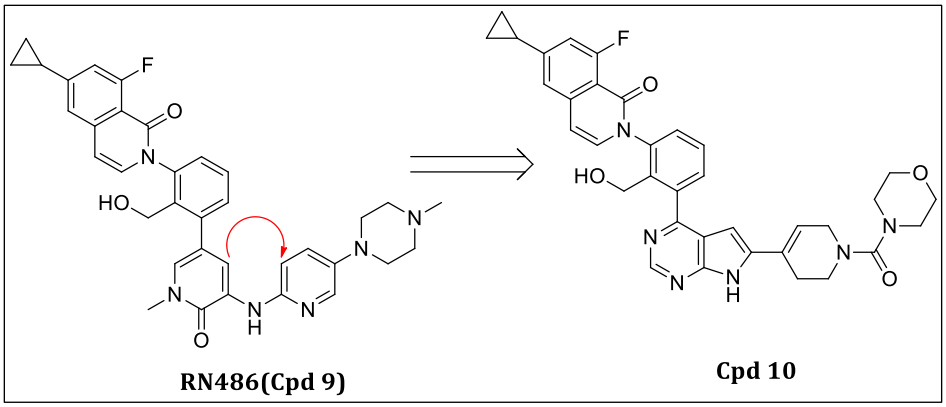


Figure 3. BTK inhibitor RN486 and designed pyrrolo[2,3-*d*]pyrimidine (Compound 10)

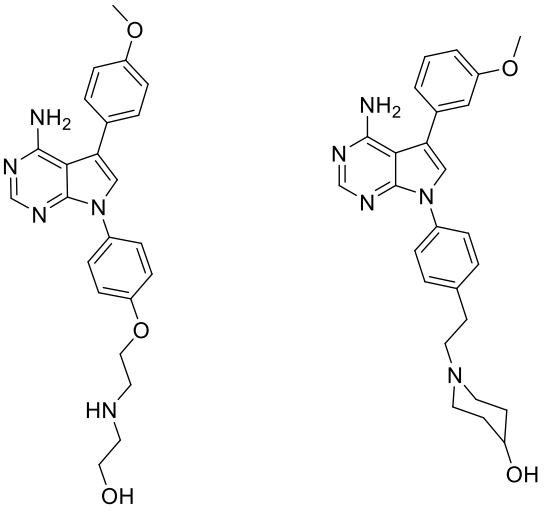
Numerous research groups are actively investigating the role and function of BTKs as well as the potent and selective BTK inhibitors as potential tool compounds and drug candidates.[57] BTK is considered a promising target for the therapeutic intervention of several diseases, including inflammatory diseases and cancer. A series of novel reversible BTK inhibitors were designed based on the structure of the recently reported preclinical drug **RN486 (Compound 9).**

Based on the study of the binding mode of **RN486**, new inhibitors that utilized pyrrolo[2,3-d]pyrimidine were designed to conformationally restrain key pharmacophoric groups within the molecule. **Compound 10** displayed superior activity both in BTK enzyme (IC_{50} = 4.8 nM) and cellular inhibition (IC_{50} = 17 nM) assays to that of **RN486** (IC_{50} = 27.3 nM) (**Figure 3**).[58].

SFKs

SFKs (Src family kinases) are a family of cytoplasmic tyrosine kinases that are overexpressed and hyperactivated in various pathologies, including cancer. Among SFKs, c-Src is involved in pathways that controls cell proliferation, migration, .and angiogenesis[59]. c-Src is a good therapeutic target, and many small molecule Src inhibitors have been developed for the treatment of different tumors.[60] Several small molecule inhibitors of SFKs are currently investigated in clinical trials. Pyrrolopyrimidine compounds **CGP76030 (Compound 11)** and **CGP77675 (Compound 12)** were reported to have inhibitory activity at nanomolar levels in osteoporosis and cancer models *in vitro* and *in vivo* against c-Src[61].

Recently in 2017, c-Src has been shown to be frequently overexpressed also in Glioblastoma multiforme (GBM), a brain tumor characterized by a high degree of proliferation, angiogenesis, necrosis, and invasiveness. A novel series of pyrrolo[2,3-d]pyrimidine compounds were found to have activity against GBM. Such compounds were able to act as ATP competitive inhibitors of Src. Enzymatic assays against a mini-panel of kinases showed an unexpected high selectivity of these pyrrolo[2,3-d]pyrimidines for Src since they did not show any activity against other tyrosine kinases. Finally, the derivatives were tested for their antiproliferative potency on U87 GBM cell line. The most active was **Compound 13**, with an IC_{50} value of 7.1 µM[62].



