Target Based Design And Synthesis of Heterocycles in the Potential Treatment of Cancer and Opportunistic Infection

Shruti Choudhary

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TARGET BASED DESIGN AND SYNTHESIS OF HETEROCYCLES IN THE
POTENTIAL TREATMENT OF CANCER AND OPPORTUNISTIC INFECTION

A Dissertation
Submitted to the Graduate School of Pharmaceutical Sciences

Duquesne University

In partial fulfillment of the requirements for
the degree of Doctor of Philosophy

By
Shruti Choudhary

December 2017
TARGET BASED DESIGN AND SYNTHESIS OF HETEROCYCLES IN THE
POTENTIAL TREATMENT OF CANCER AND OPPORTUNISTIC INFECTION

By

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Approved August 30, 2017

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ABSTRACT

TARGET BASED DESIGN AND SYNTHESIS OF HETEROCYCLES IN THE POTENTIAL TREATMENT OF CANCER AND OPPORTUNISTIC INFECTION

By
Shruti Choudhary
December 2017

Dissertation supervised by Professor Aleem Gangjee

Dose limiting toxicity and development of multidrug resistance by the tumors are the major limitations of current cancer chemotherapy. Microtubule targeting agents (MTAs) are a structurally diverse set of compounds that disrupt microtubule dynamics and exert their anticancer effect. Among the various classes of such agents, the colchicine site binding agents are particularly important as they circumvent the Pgp and β-III tubulin mediated clinical resistance. These resistance mechanisms, when manifested, are a major reason for the failure of clinically used agents such as taxanes and vinca alkaloids. A series of monocyclic pyrimidine analogs were designed and synthesized as colchicine site binding agents to overcome Pgp and β-III tubulin mediated drug resistance.

Multitargeted single agents with dual mechanism of actions, containing both cytostatic and cytotoxic components are particularly relevant to cancer chemotherapy as
they have the potential to overcome multidrug resistance and dose limiting toxicities. Antiangiogenic agents target tumor angiogenesis, an important phenomenon for tumor growth and metastasis. The angiogenic effect is mediated by receptor tyrosine kinases (RTKs) and therefore, RTK inhibitors are used widely in the treatment of various types of cancers. To overcome the limitations of current cancer chemotherapy namely, dose limiting toxicity and multidrug resistance, quinazolines were designed and synthesized as dual acting MTA and RTK inhibitors.

Toll-like receptors (TLRs) are key mediators in regulating the inflammatory response. Currently, more than 50 clinical trails of TLR agonists in the treatment of cancer are being conducted, either alone or in combination. Multitargeted single agents with dual acting MTA and TLR agonist (2,5-diaminoquinolines) were designed and synthesized as potential anticancer agents. A major part of this project covers the Pd-catalyzed cross coupling reaction optimization on the 2,5-diaminoquinolines including mechanistic details of the coupling reaction.

Opportunistic infection by *Pneumocystis jirovecii* in immunocompromised patients such as organ transplant, cancer and AIDS patients is associated with high mortality. The current treatment involving the use of a combination of trimethoprim and sulfamethoxazole is limited by drug resistance, treatment failures and adverse side effects. Dihydrofolate reductase (DHFR) is an essential enzyme that provides folate cofactor for DNA, RNA and methionine biosynthesis. Hence, selectively inhibiting *pj*DHFR is an important strategy for effective treatment of infection by the pathogen. Incorporating the key differences in the active site residues in *pj*DHFR and *h*DHFR, thieno[2,3-*d*]pyrimidines were designed and synthesized as selective *pj*DHFR inhibitors.
In addition, molecular modeling studies were performed on a series of pyrimido[4,5-\textit{b}]indoles and cyclopenta[\textit{d}]pyrimidines to explain their observed \textit{in vitro} biological activities. Molecular modeling studies were also performed on a series of furo[2,3-\textit{d}]pyrimidines and pyrrolo[3,2-\textit{d}]pyrimidines to explain their biological activities on multiple targets (colchicine site of tubulin and multiple RTKs).
This one is for you Mom…
ACKNOWLEDGEMENT

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<td>ATP binding cassette</td>
</tr>
<tr>
<td>ATP</td>
<td>adenosine triphosphate</td>
</tr>
<tr>
<td>BP</td>
<td>back pocket</td>
</tr>
<tr>
<td>CA4</td>
<td>combretastatin A4</td>
</tr>
<tr>
<td>CA4P</td>
<td>combretastatin A4 phosphate</td>
</tr>
<tr>
<td>CAM</td>
<td>chorioallantoic membrane</td>
</tr>
<tr>
<td>DC</td>
<td>dendritic cells</td>
</tr>
<tr>
<td>DFG</td>
<td>aspartate-phenylalanine-glycine</td>
</tr>
<tr>
<td>DHFR</td>
<td>dihydrofolate reductase</td>
</tr>
<tr>
<td>EGF</td>
<td>endothelial growth factor</td>
</tr>
<tr>
<td>EGFR</td>
<td>endothelial growth factor receptor</td>
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<td>GDP</td>
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<td>guanosine triphosphate</td>
</tr>
<tr>
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<td>interferon-γ</td>
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<td>leucine rich repeat</td>
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<td>microtubule associated proteins</td>
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<tr>
<td>MDR</td>
<td>multidrug resistance</td>
</tr>
<tr>
<td>MTA</td>
<td>microtubule targeting agent</td>
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<tr>
<td>NADPH</td>
<td>nicotinamide adenine dinucleotide phosphate</td>
</tr>
<tr>
<td>PDGF</td>
<td>platelet derived growth factor</td>
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<td>tumor necrosis factor</td>
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<td>vascular disrupting agents</td>
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<tr>
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<td>vascular endothelial growth factor</td>
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I. BIOCHEMICAL REVIEW

A. Microtubule targeting agents (MTAs)

A.1 Microtubules

Microtubules are long, filamentous tube-like protein fibers that form the key components of the cytoskeleton. Cytoskeletal proteins such as intermediate filaments are involved in providing mechanical support and resistance against physical stress, whereas microtubules are associated with regulating cell polarity, morphogenesis, intracellular transport and cell division during mitosis.\(^1\) Due to their dynamic behavior in dividing cells, microtubules participate in the formation of the mitotic spindle during cell division and hence, inhibiting this key step forms the basis of the efficacy of MTAs in cancer chemotherapy.\(^1\) The clinical success of these compounds is evident from the inclusion of MTAs as one of the most promising drugs in the treatment of both solid tumors as well as haematological malignancies, often as a component of combination chemotherapy.\(^2\text{-}^4\)

A.2 Microtubule structure and dynamics

Microtubules are composed of \(\alpha\)- and \(\beta\)-tubulin subunits that form the \(\alpha\beta\)-tubulin heterodimers. The tubulin dimers associate longitudinally to form the protofilaments, which in turn associate laterally to form tube like structures (Figure 1).\(^1,^5\) Tubulin dimers preferentially add to the growing microtubule protofilament at the “plus end” capped by the \(\beta\)-tubulin subunit. The “minus end” of microtubules is exposed to \(\alpha\)-tubulin subunit and is relatively stable. The two microtubule ends behave differently, such that the minus end grows more slowly and undergoes a transition from growth to shortening (catastrophe) less frequently.

*In vitro* studies have shown that the polymerizing plus end of the microtubule exhibits curved, flattened and tapered sheet-like structure, whereas during depolymerization the plus end exhibits more curved, peeling protofilament like structure (Figure 1). The switching of microtubules between phases of growth and shrinkage is termed as dynamic instability and these transitions between the curved and straight conformations of tubulin are associated with factors controlling microtubule dynamics.
The conformation of microtubules is regulated by the binding and hydrolysis of guanosine 5’-triphosphate (GTP) at the αβ-tubulin heterodimer.


Two GTP-binding sites are present within the αβ-tubulin dimer. The N-site (nonexchangeable site) is buried between the α- and β-subunits and is constitutively occupied by GTP (Figure 2A). The E-site (exchangeable site) is present at the terminal end of the microtubule plus end and is exposed to the surface. αβ-tubulin dimers in solution exchange guanosine 5’-diphosphate (GDP) for GTP so that they can undergo polymerization. When this GTP bound αβ-tubulin dimer adds to the plus end, the incoming
α-subunit interacts with the E-site of the terminal β-subunit (GTP bound) of the growing microtubule, promotes binding and facilitates hydrolysis. The microtubule continues to grow as long as the GTP-cap is present at the plus end. Once the GTP-cap is lost, rapid depolymerization occurs due to the instability of the GDP-tubulin subunits. Through the cryo-EM structure analysis (Figure 2B) of GTP-like stabilized microtubule and GDP bound microtubule it was deduced that the GTP hydrolysis and phosphate release causes the E-site to undergo compaction, which translates into an energetically unfavorable conformational strain that is released by adopting a depolymerized, bent tubulin structure.6

A.3 Microtubule binding sites


MTAs are a diverse set of cytotoxic agents that are classified under two major categories: microtubule-stabilizers and microtubule-destabilizers.1 The microtubule stabilizing agents promote microtubule polymerization at high concentration and include
taxanes/epothilones and laulimalide/peloruside A (Figure 3). The microtubule depolymerizing agents include colchicine, vinca alkaloids and maytansine and induce microtubule depolymerization at high concentrations. At high concentrations, the MTAs, depending on their mechanism, promote (microtubule-stabilizer) or inhibit (microtubule-destabilizer) the tubulin-microtubule equilibrium by acting on unassembled and/or assembled tubulin. However, at low concentration, these agents primarily target the microtubule ends, thereby affecting microtubule dynamics.

A.3.1 Taxanes and epothilones site


Taxanes and epothilones bind to the taxane site of β-tubulin, which is located on the luminal surface of the microtubules (Figure 4). Paclitaxel and its derivatives show a preferential binding to the microtubules over the soluble tubulin dimers. These compounds stabilize the microtubules by causing a conformational change that stabilizes the
longitudinal and/or lateral tubulin contacts via allostERIC mechanisms. At low concentration, taxanes are capable of causing cell cycle arrest and induce apoptosis without changing microtubule mass. This effect is evident from the observed IC₅₀ for the antimitotic effect of paclitaxel (8 nM in HeLa cells) compared to the IC₅₀ of 80 nM for increasing the microtubule polymerization. Taxanes are widely used in the treatment of breast, ovarian, non-small cell lung, prostate and head and neck cancers. In addition, ixabepilone (Ixempra®) belonging to the epothilone class of taxane-binding agents was approved for the treatment of drug-refractory metastatic breast cancer.

A.3.2 Laulimalide/peloruside A site

![Figure 5](image.png)

**Figure 5.** Representation of laulimalide/peloruside A binding site with their structures. Reprinted with permission from “Control of microtubule organization and dynamics: two ends in the limelight” by A. Akhmanova and M. O. Steinmetz, Nat. Rev. Mol. Cell Biol. 2015, 16, 711-726. Copyright 2015 Nature Publishing Group.

Laulimalide and peloruside A are marine derived macrocycles, which were shown to have microtubule stabilizing activities (Figure 5). In *in vitro* assays, both macrocycles demonstrated efficacy in cells resistant to paclitaxel and epothilones and did not compete with taxanes at the binding site, suggesting an alternate site of binding. These microtubule
stabilizing agents bind to the site adjacent to the taxane binding site, on β-tubulin at the outer surface of microtubules. Moreover, owing to their macrocyclic structure these compounds bind to two adjacent β-subunits mimicking a bridging interaction.\textsuperscript{14} High potency of peloruside A has been reported against lung and multi-drug resistant (MDR) breast cancer tumor xenografts in vivo, thus promising its further development.\textsuperscript{15} Development of laulimalide, on the other hand, is hindered owing to its high toxicity and high mortality.\textsuperscript{13}

A.3.3 Colchicine site

The compounds binding to this site comprise a diverse set of small molecules that destabilize microtubules by preventing curved to straight conformational transition of tubulin. Some of the agents binding to this site are colchicine, combretastatin A4 (CA4), podophyllotoxin and 2-methoxyestradiol.\textsuperscript{16} Colchicine binds to soluble tubulin, on β-

tubulin, at the interface of α- and β-subunits (Figure 6). On binding, it promotes formation of curved protofilament assemblies, which are not feasible for polymerization and requires straight microtubular protofilament. Several agents belonging to this class have been evaluated in phase I and II clinical trials as anticancer agents, including 2-methoxyestardiol, CA4, and verubulin, however, none of them have been clinically approved so far and are currently in the mid-to-late stages of clinical trials.

Combretastatins are potent vascular disrupting agents (VDAs), in addition to being potent antimitotic agents. VDAs are cytotoxic agents that effect the existing tumor vessels (endothelial cells and pericytes) leading to their vascular collapse and subsequent tumor death mediated via necrosis at the tumor core. VDAs selectively target the tumor vasculature without affecting the normal blood vessels. This selectivity can be attributed to significant differences between the normal blood vessels and the vessels in the tumor microenvironment. The normal vasculature is more organized as compared to the uneven, leaky vessels in the tumor microenvironment. Moreover, the tumor vasculature is more fragile due to lack of pericytes and basement membrane.

A.3.4 Vinca site

![Vinca site](image)

Figure 7. Representation of vinca site with vinca alkaloids. Reprinted with permission from “Control of microtubule organization and dynamics: two ends in the limelight” by A.
The vinca alkaloids bind to the β-subunit of two different, longitudinally aligned dimers (Figure 7).\(^5\) Vinblastine binds with high affinity to the microtubule ends, in addition to binding to soluble tubulin. Similar to the mechanism of taxol described before, at high concentration (10-100 nM in HeLa cells) vinblastine acts as a depolymerizing agent and at low concentration (1 nM) suppresses microtubule dynamics and blocks mitosis. Other agents binding to the vinca site includes cryptophycins,\(^21\) halichondrins\(^22\) and dolastatins.\(^23\)

### A.3.5 Maytansine site

Maytansine (Figure 8) was shown to bind to a recently discovered new binding site on β-tubulin reported by Prota et al.\(^24\) Binding of maytansine to the plus end of the microtubule led to microtubule depolymerization. Maytansine is also a component of the antibody drug conjugate Trastuzumab emtansine that was recently approved by the FDA for the treatment of HER-2 positive advanced breast cancer.\(^25\)

**Figure 8.** Representation of maytansine site with maytansine. Reprinted with permission from “Control of microtubule organization and dynamics: two ends in the limelight” by A.

### A.4 MTAs in cancer

The clinical efficacy of the MTAs in the treatment of cancer is attributed to their ability to inhibit cell proliferation by altering microtubule dynamics. The essential role of microtubules in dividing cells is most evident during the formation of mitotic spindle, where the participation of microtubules results in the separation of sister chromatids.\(^1\) However, according to recent literature evidence it has been suggested that even though patient tumors appear to be highly proliferative, most of the solid tumors in patients demonstrate low mitotic indices. In addition, the cytotoxicity of MTAs to non-dividing neuronal cells indicates a mechanism other than targeting mitosis. Interphase dependent alteration of the neuronal transport has been proposed as one of the reason for dose-limiting neuropathy of these chemotherapeutic agents.\(^26\) Several functions of microtubules associated with the interphase stage are affected by MTAs including centrosome clustering, mitochondrial toxicity, mRNA delivery to polysomes, and vesicular transport.\(^27\) The exact mechanism by which MTAs exert their anticancer effect currently warrants further studies but increasing evidence suggests an involvement of interphase function disruption in addition to their well-established role during mitosis.\(^26, 27\)

### A.4 Mechanism of tumor resistance to MTAs

MTAs, like other chemotherapeutic agents, are limited in their efficacy due to multiple-drug resistance (MDR). There are several mechanisms by which tumors develop
resistance to these cytotoxic agents, the clinically significant mechanisms include efflux by ATP-binding cassette (ABC) transporters and overexpression of the β-III isotype of tubulin.\textsuperscript{28, 29}

ABC transporters are transmembrane proteins that regulate the flux of hydrophobic compounds across the plasma membrane.\textsuperscript{29} The multi-drug resistance protein 1 (MDR1), also known as P-glycoprotein (Pgp), was identified as the chief transporter causing resistance to vinca alkaloids and taxanes \textit{in vitro}.\textsuperscript{30} Pgp overexpression has been associated with chemotherapy failures in clinical settings in several tumor types, including breast, kidney, colon and liver cancers, as well as leukaemias and lymphomas.\textsuperscript{29, 31} Compounds that act by inhibiting Pgp have been used in combination with MTAs to reverse Pgp-mediated drug resistance, but failed to reverse resistance in clinical trials.\textsuperscript{32} The first generation Pgp inhibitors, such as verapamil, tamoxifen and cyclosporine were non-specific and failed to demonstrate overall efficacy as Pgp inhibitors. The development of second generation inhibitors (valspodar, biricodar) with improved potency was equally disappointing as they led to drug-drug interactions ultimately leading to reduced chemotherapeutic drug clearance and therefore increased toxicity. The third generation inhibitors (tariquidar, zosuquidar) are currently being evaluated for their clinical efficacy.\textsuperscript{32, 33}

Overexpression of β-III isotype of tubulin is another mechanism that is clinically observed in the development of resistance to MTAs.\textsuperscript{28} Clinical resistance to taxanes in multiple tumor types such as non-small cell lung,\textsuperscript{34} breast,\textsuperscript{35} ovarian\textsuperscript{36} and gastric cancers\textsuperscript{37} has been strongly associated to the expression of β-III isotype of tubulin. Paclitaxel demonstrated 5-fold less activity in \textit{in vitro} studies in β-III tubulin expressing HeLa cells.\textsuperscript{38}
It was proposed that the resistance to taxanes was due to the absence of Ser275 residue on β-III tubulin, which undergoes H-bonding interaction with the 7-OH of paclitaxel and facilitates its diffusion through the pores of the microtubule wall. The loss of hydrogen bonding (H-bonding) interaction with Ala275 in β-III tubulin prevents the binding of paclitaxel in β-III expressing cell lines. In addition, β-III tubulin expression causes clinical resistance to vinca alkaloids in non-small cell lung and ovarian cancers, mediated via a different mechanism.

B. Angiogenesis and receptor tyrosine kinases (RTKs)

B.1 Angiogenesis

Angiogenesis is a process of sprouting new blood vessels from the existing vasculature, a process that is indispensable for tumor growth and metastasis. In normal tissue, angiogenesis is tightly regulated by balance between proangiogenic and antiangiogenic factors (Figure 9). The blood vessels thus formed are structurally and functionally normal. On the contrary, the tumor vasculature is highly tortuous, saccular and chaotic in its organization, with large gaps between endothelial cells, detached pericytes, and abnormally thick or thin basement membranes of the vessel walls. The tumor microenvironment, which is characterized by hypoxia, low pH and elevated fluid pressure, initiates other pathways involved in over expression of proangiogenic factors.
Factors such as vascular endothelial growth factor (VEGF), platelet derived endothelial growth factor (PDGF), and epidermal growth factor (EGF) promote endothelial cell proliferation/migration and lead to angiogenesis. These proangiogenic factors lead to hostile tumor microenvironment to chemotherapy as they promote tumor progression and treatment resistance via genetic instability, immune suppression, inflammation etc. Thus, antiangiogenic agents have been successfully used in the treatment of cancer.

**B.2 Receptor tyrosine kinases**

RTKs belong to a family of cell surface receptors that act as key regulators of critical cellular processes such as proliferation and differentiation, cell survival and metabolism, cell migration, and cell-cycle control. All the RTKs have a similar structural organization with an extracellular ligand binding domain, a single transmembrane helix and a protein tyrosine kinase domain in the cytoplasm (Figure 10). In general, the growth factor binding at the extracellular domain induces a conformational change that leads to
receptor dimerization (homodimerization or heterodimerization). This activates the intracellular tyrosine kinase domain causing autophosphorylation mediated via transfer a phosphate group from adenosine 5′-triphosphate (ATP) to the hydroxyl group of tyrosine residue of the substrate protein and triggers a cascade of downstream cell signaling pathways.47

**Figure 10.** General representation of RTKs, Ig (immunoglobulin-like). Reprinted with permission from “Cell signaling by receptor tyrosine kinases” by M. A. Lemmon and J. Schlessinger, *Cell* **2010**, *141*, 1117-1134. Copyright 2010 Elsevier Inc.

Proangiogenic growth factors such as VEGF, PDGF and EGF bind to their respective growth factor receptors VEGFR, PDGFR and EGFR, and initiate downstream signal transduction leading to regulation of cell permeability, survival, proliferation and migration.48 Under normal physiological conditions these signaling pathways are tightly...
regulated and monitored, however, dysregulation of these signaling pathways has been linked to malignancy and contributes to the hallmarks of cancer.47

Several small-molecule RTK inhibitors (RTKIs) have been approved clinically for the treatment of various types of cancers (Figure 11).49 These RTKIs broadly fall under five categories.50 Type I inhibitors bind to the active form of the kinase and reversibly compete with ATP for its binding site (eg. sunitinib).

Figure 11. Representative RTKIs in clinic

Type II inhibitors bind to the inactive form of the kinase (eg. sorafenib) and indirectly compete with ATP by reversibly occupying the catalytic DFG pocket. Type III inhibitors bind in an allosteric fashion outside the catalytic site, whereas the type IV inhibitors bind reversibly to the substrate binding site. Type V inhibitors bind covalently to cysteine residues at specific site of the tyrosine kinase (eg. afatinib).48,50
VEGFR

The VEGF family consists of five members, VEGF-A, VEGF-B, VEGF-C, VEGF-D, and placenta growth factor (PLGF) and constitute important growth factors that are involved in angiogenesis and vasculogenesis (i.e., the formation of the embryonic circulatory system). VEGFRs comprise of VEGFR-1, VEGFR-2 and VEGFR-3. Among these, VEGFR-1 and VEGFR-2 are expressed on the surface of endothelial cells, whereas VEGFR-3 is expressed on lymphatic vessels. Hypoxia mediated upregulation of VEGF-A results in activation of VEGFR-2, which further causes tumor development, progression and metastasis. VEGFRs are overexpressed in several tumor types with different expression patterns, ligand specificity and cellular/physiological effects. Thus, targeted inhibition of VEGFR-2 produces abrogation of angiogenesis, and decreased tumor growth. Sunitinib, a mutli-RTKI (VEGFR-2, PDGFR-β and c-kit) is clinically used in the treatment of renal and pancreatic cancers. Sorafenib, another mutki-RTKI (VEGFR-2, VEGFR-3, PDGFR-β and Raf kinase) is used in the treatment of renal and thyroid cancers.

PDGFR

The PDGF family consists of five growth factors namely, PDGF-AA, -BB, -CC, -DD, and -AB, which bind to two receptors, PDGFR-α and PDGFR-β. PDGFRs are structurally related to VEGFRs. These receptors are expressed on pericytes, smooth muscle cells that provide mechanical support to vasculature. Aberrant signaling of PDGF in tumor angiogenesis, either alone or in combination with VEGF, stimulates endothelial cell proliferation, migration, sprouting and tube formation in tumors. The importance of PDGF signaling in tumor angiogenesis is further supported by several studies.
demonstrating that PDGFR inhibitors improve the antitumor efficacy of VEGFR inhibitors.\textsuperscript{59}

**EGFR**

The EGFR is one of the four members of the EGFR family comprising of EGFR-1(EGFR), HER-2, HER-3, and HER-4. The ligand for EGFR, EGF, regulates cellular pathways linked to cell proliferation, migration and differentiation, such that any aberration in this signaling cascade results in increased tumor cell proliferation, survival and invasiveness.\textsuperscript{60} High expression of EGFR has been associated with primary human tumors and glioblastoma.\textsuperscript{61} As a result, EGFR has been extensively studied and targeted by small molecule inhibitors (erlotinib\textsuperscript{62}) and monoclonal antibodies (cetuximab).\textsuperscript{63}

**B.3 RTK binding site**

![RTK binding site diagram](image_url)

Most protein kinases have a common structural feature consisting of two lobes, the N-terminus lobe consisting of five antiparallel strands and one helix, and a highly helical C-terminus lobe (Figure 12).\textsuperscript{64} The ATP binding site is a narrow hydrophobic pocket located between these two lobes linked by a flexible hinge region. The hinge region usually has one hydrogen donor flanked by two hydrogen acceptors that binds to the adenine ring of ATP.\textsuperscript{64, 65}

The catalytic cleft of RTKs is composed of the N-lobe and the C-lobe. The N-lobe contains the glycine-rich loop (G-loop or P-loop) formed by highly conserved GXGXXG motif. The G-loop forms the top (roof) of the ATP binding cleft and due to its flexibility adopts various conformations, depending on the presence of ligand and the conformational state of the kinase. The catalytic cleft consists of the front cleft, where the ATP binding site is located and the back cleft, which comprises of elements important for the catalytic activity of the kinase. These two regions share a border formed by $\beta_8$-strand, an activation loop (A-segment) on which lies the Asp-Phe-Gly (DFG) motif and the $\beta_3$-strand. A gatekeeper residue at the back cleft controls the access to the back cleft depending on its size and volume. A small amino acid like Ala or Thr make the back cleft easily accessible whereas, bulkier gatekeeper residues like Phe, Leu or Met prevent the entry of small molecules into the back cleft.\textsuperscript{64, 65} $\beta_3$- Lysine is another functionally important residue that is conserved in all kinases and adopts various conformations in different protein kinase complexes. It helps anchor the $\alpha$- and $\beta$-phosphates of ATP to form a Lys-ATP complex that is stabilized by an ion pair interaction between Lys and the catalytic Glu residue (Figure 12).
In a fully active state, protein kinases adopt a DFG-in conformation where the side chain of the DFG aspartate is directed into the ATP binding site and the aromatic ring of the Phe is positioned in the back cleft. The Asp residue is required to chelate Mg$^{2+}$ and helps to orient the γ-phosphate of the ATP for its transfer in the active DFG-in conformation. The aromatic side chain of Phe in the active DFG-in motif is in contact with αC. This contact in many active kinases facilitates the formation of a Lys-Glu ion pair for kinase catalysis. In the inactive state, the DFG motif is in either a DFG-in, DFG-out or DFG-out like conformation. In the DFG-out conformation, the aromatic ring of Phe is positioned in the ATP pocket and the Asp residue of the DFG motif is in the back cleft.

The front cleft includes the ATP binding site and small, non-ATP contact regions. The ATP site is broadly divided into the following subregions depending on the binding mode.

1. Adenine region: This pocket is predominantly hydrophobic and is involved in binding various inhibitor scaffolds. This region is bordered by the hinge region and the gatekeeper. Two key hydrogen bonds are formed by the interaction of the N1 and N6 amino nitrogens of the adenine ring with the NH and carbonyl groups of the peptide backbone of the hinge region residues of the RTKs. Several RTK inhibitors use at least one of these key hydrogen bonds. Other backbone amide groups in the hinge region can serve as hydrogen bond donors or acceptors for inhibitor binding.

2. Ribose binding region: The ribose pocket accommodates the sugar moiety of ATP and is adjacent to a hydrophilic, solvent exposed region. This pocket includes includes three hydrophobic residues. These residues (Ile, Val and Leu) have traditionally been exploited in developing EGFR inhibitors.
3. Phosphate binding region: This region is covered by G-loop, Asp, Lys and Asn residues and the DFG motif. This region is highly flexible, hydrophilic and solvent exposed, and therefore, this region has been explored to improve physicochemical properties of inhibitors.\(^6^4\)

4. Hydrophobic region II: This pocket is not used by ATP and serves as an entrance for ligand binding. Variation in the residues and conformation of this pocket have been reported for various kinase targets. Thus, this region has been used to gain selectivity in the design of kinase inhibitors.

In addition to the ATP-binding site, the back cleft provides important binding regions. ATP does not bind in the pockets in the back cleft.\(^6^4,6^5\)

1. Hydrophobic region I: The hydrophobic pocket in the back cleft adjacent to the adenine pocket is called Hydrophobic region I or Back pocket I (BP-I). This pocket has been explored in the design of inhibitors to gain selectivity for kinase targets with small gatekeeper residues.

2. In addition to BP-I, additional binding pockets BP-II, BP-III, and BP-IV become available to inhibitors depending on the binding state of the RTKs.\(^6^4\)

Major progress has been made with small molecule inhibitors targeting the ATP-binding site of RTKs. The front catalytic cleft of all kinase enzymes is accessible to ligand binding. Small molecule inhibitors that target the front cleft use a core scaffold to recognize the adenine pocket. The core scaffold is then substituted to extend into the different pockets of the ATP-binding site resulting in improved binding affinity and selectivity for RTKs.\(^6^6\)
C. Toll-like receptors (TLRs)

The activation of the innate immune system is the primary cellular immune response that occurs within the body to defend against foreign agents, such as bacteria or viruses. The pattern recognition receptors (PRRs) located on the cell surface, or within the endosomes of the innate immune cells interact with the extracellular environment and modulate the immune system. The toll-like receptors (TLRs) are the key PRRs of the innate immune system.67

![Diagram of TLR Pathways]


TLRs are expressed in multiple cell types including dendritic cells (DCs, key antigen-presenting cells in adaptive immunity), macrophages, and B-cells. On agonist binding, TLRs form high order constructs (homodimers, heterodimers, and tetramers) initiating a downstream signaling cascade that causes maturation of immune cells and expression and release of immunostimulatory cytokines (Figure 13).67 The general
structure involves a horseshoe-shaped motif composed of leucine rich repeat (LRR) domain around which two TLRs form a constitutive dimer. The variability in the hydrophobic pocket of the LRR domain with its surrounding variable region determines the agonist selectivity for each TLR.67

TLRs are broadly classified based on their location in the cell. TLRs 1, 2, 4, 5, and 6 are expressed on the cell surface, whereas, TLRs 3, 7, 8, and 9 signals from the endosomes. The binding of agonists induces a conformational change that recruits adapter proteins and triggers signaling. The specific modulation is thus also dependent on the type of adapter protein. MyD88 is the predominant adapter protein and leads to activation of serine/threonine kinase IRAK4 that ultimately results in induction of pro-inflammatory cytokines. Activation of other adapter proteins such as TIR domain-containing adaptor-inducing interferon-β (TRIF) and TRIF-related adaptor molecule (TRAM) causes induction of genes encoding Type I interferons.68

Figure 14. Representative TLR agonists
TLRs1 and 2 are activated by lipids and polysaccharides found on the surface of the bacterial cell wall. Their activation results in MyD88 dependent upregulation NFκB and increase in immune cell activation. Triacylated peptides that mimic natural agonists have been reported as TLR1 agonists. In addition, small molecule agonists including thioureido-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylates (1) and N-benzyl-2-(N-phenylmethylsulfonamido)acetamide derivatives (2) have shown to activate the TLR1/2 pathway (Figure 14). TLR6 acts as a compliment to TLR1/2 heterodimer and is similarly activated by lipids, lipiddated proteins and polysaccharides. TLR4 is one of the most studied receptor and is activated by lipopolysaccharides (LPS). It can signal via both MyD88 and TRIF pathway. Recently, pyrimido[5,4-b]indoles (3) were reported as selective TLR4 agonists (Figure 14). TLR5 binds to flagellin and flagellin-related peptides derived from bacterial species.

TLR 9 is an endosomal TLR that is activated by bacterial oligonucleotides. It is particularly specific for binding to unmethylated cytosine guanine (CpG) dinucleotide sequences. The downstream signaling pathway proceeds via MyD88 pathway and results in polarized T-helper cell 1 (Th1) response in which dendritic cells interact with pre-T helper cells to produce interferon (IFN)-γ, interleukin (IL)-1, IL-2, tumor necrosis factor (TNF)-β and activation of macrophages. Similar to TLR9, TLR3 is also located in the endosomal compartment of cells such as DCs and macrophages. It is activated by double-stranded viral RNA (dsRNA) and signals exclusively through TRIF pathway. No small molecule TLR3 agonist has been reported so far.

TLR7 and 8 are endosomal receptors that are activated by single-stranded RNA (ssRNA) and heteroaromatics. The X-ray crystal structure of TLR8 was recently published.
that suggested that it exists as a homodimer prior to ligand binding and activation. The C-terminal domains of both TLR7 and 8 are 53 Å apart prior to ligand binding. However, binding of the agonists to the homodimer allows the C-terminal subunits to approach a distance of 23 Å, dimerizing the intracellular toll-interleukin-1 receptor (TIR) domain and initiating signaling cascade resulting in an immune response. Among the heteroaromatics, imidazoquinolines (imiquimod and resiquimod, Figure 14) and thiazolo[4,5-c]quinolones (4,5) have been shown to have TLR7 and 8 agonistic activity. Imiquimod works through TLR7 mediated activation of MyD88 pathway that causes secretion of proinflammatory cytokine INFα and induction of IL-6. Imiquimod is clinically approved for the treatment of basal cell carcinoma and resiquimod has shown efficacy in breast cancer model and is currently undergoing clinical trials for the treatment of melanoma. Other small molecule agonists for TLR7 and TLR8 are currently undergoing evaluation for both solid and haematological malignancies.

**Figure 15.** A) X-ray crystal structure of resiquimod in TLR7 (PDB: 5GMH). B) X-ray crystal structure of resiquimod in TLR8 (PDB: 3W3L).
The aromatic ring of resiquimod undergoes π-stacking interaction with the Phe408 in TLR7 and Phe405 in TLR8 (Figure 15). The Asp555 in TLR7 and Asp543 in TLR8 are essential for binding. A key difference in the binding site is the presence of Leu557 in TLR7, which undergoes hydrophobic interaction with the aromatic quinoline ring of resiquimod. In TLR8 an Asp545 residue is present that does not undergo hydrophobic interaction but provides a handle for design of TLR8 selective agonists by incorporating protonatable amines that can undergo ionic interaction with this residue.\(^{76}\)

Early studies demonstrated that the activation of TLR7 or TLR8 can reverse the suppressive function of the regulatory T cells. In addition, agonist binding to TLR7 and TLR8 led to the activation of dendritic cells and natural killer cells that directly kill tumor cells.\(^{77}\) Together these mechanisms resulted in strong antitumor response.\(^{68}\) On activation, the immature DCs engulf antigens from dying tumor cells and migrate to the draining lymph nodes, where they process the antigen. They mature into DCs that are capable of presenting antigens via major histocompatibility complex (MHC) class I and class II molecules on their surface. Through the MHC interaction with CD4\(^+\) T helper cells and CD8\(^+\) effector cells, activation of T cells ensues that engages in T cell surveillance and eventually result in tumor cell death.\(^{78}\)

No clinical benefit of TLR7 and 8 agonists, when used as single agents has been demonstrated so far. However, it has been suggested that combining TLR agonists with chemotherapy or radiotherapy can provide better results in clinic. The cytotoxic component of the chemotherapy can promote cancer cell death releasing antigens that can stimulate immune response.\(^{79}\) RNA-based TLR7 and TLR8 agonists when used in combination with chemotherapeutic agents demonstrated antitumor effect in mouse model of non-small cell
lung cancer, in non-human primates and in human cells in an IFN dependent manner and induction of Th1 response.\textsuperscript{68, 80} Evaluation of TLR7 agonist in combination with radiation therapy in murine T cell and B cell lymphoma model suppressed the progression of tumor growth and improved the survival rate in tumor-bearing mice.\textsuperscript{81} Administration of gardiquimod (TLR7/8 agonist) in combination therapy with NK cells stimulated with DCs was shown to significantly suppress the growth of human HepG2 liver carcinoma xenografts.\textsuperscript{82}

**D. Dihydrofolate reductase (DHFR)**

Dihydrofolate reductase, a key enzyme in folate metabolism, is present in all living organisms including humans, bacteria and protozoa. DHFR plays a crucial role in maintaining cellular levels of reduced folates (tetrahydrofolate and its derivatives). DHFR catalyzes the reduction of 7,8-dihydrofolate (FH\textsubscript{2}) to the 5,6,7,8-tetrahydrofolate (FH\textsubscript{4}) in the presence of nicotinamide adenine dinucleotide phosphate (NADPH) as a cofactor. Inhibiting DHFR interferes with thymidylate biosynthesis involved in pyrimidine synthesis and inhibition of folate-dependent formyl transferases causing inhibition of purine synthesis as well. It has long been considered as an attractive target for anti-infective therapy because of its crucial role in the biosynthesis DNA, RNA and proteins.\textsuperscript{83}

![Catalytic mechanism of DHFR](image)

**Figure 16.** Catalytic mechanism of DHFR\textsuperscript{84}
The mechanism of DHFR is not totally understood, although it has been extensively studied. DHFR catalyzes a hydride transfer from NADPH to the C6 of 7,8-FH₂ to form FH₄ (Figure 16). It is known that initial protonation of N5 followed by a hydride transfer from the C4 of the cofactor NADPH to the adjacent FH₂ reduces FH₂ to FH₄. The 3D-structures of DHFR have been used in structure-based drug design and to acquire information on inhibitor binding, enzyme-inhibitor-cofactor complexes and in particular to determine differences in amino acid location between parasites and host DHFR. The DHFR derived from *Escherichia coli* (ecDHFR) has been widely studied as a model system to understand the role of protein dynamics in enzyme catalysis and also in investigating the binding selectivity of 2,4-diaminopyrimidine based inhibitor, trimethoprim (TMP).\(^{84-87}\)

![Figure 17. X-ray crystal structure of methotrexate (MTX, purple) in ecDHFR complex with NADPH (brown) (PDB: 1RX3).\(^88\) The residues are highlighted in yellow.](image)

Evidence from NMR experiments suggests that the pKₐ of certain inhibitors such as TMP at N1, is increased from 7.5 in solution to about 10 on binding to DHFR. The Asp27 (Figure 17) gets deprotonated and protonates N1, resulting in a bidentate interaction
of Asp27 with N1 and N2 of TMP. The 4-NH$_2$ group in TMP undergoes H-bonding interactions with Ile5 and Ile94. Favorable van der Waals interactions between the trimethoxybenzene ring of TMP with Leu28, Phe31, Ser49, Ile50, Leu54 contributes to the strong affinity of TMP to ecDHFR.$^{86}$ Other factors contributing to the binding of inhibitors to DHFR include NADPH induced changes in the active site. It has been shown that NADPH binding promotes desolvation of the pocket where TMP binds and induces a conformational change of side chain Met20 ($\sim$8 Å). Depending on the conformation of the loop, the electrostatic environment around the substrate changes and alters the pK$_a$ of N5 of FH$_2$ prior to hydride transfer.$^{84}$

![Figure 18](image_url)  

**Figure 18.** Ribbon structure of ecDHFR with bound NADP$^+$ (green) and folate (blue), in closed (PDB: 3QL3, red) and occluded (PDB: 1RX6, blue) conformation of flexible Met20 loop. Reprinted with permission from “Perspectives on electrostatics and conformational motions in enzyme catalysis” by P. Hanoian, C. T. Liu, S. Hammes-Schiffer and S.
In the closed conformation, the Met20 loop interacts with the substrate (folate), while in the occluded conformation the loop occupies the NADPH binding site partially (Figure 18). The selectivity of TMP towards \( \text{ecDHFR} \) over eukaryotic DHFR (chicken liver, \( \text{clDHFR} \)) was attributed to i) the differences in residues undergoing bidentate interaction with N1 and N2 of TMP (Asp27 in \( \text{ecDHFR} \) vs Glu30 in \( \text{clDHFR} \)); ii) differences in H-bonding of 4-\( \text{NH}_2 \) of the pyrimidine ring (two H-bonding in \( \text{ecDHFR} \) vs one H-bonding in \( \text{clDHFR} \)); iii) enzyme-inhibitor interaction at trimethoxybenzyl group; iv) side chain torsion angles of the inhibitor and v) conformational change of side chain residues on TMP binding.

\[ \text{Figure 19. Chemical structure of folic acid and representative DHFR inhibitors} \]
DHFR inhibitors are classified as classical inhibitors that mimic folic acid with its glutamate side chain like methotrexate (MTX) and nonclassical DHFR inhibitors that lack the \(p\)-aminobenzoylelglutamic acid side chain like trimethoprim (TMP) and pyrimethamine (Figure 19). Nonclassical DHFR inhibitors show high potencies, which suggested that the \(p\)-aminobenzoylelglutamic acid side chain was not essential for binding to DHFR. The discovery of TMP and pyrimethamine as potential antibacterial and antimalarial agents stimulated the research for new nonclassical antifolates. These agents could be used in anti-infective therapy since bacteria and protozoa synthesize folic acid \textit{de novo} but do not utilize preformed folate. Moreover, these analogues are hydrophobic in nature and are therefore taken up by passive diffusion. The main drawback of these potent antifolates is their lack of selectivity against DHFR derived from pathogens.

![Figure 20. Pyrimethamine derived inhibitors of \textit{Plasmodium falciparum} DHFR (pfDHFR)](image)

Yuthavong \textit{et al}\textsuperscript{89} recently reported pyrimethamine derived pfDHFR inhibitors 6 and 7 (Figure 20) that were active in both wild-type and quadruple mutant DHFR. The carboxylic acid in 7 interacted with Arg122 in pfDHFR but did not make the same interaction with the corresponding Arg70 in hDHFR.
Pyrimethamine derivatives were also recently reported as selective *Toxoplasma gondii* DHFR (tgDHFR) inhibitors with 8 (Figure 21) showing 89-fold selectivity for tgDHFR over hDHFR.\(^9^0\)

![Pyrimethamine and compound 8](image)

**Figure 21.** Pyrimethamine derived inhibitors of tgDHFR

![Triazines and dihydrotriazine](image)

**Figure 22.** Triazines and dihydrotriazine based DHFR inhibitors

Traizine 9 and dihydrotriazine 10 were also recently reported as *Mycobacterium tuberculosis* DHFR (MtbDHFR) and *pf*DHFR inhibitors, respectively (Figure 22).\(^9^1, 9^2\)

*Pneumocystis jirovecii* (pj) is one of the opportunistic pathogen that infects immunocompromised patients, especially those with acquired immune deficiency syndrome (AIDS).\(^9^3\) Pneumocystis pneumonia (PCP) in humans is caused by the opportunistic pathogen *pj*, while *Pneumocystis carinii* (pc) is the pathogen that is derived from and infects rats. Due to ease in purification and crystallization, structural characterization, catalytic properties and interaction with antifolates of pcDHFR have been
reported. Cody et al. were able to isolate recombinant human-derived pneumocystis DHFR (pjDHFR) and showed that it differs from pcDHFR by 38% in amino acid sequences.