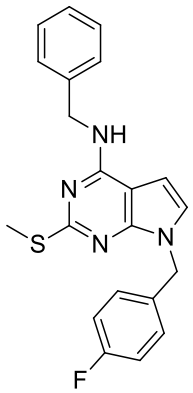
CGP76030 (Compound 11)

CGP77675 (Compound 12)

MPs 1

Monopolar spindle 1 (Mps1), also known as TTK, is a dual specificity protein kinase that phosphorylates tyrosine, serine, or threonine residues with a critical role during mitosis and is highly expressed in cancer cells. [63] Thus, Mps1 inhibition has become a promising new target of cancer research. A series of Mps1 inhibitors were identified on a purine-based lead scaffold **(Compound 14).** Since then, several promising Mps1 kinase inhibitors have been

published [64-67], **MPS-1-IN-1(Compound 15)** is a pyrrolopyridine derived Mps1 kinase inhibitor with an IC_{50}= 367 nM.[65], Due to the high molecular weight and polar surface area of these leads de novo Structure design using molecular modeling, followed by conformational restriction and scaffold hopping led to new pyrrolopyrimidine inhibitors. These scaffolds have been shown to be potent Mps1 inhibitors that possess submicromolar cellular cytotoxicity. The most active in the pyrrolopyrimidine series is **(Compound 16)** IC_{50}=20 nM (**Figure 4**). These new leads provide the basis for developing more potent, novel inhibitors of Mps1 with drug-like properties[68].



Compound 13

Various studies reported that high levels of Mps1 protein have been correlated with high histologic grade in breast cancer, while reducing Mps1 levels in BC cells resulted in induction of apoptosis, and decreased ability of BC cells to grow as xenografts in nude mice.[69,70], suggesting a potential new therapeutic target for aggressive breast cancers.[66]Mps1/TTK regulates cell cycle progression[63,72.]These observations suggest that pharmacological inhibition of Mps1/TTK may be a promising targeted cancer therapeutic. Several Mps1/TTK inhibitors have been developed in recent years. [66,73]

In 2017, novel small molecule Mps1/TTK inhibitors have been identified as potential targeted therapies for breast cancers, **(Compound 17)** inhibits in vitro kinase activity of Mps1 with an IC_{50} value of 0.809 µM and **(Compound 18)** which is the most promising in the study with an IC_{50} value of 0.356µM.[74] Future in-depth investigations of both anticancer activities and safety profiles of these Mps1 inhibitors are warranted.

CDK

Cyclin-dependent kinases (CDKs) are serine/threonine kinases that regulate cell division, transcription, apoptosis, and differentiation[75]. CDK inhibitors have attracted ther attention for their potential as anticancer therapeutics. (CDKs) 4 and 6 are enzymes that have been shown to promote cell division and multiplication in both normal and cancer cells. Many cancer cells have shown abnormalities that increase the activity of CDK, leading to the inactivation of certain tumor suppressor genes.[76,77]. This has led to the idea that inhibiting CDK4 will slow the growth of

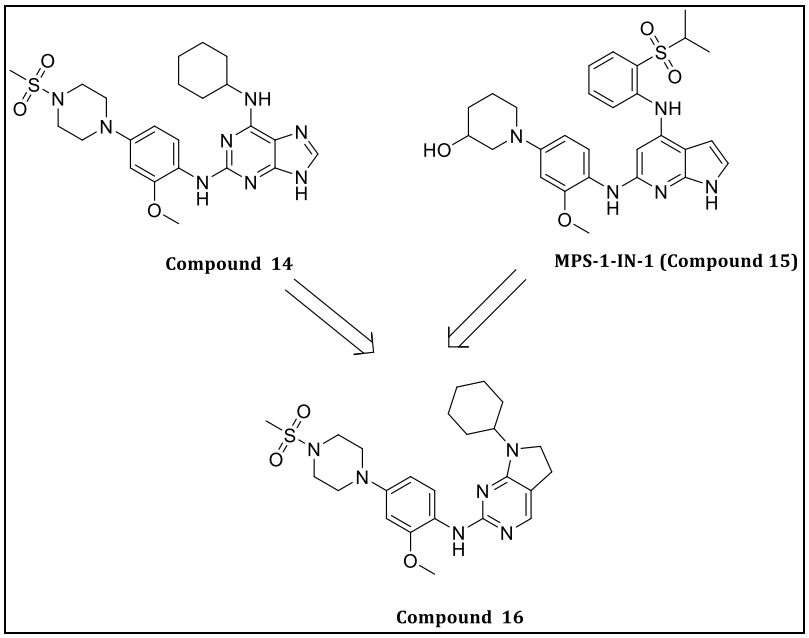
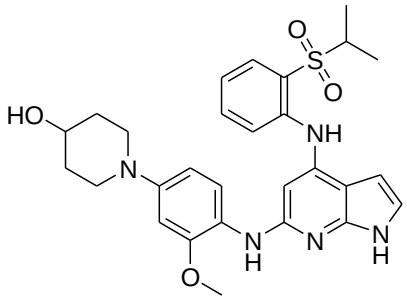
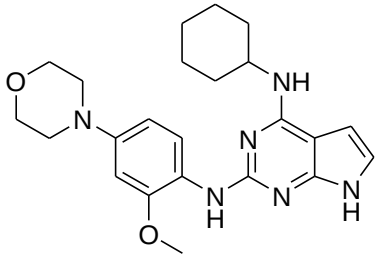


Figure 4. **Scaffold hopping to a pyrrolopyrimidine derived Mps1 kinase inhibitor**

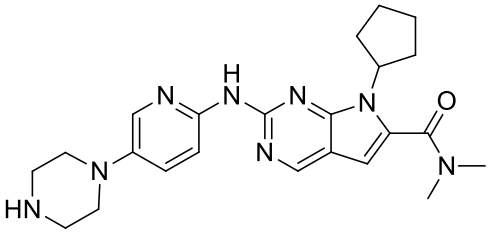


Compound 17



Compound 18

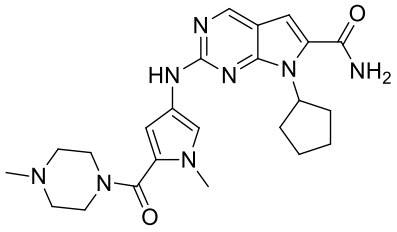
tumors by reactivating these tumor suppressors. Clinical and preclinical data support a significant role for inhibitors of the cyclin-dependent kinases (CDKs) 4 and 6 in the treatment of breast cancer.[78] The pyrrolopyrimidine based **Ribociclib (Compound 19)** previously known as LEE 011 is an inhibitor of CDK4 and CDK6, developed by Novartis and Astex Pharmaceuticals and is being studied as a treatment for drug-resistant cancers and approved in March 2017 by the US FDA as first-line treatment for metastatic breast cancer in combination with any aromatase inhibitor. This will be marketed as Novartis Kisqali®[79,80].



Novartis Kisqali® (ribociclib, LEE011)
(Compound 19)

Aurora kinase

The Aurora proteins is a small family of serine/threonine kinases that have become prominent targets for the modulation of cell cycles. Aurora-A and -B kinases are overexpressed in human tumors including breast, colon, lung, ovarian, and pancreatic cancers. J.-Y. Le Brazidec *et al*., reported a study demonstrated the conversion of the pyrazolopyrimidine to pyrrolopyrimidine core, which gave rise to a new series of dual AKA/AKB inhibitors with tunable potency against CDK1. The binding mode of this class of pyrrolopyrimidine inhibitors was elucidated by co-crystallization of Aurora-A with **(Compound 20)** (pdb code:4DHF)[81]



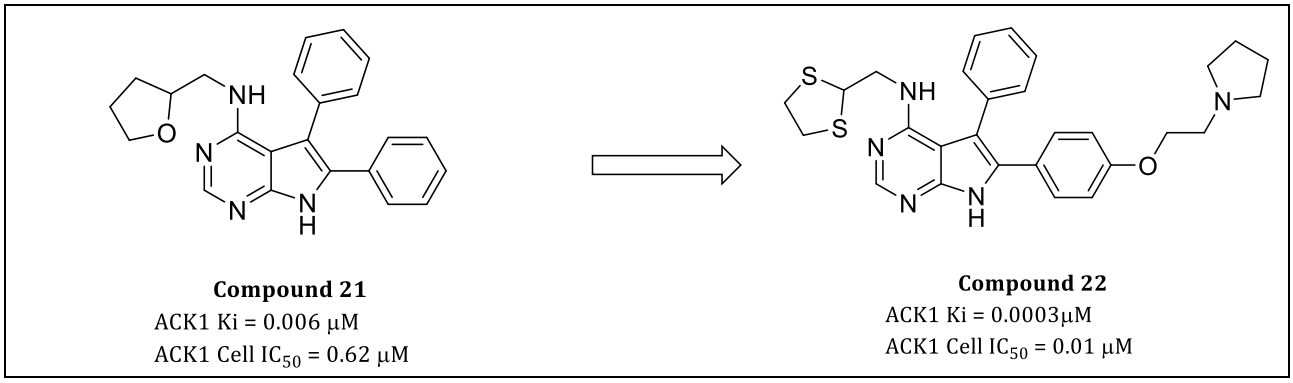
Compound 20
AKA IC_{50} = 0.028 µM
AKB IC_{50} =0.011 µM
CDK1 IC_{50} =0.5 µM

ACK1

Activated Cdc42-associated tyrosine Kinase 1(ACK1) is a non-receptor tyrosine kinase that attracted substantial interest as a target for drug discovery research in recent years.[82-85] Amplification of the ACK1 gene in primary tumors is correlated with poor prognosis [86]. A number of new heterocyclic structures were synthesized and evaluated. Pyrrolopyrimidine (**Compound 21**) proved to be a potent ACK1 inhibitor in vitro (ACK1 Ki = 0.006 µM). A large number of substituents were introduced at the para-position of the 6-phenyl ring, significant improvements in both biochemical and cellular ACK1 led to the identification of potent and selective dithiolane inhibitor **(Compound 22)** with good kinase selectivity, and a suitable in vitro metabolic profile (**Figure 5**). Unfortunately, the pharmacokinetic profile of this was poor and prevented this inhibitor from being further evaluated in tumor xenograft studies[87].