**Figure 23.** The structure of PTX and 11 and 12

Piritrexim (PTX) is a potent inhibitor of pjDHFR (IC₅₀ = 1.6 nM) but is devoid of selectivity. Gangjee et al. recently reported 11 and 12 (Figure 23) as PTX analogs that retained the potency and showed selective inhibition of pjDHFR (35-fold and 26-fold, respectively).
II. CHEMICAL REVIEW

This section will review the synthetic chemistry approaches relevant to the heterocyclic ring systems described in this dissertation.

A. Quinazolines

B. Quinolines

C. Thieno[2,3-d]pyrimidines

1. Quinazolines

Various synthetic methodologies have been employed in the synthesis of quinazolines. Few of them will be discussed here from substituted benzene precursors.

**Scheme 1. Synthesis of quinazoline from anilines**

Protection of the amino group of the aniline 13 with ethyl chloroformate (Scheme 1) and subsequent reaction with hexamethylenetetramine (HMTA) in trifluoroacetic acid (TFA) resulted in the formation of cyclized dihydrobenzoquinazoline 15. Oxidation of 15 using K\textsubscript{3}Fe(CN)\textsubscript{6} afforded the appropriate quinazoline 16 in 15-54% yield.\textsuperscript{97, 98}
Scheme 2. Synthesis of quinazoline from 2-aminoarylketones and benzylamines

Graphite oxide was used as a recyclable heterogenous catalyst (Scheme 2) in the synthesis of 2-arylquinazoline 19 (84-93%), from 2-aminoarylketones 17 and benzylamines 18. To ensure complete oxidation t-butyl hydroperoxide (TBHP) was used.98

Scheme 3. Synthesis of quinazoline from 2-iodobenzaldehydes via Ullmann reaction

Ligand free Cu-catalyzed Ullmann reaction conditions (Scheme 3) were used for preparing substituted quinazolines 22 in 61-89% yield from 2-iodobenzaldehydes 20 and amidine hydrochloride 21.98,100
Scheme 4. Synthesis of 2-aryl-4-methylquinazolines

In a similar reaction, quinazolines 25 (Scheme 4) were synthesized substituted 2-bromoacetophenone 23, aldehyde 24 and aq. ammonia in the presence of Cu catalyst (CuCl) and N-methylpyrrolidone (NMP) as a solvent. The reaction was proposed to occur via Cu(I) catalyzed amination of 23 to yield substituted 2-aminobenzophenone that further reacted with the aldehyde 24.98, 101

Scheme 5. Synthesis of 1(H)-quinazolin-2-one from alkynylanilines

The synthesis of target compounds were accomplished in two steps (Scheme 5). In the first step urea derivative 27 was obtained by reacting o-alkynylaniline 26 with potassium isocyanate. In the subsequent step acid catalyzed intramolecular cyclization of 27 yielded 28 in 28-98% yield.98, 102
Scheme 6. Synthesis of quinazolin-4(3H)-one from anthranilic acid

Treatment of anthranilic acid 29 (Scheme 6) with aliphatic anhydride yielded corresponding benzoxazinones 30, which were used further without purification to form acylaminobenzamides 31. Subsequent cyclization with formamide under microwave irradiation conditions afforded the desired quinazolin-4-one 32 in 74-87% yield.\(^{98, 103}\)

In another method, 2-nitrobenzamide 33 was reacted with aromatic aldehyde 34 in presence of sodium dithionate to yield quinazolin-4(3H)-one 38 (Scheme 7). Mechanistically, the reaction proceeded via reduction of nitro by sodium dithionate to amine 35 that cyclized with the aldehyde to yield 2,3-dihydroquinazolin-4(1H)-one 36. The sulfur dioxide from sodium dithionate subsequently participated in the oxidation to yield desired compound 38.\(^{98, 104}\)
Scheme 7. Synthesis of quinazolin-4(3H)-one from 2-nitrobenzamides

Scheme 8. Synthesis of quinazolin-4(3H)-one from isatoic anhydride

In a one-pot three-component reaction (Scheme 8) with benzyl halides 39, amines 40 and isatoic anhydride 41 quinazolin-4(3H)-one 42 were synthesized via in situ generation of aldehydes by DMSO mediated oxidation of benzyl halide in the presence of K$_2$CO$_3$ in excellent yields.$^{98, 105}$
2. Quinolines

B.1 Acid catalyzed synthesis of quinolines

Three component Povarov reaction (Scheme 9) of arylamine 43, glyoxalates 44 and phenylacetylenes 4 via aza Diels-Alder cycloaddition catalyzed led to the synthesis 4-arylquinoline-2-carboxylates 46 (78-92%). The reaction mechanism involves an initial formation of imine. Acid catalyzed protonation of imine nitrogen facilitates the attack by phenylacetylene that results in a cyclized intermediate and subsequent oxidation to give the desired quinolines in good yields. 106, 107

Scheme 9. Synthesis of quinoline-2-carboxylates catalyzed by formic acid

Scheme 10. Synthesis of 2-(2-pyridyl)quinolines

38
Trifluoride methyl etherate catalyzed three-component Pavorav reaction (Scheme 10) of arylamines 47, pyridine aldehydes 48 and ethyl vinyl ether 49 afforded 2-(2-pyridyl)quinolines 50 in moderate to good yields.\textsuperscript{107, 108}

**Scheme 11.** HCl-mediated synthesis of 4-methylquinolines

![Scheme 11](image)

Doebner type multicomponent reaction (Scheme 11) of arylamine 51, benzaldehyde 52 and acetone 53 in the presence of HCl yielded 4-methyl quinolines 54 in 70-98% yield.\textsuperscript{107}

**Scheme 12.** Synthesis of substituted quinolines from substituted arylisothiocyanates

![Scheme 12](image)

Zhao *et al.*\textsuperscript{109} reported a multicomponent reaction (Scheme 12) of arylisothiocyanates 55, alkyne 56 and alkyltriflate to yield substituted quinolines 57 in high yields.\textsuperscript{107}
Scheme 13. Triflic acid catalyzed synthesis of quinolines from arylmethyl azides

Arylmethyl azides 58 were used as precursors to generate $N$-aryl iminium ions via Schmidt rearrangement reaction (Scheme 13). Ethyl 3-ethoxyacrylate 59 was added next to yield substituted quinolines 60 in high yields.\textsuperscript{107, 110}

B.2 Base catalyzed synthesis of quinolines

2-substituted-3-nitroquinolines 66 (Scheme 14) were synthesized by the attack of the amino group of 61 on the nitro olefin 62 to form a 1,4-addition intermediate which on dehydration and oxidation with DDQ yielded the desired compounds in 25-97\% yield.\textsuperscript{107, 111}
Scheme 14. Synthesis of quinolines from nitro olefins

![Scheme 14](image)

Scheme 15. Synthesis of 2,4-disubstituted quinolines

![Scheme 15](image)

Base catalyzed cyclization of β-(2-aminophenyl)-α,β-ynones 67 (Scheme 15) via tandem nucleophilic addition annulation reaction in the presence of sodium methoxide afforded the desired quinolines 69 in 32-98% yields.\textsuperscript{107, 112}

A three-component reaction with cyanoacetic acid methyl ester 70, substituted secondary amine 71 and 2-aminobenzaldehyde 72 in the presence of sodium hydroxide yielded 2-aminoquinoline-3-carboxamides 73 in good yields (Scheme 16).\textsuperscript{107, 113}
Scheme 16. Synthesis of 2-aminoquinoline-3-carboxamides

Scheme 17. Synthesis of 7-hydroxyquinolines from N-tosylaminophenols

7-hydroxyquinoline 79 was prepared from condensation of 3-N-tosylaminophenol 74 with acrolein 75 to give intermediate 76 (Scheme 17). In the presence of ethanol, acetal 77 was formed that underwent Friedel-Craft reaction, followed by dehydration, oxidation and detosylation to yield 7-hydroxyquinoline in 60% yield.107,114
**Scheme 18.** Synthesis of 2,3,4-trisubstituted quinolines

Quinolines 81 (Scheme 18) were synthesized in good yields from o-cinnamylanilines 80 (prepared from anilines and cinnamylalcohols) via a regioselective 6-endo-trig intramolecular oxidative cyclization using potassium tert-butoxide and DMSO as an oxidant.\textsuperscript{107, 115}

B.3 Miscellaneous methods

**Scheme 19.** Iodine catalyzed synthesis of 2,4-disubstituted quinolines
Using molecular iodine anilines 82 and styrenes 83 were reacted together to form 2,4-disubstituted quinolines 84 in 64-83% yields (Scheme 19). Mechanistically, the reaction proceeds via oxidation of styrene to aldehyde, which condenses with arylamine to give an imine. Iminium ion is formed on coupling of imine with iodine that undergoes aza-Diels-Alder cycloaddition reaction and subsequent oxidation to form the desired quinolines.107, 116

Scheme 20. Synthesis of 2-amino-4-arylquinoline-3-carbonitriles

L-proline mediated synthesis of 2-amino4-arylquinoline-3-carbonitriles 4 (Scheme 20) was performed using a multi component reaction involving anilines 85, aldehydes 86 and malononitrile 87 under aqueous conditions.107, 117

Substituted quinolines 97 were synthesized from o-tosylamidobenzaldehydes 91 and activated acetylenes 90 in a phosphine-catalyzed one-pot procedure (Scheme 21). Mechanistically, nucleophilic addition of phosphine to the alkyne 89 generated phosphonium allenoalte 90, which acts as a base to activate the pro-nucleophile 91 through deprotonation, causing a subsequent base-catalyzed Michael/Morita-Baylis-Hillman tandem reaction and aromatization to yield 97.107, 118
2,4,6-trisubstituted quinolines 102 were synthesized via Au-catalyzed intramolecular cyclization of N-propargylamines 101 in methanol at room temperature in good yields (Scheme 22). The N-propargylamines were prepared from anilines 98, alkynes 99 benzaldehydes 100 using RuCl₃/CuBr. Under the reaction conditions, different substituents were tolerated at R¹ position, however, aryl substitution with electron-withdrawing group at R² position and alkyl groups at R³ position were not tolerated.
**Scheme 22.** Au-catalyzed synthesis of quinolines from \( N \)-propargylamines

\[
\begin{align*}
\textbf{98} & \quad \text{R}^2 \quad \text{H} \quad \text{R}^1 \quad \textbf{99} \\
\text{Ar} & \quad \text{NH}_2 \\
\text{CuBr} & \\
\text{RuCl}_3 & \\
\text{MeOH, rt} & \\
\text{AuCl}_3 (5 \text{ mol%}) & \\
\overrightarrow{\text{R}^3} & \\
\textbf{100} & \quad \text{R}^2 \quad \text{H} \quad \text{R}^1
\end{align*}
\]

\[
\begin{align*}
\text{RuCl}_3 & \\
\text{Cubr} & \\
\text{AuCl}_3 (5 \text{ mol%}) & \\
\text{MeOH, rt} & \\
\overrightarrow{\text{R}^3} & \\
\textbf{101} & \quad \text{R}^2 \quad \text{H} \quad \text{R}^1
\end{align*}
\]

(65-87%)

13 examples

3. Thieno[2,3-\( d \)]pyrimidines

C.1 Synthesis of thieno[2,3-\( d \)]pyrimidines from thiophene precursors

**Scheme 23.** Synthesis of thieno[2,3-\( d \)]pyrimidine 107

\[
\begin{align*}
\textbf{103} & \quad \text{X} = \text{CN}, \\
\textbf{104} & \quad \text{X} = \text{COOR}, \\
\textbf{105} & \quad \text{X} = \text{CHO} \\
\text{NH}_2 & \quad \text{R}^2 \\
\text{NH} & \quad \text{R}^1
\end{align*}
\]

\[
\begin{align*}
\textbf{104} & \quad \text{R} = \text{NH}_2, \text{Cl} \\
\textbf{105} & \quad \text{Y} = \text{NH}_2, \text{OH}, \text{H} \\
\textbf{106} & \quad \text{R}^2 \\
\textbf{107} & \quad \text{Y} \quad \text{R}^1
\end{align*}
\]

2-Amino-5,6-disubstituted thieno[2,3-\( d \)]pyrimidines 107 (Scheme 23) were synthesized via cyclocondensation of appropriate thiophenes 103-105 with an amidine derivative 106, (guanidine \( \text{R} = \text{NH}_2 \) or chloroformamidine hydrochloride \( \text{R} = \text{Cl} \)). The nature of the X substitution in thiophene 103-105 determined the substitution pattern at 4-
position (Y at the C4-position) in 107; when X = CN, COOR or CHO, the cyclization reaction afforded 107 with Y = NH₂, OH or H respectively.¹²⁰,¹²¹

**Scheme 24.** Synthesis of thieno[2,3-d]pyrimidine 110

A novel synthesis of 2-amino-4-phenyl substituted thieno[2,3-d]pyrimidines 110 (Scheme 24) was reported by Ishikawa and coworkers. Urea 109 was utilized for condensation with aminocarbonyl thiophenes 108 to afford 110.¹²²

**Scheme 25.** Synthesis of thieno[2,3-d]pyrimidine 113

The 2-substituted-4-oxo-thieno[2,3-d]pyrimidines 113 (Scheme 25) were synthesized by the condensation of thiophene 111 and aldehyde 112. The R group of 122 can be an alkyl or aromatic substitution. 2-Methyl-5,6-disubstituted-thieno[2,3-d]pyrimidines 113 were obtained when the R group is a methyl group.¹²³
A modified approach was reported by Corral and coworkers$^{124}$ for the synthesis of thieno[2,3-$d$]pyrimidine, using acid chlorides as the cyclization reagent instead of an aldehyde (Scheme 26). The amino group of the thiophene 114 attacked the carbonyl group of acid chloride 115 to form the intermediate amide 116, which cyclized with NH$_3$ to afford thieno[2,3-$d$]pyrimidines 117. Good leaving groups, such as OCH$_3$, at R$_3$ position in 114 resulted in OH substitutions at the 4-position in 117. When acetyl chloride was used as the reactant, 2-methyl-5,6-disubstituted thieno[2,3-$d$]pyrimidines 117 was obtained after cyclization.

Horiuchi and coworkers$^{125}$ reported the one-pot synthesis of 119. Thiophene 118 was reacted with formamide to afford 119 in good yields (Scheme 27).
Scheme 28. Synthesis of thieno[2,3-d]pyrimidine 122

![Scheme 28](attachment:Scheme_28.png)

Briel, D.\textsuperscript{126} reported the synthesis of 2-phenyl substituted thieno[2,3-d]pyrimidine 122 via the condensation of thiophene 120 with benzamidine hydrochloride 121 (Scheme 28).

C.2 Synthesis of thieno[2,3-d]pyrimidines from pyrimidine precursors

Scheme 29. Synthesis of thieno[2,3-d]pyrimidine 126

![Scheme 29](attachment:Scheme_29.png)

Taylor and coworkers\textsuperscript{127} reported the synthesis of the classical TS inhibitor 126 as an analogue of pemetrexed (Scheme 29). Condensation of 2-substituted-4-hydroxy-6-mercaptopypyrimidine 123 with the α-chloroketone 124 gave pyrimidine sulfide 125, which was further cyclized in the presence of p-toluenesulfonic acid to give 126.
Scheme 30. Synthesis of thieno[2,3-d]pyrimidine 130

A novel approach for the synthesis of thieno[2,3-d]pyrimidine 130 was reported by Sakamoto and coworkers\(^\text{128}\) (Scheme 30). The intermediate 129 was synthesized by Sonogashira coupling between iodopyrimidines 127 and ethynyltrimethylsilane 128. Compound 129 then cyclized to give 130 in the presence of NaSH.

Scheme 31. Synthesis of thieno[2,3-d]pyrimidine 134

A novel approach for the synthesis of the thieno[2,3-d]pyrimidine 134 was reported via a cyclization reaction of 5-carbonitrile-6-mercaptopyrimidines 131 and chloroacetone 132 (Scheme 31).\(^\text{129}\) Compound 133 was the key intermediate in the reaction, which was further cyclized in the presence of NaSH to give 134.

Scheme 32. Synthesis of thieno[2,3-d]pyrimidine 136

...
Similarly, chloroacetonitrile 135 was utilized instead of chloroacetone 132, with 131 to afford 5-amino-4-methyl-2-phenyl-thieno[2,3-\textit{d}]pyrimidine-6-carbonitrile 136 (Scheme 32).^{130}

**Scheme 33.** Synthesis of thieno[2,3-\textit{d}]pyrimidine 139

\[
\begin{align*}
137 & \quad \text{Ph} \quad \text{CO}_2\text{Et} \\
138 & \quad \text{MeOCl} \\
139 & \quad \text{Ph} \quad \text{OH} \quad \text{CO}_2\text{Me}
\end{align*}
\]

Briel and coworkers\textsuperscript{129} reported a strategy to synthesize 139 (Scheme 33). 5-ethylester-6-mercaptopyrimidines 137 was cyclized with chloroacetic acid methyl ester 138 to afford thieno[2,3-\textit{d}]pyrimidine 139 with a 5-hydroxy.

**Scheme 34.** Synthesis of thieno[2,3-\textit{d}]pyrimidine 147

\[
\begin{align*}
\text{140} + \text{141} \quad \text{NaOH} & \quad \text{R}^1\text{CO} \\
\text{142} & \quad \text{H}_2\text{N} \quad \text{143} \\
\text{144} & \quad \text{EtO} \quad \text{145}
\end{align*}
\]

van Straten and coworkers\textsuperscript{131} reported the synthesis of 147 by the procedure depicted in Scheme 34. \(\alpha,\beta\)-unsaturated ketones 142, obtained by aldol condensations, were reacted with 2-cyanothioacetamide 143 to afford thiopyridones 144, which underwent nucleophilic substitution and cyclization to afford 147.
Scheme 35. Synthesis of thieno[2,3-d]pyrimidine 151

Based on the cyclization reaction shown in Scheme 3, a modified method was developed by Ried and coworkers (Scheme 35).\textsuperscript{132} 5-Carbonitrile-6-chloropyrimidine 148 reacted with ethyl 2-mercaptoacetate 149 to form intermediate 150, which was further cyclized to afford 151.
III. STATEMENT OF THE PROBLEM

1. The present work deals with the design and molecular modeling studies in the following three major areas:

1.A Microtubule targeting agents: Monocyclic pyrimidine analogs as microtubule targeting agents binding to the colchicine site

1.B Single agents with combination chemotherapy potential

1.B.1 Inhibition of multiple tyrosine kinases and microtubule targeting agents: Quinazoline analogs as microtubule targeting agents and multiple receptor tyrosine kinase inhibitors

1.B.2 Dual acting microtubule targeting agents and toll-like receptor agonists: Quinoline-2,5-diamines as microtubule targeting agents and toll like receptor agonists

1.C Selective inhibition of \textit{P. jirovecii} dihydrofolate reductase: Thieno[2,3-\textit{d}]pyrimidines as selective inhibitors of \textit{pj}DHFR

2. In addition, molecular modeling studies were performed to rationalize the observed biological activities for the following projects:

2.A Microtubule targeting agents: Pyrimido[4,5-\textit{b}]indoles and cyclopenta[\textit{d}]pyrimidines in the colchicine site of tubulin

2.B Single agents with combination chemotherapy potential: 5-Methyl-\textit{N}-(substituted)-furo[2,3-\textit{d}]pyrimidin-4-amines and 7-benzyl-\textit{N}-(substituted)-pyrrolo[3,2-\textit{d}]pyrimidin-4-amines as dual acting microtubule targeting agents and multiple tyrosine kinase inhibitors
1. Microtubule targeting agents (MTAs)

MTAs have a long history of utility in the treatment of both solid tumors and hematological malignancies.\(^1\),\(^{133}\),\(^{134}\) The tumor suppressor p53 regulates transcription of a wide variety of genes involved in DNA repair, metabolism, cell cycle arrest, apoptosis and senescence. More than half of all human tumors carry mutations in this gene making it one of the most frequently mutated gene.\(^{135}\) Recent efforts have led to development of drugs that could activate or restore the p53 pathway, particularly inhibitors of MDM2, a negative regulator of p53.\(^{136}\) Many of the inhibitors of MDM2 are still in early stages of clinical trials and therefore, in the absence of such agents the most effective drugs in p53 mutant cell lines are MTAs.\(^{137}\) Their efficacy in the treatment of a wide variety of tumors is attributed to the disruption of microtubule dynamics during mitosis.\(^1\),\(^{133}\) Their ability to disrupt cellular transport and protein trafficking has also been implicated as an important contribution towards the anticancer effect of these agents in the interphase.\(^{26},^{27}\)

The clinical efficacy of the most widely used MTAs, taxanes and vinca alkaloids, is limited by two major mechanisms of drug resistance, namely, overexpression of Pgp and expression of β-III tubulin.\(^{138}\) Pgp overexpression is particularly observed in patients who have received prior chemotherapy and is also associated with poor response to taxol-based chemotherapy in patients with non-small cell lung cancer.\(^{139},^{140}\) Additionally, circumventing Pgp-mediated resistance by using Pgp inhibitors failed to show any success due to intolerable side effects.\(^{141}\) Therefore, MTAs that are not substrates for Pgp (e.g. epothiolones) represents a promising alternate strategy for overcoming Pgp-mediated drug resistance.\(^{138}\)
In addition to Pgp overexpression, clinical resistance to taxanes and vinca alkaloids is also observed in non-small cell lung, breast, ovarian and gastric cancers due to expression of β-III tubulin. Several colchicine site binding agents such as CA4P (Fosbretabulin®) and OXi4503 have been evaluated in clinical trials and verubulin (Azixa®) has been granted orphan drug status for the treatment of glioblastoma multiforme. Development of MTAs binding to the colchicine site, therefore, offers the advantage of overcoming Pgp and/or β-III tubulin resistance and could result in broader antitumor activity and improved survival rates.

1.A Monocyclic pyrimidine analogs as microtubule targeting agents binding to the colchicine site

![Figure 24. Tubulin inhibitor 152](image)

Pyrrolo[2,3-d]pyrimidine 152 (Figure 24) was reported by Gangjee et al. as a potent microtubule depolymerizing agents which inhibited the growth of 51 cancer cell lines in the NCI 60 cell line panel assay in the submicromolar range. It was also shown to bind at the colchicine site of tubulin (Table 1) and was capable of overcoming Pgp and β-III tubulin mediated drug resistance.
Table 1. Effects of 152 on proliferation of MDA-MB-435 cell lines, tubulin assembly and
[^3H] colchicine binding.38

<table>
<thead>
<tr>
<th>Compd</th>
<th>IC(_{50}) ± SD (nM) (MDA-MB-435)</th>
<th>Inhibition of tubulin assembly (µM)</th>
<th>Inhibition of colchicine binding (% inhibition ± SD) at 5 µM</th>
</tr>
</thead>
<tbody>
<tr>
<td>152</td>
<td>183 ± 3.4</td>
<td>2.6 ± 0.05</td>
<td>70 ± 2</td>
</tr>
<tr>
<td>CA4</td>
<td>4.4 ± 0.5</td>
<td>1.0 ± 0.09</td>
<td>99 ± 0.2</td>
</tr>
</tbody>
</table>

Figure 25. Series I (compounds 153-154)

In an attempt to improve activity, it was of interest to design structurally simplified
analogs of 152 followed by optimization studies. Compounds 153 and 154 (Figure 25)
were designed as structurally simplified derivatives of 152 to determine the minimal
structural requirement for a compound to be a MTA binding at the colchicine site.
Compound 153 with a 5-Me substitution was designed to mimic the pyrrole ring of the
pyrrolo[2,3-\(d\)]pyrimidine scaffold of 152. The hydrophobicity imparted by a chloride
group and its similarity in size to a methyl group led to the design of 154 with a 5-Cl
substitution to evaluate the effect of an electron withdrawing group on the pyrimidine ring.
In addition, the acidic N-7 proton in 152 was replaced by a basic 6-NH\(_2\) group in
compounds 153 and 154. On the basis of the preliminary activities of compounds 153 (IC\(_{50}\)
MDA-MB-435 = 206.4 ± 8.9 nM) and 154 (IC\(_{50}\) MDA-MB-435 = 71.3 ± 6.1 nM), scaffold
154 was chosen for further optimization studies.
Figure 26. Series II

Compounds 155-160 (Figure 26) were designed to optimize the substitution at the N4-position. N4-desmethyl compound 155 was designed to evaluate the contribution of N4-Me group towards the antitubulin activity of 154. Additionally, the N4-Me group in 154 can impart activity due to hydrophobic interactions and/or conformational restriction about ‘a’ bond, which would be absent in 155. This was confirmed by performing a conformational search for 154 and 155 (SYBYL-X 2.1.1) by rotating bond ‘a’ by 1° within 5 kcal/mol, which yielded 91 and 157 conformations, respectively.33 Compounds 156 with 4´-SMe substitution and 157 with 3´-OMe substitution, were designed to determine the effect of H-bond strength and the position of H-bond acceptor on the phenyl ring on tubulin inhibitory activity, respectively. Due to the lower electronegativity of sulfur atom, the 4´-SMe group in 156 is a weaker H-bond acceptor than 4´-OMe group in 154.147 Compounds 158 and 159 were designed to assess the effect of conformational restriction on different parts of the 4-anilino substitution (bond b in 158 and bond c in 159) on the antitubulin activity, along with the additional hydrophobic properties of the added ring. Cyclopenta[d]pyrimidines with regioisomeric methoxy substituted naphthylamines were synthesized by Gangjee and coworkers as microtubule targeting agents in which 5-methoxy-N-methylnaphthalen-2-amine substituted compound was most potent inhibitor of tubulin.
polymerization. Therefore, compound 160 was designed to determine the steric bulk tolerance at the $N^4$-position by replacing a 4'-OMe phenyl with a 5'-OMe naphthyl moiety for the monocyclic pyrimidine scaffold.

Figure 27. Series III

The colchicine binding site on tubulin is a large site with dimensions ~10 Å ~10 Å ~4-5 Å. To mimic the steric effects of the pyrrole ring of the lead pyrrolo[2,3-$d$]pyrimidine 152 and to improve the tubulin inhibition of the monocyclic pyrimidine analogs by optimizing hydrophobic interactions in the pocket, compounds 161-169 (Figure 27) were designed. Substitution of the 6-NH$_2$ with alkyl and benzyl groups led to the design of 161-167 with 4-methoxy-$N$-methylaniline substitution at the C4-position. Due to similar activity of 160 (Figure 26, IC$_{50}$ MDA-MB-435 = 56.1 ± 5.0 nM) compared to 154 (Figure 25), substituted derivatives 168 and 169 with 5-methoxy-$N$-methylnaphthalen-2-amine at the C4-position and alkylamines at the 6-position were also proposed.
Figure 28. Superimposition of the docked poses of 152 (green, Figure 25) and 163 (magenta, Figure 27) in the colchicine (pink) binding site of tubulin (PDB ID: 4O2B).  

Figure 28 shows the docked conformations of lead compound 152 (green) and proposed analog 163 (magenta), superimposed on the co-crystallized ligand, colchicine (pink). The pyrimidine ring of 163 superimposes on the pyrimidine ring of the lead compound 152 and interacts with residues Leuβ248, Asnβ258 and Lysβ352 in the binding site. The 2-Me moiety lies in a pocket lined by residues Serα178, Alaα180, Leuβ248 and Lysβ352. The N₄-Me group mimics the bridging interaction of C5-C6 atoms of colchicine and interacts with Lysβ254. The N₄-phenyl group undergoes hydrophobic interactions with Cysβ241, Leuβ248, Leuβ255 and Alaβ316. The 4'-OMe substitution in 152 and 163
superimposes on the 2-OMe substitution on the A ring of colchicine. These OMe groups undergo a water mediated H-bond with Valβ238 and Cysβ241. This interaction has been described in the literature as an important interaction for compounds binding at the colchicine site. Additionally, the N-propyl substitution at the N6-position in 163 undergoes hydrophobic interactions with Asnβ258, Metβ259, Thrβ314 and Lysβ352 and accesses the pocket more completely than the rigid pyrrole group in 152. The docked score of 152 was -7.48 kcal/mol whereas, the docked score of 163 was -8.67 kcal/mol. The docked scores for compounds 161-169 in Series III were -7.65 kcal/mol to -9.26 kcal/mol.

Figure 29. Series IV (compounds 170-171)

Compounds in Series IV (Figure 29) were designed as pKₐ mimics of the pyrrole ring in the scaffold pyrrolo[2,3-d]pyrimidine of compound 152 (calc. pKₐ = 13.5). Compound 170 was N-acetyl derivative of 154 with a calc. pKₐ of 11.5. Compound 171 with an N-pivaloyl substitution was an intermediate in the synthesis of 158 and had a calc. pKₐ of 11.3, and was also tested for its antitubulin activity as a pKₐ mimic of 152.
1.B Single agents with combination chemotherapy potential

1.B.1 Inhibition of multiple tyrosine kinases and microtubule targeting agents: Quinazoline analogs as microtubule targeting agents and multiple receptor tyrosine kinase inhibitors

Angiogenesis is an essential process for tumor growth and metastases and involves the formation of new blood vessels from existing vasculature\textsuperscript{153}. Proangiogenic growth factors such as VEGF, PDGF, and EGF are released by the tumors under hypoxic conditions which bind to their respective RTKs (VEGFR, PDGFR-\(\beta\) and EGFR). This results in a cascade of events leading to angiogenesis and further tumor growth, survival and metastases. RTK inhibitors that circumvent angiogenesis have established a new paradigm in cancer chemotherapy\textsuperscript{49, 154}. Utility of multiple RTK inhibitors in cancer chemotherapy offers an advantage in overcoming resistance due to single RTK inhibitors such as point mutations in the ATP binding site and upregulation of additional RTKs\textsuperscript{155, 156}. Subsequently, the use of multiple RTK inhibitors has emerged as an important approach in cancer chemotherapy and is validated by the approval of several multi-RTK inhibitors such as axitinib\textsuperscript{157, 158} (VEGFR, PDGFR, c-kit), pazopanib\textsuperscript{159} (PDGFR, VEGFR, c-kit, FGFR), vandetanib\textsuperscript{160} (VEGFR, EGFR, RET kinase), sunitinib\textsuperscript{161, 162} (VEGFR, PDGFR and c-kit) and sorafenib\textsuperscript{163, 164} (VEGFR-2, PDGFR \(\beta\) and Raf kinase). The cytostatic activity of RTK inhibitors does not kill cancer cells and thus, needs to be combined with cytotoxic chemotherapeutic or radiotherapeutic agents to provide a synergistic effect and improve therapy outcomes\textsuperscript{165}. 
Microtubules are essential cytoskeletal component in eukaryotic cells which are involved in cellular process during both the interphase as well as the dividing phase of the cell.\textsuperscript{1} In the interphase stage microtubules are involved in intracellular trafficking of proteins and nucleic acid cargo and in dividing cells they make up the mitotic spindle and take part separation of daughter chromosomes.\textsuperscript{27} Microtubule targeting agents act by interfering with crucial processes involved in cell division in mitotic phase as well as by disrupting cellular transport and protein trafficking in the interphase.\textsuperscript{1, 26, 133}

The strategy of using a combination of drugs, each acting at a different biological target has been successfully applied in the treatment of many diseases.\textsuperscript{166} The basic premise for such an approach is to overcome alternate signaling pathways which may get activated when only a single pathway is inhibited. In the same way, combination chemotherapy with antiangiogenic and cytotoxic agents acts by simultaneously inhibiting multiple pathways, thereby, inhibiting the growth of tumor cells dependent on alternate signaling pathways.\textsuperscript{165, 167, 168}

The vascular normalization theory proposes that the antiangiogenic effect of RTK inhibitors causes pruning of abnormal blood vessels and transiently normalizes the structural and functional integrity of the vasculature. This normalization restores blood flow to the tumors, aids in the improved delivery of co-administered cytotoxic agents and thus, explains, in part, the synergistic effect seen with such a combination.\textsuperscript{169, 170} Multi-target directed ligands are rationally designed analogs which incorporate essential pharmacophores for inhibiting two or more biological targets.\textsuperscript{171, 172} In cancer chemotherapy, such agents may help in preventing cancer cells to develop resistance, lower
the risk of drug-drug interactions, improve patient compliance and have low side effect profile due to efficacy of low affinity of cytotoxic component of the drug to its target.

Single agents with dual microtubule targeting activity and antiangiogenic activity are highly desirable as these agents can act synergistically and exert their cytotoxic effect as soon as the tumor vasculature normalization is achieved via the antiangiogenic component.\textsuperscript{42} Due to their mode of action, these agents need not be as potent as the currently used chemotherapeutic agents. The lower cytotoxic activity of these agents would mimic metronomic chemotherapy, which allows for low doses of cytotoxic agent to be administered compared to conventional chemotherapy.\textsuperscript{173} Clinical evaluation of the safety and efficacy of metronomically dosed cytotoxic agents in conjunction with antiangiogenic agents such as sunitinib and sorafenib has also been explored.\textsuperscript{168, 174, 175} The dose limiting cytotoxicity of conventional chemotherapeutic agents could also be avoided by using these single agents as these agents work by synergistic mechanisms and the cytotoxic component need not be as potent, thus minimizing toxicity.

Multi-target directed ligands with dual cytotoxic and antiangiogenic activities have shown to significantly reduce tumor growth, metastasis and angiogenesis in tumor xenograft mice models, without any significant toxicity.\textsuperscript{176, 177} Because of their crucial role in angiogenesis VEGFR-2, PDGFR-β and EGFR were chosen as targets in the current studies. Due to the clinical utility of cytotoxic tubulin inhibitors, such as paclitaxel, in combination with antiangiogenic agents, tubulin was chosen as the potential cytotoxic target.\textsuperscript{178-180}
Compound 172 (Figure 30) was reported as a potent inducer of apoptosis, binding at the colchicine site and an inhibitor of tubulin polymerization (EC\textsubscript{50} T47D = 2 ± 0.1 nM).\textsuperscript{181} The compound was also shown to overcome resistance due to multidrug resistant transporters such as P-glycoprotein (Pgp), breast cancer resistance protein (BCRP1) and was able to cross the BBB to attain 30-fold higher concentration in the brain compared to the plasma. These pharmacokinetic parameters led to the evaluation of 172 in glioblastoma multiforme (GBM) and other cancers, and it is currently approved by FDA as an orphan drug in the treatment of GBM.\textsuperscript{182}

The quinazoline scaffold has also been widely explored for its RTK inhibitory activity as exemplified by gefitinib (EGFR), erlotinib (EGFR) and vandetanib (VEGFR-2, VEGFR-3 and EGFR) (Figure 31). Thorough structure-activity relationships have been performed on the quinazoline scaffold with N\textsuperscript{4}-desmethyl analogs as EGFR inhibitors which suggested that the quinazoline scaffold showed bulk tolerance at the 6- and 7-
However, the corresponding $N^4$-Me analogs were not explored for their RTK inhibitory activities. Separately, various scaffolds such as furo[2,3-$d$]pyrimidines and pyrrolo[3,2-$d$]pyrimidines with $N^4$-Me substitution have been shown by Gangjee and coworkers to have dual acting antitubulin and multiple RTK inhibitory activities. Based on literature evidence and the structural similarities between 172 and the quinazoline based RTK inhibitors, it was predicted that 172 would have RTK inhibitory and thus antiangiogenic effects as well.

**Figure 32.** Docked pose of lead compound 172 (green) superimposed on the crystallized ligand gefitinib (pink) in EGFR (PDB: 4WKQ)

Compound 172 was docked in the binding site of EGFR (Figure 32) and had a docked pose similar to gefitinib. Due to the presence of a 2-Me group, the hinge region binding interaction with Met793 and the interaction of N3 with HOH1320 does not occur as seen with gefitinib but the 4-position aniline substitution is oriented in the pocket similar to the 3-chloro-4-fluoro substituted aniline in gefitinib and lies in a pocket lined by residues Lys745 and Asp855. The quinazoline scaffold of 194 is stabilized by hydrophobic
interactions with Leu718, Leu844 and Val726 (not shown). The 2-Me group lies in a hydrophobic pocket formed by residues Leu718, Met793 and Leu844. The docked score of 194 was -6.36 kcal/mol compared to gefitinib which had a docked score of -7.85 kcal/mol.

Figure 3. Docked pose of 172 (magenta) superimposed on the crystallized ligand axitinib (pink) in VEGFR-2 (PDB: 4AG8)

The docked pose of 172 in the binding site of VEGFR-2 (Figure 3) shows that the quinazoline scaffold binds to the pocket where the thiobenzamide of axitinib binds and interacts with residues Lys868, Val899 and Val916. The 4-position aniline substituent of 172 interacts with Val848, Ala866, Leu1035 and Phe1047, and binds in the pocket where the indazole scaffold of axitinib binds. The docked score of 172 in VEGFR-2 was -5.82 kcal/mol compared to axitinib which had a docked score of -10.00 kcal/mol.

As expected, preliminary evaluation of 172 in in vitro assay for inhibition of EGFR, VEGFR-2 and PDGFR-β resulted in potent activities against all three RTKs (Table 2).
Compound 172 was more potent than clinically used agent sunitinib (EGFR ~61-fold, VEGFR-2 ~2-fold and PDGFR-β ~15-fold). Compared to erlotinib, 172 was 2-fold less potent in EGFR but ~15-fold and ~2-fold more potent in inhibiting VEGFR-2 and PDGFR-β, respectively.

**Table 2.** EGFR, VEGFR-2 and PDGFR-β inhibitory activity of compound 172

<table>
<thead>
<tr>
<th>Compd</th>
<th>EGFR kinase inhibition (nM)</th>
<th>Flk-1 (VEGFR-2) kinase inhibition (nM)</th>
<th>PDGFR kinase inhibition (nM)</th>
<th>A431 Cytotoxicity (nM)</th>
<th>U251 cytotoxicity (nM)</th>
<th>CAM angiogenesis inhibition (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>172</td>
<td>2.8 ± 1.1</td>
<td>8.4 ± 2.2</td>
<td>5.6 ± 0.4</td>
<td>0.9 ± 0.2</td>
<td>2.8 ± 0.6</td>
<td>2.8 ± 1.1</td>
</tr>
<tr>
<td>Sunitinib</td>
<td>172.1 ± 19.4</td>
<td>18.9 ± 2.7</td>
<td>83.1 ± 10.1</td>
<td>-</td>
<td>-</td>
<td>1.3 ± 0.07</td>
</tr>
<tr>
<td>Erlotinib</td>
<td>1.2 ± 0.2</td>
<td>124.7 ± 18.2</td>
<td>12.2 ± 1.9</td>
<td>-</td>
<td>-</td>
<td>29.1 ± 1.9</td>
</tr>
</tbody>
</table>

**Figure 34.** Series V (173-175), Series VI (176-178), Series VII (179-182)
Compounds 173-175, Series V, (Figure 34) with a 2-Me substitution were designed to optimize the substitution at the 4-position for activity at multiple RTKs and at the colchicine site of tubulin. The clinically used quinazoline RTK inhibitors (Figure 31) all had a 2-H substitution and hence compounds in Series VI (176-178) were designed to mimic the 2-position substitution of the known RTK inhibitors and determine its effect on microtubule polymerization activity. Due to the loss of bulk at 2-position, compounds in Series VI were expected to bind at the active sites of EGFR and VEGFR-2 by maintaining the hinge region binding interactions as seen for gefitinib in EGFR (Figure 32) and axitinib in VEGFR-2 (Figure 33) while still retaining interaction at the colchicine site of tubulin.

Figure 35. Superimposition of the docked poses of 172 (green) and 176 (magenta) in the colchicine binding site of tubulin (PDB ID: 4O2B).
The docked pose of 176 in the binding site of tubulin (Figure 35) is very similar to the lead compound 172. The quinazoline scaffold in 172 and 176 undergoes hydrophobic interactions with Alaα180, Asnβ258, Metβ259 and Lysβ352. The N4-Me in 172 is oriented towards and interacts with Lysβ254 and in 176 it interacts with Lysβ248 and Alaβ354. The N4-phenyl group hydrophobically interacts with Leuβ248, Leuβ255, Alaβ316 and Ileβ318. The 4'-OMe is oriented towards Valβ238 and Cysβ241 and can potentially undergo water mediated H-bonding interaction. The docked score of 172 in the colcicine site of tubulin was -7.65 kcal/mol and that of 176 was -7.16 kcal/mol. Compounds in Series V (173-175) had docked scores in the range of -7.62 to -8.82 kcal/mol and compounds in Series VI (176-178) had docked scores in the range of -7.16 to 8.33 kcal/mol suggesting these compounds to have similar or better activity than 172 as MTAs binding to colchicine site.

**Figure 36.** Superimposition of the docked poses of 172 (green) and 176 (magenta) in EGFR (PDB: 4WKQ).\textsuperscript{186}
As mentioned above, due to the loss of bulk at 2-position compound 176 (Figure 36) is able to undergo hinge region binding interaction with Met793 while maintain other interactions at the binding site of EGFR as seen with 172 (Figure 34). Compounds in Series V docked similar to compound 172 and had docked scores in the range of -7.06 to -7.32 kcal/mol. Compounds in Series VI docked similar to 176 and had docked scores of -7.22 to -7.70 kcal/mol.