JAK

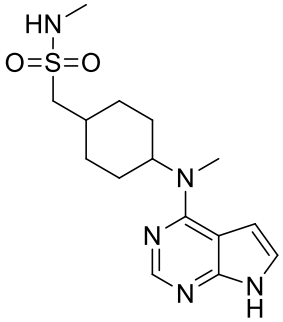
Janus kinases (JAKs) are non-receptor tyrosine kinases that play important roles in signaling processes in mammalian cells such as cell growth, survival, development and differentiation. [88,89]. Four isoforms of JAK have been identified, including JAK1, JAK2, JAK3 and tyrosine kinase 2 TYK2.[90] Each JAK kinase has significant role in the pathogenesis of immune-related diseases and in hematological cancer signaling in various cells.[91] Accordingly, the JAK family have been investigated for their potential as therapeutic targets in drug discovery. The Jak kinase inhibitors Oclacitinib **(Compound 23)**[92], Ruxolitinib **(Compound 24)**[93], and Tofacitinib **(Compound 25)**[94] have been approved by the US FDA and in other countries for the treatment of rheumatoid arthritis, canine allergic dermatitis and



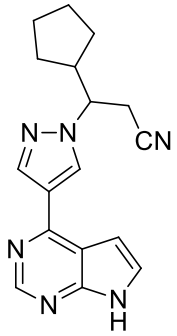
Compound 21
ACK1 Ki = 0.006 μM
ACK1 Cell IC_{50} = 0.62 μM

Compound 22
ACK1 Ki = 0.0003μM
ACK1 Cell IC_{50} = 0.01 μM

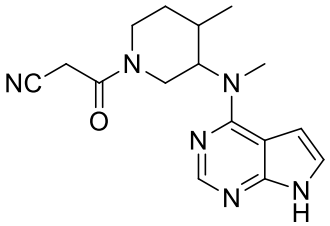
Figure 5. Initial hit (Compound 21) and subsequent derivative (Compound 22) resulting from phenyl ring substitution.



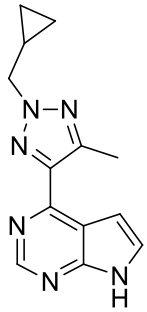
Oclacitinib (Compound 23)



Ruxolitinib (Compound 24)
JAK1 3.2 nM
JAK2 4.1 nM
JAK3 428 nM



Tofacitinib (Compound 25)
JAK1 3.2 nM
JAK2 4.1 nM
JAK3 1.6nM



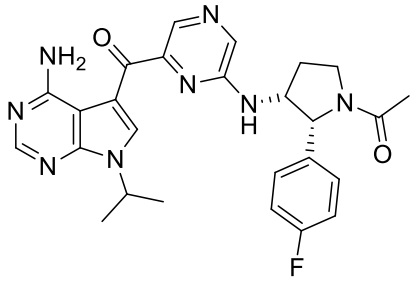
Compound 26
JAK1 444nM
JAK2 41.9
JAK3 2400nM

myelofibrosis, other Jak inhibitors are in clinical trials.[95] The pyrrolopyrimidine group of Ruxolitinib **(Compound 24)** and Tofacitinib **(Compound 25)**, are FDA approved as a JAK1/2 inhibitor and a JAK3 inhibitor, respectively.[96]

In 2016, various analogues were designed and synthesized based on a 4-triazole-pyrrolopyrimidine skeleton for the discovery of selective and novel JAK2 inhibitors. **Compound 26** (IC_{50} = 41.9 nM) and with fold selectivity: JAK1/2 = 10.6 and JAK3/2= 58.1 was found to be a novel potent and selective JAK2 inhibitor.[97] This novel JAK2 selective inhibitor may be a potential lead for new drug discovery via development of more potent and selective JAK2 inhibitors.

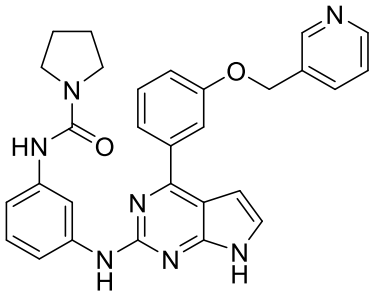
PDK1

3-Phosphoinositide-dependent kinase 1 (PDK1) is a biological target that has attracted a considerable interest in recent years. As PDK1 plays a key regulatory role within the PI3K/Akt signalling pathway, targeted inhibition of this kinase provides an attractive target for the treatment of cancer.[98] In 2011, a study involved the discovery of pyrrolopyrimidine analogues as potent PDK1 inhibitors. The study revealed that **Compound 27** was selective inhibitor of PDK1 with Ki of 1 nM and >100-fold selectivity against PI3K/AKT-pathway kinases.[99]

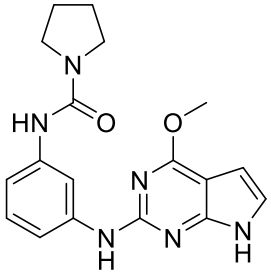


Compound 27

Later on 2014, a first-generation series of substituted 2-anilino-7H-pyrrolopyrimidines **(Compound 28)**[100] were designed by a molecular modelling study in which the core of the inhibitor was bound to the ATP binding site of PDK1. Biological screening against PDK1 was performed, but only modest inhibition was observed. a series of second-generation analogues, with variation at the 4-position of the 7H-pyrrolopyrimidine scaffold were developed. Biological evaluation showed an overall increase in activity towards the PDK1 in comparison to the first-generation set. 4-Methoxy derivative **(Compound 29)** provides a potentially useful lead for the development of new PDK1 inhibitors[101].



Compound 28



Compound 29
(IC_{50} =1.2 µM)

AKT

Akt is a serine threonine kinase that is frequently deregulated in cancer, making it an attractive anticancer drug target.[102] Due to the strong rationale for targeting Akt in cancer, much effort has been made to identify Akt inhibitors with acceptable pharmaceutical properties. The most common approaches described to date have been through the development of compounds that are either ATP-competitive or that prevent the formation of the active enzyme.[103] A number of Akt inhibitors are currently being tested in clinical trials, including allosteric inhibitors of inactive Akt.[104-106] **CCT128930 (Compound 30)** is a potent ATP-competitive AKT inhibitor discovered using fragment and structure-based approaches. It is a lead pyrrolopyrimidine exhibiting selectivity for AKT2 with IC_{50} = 6nM. **CCT128930** exhibited marked antiproliferative activity and inhibited the phosphorylation of a range of AKT substrates in multiple tumor cell lines in vitro.[107]

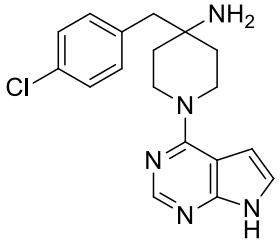
Recently, in 2013 the benzylamide **(Compound 31)** that previously reported as an orally bioavailable inhibitor of Akt and was identified as a suitable starting point for further optimization as a result of collaboration of AstraZeneca, with Astex Therapeutics Ltd and the Institute of Cancer Research.[108,109] **Compound 31** served as a lead Akt inhibitor. A crystal structure of this bound to Akt1 suggested a possible vector for further substitution (PDB code 2X39), and was explored with a range of diverse substituents and chain lengths, leading to **AZD5363 (Compound 32)**. This agent inhibits all Akt isoforms with a potency of <10 nM in vitro, and it showed pharmacodynamic and xenograft activity in vivo. It has potential in cancer therapy and is under clinical trials[110]

EGFR

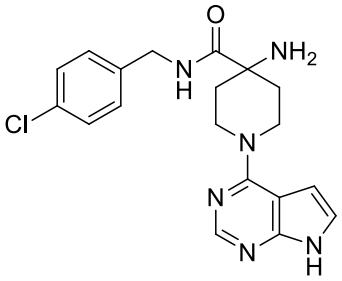
The membrane bound epidermal growth factor receptor tyrosine kinase (EGFR) has been one of the most investigated kinases, since EGFR amplification or mutation has been noted in various cancer types. Only three pyrroloyrimidine based EGFR inhibitors have reached clinical trials, namely **PKI-166(Compound 33)[111]**, **AEE-788 (Compound 34)**[112,113], and **TAK-285 (Compound 35)**[114,115].

In 2014, S.J. Kaspersen *et al.* performed a study on the enzymatic inhibition potential of a series of chiral and non-chiral pyrrolopyrimidine based derivatives that have been investigated and optimized. New structures were identified having enzymatic IC_{50} values comparable to the commercial drug Erlotinib. These compounds were further evaluated towards a panel of 52 kinases revealing interesting Src-family kinase inhibitory activity, (**Compound 36**), the most active in the designed series against EGFR with IC_{50}=0.1nM, confirming pyrrolopyrimidines as attractive drug candidates or lead structures.[116] A late focus on absorption, distribution, metabolism, excretion and toxicology (ADMET) is regarded as one of the main reasons for drug failure.[117,118] Thus in 2015, the pyrrolopyrimidine **(compound 36**) have been evaluated and compared with the established drug Erlotinib in cellular assays towards 25 cancer cell lines and by ADME/tox profiling. This performed equally or better than the Erlotinib in most of the cases and the highest activity was seen towards lung and breast EGFR and HER2[119].