**Figure 37.** Superimposition of the docked poses of 172 (green) and 176 (magenta) in VEGFR-2 in two different binding modes (PDB: 4AG8).187

Compound 176 (Figure 37) when docked in the X-ray crystal structure of VEGFR-2 showed two modes of binding, which corroborate with the similar findings reported for a series of furo[2,3-d]pyrimidine analogs.176 In Figure 37A 176 binds to VEGFR-2 in a binding mode similar to that seen for compound 172 (Figure 33) and has a docking score of -7.61 kcal/mol. In addition, due to the absence of bulk at 2-position, 176 binds to VEGFR-2 in an alternate binding mode where the N1 of the quinazoline scaffold in 176 undergoes H-bonding with Cys919 at the hinge region of VEGFR-2 and has a similar docking score of -7.51 kcal/mol. Compounds in Series V docked similar to compound 172 and had docked scores in the range of -5.8 to -6.51 kcal/mol. Compounds in Series VI docked similar to 176 and had docked scores of -4.87 to -8.40 kcal/mol.
The 2-Cl substituted analog of \textbf{172} was reported to be an equipotent inhibitor of microtubule polymerization (EC\textsubscript{50} T47D = 2 ± 0.1 nM) as \textbf{172} and hence by varying the bulk and electronics at the 2-position, compounds \textbf{179-182} (Series VII, Figure 34) were designed to assess the inhibitory effect on multiple RTKs. These compounds docked at the colchicine site of tubulin (docked scores, -7.50 to -8.61 kcal/mol), EGFR (docked scores, -6.49 to -7.39 kcal/mol) and VEGFR-2 (docked scores, -4.34 to -7.94 kcal/mol) in a mode similar to that shown for lead compound \textbf{172}.

![Chemical structure](image)

**Figure 38.** Pyrido[2,3-\textit{d}]pyrimidine derivative Series VIII (\textbf{183})

Compound \textbf{183} was designed to determine the effect of changing the electronics of the quinazoline scaffold on the inhibitory activities towards microtubule polymerization and RTKs. Introduction of nitrogen atom at 8-position of the quinazoline ring changes the evenly distributed electron density in a benzene ring with variable electron density in a pyridine ring which has a dipole moment of 2.2 D. Due to the presence of pyridine ring, the pyrido[2,3-\textit{d}]pyrimidine scaffold is electron deficient as compared to the electron rich quinzoline scaffold.\textsuperscript{188} Compound \textbf{183} docks in the colchicine site of tubulin in a binding mode similar to that observed for \textbf{176} (Figure 35) in which the N8 atom can potentially interact with the NH backbone of Val\textsubscript{a181} via H-bonding. The docked score of \textbf{183} in the colchicine site of tubulin was -7.24 kcal/mol. The docked scores in EGFR and VEGFR-2 were -7.28 kcal/mol and -7.63 kcal/mol, respectively.
1.B.2 Dual acting microtubule targeting agents and toll-like receptor agonists: Quinoline-2,5-diamines as microtubule targeting agents and toll like receptor agonists

Cancer and immunotherapy

The fundamental principle underlining the use of immune therapy in the treatment of cancer lies in the fact that the cancer cells express a variety of proteins that can be recognized by the immune system and is currently a well-established component of many cancer treatment regimens.\textsuperscript{189} The anti-cancer effect of such therapy is attained by one of the following methods: 1) passive immunization by monoclonal antibodies, 2) systemic delivery of cytokines and, 3) introducing adjuvants to the tumor microenvironment. These protective anti-tumor effects are mediated by the regulation of key signaling pathways in the innate (early, general attack) and/or the adaptive (specific attack) immune system.\textsuperscript{189}

Toll-like receptors (TLRs) are pattern recognition receptors which are expressed on the members of the innate or the adaptive immune system such as DCs, macrophages, granulocytes, T cells, B cells, natural killer (NK) cells and mast cells.\textsuperscript{190} They are responsible for recognizing conserved signature patterns, either pathogen-associated molecular patterns (PAMPs) or microbe-associated molecular patterns (MAMPs), and initiate and propagate inflammatory response.\textsuperscript{191} Ten subtypes (TLR1-10) have been identified in humans which are activated by various ligands.\textsuperscript{192} The role of TLRs in eliciting antitumor response in bone sarcomas was first reported by repeated injections of Coley’s toxin (mixture of lipopolysaccharides and bacterial DNA products).\textsuperscript{193} This effect was due to TLR4 and TLR9 mediated cytokine production, stimulation of active immunity and
activation of NK and cytotoxic T cells. Due to recent efforts in identifying the role of innate immunity in cancer treatment, immunotherapeutic agents such as TLR agonists are now considered by the National Cancer Institute (NCI) as one of the few agents with highest potential in the treatment of cancer. 

In response to cellular stress signals (DNA damage, viral infection etc.), the tumor suppressor p53 initiates a transcriptional process that regulates transcription of genes involved in many biological processes. In cancer, in addition to the tumor suppressor response, p53 increases the expression of key mediators of innate immunity to improve the clearance of damaged cells. Amongst various targets many members of the TLR family are also regulated by p53. The utility of TLR agonists in cancer treatment can thus be exploited, depending on the TLR gene expression profile and tumor microenvironment, to increase the immune response to tumor antigens or as adjuvants in cancer chemotherapy. Accumulating amount of evidence suggests that by activating TLRs present on monocytes, macrophages and DCs, the immune effector functions such as phagocytosis, antigen presentation and cytokine secretion, are improved which lead to efficient anticancer immunity. Calreticulin (CRT) is an endoplasmic reticulum (ER) protein which is associated with phagocytic eat-me signal. CRT expression on the surface of macrophages is controlled by TLR, activation of which leads to phosphorylation of CRT, cleavage from the ER and subsequent increased expression on the cell surface. Higher surface CRT on macrophages resulted in stronger phagocytic ability towards human colon cancer cells in vitro. Thus, enhanced immunosurveillance of tumor cells can be achieved by increasing TLR mediated surface expression of CRT on macrophages.
Bacillus Calmette-Guérin (BCG), an attenuated form of \textit{Mycobacterium bovis}, is currently approved by the US FDA in the treatment of non-invasive transitional cell carcinomas of the bladder.\textsuperscript{200} Lyophilized preparation of \textit{Streptococcus pyogenes}, picibanil, is approved in Japan for the treatment of many carcinomas.\textsuperscript{201} Monophosphoryl lipid A (MPL) is a derivative of \textit{Salmonella minnesota} lipopolysaccharide which is used as an adjuvant in the treatment of cervical carcinoma associated with human papillomavirus (HPV) infection.\textsuperscript{202} Imiquimod, a small molecule TLR7/8 agonist, is used in the treatment of basal cell carcinoma.\textsuperscript{203} More than 50 clinical trials of TLR agonists have been initiated to assess their safety and efficacy as immunotherapeutic agents in a number of cancers, as a standalone therapy as well as in combination with other agents/interventions.\textsuperscript{204} Gliomas are one of the most aggressive types of tumors which occur in the central nervous system. Since these gliomas escape the immune surveillance by creating immune suppressed environment, immunotherapy is one of the more promising strategy for their treatment.\textsuperscript{205}

The expression of different TLR subtypes has different biological responses. TLR2, TLR4 and TLR9 subtypes in glioma have shown to be tumor promoting as they contribute to tumor development.\textsuperscript{206-208} On the other hand, it has been shown that imiquimod (TLR7/8 agonist) leads to eradication of intracranial tumor when applied to mouse glioma model.\textsuperscript{209} Additionally, a number of clinical trials are currently underway which utilize imiquimod as an adjuvant in the treatment of glioblastoma multiforme (GBM), the most aggressive form of human glioma.\textsuperscript{205} The efficacy of TLR7 agonist in cancer is due to NF-κB mediated induction of proinflammatory chemokines and activation of cytotoxic activity of NK cells which are capable of inducing tumor apoptosis.\textsuperscript{194}
Few studies have explored the potential of exploiting combination of TLR agonists as adjuvants in combination with radiation, chemotherapy and/or, cancer vaccines and it has been suggested that the combination has better therapeutic success over monotherapy with TLR agonists in the treatment of cancer. The efficacy arises due to the ability of TLR agonist to enhance Th1 mediated cytotoxic effect via activating immune cells involved in adaptive immunity. Conventional cytotoxic chemotherapeutic agents are capable of reducing the tumor burden but as such are unable to activate signaling pathways involved in enhancing immunogenicity of cancer cells. The improved immunogenicity of cancer cells can increase the chances of T-cell mediated susceptibility to tumors and higher remission rates. It was observed that vincristine, a microtubule destabilizer, killed chronic lymphocytic leukemia (CLL) cells rapidly in vitro and also had weak T cell stimulatory properties. The presence of protein kinase C (PKC) agonist, bryostatin, in addition to vincristine, increased the immunogenicity of CLL cells and had effects similar to that seen with of TLR agonist. The use of combining vascular disrupting agents (VDAs) (CA-4P) with one or more antibodies capable of improving immunomodulatory responses in cancer cells (PD-1, CTLA-4) are also being assessed.

![Figure 39. Structure of lead compounds (172, 184-185)](image)

A series of single agents were designed that incorporate structural features to interact with essential pharmacophores for both MTA binding at the colchicine site and
TLR7/8 agonists, to explore their effect in the treatment of cancer, particularly gliomas. Quinazoline 172 (Figure 39) is a MTA that binds at the colchicine site of tubulin (EC$_{50}$ T47D cells = 2 ± 0.1 nM)$^{181}$ and was granted orphan drug status for the treatment of gliomas. The efficacy was attributed due to its vascular disrupting effect. A number of scaffolds with varying substitutions at 2-position (pyrrolo[2,3-\(d\)]pyrimidines$^{38}$, pyrrolo[3,2-\(d\)]pyrimidines$^{185}$, furo[2,3-\(d\)]pyrimidines$^{212}$, pyrimido[4,5-\(b\)]indoles$^{213}$) and bearing the 4-methoxy-\(N\)-methylaniline substitution have been reported by Gangjee and coworkers, of which the aniline substitution at the C4-position is considered essential for the antitubulin activity. Additionally, 2-aminoquinolines (184 TLR8 EC$_{50}$ = 200 nM, 185 TLR8 EC$_{50}$ = 49 nM) were recently reported as TLR8 agonists.$^{76, 214}$

![Chemical structure of 2-aminoquinoline scaffold with varying alkyl substitutions](image)

**Figure 40. Series IX**

Maintaining the 2-aminoquinoline scaffold for TLR agonistic activity (Figure 40), 4-methoxy-\(N\)-methyl substituted compounds (186-190) Series IX, with varying alkyl substitutions at the 3-position are proposed. These analogs incorporate structural determinants for both MTAs and TLR agonists. Compound 184 (Figure 39) with \(n\)-pentyl substitution at the 3-position was a selective TLR8 agonistic activity. However, by varying substitutions at the 3- and 4-positions of the 2-aminoquinoline scaffold dual acting TLR7/8 agonists can be obtained.$^{215, 216}$ These homologated analogs will also help in optimizing the 3-position substitution for binding at the colchicine site of tubulin as well as TLR7/8.
Compounds 186-190 were docked in the X-ray crystal structure of TLR8 (PDB: 4QBZ) using MOE 2016.08. Figure 41 shows the docked conformations of compound 190 (green) superimposed on the co-crystallized ligand D80 (pink). The 2-aminoquinoline scaffold retains the interactions with Asp543. The n-pentyl group of 190 orients similar to the butyl group in D80 and interacts with Phe346, Tyr348 and Val378. The N4-Me moiety interacts with Tyr353 and Val378. The N4-Ph with the 4'-OMe group lies in the solvent exposed region of the binding pocket. The docked score of 190 in TLR8 was -8.11 kcal/mol which was similar to the docked score of the crystalized ligand, -8.35 kcal/mol, indicating that 190 is anticipated to be active as a TLR8 agonist.
Compounds 186-190 were also docked in the X-ray crystal structure of colchicine in tubulin (PDB: 4O2B) using MOE 2016.08. Figure 42 shows the docked conformations of the proposed compound 190 (green) superimposed on the co-crySTALLized ligand, colchicine (pink). The quinoline scaffold interacts with Alaα180, Leuβ248, Asnβ258 whereas the 2-NH$_2$ undergoes H-bonding with Thrα179. The n-pentyl group at the 3-position lies in a pocket lined by residues Serα178 and Leuβ248. The conformational orientation of the N$^4$-substituted aniline superimposes on to the A-ring of colchicine with the N$^4$-Me group in 190 oriented towards the bridging C5 and C6 atoms of colchicine. The docked score of 190 was -7.05 kcal/mol compared to the score of colchicine which was -10.73 kcal/mol, indicating that there is a reasonable probability that 190 will have colchicine site binding and thus antitubulin activity.
Figure 43. Series X

The \( p \)-anisidine analog 191 was designed to determine the effect of conformational restriction provided by the \( N^4 \)-Me substitution of 186 with respect to the quinoline scaffold on the activity at both TLR8 and the colchicine site of tubulin. The conformational restriction provided by \( N^4 \)-Me group was confirmed by performing conformational search on C5-N bond of 186 (91 conformations) and 191 (133 confirmations) within 5 kcal/mol using SYBYL-X 2.1.1.

C. Selective inhibition of \( P. jirovecii \) dihydrofolate reductase

Pneumocystis pneumonia (PCP) is an opportunistic infection caused by fungus \( Pneumocystis jirovecii \) (\( Pj \)). The pathogen is present in the lungs of a majority of the population around the world. In healthy individuals the infection is kept under check due to normal functioning of the immune system.\(^9\) In immunocompromised patients, such as those suffering from HIV/AIDS, undergoing cancer chemotherapy, and organ transplantation, and where the CD4 count is below 200 cells/mm\(^3\) PCP infection, without treatment, is fatal.\(^\text{217, 218}\) Current prophylaxis and treatment for PCP includes administration of co-trimoxazole, a combination of trimethoprim (TMP) (targets dihydrofolate reductase, DHFR) and sulfamethoxazole (SMX) (targets dihydropteroate synthase, DHPS). In several cases the failure of co-trimoxazole therapy occurs due to development of resistance or due
to allergy/toxicity caused by SMX.\textsuperscript{219-221} The potent and non-selective inhibitors, such as, trimetrexate (TMQ) or piritrexim (PTX), require co-administration of leucovorin which is associated with high treatment cost, drug interactions and lower efficacy.\textsuperscript{222, 223} Thus, therapeutic agents (DHFR inhibitors) that have the potential of combining the selectivity of TMP for pathogenic DHFR with the potency offered by TMQ or PTX are highly desirable and present a significant advantage of improving patient compliance and can be used to target multiple opportunistic infections.

1. C Selective inhibition of \textit{P. jirovecii} dihydrofolate reductase: Thieno[2,3-\textit{d}]pyrimidines as selective inhibitors of \textit{pj}DHFR

\begin{figure}[h]
\centering
\includegraphics[width=1\textwidth]{structure.png}
\caption{General structure of pyrrolo[2,3-\textit{d}]pyrimidines, thieno[2,3-\textit{d}]pyrimidines and TMQ}
\end{figure}

\textit{Gangjee et al.}\textsuperscript{224, 225} reported 5-methyl-6-substituted pyrrolo[2,3-\textit{d}]pyrimidines (Figure 44) as inhibitors of \textit{pneumocystis carinii} (\textit{pc}) and \textit{pj}DHFR The compounds in the series were 3-12 fold more selective for \textit{pj}DHFR and had good clogP (2.31-3.91) values.
**Figure 45.** Superimposed poses of quinazoline ring of TMQ (teal) with pyrrolo[2,3-\(d\)]pyrimidine (orange) and thieno[2,3-\(d\)]pyrimidine (purple)

The larger size of sulfur as compared to nitrogen allows the thieno[2,3-\(d\)]pyrimidines to mimic the 6,6-ring system of the potent inhibitor TMQ more closely than the NH in a pyrrolo[2,3-\(d\)]pyrimidine (Figure 45). The replacement of the pyrrole NH with S would also allow for the evaluation of the effect of HBD to a HBA, at the 7-position, on the biological activity. In addition, since these compounds are non-classical antifolates lacking the glutamic acid chain and are taken up by the pathogen via passive diffusion, the thieno[2,3-\(d\)]pyrimidines were found to have higher clogP values (3.04-4.65), compared to the pyrrolo[2,3-\(d\)]pyrimidine analogs (2.31-3.91). The thieno[2,3-\(d\)]pyrimidine scaffold affords another advantage as it has already been explored for antibacterial and antimalarial activities\(^{120, 226-228}\) and thus, these compounds can also be tested as potential DHFR inhibitors of other pathogens.
Compounds 192-199 Series XI (Figure 46) were designed to explore the effect of electron donating groups in the side chain thiophenyl group at the 6-position on the potency and selectivity towards \(\text{pjDHFR}\). The substitutions were varied to have mono-(193-195), and di-(196-199) substituted electron donating groups. Similarly, compounds 200-207 in Series XII were designed to assess the effect of electron withdrawing groups on activity.

Compounds 208-210 Series XIII (Figure 47) with bulkier 1-naphthyl, 2-naphthyl and biphenyl groups respectively, were designed to explore the bulk tolerance in the side pocket and its effect on \(\text{pjDHFR}\) and \(\text{hDHFR}\) inhibitory activity.
Figure 48. Docked pose of 209 (purple) in the homology model of \(pj\)DHFR\(^{225}\) (green), superimposed on to the X-ray crystal structure of \(h\)DHFR (PDB: 1U72\(^{229}\), gray) to emphasize the difference in residues in the binding pocket. NADPH is in black. The key differences in the binding site residues are highlighted in red circles.

The docked pose of 209 in the \(pj\)DHFR homology model (Figure 48) is a representative example of the mode of binding of thieno[2,3-\(d\)]pyrimidines in the active site. The key interaction involves the bidentate ionic interaction of the N1 and 2-NH\(_2\) of the thieno[2,3-\(d\)]pyrimidine scaffold with Asp32 in \(pj\)DHFR and Glu30 in \(h\)DHFR. The 4-NH\(_2\) undergoes H-bonding with Ile10 and Ile7 in \(pj\)DHFR and \(h\)DHFR, respectively. The rationale for introducing bulk at the 6-position can be observed from the docked pose which suggests that the bulky group can be accommodated by the Met33 in \(pj\)DHFR. In \(h\)DHFR, the presence of a Phe31 results in a steric clash of the bulky substituent with the Phe31 and hence improved selectivity of binding can be achieved for \(pj\)DHFR.
Figure 49. Series XIV

Compounds 211 and 212 Series XIV (Figure 49) were designed to exploit the difference in the residues Ile123 in \( pj \)DHFR and the corresponding Val115 in \( h \)DHFR (Figure 48). The homologated analogs 211 and 212 can interact with Ile123 in \( pj \)DHFR but Val115 in \( h \)DHFR being shorter is unable to interact with the substitutions at 5-position of 211 and 212. This allows for increased hydrophobic binding of 211 and 212 with \( pj \)DHFR compared to \( h \)DHFR.

2. Molecular modeling studies

2.A.1 Pyrimido[4,5-\( b \)]indoles as microtubule targeting agents
Figure 50. Structures of pyrimido[4,5-b]indole tubulin inhibitors (compounds 213-220)

Compounds 213–220 (Figure 30) were synthesized by Gangjee and coworkers to explore the SAR and identify key structural features of 213 responsible for microtubule depolymerizing activity in the colchicine site.\textsuperscript{230}

Docking studies of compounds 213-220 were carried out in the reported X-ray crystal structure of colchicine in tubulin (PDB: 4O2B)\textsuperscript{152} to explain the microtubule depolymerization activities observed for these series of compounds.\textsuperscript{230}

2.A.2 Cyclopenta[d]pyrimidines as microtubule targeting agents

![Chemical structures]

Figure 51. Structures of cyclopenta[d]pyrimidine tubulin inhibitors (compounds 221-234)

Compounds 221–234 (Figure 51) were synthesized by Gangjee and coworkers to explore the SAR of cyclopenta[d]pyrimidines for microtubule depolymerizing activity in the colchicine site.\textsuperscript{148}
Docking studies of compounds 221-234 were carried out in the colchicine binding site in tubulin (PDB: 4O2B). Additionally, study of the electrostatic surfaces as well as conformational search were also performed for these compounds to correlate the structures with their in vitro activities.

2.B.1 5-Methyl-N-(substituted)-furo[2,3-d]pyrimidin-4-amines as single agents with combination chemotherapy potential

![Chemical structures](image)

**Figure 52.** Structures of dual antitubulin and antiangiogenic compounds 236-246

Based on lead compounds 235-238, compounds 239-246 (Figure 52) were synthesized by Gangjee and coworkers to determine the effect of bulky substitutions at the N⁴-position towards binding at the ATP site of multiple RTKs and at the colchicine site of tubulin.²³¹

Docking studies of compounds 236-246 were carried out in the reported X-ray crystal structure of colchicine in tubulin (PDB: 4O2B, 2.3 Å)¹⁵² to explain the microtubule depolymerization activities observed for these series of compounds.²¹² To elucidate the RTK inhibitory activities, these compounds were docked in in the published X-ray crystal
structures of EGFR (PDB: 4JQ7, \(2.73 \, \text{Å}\)) and VEGFR-2 (PDB: 4AG8, \(1.95 \, \text{Å}\)), and in a homology model of PDGFR-β.\(^{233}\)

2.B.2 7-Benzyl-\(N\)-(substituted)-pyrrolo[3,2-\(d\)]pyrimidin-4-amine as single agents with combination chemotherapy potential

Figure 53. Dual acting pyrrolo[3,2-\(d\)]pyrimidines 249-256

Based on lead compounds 247-249, compounds 250-256 (Figure 53) were synthesized by Gangjee and coworkers to explore the SAR of pyrrolo[3,2-\(d\)]pyrimidines towards binding at the ATP site of multiple RTKs and at the colchicine site of tubulin.\(^{185}\)

Docking studies of compounds 249-256 were carried out in the reported X-ray crystal structure of colchicine in tubulin (PDB: 4O2B, 2.3 \(\text{Å}\))\(^{152}\) to explain the microtubule
depolymerization activities observed and the RTK inhibitory activities (VEGFR-2 PDB: 4AG8,\textsuperscript{187} 1.95 Å; EGFR PDB: 4WKQ,\textsuperscript{186} 1.85 Å; and PDGFR-β homology model\textsuperscript{233}) of these series of compounds.\textsuperscript{185}
IV. CHEMICAL DISCUSSION

Synthesis of monocyclic pyrimidine analogs 155-171 and lead compounds 153 and 154

Lead compounds 155 and 154 (Scheme 36) were synthesized from the commercially available 6-chloro-2,5-dimethylpyrimidin-4-amine (261) and 5,6-dichloro-2-methylpyrimidin-4-amine (257), respectively. Further synthesis of compounds 155-171 was accomplished based on the synthesis of lead compound 154.

Scheme 36. Synthesis of lead compounds 153 and 154 (Series I)

Compound 154 (Scheme 36) was initially synthesized over three steps starting from commercially available pyrimidine 257. The free amine in 257 was protected using pivalic anhydride to give pivaloyl protected pyrimidine 258. Nucleophilic aromatic displacement of the 4-chloro in 258 with 4-methoxy-N-methyl aniline 259 under microwave conditions in 1,4-dioxane gave intermediate 260 which was deprotected under basic conditions to give the final compound 154 in 74% yield. The $^1$H NMR of 154 showed the presence of NH$_2$.
peak at 6.61 ppm, 4-NCH$_3$ peak at 3.27 ppm and 4’-OCH$_3$ peak at 3.73 ppm, confirming the formation of 154. Since compound 154 was required in bulk for the synthesis of compounds in Series III, an alternate synthetic route utilizing benchtop conditions was desired which would be easily amenable to scale up conditions. Therefore, compounds 153 and 154 were synthesized in one step via S$_{\text{N}}$Ar displacement of the 4-chloro of 261 and 257 with aniline 259 under benchtop conditions to give final compounds in 27% and 85% yield, respectively. $n$-Butanol being more polar than 1,4-dioxane ($\varepsilon$ 17.8 vs 2.21) was chosen as a solvent for the reaction on polar starting material 257 and 261 ($R_f$ of 257 in 10:1 CHCl$_3$/MeOH = 0.52 compared to $R_f$ of 258 in 10:1 CHCl$_3$/MeOH = 0.76 ).

![13C NMR of compound 154](image)

**Figure 54.** $^{13}$C NMR of compound 154

To ensure that the S$_{\text{N}}$Ar reaction in Scheme 36 on 257 results in the 4-substituted compound 154 and not the 5-substituted compound 154a, the predicted $^{13}$C NMR values (ACD Labs) were compared with the values obtained experimentally (Figure 54). Clearly the presence of the 4C signal at 163 ppm and 5C peak at 94 ppm correlates with the 4-substituted compound 154 and confirmed that the S$_{\text{N}}$Ar displacement occurs, as expected,
at the more electrophilic and activated 4-position of the 5,6-dichloro-2-methylpyrimidin-4-amine 257.

**Scheme 37.** Synthesis of N-methylated anilines 263, 265 and 269

To synthesize compounds 156, 159 and 160 (Series II), the respective commercially available primary anilines (262, 264 and 266) were first methylated (Scheme 37). For synthesis of N-methylated anilines 263 and 265, anilines 262 and 264 were formylated using paraformaldehyde in the presence of sodium methoxide in methanol. Subsequent reduction of the N-formyl group using NaBH₄ yielded anilines 263 (39%) and 265 (46%).²³⁴ To synthesize the aniline 269 from 6-aminonaphthalen-1-ol 266, both N- and O-methylation was required and a different strategy was necessary. 6-Aminonaphthalen-1-ol 266 was first boc protected to give a N-Boc protected intermediate 267, which was subsequently methylated (both N- and O-) with methyl iodide in the presence of sodium
hydride$^{235}$ and deprotected using trifluoroacetic acid to give 5-methoxy-N-methylnaphthyl-2-amine 269 (55% over 3 steps).$^{236}$

**Scheme 38.** Synthesis of target compounds 155, 156, 158, 159 and 171

Compounds 155, 156, 158, and 159 (Scheme 38) were synthesized by $S_N$Ar displacement of the pivaloyl protected chloro intermediate 258 with appropriate anilines to give the pivaloyl protected intermediates, which were deprotected under basic conditions to afford the final compounds in 43%, 32%, 26% and 32% respectively. The pivaloyl protected intermediate 171 was also obtained in 11% yield via the $S_N$Ar displacement of 258 with 6-methoxy-1,2,3,4-tetrahydroquinoline 271.
Compounds 157 and 160 (Scheme 39) were similarly synthesized by the nucleophilic displacement of the chloro group of 157 by 3-methoxy-N-methylaniline 272 and 5-methoxy-N-methylnaphthalen-2-amine 269, respectively.

**Scheme 39. Synthesis of target compounds 157 and 160**

![Synthesis of target compounds 157 and 160](image)

**Scheme 40. Synthesis of target compounds 161-169 (Series III)**

![Synthesis of target compounds 161-169 (Series III)](image)

Compounds 161-167 (Scheme 40) were synthesized by first reacting 154 with sodium hydride to generate the anion which was then treated with the appropriate alkyl
iodide or benzyl bromide to yield compounds 161-167. The low yield of these reactions can be attributed to the formation of a dialkylated side product which was isolated for the reaction of 154 with benzyl bromide as the dibenzylated analog 167a. Similarly, compounds 168 and 169 were synthesized by displacing the anion obtained from 160 with ethyl iodide and propyl iodide, respectively.

**Scheme 41. Synthesis of compound 170 (Series IV)**

![Scheme 41](image)

Compound 154 was acetylated using acetic anhydride in acetic acid to give the final compound 170 in 30% yield (Scheme 41). For biological activities of 155-171, see Appendix Table 12, pg 219.

**Synthesis of N<sup>4</sup>-anilinoquinazolines 172-182 and pyrido[2,3-d]pyrimidine 183**

The synthesis of N<sup>4</sup>-anilinoquinazolines 172-182 and the pyrido[2,3-d]pyrimidine 183 (Figure 55), was envisioned via the chloro intermediate 273(a-d) which could be displaced via nucleophilic reaction with appropriate anilines to give the target compounds. Since 273c was commercially available, the 4-chloro intermediate 273(a, b, d) were considered to be synthesized via chlorination using phosphorus oxychloride on the 4-oxo intermediates 274(a, b, d). Intermediates 274a and 274b were commercially available. For synthesizing the 4-oxo intermediate 274d, it was of interest to carry out a
condensation of 2-aminonicotinic acid 275 in the presence of formamide under microwave irradiation conditions. A similar method was reported for the synthesis of 2-H substituted quinazoline (274b), which gave the target compound in 60% yield.\textsuperscript{238}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{example.png}
\caption{Retrosynthesis of target compounds 172-183}
\end{figure}

\textbf{Scheme 42}. Reaction optimization for synthesis of intermediate 4-chloroquinazoline (273b)
The synthesis of 4-chloroquinazoline was attempted by the reaction of commercially available quinazolin-4(3H)-one 274b in phosphorus oxychloride at reflux (Scheme 42). After 4 hours, monitoring the reaction using thin-layer chromatography (TLC) suggested completion of the reaction. The work up was carried out by evaporating the solvent in vacuo and adjusting the pH to 8 using ammonia solution. This resulted in the conversion of 273b back to the 4-oxo starting material 274b. In another case, after the reaction was completed, the neutralization was carried out under ice cold conditions using 7N NH₃ in methanol which resulted in the formation of 4-methoxyquinazoline 276 (appearance of singlet at 4.24 ppm accounting for 3H).

In the next set of condition, 274b was reacted with phosphorus oxychloride (1 eq) in toluene with pyridine (1 eq) as a base at reflux (Scheme 41). This resulted in the formation of the dimer 277 (¹H NMR spectrum suggested 8 aromatic peaks between 7.68-8.26 ppm, singlets at 8.63 and 9.46 ppm, accounting for the 2-H protons of the quinazoline ring).

![Figure 56. Mechanism for the formation of dimer 277](image)

The dimer formation was also documented by Arnott et al., who suggested that the formation of the dimer occurs as a result of the reaction between quinazolinone 274b and the phosphate intermediate 278 (Figure 56). They proposed that the side reaction could
be overcome by adding phosphorus oxychloride to 274b under basic conditions (pK_a > 9) and running the reaction at room temperature for 1 hour to ensure complete conversion of 274b to 278. Once all the starting material is converted to the phosphate intermediate 278, heating the reaction mixture to 95 °C for 2-3 hours results in the formation of the desired 4-chloroquinoline 273b.

**Scheme 43. Synthesis of 4-chloroquinazoline 273b**

![Scheme 43. Synthesis of 4-chloroquinazoline 273b]

Based on the above description, 4-chloroquinazoline 273b (Scheme 43) was synthesized in 80% yield using a stoichiometric concentration of phosphorus oxychloride and triethylamine in toluene. The initial reaction solution at room temperature underwent a color change from pink to orange brown, which on reflux resulted in a clear brown solution. During the reaction work up and at later steps, care was taken to use ethyl acetate and not methanol to dissolve the compound, as the presence of methanol could result in the formation of 276 (Scheme 41).

**Scheme 44. Synthesis of 4-chloro-2-methylquinazoline 273a**

![Scheme 44. Synthesis of 4-chloro-2-methylquinazoline 273a]

Similar to the synthesis of 273b, 4-chloro-2-methylquinazoline 273a was synthesized from 2-methylquinazolin-4(3H)-one 274a in 50% yield (Scheme 44).
Compounds 172-180 and 182 were synthesized by nucleophilic displacement reaction of 4-chloroquinazolines (273a, 273b and 273c) by respective anilines in isopropyl alcohol and a drop of conc. HCl at room temperature for 12 hours (Scheme 45).

Yields of compounds in Series V were 58% (172, reported yield 71%$^{181}$, 65% (173, reported yield 79%$^{241}$), 70% (174, reported yield 41%$^{181}$), 68% (175). Yields of compounds in Series VI were 76% (176, reported yield 79%$^{242}$), 70% (177, reported yield 69%$^{243}$), 72% (178). Yields of compounds in Series VII were 10% (179, reported yield 87%$^{242}$), 25% (180, reported yield 87%$^{241}$). The synthesis of 181 was unsuccessful under the reaction conditions mentioned above which led to the formation of dimer 279 ($^1$H NMR...
indicated four methyl singlets, two for \( \text{SCH}_3 \) at 2.48 and 2.49 ppm and two for \( \text{NCH}_3 \) at 3.29 and 3.57 ppm). Compound 182 was synthesized over two steps. The first step involved the nucleophilic displacement of quinazoline 273c with 6-aminonaphthalen-1-ol 266 to yield 6-((2-chloroquinazolin-4-yl)amino)naphthalen-1-ol 280. \( \text{N} \)- and \( \text{O} \)-methylation of 280 using methyl iodide and sodium hydride yielded the desired compound 182 in 47% yield.

**Scheme 46. Synthesis of target compound 183 (Series VII)**

Synthesis of compound 183 was accomplished starting with the cyclization of 2-aminonicotinic acid 275 with formamide to pyrido[2,3-\(d\)]pyrimidin-4(3\(H\))-one 274d (70%). Chlorination of 274d in the presence of phosphorus oxychloride, as mentioned above, led to 4-chloropyrido[2,3-\(d\)]pyrimidine 273d in 20% yield. Subsequent nucleophilic displacement of 273d with 4-methoxy-\( \text{N} \)-methylaniline yielded target compound 183 in 29% yield.\(^{238} \) For biological activities of 172-183, see Appendix Table 13, pg 221.
Synthesis of quinoline-2,5-diamines 186-191

![Chemical structure](image)

**Figure 57.** Retrosynthesis of target compounds 186-191.

The synthesis of target compounds 186-191 (Figure 57) was envisioned via palladium-catalyzed cross coupling reactions of intermediate 5-chloro- or 5-bromo-2-aminoquinoline 281a or 281b, which could be synthesized via a modified Friedlander synthesis involving condensation of 2-amino-6-chlorobenzaldehyde or 2-amino-6-bromobenzaldehyde 282a or 282b with appropriate alkanenitriles. Since the palladium-catalyzed coupling reaction of intermediate 281 with anilines has not been reported, it was of interest to devise an efficient synthetic strategy for the target compounds 186-191. To accomplish the same goal the synthesis of 190 was performed for reaction optimization studies.

2-amino-6-chlorobenzaldehyde 282a was synthesized via oxidation of (2-amino-6-chlorophenyl)methanol 283. Oxidation using manganese dioxide afforded a better yield (83%)\textsuperscript{244} compared to the oxidation under PCC conditions (13%). Condensation of 282a with heptanenitrile in the presence of potassium tert-butoxide under a modified Friedlander synthesis yielded 2-amino-5-chloroquinoline 281a in 48% yield.\textsuperscript{76} 2-Amino-5-bromoquinoline 281b was similarly synthesized in 30% yield from 2-amino-6-bromobenzaldehyde 282b.
Scheme 47. Synthesis of 2-amino-5-chloro-quinoline intermediate 281a and 2-amino-5-bromo-quinoline intermediate 281b

Since the 2-NH$_2$ group can interfere in the subsequent palladium-catalyzed coupling reactions and lead to a self-coupled product, it was envisioned to synthesize pivaloyl protected intermediates 284 (58%) and 285 (78%) by reacting 2-amino-6-chlorobenzaldehyde 281a and 2-amino-6-bromobenzaldehyde 281b with pivalic anhydride under reflux, respectively.

Scheme 48. Attempted synthesis of 190 using intermediate 284
In an attempt to synthesize 190, palladium-catalyzed amination of pivaloyl protected 5-chloro-3-pentylquinolin-2-amine 284 was carried out under microwave irradiation conditions in the presence of catalyst bis(dibenzylideneacetone)palladium(0) (Pd(dba)$_2$) and BINAP as a ligand.

Table 3. Reaction conditions for Pd-catalyzed coupling reaction on 284

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Catalyst</th>
<th>Ligand</th>
<th>Base</th>
<th>Solvent</th>
<th>Temperature</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Pd(dba)$_2$</td>
<td>BINAP</td>
<td>NaOrBu</td>
<td>1,4-dioxane</td>
<td>110 °C, µW, 4 h</td>
<td>No reaction</td>
</tr>
</tbody>
</table>

The reaction, after 4 hours, generated no new spots. A similar reaction was successfully utilized for palladium catalyzed amination reaction of chloroanthracenes and chloroanthraquinones. Due to commercial availability of 2-amino-6-bromobenzaldehyde 282b and reduction in the number of steps involved in the synthesis, further optimization studies were carried out using 5-bromo-3-pentylquinolin-2-amine 281b or its pivaloyl protected intermediate 285.

Scheme 49. Attempted synthesis of 190 using intermediate 281b
Table 4. Reaction conditions for Pd-catalyzed coupling reaction on 281b

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Catalyst</th>
<th>Ligand</th>
<th>Base</th>
<th>Solvent</th>
<th>Temperature</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
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<td>RuPhos Pd G3</td>
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<td>NaOrBu</td>
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<td>No reaction</td>
</tr>
<tr>
<td></td>
<td>1 mol%</td>
<td>1 mol%</td>
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<td></td>
</tr>
<tr>
<td>2</td>
<td>RuPhos Pd G3</td>
<td>RuPhos</td>
<td>NaOrBu</td>
<td>1,4-dioxane</td>
<td>110 °C, 24 h</td>
<td>No reaction</td>
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<tr>
<td></td>
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<td>2 mol%</td>
<td>2.0 eq</td>
<td></td>
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<td></td>
</tr>
<tr>
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<td>SPhos</td>
<td>NaOrBu</td>
<td>1,4-dioxane</td>
<td>110 °C, 24 h</td>
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</tr>
<tr>
<td></td>
<td>2 mol%</td>
<td>2 mol%</td>
<td>2.0 eq</td>
<td></td>
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<tr>
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In another attempt to synthesize 190, 5-bromo-3-pentylquinolin-2-amine 281b was reacted with 4-methoxy-N-methylaniline 259 under a variety of reaction conditions by varying the precatalysts (RuPhos Pd G3/SPhos Pd G1/XPhos Pd G3), ligands (RuPhos/SPhos/XPhos), base (NaOrBu/Cs2CO3), solvent (THF/1,4-dioxane/tert-butanol) and temperature. However, none of the above mentioned reactions gave a new spot on TLC.247, 248

Scheme 50. Attempted synthesis of 190 using intermediate 285
Table 5. Reaction conditions for Pd-catalyzed coupling reaction on 285  

<table>
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<th>S. No.</th>
<th>Catalyst</th>
<th>Ligand</th>
<th>Base</th>
<th>Solvent</th>
<th>Temperature</th>
<th>Result</th>
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<td>Dimer 287</td>
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</tbody>
</table>

Amination of pivaloyl protected intermediate 285 with tris(dibenzylideneacetone)dipalladium(0) (Pd$_2$(dba)$_3$) catalyst with ligands SPhos or RuPhos in the presence of strong bases such as LiHMDS (entries 1 and 2, Table 5) led to the formation of dimer 287 ($^1$H NMR indicated protons at 6.48 ppm, 1H, NH and 6.59 ppm 2H, NH$_2$ both of which were exchangeable in D$_2$O). Using another set of reaction
conditions with Pd(dba)$_2$ and BINAP as a ligand did not succeed (entries 3 and 4). Changing the catalyst back to Pd$_2$(dba)$_3$ and by using different bases in THF led to no reaction in entry 5 and formation of dimer entry 6. By using LiHMDS under low temperature conditions (entry 7) did not form the dimer as seen in entry 1. Use of DavePhos as a ligand under similar reaction conditions failed to give any product (entry 8). In addition, reaction in the presence of NaO$_2$Bu with XPhos Pd G3 catalyst led to the formation of dimer 287.

**Scheme 51. Synthesis of $N$-(5-((4-methoxyphenyl)amino)-3-pentylquinolin-2-yl)pivalamide 288**

![Scheme 51](image_url)

Activated palladium precatalysts (XPhos Pd G3, RuPhos Pd G3 and SPhos Pd G1) offer an advantage as they are air moisture stable Pd(II) sources which readily form ligated Pd(0) *in situ* when exposed to base. In addition, XPhos is used as a preferred ligand for amination of primary amines/anilines, whereas, SPhos or RuPhos are preferred ligands for amination of secondary amines/anilines. To test the precatalyst, intermediate 285 (Scheme 51) was reacted with $p$-anisidine 270 (primary aniline) in the presence of XPhos Pd G3 precatalyst which gave a new spot on TLC ($R_f = 0.61$ in 10:1 CHCl$_3$/MeOH). $^1$H NMR of 288 confirmed the formation of the desired product as it indicated presence of $4´$-
OMe at 3.72 ppm as a singlet accounting for three protons and the aniline aromatic peaks at 6.87-6.89 ppm and 7.04-7.06 ppm as doublets accounting for four protons.

**Scheme 52.** Attempted synthesis of target compound 286 using precatalysts

![Scheme 52](image)

**Table 6.** Reaction conditions for Pd-catalyzed coupling reaction on 285

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Catalyst</th>
<th>Ligand</th>
<th>Base</th>
<th>Solvent</th>
<th>Temperature</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>RuPhos Pd G3</td>
<td>RuPhos</td>
<td>NaO\text{Bu}</td>
<td>1,4-dioxane</td>
<td>110 °C, 24 h</td>
<td>281b</td>
</tr>
<tr>
<td></td>
<td>2 mol%</td>
<td>2 mol%</td>
<td>2.5 eq</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>SPhos Pd G1</td>
<td>SPhos</td>
<td>NaO\text{Bu}</td>
<td>1,4-dioxane</td>
<td>110 °C, 24 h</td>
<td>281b</td>
</tr>
<tr>
<td></td>
<td>2 mol%</td>
<td>2 mol%</td>
<td>2.5 eq</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>RuPhos Pd G3</td>
<td>RuPhos</td>
<td>Cs$_2$CO$_3$</td>
<td>tert-butanol</td>
<td>110 °C, 24 h</td>
<td>281b</td>
</tr>
<tr>
<td></td>
<td>2 mol%</td>
<td>2 mol%</td>
<td>2.0 eq</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Considering that the reaction conditions with XPhos Pd G3 in Scheme 51 worked for amination with $p$-anisidine, RuPhos Pd G3 and SPhos Pd G1 were used for amination of secondary aniline (Scheme 52, Table 6) which, however, gave deprotected 281b as the product after 24 h (entry 1-3).

In a final attempt intermediate 285 was reacted with 4-methoxy-$N$-methylaniline 259 using XPhos Pd G3 with Cs$_2$CO$_3$ as a base in tert-butanol to give the aminated intermediate 286 in 55 % crude yield. Subsequent base hydrolysis of the pivaloyl group yielded the desired compound 190 in 83% yield.
Scheme 53. Synthesis of target compound 190

It was of further interest to mechanistically determine the reason as to why RuPhos (Figure 58) and SPhos based precatalysts, which are conventionally used for aminations of secondary amines/anilines did not work for the 2,5-diaminoquinoline scaffold and the reason why XPhos Pd G3 worked in the palladium catalyzed amination reactions. Therefore, further mechanistic studies of the Pd-catalyzed cross coupling reactions with precatalysts were carried out.

Figure 58. Structures of ligands used in Pd-catalyzed cross coupling reactions
Figure 59. Proposed mechanism of SPhos-Pd-catalyzed amination of chlorobenzene with dimethylamine (modified from ref. 150).

The catalytic cycle in Pd-catalyzed cross coupling reactions begins with the formation of an active ligand-Pd complex. The ligand-Pd complexes prior to oxidative addition have geometries which favors Pd-O interaction (in the case of SPhos, interaction of Pd with OMe group) or Pd-arene interaction (in the case of XPhos, interaction with the aromatic ring). These interactions provide stability to the palladium complex and increase the electron density at the palladium center. The oxidative addition of aryl halides results in formation of active Pd(II) complexes with the Pd center saturated with four ligands: the phosphorus center, the aryl group, the halide group, and a Pd-arene interaction with the ipso carbon of the lower aryl ring (Figure 59). The oxidative addition complex has the chloride atom trans to the phosphorus atom. For the favorable binding of the amine (dimethylamine), rotation of the Pd(II) complex about the C-P bond of the upper aryl ring occurs, which makes the subsequent deprotonation by base more facile due to a lack of steric bulk from the lower aryl ring. Once the deprotonation has taken place, the C-P bond
again rotates back to establish the Pd-arene interaction with the ipso carbon and the steric bulk of the lower aryl ring forces the aryl and the amido ligand in closer proximity such that it can more closely resemble the transition state for reductive elimination.\textsuperscript{254}

Based on the above mentioned mechanistic pathway, two steps, namely, oxidative addition and reductive elimination were probed for their involvement in the failure of the amination of the 2,5-diaminoquinoline scaffold with secondary anilines in the presence of RuPhos and XPhos based precatalysts.

It has been suggested that the electronic properties of the lower aryl ring strongly influence the rate of reductive elimination.\textsuperscript{255} XPhos with its isopropyl group in the lower aryl ring is considered electron-deficient, as this substitution does not donate electron density into the $\pi$-system. On the other hand, RuPhos with isopropoxy substitution is considered as an electron-rich ligand as the oxygen lone pair can easily donate electron density into the $\pi$-system of the lower aryl ring.\textsuperscript{255} For the RuPhos.Pd complex (Figure 60), the Pd center in the reductive elimination complex can be stabilized by interaction with the ipso carbon as well as the interaction with the oxygen atom of the isopropoxy group in RuPhos. However, for XPhos the Pd center is stabilized by interaction with the ipso carbon only. The additional stabilization of the RuPhos.Pd complex could therefore prevent reductive elimination with the use of RuPhos precatalyst. In addition, the bulky isopropyl groups in XPhos can promote reductive elimination by forcing the arene and amido group in close proximity to each other. This could result in the formation of the desired coupled product with the use of XPhos precatalyst.
**Figure 60.** Reductive elimination complexes of 2,5-diaminoquinolines and 4-methoxy-N-methylaniline with RuPhos.Pd complex and XPhos.Pd complex. PG(protecting group)

The use of RuPhos, however, did not yield the desired product. In addition, no dehalogenated side product was obtained in any of the reactions involving the use of SPhos or RuPhos, which suggested that the problem probably exists in the oxidative addition step of the catalytic cycle.
Figure 61. Formation of the oxidative addition complex of 285 with RuPhos precatalyst.

To analyze the formation of the oxidative addition complex with RuPhos Pd G3 precatalyst (Figure 61), compound 285 was reacted with stoichiometric quantities of the RuPhos Pd G3 precatalyst and RuPhos ligand under the same reaction conditions mentioned above (Cs$_2$CO$_3$, tert-butanol).\(^{252}\)

Figure 62. TLC of oxidative addition reaction of compound 285 with precatalysts

When the reaction was carried out with RuPhos precatalyst at 40 °C for 5 min, the TLC shows that after the reaction was stopped starting material was still present (Figure 62, left). The same trend was observed when the reaction was carried out at 100 °C for 5 min, with RuPhos precatalyst (Figure 62, middle). However, when the same reaction was
carried out with XPhos precatalyst (Figure 62, right), almost complete conversion of compound 285 to new spot was seen on the TLC at 100 °C.

These set of experiments suggest that the reaction of compound 285 with 4-methoxy-N-methylaniline 259 in the presence of RuPhos precatalyst failed due to a low rate of oxidative addition when compared to that observed with the use of XPhos precatalyst. However, no characterization was carried out to determine the structural identity of the new spot seen on TLC.

**Scheme 54.** Synthesis of target compounds 186-191

As mentioned for compound 190 above (Scheme 53), the synthesis of compounds 186-189 was achieved via condensation of benzaldehyde 282b with alkynitriles (289a-d) in the presence of potassium tert-butoxide under modified Friedlander synthesis, which were subsequently protected with pivalic anhydride to yield the 5-bromo-3-alkylquinolin-
2-amines 290a (3-Me: 50%), 290b (3-Et: 36%), 290c (3-Pr: 33%), and 290d (3-Bu: 24%). Further palladium catalyzed cross coupling aminations using XPhos Pd G3 precatalyst, XPhos ligand in the presence of Cs₂CO₃ as base in tert-butanol led to aminated products 186 (20%), 291b (60%, crude yield) 291c (59%, crude yield) and 291d (40%, crude yield). Due to the relatively less bulky methyl group at the 3-position in 290a, microwave irradiation conditions were feasible to afford deprotection of the pivaloyl group to yield 186 directly. For the bulkier substitutions in compounds 290b-d, the amination led to the pivaloyl protected compounds 291b-d, which were subsequently hydrolyzed under basic conditions to afford target compounds 187-189.

Scheme 55. Synthesis of target compounds 191

Similarly, target compound 191 was synthesized in 64% yield via the palladium catalyzed amination reaction of 290a with p-anisidine 270.

Synthesis of thieno[2,3-d]pyrimidines 192-210

The synthesis of 192-210 were accomplished as shown in Scheme 56. Reaction of acetone 292 with malononitrile and sulfur in the presence of morpholine was initially carried out according to the general procedure of Gewald and coworkers²⁵⁶ which gave very low yield of 11%. Optimization studies were carried out to synthesize the thiophene
using different bases in DMF (imidazole, 0.1 equivalent, 10%; morpholine, 0.1 equivalent, 15%; L-proline, 0.1 equivalent, 23%; L-lysine, 0.1 equivalent, 42%).

Scheme 56. Synthesis of target compounds 192-210

L-lysine was chosen as the base for the synthesis of thiophene aminonitrile 293, which was subsequently condensed with chlorformamidine hydrochloride 294 in DMSO to give the key thieno[2,3-d]pyrimidine intermediate 295 in 73% yield. Synthesis of target compounds 192-210 (6-61%) was accomplished by reacting 1 equivalent of 295 with 2 equivalents of substituted benzenethiol under oxidative thiolation conditions in the presence of iodine. The oxidative thiolation process was confirmed by disappearance of the C6 proton 6.51 ppm and the appearance of suitable aromatic protons for compounds 192-210. Compounds 192, 195, 196, 199, and 203 have been synthesized previously and tested for pcDHFR inhibitory activity. They were resynthesized to determine the pfDHFR inhibitory activities.
Scheme 57. Synthesis of substituted benzenethiols from anilines

![Chemical structure](image)

297a R = 2',4'-diOCH₃  
297b R = 2',6'-diOCH₃  
297c R = 4'-C₆H₅  
298a R = 2',4'-diOCH₃  
298b R = 2',6'-diOCH₃  
298c R = 4'-C₆H₅  
299a R = 2',4'-diOCH₃ (26%)  
299b R = 2',6'-diOCH₃ (15%)  
299c R = 4'-C₆H₅ (64%)

Yields calculated over 2 steps

Benzenethiols 299a-c were not available commercially and were therefore synthesized from the precursor anilinies (297a-c) via diazotization and displacement of nitrogen using potassium ethyl xanthate to afford intermediate O-ethyl S-aryl carbonodithioate 298a-c, which were subsequently hydrolyzed under basic conditions to afford the benzenethiols 299a-c.²⁶⁰

Scheme 58. Attempted synthesis of 211

![Chemical structure](image)

An attempt to synthesize 301 (Scheme 58) using 2-butanone 300 under Gewald reaction as mentioned above in Scheme 56 led to formation of a mixture of 301 and 301a (301/301a 1:5.6 ratio as determined from ¹H NMR). Since it was difficult to obtain pure 301, further synthesis of compound 211 was not carried out. It was of interest to determine the mechanism of formation of thiophenes 301 and 301a.
Figure 63. Mechanism for the formation of 301 and 301a

The formation of critical intermediate ‘vi’ (Figure 63) in the base catalyzed mechanism for the Gewald reaction results in the formation of two products when an asymmetrical ketone, 2-butanone 300 is used. Abstraction of the proton in ‘vi’ by the base
to generate a less stable, primary anion ‘vii’ results in the formation of the desired thiophene 301. On the other hand, the abstraction of a proton by the base to form a more stable secondary carbanion in ‘x’ eventually results in the formation of thiophene 301a in higher proportion.

**Scheme 59 Synthesis of desired compound 212**

3-amino-4-isopropylthiophene-2-carbonitrile 303 was obtained via Gewald synthesis on 3-methylbutan-2-one 302 in 52% yield (Scheme 59). Subsequent cyclization using chlorformamide hydrochloride 294 gave the thieno[2,3-d]pyrimidine 304 (85%). Iodination of 304 with N-iodosuccinimide (NIS) was carried out in the presence of acetic acid to yield 6-iodo-5-isopropylthiopheno[2,3-d]pyrimidine-2,4-diamine 305 (75%) as indicated by the disappearance of the 6-H peak at 6.52 ppm in 304. Ullman coupling of the iodo derivative 305 with the naphthalene-2-thiol 306 resulted in the formation of 212 (25%). Under microwave irradiation conditions, at 180 °C for 45 min resulted in dehalogenation (50% dehalogenated compound, 50% desired compound, as observed on
TLC). However if the reaction is carried out at 150 °C for 90 min less than 10% of the dehalogenated product is observed on TLC. For biological activities of 192-210 see Appendix Table 14, pg 222.

Molecular modeling studies in the colchicine site of tubulin

Pyrimido[4,5-b]indoles

Table 7. Biological activities of compounds 213-220

<table>
<thead>
<tr>
<th>Compd</th>
<th>EC₅₀ for microtubule depolymerization (nM)</th>
<th>MDA-MB-435 IC₅₀ ± SD (nM)</th>
<th>EC₅₀/IC₅₀ Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>213ᵃ</td>
<td>130</td>
<td>14.7 ± 1.5</td>
<td>8.8</td>
</tr>
<tr>
<td>214</td>
<td>130</td>
<td>33.9 ± 3.4</td>
<td>3.8</td>
</tr>
<tr>
<td>215</td>
<td>1200</td>
<td>130 ± 7.8</td>
<td>9.2</td>
</tr>
<tr>
<td>216</td>
<td>1100</td>
<td>89.1 ± 10</td>
<td>12</td>
</tr>
<tr>
<td>217</td>
<td>1400</td>
<td>220 ± 25</td>
<td>6.5</td>
</tr>
<tr>
<td>218</td>
<td>No effects up to 40 µM</td>
<td>3,900 ± 200</td>
<td>-</td>
</tr>
<tr>
<td>219</td>
<td>150</td>
<td>54.4 ± 4</td>
<td>2.8</td>
</tr>
<tr>
<td>220</td>
<td>3700</td>
<td>271 ± 4</td>
<td>15</td>
</tr>
<tr>
<td>paclitaxelᵇ</td>
<td>-</td>
<td>4.5 ± 0.5</td>
<td>-</td>
</tr>
<tr>
<td>CA-4ᵇ</td>
<td>9.8</td>
<td>4.4 ± 0.5</td>
<td>2.2</td>
</tr>
</tbody>
</table>

ᵃResults previously published.ᵇResults previously published.

Docking studies were performed for compounds 213-220 to rationalize the biological activities observed for the pyrimido[4,5-b]indoles as shown above (Table 7).
Protein and ligand preparation and docking validation

The X-ray crystal structure of colchicine in tubulin (PDB: 4O2B) was obtained from protein database (PDB) and imported into Molecular Operating Environment (MOE 2013.0801). Default settings were used for the protein preparation using LigX function and Amber99 forcefield. After the preparation of the protein using the LigX function, chains C, D, E, and F were deleted along with Ca	extsuperscript{2+}, Mg	extsuperscript{2+}, GDP, GTP, and the active site was defined using colchicine as the reference ligand. Ligands were sketched in MOE (MOE 2015.10) using the builder function and minimized using Amber10:EHT forcefield. The ligands were then docked into the colchicine binding site using Triangle Matcher placement method and scored using London dG. The refinement was carried out Rigid Receptor and scored using GBVI/WSA dG function, under default settings.

To validate the docking study, colchicine was redocked in the binding site using the same set of parameters specified above and generated a docked pose with a rmsd of 0.34 Å.

The pyrimido[4,5-b]indole scaffold of 213, 214 and 215 (Figure 64) overlaps with the C-ring of colchicine and undergoes hydrophobic interactions with residues Alaα180, Valα181, Leuβ248, Asnβ258, Metβ259, Thrβ314 and Lysβ352. The N\textsuperscript{4}-Me group interacts with Leuβ248 and Alaβ354, whereas, the N\textsuperscript{4}-aryl moiety undergoes hydrophobic interactions with Cysβ241, Alaβ250, Leuβ255, and Alaβ316 and overlaps with ring A of colchicine. The 4'-OMe group superimposes with the 3-OMe group in colchicine and is oriented towards residues Cysβ241, Leuβ242 and Leuβ255 in the binding pocket. The 2-NH\textsubscript{2} of 213 makes a H-bond interaction with HOH623. The 2-Me group in 214 retains hydrophobic interactions with Leuβ248 (3.90 Å) and Serα178 (3.99 Å), however, for
compound 215, the 2-H group is incapable of making a H-bond with HOH623 or undergoing hydrophobic interactions with the surrounding residues, which could explain a 10-fold loss in both the IC50 and EC50 compared to 213 with its 2-NH2 moiety.

**Figure 64.** Superimposition of the docked poses of 213 (black), 214 (green) and 215 (magenta) in the colchicine (pink) binding site of tubulin (PDB ID: 4O2B).213

The best docked poses of 213, 214 and 215 were scored at -7.08 kcal/mol, -7.18 kcal/mol and -6.84 kcal/mol, respectively, which suggested slightly lower affinity of 215 when compared to 213 and 214 in the colchicine site.

Similar to compound 215, 217 also results in almost 10-fold loss in the EC50 value and almost 16-fold loss in IC50 value when compared to 213 (Table 4). To explain the observed activity the docked poses of 213 and 217 were superimposed (Figure 65).
Figure 65. Superimposition of the docked poses of 213 (black) and 217 (cyan) in the colchicine (pink) site of tubulin (PDB ID: 4O2B). Hydrophobic surface is in green and hydrophilic surface is in pink.  

The key binding interactions of 213 as shown above in Figure 64 were retained by 217 and the 2-NH$_2$ afforded H-bond interactions with HOH606. Examining the lipophilic surface area of the pocket (Figure 65), the lower activities of compounds 217 and 218 (not shown), can be rationalized, in part, on the basis of the small hydrophobic pocket where the 5-Me and 5-Cl groups of 217 and 218, respectively, bind in the site. This hydrophobic pocket is lined by residues Metβ259, Alaβ316 and Lysβ352 which could result in steric hindrance and hence lower the activities of 217 and 218 compared to 213. The docked score of 217 and 218 were -5.96 kcal/mol and -6.26 kcal/mol, respectively, which was worse than the best docked pose of 213 (-7.08 kcal/mol).
Cyclopenta[d]pyrimidines

Docking studies were performed for compounds 221-234 to rationalize the biological activities observed for the cyclopenta[d]pyrimidines synthesized in the Gangjee lab as shown below (Table 8).

Table 8. Biological activities of compounds 221-234

<table>
<thead>
<tr>
<th>Compd</th>
<th>EC&lt;sub&gt;50&lt;/sub&gt; for microtubule depolymerization (nM)</th>
<th>MDA-MB-435 IC&lt;sub&gt;50&lt;/sub&gt; ± SD (nM)</th>
<th>EC&lt;sub&gt;50&lt;/sub&gt;/IC&lt;sub&gt;50&lt;/sub&gt; Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>221</td>
<td>25.9</td>
<td>7.0 ± 0.7</td>
<td>3.7</td>
</tr>
<tr>
<td>222</td>
<td>5,560</td>
<td>667</td>
<td>8.3</td>
</tr>
<tr>
<td>223</td>
<td>12.6</td>
<td>4.6 ± 0.5</td>
<td>3</td>
</tr>
<tr>
<td>224</td>
<td>40</td>
<td>8.2 ± 0.62</td>
<td>4.9</td>
</tr>
<tr>
<td>225</td>
<td>31</td>
<td>7.9 ± 0.49</td>
<td>3.9</td>
</tr>
<tr>
<td>226</td>
<td>&gt;10,000</td>
<td>ND</td>
<td>-</td>
</tr>
<tr>
<td>227</td>
<td>45</td>
<td>20 ± 1.9</td>
<td>2.25</td>
</tr>
<tr>
<td>228</td>
<td>5,116</td>
<td>1,258</td>
<td>4.1</td>
</tr>
<tr>
<td>229</td>
<td>70</td>
<td>12.1 ± 0.9</td>
<td>5.8</td>
</tr>
<tr>
<td>230</td>
<td>56</td>
<td>18.4 ± 1.9</td>
<td>3.0</td>
</tr>
<tr>
<td>231</td>
<td>&gt;10,000</td>
<td>ND</td>
<td>-</td>
</tr>
<tr>
<td>232</td>
<td>11,300</td>
<td>450.0 ± 13.5</td>
<td>25</td>
</tr>
<tr>
<td>233</td>
<td>&gt;10,000</td>
<td>ND</td>
<td>-</td>
</tr>
<tr>
<td>234</td>
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</tr>
<tr>
<td>CA-4</td>
<td>13.0</td>
<td>3.4 ± 0.6</td>
<td>3.8</td>
</tr>
</tbody>
</table>

Protein and ligand preparation and docking validation

The X-ray crystal structure of colchicine in tubulin (PDB: 4O2B) was obtained from protein database and imported into Molecular Operating Environment (MOE
Default settings were used for the protein preparation using QuickPrep function and Amber10:EHT forcefield. After the preparation of the protein using the QuickPrep function, chains C, D, E, and F were deleted along with Ca\(^{2+}\), Mg\(^{2+}\), GDP, GTP, and the active site was defined using colchicine as the reference ligand. Ligands were sketched in MOE (MOE 2016.08) using the builder function and minimized using Amber10:EHT forcefield. The ligands were then docked into the colchicine binding site using Triangle Matcher placement method and scored using London dG. The refinement was carried out using Induced Fit, the side chain was set free, cutoff was increased to 8 Å and radius offset was set to 0.6.

To validate the docking study, colchicine was redocked in the binding site using the same set of parameters specified above and generated a docked pose with a rmsd of 0.36 Å.

**Figure 66.** Superimposition of the docked poses of 221 (green), 225 (orange) and colchicine (pink) in the colchicine binding site of tubulin (PDB ID: 4O2B).
The docked poses of compounds 221 and 225 are shown as representative examples of the mode of binding mode of the cyclopenta[d]pyrimidines 221-234. The cyclopenta[d]pyrimidine scaffold undergoes hydrophobic interactions with Alaα180, Valα181, Asnβ258, Metβ259 and Lysβ352. The \( N^4 \)-Me group in both 221 and 225 interacts with Alaα180, Valα181, Asnβ258, Metβ259 and Lysβ352 and superimposes on the B-ring of colchicine. The \( N^4 \)-phenyl moiety undergoes hydrophobic interaction with Leuβ248, Alaβ250, Leuβ255 and Alaβ316 and superimposes on the A-ring of colchicine. The 4'-OMe group in 221 superimposes with the 2-OMe in colchicine and interacts with residues Valβ238, Cysβ241, and Ileβ378 and lies within H-bond distance with HOH728 near Cysβ241 (3.0 Å). In compound 225, the 4'-SOMe group undergoes H-bond interaction with HOH728. Compounds 221 and 225 had docked scores of -7.91 kcal/mol and -8.19 kcal/mol, respectively. Compounds 222 and 226 failed to dock, suggesting that these compounds may show reduced binding at the colchicine site.
Figure 67 A. Electrostatic surface of the colchicine binding site pocket in tubulin. B. Electrostatic surface map of colchicine. C. Electrostatic surfaces of the compounds 221-228. Red surface indicates electron rich surface, blue surface indicates electron deficient surface, and white surface represents hydrophobic surface.

The docking studies performed on compounds 221-234, except 222 and 226, suggested these compounds to be potent inhibitors of tubulin. To rationalize the low activities observed for compounds 222, 226, 227 and 228, an electrostatic surface map was generated for the colchicine site pocket, colchicine and compounds 221-228 using MOE2016.08. The blue surface observed for the colchicine pocket (Figure 67A) signifies an electron deficient surface and suggests that compounds like colchicine which have electron rich methoxy substituents will show surface complementarity (red surface, figure 67B) and will bind more favorably. This was well corroborated by the activities of compounds 221, 223, 224 and 225 which, like colchicine, have electron rich surfaces. The 4′-SO₂Me in 226 makes the aryl ring highly electron deficient. In addition, the carbonyl backbone of Cysβ241, Valβ248 and Aspβ251 lead to an unfavorable interaction with the sulfone group and is probably the reason for the failure of this compound to dock in the colchicine site and reflects its inactivity.

The low activities of 222 (compared to 221), 227 and 228 (compared to 225) can also be rationalized based on the electrostatic surface complementarity (Figure 67C). The presence of a H-bond donor at the 4′-position in 222, 227 and 228 (circled blue surfaces in Figure 67C) can result in an unfavorable interaction in an already electron deficient region of the binding site and may provide an explanation of the poor activity of these compounds compared to lead compound 221. Targeting electrostatic surface complementarity has been
successfully utilized in obtaining target selective compounds\textsuperscript{264} and based on the above discussion can also be used to explain the biological activities of compounds \textbf{221-228}.

The above-mentioned premise, however, was not sufficient to justify the inactivity of conformationally flexible compounds \textbf{230-234} which had electron rich substituents at the 4’-position. To justify the observed activities, a conformational search for compounds \textbf{221-234} was carried out using MOE2016.08 within 7 kcal/mol using the default settings.

\textbf{Table 9.} Conformations generated for compounds \textbf{221-234} using MOE2016.08.

<table>
<thead>
<tr>
<th>Compd</th>
<th>No. of conformations within 7 kcal/mol</th>
<th>Compd</th>
<th>No. of conformations within 7 kcal/mol</th>
</tr>
</thead>
<tbody>
<tr>
<td>221</td>
<td>4</td>
<td>228</td>
<td>2</td>
</tr>
<tr>
<td>222</td>
<td>2</td>
<td>229</td>
<td>4</td>
</tr>
<tr>
<td>223</td>
<td>4</td>
<td>230</td>
<td>4</td>
</tr>
<tr>
<td>224</td>
<td>5</td>
<td>231</td>
<td>42</td>
</tr>
<tr>
<td>225</td>
<td>4</td>
<td>232</td>
<td>34</td>
</tr>
<tr>
<td>226</td>
<td>4</td>
<td>233</td>
<td>36</td>
</tr>
<tr>
<td>227</td>
<td>8</td>
<td>234</td>
<td>34</td>
</tr>
</tbody>
</table>

Comparing the low energy conformations (within 7 kcal/mol) for \textbf{221-234}, compounds \textbf{221-230} show very low number of conformations (2-8). However, the conformationally flexible compounds \textbf{231-234} had 5 to 21-fold greater number of conformations. Such increased conformational flexibility can prove to be detrimental for these compounds to adopt the bioactive conformation and therefore could be responsible for in the low activities of these compounds.
Molecular modeling studies in the colchicine site of tubulin and multiple receptor tyrosine kinases (EGFR, VEGFR-2 and PDGFR-β)

5-Methyl-N-(substituted)-furo[2,3-d]pyrimidin-4-amines

Docking studies were performed for compounds 236-246 to rationalize the biological activities observed for the furo[2,3-d]pyrimidines as shown below (Table 10).

**Table 10. Biological activities of compounds 236-246**

<table>
<thead>
<tr>
<th>Compd</th>
<th>IC₅₀ ± SD (nM)</th>
<th>EC₅₀ for MT depolymerization</th>
<th>Inhibition of EGFR (nM)</th>
<th>Inhibition of VEGFR-2 (nM)</th>
<th>Inhibition of PDGFR-β (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>236</td>
<td>ND</td>
<td>&gt; 10 µM</td>
<td>283.1 ± 40.0</td>
<td>38.1 ± 4.2</td>
<td>57.3 ± 8.0</td>
</tr>
<tr>
<td>237</td>
<td>4.3 ± 0.3</td>
<td>24 nM</td>
<td>15.5 ± 2.0</td>
<td>9.3 ± 0.72</td>
<td>12.3 ± 2.0</td>
</tr>
<tr>
<td>238</td>
<td>17.1 ± 1.5</td>
<td>103 nM</td>
<td>68.2 ± 6.2</td>
<td>19.1 ± 3.0</td>
<td>22.8 ± 4.9</td>
</tr>
<tr>
<td>239</td>
<td>8.1 ± 0.5</td>
<td>53 nM</td>
<td>92.5 ± 9.9</td>
<td>67.3 ± 6.9</td>
<td>51.4 ± 7.2</td>
</tr>
<tr>
<td>240</td>
<td>504 ± 28</td>
<td>&gt; 10 µM</td>
<td>10.1 ± 0.91</td>
<td>23.4 ± 4.1</td>
<td>42.6 ± 5.6</td>
</tr>
<tr>
<td>241</td>
<td>27.3 ± 4.5</td>
<td>306 nM</td>
<td>7.1 ± 0.74</td>
<td>12.0 ± 1.6</td>
<td>38.9 ± 6.6</td>
</tr>
<tr>
<td>242</td>
<td>183 ± 13</td>
<td>750 nM</td>
<td>4.0 ± 0.39</td>
<td>7.8 ± 0.94</td>
<td>28.9 ± 4.0</td>
</tr>
<tr>
<td>243</td>
<td>12.3 ± 0.9</td>
<td>45 nM</td>
<td>7.2 ± 0.81</td>
<td>13.6 ± 2.0</td>
<td>62.2 ± 9.6</td>
</tr>
<tr>
<td>244</td>
<td>ND</td>
<td>240 nM</td>
<td>6.4 ± 0.97</td>
<td>12.7 ± 1.8</td>
<td>57.8 ± 9.9</td>
</tr>
<tr>
<td>245</td>
<td>ND</td>
<td>140 nM</td>
<td>3.1 ± 0.06</td>
<td>15.6 ± 2.2</td>
<td>63.0 ± 10.7</td>
</tr>
<tr>
<td>246</td>
<td>ND</td>
<td>&gt; 10 µM</td>
<td>&gt;200</td>
<td>&gt;200</td>
<td>&gt;200</td>
</tr>
</tbody>
</table>

ND = Not Determined
Protein and ligand preparation and docking validation in the colchicine binding site on tubulin

The X-ray crystal structure of colchicine in tubulin (PDB: 4O2B) was obtained from protein database and imported into Molecular Operating Environment (MOE 2013.0801). The protein was prepared using LigX function and Amber99 forcefield under default settings for protein minimization. After the preparation of the protein using the QuickPrep function, chains C, D, E, and F were deleted along with Ca\(^{2+}\), Mg\(^{2+}\), GDP, GTP, and the active site was defined using colchicine as a reference ligand. Ligands were sketched using the builder function in MOE and minimized using MMF94x forcefield. The ligands were then docked in the binding site using the Alpha triangle placement method. Refinement was carried out using Forcefield and scored using the Affinity dG scoring system.

To validate the docking study, colchicine was redocked in the binding site using the same set of parameters specified above and generated a docked pose with a rmsd of 0.53 Å.
Compounds 236-246 were docked in the colchicine binding site of tubulin and the docked pose of 237 in the colchicine site is shown as a representative example for this series (Figure 68). The furo[2,3-d]pyrimidine scaffold forms hydrophobic interactions with Alaα180, Asnβ258 and Lysβ352 and overlaps the C-ring of colchicine. The 5-Me group of 237 lies in a pocket lined by residues Leuβ255, Asnβ258 and Metβ259. The N4-Me moiety of 237 mimics the bridged C5- and C6-positions of the B-ring of colchicine and interacts with Leuβ248, Alaβ250 and Lysβ254. The N4-phenyl group superposes on to the A-ring of colchicine and interacts with Leuβ248, Leuβ255, Alaβ316 and Alaβ354, the 4′-OMe is oriented towards hydrophobic pocket lined by residues Thrβ240, Cysβ241, Alaβ316, Ileβ318 and Ileβ378.
Protein and ligand preparation and docking validation in RTKs

Compounds 236-246 were docked in the reported X-ray crystal structures of EGFR (PDB: 4JQ7, 2.73 Å)\textsuperscript{265}, VEGFR-2 (PDB: 4AG8, 1.95 Å)\textsuperscript{187} and in the homology model of PDGFR-\(\beta\)\textsuperscript{233} using Molecular Operating Environment (MOE 2016.10). The crystal structures of EGFR and VEGFR-2 were obtained from the protein database and imported into MOE 2016.10. The proteins were prepared using the QuickPrep function and the Amber10:EHT forcefield for energy minimization under default settings. Ligands were sketched using the builder function in MOE and minimized using Amber10:EHT forcefield. The ligands docked in the binding site of EGFR were placed using Alpha Triangle and scored using Affinity dG. The refinement was carried out using Rigid Receptor and scored using GBVI/WSA dG. The ligands were docked in the binding site of VEGFR-2 using a different protocol. The placement was carried using Alpha Triangle and scored using Affinity dG. The refinement was carried out using Induced Fit and scored using GBVI/WSA dG. The side chain was set free, cutoff was increased to 8 Å and radius offset was set to 0.6. For docking in PDGFR- \(\beta\) the placement was carried out using Triangle Matcher and scored using London dG. The refinement was carried out using Rigid Receptor and scored using GBVI/WSA dG.

To validate our docking studies, the co-crystallized ligands were re-docked into the binding sites of EGFR and VEGFR-2, respectively, using the same set of parameters as described above. The rmsd of the best docked pose of co-crystallized ligands in EGFR and VEGFR-2 were 0.22 Å and 0.76 Å, respectively, thus validating the docking.
Figure 69. Superimposition of docked pose of 237 (green) and 238 (magenta) in the ATP-binding site of EGFR (PDB: 4JQ7).

The docked poses of compounds 239-246 docked at the binding site of EGFR similar to lead compound 237, which was compared with binding mode for another lead compound 238 (Figure 69). The furo[2,3-\textit{d}]pyrimidine scaffold of 237 forms hydrophobic interactions with Leu694, Ala719 and Leu820 and binds at the hinge region of the ATP binding pocket \textit{via} H-bonding interactions of N1 with backbone NH of Met769 and N3 with HOH2015. The N4-phenyl substitution is oriented towards pocket lined by residues Phe699, Val702, Leu820, Thr830, and carbon atoms of Arg817. The docked conformation of 238 is slightly different due to the presence of 2-Me substitution, which prevents it from making the hinge region binding interaction with Met769, but otherwise retains similar interactions as 237 in the binding site. The docked scores for 237 and 238 were -6.08 kcal/mom and -5.94 kcal/mol respectively.
Figure 70. Superimposition of docked pose of 237 (green) and 238 (magenta) in the ATP-binding site of VEGFR-2 (PDB: 4AG8).

Figure 62 shows the docked poses of compounds 237 and 238 in the ATP binding site of VEGFR-2 (PDB: 4AG8). Due to presence of steric bulk of the 2-Me group in 238, the scaffold binds in a flipped mode when compared to the scaffold in 237. The furo[2,3-d]pyrimidine scaffold in 237 binds at the hinge region of ATP binding pocket in VEGFR-2 via H-bonding of N1 with backbone NH of Cys919. Additional interactions of the scaffold are formed with hydrophobic residues Leu840, Ala866 and Leu1035 with the 5-Me group oriented towards Phe1047. The N<sup>4</sup>-phenyl substitution lies in a hydrophobic pocket formed by residues Leu889, Val914, Val916, Cys1045 and carbon atoms of Lys868. For compound 238, the alternate binding mode results in formation of H-bonding interaction of N1 with HOH2085. The docked scores for 237 and 238 were -8.17 kcal/mom.
and -7.84 kcal/mol respectively. The docked poses of compounds **239-246** docked at the binding site of VEGFR-2 were similar to lead compound **237**.

**Figure 71.** Superimposition of docked pose of **237** (green) and **238** (magenta) in PDGFR-β homology model.

Docking in the PDGFR-β suggests that the furo[2,3-d]pyrimidine scaffold binds at the hinge region via H-bonding of N1 and O7 with backbone NH of Cys684 (Figure 71). The scaffold is additionally stabilized by interactions with Ala632, Tyr683 and Leu833. The $N^1$-phenyl substitution lies in a hydrophobic pocket lined by residues Leu606, Val614, Val615 and Tyr683. Compound **238** binds in a different mode where the scaffold flips to accommodate the bulk at the 2-position. The docked scores of **237** and **238** were -6.13 kcal/mol and -6.55 kcal/mol respectively. Compounds **239-246** docked at the active site of PDGFR-β with a similar binding mode.
Figure 72. A) Docked pose of 246 (magenta) in the ATP-binding site of EGFR. B) Docked pose of 246 (orange) in the ATP-binding site of VEGFR-2. Electrostatic surface of pocket, blue indicates electropositive regions, red indicates electronegative regions and white indicates lipophilic regions.

The loss of activity of the 4'-NO₂ analog 246 (Table 10) in EGFR and VEGFR-2 can be rationalized using Figure 64A and 64B. The docked pose of 246 in EGFR and VEGFR-2 are similar to those shown for compound 237 (Figures 69 and 70). In EGFR the 4'-NO₂ group lies in a highly electronegative pocket formed by the side chain carboxylate group of Asp831 and the backbone carboxyls of Arg817 and Asn818. Similarly, in VEGFR-2 the 4'-NO₂ group lies in the electronegative pocket formed by the carboxylate group Glu885 and backbone carbonyl of Val914. These unfavorable interactions are reflected in the lower activity of 246 in EGFR and VEGFR-2.
7-benzyl-N-(substituted)-pyrrolo[3,2-d]pyrimidin-4-amines

Docking studies were performed for compounds 249-256 to rationalize the biological activities observed for the pyrrolo[3,2-d]pyrimidines as shown below (Table 11).

**Table 11. Biological activities of compounds 249-256**

<table>
<thead>
<tr>
<th>Compd</th>
<th>Tubulin assembly IC₅₀ (µM) ± SD</th>
<th>[%] H Colchicine binding 5 µM inhibitor</th>
<th>Inhibition of EGFR (nM)</th>
<th>Inhibition of VEGFR-2 (nM)</th>
<th>Inhibition of PDGFR-β (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>249</td>
<td>21 ± 1</td>
<td>ND</td>
<td>23.6 ± 5.8</td>
<td>21.3 ± 3.2</td>
<td>&gt;200</td>
</tr>
<tr>
<td>250</td>
<td>&gt; 20</td>
<td>ND</td>
<td>1.1 ± 0.2</td>
<td>27.3 ± 5.1</td>
<td>8.1 ± 0.91</td>
</tr>
<tr>
<td>251</td>
<td>&gt; 20</td>
<td>ND</td>
<td>2.6 ± 0.42</td>
<td>26.7 ± 4.6</td>
<td>7.2 ± 0.9</td>
</tr>
<tr>
<td>252</td>
<td>0.48 ± 0.008</td>
<td>92 ± 0.2</td>
<td>2.3 ± 0.30</td>
<td>33.0 ± 5.0</td>
<td>10.3 ± 1.7</td>
</tr>
<tr>
<td>253</td>
<td>&gt; 20</td>
<td>ND</td>
<td>167.1 ± 27.6</td>
<td>&gt;200</td>
<td>&gt;200</td>
</tr>
<tr>
<td>254</td>
<td>3.3 ± 0.3</td>
<td>43 ± 3</td>
<td>33.6 ± 5.6</td>
<td>38.7 ± 7.1</td>
<td>90.3 ± 18.3</td>
</tr>
<tr>
<td>255</td>
<td>0.91 ± 0.03</td>
<td>72 ± 2</td>
<td>4.9 ± 0.6</td>
<td>32.9 ± 4.9</td>
<td>30.2 ± 7.2</td>
</tr>
<tr>
<td>256</td>
<td>13 ± 1</td>
<td>ND</td>
<td>47.1 ± 5.0</td>
<td>84.3 ± 3.9</td>
<td>&gt;200</td>
</tr>
<tr>
<td>CA</td>
<td>1.2 ± 0.01</td>
<td>99 ± 0.3</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Semaxinib</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>12.9</td>
<td>ND</td>
</tr>
<tr>
<td>Sunitinib</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>18.9 ± 2.7</td>
<td>83.1 ± 10.1</td>
</tr>
<tr>
<td>Erlotinib</td>
<td>ND</td>
<td>ND</td>
<td>1.2 ± 0.2</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

ND = Not Determined
Protein and ligand preparation and docking validation in the colchicine binding site in tubulin and RTKs

Docking of compounds 249–256 were performed using Molecular Operating Environment (MOE 2015.10) in the published X-ray crystal structures of colchicine in tubulin (PDB: 4O2B, 2.3 Å), gefitinib in EGFR (PDB: 4WKQ, 1.85 Å), axitinib in VEGFR-2 (PDB: 4AG8, 1.95 Å), and in a homology model of PDGFR-β. For tubulin, EGFR and VEGFR-2, the crystal structures were obtained from the protein database and imported into MOE 2015.10. The proteins were then prepared using the QuickPrep function and the Amber10:EHT forcefield for energy minimization under default settings. For preparation of tubulin, prior to setting up the protein for QuickPrep, chains C, D, E and F were deleted to reduce the time for protein preparation. After the preparation of the protein, Ca²⁺, Mg²⁺, GDP, GTP and all other bound ligands except for colchicine were deleted. Ligands were sketched using the builder function in MOE and minimized using Amber10:EHT forcefield. The ligands were docked in the binding site of tubulin, EGFR and PDGFR-β using a docking protocol to include the key interactions with water molecules in the binding site (for tubulin and EGFR). The placement was carried using Triangle Matcher and scored using London dG. The refinement was carried out using Induced Fit, the side chain was set free, cutoff was increased to 8 Å and radius offset was set to 0.6. The ligands were docked in the binding site of VEGFR-2 using the default settings in the docking protocol. The placement was carried using Triangle Matcher and scored using London dG. The refinement was carried out using Rigid Receptor and scored using GBVI/WSA dG.
To validate our docking studies, the native ligands colchicine, axitinib and gefitinib were re-docked into the binding sites of tubulin, EGFR and VEGFR-2, respectively, using the same set of parameters as described above. The rmsd of the best docked pose of colchicine in tubulin, gefitinib in EGFR and axitinib in VEGFR-2 were 0.36 Å, 0.85 Å and 0.51 Å, respectively, thus validating the docking.

Figure 73. Superimposition of the docked pose of 249 (magenta) and colchicine (pink) in tubulin (PDB ID: 4O2B).\textsuperscript{185}

Compounds 249-256 were docked in the X-ray crystal structure of colchicine in tubulin. The pyrrolo[3,2-\textit{d}]pyrimidine scaffold partially occupies the region where the C-ring of colchicine binds and undergoes hydrophobic interactions with Ala\textalpha{}180, Val\textalpha{}181, Leu\textbeta{}248, Asn\textbeta{}258 and Lys\textbeta{}352. The \textit{N}\textsuperscript{4}-Me interacts with Leu\textbeta{}255, Asn\textbeta{}258, Met\textbeta{}259 and Ala\textbeta{}316 and the \textit{N}\textsuperscript{4}-phenyl moiety interacts with Cys\textbeta{}241, Leu\textbeta{}248, Ala\textbeta{}250, and Leu\textbeta{}255 and superimposes with the A-ring of colchicine. The 4´-OMe group lies within H-bond distance with a water molecule (3.16 Å) present in the crystal structure in the
vicinity of Cysβ241. The benzylic group occupies a pocket lined by residues Serα178, Glnβ247, Leuβ248 and Lysβ352.

**Figure 74.** A) Superimposition of docked pose of 249 (cyan) and 252 (orange) in the colchicine site of tubulin (PDB ID: 4O2B). B) Space fill view of selected residues (N5-Me of compound 252, Leuβ248 and Alaβ354) in the colchicine site of tubulin (PDB ID: 4O2B). The green surface indicates hydrophobic regions and the pink surface indicates hydrophilic region in the pocket.\(^{185}\)

To rationalize the 40-fold improvement in the inhibition of tubulin assembly (comparing 249 and 252), the docked poses were superimposed and compared (Figure 66A and 66B). The N5-Me group in 252 makes additional hydrophobic interaction with Leuβ248 and Alaβ354. To compare the number of low energy conformations, a conformational search was carried out by a \(1^\circ\) rotation about the C4-N bond using Sybyl-X 2.1.1\(^{33}\) within 5 kcal/mol. Compound 249 afforded 111 conformers whereas 252 afforded 53 conformers. Similar improvement in activity has been attributed to a combination of conformational, hydrophobic and desolvation effects afforded by introducing methyl groups.\(^{266}\) Therefore, improved binding of 252 over 249 could also be attributed, in part,
to the conformational restriction to the C4-N bond due to the presence of the N5-Me in 252 and is absent in 249.

The 2-NH₂ substituted compound 250 was proposed to improve the inhibitory potency at RTKs by undergoing H-bond interaction at the hinge region of RTKs. The observed inhibitory activity of 250 improved 23-fold for EGFR and >15-fold for PDGFR-β over 249 (Table 11).