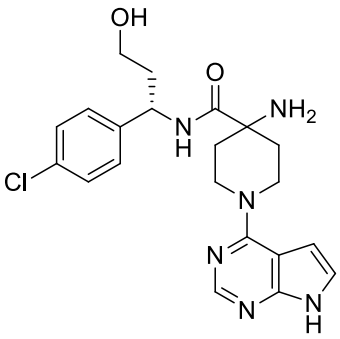
In 2016, in an attempt to improve the ADMET properties of the designed structures, starting from lead



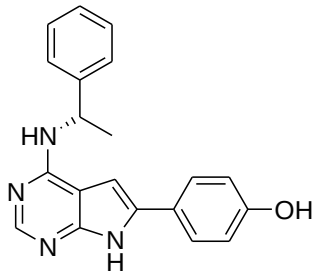
CCT128930(Compound 30)



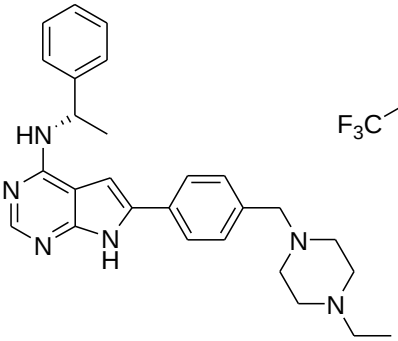
Compound 31



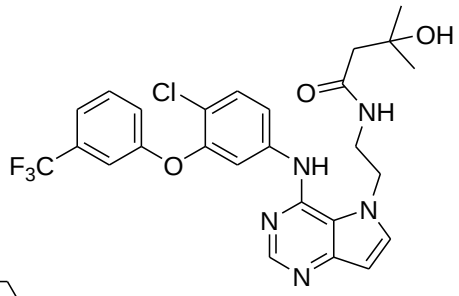
AZD536 (Compound 32)



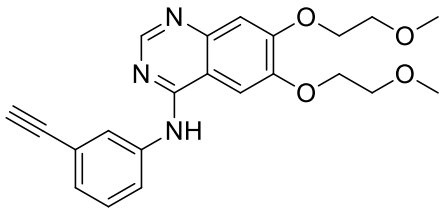
PKI-166(Compound 33)



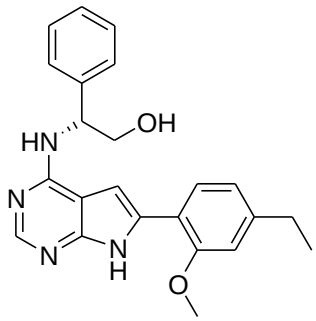
AEE-788 (Compound 34)



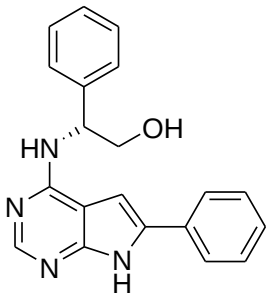
TAK-285 (Compound 35)



Erlotinib



Compound 36



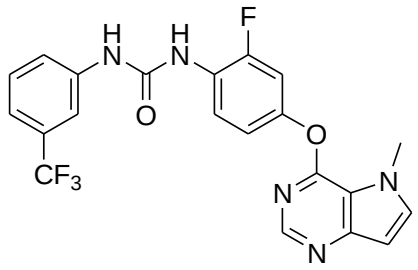
Compound 37

structure **(Compound 36)**, a SAR study was evolved to identify the most promising drug candidate **(Compound 37)**. The high potency displayed in Ba/F3-EGFRL858R reporter cells, PC9, and A-431 cell proliferation studies indicated that this have a potential therapeutic use in EGFR driven diseases. [120]

VEGFR

Vascular endothelial growth factor (VEGF) is considered to be one of the most important regulators of angiogenesis and a key target in anticancer treatment.

VEGFR-2 is the predominant receptor in angiogenic signaling. differentiation, and survival as well as vessel permeability and dilation.[121] In order to develop new VEGFR inhibitors a series of pyrrolo[3,2-d]pyrimidine derivatives were designed and synthesized. The diphenylurea substitution at the C4-position of the pyrrolo[3,2-d]pyrimidine core via an oxygen linker afforded potent VEGFR2 kinase inhibitors. additionally, meta-substitution of the urea terminal benzene ring with a small lipophilic group at the 2-position of the central benzene ring improved HUVEC inhibitory activity. **Compound 38** possessed an IC_{50} value of 4.4 nM.[122]



Compound 38

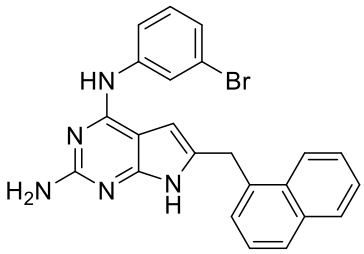
Pyrrolopyrimidines as multiple receptor tyrosine kinase inhibitors