**Figure 75.** Docked pose of 250 (green) in the binding site of gefitinib (pink) in EGFR (PDB ID: 4WKQ).¹⁸⁵

The docked pose of 250 shows that the 2-NH₂ group makes an additional H-bond interaction in the hinge region with the carbonyl backbone of Gln791, as predicted in the statement of the problem (Figure 75).

Similar to the docked pose of 250 in EGFR, the 2-NH₂ makes an additional H-bond interaction with Cys684 in the hinge region of PDGFR-β while retaining the other interactions at the binding site (Figure 76).
Figure 76. Docked pose of 250 (green) in the binding site of homology model of PDGFR-β.185
V. EXPERIMENTAL

All evaporations were carried out in vacuum with a rotary evaporator. Analytical samples were dried in vacuo in a CHEM-DRY drying apparatus over P$_2$O$_5$ at 50 °C. Melting points were determined either using a MEL-TEMP II melting point apparatus with FLUKE 51 K/J electronic thermometer or using an MPA100 OptiMelt automated melting point system and are uncorrected. Thin-layer chromatography (TLC) was performed on Whatman® PE SIL G/UV254 flexible silica gel plates or Sorbetch silica g TLC plates w/UV254 and the spots were visualized under 254 and 365 nm ultraviolet illumination. Proportions of solvents used for TLC are by volume. All analytical samples were homogeneous on TLC in at least two different solvent systems. Flash chromatography was carried out on the CombiFlash® Rf 200 (Teledyne ISCO) automated flash chromatography system with prepacked RediSep® Rf normal-phase flash columns (230 to 400 meshes) of various sizes were used. The amount (weight) of silica gel for column chromatography was in the range of 50-100 times the amount (weight) of the crude compounds being separated. Nuclear magnetic resonance spectra for proton (¹H NMR) were recorded on the Bruker Avance II 400 (400 MHz) or Bruker Avance II 500 (500 MHz) NMR systems and were analyzed using MestReC NMR data processing software. The chemical shift values (δ) are expressed in ppm (parts per million) relative to tetramethylsilane as an internal standard: s, singlet; d, doublet; dd, doublet of doublet; t, triplet; q, quartet; m, multiplet; br, broad singlet; td, triplet of doublet; dt, doublet of triplet; quin, quintet. Elemental analyses were performed by Atlantic Microlab, Inc., Norcross, GA. Element compositions are within ±0.4% of the calculated values. Fractional moles of water or organic solvents frequently found in some analytical samples could not be prevented despite 24 to 48 hours of drying.
in vacuo and were confirmed where possible by their presence in the $^1$H NMR spectra. Mass spectrum data were acquired on an Advion expression CMS system using the ESI probe. All solvents and chemicals were purchased from Sigma-Aldrich or Fisher Scientific and were used as received.

**N-(5,6-Dichloro-2-methylpyrimidin-4-yl)pivalamide (258)**

5,6-dichloro-2-methylpyrimidin-4-amine **257** (1.0 g, 5.62 mmol) was refluxed in pivalic anhydride (10 mL) for 12 h. Pivalic anhydride was then evaporated, hexanes was added to give a precipitate which was then filtered, washed with hexanes and dried to give **258** as white colored solid (1.43 g, 97%). TLC $R_f = 0.87$ (CH$_3$OH: CHCl$_3$; 1:10); mp, 105.1-106.2 °C; $^1$H NMR, DMSO-$d_6$ (400 MHz): 1.23 (s, 9 H, ($CH_3$)$_3$), 2.58 (s, 3 H, 2-CH$_3$), 10.34 (s, 1 H, exch, NH). The compound was carried on directly without further characterization.

**N-(5-Chloro-6-((4-methoxyphenyl)(methyl)amino)-2-methylpyrimidin-4-yl)pivalamide (260)**

Compound **258** (0.478g, 1.82 mmol) and 4-methoxy-$N$-methyl aniline **259** (0.3g, 2.19 mmol) were added to a 2-5 mL Biotage microwave vial. 1,4-dioxane (3 mL) was added as the solvent and 1 drop of HCl was added. The reaction was run in a Biotage Initiator at 120 °C for 1 hour. Silica gel was added, solvent was removed under reduced pressure and the crude product was purified by column chromatography using 10% MeOH in CHCl$_3$ to yield light brown colored solid (0.067 g, 10%). TLC $R_f = 0.62$ (CH$_3$OH: CHCl$_3$; 1:10); mp, 149.1-150.4 °C; $^1$H NMR, DMSO-$d_6$ (400 MHz): 1.15 (s, 9 H, ($CH_3$)$_3$), 2.47 (s, 3 H, 2-
CH₃), 3.38 (s, 3 H, NCH₃), 3.75 (s, 3 H, OCH₃), 6.90-6.92 (d, 2 H, J = 8.91, Ar), 7.04-7.06 (d, 2 H, J = 8.90, Ar), 9.65 (s, 1 H, exch, NH). The compound was carried on directly without further characterization.

**5-Chloro-N⁴-(4-methoxyphenyl)-N⁴,2-dimethylpyrimidine-4,6-diamine (154)**

To a solution of 260 (0.050g, 0.0138 mmol) in EtOH, 1N NaOH (1 mL) was added and heated at 80 °C for 4 h. EtOH was evaporated, water was added and NaOH was neutralized using HCl. The compound precipitated, which was filtered and dried to give 154 as a brown colored solid (0.028 g, 74%). TLC Rf = 0.58 (CH₃OH: CHCl₃; 1:5); mp, 146.8-148.0 °C; ¹H NMR, DMSO-d₆ (400 MHz): 2.28 (s, 3 H, 2-CH₃), 3.27 (s, 3 H, NCH₃), 3.73 (s, 3 H, OCH₃), 6.61 (s, 2 H, exch, NH₂), 6.86-6.88 (d, 2 H, J = 8.36, Ar), 6.97-6.99 (d, 2 H, J = 8.28, Ar). Anal. Calcd. for C₁₃H₁₅ClN₄O : C, 56.02; H, 5.42; N, 20.10; Cl, 12.72. Found C, 56.06; H, 5.38; N, 19.99; Cl, 12.68.

**N⁴-(4-Methoxyphenyl)-N⁴,2,5-trimethylpyrimidine-4,6-diamine (153)**

6-chloro-2,5-dimethylpyrimidin-4-amine 261 (0.150 g, 0.951 mmol) and aniline 259 were dissolved in n-butanol (15 mL) and heated at reflux for 12 h, after which the solvent was evaporated in vacuo. Silica gel and methanol were added and the solvent was evaporated to afford a plug. The crude product was purified by column chromatography using 10% MeOH in CHCl₃ to yield light brown colored solid (0.067 g, 27%). TLC Rf = 0.57 (CH₃OH: CHCl₃; 1:10); mp, 95.1-96.4 °C; ¹H NMR, DMSO-d₆ (400 MHz): 1.33 (s, 3 H, 5-CH₃), 2.37 (s, 3 H, 2-CH₃), 3.34 (s, 3 H, NCH₃), 3.75 (s, 3 H, OCH₃), 6.90 (br, 2 H, exch, NH₂),
6.90-6.92 (d, 2 H, J = 8.97, Ar), 7.00-7.02 (d, 2 H, J = 8.91, Ar). Anal. Calcd. for C_{14}H_{18}N_{4}O. 0.25 CHCl₃: C, 59.43; H, 6.39; N, 19.46. Found C, 59.52; H, 6.77; N, 19.12; Cl, 6.80.

**N-Methyl-4-(methylthio)aniline (263)**

4-(methylthio)aniline 262 (1.13 g, 8.12 mmol) was added to a suspension of sodium methoxide (2.193 g, 40.6 mmol) in MeOH (10 mL). This mixture was then poured to another suspension of paraformaldehyde (0.342 g, 11.368 mmol) in MeOH (10 mL). The reaction mixture was then stirred at room temperature. After 5 h, sodium borohydride (0.307 g, 8.12 mmol) was added and the mixture was refluxed for 2 h. After the reaction, solvent was evaporated and 1M KOH (10 mL) was then added. The sample was then extracted 3 times using ether and dried over MgSO₄. Combined fractions were evaporated, silica plug was made and was purified by column chromatography using 10% EtOAc in hexanes to yield 263 as brown colored liquid (0.49 g, 39%). TLC \( R_f = 0.76 \) (Hex: EtOAc; 1:1); \(^1\)H NMR, DMSO-\( d_6 \) (400 MHz): 2.33 (s, 3 H, S\( CH_3 \)), 2.64-2.66 (d, 3 H, J = 5.09, NH\( CH_3 \)), 5.72-5.73 (m, 1 H, exch, NH), 6.49-6.52 (d, 2 H, J = 8.58, Ar), 7.11-7.13 (d, 2 H, J = 8.57, Ar). \(^1\)H NMR agreed well with the literature\(^{230}\) reported values.

**N-Methyl-2,3-dihydrobenzofuran-5-amine (265)**

Using the same synthetic method described for 263, compound 265 was obtained from 2,3-dihydrobenzofuran-5-amine 264 (2.0 g, 14.80 mmol) as brown colored semi-solid (1.0 g, 46%). TLC \( R_f = 0.77 \) (CH₃OH: CHCl₃; 1:10); \(^1\)H NMR, DMSO-\( d_6 \) (400 MHz): 2.62 (s, 3 H, N\( CH_3 \)), 3.04-3.08 (t, 2 H, J = 8.55, Ar\( CH_2 \)), 4.36-4.40 (t, 2 H, J = 8.56, O\( CH_2 \)), 5.08 (s, 1 H, exch, NH), 6.25-6.28 (dd, 1 H, \( J_1 = 2.41, J_2 = 8.41, 6\)-Ar), 6.48-6.49 (d, 1H, \( J = 1.77, \)
4-Ar), 6.52-6.54 (d, 1 H, J = 8.40, 7-Ar). \(^1\)H NMR agreed well with the literature\(^{148}\) reported values.

**5-Methoxy-\(N\)-methylnaphthalen-2-amine (269)**

Compound 269 was prepared over 3 steps. 6-aminonaphthalen-1-ol 266 (1.25 g, 7.85 mmol) was stirred with boc anhydride (5 mL) in ethyl acetate (15 mL) at room temperature. After 12 h, boc anhydride was evaporated and the residue was dissolved in ethyl acetate and purified using flash chromatography in CHCl\(_3\)/MeOH to afford 267 as a brown colored liquid (1.74 g, 85%). TLC \(R_f = 0.67\) (CH\(_3\)OH: CHCl\(_3\); 1:5); \(^1\)H NMR, DMSO-\(d_6\) (400 MHz): 1.51 (s, 9 H, (CH\(_3\))\(_3\)), 6.71-6.73 (dd, 1 H, \(J_1 = 7.13\), \(J_2 = 1.21\), Ar), 7.17-7.25 (m, 2 H, Ar), 7.43-7.46 (dd, 1 H, \(J_1 = 1.97\), \(J_2 = 9.14\), Ar), 8.00-8.01 (d, 2 H, \(J = 8.87\), Ar), 9.54 (s, 1 H, exch., OH), 10.00 (s, 1 H, exch., NH). Compound 267 (1.70 g, 6.79 mmol) was dissolved in DMF (30 mL) under argon. Sodium hydride (488 mg, 20.36 mmol) was added next and the mixture was stirred at 0 °C for 15 min. Methyl iodide (2.12 g, 14.93 mmol) was added and the reaction was stirred at room temperature for 2 h. At the end of the reaction, solvent was evaporated, silica gel was added with methanol to make a plug and the mixture was purified using flash chromatography in hexanes/ethyl acetate to afford 268 (1.36 g, 72%) as a yellow colored liquid. TLC \(R_f = 0.8\) (Hex: EtOAc; 1:1); \(^1\)H NMR, DMSO-\(d_6\) (400 MHz): 1.41 (s, 9 H, (CH\(_3\))\(_3\)), 3.28 (s, 3 H, NCH\(_3\)), 3.96 (s, 3 H, OCH\(_3\)), 6.92-6.94 (m, 1 H, Ar), 7.42-7.43 (m, 2 H, Ar), 7.45-7.46 (d, 1 H, \(J = 2.19\), Ar), 7.72-7.73 (d, 1 H, \(J = 2.12\), Ar), 8.07-8.09 (d, 1 H, \(J = 9.03\), Ar). Boc deprotection of 268 (1.3 g, 4.52 mmol) was carried out in trifluoroacetic acid (TFA, 15 ml) at room temperature for 2 h. At the end of the reaction, TFA was evaporated and the mixture was neutralized using aq.
solution of K$_2$CO$_3$. Extraction with ethyl acetate (20 mL × 3) was concentrated and dried over Na$_2$SO$_4$ to yield 269 (0.810 g, 95%) as a green colored solid. TLC $R_f$ = 0.77 (Hex: EtOAc; 1:1); $^1$H NMR, DMSO-$d_6$ (400 MHz): 2.75-2.76 (d, 3 H, NCH$_3$), 3.89 (s, 3 H, OCH$_3$), 5.98-5.99 (q, 1 H, NHCH$_3$, exch.), 6.57-6.59 (d, 1 H, J = 7.43, Ar), 6.61-6.62 (d, 1 H, J = 1.87, Ar), 6.88-6.90 (dd, 1 H, J$_1$ = 1.42, J$_2$ = 9.05, Ar), 7.15-7.23 (m, 2 H, Ar), 7.83-7.86 (d, 1 H, J = 9.05, Ar). The compound was carried on directly without further purification.

**General procedure for the synthesis of 5-chloro-$N^4$-(substituted)-2-methylpyrimidine-4,6-diamines 155, 156, 158 and 159**

To a 2-5 mL Biotage microwave vial were added 258 (1 equivalent), appropriate aniline (1.2 equivalents), 1,4-dioxane (3 mL) and catalytic amount of conc. HCl (1 drop). The resulting mixture was heated in microwave at 140 °C for 3 h. The solvent was removed under reduced pressure to obtain a mixture that was dissolved in methanol and to which silica gel was added. The solvent was removed by evaporation to yield a dry plug which was purified by flash chromatography using chloroform/methanol. The fractions containing the desired product (TLC) were pooled and evaporated to obtain the desired compounds 155, 156, 158 and 159.

**5-Chloro-$N^4$-(4-methoxyphenyl)-2-methylpyrimidine-4,6-diamine (155)**

Using the general procedure described above, compound 258 (0.750 g, 2.86 mmol), $p$-anisidine 270 (0.423 g, 3.43 mmol) were reacted to afford 155 as light pink-brown colored solid (0.325 g, 43%). Under the microwave irradiation conditions, the pivaloyl protected
intermediate was not obtained. TLC \( R_f = 0.7 \) (CH\(_3\)OH: CHCl\(_3\); 1:10); mp, 167.6-168.8 °C; \(^1\)H NMR, DMSO-\(d_6\) (400 MHz): 2.18 (s, 3 H, 2-CH\(_3\)), 3.73 (s, 3 H, OCH\(_3\)), 6.59 (s, 2 H, exch, NH\(_2\)), 6.85-6.87 (d, 2 H, \( J = 8.93\), Ar), 7.48-7.50 (d, 2 H, \( J = 8.93\), Ar), 8.18 (s, 1 H, exch, NH). Anal. Calcd. for C\(_{12}\)H\(_{13}\)ClN\(_4\)O : C, 54.45; H, 4.95; N, 21.16; Cl, 13.39. Found C, 54.54; H, 4.99; N, 21.08; Cl, 13.22.

**5-Chloro-N\(^4\),2-dimethyl-N\(^4\)-(4-(methylthio)phenyl)pyrimidine-4,6-diamine (156)**

Using the general procedure described above, compound 258 (0.3 g, 1.14 mmol), N-methyl-4-(methylthio)aniline 263 (0.21 g, 1.37 mmol) were reacted to afford 156 as white colored solid (0.11 g, 32%). Under the microwave irradiation conditions, the pivaloyl protected intermediate was not obtained. TLC \( R_f = 0.71 \) (CH\(_3\)OH: CHCl\(_3\); 1:10); mp, 134.8-136.9 °C; \(^1\)H NMR, DMSO-\(d_6\) (400 MHz): 2.30 (s, 3 H, 2-CH\(_3\)), 2.45 (s, 3 H, SCH\(_3\)), 3.30 (s, 3 H, NCH\(_3\)), 6.75 (s, 2 H, exch., NH\(_2\)), 6.93-6.95 (d, 2 H, \( J = 8.69\), Ar), 7.18-7.20 (d, 2 H, \( J = 8.73\), Ar). Anal. Calcd. for C\(_{13}\)H\(_{15}\)ClN\(_4\)S : C, 53.68; H, 5.39; N, 18.56; Cl, 11.75; S, 10.62. Found C, 53.61; H, 5.16; N, 18.83; Cl, 11.63; S, 10.69.

**5-Chloro-6-(6-methoxy-3,4-dihydroquinolin-1(2\(H\))-yl)-2-methylpyrimidin-4-amine (158)**

Using the general procedure described above, compound 258 (0.750 g, 2.86 mmol), 6-methoxy-1,2,3,4-tetrahydroquinoline 271 (0.561 g, 3.43 mmol) were reacted to afford a mixture with two spots. After running the flash chromatography the pivaloyl protected intermediate 171 was isolated in addition to the desired compound 158. Compound 158 was obtained as off-white colored solid (0.225 g, 26%). TLC \( R_f = 0.61 \) (CH\(_3\)OH: CHCl\(_3\);
1:10; mp, 192.9-194.7 °C; $^1$H NMR, DMSO-$d_6$ (400 MHz): 1.85-1.91 (m, 2 H, 3'-CH$_2$), 2.25 (s, 3 H, 2-CH$_3$), 2.73-2.76 (t, 2 H, 4'-CH$_2$), 3.60-3.63 (t, 2 H, N-CH$_2$), 3.68 (s, 3 H, OCH$_3$), 6.44-6.46 (d, 1 H, J = 8.80, 8'-Ar), 6.57-6.60 (dd, 1 H, J$_1$ = 8.75, J$_2$ = 2.52, 7'-Ar), 6.68-6.69 (d, 1 H, J = 2.15, 5'-Ar), 6.87 (s, 2 H, exch., NH$_2$). Anal. Calcd. for C$_{15}$H$_{17}$ClN$_4$O: C, 59.11; H, 5.62; N, 18.38; Cl, 11.63. Found C, 59.41; H, 5.61; N, 18.41; Cl, 11.33.

$N$-(5-Chloro-6-(6-methoxy-3,4-dihydroquinolin-1(2H)-yl)-2-methylpyrimidin-4-yl)pivalamide (171)

As mentioned above 171 was obtained as buff colored solid (0.125 g, 11%). TLC $R_f$ = 0.80 (CH$_3$OH: CHCl$_3$; 1:10); mp, 111.5-114.1 °C; $^1$H NMR, DMSO-$d_6$ (400 MHz): 1.20 (s, 9 H, CH$_3$), 1.89-1.97 (m, 2 H, 3'-CH$_2$), 2.45 (s, 3 H, 2-CH$_3$), 2.75-2.78 (t, 2 H, 4'-CH$_2$), 3.72 (s, 3 H, OCH$_3$), 3.77-3.80 (t, 2 H, N-CH$_2$), 6.62-6.63 (m, 2 H, Ar), 6.75-6.76 (d, 1 H, Ar), 9.89 (s, 1 H, exch., NH). Anal. Calcd. for C$_{20}$H$_{25}$ClN$_4$O$_2$: C, 61.77; H, 6.48; N, 14.41; Cl, 9.12. Found C, 61.95; H, 6.40; N, 14.22; Cl, 8.85.

5-Chloro-$N^4$-(2,3-dihydrobenzofuran-5-yl)-$N^4,2$-dimethylpyrimidine-4,6-diamine (159)

Using the general procedure described above, compound 258 (0.5 g, 1.91 mmol), $N$-methyl-2,3-dihydrobenzofuran-5-amine 265 (0.341 g, 2.29 mmol) were reacted to afford 159 as buff colored solid (0.22 g, 32%). Under the microwave irradiation conditions, the pivaloyl protected intermediate was not obtained. TLC $R_f$ = 0.63 (CH$_3$OH: CHCl$_3$; 1:10); mp, 192.8-194.1 °C; $^1$H NMR, DMSO-$d_6$ (400 MHz): 2.28 (s, 3 H, CH$_3$), 3.12-3.16 (t, 2 H, 3'-CH$_2$), 3.25 (s, 3 H, NCH$_3$), 4.49-4.54 (t, 2 H, O-CH$_2$), 6.57 (s, 2 H, exch., NH$_2$), 6.66-
6.68 (d, 1 H, J = 8.40, 7'-Ar), 6.75-6.77 (dd, 1 H, J1 = 8.40, J2 = 2.34, 6'-Ar), 6.98-6.99 (d, 1 H, J = 1.36, 4'-Ar). Anal. Calcd. for C14H15ClN4O 0.07 C4H8O2: C, 57.76; H, 5.28; N, 18.84; Cl, 11.92. Found C, 58.15; H, 5.27; N, 18.81; Cl, 11.83.

5-Chloro-N4-(3-methoxyphenyl)-N4,2-dimethylpyrimidine-4,6-diamine (157)

To a 100 mL round-bottomed flask were added 257 (0.2 g, 1.12 mmol), 3-methoxy-N-methylaniline 272 (1.2 equivalents), n-butanol (15 mL) and catalytic amount of conc. HCl (1 drop). The resulting mixture was refluxed for 12 h. The solvent was removed under reduced pressure to obtain a mixture that was dissolved in methanol and to which silica gel was added. The solvent was removed by evaporation to yield a dry plug which was purified by flash chromatography using chloroform/methanol. The fractions containing the desired product (TLC) were pooled and evaporated to afford 157 as light brown colored solid (0.101 g, 32%). TLC Rf = 0.68 (CH3OH: CHCl3; 1:10); mp, 150.8-151.9 °C; 1H NMR, DMSO-d6 (400 MHz): 2.30 (s, 3 H, 2-CH3), 3.32 (s, 3 H, NCH3), 3.71 (s, 3 H, OCH3), 6.51-6.53 (d, 2 H, Ar), 6.60-6.63 (m, 1 H, Ar), 6.80 (s, 2 H, exch., NH2), 7.15-7.19 (t, 1 H, J = 8.05, Ar). Anal. Calcd. for C13H15ClN4O: C, 56.01; H, 5.42; N, 20.10; Cl, 12.72. Found C, 56.40; H, 5.75; N, 19.80; Cl, 12.37.

5-Chloro-N4-(5-methoxynaphthalen-2-yl)-N4,2-dimethylpyrimidine-4,6-diamine (160)

To a 100 mL round-bottomed flask were added 257 (0.25 g, 1.40 mmol), 5-methoxy-N-methylnaphthalen-2-amine 269 (0.315 g, 1.69 mmol), n-butanol (15 mL) and catalytic amount of conc. HCl (1 drop). The resulting mixture was refluxed for 12 h. The solvent was
removed under reduced pressure to obtain a mixture that was dissolved in methanol and to which silica gel was added. The solvent was removed by evaporation to yield a dry plug which was purified by flash chromatography using hexanes/ethyl acetate. The fractions containing the desired product (TLC) were pooled and evaporated to afford 160 as light pink colored solid (0.06 g, 13%). TLC $R_f = 0.78$ (CH$_3$OH: CHCl$_3$; 1:10); mp, 138.3-140.1 °C; $^1$H NMR, DMSO-$d_6$ (400 MHz): 2.39 (s, 3 H, CH$_3$), 3.43 (s, 3 H, NCH$_3$), 3.95 (s, 3 H, OCH$_3$), 6.84-6.86 (m, 3 H, Ar, NH$_2$, exch.), 7.15-7.18 (dd, 1 H, $J_1 = 2.33$, $J_2 = 9.07$, Ar), 7.35-7.36 (m, 3 H, Ar), 8.02-8.04 (d, 1 H, $J = 9.07$, Ar). Anal. Calcd. for C$_{17}$H$_{17}$ClN$_4$O : 0.33CH$_3$CO$_2$C$_2$H$_5$: C, 61.48; H, 5.54; N, 15.64; Cl, 9.89. Found C, 61.52; H, 5.34; N, 15.69; Cl, 9.70.

**General procedure for the synthesis of 5-chloro-N$^4$-substituted-N$^6$-(4-methoxyphenyl)-N$^6$2-dimethylpyrimidine-4,6-diamines 161-167a**

Compound 154 (1 equivalent) was dissolved in anhydrous DMF in a three necked round-bottomed flask and was stirred at 0 °C under argon. Sodium hydride (2 equivalents) was added the above mixture and stirred at 0 °C for additional 15 min followed by addition of suitable alkyl halide (1.1 equivalents) while maintaining the reaction at room temperature for 4 h. Methanol was added at the end of the reaction, solvent was evaporated and the reaction mixture was then extracted with water and ethyl acetate (25 mL x 3) which were dried over Na$_2$SO$_4$. Silica gel was added next to the combined fractions, the solvent was removed by evaporation to yield a dry plug and column was the run using hexanes/ethyl acetate. The fractions containing the desired product (TLC) were pooled and evaporated to obtain the desired compounds 161-167a.
5-Chloro-N⁴-(4-methoxyphenyl)-N⁴,N⁶,2-trimethylpyrimidine-4,6-diamine (161)

Using the general procedure described above, compound 154 (0.113 g, 0.405 mmol) was dissolved in DMF (10 mL), to which sodium hydride (0.019 g, 0.810 mmol) was added at 0 °C. Methyl iodide (0.064 g, 0.446 mmol) was added next and after following the work up procedure mentioned above afforded 161 as off-white colored (0.036 g, 30%). TLC $R_f = 0.89$ (CH₃OH: CHCl₃; 1:10); mp, 104-105 °C; $^1$H NMR, DMSO-d₆ (400 MHz): 2.34 (s, 3 H, CH₃), 2.81-2.82 (d, 3 H, J = 4.61, NH-CH₃), 3.27 (s, 3 H, NCH₃), 3.73 (s, 3 H, OCH₃), 6.71-6.75 (q, 1 H, J = 4.30, exch., NH), 6.85-6.87 (d, 2 H, J = 8.99, Ar), 6.95-6.97 (d, 2 H, J = 8.98, Ar). Anal. Calcd. for C₁₄H₁₇ClN₄O: C, 57.43; H, 5.85; N, 19.14; Cl, 12.11. Found C, 57.58; H, 6.02; N, 18.92; Cl, 11.88.

5-Chloro-N⁴-ethyl-N⁶-(4-methoxyphenyl)-N⁶,2-dimethylpyrimidine-4,6-diamine (162)

Using the general procedure described above, compound 154 (0.150 g, 0.538 mmol) was dissolved in DMF (10 mL), to which sodium hydride (0.026 g, 1.08 mmol) was added at 0 °C. Iodoethane (0.092 g, 0.591 mmol) was added next and after following the work up procedure mentioned above afforded 162 as pink colored crystalline solid (0.060 g, 36%). TLC $R_f = 0.92$ (CH₃OH: CHCl₃; 1:10); mp, 105.4-106.5 °C; $^1$H NMR, DMSO-d₆ (400 MHz): 1.07-1.11 (t, 3 H, CH₂CH₃), 2.33 (s, 3 H, 2-CH₃), 3.27 (s, 3 H, NCH₃), 3.36-3.40 (m, 2 H, NH-CH₂), 3.73 (s, 3 H, OCH₃), 6.69-6.72 (t, 1 H, exch., NH), 6.85-6.87 (d, 2 H, J = 8.96, Ar), 6.95-6.98 (d, 2 H, J = 8.92, Ar). Anal. Calcd. for C₁₅H₁₉ClN₄O: C, 58.72; H, 6.24; N, 18.26; Cl, 11.56. Found C, 58.79; H, 6.25; N, 18.09; Cl, 11.42.
5-Chloro-N^4-(4-methoxyphenyl)-N^4,2-dimethyl-N^6-propylpyrimidine-4,6-diamine (163)

Using the general procedure described above, compound 154 (0.2 g, 0.717 mmol) was dissolved in DMF (10 mL), to which sodium hydride (0.034 g, 1.44 mmol) was added at 0 °C. 1-Iodopropane (0.134 g, 0.789 mmol) was added next and after following the work up procedure mentioned above afforded 163 as a semi-solid. HCl salt was prepared by bubbling HCl gas through the solution of compound in ether to afford 163.HCl as light pink colored solid (0.078 g, 30%). TLC $R_f = 0.95$ (CH$_3$OH: CHCl$_3$; 1:10); mp, 165.7-167.8 °C; $^1$H NMR, DMSO-$d_6$ (400 MHz): 0.84-0.88 (t, 3 H, CH$_2$CH$_2$CH$_3$), 1.50-1.59 (m, 2 H, CH$_2$CH$_2$CH$_3$), 2.56 (s, 3 H, 2-CH$_3$), 3.44-3.47 (m, 5 H, NCH$_3$, CH$_2$CH$_2$CH$_3$), 3.75 (s, 3 H, OCH$_3$), 6.91-6.93 (d, 2 H, J = 8.97, Ar), 7.13-7.16 (d, 2 H, J = 8.90, Ar), 8.02 (br, s, 1 H, exch., NH). Anal. Calcd. for C$_{16}$H$_{21}$ClN$_4$O, 0.96 HCl. 0.51H$_2$O: C, 52.65; H, 6.35; N, 15.35; Cl, 19.02. Found C, 52.68; H, 6.03; N, 15.7; Cl, 18.99.

5-Chloro-N^4-isopropyl-N^6-(4-methoxyphenyl)-N^6,2-dimethylpyrimidine-4,6-diamine (164)

Using the general procedure described above, compound 154 (0.150 g, 0.538 mmol) was dissolved in DMF (10 mL), to which sodium hydride (0.026 g, 1.08 mmol) was added at 0 °C. Isopropyl iodide (0.1 g, 0.592 mmol) was added next and after following the work up procedure mentioned above afforded 164 as white colored crystalline solid (0.079 g, 46%). TLC $R_f = 0.77$ (Hex:EtOAc; 1:1); mp, 94-95.6 °C; $^1$H NMR, DMSO-$d_6$ (400 MHz): 0.13-1.15 (d, 6 H, 2 CH$_3$), 2.33 (s, 3 H, 2-CH$_3$), 3.27 (s, 3 H, NCH$_3$), 3.73 (s, 3 H, OCH$_3$), 4.28-4.29 (m, 1 H, CH(CH$_3$)$_2$), 6.21-6.23 (d, 1 H, exch., NH), 6.85-6.87 (d, 2 H, J = 9.0, Ar),
6.95-6.98 (d, 2 H, J = 9.04, Ar). Anal. Calcd. for C\textsubscript{16}H\textsubscript{21}ClN\textsubscript{4}O: C, 59.90; H, 6.60; N, 17.46; Cl, 11.05. Found C, 60.17; H, 6.72; N, 17.51; Cl, 10.80.

\textit{N}^4\text{-Butyl-5-chloro-N}^6\text{(4-methoxyphenyl)-N}^6\text{,2-dimethylpyrimidine-4,6-diamine (165)}

Using the general procedure described above, compound 154 (0.150 g, 0.538 mmol) was dissolved in DMF (10 mL), to which sodium hydride (0.026 g, 1.08 mmol) was added at 0 °C. 1-Iodobutane (0.109 g, 0.592 mmol) was added next and after following the work up procedure mentioned above afforded 165 as a semisolid. HCl salt was prepared by bubbling HCl gas through the solution of compound in ether to afford 165.HCl as light pink colored crystalline solid (0.042 g, 21%). TLC \( R_f = 0.97 \) (CH\textsubscript{3}OH: CHCl\textsubscript{3}; 1:10); mp, 144.8-147.1 °C; \(^1\text{H} \text{NMR, DMSO-}d_6 (400 MHz): 0.87-0.91 \) (t, 3 H, (CH\textsubscript{2})\textsubscript{3}CH\textsubscript{3}), 1.25-1.34 (m, 2 H, CH\textsubscript{2}CH\textsubscript{2}CH\textsubscript{2}CH\textsubscript{3}), 1.48-1.55 (m, 2 H, CH\textsubscript{2}CH\textsubscript{2}CH\textsubscript{2}CH\textsubscript{3}), 2.53 (s, 3 H, 2-CH\textsubscript{3}), 3.43 (s, 3 H, NCH\textsubscript{3}), 3.46-3.48 (m, 2 H, NH-CH\textsubscript{2}), 3.75 (s, 3 H, OCH\textsubscript{3}), 6.91-6.93 (d, 2 H, J = 8.98, Ar), 7.12-7.14 (d, 2 H, J = 8.91, Ar), 7.91 (s, 1 H, exch., NH). Anal. Calcd. for C\textsubscript{17}H\textsubscript{23}ClN\textsubscript{4}O. 0.97 HCl: C, 55.16; H, 6.52; N, 15.13; Cl, 18.86. Found C, 55.27; H, 6.66; N, 14.97; Cl, 18.91.

\textit{5-Chloro-N}^4\text{-isobutyl-N}^6\text{(4-methoxyphenyl)-N}^6\text{,2-dimethylpyrimidine-4,6-diamine (166)}

Using the general procedure described above, compound 154 (0.150 g, 0.538 mmol) was dissolved in DMF (10 mL), to which sodium hydride (0.026 g, 1.08 mmol) was added at 0 °C. Isobutanol (0.109 g, 0.592 mmol) was added next and after following the work up
procedure mentioned above afforded 166 as a semisolid. HCl salt was prepared by bubbling HCl gas through the solution of compound in ether to afford 166.HCl as yellow colored crystalline solid (0.026 g, 13%). TLC $R_f = 0.95$ (CH$_3$OH: CHCl$_3$; 1:10); mp, 143.1-145.9 °C; $^1$H NMR, DMSO-$d_6$ (400 MHz): 0.85-0.87 (d, 6 H, CH(CH$_3$)$_2$), 1.85-1.95 (m, 1 H, CH(CH$_3$)$_2$), 2.52 (s, 3 H, 2-CH$_3$), 3.29-3.33 (t, 2 H, NH-CH$_2$), 3.43 (s, 3 H, NCH$_3$), 3.76 (s, 3 H, OCH$_3$), 6.91-6.94 (d, 2 H, J = 8.90, Ar), 7.13-7.15 (d, 2 H, J = 8.77, Ar), 7.84 (s, 1 H, exch., NH). Anal. Calcd. for C$_{17}$H$_{23}$ClN$_4$O. 0.96HCl. 0.40H$_2$O: C, 54.13; H, 6.62; N, 14.85; Cl, 18.46. Found C, 54.14; H, 6.31; N, 14.77; Cl, 18.44.

$N^4$-Benzyl-5-chloro-$N^6$-(4-methoxyphenyl)-$N^6$-2-dimethylpyrimidine-4,6-diamine (167)

Using the general procedure described above, compound 154 (0.300 g, 1.08 mmol) was dissolved in DMF (20 mL), to which sodium hydride (0.052 g, 2.15 mmol) was added at 0 °C. Benzyl bromide (0.203 g, 1.18 mmol) was added next and after following the work up procedure mentioned above afforded 167 as off-white colored crystalline solid (0.115 g, 29%). TLC $R_f = 0.95$ (CH$_3$OH: CHCl$_3$; 1:10); mp, 95.3-96.1 °C; $^1$H NMR, DMSO-$d_6$ (400 MHz): 2.31 (s, 3 H, 2-CH$_3$), 3.29 (s, 3 H, NCH$_3$), 3.74 (s, 3 H, OCH$_3$), 4.57-4.58 (d, 2 H, CH$_2$C$_6$H$_5$), 6.86-6.88 (d, 2 H, J = 9.00, Ar), 6.98-7.00 (d, 2 H, J = 8.97, Ar), 7.20-7.23 (m, 1 H, exch., NH), 7.28-7.33 (m, 5 H, Ar). Anal. Calcd. for C$_{20}$H$_{21}$ClN$_4$O: C, 65.12; H, 5.74; N, 15.19; Cl, 9.61. Found C, 65.12; H, 5.68; N, 15.13; Cl, 9.47.
$N^4, N^4$-Dibenzyl-$N^6$-(4-methoxyphenyl)-$N^6, 2$-dimethylpyrimidine-$4, 6$-diamine (167a)

Compound 167a was isolated as a white semisolid. TLC $R_f = 0.97$ (CH$_3$OH: CHCl$_3$; 1:10);
$^1$H NMR, DMSO-$d_6$ (400 MHz): 2.35 (s, 3 H, 2-CH$_3$), 3.32 (s, 3 H, NCH$_3$), 3.71 (s, 3 H, OCH$_3$), 4.65 (s, 4 H, CH$_2$C$_6$H$_5$), 6.75-6.77 (dd, 2 H, J = 8.95, J = 1.66, Ar), 6.80-6.83 (dd, 2 H, J = 8.71, J = 1.62, Ar), 7.20-7.22 (d, 4 H, Ar), 7.25-7.27 (d, 2 H, Ar), 7.30-7.34 (m, 4 H, Ar). The compound was carried on directly without further characterization.

5-Chloro-$N^4$-ethyl-$N^6$-(5-methoxynaphthalen-2-yl)-$N^6, 2$-dimethylpyrimidine-$4, 6$-diamine (168)

Using the general procedure described above, compound 160 (0.130 g, 0.395 mmol) was dissolved in DMF (10 mL), to which sodium hydride (0.014 g, 0.593 mmol) was added at 0 °C. Iodoethane (0.068 g, 0.435 mmol) was added next and after following the work up procedure mentioned above afforded 168 as pale yellow colored solid (0.040 g, 28%). TLC $R_f = 0.92$ (CH$_3$OH: CHCl$_3$; 1:10); $^1$H NMR, DMSO-$d_6$ (400 MHz): 1.10-1.14 (t, 3 H, CH$_2$CH$_3$), 2.37 (s, 3 H, 2-CH$_3$), 3.39-3.43 (m, 5 H, CH$_2$CH$_3$, NCH$_3$), 3.95 (s, 3 H, OCH$_3$), 6.84-6.85 (m, 1 H, Ar), 6.98-7.00 (t, 1 H, exch., NH), 7.12-7.15 (d, 1 H, Ar), 7.34-7.36 (m, 3 H, Ar), 8.00-8.02 (d, 1 H, Ar). Anal. Calcd. for C$_{19}$H$_{21}$ClN$_4$: C, 63.85; H, 6.04; N, 15.39; Cl, 9.67. Found C, 63.85; H, 6.04; N, 15.39; Cl, 9.67.
5-Chloro-N⁴-(5-methoxynaphthalen-2-yl)-N⁴,2-dimethyl-N⁶-propylpyrimidine-4,6-diamine (169)

Using the general procedure described above, compound 160 (0.130 g, 0.395 mmol) was dissolved in DMF (10 mL), to which sodium hydride (0.019 g, 0.791 mmol) was added at 0 °C. Iodopropane (0.074 g, 0.435 mmol) was added next and after following the work up procedure mentioned above afforded 169 as a semisolid. HCl salt was prepared by bubbling HCl gas through the solution of compound in ether to afford 169.HCl as light pink colored solid (0.083 g, 51%). TLC Rf = 0.95 (CH₃OH: CHCl₃; 1:10); mp, 180.6-182.8 °C; ¹H NMR, DMSO-d₆ (400 MHz): 0.86-0.90 (t, 3 H, CH₂CH₂CH₃), 1.53-1.62 (m, 2 H, CH₂CH₂CH₃), 2.48 (s, 3 H, 2-CH₃), 3.40-3.44 (q, 2 H, CH₂CH₂CH₃), 3.50 (s, 3 H, NCH₃), 3.96 (s, 3 H, OCH₃), 6.87-6.89 (dd, 1 H, Ar), 7.22-7.25 (dd, 1 H, Ar), 7.36-7.40 (m, 2 H, Ar), 7.47-7.48 (d, 1 H, Ar), 7.83 (s, br, 1 H, exch., NH), 8.04-8.06 (d, 1 H, Ar). Anal. Calcd. for C₂₀H₂₅ClN₄O . 0.97HCl . 0.97H₂O: C, 56.66; H, 6.16; N, 13.22; Cl, 16.51. Found C, 56.71; H, 6.08; N, 12.99; Cl, 16.50.

N-(5-Chloro-6-(((4-methoxyphenyl)(methyl)amino)-2-methylpyrimidin-4-yl)acetamide (170)

Compound 154 (0.120 g, 0.430 mmol) was dissolved in acetic acid (5 mL) and acetic anhydride (2 mL) was added to the above mixture and heated to 90 °C for 12 h. After the completion of reaction (monitored by TLC), the mixture was cooled to room temperature and water (5 mL) was added over 10 min. The mixture was then filtered, and the filter cake was washed with water, dried over P₂O₅ to yield 170 as white colored solid (0.040 g, 30%). TLC Rf = 0.55 (MeOH:CHCl₃; 1:10); mp, 143.0-144.2 °C; ¹H NMR, DMSO-d₆ (400
MHz): 2.04 (s, 3 H, COCH$_3$), 2.45 (s, 3 H, 2-CH$_3$), 3.38 (s, 3 H, NCH$_3$), 3.76 (s, 3 H, OCH$_3$), 6.89-6.92 (d, 2 H, J = 8.75, Ar), 7.05-7.07 (d, 2 H, J = 8.73, Ar), 9.98 (s, 1 H, exch., NH). Anal. Calcd. for C$_{15}$H$_{17}$ClN$_4$O$_2$: C, 56.16; H, 5.34; N, 17.47; Cl, 11.05. Found C, 56.19; H, 5.42; N, 17.20; Cl, 11.21.

4-Methoxyquinazoline (276)

Quinazolin-4(3H)-one 274b (1.0 g, 6.84 mmol) was dissolved in phosphorus oxychloride (15 mL) and set for reflux for 4 h. The solvent was evaporated in vacuo, and the residue was adjusted to pH 8 with 7N ammonia solution in MeOH. The reaction mixture was extracted with ethyl acetate (20 mL $\times$ 3), the fractions were pooled, dried over Na$_2$SO$_4$ and the solvent was evaporated in vacuo to afford 276 as yellow colored liquid.$^{267}$ TLC $R_f = 0.57$ (MeOH:CHCl$_3$; 1:5); $^1$H NMR, DMSO-$d_6$ (400 MHz): 4.24 (s, 3 H, OCH$_3$), 7.80-7.84 (m, 1 H, Ar), 8.06-8.10 (m, 2 H, Ar), 8.24-8.26 (m, 1 H, Ar), 9.12 (s, 1 H, Ar). The compound was not characterized further.

4H-[3,4'-Biquinazolin]-4-one (277)

Quinazolin-4(3H)-one 274b (1.0 g, 6.84 mmol) was dissolved in toluene (25 mL) to which phosphorus oxychloride (1 mL) and pyridine (0.8 mL) were added and the reaction mixture was refluxed for 1 h. After cooling, the reaction mixture to room temperature and 20 mL of cold water was added. The reaction mixture was extracted with ethyl acetate (20 mL $\times$ 3), the fractions were pooled, dried over Na$_2$SO$_4$ and the solvent was evaporated in vacuo to afford 277 as off-white colored solid (0.200 g, 11%). TLC $R_f = 0.77$ (MeOH:CHCl$_3$; 1:5); mp, 210.7-212.0 °C (lit.$^{268}$ 229-230 °C); $^1$H NMR, DMSO-$d_6$ (400 MHz): 7.68-7.82
(m, 2 H, Ar), 7.85-7.87 (d, 1 H, Ar), 7.98-8.05 (m, 2 H, Ar), 8.14-8.26 (m, 3 H, Ar), 8.63 (s, 1 H, Ar), 9.46 (s, 1 H, Ar). The compound was not characterized further.

4-Chloroquinazoline (273b)

To a 250 mL round-bottomed flask were added quinazolin-4(3H)-one 274b (2.0 g, 13.68 mmol), trimethylamine (3 mL), and toluene (30 mL). The mixture was cooled to 0 °C to which phosphorus oxychloride (2 mL) was added and stirred at room temperature for 1 h. The reaction was heated to 95 °C for 3 h. After monitoring the reaction on TLC, the reaction was cooled to room temperature and diluted with 30 mL of ethyl acetate. The solution was then washed with 10 mL of ice cold water, 10 mL of saturated NaHCO₃, 10 mL of water, 1N HCl (5 mL), 10 mL of water, 10 mL of saturated NaHCO₃ and 10 mL of saturated NaCl. The organic layer was dried over Na₂SO₄, filtered and concentrated to obtain 273b as yellow colored solid (1.79 g, 80%). TLC $R_f = 0.85$ (MeOH:CHCl₃; 1:5); mp, 144.2-146.1 °C (lit.239 145-147.5 °C); $^1$H NMR, DMSO-$d_6$ (400 MHz): 7.59-7.63 (dt, 1 H, $J_1 = 1.09$, $J_2 = 7.23$, $J_3 = 8.01$, Ar), 7.76-7.78 (d, 1 H, $J = 8.24$, Ar), 7.89-7.92 (dt, 1 H, $J_1 = 1.47$, $J_2 = 7.24$, $J_3 = 8.29$, Ar), 8.15-8.17 (d, 1 H, $J = 7.03$, Ar), 8.58 (s, 1 H, Ar).

4-Chloro-2-methylquinazoline (273a)

Similar to the method described for compound 273b, 273a was synthesized from 2-methylquinazolin-4(3H)-one 274a (1.0 g, 6.24 mmol) as yellow colored solid (0.600 g, 54%). TLC $R_f = 0.8$ (MeOH:CHCl₃; 1:5); mp, 83.9-85.3 °C (lit.269 85-86 °C); $^1$H NMR, DMSO-$d_6$ (400 MHz): 2.58 (s, 3 H, CH₃), 7.59-7.63 (t, 1 H, $J_1 = 7.58$, $J_2 = 7.58$, Ar), 7.78-
7.80 (d, 1 H, J = 7.98, Ar), 7.90-7.95 (t, 1 H, J<sub>1</sub> = 7.67, J<sub>2</sub> = 7.67, Ar), 8.13-8.15 (d, 1 H, J = 7.65, Ar).

**General method for the synthesis of compounds 172-180 and 182**

To a 100 mL round-bottomed flask substituted-4-chloroquinazolines (1 equivalent), appropriate anilines (1.2 equivalents) and iso-propanol (10 mL) were added and stirred at room temperature in isopropanol for 12 h. The compounds precipitated as HCl salts, which were filtered and dried in vacuo over P<sub>2</sub>O<sub>5</sub> to afford the desired compounds.

**N-(4-Methoxyphenyl)-N,N-dimethylquinazolin-4-amine (172)**

Using the general method described above, 273a (0.150 g, 0.840 mmol) and 4-methoxy-N-methylaniline 259 (0.138 g, 1.01 mmol) were reacted for 12 h. At the end of the reaction, solvent was evaporated, silica gel and methanol were added and evaporated in vacuo to give a dried plug. The reaction mixture was purified using flash chromatography using chloroform/methanol to afford 172 as brown colored solid (0.136 g, 58%). TLC <i>R_f</i> = 0.8 (MeOH:CHCl<sub>3</sub>; 1:5); mp, 99.1-101.2 °C; <sup>1</sup>H NMR, DMSO-<i>d</i><sub>6</sub> (400 MHz): 2.66 (s, 3 H, 2-CH<sub>3</sub>), 3.60 (s, 3 H, NCH<sub>3</sub>), 3.81 (s, 3 H, OCH<sub>3</sub>), 6.88-6.90 (d, 1 H, J = 8.35, Ar), 7.04-7.06 (d, 2 H, J = 8.89, Ar), 7.14-7.18 (t, 1 H, J<sub>1</sub> = 7.49, J<sub>2</sub> = 7.49, Ar), 7.30-7.32 (d, 2 H, J = 8.78, Ar), 7.68-7.75 (m, 2 H, Ar). Anal. Calcd. for C<sub>17</sub>H<sub>17</sub>N<sub>3</sub>O .0.52 HCl: C, 68.46; H, 5.92; N, 14.08. Found C, 68.50; H, 6.24; N, 13.80; Cl, 4.54.

**4-(6-Methoxy-3,4-dihydroquinolin-1(2H)-yl)-2-methylquinazoline (173)**

160
Using the general method described above, 273a (0.150 g, 0.840 mmol) and 6-methoxy-1,2,3,4-tetrahydroquinoline 271 (0.164 g, 1.01 mmol) were reacted to afford 173 as a yellow colored precipitate (0.188 g, 65%). TLC RF = 0.85 (MeOH:CHCl₃; 1:5); mp, 255.8-257.9 °C; ¹H NMR, DMSO-d₆ (400 MHz): 2.1 (quin, 2 H, CH₂), 2.75 (s, 3 H, 2-CH₃), 2.87-2.90 (t, 2 H, CH₂), 3.79 (s, 3 H, OCH₃), 4.20 (t, 2 H, NCH₂), 6.68-6.71 (dd, 1 H, J₁ = 2.88, J₂ = 8.85, Ar), 7.02-7.03 (d, 1 H, J = 2.81, Ar), 7.05-7.08 (d, 1 H, J = 8.87, Ar), 7.26-7.28 (d, 1 H, J = 8.53, Ar), 7.36-7.40 (dt, J₁ = 1.38, J₂ = 6.83, J₃ = 8.28, Ar), 7.88-7.96 (m, 2 H, Ar), 15.28 (s, 1 H, exch., NH). Anal. Calcd. for C₁₉H₁₉N₃O. 1.07HCl: C, 66.26; H, 5.87; N, 12.20. Found C, 66.28; H, 5.75; N, 12.11; Cl, 10.49.

**N₂-Dimethyl-N-(4-(methylthio)phenyl)quinazolin-4-amine (174)**

Using the general method described above, 273a (0.135 g, 0.756 mmol) and N-methyl-4-(methylthio)aniline 263 (0.135 g, 0.907 mmol) were reacted to afford 174 as white colored precipitate (0.175 g, 70%). TLC RF = 0.9 (MeOH:CHCl₃; 1:5); mp, 235.2-236.4 °C; ¹H NMR, DMSO-d₆ (400 MHz): 2.55 (s, 3 H, 2-CH₃), 2.77 (s, 3 H, SCH₃), 3.74 (s, 3 H, NCH₃), 6.87-6.89 (d, 1 H, J = 8.39, Ar), 7.30-7.34 (dt, 1 H, J₁ = 1.33, J₂ = 7.0, J₃ = 8.48, Ar), 7.43-7.50 (m, 4 H, Ar), 7.85-7.89 (dt, 1 H, J₁ = 1.14, J₂ = 7.05, J₃ = 8.34, Ar), 7.93-7.95 (d, 1 H, J = 8.32, Ar), 15.22 (s, 1 H, exch., NH). Anal. Calcd. for C₁₇H₁₇N₃S. 1.34 HCl: C, 59.28; H, 5.37; N, 12.20; S, 9.31. Found C, 59.37; H, 5.62; N, 11.93; S, 9.03; Cl, 10.55.
**N-(5-Methoxynaphthalen-2-yl)-N,2-dimethylquinazolin-4-amine (175)**

Using the general method described above, 273a (0.135 g, 0.840 mmol) and 5-methoxy-N-methylnaphthalen-2-amine 269 (0.156 g, 0.831 mmol) were reacted for 12 h. At the end of reaction, solvent was evaporated, silica gel and methanol were added and evaporated in vacuo to give a dried plug. The reaction mixture was purified using flash chromatography using chloroform/methanol to afford 175 as a yellow colored solid (0.170 g, 68%). TLC $R_f = 0.9$ (MeOH:CHCl$_3$; 1:10); mp, 203.0-204.4 °C; $^1$H NMR, DMSO-$d_6$ (400 MHz): 2.70 (s, 3 H, 2-CH$_3$), 3.72 (s, 3 H, NCH$_3$), 3.99 (s, 3 H, OCH$_3$), 6.91-6.93 (d, 1 H, J = 8.26, Ar), 7.00-7.02 (d, 1 H, J = 7.46, Ar), 7.05-7.09 (t, 1 H, J$_1$ = 7.70, J$_2$ = 7.70, Ar), 7.40-7.49 (m, 3 H, Ar), 7.66-7.69 (t, 1 H, J$_1$ = 7.59, J$_2$ = 7.59, Ar), 7.74-7.76 (d, 1 H, J = 8.33, Ar), 7.84 (s, 1 H, Ar), 8.21-8.23 (d, 1 H, J = 8.99, Ar). Anal. Calcd. for C$_{21}$H$_{19}$N$_3$O. 0.41 HCl: C, 73.22; H, 5.68; N, 12.20. Found C, 73.19; H, 5.82; N, 12.35; Cl, 2.22.

**N-(4-Methoxyphenyl)-N-methylquinazolin-4-amine (176)**

Using the general method described above, 273b (0.150 g, 0.911 mmol) and N-methyl-4-methoxyaniline 259 (0.150 g, 1.09 mmol) were reacted to afford 176 as a pale yellow colored precipitate (0.210 g, 76%). TLC $R_f = 0.8$ (MeOH:CHCl$_3$; 1:5); mp, 244.3-245.3 °C; $^1$H NMR, DMSO-$d_6$ (400 MHz): 3.75 (s, 3 H, NCH$_3$), 3.84 (s, 3 H, OCH$_3$), 6.84-6.86 (d, 1 H, J = 8.43, Ar), 7.12-7.16 (d, 2 H, J = 8.97, Ar), 7.32-7.36 (dt, 1 H, J$_1$ = 1.12, J$_2$ = 6.93, J$_3$ = 8.45, Ar), 7.46-7.50 (d, 2 H, J = 8.96, Ar), 7.87-7.91 (dt, 1 H, J$_1$ = 1.05, J$_2$ = 6.94, J$_3$ = 8.25, Ar), 7.95-7.97 (d, 1 H, J = 8.15, Ar), 9.06 (s, 1 H, Ar). Anal. Calcd. for C$_{16}$H$_{15}$N$_3$O. 1.10HCl: C, 62.89; H, 5.31; N, 13.75. Found C, 62.89; H, 5.33; N, 13.75; Cl, 11.43.
4-(6-Methoxy-3,4-dihydroquinolin-1(2H)-yl)quinazoline (177)

Using the general method described above, 273b (0.150 g, 0.911 mmol) and 6-methoxy-1,2,3,4-tetrahydroquinoline 271 (0.179 g, 1.09 mmol) were reacted to afford 177 as a yellow colored precipitate (0.211 g, 70%). TLC $R_f = 0.8$ (MeOH:CHCl$_3$; 1:5); mp, 234.2-235.3 °C; $^1$H NMR, DMSO-$d_6$ (400 MHz): 2.05-2.13 (quint, 2 H, CH$_2$), 2.87-2.91 (t, 2 H, CH$_2$), 3.79 (s, 3 H, OCH$_3$), 4.20-4.21 (t, 2 H, NCH$_3$), 6.67-6.70 (dd, 1 H, J$_1 = 2.91$, J$_2 = 8.85$, Ar), 7.02-7.03 (d, 1 H, J = 2.82, Ar), 7.08-7.10 (d, 1 H J = 8.85, Ar), 7.33-7.35 (d, 1 H, J = 8.50, Ar), 7.42-7.46 (dt, 1 H, J$_1 = 1.55$, J$_2 = 6.67$, J$_3 = 8.32$, Ar), 7.91-7.98 (m, 2 H, Ar), 9.04 (s, 1 H, Ar). Anal. Calcd. for C$_{18}$H$_{17}$N$_3$O. 1.22HCl: C, 64.38; H, 5.47; N, 12.51. Found C, 64.39; H, 5.47; N, 12.45; Cl, 10.41.

N-Methyl-N-(4-(methylthio)phenyl)quinazolin-4-amine (178)

Using the general method described above, 273b (0.150 g, 0.911 mmol) and N-methyl-4-(methylthio)aniline 263 (0.168 g, 1.09 mmol) were reacted to afford 178 as a pale yellow colored precipitate (0.209 g, 72%). TLC $R_f = 0.8$ (MeOH:CHCl$_3$; 1:5); mp, 233.4-235.9 °C; $^1$H NMR, DMSO-$d_6$ (400 MHz): 2.54 (s, 3 H, SCH$_3$), 3.75 (s, 3 H, NCH$_3$), 6.94-6.96 (d, 1 H, J = 8.74, Ar), 7.35-7.39 (t, 1 H, J$_1 = 7.69$, J$_2 = 7.69$, Ar), 7.43-7.45 (d, 2 H, J = 8.70, Ar), 7.48-7.50 (d, 2 H, J = 8.68, Ar), 7.88-7.92 (t, 1 H, J$_1 = 7.55$, J$_2 = 7.55$, Ar), 7.95-7.97 (d, 1 H, J = 7.68, Ar), 9.08 (s, 1 H, Ar). Anal. Calcd. for C$_{16}$H$_{15}$N$_3$S. 1.13HCl: C, 59.59; H, 5.04; N, 13.03; S, 9.94. Found C, 59.56; H, 5.13; N, 12.97; S, 10.22; Cl, 10.22.
2-Chloro-N-(4-methoxyphenyl)-N-methylquinazolin-4-amine (179)

Using the general method described above, 273c (0.500 g, 2.51 mmol) and 4-methoxy-N-methylaniline 259 (0.413 g, 3.01 mmol) were reacted for 12 h. At the end of reaction, solvent was evaporated, silica gel and methanol were added and evaporated in vacuo to give a dried plug. The reaction mixture was purified using flash chromatography using chloroform/methanol to afford 179 as buff colored solid (0.070 g, 9%). TLC $R_f = 0.95$ (MeOH:CHCl$_3$; 1:5); mp, 146.3-148.7 °C; $^1$H NMR, DMSO-$d_6$ (400 MHz): 3.52 (s, 3 H, NCH$_3$), 3.81 (s, 3 H, OCH$_3$), 6.86-6.88 (d, 1 H, Ar), 7.03-7.06 (d, 2 H, J = 8.96, Ar), 7.11-7.16 (m, 1 H, Ar), 7.32-7.35 (d, 2 H, J = 8.95, Ar), 7.66-7.67 (m, 2 H, Ar). Anal. Calcd. for C$_{16}$H$_{14}$ClN$_3$O: C, 64.11; H, 4.71; N, 14.02; Cl, 11.83. Found C, 64.08; H, 4.77; N, 13.93; Cl, 11.97.

2-Chloro-4-(6-methoxy-3,4-dihydroquinolin-1(2H)-yl)quinazoline (180)

Using the general method described above, 273c (0.300 g, 1.51 mmol) and 6-methoxy-1,2,3,4-tetrahydroquinoline 271 (0.295 g, 1.81 mmol) were reacted for 12 h. At the end of reaction, solvent was evaporated, silica gel and methanol were added and evaporated in vacuo to give a dried plug. The reaction mixture was purified using flash chromatography using chloroform/methanol to afford 180 as yellow colored solid (0.121 g, 25%). TLC $R_f = 0.97$ (MeOH:CHCl$_3$; 1:5); mp, 135.9-137.0 °C (lit.$^{241}$ 136-138 °C); $^1$H NMR, DMSO-$d_6$ (400 MHz): 2.13-2.18 (m, 2 H, CH$_2$), 2.88-2.91 (t, 2 H, CH$_2$), 3.85 (s, 3 H, OCH$_3$), 4.11-4.16 (m, 2 H, CH$_2$), 6.59-6.62 (dd, 1 H, J$_1$ = 2.89, J$_2$ = 8.81, 7'-Ar), 6.75-6.77 (d, 1 H, J = 8.85, 8'-Ar), 6.857-6.863 (d, 1 H, J = 2.86, 5'-Ar), 7.16-7.19 (m, 1 H, J$_1$ = 1.26, J$_2$ = 6.96, J$_3$ = 8.43, Ar), 7.31-7.33 (dd, 1 H, J$_1$ = 0.90, J$_2$ = 8.53, Ar), 7.68-7.71 (m, 1 H, J$_1$ = 1.36,
\[ J_2 = 6.97, J_3 = 8.39 \text{ (Ar)}, 8.05-8.07 \text{ (d, 1 H, Ar)} \].

Anal. Calcd. for C_{18}H_{16}ClN_3O .

0.24(CH_3)_2CHOH: C, 66.08; H, 5.31; N, 12.34; Cl, 10.41. Found C, 66.30; H, 5.19; N, 12.35; Cl, 10.17.

\[ N^2, N^4- \text{Dimethyl-} N^2, N^4- \text{bis(4-(methylthio)phenyl)quinazoline-2,4-diamine (279)} \]

Compound 273c (0.200 g, 1.00 mmol) and N-methyl-4-(methylthio)aniline 263 (0.185 g, 1.21 mmol) were stirred at room temperature for 12 h. At the end of reaction, solvent was evaporated, silica gel and methanol were added and evaporated in vacuo to give a dried plug. The reaction mixture was purified using flash chromatography using chloroform/methanol which afforded dimer 279 as a yellow colored semisolid (0.195 g, 45%). TLC \( R_f = 0.87 \) (Hex:EtOAc; 1:1); \(^1\)H NMR, DMSO-\(d_6\) (400 MHz): 2.48 (s, 3 H, SCH_3), 2.49 (s, 3 H, SCH_3), 3.29 (s, 3 H, NCH_3), 3.57 (s, 3 H, NCH_3), 6.76-6.81 (m, 1 H, Ar), 6.88-6.90 (dd, 1 H, J = 0.89, J = 8.43, Ar), 7.14-7.16 (d, 2 H, J = 8.68, Ar) 7.25-7.29 (m, 4 H, Ar), 7.37-7.45 (m, 4 H, Ar). The compound was carried on directly without further characterization.

\[ 6-((2-\text{Chloroquinazolin-4-yl)amino})naphthalen-1-ol (280) \]

Using the general method described above, 273c (0.200 g, 1.00 mmol) and 6-aminonaphthalen-1-ol 266 (0.144 g, 0.904 mmol) were reacted for 12 h. At the end of reaction, solvent was evaporated, silica gel and methanol were added and evaporated in vacuo to give a dried plug. The reaction mixture was purified using flash chromatography using chloroform/methanol to afford 280 as white colored solid (0.070 g, 22%). TLC \( R_f = 0.67 \) (MeOH:CHCl_3; 1:5); \(^1\)H NMR, DMSO-\(d_6\) (400 MHz): 6.83-6.87 (t, 1 H, Ar),
7.32-7.33 (d, 2 H, J = 4.55, Ar), 7.67-7.71 (dt, 1 H, J₁ = 1.13, J₂ = 6.96, J₃ = 8.09, Ar), 7.74-7.76 (dd, 1 H, J₁ = 0.79, J₂ = 8.31, Ar), 7.81-7.84 (dd, 1 H, J₁ = 2.11, J₂ = 9.07, Ar), 7.90-7.94 (dt, 1 H, J₁ = 1.13, J₂ = 7.08, J₃ = 8.35, Ar), 8.16-8.18 (d, 1 H, J = 9.02, Ar), 8.24-8.25 (d, 1 H, J = 2.03, Ar), 8.63-8.65 (dd, 1 H, J₁ = 1.35, J₂ = 8.66, Ar), 10.16 (s, 1 H, exch., NH), 10.40 (s, br, 1 H, exch., OH). The compound was used without further characterization.

2-Chloro-N-(5-methoxynaphthalen-2-yl)-N-methylquinazolin-4-amine (182)

Compound 280 (0.070 g, 0.218 mmol) was dissolved in anhydrous DMF (10 mL) in a three necked round-bottomed flask and was stirred at 0 °C under argon. Sodium hydride (0.016 g, 0.653 mmol) was added to the above mixture and stirred at 0 °C for additional 15 min followed by addition of methyl iodide (0.068 g, 0.479 mmol) while maintaining the reaction at room temperature for 4 h. Methanol was added at the end of the reaction, solvent was evaporated and the reaction mixture was then extracted with water and ethyl acetate (25 mL × 3) and dried over Na₂SO₄. Silica gel was added next to the combined fractions, the solvent was removed by evaporation to yield a dry plug and column was the run using hexanes/ethyl acetate. The fractions containing the desired product (TLC) were pooled and evaporated to obtain the desired compounds 182 as off-white colored solid (0.036 g, 47%).

TLC Rₛ = 0.82 (MeOH:CHCl₃; 1:5); mp, 171.9-173.8 °C; ¹H NMR, DMSO-d₆ (400 MHz):
3.64 (s, 3 H, NCH₃), 3.99 (s, 3 H, OCH₃), 6.86-6.88 (d, 1 H, J = 8.54, Ar), 7.00-7.06 (m, 2 H, Ar), 7.40-7.49 (m, 3 H, Ar), 7.63-7.71 (m, 2 H, Ar), 7.88-7.89 (d, 1 H, J = 2.02, Ar), 8.21-8.23 (d, 1 H, J = 8.92, Ar). Anal. Calcd. for C₂₀H₁₆ClN₃O . 0.11CH₃(CH₂)₄CH₃: C, 69.07; H, 4.93; N, 11.68; Cl, 9.86. Found C, 68.84; H, 5.06; N, 11.69; Cl, 9.64.
Pyrido[2,3-\textit{d}]pyrimidin-4(3\textit{H})-one (274d)

To a 10-20 mL Biotage microwave vial were added 2-aminopicolinic acid 275 (2 g, 14.5 mmol) and formamide (3.26 g, 72.5 mmol) which were set up for microwave irradiation at 170 °C for 90 min to yield 274d as buff colored solid (1.48 g, 70%). TLC \( R_f = 0.43 \) (MeOH:CHCl\textsubscript{3}; 1:5); mp, >250 °C (lit.\textsuperscript{270} 255-257 °C); \(^1\text{H} NMR,\) DMSO-\textit{d}\textsubscript{6} (400 MHz): 7.54-7.57 (dd, 1 H, \( J_1 = 7.92, J_2 = 4.57, 6\)-Ar), 8.32 (s, 1 H, 2-H), 8.50-8.53 (dd, 1 H, \( J_1 = 7.85, J_2 = 2.02, 5\)-Ar), 8.95-8.97 (dd, 1 H, \( J_1 = 1.98, J_2 = 4.54, 7\)-Ar), 12.57 (s, br, exch., NH).

4-Chloropyrido[2,3-\textit{d}]pyrimidine (273d)

To a 250 mL round-bottomed flask were added quinazolin-4(3\textit{H})-one 273d (2.5 g, 16.99 mmol), trimethylamine (3.5 mL), and toluene (30 mL). The mixture was cooled to 0 °C to which phosphorus oxychloride (4.2 mL) was added and stirred at room temperature for 1 h. The reaction was heated to 95 °C for 3 h. After monitoring the reaction on TLC, the reaction was cooled. The work up procedure was similar to that provided for compound 273b above. Compound 273d was obtained as brown colored solid (0.455 g, 20%). TLC \( R_f = 0.48 \) (MeOH:CHCl\textsubscript{3}; 1:5); mp, 250.0-251.1 °C (lit.\textsuperscript{230} 240 °C). \(^1\text{H} NMR,\) DMSO-\textit{d}\textsubscript{6} (400 MHz): 7.65-7.68 (q, 1 H, \( J_1 = 7.90, J_2 = 4.75, 6\)-Ar), 8.54 (s, 1 H, 2-H), 8.62-8.64 (dd, 1 H, \( J_1 = 7.90, J_2 = 1.90, 5\)-Ar), 9.00-9.01 (dd, 1 H, \( J_1 = 1.90, J_2 = 4.73, 7\)-Ar).

\textit{N}-(4-methoxyphenyl)-\textit{N}-methylpyrido[2,3-\textit{d}]pyrimidin-4-amine (183)

Compound 273d (0.100 g, 0.604 mmol) and 4-methoxy-\textit{N}-methylaniline 259 (0.099 g, 0.725 mmol) were stirred at room temperature for 12 h. At the end of reaction, solvent was
evaporated, silica gel and methanol were added and evaporated in vacuo to give a dried plug. The reaction mixture was purified using flash chromatography using chloroform/methanol which afforded 183 as yellow colored solid (0.047 g, 29%). TLC $R_f$ = 0.67 (MeOH:CHCl$_3$; 1:5); mp, 105.4-106.4 °C; $^1$H NMR, DMSO-$d_6$ (400 MHz): 3.55 (s, 3 H, NCH$_3$), 3.80 (s, 3 H, OCH$_3$), 7.02-7.05 (d, 2 H, J = 8.88, Ar), 7.16-7.19 (q, 1 H, J$_1$ = 4.25, J$_2$ = 8.51, 6-Ar), 7.27-7.30 (m, 1 H, 5-Ar), 7.29-7.31 (d, 2 H, J = 8.85, Ar), 8.82 (s, 1 H, Ar), 8.86-8.88 (dd, 1 H, J$_1$ = 1.91, J$_2$ = 4.23, 7-Ar). Anal. Calcd. for C$_{15}$H$_{14}$N$_4$O. 0.57 H$_2$O: C, 65.16; H, 5.52; N, 20.26. Found C, 65.17; H, 5.58; N, 20.24.

2-Amino-6-chlorobenzaldehyde (282a)

To a solution of (2-amino-6-chlorophenyl)methanol 283 (0.4 g, 2.54 mmol) in CH$_2$Cl$_2$ (10 mL) was added MnO$_2$ (0.883 g, 10.15 mmol) and stirred at room temperature for 24 h. After the completion of reaction silica gel was added and the solvent was dried in vacuo to give a dry plug. The reaction mixture was purified using flash chromatography with chloroform/methanol to yield pure 282a as yellow needles (0.326 g, 83%). TLC $R_f$ = 0.78 (Hex:EtOAc; 1:1); mp, 92.4-95.0 °C (lit.$^{244}$ 96-97 °C); $^1$H NMR, DMSO-$d_6$ (400 MHz): 6.62-6.64 (d, 1 H, J = 7.58, Ar), 6.75-6.77 (dd, 1 H, J$_1$ = 0.51, J$_2$ = 8.75, Ar), 7.24-7.28 (t, 1 H, J = 8.33, Ar), 7.68 (s, br, 2 H, exch., NH$_2$), 10.32 (s, 1 H, CHO).

5-Chloro-3-pentylquinolin-2-amine (281a)

To a solution of compound 282a (0.200 g, 1.29 mmol) in DMSO (5 mL) were added heptanenitrile (0.286 g, 2.57 mmol) and potassium tert-butoxide (0.289 g, 2.57 mmol). The reaction mixture was stirred at 60 °C for 3 h and was subsequently diluted with water and
extracted with ethyl acetate (50 × 3 mL) and dried over Na₂SO₄. The combined organic layer was pooled, silica gel was added and concentrated under reduced pressure to give a dry plug. The crude mixture was purified by flash chromatography (hexanes/ethyl acetate) to yield pure 281a as an off-white solid (0.152 g, 48%). TLC $R_f = 0.45$ (Hex:EtOAc; 1:1); mp, 104.2-106.0 °C; $^1$H NMR, DMSO-$d_6$ (400 MHz): 0.87-0.90 (t, 3 H, CH$_3$), 1.34-1.35 (m, 4 H, CH$_2$), 1.58-1.65 (quin, 2 H, CH$_2$), 2.60-2.64 (t, 2 H, CH$_2$), 6.58 (s, 2 H, exch., NH$_2$), 7.25-7.27 (dd, 1 H, J$_1$ = 6.89, J$_2$ = 1.69, 7-Ar), 7.38-7.45 (m, 2 H, Ar), 7.88 (s, 1 H, 4-Ar). The compound was used directly without further characterization.

$N$-(5-Chloro-3-pentylquinolin-2-yl)pivalamide (284)

Compound 281a (0.200 g, 0.804 mmol) was dissolved in ethyl acetate (10 mL) to which pivalic anhydride (3 mL) was added and heated at 60 °C for 12 h. After the reaction completion, silica gel was added and the solvent was evaporated in vacuo to afford a dry plug. Flash chromatography of the crude mixture in hexanes/ethyl acetate yielded pure 284 as buff colored solid (0.150 g, 58%). TLC $R_f = 0.74$ (Hex:EtOAc; 1:1); mp, 105.4-106.4 °C; $^1$H NMR, DMSO-$d_6$ (400 MHz): 0.85-0.88 (t, 3 H, CH$_3$), 1.28 (m, 13 H, 2 CH$_2$, (CH$_3$)$_3$), 1.57-1.64 (quin, 2 H, CH$_2$), 2.69-2.73 (t, 2 H, CH$_2$), 7.68-7.72 (t, 1 H, 7-Ar), 7.74-7.76 (dd, 1 H, J$_1$ = 1.30, J$_2$ = 7.55, Ar), 7.93-7.95 (dd, 1 H, J$_1$ = 8.18, J$_2$ = 0.71, Ar), 8.35 (s, 1 H, 4-Ar), 9.96 (s, 1 H, exch., NH). Anal. Calcd. for C$_{19}$H$_{25}$ClN$_2$O: C, 68.56; H, 7.57; N, 8.41; Cl, 10.65. Found C, 68.48; H, 7.47; N, 8.22; Cl, 10.36.
5-Bromo-3-pentylquinolin-2-amine (281b)

To a solution of 2-amino-6-bromobenzaldehyde 282a (0.500 g, 2.5 mmol) in DMSO (10 mL) were added heptanenitrile (0.556 g, 5 mmol) and potassium tert-butoxide (0.561 g, 5 mmol). The reaction mixture was stirred at 60 °C for 3 h and was subsequently diluted with water and extracted with ethyl acetate (50 × 3 mL) and dried over Na₂SO₄. The combined organic layer was pooled, silica gel was added and concentrated under reduced pressure to give a dry plug. The crude mixture was purified by flash chromatography (hexanes/ethyl acetate) to yield pure 281b as light brown colored solid (0.217 g, 30%). TLC $R_f = 0.45$ (Hex:EtOAc; 1:1); mp, 108.8-111.1 °C; $^1$H NMR, DMSO-$d_6$ (400 MHz): 0.88-0.91 (t, 3 H, CH₃), 1.35-1.37 (m, 4 H, CH₂), 1.59-1.66 (quin, 2 H, CH₂), 2.60-2.64 (t, 2 H, CH₂), 6.58 (s, 2 H, exch., NH₂), 7.32-7.36 (t, 1 H, Ar), 7.43-7.47 (m, 2 H, Ar), 7.83 (s, 1 H, 4-Ar). $^1$H NMR agreed well with the literature reported values.

N-(5-Bromo-3-pentylquinolin-2-yl)pivalamide (285)

Compound 281b (0.200 g, 0.682 mmol) was dissolved in ethyl acetate (10 mL) to which pivalic anhydride (3 mL) was added and heated at 60 °C for 12 h. After the reaction completion, silica gel was added and the solvent was evaporated in vacuo to afford a dry plug. Flash chromatography of the crude mixture in hexanes/ethyl acetate yielded pure 285 as buff colored solid (0.202 g, 68%). TLC $R_f = 0.74$ (Hex:EtOAc; 1:1); mp, 109.5-112.1 °C; $^1$H NMR, DMSO-$d_6$ (400 MHz): 0.85-0.88 (t, 3 H, CH₃), 1.28-1.32 (m, 13 H, 2 CH₂, (CH₃)₃), 1.57-1.64 (quin, 2 H, CH₂), 2.69-2.73 (t, 2 H, CH₂), 7.61-7.65 (t, 1 H, 7-Ar), 7.91-7.93 (dd, 1 H, J₁ = 7.51, J₂ = 0.80, Ar), 7.96-7.98 (d, 1 H, J = 8.42, Ar), 8.29 (s, 1 H, 4-Ar), 9.97 (s, 1 H, exch., NH). The compound was used directly without further characterization.
General method for Pd-catalyzed cross coupling reactions

To a 2-5 mL Biotage microwave vial were added 5-haloquinoline (1 equivalent), aniline (1.2-1.4 equivalents), Pd-catalyst (1-10 mol%), ligand (1-10 mol%) and the base (1.5-3.2 equivalents) and the vial was sealed. Vacuum was applied to the vial and was back filled with argon. The procedure was repeated 2 more times. Suitable solvent (1-3 mL) was added and the reaction was set for microwave irradiation. At the end of the reaction, the solvent was evaporated and the reaction mixture was extracted with ethyl acetate (10 × 3 mL) and dried over Na₂SO₄. The fractions were combined to which silica gel was added and the solvent was evaporated to give a dry plug. Flash chromatography of the crude mixture in hexanes/ethyl acetate was carried out to afford pure compounds.

N⁵-(5-Bromo-3-pentylquinolin-2-yl)-3-pentylquinoline-2,5-diamine (287)

Using the general method specified above, 285 (0.020 g, 0.053 mmol), 4-methoxy-N-methylaniline 259 (0.013 g, 0.074 mmol), Pd₂(dba)₃ (0.001 g, 2 mol%), SPhos (0.008 g, 8 mol%) and sodium tert-butoxide (0.013 g, 0.132 mmol) were added to a microwave vial. 2 mL of THF was added and the reaction was carried out at 85 °C for 4 h. TLC Rₜ = 0.40 (Hex:EtOAc; 1:1); ¹H NMR, DMSO-d₆ (400 MHz): 0.87-0.90 (m, 6 H, CH₃), 1.34-1.38 (m, 8 H, CH₂), 1.60-1.69 (m, 4 H, CH₂), 2.57-2.64 (m, 4 H, CH₂), 6.48 (s, 1 H, exch., NH), 6.59 (s, 2 H, exch., NH₂), 7.30-7.51 (m, 6 H, Ar), 7.83 (s, 1 H, Ar), 7.94 (s, 1 H, Ar). The compound was used directly without further characterization.
**N-(5-((4-Methoxyphenyl)amino)-3-pentylquinolin-2-yl)pivalamide (288)**

Using the general method specified above, 285 (0.020 g, 0.053 mmol), p-anisidine 270 (0.009 g, 0.074 mmol), XPhos Pd G3 (0.009 g, 2 mol%), XPhos (0.005 g, 2 mol%) and sodium tert-butoxide (0.013 g, 0.132 mmol) were added to a microwave vial. A 2 mL volume of 1,4-dioxane was added and the reaction was carried out at 110 °C for 24 h. New spot of 288 appeared when the reaction mixture was monitored on TLC. TLC $R_f = 0.61$ (MeOH: CHCl$_3$; 1:1); $^1$H NMR, DMSO-$d_6$ (400 MHz): 0.87-0.90 (t, 3 H, CH$_3$), 1.12 (s, 9 H, (CH$_3$)$_3$), 1.33-1.37 (m, 4 H, CH$_2$), 1.58-1.66 (quin, 2 H, CH$_2$), 2.54-2.57 (t, 2 H, CH$_2$), 3.72 (s, 3 H, OCH$_3$), 6.20 (s, 1 H, exch., NH), 6.68-6.70 (d, 1 H, J = 7.68, 6-Ar), 6.87-6.89 (d, 2 H, J = 8.89, Ar), 6.94-6.96 (d, 1 H, J = 8.15, 8-Ar), 7.04-7.06 (d, 2 H, J = 8.89, Ar), 7.18-7.22 (t, 1 H, J$_1$ = J$_2$ = 8.00, 7-Ar), 7.81 (s, 1 H, exch., NH), 8.01 (s, 1 H, 4-Ar). The compound was used directly without further characterization.

**N$^5$-(4-Methoxyphenyl)-N$^5$-methyl-3-pentylquinoline-2,5-diamine (190)**

Using the general method specified above, 285 (0.150 g, 0.397 mmol), 4-methoxy-N-methylaniline 259 (0.076 g, 0.557 mmol), XPhos Pd G3 (0.034 g, 10 mol%), XPhos (0.019 g, 10 mol%) and cesium carbonate (0.388 g, 1.19 mmol) were added to a microwave vial. 2 mL of tert-butanol was added and the reaction was carried out at 100 °C for 24 h. Following the work up procedure mentioned above and purification afforded 286 as a yellow colored semisolid (0.095 g, 55%), TLC $R_f = 0.61$ (Hex:EtOAc; 1:1), which was used without further characterization. To a solution of 286 (0.062 g, 0.143 mmol) in methanol (10 mL), 1N NaOH was added (2 mL) and the reaction was stirred at reflux for 8 h. At the end of the reaction the solvent was evaporated in vacuo, the reaction mixture
was extracted with ethyl acetate (10 × 3 mL), dried over Na₂SO₄ and the fractions were pooled. After evaporating the solvent, the compound was dried over P₂O₅ to yield 190 as a brown colored powder (0.063 g, 83%). TLC \( R_f = 0.24 \) (Hex:EtOAc; 1:1); mp, 132.6-133.9 °C; \(^1\)H NMR, DMSO-\(d_6\) (400 MHz): 0.78-0.82 (t, 3 H, CH₃), 1.13-1.26 (m, 4 H, CH₂), 1.39-1.46 (quin, 2 H, CH₂), 2.42-2.46 (t, 2 H, CH₂), 3.26 (s, 3 H, NCH₃), 3.65 (s, 3 H, OCH₃), 6.31 (s, 2 H, exch., NH₂), 6.57-6.59 (d, 2 H, J = 9.0, Ar), 6.74-6.76 (d, 2 H, J = 9.03, Ar), 6.93-6.95 (d, 1 H, J = 7.32, Ar), 7.31-7.33 (d, 1 H, J = 8.28, Ar), 7.41-7.45 (t, 1 H, \( J_1 = J_2 = 7.89, \) Ar), 7.50 (s, 1 H, 4-Ar). Anal. Calcd. for C₂₂H₂₇N₃O: C, 75.61; H, 7.79; N, 12.02. Found C, 75.40; H, 7.96; N, 11.77.

\( N\)-(5-((4-Methoxyphenyl)(methyl)amino)-3-methylquinolin-2-yl)pival amide (290a)

To a solution of 2-amino-6-bromobenzaldehyde 282b (1.0 g, 5 mmol) in DMSO (20 mL) were added propionitrile 289a (0.551 g, 10 mmol) and potassium tert-butoxide (1.12 g, 10 mmol). The reaction mixture was stirred at 60 °C for 3 h and was subsequently diluted with water and extracted with ethyl acetate (50 × 3 mL) and dried over Na₂SO₄. The combined organic layer was pooled, silica gel was added and concentrated under reduced pressure to give a dry plug. The crude mixture was purified by flash chromatography (hexanes/ethyl acetate) to yield semi-solid intermediate which was pivaloyl protected by dissolving the intermediate in ethyl acetate (20 mL) to which pivalic anhydride (4 mL) was added and heated at 60 °C for 12 h. After the reaction completion, silica gel was added and the solvent was evaporated in vacuo to afford a dry plug. Flash chromatography of the crude mixture in hexanes/ethyl acetate yielded pure 290a as buff colored solid (0.788 g, 49%). TLC \( R_f = 0.80 \) (Hex:EtOAc; 1:1); mp, 103.7-105.5 °C; \(^1\)H NMR, DMSO-\(d_6\) (500 MHz): 1.29 (s, 9 H,
(CH$_3$)$_3$, 2.367-2.369 (d, 3 H, CH$_3$), 7.62-7.65 (dd, 1 H, $J_1 = 7.58$, $J_2 = 8.38$, Ar), 7.90-7.92 (dd, 1 H, $J_1 = 1.03$, $J_2 = 7.53$, Ar), 7.95-7.97 (m, 1 H, $J_1 = 0.93$, $J_2 = 0.93$, $J_3 = 8.43$, Ar), 8.32 (s, 1 H, Ar), 10.02 (s, 1 H, exch., NH). The compound was used directly without further characterization.