Receptor tyrosine kinases (RTKs) such as vascular endothelial growth factor receptor (VEGFR), epidermal growth factor receptor (EGFR), and platelet-derived growth factor receptor (PDGFR) have important functions in signal transduction pathways that regulate cell growth proliferation and differentiation under normal cell function, as well as under abnormal conditions. RTK receptors are overexpressed in several tumors and are directly and indirectly involved in cancer.[123-125] where they play a pivotal role in tumor angiogenesis.[123,124] Angiogenesis is the formation of new blood vessels from existing Vasculature, the newly sprouted blood vessels provide supply of nutrients to the tumor to grow beyond 1–2 mm.[126] Angiogenesis is essential step in the transition of solid tumors from a dormant state to a malignant state and it also provides metastatic pathways for solid tumors[127]. Small molecule RTK inhibitors targeting the ATP binding site of tyrosine kinases are currently in clinical use while others are in clinical trials as antitumor agents.[128-130]

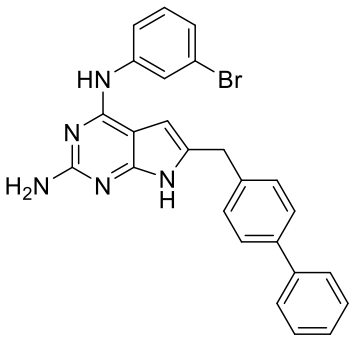
There has been considerable discussion in the literature regarding the use of RTK inhibitors for cancer as monotherapy or the combination of multiple RTK inhibitors. Initial strategies for RTK inhibition focused on single RTK inhibitors. However, tumors have redundant signaling pathways for angiogenesis and often develop resistance to agents that target one specific pathway.[131] A multitargeted approach that inhibits multiple signaling pathways proved to be more effective than the inhibition of a single target. The inhibition of multiple RTKs retard tumor resistance by blocking potential 'escape routes'. Since RTKs are present in endothelial cells (VEGFR, PDGFR), tumor cells (FGFR, PDGFR), and pericytes/smooth muscle cells (FGFR, PDGFR), inhibition of more than one RTKs provides synergistic inhibitory effects against solid tumors.[132]

Many derivatives bearing pyrrolopyrimidine core have been developed as RTKIs. pyrrolopyrimidine derivatives, which are useful as RTK inhibitors are reviewed herein.

In 2003 based on a pharmacophore model, A. Gangjee *et al*. designed RTKIs using the pyrrolo[2,3-*d*]pyrimidine scaffold with a 2-NH2 moiety.[133] These compounds were designed, synthesized and evaluated as VEGFR 1/2 inhibitors, EGFR, and PDGFRβ. Both **Compound 39** and **40** exhibited high antiangiogenic activity.

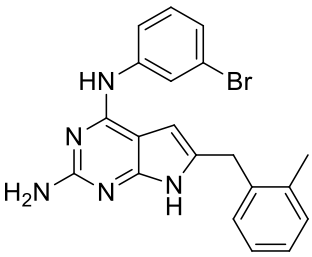


Compound 39

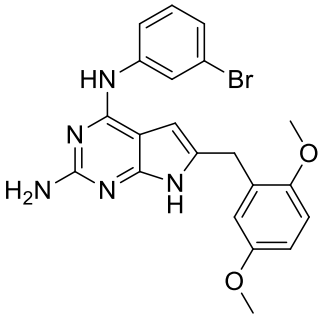


Compound 40

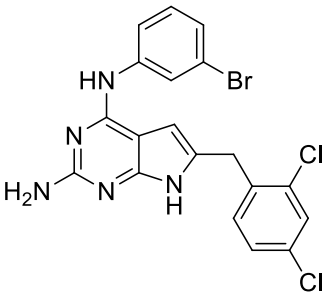
In 2004 A. Gangjee *et al.* reported a series of N4-(3-bromophenyl)-6-phenylmethylsubstituted- 7H-pyrrolo[2,3-*d*]pyrimidine-2,4-diamines compounds as multiple RTK inhibitors. **Compound 41** was a potent VEGFR-2 inhibitor and moderate EGFR inhibitor in the cellular assays**.** On the other hand**, (Compound 42)** was a potent VEGFR-2 inhibitor and a moderate PDGFRb and VEGFR-1 inhibitor, while, **(Compound 43)**, was a potent EGFR inhibitor, with poor VEGFR-2 inhibition.[134]