$\textit{N}$(3-ethyl-5-((4-methoxyphenyl)(methyl)amino)quinoline-2-yl)pivalamide (290b)

To a solution of 2-amino-6-bromobenzaldehyde 282b (0.500 g, 2.5 mmol) in DMSO (20 mL) were added butyronitrile 289b (0.345 g, 5 mmol) and potassium tert-butoxide (0.561 g, 5 mmol). The reaction mixture was stirred at 60 °C for 3 h and was subsequently diluted with water and extracted with ethyl acetate (25 × 3 mL) and dried over Na$_2$SO$_4$. The combined organic layer was pooled, silica gel was added and concentrated under reduced pressure to give a dry plug. The crude mixture was purified by flash chromatography (hexanes/ethyl acetate) to yield semi-solid intermediate which was pivaloyl protected by dissolving the intermediate in ethyl acetate (20 mL) to which pivalic anhydride (4 mL) was added and heated at 60 °C for 12 h. After the reaction completion, silica gel was added and the solvent was evaporated in vacuo to afford a dry plug. Flash chromatography of the crude mixture in hexanes/ethyl acetate yielded pure 290b as buff colored solid (0.288 g, 36%). TLC $R_f = 0.80$ (Hex:EtOAc; 1:1); mp, 112.7-133.3 °C; $^1$H NMR, DMSO-$d_6$ (400 MHz): 1.23-1.26 (t, 3 H, CH$_3$), 1.29 (s, 9 H, (CH$_3$)$_3$), 2.72-2.78 (q, 2 H, CH$_2$), 7.62-7.66 (t, 1 H, Ar), 7.91-7.93 (d, 1 H, $J = 7.48$, Ar), 7.97-7.99 (d, 1 H, $J = 8.41$, Ar), 8.30 (s, 1 H, Ar), 9.97 (s, 1 H, exch., NH). The compound was used directly without further characterization.
**N-(5-((4-Methoxyphenyl)(methyl)amino)-3-propylquinolin-2-yl)pivalamide (290c)**

To a solution of 2-amino-6-bromobenzaldehyde 282b (1.0 g, 5 mmol) in DMSO (20 mL) were added pentanenitrile 289c (0.831 g, 10 mmol) and potassium tert-butoxide (1.12 g, 10 mmol). The reaction mixture was stirred at 60 °C for 3 h and was subsequently diluted with water and extracted with ethyl acetate (50 × 3 mL) and dried over Na₂SO₄. The combined organic layer was pooled, silica gel was added and concentrated under reduced pressure to give a dry plug. The crude mixture was purified by flash chromatography (hexanes/ethyl acetate) to yield an intermediate which was pivaloyl protected by dissolving the intermediate in ethyl acetate (20 mL) to which pivalic anhydride (4 mL) was added and heated at 60 °C for 12 h. After the reaction completion, silica gel was added and the solvent was evaporated in vacuo to afford a dry plug. Flash chromatography of the crude mixture in hexanes/ethyl acetate yielded pure 290c as a brown colored semisolid (0.788 g, 32%).

TLC $R_f = 0.80$ (Hex:EtOAc; 1:1); $^1$H NMR, DMSO-$d_6$ (500 MHz): 0.91-0.94 (t, 3 H, CH₃), 1.29 (s, 9 H, (CH₃)₃), 1.59-1.67 (m, 2 H, CH₂), 2.68-2.71 (t, 2 H, CH₂), 7.62-7.66 (dd, 1 H, $J_1 = 7.56$, $J_2 = 8.41$, Ar), 7.92-7.94 (dd, 1 H, $J_1 = 1.02$, $J_2 = 7.54$, Ar), 7.97-7.99 (m, 1 H, $J_1 = J_2 = 0.91$, $J_3 = 8.43$, Ar), 8.29 (s, 1 H, Ar), 9.96 (s, 1 H, exch., NH). The compound was used directly without further characterization.

**N-(3-Butyl-5-((4-methoxyphenyl)(methyl)amino)quinolin-2-yl)pivalamide (290d)**

To a solution of 2-amino-6-bromobenzaldehyde 282b (1.0 g, 5 mmol) in DMSO (20 mL) were added hexanenitrile 289d (0.971 g, 10 mmol) and potassium tert-butoxide (1.12 g, 10 mmol). The reaction mixture was stirred at 60 °C for 3 h and was subsequently diluted with water and extracted with ethyl acetate (50 × 3 mL) and dried over Na₂SO₄. The combined
organic layer was pooled, silica gel was added and concentrated under reduced pressure to give a dry plug. The crude mixture was purified by flash chromatography (hexanes/ethyl acetate) to yield an intermediate which was pivaloyl protected by dissolving the intermediate in ethyl acetate (20 mL) to which pivalic anhydride (4 mL) was added and heated at 60 °C for 12 h. After the reaction completion, silica gel was added and the solvent was evaporated in vacuo to afford a dry plug. Flash chromatography of the crude mixture in hexanes/ethyl acetate yielded pure 290d as a brown colored semisolid (0.436 g, 24%). TLC $R_f = 0.80$ (Hex:EtOAc; 1:1); $^1$H NMR, DMSO-$d_6$ (500 MHz): 0.90-0.92 (t, 3 H, CH$_3$), 1.29 (s, 9 H, (CH$_3$)$_3$), 1.32-1.36 (m, 2 H, CH$_2$), 1.56-1.62 (m, 2 H, CH$_2$), 2.71-2.74 (t, 2 H, CH$_2$), 7.62-7.65 (dd, 1 H, $J_1 = 7.72$, $J_2 = 8.25$, Ar), 7.92-7.93 (dd, 1 H, $J_1 = 0.86$, $J_2 = 7.51$, Ar), 7.96-7.98 (d, 1 H, $J = 8.41$, Ar), 8.29 (s, 1 H, Ar), 9.96 (s, 1H, exch., NH). The compound was used directly without further characterization.

$N^5$-(4-Methoxyphenyl)-$N^5$,3-dimethylquinoline-2,5-diamine (186)

Using the general method for Pd-catalyzed cross coupling reaction specified above, 290a (0.150 g, 0.467 mmol), 4-methoxy-$N$-methylaniline 259 (0.090 g, 0.654 mmol), XPhos Pd G3 (0.040 g, 10 mol%), XPhos (0.022 g, 10 mol%) and cesium carbonate (0.456 g, 1.4 mmol) were added to a microwave vial. A 2 mL volume of tert-butanol was added and the reaction was carried out at 110 °C for 24 h. Following the work up procedure mentioned above subsequent separation and purification afforded 186 as a brown colored solid (0.027 g, 20%). TLC $R_f = 0.12$ (Hex:EtOAc; 1:1); $^1$H NMR, DMSO-$d_6$ (400 MHz): 2.13 (s, 3 H, CH$_3$), 3.24 (s, 3 H, NCH$_3$), 3.66 (s, 3 H, OCH$_3$), 6.40 (s, 2 H, exch., NH$_2$), 6.53-6.56 (d, 2 H, $J = 9.07$, Ar), 6.74-6.76 (d, 2 H, $J = 9.04$, Ar), 6.93-6.95 (d, 1 H, $J = 7.31$, Ar), 7.35-
7.37 (d, 1 H, \( J = 7.88 \), Ar), 7.43-7.47 (t, 1 H, Ar), 7.61 (s, 1 H, Ar). Anal. Calcd. for C_{18}H_{19}N_{3}O: C, 73.69; H, 6.53; N, 14.32. Found C, 76.39; H, 8.42; N, 6.36. Sample did not pass CHN analysis. MS (ESI): m/z calculated for C_{18}H_{19}N_{3}O + H+ [M+H+]: 294.15. Found: 294.2. The compound was not sent for biological evaluation and requires further characterization.

3-Ethyl-N^5-(4-methoxyphenyl)-N^5-methylquinoline-2,5-diamine (187)

Using the general method for Pd-catalyzed cross coupling reaction specified above, 290b (0.150 g, 0.447 mmol), 4-methoxy-N-methylaniline 259 (0.086 g, 0.626 mmol), XPhos Pd G3 (0.038 g, 10 mol%), XPhos (0.021 g, 10 mol%) and cesium carbonate (0.437 g, 1.34 mmol) were added to a microwave vial. 2 mL of tert-butanol was added and the reaction was carried out at 110 °C for 24 h. Following the work up procedure mentioned above and purification afforded a yellow colored semisolid intermediate 291b (0.106 g, crude yield 60%). To a solution of the intermediate in methanol (10 mL), 1N NaOH was added (2 mL) and the reaction was stirred at reflux for 8 h. At the end of the reaction the solvent was evaporated in vacuo, the reaction mixture was extracted with ethyl acetate (10 × 3 mL), dried over Na_{2}SO_{4} and the fractions were pooled. After evaporating the solvent, the compound was dried over P_{2}O_{5} to yield 187 as a brown colored powder (0.045 g, 54%). TLC \( R_{f} = 0.15 \) (Hex:EtOAc; 1:1); mp, 163.9-166.7 °C; \(^1\)H NMR, DMSO-\( d_6 \) (400 MHz): 1.03-1.06 (t, 3 H, CH\(_3\)), 2.44-2.48 (q, 2 H, CH\(_2\)), 3.25 (s, 3 H, NCH\(_3\)), 3.66 (s, 3 H, OCH\(_3\)), 6.32 (s, 2 H, exch., NH\(_2\)), 6.58-6.60 (d, 2 H, J = 8.84, Ar), 6.75-6.77 (d, 2 H, J = 8.88, Ar), 6.92-6.94 (d, 1 H, J = 7.24, Ar), 7.32-7.34 (d, 1 H, J = 8.14, Ar), 7.41-7.45 (t, 1 H, Ar),
7.55 (s, 1 H, Ar). Anal. Calcd. for C_{10}H_{21}N_{3}O. 0.27CH_{3}CO_{2}C_{2}H_{5}: C, 72.83; H, 7.05; N, 12.69. Found C, 73.10; H, 7.19; N, 12.30.

**N^5-(4-Methoxyphenyl)-N^5-methyl-3-propylquinoline-2,5-diamine (188)**

Using the general method for Pd-catalyzed cross coupling reaction specified above, 290c (0.150 g, 0.429 mmol), 4-methoxy-N-methylaniline (0.082 g, 0.601 mmol), XPhos Pd G3 (0.036 g, 10 mol%), XPhos (0.020 g, 10 mol%) and cesium carbonate (0.420 g, 1.29 mmol) were added to a microwave vial. 2 mL of tert-butanol was added and the reaction was carried out at 110 °C for 24 h. Following the work up procedure mentioned above and purification afforded a yellow colored semisolid intermediate 291c (0.102 g, crude yield 59%). To a solution of the intermediate in methanol (10 mL), 1N NaOH was added (2 mL) and the reaction was stirred at reflux for 8 h. At the end of the reaction the solvent was evaporated in vacuo, the reaction mixture was extracted with ethyl acetate (10 × 3 mL), dried over Na$_2$SO$_4$ and the fractions were pooled. After evaporating the solvent, the compound was dried over P$_2$O$_5$ to yield 188 as a brown colored powder (0.042 g, 52%).

TLC $R_f$ = 0.18 (Hex:EtOAc; 1:1); mp, 168.1-170 °C; $^1$H NMR, DMSO-$d_6$ (500 MHz): 0.79-0.82 (t, 3 H, CH$_3$), 1.43-1.51 (m, 2 H, CH$_2$), 2.42-2.45 (t, 2 H, CH$_2$), 3.25 (s, 3 H, NCH$_3$), 3.65 (s, 3 H, OCH$_3$), 6.34 (s, 2 H, exch., NH$_2$), 6.57-6.59 (d, 2 H, J = 9.06, Ar), 6.75-6.77 (d, 2 H, J = 9.09, Ar), 6.93-6.95 (dd, 1 H, J$_1$ = 0.77, J$_2$ = 7.39, Ar), 7.31-7.33 (d, 1 H, J = 8.32, Ar), 7.42-7.46 (t, 1 H, Ar), 7.53 (s, 1 H, Ar). Anal. Calcd. for C$_{20}$H$_{23}$N$_3$O. 0.36CH$_3$COCH$_3$: C, 73.96; H, 7.41; N, 12.28. Found C, 74.33; H, 7.69; N, 11.97.
3-Butyl-N⁵-(4-methoxyphenyl)-N⁵-methylquinoline-2,5-diamine (189)

Using the general method for Pd-catalyzed cross coupling reaction specified above, 290d (0.150 g, 0.429 mmol), 4-methoxy-N-methylaniline (0.079 g, 0.578 mmol), XPhos Pd G3 (0.035 g, 10 mol%), XPhos (0.020 g, 10 mol%) and cesium carbonate (0.403 g, 1.24 mmol) were added to a microwave vial. 2 mL of tert-butanol was added and the reaction was carried out at 110 °C for 24 h. Following the work up procedure mentioned above and purification afforded a yellow colored semisolid intermediate 291b (0.070 g, crude yield 40%). To a solution of the intermediate in methanol (10 mL), 1N NaOH was added (2 mL) and the reaction was stirred at reflux for 8 h. At the end of the reaction the solvent was evaporated in vacuo, the reaction mixture was extracted with ethyl acetate (10 × 3 mL), dried over Na₂SO₄ and the fractions were pooled. After evaporating the solvent, the compound was dried over P₂O₅ to yield 189 as a brown colored powder (0.038 g, 68%).

TLC Rf = 0.18 (Hex:EtOAc; 1:1); mp, 97.7-100.1 °C; ¹H NMR, DMSO-d₆ (500 MHz): 0.79-0.82 (t, 3 H, CH₃), 1.15-1.20 (m, 2H, CH₂), 1.38-1.44 (m, 2 H, CH₂), 2.43-2.46 (t, 2 H, CH₂), 3.26 (s, 3 H, CH₃), 3.65 (s, 3 H, OCH₃), 6.29 (s, 2 H, exch., NH₂), 6.58-6.60 (d, 2 H, J = 8.93, Ar), 6.75-6.77 (d, 2 H, J = 9.01, Ar), 6.93-6.95 (dd, 1 H, J₁ = 0.90, J₂ = 7.41, Ar), 7.31-7.32 (d, 1 H, J = 7.78, Ar), 7.42-7.45 (t, 1 H, Ar), 7.51 (s, 1 H, Ar). Anal. Calcd. for C₂₁H₂₅N₃O. 0.13CH₃(CH₂)₄CH₃: C, 75.41; H, 7.77; N, 12.18. Found C, 75.02; H, 7.63; N, 11.80.

N⁵-(4-Methoxyphenyl)-3-methylquinoline-2,5-diamine (191)

Using the general method for Pd-catalyzed cross coupling reaction specified above, 290a (0.125 g, 0.389 mmol), p-anisidine 270 (0.067 g, 0.545 mmol), XPhos Pd G3 (0.034 g, 10
mol%), XPhos (0.019 g, 10 mol%) and cesium carbonate (0.380 g, 1.17 mmol) were added to a microwave vial. 2 mL of tert-butanol was added and the reaction was carried out at 110 °C for 12 h. Following the work up procedure mentioned above and purification afforded deprotected 191 as a brown colored solid (0.070 g, 64%). TLC $R_f = 0.05$ (Hex:EtOAc; 1:1); mp, 189.1-190.9 °C; $^1$H NMR, DMSO-$d_6$ (400 MHz): 2.22 (s, 3 H, CH$_3$), 3.72 (s, 3 H, OCH$_3$), 6.20 (s, 2 H, exch., NH$_2$), 6.70-6.72 (d, 1 H, J = 9.06, Ar), 6.86-6.88 (d, 2 H, J = 9.09, Ar), 6.95-6.97 (d, 1 H, J = 7.45, Ar), 7.04-7.06 (d, 1 H, J = 8.32, Ar), 7.18-7.22 (t, 1 H, Ar), 7.79 (s, 1 H, exch., NH), 8.06 (s, 1 H, Ar). Anal. Calcd. for C$_{17}$H$_{17}$N$_3$O. 0.13CH$_3$CO$_2$C$_2$H$_5$: C, 72.34; H, 6.26; N, 14.44. Found C, 72.33; H, 6.24; N, 14.45.

2-Amino-4-methylthiophene-3-carbonitrile (293)

To a 250 ml round-bottomed flask were added acetone 292 (0.500 g, 8.61 mmol), sulfur (2.21 g, 8.61 mmol), malononitrile (0.567 g, 8.61 mmol) and 25 mL of DMF. The reaction was put to stirring at 60 °C. L-lysine (0.189 g, 1.29 mmol) was added after 15 min. After 5 h, the reaction was run at room temperature for approx. 12 h. At end of the reaction, solvent was evaporated in vacuo and ethyl acetate (30 mL) was added. The reaction mixture was filtered to remove excess sulfur and the filtrate was extracted with water. The combined fractions were pooled, silica gel was added and the solvent was evaporated to afford a dry plug. The crude mixture was purified using hexanes/ethyl acetate to yield pure 293 as pale orange colored solid (0.600 g, 42%). TLC $R_f = 0.70$ (Hex:EtOAc; 1:1); mp, 116.8-118.7 °C (lit.$^{271}$ 118-119 °C); $^1$H NMR, DMSO-$d_6$ (400 MHz): 2.047-2.05 (d, 3 H, J = 1.22, 4-CH$_3$), 6.035-6.038 (d, 1 H, J = 1.22, 5-Ar), 7.07 (s, 2 H, exch., NH$_2$).
5-Methylthieno[2,3-\textit{d}]pyrimidin-2,4-diamine (295)

To a 500 ml round-bottomed flask cyanamide 296 (2.5 g, 0.060 mol) and 60 mL of anhydrous ether were added and stirred under argon. 120 mL of 1N HCl in ether (0.120 mol) was added to round-bottomed flask using dropping funnel at room temperature with continuous stirring. After 2 h, the compound was filtered, washed with ether and dried over P$_2$O$_5$ to yield 294 as a chalky white solid (6.746 g, 98%). To a 100 ml round-bottomed flask were added 293 (0.165 g, 1.20 mmol), 294 (0.275 g, 2.40 mmol) and DMSO$_2$ (2 g) were added. The reaction was run at 120 °C for 1 h. The reaction mixture was cooled down to room temperature. 20 mL of methanol was added to the round-bottomed flask and the solution was brought to neutral pH using ammonium hydroxide solution. Silica gel was added to the above mixture, solvent was evaporated to afford a dry plug. Flash chromatography on the crude mixture was carried out using chloroform/methanol to yield pure 295 as a pale yellow colored solid (0.215 g, 73%). TLC $R_f = 0.63$ (MeOH:CHCl$_3$; 1:5); mp, 213.7-214.7 °C (lit.$^{272}$ 210-212 °C); $^1$H NMR, DMSO-$d_6$ (400 MHz): 2.412-2.416 (d, 3 H, J = 1.27, 5-CH$_3$), 5.98 (s, 2 H, exch., NH$_2$), 6.40 (s, 2 H, exch., NH$_2$), 6.508-6.511 (q, 1 H, J = 1.26, 6-Ar).

**General method for oxidative iodination to synthesize 192-210**

To a 100 mL round-bottomed flask were added 295 (1 equivalent), substituted benzenethiol (2 equivalents) in EtOH/H$_2$O (2:1) and set for reflux. Iodine (2 equivalents) was added after 10 min and the reaction was refluxed for 12 h. At the end of reaction, solvent was evaporated in vacuo and the residue was treated with saturated solution of sodium thiosulfate followed by extraction with ethyl acetate. Combined ethyl acetate fractions
were pooled, dried over Na$_2$SO$_4$, a plug was made using silica gel and flash chromatography was run using chloroform/methanol to yield pure compounds.

5-Methyl-6-(phenylthio)thieno[2,3-$d$]pyrimidine-2,4-diamine (192)

Compound 295 (0.100 g, 0.555 mmol), benzenethiol (0.122 g, 1.11 mmol) and iodine (0.282 g, 1.11 mmol) were reacted as mentioned above using the general method to afford 192 as off-white colored solid (0.051 g, 32%). TLC $R_f = 0.7$ (MeOH:CHCl$_3$; 1:5); mp, 243.9-244.8 °C (lit.$^{259}$ 238.6-239.1 °C); $^1$H NMR, DMSO-$d_6$ (400 MHz): 2.50 (s, 3 H, 5-CH$_3$), 6.27 (s, 2 H, exch., NH$_2$), 6.67 (s, 2 H, exch., NH$_2$), 7.10-7.12 (d, 2 H, J = 7.44, Ar), 7.19-7.20 (d, 1 H, J = 6.77, Ar), 7.30-7.34 (t, 2 H, ArH, $J_1 = J_2 = 6.89$, Ar). Anal. Calcd. for C$_{13}$H$_{12}$N$_4$S$_2$. 0.1C$_2$H$_5$OH: C, 54.11; H, 4.33; N, 19.13; S, 21.90. Found C, 54.30; H, 4.13; N, 19.00; S, 21.91. $^1$H NMR agreed well with the literature$^{259}$ reported values.

6-((2-Methoxyphenyl)thio)-5-methylthieno[2,3-$d$]pyrimidine-2,4-diamine (193)

Compound 295 (0.100 g, 0.555 mmol), 2-methoxybenzenethiol (0.156 g, 1.11 mmol) and iodine (0.282 g, 1.11 mmol) were reacted as mentioned above using the general method to afford 193 as off-white colored solid (0.073 g, 41%). TLC $R_f = 0.7$ (MeOH:CHCl$_3$; 1:5); mp, 201.3-203.4 °C; $^1$H NMR, DMSO-$d_6$ (400 MHz): 2.46 (s, 3 H, 5-CH$_3$), 3.86 (s, 3 H, OCH$_3$), 6.26 (s, 2 H, exch., NH$_2$), 6.61-6.63 (dd, 1 H, $J_1 = 1.47$, $J_2 = 7.78$, Ar), 6.65 (s, 2 H, exch., NH$_2$), 6.85-6.89 (t, 1 H, $J_1 = J_2 = 7.34$, Ar), 7.01-7.03 (d, 1 H, J = 7.86, Ar), 7.14-7.17 (t, 1 H, $J_1 = J_2 = 7.75$, Ar). Anal. Calcd. for C$_{14}$H$_{14}$N$_4$OS$_2$. 0.72CH$_3$OH: C, 51.77; H, 4.99; N, 16.40; S, 18.77. Found C, 51.99; H, 4.66; N, 16.23; S, 18.61.
6-((3-Methoxyphenyl)thio)-5-methylthieno[2,3-d]pyrimidine-2,4-diamine (194)

Compound 295 (0.070 g, 0.389 mmol), 3-methoxybenzenethiol (0.109 g, 0.778 mmol) and iodine (0.198 g, 0.778 mmol) were reacted as mentioned above using the general method to afford 194 as white colored solid (0.020 g, 16%). TLC $R_f = 0.7$ (MeOH:CHCl$_3$; 1:5); mp, 205.5-206.8 °C; $^1$H NMR, CDCl$_3$ (400 MHz): 2.61 (s, 3 H, 5-Me), 3.77 (s, 3 H, OCH$_3$), 4.84 (s, 2 H, exch., NH$_2$), 5.31 (s, 2 H, exch., NH$_2$), 6.69-6.75 (m, 3 H, Ar), 7.17-7.21 (t, 1 H, $J_1 = J_2 = 7.88$, Ar). Anal. Calcd. for C$_{14}$H$_{14}$N$_4$O$_2$: C, 52.81; H, 4.43; N, 17.59; S, 20.14. Found C, 52.79; H, 4.56; N, 17.35; S, 19.98.

6-((4-Methoxyphenyl)thio)-5-methylthieno[2,3-d]pyrimidine-2,4-diamine (195)

Compound 295 (0.060 g, 0.333 mmol), 4-methoxybenzenethiol (0.093 g, 0.666 mmol) and iodine (0.169 g, 0.666 mmol) were reacted as mentioned above using the general method to afford 195 as colored solid (0.018 g, 17%). TLC $R_f = 0.7$ (MeOH:CHCl$_3$; 1:5); mp, 218.1-219.0 °C (lit.$^{259}$ 208.2-208.5 °C); $^1$H NMR, DMSO-$d_6$ (400 MHz): 2.53 (s, 3 H, 5-Me), 3.72 (s, 3 H, OCH$_3$), 6.22 (s, 2 H, exch., NH$_2$), 6.62 (s, 2 H, exch., NH$_2$), 6.90-6.92 (d, 2 H, $J = 8.84$, Ar), 7.14-7.16 (d, 2H, $J = 8.84$, Ar). Anal. Calcd. for C$_{14}$H$_{14}$N$_4$O$_2$: C, 51.25; H, 5.26; N, 15.81; S, 18.10. Found C, 51.54; H, 4.88; N, 15.53; S, 17.96. $^1$H NMR agreed well with the literature$^{259}$ reported values.

6-((2,5-Dimethoxyphenyl)thio)-5-methylthieno[2,3-d]pyrimidine-2,4-diamine (196)

Compound 295 (0.044 g, 0.244 mmol), 2,5-dimethoxybenzenethiol (0.083 g, 0.488 mmol) and iodine (0.124 g, 0.488 mmol) were reacted as mentioned above using the general method to afford 196 as buff colored solid (0.014 g, 17%). TLC $R_f = 0.7$ (MeOH:CHCl$_3$;
1:5); mp, 216.7-219.3 °C (lit.\textsuperscript{259} 213.8-214.0 °C); \textsuperscript{1}H NMR, DMSO-\textit{d}_6 (400 MHz): 2.45 (s, 3 H, 5-CH\textsubscript{3}), 3.57 (s, 3 H, OCH\textsubscript{3}), 3.80 (s, 3 H, OCH\textsubscript{3}), 6.10-6.11 (d, 1 H, J = 2.95, 6´-Ar), 6.26 (s, 2 H, exch., NH\textsubscript{2}), 6.66 (s, 2 H, exch., NH\textsubscript{2}), 6.70-6.73 (dd, 1 H, J = 2.95, J\textsubscript{1} = 8.79, 4´-Ar), 6.94-6.96 (d, 1 H, J = 8.81, 3´-Ar). Anal. Calcd. for C\textsubscript{15}H\textsubscript{16}N\textsubscript{4}O\textsubscript{2}S\textsubscript{2}: C, 51.71; H, 4.63; N, 16.08; S, 18.40. Found C, 51.51; H, 4.77; N, 15.15; S, 18.20. \textsuperscript{1}H NMR agreed well with the literature\textsuperscript{259} reported values.

6-((2,4-Dimethoxyphenyl)thio)-5-methylthieno[2,3-d]pyrimidine-2,4-diamine (197)

Compound 295 (0.080 g, 0.444 mmol), 2,4-dimethoxybenzenethiol (0.151 g, 0.888 mmol) and iodine (0.225 g, 0.888 mmol) were reacted as mentioned above using the general method to afford 197 as buff colored solid (0.046 g, 30%). TLC \textit{R}_f = 0.7 (MeOH:CHCl\textsubscript{3}; 1:5); mp, 174.1-177.8 °C; \textsuperscript{1}H NMR, DMSO-\textit{d}_6 (400 MHz): 2.49 (s, 3 H, 5-CH\textsubscript{3}), 3.73 (s, 3 H, OCH\textsubscript{3}), 3.84 (s, 3 H, OCH\textsubscript{3}), 6.20 (s, 2 H, exch., NH\textsubscript{2}), 6.48-6.50 (dd, 1 H, J\textsubscript{1} = 2.44, J\textsubscript{2} = 8.59, 5´Ar), 6.60-6.11 (d, 3 H, J = 2.38, 5´Ar, NH\textsubscript{2}, exch.), 6.74-6.77 (d, 1 H, J = 8.60, 6´Ar). Anal. Calcd. for C\textsubscript{15}H\textsubscript{16}N\textsubscript{4}O\textsubscript{2}S\textsubscript{2}: C, 51.15; H, 4.94; N, 15.46; S, 17.69. Found C, 51.06; H, 4.71; N, 15.26; S, 17.83.

6-((2,6-Dimethoxyphenyl)thio)-5-methylthieno[2,3-d]pyrimidine-2,4-diamine (198)

Compound 295 (0.125 g, 0.694 mmol), 2,6-dimethoxybenzenethiol (0.236 g, 1.388 mmol) and iodine (0.352 g, 1.388 mmol) were reacted as mentioned above using the general method to afford 198 as off-white colored solid (0.050 g, 21%). TLC \textit{R}_f = 0.7 (MeOH:CHCl\textsubscript{3}; 1:5); mp, 214.2-215.9 °C; \textsuperscript{1}H NMR, DMSO-\textit{d}_6 (400 MHz): 2.59 (s, 3 H, 5-CH\textsubscript{3}), 3.73 (s, 3 H, OCH\textsubscript{3}), 3.81 (s, 6 H, OCH\textsubscript{3}), 6.04 (s, 2 H, exch., NH\textsubscript{2}), 6.45 (s, 2 H,
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6-((3,4-Dimethoxyphenyl)thio)-5-methylthieno[2,3-d]pyrimidine-2,4-diamine (199)

Compound 295 (0.420 g, 2.33 mmol), 3,4-dimethoxybenzenethiol (0.793 g, 4.66 mmol) and iodine (1.18 g, 4.66 mmol) were reacted as mentioned above using the general method to afford 199 as light brown colored solid (0.290 g, 36%). TLC Rf = 0.7 (MeOH:CHCl3; 1:5); mp, 169.5-170.0 °C (lit.259 182.8-183.6 °C); 1H NMR, DMSO-d6 (400 MHz): 2.54 (s, 3 H, 5-CH3), 3.71 (s, 6 H, OCH3), 6.22 (s, 2 H, exch., NH2), 6.62 (s, 2 H, exch., NH2), 6.65-6.68 (dd, 1 H, J1 = 2.18, J2 = 8.37, 6´Ar), 6.84-6.85 (d, 1 H, J = 2.17, 2´Ar), 6.91-6.93 (d, 1 H, J = 8.49, 5´Ar). Anal. Calcd. for C15H16N4O2S2.04CHCl3: C, 47.09; H, 4.21; N, 14.30; S, 16.37. Found C, 47.05; H, 4.40; N, 14.49; S, 16.38; Cl, 9.44. 1H NMR agreed well with the literature259 reported values.

5-Methyl-6-((4-(trifluoromethyl)phenyl)thio)thieno[2,3-d]pyrimidine-2,4-diamine (200)

Compound 295 (0.250 g, 1.39 mmol), 4-(trifluoromethyl)benzenethiol (0.494 g, 2.77 mmol) and iodine (0.704 g, 2.77 mmol) were reacted as mentioned above using the general method to afford 200 as off-white colored solid (0.125 g, 26%). TLC Rf = 0.7 (MeOH:CHCl3; 1:5); mp, 208.0-209.1 °C; 1H NMR, DMSO-d6 (400 MHz): 2.50 (s, 3 H, 5-CH3), 6.01 (s, 2 H, CH2), 6.32 (s, 2 H, exch., NH2), 6.72 (s, 2 H, exch., NH2), 7.26-7.28 (d, 2 H, J = 8.48, Ar), 7.65-7.67 (d, 2 H, J = 8.59, Ar). Anal. Calcd. for C14H11F3N4S2: C,
47.18; H, 3.11; N, 15.72; S, 17.99; F, 15.99. Found C, 47.28; H, 3.26; N, 15.65; S, 18.23; F, 15.71.

5-Methyl-6-((3-(trifluoromethoxy)phenyl)thio)thieno[2,3-d]pyrimidine-2,4-diamine (201)

Compound 295 (0.200 g, 1.11 mmol), 3-(trifluoromethoxy)benzenethiol (0.431 g, 2.22 mmol) and iodine (0.563 g, 2.22 mmol) were reacted as mentioned above using the general method to afford 201 as off-white colored solid (0.055 g, 13%). TLC \( R_f = 0.7 \) (MeOH:CHCl₃; 1:5); mp, 195.3-197.4 °C; \(^1\)H NMR, DMSO-\( d_6 \) (400 MHz): 2.49 (s, 3 H, 5-CH₃), 6.33 (s, 2 H, exch., NH₂), 6.72 (s, 2 H, exch., NH₂), 7.03 (s, 1 H, 2’Ar), 7.09-7.11 (d, 1 H, J = 7.96, 6’Ar), 7.17-7.20 (d, 1 H, J = 8.27, 4’Ar), 7.43-7.47 (t, 1 H, J = 8.08, 5’Ar). Anal. Calcd. for C₁₄H₁₁F₃N₄OS₂: C, 45.29; H, 3.17; N, 14.76; S, 16.89; F, 15.01. Found C, 45.56; H, 3.10; N, 14.73; S, 16.98; F, 15.02.

5-Methyl-6-((4-(trifluoromethoxy)phenyl)thio)thieno[2,3-d]pyrimidine-2,4-diamine (202)

Compound 295 (0.200 g, 1.11 mmol), 4-(trifluoromethoxy)benzenethiol (0.431 g, 2.22 mmol) and iodine (0.563 g, 2.22 mmol) were reacted as mentioned above using the general method to afford 202 as a off-white colored solid (0.100 g, 24%). TLC \( R_f = 0.7 \) (MeOH:CHCl₃; 1:5); mp, 197.6-198.7 °C; \(^1\)H NMR, DMSO-\( d_6 \) (400 MHz): 2.50 (s, 3 H, 5-CH₃), 6.30 (s, 2 H, exch., NH₂), 6.69 (s, 2 H, exch., NH₂), 7.19-7.22 (dd, 2 H, J₁ = 2.10, J₂ = 8.85, Ar), 7.32-7.34 (d, 2 H, J = 7.37, Ar). Anal. Calcd. for C₁₄H₁₁F₃N₄OS₂: C, 45.15; H, 2.98; N, 14.05; S, 17.22; F, 15.31. Found C, 45.28; H, 3.05; N, 14.82; S, 17.37; F, 15.16.
6-((3,4-Dichlorophenyl)thio)-5-methylthieno[2,3-d]pyrimidine-2,4-diamine (203)

Compound 295 (0.150 g, 0.832 mmol), 3,4-dichlorobenzenethiol (0.298 g, 1.664 mmol) and iodine (0.422 g, 1.664 mmol) were reacted as mentioned above using the general method to afford 203 as white colored solid (0.087 g, 29%). TLC $R_f = 0.7$ (MeOH:CHCl$_3$; 1:5); mp, 245.1-246.0 °C (lit.$^{259}$ 238.6-238.9 °C); $^1$H NMR, DMSO-$d_6$ (400 MHz): 2.50 (s, 3 H, 5-CH$_3$), 6.33 (s, 2 H, exch., NH$_2$), 6.72 (s, 2 H, exch., NH$_2$), 7.02-7.05 (dd, 1 H, $J_1 = 2.26$, $J_2 = 8.52$, 6’Ar), 7.34-7.35 (d, 1 H, $J = 2.20$, 2´Ar), 7.56-7.58 (d, 1 H, $J = 8.53$, 5´Ar). Anal. Calcd. for C$_{13}$H$_{10}$Cl$_2$N$_4$S$_2$.0.32C$_2$H$_5$OH: C, 44.03; H, 3.22; N, 15.06; S, 17.24; Cl, 19.06. Found C, 44.12; H, 2.84; N, 15.22; S, 17.22; Cl, 18.79. $^1$H NMR agreed well with the literature$^{259}$ reported values.

6-((3,4-Difluorophenyl)thio)-5-methylthieno[2,3-d]pyrimidine-2,4-diamine (204)

Compound 295 (0.250 g, 1.39 mmol), 3,4-difluorobenzenethiol (0.405 g, 2.77 mmol) and iodine (0.704 g, 2.77 mmol) were reacted as mentioned above using the general method to afford 204 as off-white colored solid (0.255 g, 57%). TLC $R_f = 0.7$ (MeOH:CHCl$_3$; 1:5); mp, 245.0-245.9 °C; $^1$H NMR, DMSO-$d_6$ (400 MHz): 2.50 (s, 3 H, 5-CH$_3$), 6.31 (s, 2 H, exch., NH$_2$), 6.70 (s, 2 H, exch., NH$_2$), 6.93-6.94 (m, 1 H, Ar), 7.18-7.22 (m, 1 H, Ar), 7.37-7.44 (d, 1 H, Ar). Anal. Calcd. for C$_{13}$H$_{10}$F$_2$N$_4$S$_2$.0.05CH$_3$OH: C, 48.14; H, 3.11; N, 17.27; S, 19.77; F, 11.71. Found C, 48.43; H, 3.24; N, 16.81; S, 20.02; F, 11.32.

5-Methyl-6-((3,4,5-trifluorophenyl)thio)thieno[2,3-d]pyrimidine-2,4-diamine (205)

Compound 295 (0.200 g, 1.11 mmol), 3,4,5-trifluorobenzenethiol (0.364 g, 2.22 mmol) and iodine (0.563 g, 2.22 mmol) were reacted as mentioned above using the general method
to afford 205 as pale yellow colored solid (0.140 g, 37%). TLC $R_f = 0.7$ (MeOH:CHCl$_3$; 1:5); mp, 230.2-230.8 °C; $^1$H NMR, DMSO-$d_6$ (400 MHz): 2.50 (s, 3 H, 5-CH$_3$), 6.32 (s, 2 H, exch., NH$_2$), 6.70 (s, 2 H, exch., NH$_2$), 7.01-7.05 (dd, 2 H, J$_1 = 6.45$, J$_2 = 8.17$, Ar).

Anal. Calcd. for C$_{13}$H$_9$F$_3$N$_4$S$_2$: C, 45.61; H, 2.65; N, 16.36; S, 18.73; F, 16.65. Found C, 45.78; H, 2.61; N, 16.28; S, 18.89; F, 16.39.

5-Methyl-6-((2,3,4-trifluorophenyl)thio)thieno[2,3-$d$]pyrimidine-2,4-diamine (206)

Compound 295 (0.200 g, 1.11 mmol), 2,3,4-trifluorobenzenethiol (0.364 g, 2.22 mmol) and iodine (0.563 g, 2.22 mmol) were reacted as mentioned above using the general method to afford 206 as off-white colored solid (0.230 g, 61%). TLC $R_f = 0.7$ (MeOH:CHCl$_3$; 1:5); mp, 240.7-242.0 °C; $^1$H NMR, DMSO-$d_6$ (400 MHz): 2.53 (s, 3 H, 5-CH$_3$), 6.32 (s, 2 H, exch., NH$_2$), 6.71 (s, 2 H, exch., NH$_2$), 6.82-6.88 (m, 1 H, Ar), 7.28-7.33 (m, 1 H, Ar).

Anal. Calcd. for C$_{13}$H$_9$F$_3$N$_4$S$_2$: C, 45.61; H, 2.65; N, 16.36; S, 18.73; F, 16.36. Found C, 45.93; H, 2.66; N, 16.23; S, 18.59; F, 16.27.

5-Methyl-6-((perfluorophenyl)thio)thieno[2,3-$d$]pyrimidine-2,4-diamine (207)

Compound 295 (0.200 g, 1.11 mmol), 2,3,4,5,6-pentafluorobenzenethiol (0.444 g, 2.22 mmol) and iodine (0.563 g, 2.22 mmol) were reacted as mentioned above using the general method to afford 207 as light pink colored solid (0.152 g, 36%). TLC $R_f = 0.7$ (MeOH:CHCl$_3$; 1:5); mp, 258.3-260.5 °C; $^1$H NMR, DMSO-$d_6$ (400 MHz): 2.62 (s, 3 H, 5-CH$_3$), 6.30 (s, 2 H, exch., NH$_2$), 6.68 (s, 2 H, exch., NH$_2$). Anal. Calcd. for C$_{13}$H$_7$F$_5$N$_4$S$_2$: C, 41.27; H, 1.86; N, 14.80; S, 16.95; F, 25.11. Found C, 41.44; H, 1.95; N, 14.62; S, 16.80; F, 25.14.
5-Methyl-6-(naphthalen-1-ylthio)thieno[2,3-d]pyrimidine-2,4-diamine (208)

Compound 295 (0.200 g, 1.11 mmol), naphthalene-1-thiol (0.354 g, 2.22 mmol) and iodine (0.563 g, 2.22 mmol) were reacted as mentioned above using the general method to afford 208 as light brown colored solid (0.037 g, 10%). TLC $R_f = 0.7$ (MeOH:CHCl$_3$; 1:5); mp, 236-238.7 °C (lit.$^{259}$ 233.8-234.4 °C); $^1$H NMR, DMSO-$d_6$ (400 MHz): 2.55 (s, 3 H, 5-CH$_3$), 6.29 (s, 2 H, exch., NH$_2$), 6.70 (s, 2 H, exch., NH$_2$), 7.05-7.07 (d, 1 H, J= 6.71, Ar), 7.41-7.45 (t, 1 H, J = 7.31, Ar), 7.61-7.66 (m, 2 H, Ar), 7.79-7.81 (d, 1 H, J =7.80, Ar), 7.98-8.00 (d, 1 H, J = 8.07, Ar), 8.24-8.26 (d, 1 H, J =8.15, Ar). Anal. Calcd. for C$_{17}$H$_{14}$N$_4$S$_2$. 0.43CH$_3$OH: C, 59.43; H, 4.50; N, 15.9; S, 18.20. Found C, 59.59; H, 4.29; N, 15.76; S, 18.04. $^1$H NMR agreed well with the literature$^{259}$ reported values.

5-Methyl-6-(naphthalen-2-ylthio)thieno[2,3-d]pyrimidine-2,4-diamine (209)

Compound 295 (0.300 g, 1.67 mmol), naphthalene-2-thiol (0.534 g, 3.33 mmol) and iodine (0.845 g, 3.33 mmol) were reacted as mentioned above using the general method to afford 209 as light brown colored solid (0.0.29 g, 7%). TLC $R_f = 0.7$ (MeOH:CHCl$_3$; 1:5); mp, 254.4-256.0 °C; $^1$H NMR, DMSO-$d_6$ (400 MHz): 2.56 (s, 3 H, 5-CH$_3$), 6.29 (s, 2 H, exch., NH$_2$), 6.70 (s, 2 H, exch., NH$_2$), 7.26-7.29 (dd, 1 H, J$_1$ = 1.90, J$_2$ = 8.68, 8´Ar), 7.46-7.50 (m, 2 H, 6´Ar, 7´Ar), 7.62 (s, 1 H, 1´Ar), 7.81-7.83 (d, 1 H, J = 8.70, 3´Ar), 7.86-7.89 (d, 2 H, J = 8.34, 4´Ar, 5´Ar). Anal. Calcd. for C$_{17}$H$_{14}$N$_4$S$_2$. 0.15CH$_3$OH: C, 60.00; H, 4.29; N, 16.31; S, 18.67. Found C, 60.31; H, 4.12; N, 15.93; S, 18.54.
6-[(1,1'-Biphenyl)-4-ylthio]-5-methylthieno[2,3-d]pyrimidine-2,4-diamine (210)

Compound 295 (0.200 g, 1.11 mmol), naphthalene-2-thiol (0.413 g, 2.22 mmol) and iodine (0.563 g, 2.22 mmol) were reacted as mentioned above using the general method to afford 210 as buff colored solid (0.019 g, 5%). TLC Rf = 0.7 (MeOH:CHCl3; 1:5); mp, 261.3-264.3 °C; 1H NMR, DMSO-d6 (400 MHz): 2.54 (s, 3 H, 5-CH3), 6.32 (s, 2 H, exch., NH2), 6.72 (s, 2 H, exch., NH2), 7.18-7.20 (d, 2 H, J = 8.52, Ar), 7.34-7.38 (t, 1 H, J = 7.31, Ar), 7.43-7.47 (t, 2 H, J = 7.91, Ar), 7.61-7.63 (d, 4 H, J = 8.49, Ar). Anal. Calcd. for C19H16N4S2. 0.34CH3OH: C, 61.88; H, 4.66; N, 14.93; S, 17.09. Found C, 62.08; H, 4.33; N, 14.77; Cl, 16.78.

General procedure for the synthesis of benzenethiols 299a-c from anilines 297a-c

Aniline (1 equivalent), MeOH and 10% HCl were added to a round-bottomed flask at 0 °C. A solution of sodium nitrite (1.3 equivalent) in water was added dropwise to the aniline solution over a period of 15 min at 0 °C to generate diazonium salt. The above mixture was added to another 100 mL round-bottomed flask containing a solution of potassium ethyl acetate (2 equivalents) in water at 65 °C. The reaction was stirred at 65 °C for 15 min after which the reaction was cooled down to room temperature. Solvent was evaporated in vacuo and the compound was extracted with ethyl acetate (3 times), washed with brine and dried over Na2SO4. The combined fractions were pooled, silica gel was added solvent was removed to afford a dry plug. Crude compound was purified using flash chromatography in hexanes/ethyl acetate to afford S-(substitutedphenyl) O-ethyl carbonothioate 298a-c.

Hydrolysis of compounds 298a-c in KOH/NaOH was carried out at 65 °C for 2 h. The crude mixture was extracted with ethyl acetate, washed with brine, dried over Na2SO4 and
the solvent was evaporated in vacuo to yield **299a-c** which were used further without purification.

### 2,4-Dimethoxybenzenethiol (299a)

2,4-dimethoxyaniline **297a** (0.500 g, 3.264 mmol) was dissolved in MeOH (10 mL) to which 10% HCl (5 mL) was added and stirred at 0 °C as specified in the general procedure above. Sodium nitrite (0.293 g, 4.243 mmol) was dissolved in water (10 mL) and added to the aniline solution dropwise. Potassium ethyl xanthate (1.046 g, 6.528 mmol) was used to give the **298a** (0.224 g, 26%) as pale white colored semisolid. TLC \( R_f = 0.7 \) (Hex:EtOAc; 1:1); \(^1\)H NMR, DMSO-\( d_6 \) (400 MHz): 1.21-1.24 (t, 3 H, CH\(_3\)), 3.80 (s, 3 H, OCH\(_3\)), 3.83 (s, 3 H, OCH\(_3\)), 4.52-4.58 (q, 2 H, CH\(_2\)), 6.60-6.62 (dd, 1 H, \( J_1 = 2.51, J_2 = 8.53, 5\)-Ar), 6.68-6.69 (d, 1 H, \( J = 2.48, 3\)-Ar), 7.34-7.36 (d, 1 H, \( J = 8.50, 6\)-Ar). Compound **298a** (0.220 g, 0.908 mmol) was dissolved in EtOH (10 mL) to which 1N NaOH (3 mL) was added and heated at 65 °C for 2 h. Following the general procedure mentioned above, **299a** (0.145 g, 94%) was obtained as a pale yellow liquid which was used further without purification. TLC \( R_f = 0.87 \) (Hex:EtOAc; 1:1); \(^1\)H NMR, DMSO-\( d_6 \) (400 MHz): 3.73 (s, 3 H, OCH\(_3\)), 3.80 (s, 3 H, OCH\(_3\)), 4.51 (s, 1 H, exch., SH), 6.46-6.49 (dd, 1 H, \( J_1 = 2.54, J_2 = 8.48, 5\)-Ar), 6.57-6.58 (d, 1 H, \( J = 2.52, 3\)-Ar), 7.19-7.21 (d, 1 H, \( J = 8.46, 6\)-Ar). The compound was carried without additional characterization.

### 2,6-Dimethoxybenzenethiol (299b)

2,6-dimethoxyaniline **297b** (2.0 g, 13.056 mmol) was dissolved in MeOH (30 mL) to which 10% HCl (5 mL) was added and stirred at 0 °C as specified in the general procedure above.
Sodium nitrite (1.15 g, 16.972 mmol) was dissolved in water (15 mL) and added to the aniline solution dropwise. Potassium ethyl xanthate (4.186 g, 26.112 mmols) was used to give the **298b** (2.068 g, 47%) as pale yellow colored semisolid. TLC $R_f = 0.77$ (Hex:EtOAc; 1:1); $^1$H NMR, DMSO-$d_6$ (400 MHz): 1.18-1.21 (t, 3 H, CH$_3$), 3.80 (s, 6 H, OCH$_3$), 4.50-4.56 (q, 2 H, CH$_2$), 6.76-6.78 (d, 2 H, J = 8.38, Ar), 7.46-7.50 (t, 1 H, J = 8.41, Ar). Compound **298b** (0.500 g, 2.06 mmol) was dissolved in EtOH (10 mL) to which 1N NaOH (3 mL) was added and heated at 65 °C for 2 h. Following the general procedure mentioned above, **299b** (0.339 g, 96%) was obtained as a pale yellow colored solid which was used further without purification. TLC $R_f = 0.85$ (Hex:EtOAc; 1:1); $^1$H NMR, DMSO-$d_6$ (400 MHz): 3.83 (s, 6 H, OCH$_3$), 4.28 (s, 1 H, exch., SH), 6.68-6.70 (d, 2 H, J = 8.33, Ar), 7.08-7.12 (t, 1 H, J$_1$ = J$_2$ = 8.32, Ar). The compound was carried without additional characterization.

**[1,1'-Biphenyl]-4-thiol (299c)**

[1,1'-biphenyl]-4-amine **297c** (1.0 g, 5.91 mmol) was dissolved in MeOH (35 mL) to which 10% HCl (10 mL) was added and stirred at 0 °C as specified in the general procedure above. Sodium nitrite (0.530 g, 7.68 mmol) was dissolved in water (20 mL) and added to the aniline solution dropwise. Potassium ethyl xanthate (1.89 g, 11.82 mmols) was used to give the **298c** which was dissolved in EtOH (20 mL) and hydrolyzed using 1N NaOH (5 mL) at 65 °C for 2 h. Following the general procedure mentioned above, **299c** (0.707 g, 64% over 2 steps) was obtained as a pale yellow liquid. TLC $R_f = 0.9$ (Hex:EtOAc; 1:1); $^1$H NMR, DMSO-$d_6$ (400 MHz): 5.35 (s, 1 H, exch., SH), 7.36-7.41 (m, 1 H, Ar), 7.45-
7.50 (m, 2 H, Ar), 7.64-7.69 (m, 4 H, Ar), 7.71-7.74 (m, 2 H, Ar). The compound was carried directly without additional characterization.

3-Amino-4-ethylthiophene-2-carbonitrile 301 and 3-amino-4,5-dimethylthiophene-2-carbonitrile 301a

To a 100 ml round-bottomed flask were added 2-butanone 300 (0.100 g, 1.39 mmol), sulfur (0.067 g, 2.085 mmol), malononitrile (0.101 g, 1.53 mmol) and 15 mL of DMF. The reaction was put to stirring at 60 °C. L-proline (0.016 g, 0.139 mmol) was added after 15 min. After 5 h, the reaction was run at room temperature for approx. 12 h. At end of the reaction, solvent was evaporated in vacuo and ethyl acetate (30 mL) was added. The reaction mixture was filtered to remove excess sulfur and the filtrate was extracted with water. The combined fractions were pooled, silica gel was added and the solvent was evaporated to afford a dry plug. The crude mixture was purified using hexanes/ethyl acetate. The purified fraction (TLC Rf = 0.77 (Hex:EtOAc; 1:1)) showed presence of two compounds 301 and 301a in 1:5.6 ratio. 1H NMR of 301, DMSO-d6 (400 MHz): 1.12-1.16 (t, 3 H, CH₃), 2.38-2.44 (q, 2 H, CH₂), 6.02 (s, 1 H, Ar), 7.06 (s, 2 H, exch., NH₂). 1H NMR of 301a, DMSO-d6 (400 MHz): 1.94 (s, 3 H, CH₃), 2.10 (s, 3 H, CH₃), 6.89 (s, 2 H, exch., NH₂). The compound was carried without additional characterization.

2-Amino-4-isopropylthiophene-3-carbonitrile (303)

To a 500 ml round-bottomed flask were added 3-methylbutan-2-one 302 (5.0 g, 58.05 mmol), sulfur (1.86 g, 58.05 mmol), malononitrile (3.83 g, 58.05 mmol) and 50 mL of DMF. The reaction was put to stirring at 60 °C. L-lysine (0.848 g, 5.80 mmol) was added
after 15 min. After 5 h, the reaction was run at room temperature for approx. 12 h. At end of the reaction, solvent was evaporated in vacuo and ethyl acetate (30 mL) was added. The reaction mixture was filtered to remove excess sulfur and the filtrate was extracted with water. The combined fractions were pooled, silica gel was added and the solvent was evaporated to afford a dry plug. The crude mixture was purified using hexanes/ethyl acetate to yield pure 303 as brown solid (5.04 g, 52%). TLC \( R_f = 0.78 \) (Hex:EtOAc; 1:1); mp, 67.1-68.7 °C; \(^1\)H NMR, DMSO-\textit{d}_6 (400 MHz): 1.16-1.18 (d, 6 H, (CH\textsubscript{3})\(_2\)), 2.68-2.78 (m, 1 H, CH), 6.00 (s, 1 H, Ar), 7.05 (s, 2 H, exch, NH\(_2\)). The compound was carried directly without additional characterization.

**5-Isopropylthieno[2,3-\textit{d}]pyrimidine-2,4-diamine (304)**

To a 100 ml round-bottomed flask were added 303 (1.12 g, 6.74 mmol), 294 (1.55 g, 13.47 mmol) and DMSO\(_2\) (4 g) were added. The reaction was run at 120 °C for 1 h. The reaction mixture was cooled down to room temperature. 20 mL of methanol was added to the round-bottomed flask and the solution was brought to neutral pH using ammonium hydroxide solution. Silica gel was added to the above mixture, solvent was evaporated to afford a dry plug. Flash chromatography on the crude mixture was carried out using chloroform/methanol to yield pure 304 as buff colored solid (1.20 g, 85%). TLC \( R_f = 0.6 \) (MeOH:CHCl\(_3\); 1:5); mp, 221.8-224.6 °C; \(^1\)H NMR, DMSO-\textit{d}_6 (400 MHz): 1.20-1.22 (d, 6 H, (CH\textsubscript{3})\(_2\)), 3.27-3.31 (m, 1 H, CH), 6.06 (s, 2 H, exch., NH\(_2\)), 6.50 (s, 2 H, exch., NH\(_2\)) 6.59 (s, 1 H, Ar). Anal. Calcd. for C\(_9\)H\(_{12}\)N\(_4\)S: C, 51.90; H, 5.81; N, 26.90; S, 15.39. Found C, 51.93; H, 5.66; N, 27.05; S, 15.18.
6-Iodo-5-isopropylthieno[2,3-d]pyrimidine-2,4-diamine (305)

To a 100 mL round-bottomed flask were added 304 (0.500 g, 2.40 mmol), N-iodosuccinimide (0.594 g, 2.64 mmol) and acetic acid (10 mL). The reaction mixture was heated at 50 °C for 2 h and then cooled to room temperature. Water (20 mL) was added and the mixture was stirred at room temperature for 15 min after which the compound was filtered, washed with water and dried over P₂O₅ to yield 305 (0.600 g, 75%) as pale yellow colored solid. TLC $R_f = 0.62$ (MeOH:CHCl₃; 1:5); $^1$H NMR, DMSO-$d_6$ (500 MHz): 1.36-1.37 (d, 6 H, (CH₃)₂), 3.47-3.53 (m, 1 H, CH), 6.13 (s, 2 H, exch., NH₂), 6.41 (s, 2 H, exch., NH₂). The compound was used directly without further characterization.

5-Isopropyl-6-(naphthalen-2-ylthio)thieno[2,3-d]pyrimidine-2,4-diamine (212)

To a 2-5 mL Biotage vial were added 305 (0.300 g, 0.898 mmol), Cs₂CO₃ (0.877 g, 2.69 mmol), naphthalene-2-thiol 306 (0.575 g, 3.59 mmol), CuI (2.22 g, 1.17 mmol) and DMF (3 mL). The vial was sealed, the mixture was evacuated and back filled with argon (three cycles). The reaction mixture was irradiated in microwave at 150 °C for 90 min. After the reaction was cooled to room temperature, the solvent was evaporated and extracted with ethyl acetate. The fractions were pooled, dried over Na₂SO₄ and silica gel was added to afford a dry plug. Crude compound was purified using flash chromatography in chloroform/methanol to afford 212 (0.082 g, 25%) as pale brown colored solid. TLC $R_f = 0.67$ (MeOH:CHCl₃; 1:5); mp, 211.8-214.5 °C; $^1$H NMR, DMSO-$d_6$ (400 MHz): 1.36-1.38 (d, 6 H, (CH₃)₂), 3.67-3.76 (m, 1 H, CH), 6.27 (s, 2 H, exch., NH₂), 6.60 (s, 2 H, exch., NH₂), 7.29-7.32 (dd, 1 H, J₁ = 1.92, J₂ = 8.68, Ar), 7.44-7.51 (m, 2 H, Ar), 7.62-7.63 (d, 1 H, J = 1.64, Ar), 7.81-7.83 (dd, 1 H, J₁ = 2.21, J₂ = 7.03, Ar), 7.86-7.90 (m, 2 H, Ar). Anal.
Calcd. for C_{19}H_{18}N_{4}S_{2}·0.35HCON(CH_{3})_{2}: C, 61.42; H, 5.25; N, 15.53; S, 16.36. Found C, 61.19; H, 5.11; N, 15.33; S, 16.44.
VI. SUMMARY

This dissertation describes the design and synthesis of heterocycles (pyrimidines, quinazolines, quinoline-2,5-diamines and thieno[2,3-d]pyrimidines) as agents with cancer chemotherapeutic and anti-infective potential. For these projects a total of 44 novel compounds were synthesized and characterized, of which 37 were submitted for evaluation in \textit{in vitro} biological assays. In addition, 7 compounds were resynthesized in the quinazolines series for additional evaluation of these previously published compounds as RTK inhibitors. 6 compounds were resynthesized in the thieno[2,3-d]pyrimidine series for their evaluation as \textit{pJ}DHFR inhibitors. The novel target compounds synthesized as part of this dissertation are listed below:

1. 5-Chloro-\textit{N}^{4}-(4-methoxyphenyl)-\textit{N}^{4},2-dimethylpyrimidine-4,6-diamine (154)
2. \textit{N}^{4}-(4-Methoxyphenyl)-\textit{N}^{4},2,5-trimethylpyrimidine-4,6-diamine (153)
3. 5-Chloro-\textit{N}^{4}-(4-methoxyphenyl)-2-methylpyrimidine-4,6-diamine (155)
4. 5-Chloro-\textit{N}^{4},2-dimethyl-\textit{N}^{4}-(4-(methylthio)phenyl)pyrimidine-4,6-diamine (156)
5. 5-Chloro-6-(6-methoxy-3,4-dihydroquinolin-1(2\textit{H})-yl)-2-methylpyrimidin-4-amine (158)
6. \textit{N}-(5-Chloro-6-(6-methoxy-3,4-dihydroquinolin-1(2\textit{H})-yl)-2-methylpyrimidin-4-yl)pivalamide (171)
7. 5-Chloro-\textit{N}^{4}-(2,3-dihydrobenzofuran-5-yl)-\textit{N}^{4},2-dimethylpyrimidine-4,6-diamine (159)
8. 5-Chloro-\textit{N}^{4}-(3-methoxyphenyl)-\textit{N}^{4},2-dimethylpyrimidine-4,6-diamine (157)
9. 5-Chloro-\textit{N}^{4}-(5-methoxynaphthalen-2-yl)-\textit{N}^{4},2-dimethylpyrimidine-4,6-diamine (160)
10. 5-Chloro-\textit{N}^{4}-(4-methoxyphenyl)-\textit{N}^{4},\textit{N}^{6},2-trimethylpyrimidine-4,6-diamine (161)
11. 5-Chloro-\(N^4\)-ethyl-\(N^6\)-(4-methoxyphenyl)-\(N^6\),2-dimethylpyrimidine-4,6-diamine
   (162)
12. 5-Chloro-\(N^4\)-(4-methoxyphenyl)-\(N^4\),2-dimethyl-N\(^6\)-propylpyrimidine-4,6-diamine
   (163)
13. 5-Chloro-\(N^4\)-isopropyl-\(N^6\)-(4-methoxyphenyl)-\(N^6\),2-dimethylpyrimidine-4,6-diamine
   (164)
14. \(N^4\)-Butyl-5-chloro-\(N^6\)-(4-methoxyphenyl)-\(N^6\),2-dimethylpyrimidine-4,6-diamine
   (165)
15. 5-Chloro-\(N^4\)-isobutyl-\(N^6\)-(4-methoxyphenyl)-\(N^6\),2-dimethylpyrimidine-4,6-diamine
   (166)
16. \(N^4\)-Benzyl-5-chloro-\(N^6\)-(4-methoxyphenyl)-\(N^6\),2-dimethylpyrimidine-4,6-diamine
   (167)
17. 5-Chloro-\(N^4\)-ethyl-\(N^6\)-(5-methoxynaphthalen-2-yl)-\(N^6\),2-dimethylpyrimidine-4,6-
   diamine (168)
18. 5-Chloro-\(N^4\)-(5-methoxynaphthalen-2-yl)-\(N^4\),2-dimethyl-\(N^6\)-propylpyrimidine-4,6-
   diamine (169)
19. \(N\)-(5-Chloro-6-((4-methoxyphenyl)(methyl)amino)-2-methylpyrimidin-4-y1)acetamide (170)
20. \(N\)-(5-Methoxynaphthalen-2-yl)-\(N\),2-dimethylquinazolin-4-amine (175)
21. \(N\)-Methyl-\(N\)-(4-(methylthio)phenyl)quinazolin-4-amine (178)
22. 2-Chloro-\(N\)-(5-methoxynaphthalen-2-yl)-\(N\)-methylquinazolin-4-amine (182)
23. \(N\)-(4-methoxyphenyl)-\(N\)-methylpyrido[2,3-\(d\)]pyrimidin-4-amine (183)
24. \(N\)-(5-Chloro-3-pentylquinolin-2-yl)pivalamide (284)
25. \(N^5-(4\text{-Methoxyphenyl})-N^5\text{-methyl-3-pentylquinoline-2,5-diamine} (190)\)
26. \(3\text{-Ethyl-}N^5-(4\text{-methoxyphenyl})-N^5\text{-methylquinoline-2,5-diamine} (187)\)
27. \(N^5-(4\text{-Methoxyphenyl})-N^5\text{-methyl-3-propylquinoline-2,5-diamine} (188)\)
28. \(3\text{-Butyl-}N^5-(4\text{-methoxyphenyl})-N^5\text{-methylquinoline-2,5-diamine} (189)\)
29. \(N^5-(4\text{-Methoxyphenyl})-3\text{-methylquinoline-2,5-diamine} \) (191)
30. \(6\text{-((2\text{-Methoxyphenyl})thio)-5-methylthieno[2,3-\text{d}]pyrimidine-2,4-diamine} (193)\)
31. \(6\text{-((3\text{-Methoxyphenyl})thio)-5-methylthieno[2,3-\text{d}]pyrimidine-2,4-diamine} (194)\)
32. \(6\text{-((2,4\text{-Dimethoxyphenyl})thio)-5-methylthieno[2,3-\text{d}]pyrimidine-2,4-diamine} (197)\)
33. \(6\text{-((2,6\text{-Dimethoxyphenyl})thio)-5-methylthieno[2,3-\text{d}]pyrimidine-2,4-diamine} (198)\)
34. \(5\text{-Methyl-6-((4\text{-trifluoromethyl} \text{phenyl})thio)thieno[2,3-\text{d}]pyrimidine-2,4-diamine} \) (200)
35. \(5\text{-Methyl-6-((3\text{-trifluoromethoxy} \text{phenyl})thio)thieno[2,3-\text{d}]pyrimidine-2,4-diamine} \) (201)
36. \(5\text{-Methyl-6-((4\text{-trifluoromethoxy} \text{phenyl})thio)thieno[2,3-\text{d}]pyrimidine-2,4-diamine} \) (202)
37. \(6\text{-((3,4\text{-Difluorophenyl})thio)-5-methylthieno[2,3-\text{d}]pyrimidine-2,4-diamine} (204)\)
38. \(5\text{-Methyl-6-((3,4,5\text{-trifluorophenyl})thio)thieno[2,3-\text{d}]pyrimidine-2,4-diamine} (205)\)
39. \(5\text{-Methyl-6-((2,3,4\text{-trifluorophenyl})thio)thieno[2,3-\text{d}]pyrimidine-2,4-diamine} (206)\)
40. \(5\text{-Methyl-6-((perfluorophenyl)thio)thieno[2,3-\text{d}]pyrimidine-2,4-diamine} (207)\)
41. \(5\text{-Methyl-6-((naphthalen-2-ylthio)thieno[2,3-\text{d}]pyrimidine-2,4-diamine} (209)\)
42. \(6\text{-((1,1\text{'-Biphenyl}-4-ylthio)-5-methylthieno[2,3-\text{d}]pyrimidine-2,4-diamine} (210)\)
43. \(5\text{-Isopropylthieno[2,3-\text{d}]pyrimidine-2,4-diamine} (304)\)
44. \(5\text{-Isopropyl-6-((naphthalen-2-ylthio)thieno[2,3-\text{d}]pyrimidine-2,4-diamine} (212)\)
Among these compounds 153-170 were evaluated as microtubule targeting agents binding at the colchicine site of tubulin. Compounds 175, 178, 182 and 183 were evaluated as single agents with combination chemotherapy potential and tested for antitubulin and RTK inhibitory assays in vitro. Compounds 193-210 and 212 were evaluated for selective inhibition of \( p_j \)DHFR over \( h \)DHFR. During this study, palladium catalyzed cross coupling amination reactions on quinoline-2,5-diamines were also optimized and mechanistic details were explored. XPhos precatalyst (XPhos Pd G3) was effectively used for the synthesis of target compounds 186-191.

In addition, molecular modeling studies were performed on analogs of pyrimido[4,5-b]indoles, cyclopenta[d]pyrimidines, furo[2,3-d]pyrimidines and pyrrolo[3,2-d]pyrimidines to rationalize the biological activities of these compounds.
VII. BIBLIOGRAPHY


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VIII. APPENDIX

Biological Evaluation

The tumor inhibitory assays, microtubule depolymerization assays were performed by Dr. Susan L. Mooberry (Department of Pharmacology, University of Texas Health Science Center at San Antonio, San Antonio, TX 78229). The colchicine binding assays and bovine brain tubulin polymerization assays were performed by Dr. Ernest Hamel (Screening Technologies Branch, Developmental Therapeutics Program, Division of Cancer Treatment and Diagnosis, Frederick National Laboratory for Cancer Research, National Cancer Institute, Frederick, MD 21702). RTK inhibitory assays, CAM assays were performed by Dr. Michael Ihnat (Department of Pharmaceutical Sciences, University of Oklahoma College of Pharmacy, Oklahoma City, OK 73117). DHFR inhibitory assays were performed by Dr. Vivian Cody (Hauptman-Woodward Medical Research Institute, Buffalo, NY 14203).

1. Biological evaluation of pyrimidine analogs as microtubule targeting agents

Compounds 153 and 154 were initially evaluated for their ability to depolymerize microtubules (Table 12). These compounds were also tested for their antiproliferative activity in MDA-MB-435 cells lines. Compound 154 with 5-Cl group was 3-fold more potent than the 5-Me analog 153, suggesting that the EWG at 5-position was conducive towards improving potency. Compounds 155 and 157 with p-anisidine and 3-methoxy-N-methyl aniline substitutions at N4-position led to complete loss of depolymerizing
activity, suggesting that the conformational restriction afforded by the N⁴-Me group in 154 and the position of HBA at 4´-position of the N⁴-phenyl group was essential for activity.

Table 12. Biological data for monocyclic pyrimidine analogs 153-171

<table>
<thead>
<tr>
<th>Compd</th>
<th>IC⁵₀ ± SD in MDA-435 Cells</th>
<th>EC⁵₀ for Microtubule Depolymerization</th>
<th>Inhibition of colchicine binding % Inhibition ± SD 5 µM inhibitor</th>
</tr>
</thead>
<tbody>
<tr>
<td>153</td>
<td>206.4 ± 8.9 nM</td>
<td>&gt; 8000 nM</td>
<td>45 ± 0.3</td>
</tr>
<tr>
<td>154</td>
<td>71.3 ± 6.1 nM</td>
<td>1.5 µM</td>
<td>76 ± 2</td>
</tr>
<tr>
<td>155</td>
<td>ND</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>156</td>
<td>140 ± 12 nM</td>
<td>3.3 µM</td>
<td>71 ± 4</td>
</tr>
<tr>
<td>157</td>
<td>ND</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>158</td>
<td>220 ± 25 nM</td>
<td>1.4 µM</td>
<td>61 ± 5</td>
</tr>
<tr>
<td>159</td>
<td>620 ± 89 nM</td>
<td>9.2 µM</td>
<td>60 ± 3</td>
</tr>
<tr>
<td>160</td>
<td>56.1 ± 5.0 nM</td>
<td>831 nM</td>
<td>74 ± 4</td>
</tr>
<tr>
<td>161</td>
<td>103.3 ± 11.8 nM</td>
<td>2.1 µM</td>
<td>68 ± 4</td>
</tr>
<tr>
<td>162</td>
<td>50.4 ± 8.0 nM</td>
<td>305 nM</td>
<td>74 ± 3</td>
</tr>
<tr>
<td>163</td>
<td>24.4 ± 1.4 nM</td>
<td>123 nM</td>
<td>82 ± 0.1</td>
</tr>
<tr>
<td>164</td>
<td>684.8 ± 53.7 nM</td>
<td>7.4 µM</td>
<td>-</td>
</tr>
<tr>
<td>165</td>
<td>84.4 ± 8.0 nM</td>
<td>432 nM</td>
<td>84 ± 2</td>
</tr>
<tr>
<td>166</td>
<td>78.1 ± 2.8 nM</td>
<td>394 nM</td>
<td>57 ± 0.1</td>
</tr>
<tr>
<td>167</td>
<td>ND</td>
<td>&gt; 10,000 nM</td>
<td>14 ± 0.02</td>
</tr>
<tr>
<td>170</td>
<td>3,770 ± 350 nM</td>
<td>&gt; 15 µM</td>
<td>-</td>
</tr>
<tr>
<td>171</td>
<td>2.08 ± 0.46 µM</td>
<td>&gt; 10 µM</td>
<td>-</td>
</tr>
<tr>
<td>CA4</td>
<td>4.4 ± 0.46 nM</td>
<td>9.8 nM</td>
<td>97 ± 0.8</td>
</tr>
<tr>
<td>Paclitaxel</td>
<td>4.5 ± 0.52 nM</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

ND-Not Determined
Compounds 156 and 158 with the N-methyl-4-(methylthio)aniline and 6-methoxy-1,2,3,4-tetrahydroquinoline substitutions at 4-position were 2-fold and 3-fold less potent than 154 in the antiproliferative activity, respectively. The N⁶-alkylated analogs 161-166 led to an improvement in both depolymerizing and antiproliferative activities (except 161 and 164), suggesting that these alkyl groups are able to occupy the binding site by undergoing hydrophobic interactions with residues in the binding pocket more completely than 154. Compounds 163 and 165 were able to displace [³H] colchicine similar to that seen for CA4. Benzyl substitution at N⁶-position in 167 was not tolerated and led to loss of activity. Compounds 170 and 171 with N⁶-acetyl and N⁶-pivaloyl substitutions also led to a loss in activity suggesting that EWG in these compounds is not tolerated.

2. Biological evaluation of quinazolines (172-183) as MTAs and RTK inhibitors

In the antiproliferative assays and the microtubule depolymerization assays compounds 172-180 displayed potent inhibitory activities (Table 13). The 2-H substituted compounds (176-178) were 7- to 10-fold less potent than the 2-Me (172-175) and 2-Cl (179-180) substituted compounds, suggesting bulk is important for antitubulin activity at the 2-position of the quinazoline scaffold. The pyrido[2,3-d]pyrimidine analog 183 lost activity in the antiproliferative assay suggesting that replacement of fused benzene ring with electron withdrawing pyridine ring is not conducive for activity. In the RTK inhibitory assays, the 2-Me and 2-Cl substituted compounds were more potent than 2-H substituted compounds. For 2-substituted quinazolines (172-175), varying the anilne substitution at 4-
position was well tolerated. The 2-Cl substituted compound 179 was a potent inhibitor of EGFR but resulted in loss of 10-fold and 15-fold activity in VEGFR-2 and PDGFR-β, when compared to corresponding 2-Me compound 172. As seen with tubulin activity, compound 183 was inactive in RTK inhibitory assays.

Table 13. Biological activities of quinazolines 172-183

<table>
<thead>
<tr>
<th>Compd</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt; ± SD in MDA-MB-435 Cells (nM)</th>
<th>EC&lt;sub&gt;50&lt;/sub&gt; microtubule depolymerization (nM)</th>
<th>EGFR kinase inhibition (nM)</th>
<th>VEGFR-2 kinase inhibition (nM)</th>
<th>PDGFR kinase inhibition (nM)</th>
<th>CAM inhibition (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>172</td>
<td>1.7 ± 0.1</td>
<td>2.1</td>
<td>2.8 ± 1.1</td>
<td>8.4 ± 2.2</td>
<td>5.6 ± 0.4</td>
<td>2.8 ± 1.1</td>
</tr>
<tr>
<td>173</td>
<td>1.1 ± 0.2</td>
<td>3</td>
<td>5.0 ± 0.4</td>
<td>9.3 ± 3.9</td>
<td>7.8 ± 0.5</td>
<td>3.1 ± 1.3</td>
</tr>
<tr>
<td>174</td>
<td>1.2 ± 0.2</td>
<td>2</td>
<td>8.7 ± 1.6</td>
<td>14.7 ± 2.8</td>
<td>231.5 ± 29.9</td>
<td>9.8 ± 0.7</td>
</tr>
<tr>
<td>175</td>
<td>2.0 ± 0.4</td>
<td>2.5</td>
<td>10.6 ± 1.8</td>
<td>16.8 ± 4.1</td>
<td>29.4 ± 5.1</td>
<td>ND</td>
</tr>
<tr>
<td>176</td>
<td>7.1 ± 0.8</td>
<td>8.15</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>177</td>
<td>12.4 ± 1.7</td>
<td>23.9</td>
<td>48.9 ± 6.0</td>
<td>111.1 ± 19.2</td>
<td>54 ± 14.1</td>
<td>ND</td>
</tr>
<tr>
<td>178</td>
<td>7.8 ± 0.8</td>
<td>12.3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>179</td>
<td>0.6 ± 0.1</td>
<td>2</td>
<td>3.6 ± 0.6</td>
<td>97.5 ± 10.1</td>
<td>76.6 ± 16.2</td>
<td>130.0 ± 22.2</td>
</tr>
<tr>
<td>180</td>
<td>0.7 ± 0.2</td>
<td>2</td>
<td>5.4 ± 0.3</td>
<td>55.2 ± 11.1</td>
<td>80.6 ± 12.1</td>
<td>41.4 ± 6.1</td>
</tr>
<tr>
<td>183</td>
<td>324.0 ± 47.4</td>
<td>2.7 µM</td>
<td>1.8 ± 0.5 µM</td>
<td>3.3 ± 1.0 µM</td>
<td>6 ± 3 µM</td>
<td>1.1 ± 0.3 µM</td>
</tr>
<tr>
<td>CA4</td>
<td>4.4 ± 0.46</td>
<td>9.8</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sunitinib</td>
<td>-</td>
<td>-</td>
<td>172.1 ± 19.4</td>
<td>18.9 ± 2.7</td>
<td>83.1 ± 10.1</td>
<td>1.3 ± 0.07</td>
</tr>
<tr>
<td>Erlotinib</td>
<td>-</td>
<td>-</td>
<td>1.2 ± 0.2</td>
<td>124.7 ± 18.2</td>
<td>12.2 ± 1.9</td>
<td>29.1 ± 1.9</td>
</tr>
</tbody>
</table>

ND-Not Determined
3. Biological evaluation of thieno[2,3-\textit{d}]pyrimidines as selective \textit{pj}DHFR inhibitors

Table 14. Biological activities of 192-209

<table>
<thead>
<tr>
<th>Compd</th>
<th>\textit{pj}DHFR IC_{50} (\mu M)</th>
<th>\textit{h}DHFR IC_{50} (\mu M)</th>
<th>\textit{h/pj}</th>
</tr>
</thead>
<tbody>
<tr>
<td>192</td>
<td>0.399</td>
<td>0.755</td>
<td>1.89</td>
</tr>
<tr>
<td>193</td>
<td>0.7</td>
<td>1.5</td>
<td>2.14</td>
</tr>
<tr>
<td>194</td>
<td>2.3</td>
<td>4.7</td>
<td>2.04</td>
</tr>
<tr>
<td>195</td>
<td>0.069</td>
<td>1.035</td>
<td>15.00</td>
</tr>
<tr>
<td>196</td>
<td>0.124</td>
<td>1.233</td>
<td>9.94</td>
</tr>
<tr>
<td>197</td>
<td>2.95</td>
<td>2.1</td>
<td>0.71</td>
</tr>
<tr>
<td>198</td>
<td>0.21</td>
<td>0.059</td>
<td>0.28</td>
</tr>
<tr>
<td>199</td>
<td>0.133</td>
<td>0.982</td>
<td>7.38</td>
</tr>
<tr>
<td>200</td>
<td>0.8</td>
<td>1.5</td>
<td>1.9</td>
</tr>
<tr>
<td>201</td>
<td>0.528</td>
<td>5.05</td>
<td>9.6</td>
</tr>
<tr>
<td>202</td>
<td>0.55</td>
<td>19.7</td>
<td>35.8</td>
</tr>
<tr>
<td>203</td>
<td>0.148</td>
<td>1.054</td>
<td>7.12</td>
</tr>
<tr>
<td>204</td>
<td>0.064</td>
<td>1.5</td>
<td>23.4</td>
</tr>
<tr>
<td>205</td>
<td>0.32</td>
<td>0.52</td>
<td>1.6</td>
</tr>
<tr>
<td>206</td>
<td>0.12</td>
<td>0.93</td>
<td>7.8</td>
</tr>
<tr>
<td>207</td>
<td>1.199</td>
<td>28.95</td>
<td>24.1</td>
</tr>
<tr>
<td>208</td>
<td>1.055</td>
<td>0.394</td>
<td>0.4</td>
</tr>
<tr>
<td>209</td>
<td>0.599</td>
<td>32.9</td>
<td>54.9</td>
</tr>
</tbody>
</table>

Thieno[2,3-\textit{d}]pyrimidines 192-209 were evaluated for their inhibitory activities in \textit{pj}DHFR and \textit{h}DHFR. Compound 192 with phenyl and compounds 193 and 194 with 2´-
OMe phenyl and 3'-OMe phenyl substitutions at 6-position respectively, had similar inhibitory activities in hDHFR and pjDHFR. The 4'-OMe substituted compound 195 was however, 15-fold selective for inhibition of pjDHFR with an IC$_{50}$ value of 69 nM suggesting that bulk at 4'-position is not conducive for binding in hDHFR. The disubstituted compounds 196-199 and the 4'-CF$_3$ substituted compound 200 inhibited pjDHFR with low potencies and displayed low selectivity for inhibition. Moving the trifluoromethoxy substitution from 3'-position in 201 to 4'-position in 202 led to 3 fold improvement in selectivity ratio. While the inhibition of pjDHFR was similar for 201 and 202, the change led to 4-fold loss of activity in hDHFR. Similarly for compounds 204 (3',4'-diF) and 207 (2',3',4',5',6'-pentaF), substitution at 4'-position led to improvement in selectivity ratios, with improved binding of 204 at pjDHFR (64 nM). The 2-naphthyl substituted compound 209 was the most selective compound in the series (55-fold), suggesting bulk in the side chain of the thieno[2,3-d]pyrimidine scaffold is not conducive for binding in hDHFR. Compared to unsubstituted compound 192, the 2-naphthyl substitution in 209 causes a 1.5-fold loss in potency in pjDHFR but results in 44-fold loss in activity in hDHFR.

**Methods**

**Effects of compounds on cellular microtubules:** The effect of the compounds on interphase and mitotic microtubules were evaluated in A-10 cells using indirect immunofluorescence techniques, and the EC$_{50}$ (concentration required to cause 50% loss of cellular microtubules) values were calculated from a minimum of three experiments.
**Sulforhodamine B (SRB) assay:** The cytotoxic and antiproliferative effects of the compounds against cancer cells was evaluated using SRB assay. MDA-MB-435 cells were used for determining IC$_{50}$ values (concentration required to cause 50% inhibition of proliferation).

**[$^3$H] Colchicine displacement assay:** The binding of [$^3$H]colchicine to tubulin was performed by the DEAE-cellulose filter technique with a stack of two filters, as described in detail previously. In brief, reaction mixtures contained, in a 0.10 mL volume, 1.0 µM purified tubulin, 5.0 µM [$^3$H]colchicine, potential inhibitor at 5.0 µM, 5% (v/v) dimethyl sulfoxide (the compound solvent), and other components previously found to stabilize the colchicine binding activity of tubulin for prolonged periods at 37°C. Incubation was at 37°C for 10 min, at which time samples were diluted with 2 mL of ice-cold water and poured over the DEAE-filters under mild suction, with several rinses of the reaction vessel and of the filtration chamber. The amount of radiolabel bound to the filters was determined by liquid scintillation counting, and samples containing test compounds were compared to reaction mixtures without compound. The percent inhibition relative to the control was determined for each compound in 2-4 independent experiments.

**Phosphotyrosine ELISA:** Cells used were tumor cell lines naturally expressing high levels of VEGFR-2 (U251). Expression levels at the RNA level were derived from the NCI Developmental Therapeutics Program (NCI-DTP) web site public molecular target information. Briefly, cells at 60–75% confluence are placed in serum-free medium for 18 h
to reduce the background phosphorylation. Cells were always >98% viable by trypan blue exclusion. Cells were then pretreated for 60 min with a dose-response relation of 100-1.4 µM compound followed in ½ Log increments by 100 ng/mL VEGF for 10 min. The reaction was stopped and cells permeabilized by quickly removing the media from the cells and adding ice-cold Tris-buffered saline (TBS) containing 0.05% Triton X-100, protease inhibitor cocktail and tyrosine phosphatase inhibitor cocktail. t was then removed and cells fixed to the plate for 30 min at 60 C with a further incubation in 70% ethanol for 30 min. Cells were exposed to a blocking solution (TBS with 1% BSA) for 1 h, washed, and then a horseradish peroxidase (HRP)-conjugated phosphotyrosine (PY) antibody was added overnight. The antibody was removed, and the cells were washed again in TBS, exposed to an enhanced luminol ELISA substrate (Pierce Chemical EMD, Rockford, IL), and light emission was measured using a UV Products (Upland, CA) BioChemi digital darkroom. Data were graphed as a percent of cells receiving growth factor alone and IC$_{50}$ values were determined from two to three separate experiments (n = 8-24) using non-linear regression dose–response analysis with Prism 5.0 software (GraphPad, San Diego, CA). In each case, the activity of a positive control inhibitor did not deviate more than 10% from the IC$_{50}$ values listed in the text.

Chorioallantoic membrane assay of angiogenesis: The CAM assay is a standard assay for testing antiangiogenic agents. Briefly, fertile leghorn chicken eggs (CBT Farms, Chestertown, MD) were incubated for 10 days. The proangiogenic factors human VEGF-165 and bFGF (100 ng each) were then added at saturation to a 6 mm microbial testing disk (BBL, Cockeysville, MD) and placed onto the CAM by breaking a small hole in the
superior surface of the egg. Antiangiogenic compounds were added 8 h after the VEGF/bFGF at saturation to the same microbial testing disk and embryos allowed to incubate for an additional 40 h. After 48 h, the CAMs were perfused with 2% paraformaldehyde/3% glutaraldehyde containing 0.025% Triton X-100 for 20 s, excised around the area of treatment, fixed again in 2% paraformaldehyde/3% glutaraldehyde for 30 min, placed on Petri dishes, and a digitized image taken using a dissecting microscope (Wild M400; Bannockburn, IL) at 7.5X and a SPOT enhanced digital imaging system (Diagnostic Instruments, Sterling Heights, MI). A grid was then added to the digital CAM images and the average number of vessels within 5–7 grids counted as a measure of vascularity. Sunitinib and semaxanib were used as a positive control for antiangiogenic activity. Data were graphed as a percent of CAMs receiving bFGF/VEGF only and IC50 values calculated from two to three separate experiments (n = 5–11) using non-linear regression dose-response relation analysis.

**DHFR inhibitory assays:** For analysis of inhibitor susceptibility, DHFR was assayed using a continuous spectrophotometric assay. The assay contains a final concentration of 9 µM dihydrofolic acid, 117 µM NADPH, and 6–8 IU of enzyme activity (1 IU D0.005 optical density units/min). The reaction is incubated at 37 °C and change in absorbance at 340 nM, which is related to oxidation of NADPH, is measured over 3 min. The assay under standard conditions is linear over this time span and linear with protein concentration. KCl 150 mM is included in assays of mammalian DHFR. The standard error of the means for these values are 12% or less than the mean value.