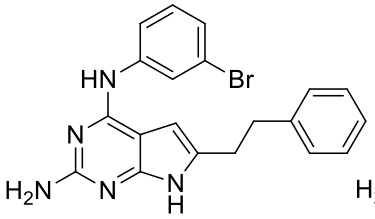
Compound 41



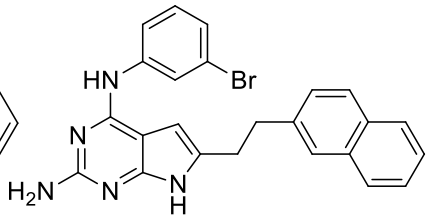
Compound 42



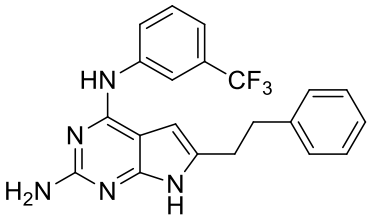
Compound 43



Compound 44



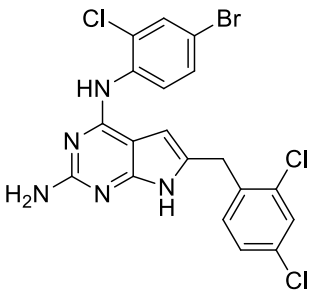
Compound 45



Compound 46

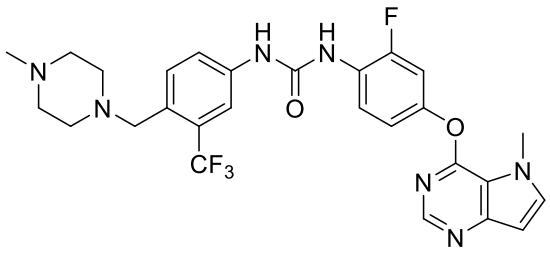
In 2008, A. Gangjee *et al* developed a novel class of substituted pyrrolo[2,3- *d*]pyrimidines as multiple RTK inhibitors and antiangiogenic agents.[135] which lead to discovery of novel multi-RTK inhibitors and potent angiogenesis inhibitors (**Compound 44, 45)**. **Compound 46** showed excellent potency with an IC_{50} value of 30 nM in the CAM angiogenesis inhibition assay.

In 2010 in an attempt to structurally design either a dual EGFR/VEGFR-2 inhibitor or even a selective VEGFR-2 inhibitor, **(Compound 43**) was previously synthesized as multiple RTK inhibitor, and the VEGFR-2 inhibitor (**Compound 47**), were chosen for further evaluation and showed significant inhibition of tumor growth, angiogenesis and metastasis.[136]



Compound 47

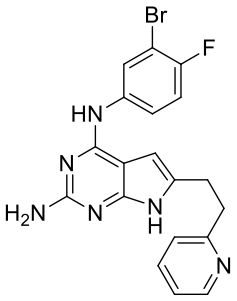
Another study based on the pyrrolo[3,2-d] pyrimidine derivative mentioned before as potent anti-angiogenic kinase inhibitors **(Compound 38)** was performed.[122] This exhibited inhibitory activities against VEGFR, PDGFR, and Tie-2 with IC_{50} values of <100 nM but it was a weak inhibitor of FGFR, Since FGF-FGFR signaling has been described as the escape mechanism for resistant tumors to VEGF treatment[137], Y. Oguro *et al.* thought of the development of compounds that would inhibit both VEGFR and FGFR to avoid resistance over long-term treatment. Also, these compounds were expected to be more soluble than **(Compound 38)**, which have poor aqueous solubility. Among the compounds synthesized, urea derivative **(Compound 48)** with a piperazine ring on the terminal benzene ring strongly inhibited FGFR1 kinase as well as VEGFR2 kinase with IC_{50} values of 14 and 9 nm, respectively.[138]



Compound 48

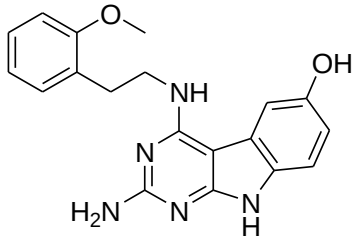
In 2013 A. Gangjee *et al*. continued their studies on pyrrolopyrimidine 2,4 diamines as multikinase inhibitors, thus a general RTK pharmacophore model was utilized in the design of these s. The synthesized

compounds were evaluated in cell-based assay for their inhibitory activity against EGFR, VEGFR-1, 2 and PDFGR-b. Minor variations in the substitution on the 4-anilino ring led to significant differences in inhibition of the different RTKs. These variations in activity may be due to the specific requirements for binding in the individual RTKs or due to differences in cell permeability of these structures. **(Compound 49)** was evaluated *in vivo* in B16-F10 syngeneic mouse tumor model. In this study a significant reduction in tumor growth rate, vascular density and metastases of the tumor to the lung was observed for this .[139]



Compound 49

The most recent study in 2017 described the development of a series of novel benzo-pyrrolopyrimidines as dual inhibitors of both EGFR and VEGFR2. Of the different compounds were identified (**Compound 50**) exhibited very good kinase inhibitory activity for both targets (EGFR Ki 1.87 uM, VEGFR Ki 0.85 uM). These structures can be used as lead compounds for further drug development studies.[140]



Compound 50

CONCLUSION

In this review we have provided a comprehensive and up-to-date account on the most recent development in the medicinal chemistry of pyrrolopyrimidine derivatives with significant anticancer activity through inhibition of different kinase enzymes. This review described the various pyrrolopyrimidine derivatives that have been published in the literature. This could provide a guide for a comprehensive and target oriented information for development of clinically approved pyrrolopyrimidine-based anticancer agents for novel anticancer drug discovery starting from the last exhaustive publication in this field.

Conflict of Interest

The authors declare that they don't have any conflict of interest.

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