Repurposing of human tyrosine kinase inhibitors for neglected diseases: lead discovery for Chagas disease

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Abstract

Chapter 1: Neglected tropical disease, Chagas disease and target repurposing Approach

The term "Neglected Tropical Diseases" (NTDs) most commonly refers to a group of parasitic and bacterial infectious disease affecting more than one billion people globally. There are 13 core diseases in the NTD group that are widespread across the countries from sub-Saharan Africa, Asia and to Latin America. These diseases primarily occur in rural areas, urban slums or conflict zones and affect 2.7 billion people who live on less than $2 per day, causing considerable morbidity and mortality. NTDs cause severe impact on physical and cognitive development, long-term illness, impaired childhood growth and development, adverse outcomes of pregnancy and trap the poor population in a cycle of poverty and disease. Many of these diseases are neglected at the global and local level by the government and private sector owing to the poor return of drug discovery research costs. Also, the emerging resistance to the current therapies can add more burdens to the poor nations.

The focus of this thesis is upon finding new leads for targeting protozoan parasite *Trypanosoma cruzi*, responsible for Chagas disease (CD) by using Target repurposing approach. CD affects nearly 8 million people in Latin America and increasing number of cases reported across Europe and United States. Only two FDA approved drugs are currently available for treating CD. Target repurposing approach is a viable strategy to reduce the time and expense of discovering new therapeutic molecules. In this approach a molecular target in the parasites are matched with homologous human targets that has been previously pursued for drug discovery. We can utilize this knowledge of "druggable" targets in one organism to pursue new potential drug targets in the parasites.

Chapter 2 & 3: Scaffold replacements and head group truncation using *in silico* Models

Our lab has applied target repurposing approach to identify several novel leads against protozoan parasites responsible for causing CD, human African sleeping sickness, leishmaniasis and malaria. Kinases are an extensively pursued class of drug targets to treat several diseases like cancer, immunological and neurological disorders, inflammation and infectious diseases. Lapatinib, an FDA
approved dual tyrosine kinase inhibitor (EGFR/HER2) drug for treating breast cancer was repurposed for its anti-trypanosomal properties. Extensive SAR studies of lapatinib analogs led to sub-micromolar potent compound 1.25 (Figure 1.9) against the parasite T. brucei. Hypothesizing that other parasites express similar kinase pathways, these libraries of compound were screened against other related parasites, which resulted in several lead compounds of micromolar potency. Encouraged by these results, we advanced our work to a scaffold replacement strategy. This resulted in the discovery of a potent lead 2.25b (Figure 2.6) with 0.09 µM activity against T. cruzi. Despite having a good efficacy, the lead compound failed in the in vivo animal studies due to poor physicochemical properties. In an attempt to improve the physicochemical properties, we adopted a head group replacement strategy for the lead compound based on molecular modelling and virtual screening tools. The concept of ligand efficiency (LE) and lipophilic ligand efficiency (LLE/LiPE) were introduced with an aim to identify new leads. Finally, an attempt was made to establish a possible structure activity relationship (SAR) from the screening data of all the library series.

Chapter 4: Future directions

Final chapter summarizes the results of this thesis and provides the futuristic plan for the advancement of the current work to identify potent leads for Chagas disease.
## Table of contents

Acknowledgements**************************************************************************ii

Abstract******************************************************************************iv

Table of Contents***************************************************************************vi

List of Abbreviations***************************************************************************ix

List of Figures******************************************************************************xiii

List of Schemes******************************************************************************xv

List of Tables******************************************************************************xvi

### Chapter 1: Introduction and background

1.1. Neglected tropical disease (NTDs)

  1.1.1. Background of NTDs************************************************************************1

1.2. Chagas disease

  1.2.1. Introduction to Chagas disease************************************************************************3

  1.2.2. Current treatment for Chagas disease************************************************************************5

  1.2.3. Target Product profile for Chagas disease************************************************************************8

1.3. Target repurposing approach

  1.3.1. Target repurposing approach for NTDs************************************************************************8

  1.3.2. Discovery and development of EGFR kinase inhibitors************************************************************************10

  1.3.3. Lapatinib as anti-trypanosomal drug and discovery of 1.25************************************************************************14

  1.3.4. Cross-screening of lapatinib analogs, discovery of 1.26************************************************************************17

1.4. Summary******************************************************************************18

### Chapter 2: Scaffold replacements
2.1. Introduction

2.1.1. Rationale for 3-cyanoquinoline template as EGFR/ErbB kinase inhibitor

2.2. Design and synthesis of 3-cyanoquinoline analogs

2.2.1. Optimization of 3-cyanoquinoline core synthesis

2.2.2. Optimization of head group chemistry and Suzuki coupling reaction

2.3. Biological screening results and discussions

2.4. Pharmacokinetic and animal efficacy studies in Chagas infection model

2.5. Future directions

Chapter 3: Head group truncation and replacement for 2.25b

3.1. Introduction

3.1.1. Design strategy

3.1.2. Conformational model generators: shape/electrostatic similarity search

3.1.3. Results of in silico virtual screening models

3.1.4. Library shaping based on properties

3.1.5. Enumeration of virtual library of head group truncation analogs and computed properties

3.2. Library pilot chemistry using manually-selected headgroup replacements

3.2.1. Design and synthesis

3.2.2. Results and discussion

3.3. Head group truncation library synthesis

3.3.1. Optimization of library synthesis

3.3.2. Execution of library

3.4. Results and discussions

3.5. Summary

vii
Chapter 4: Future directions

4.1. Status and success achieved with lapatinib analogs for anti-typanosomal drug discovery---------58

4.2. Next step for the development of SAR on 3-cyanoquinoline template for Chagas disease---------58

4.2.1. More focused library of head group truncations for better efficacy and physicochemical Properties---------------------------------------------------------------------------------------------------------------------------59

4.2.2 Optimization of the tail end of the lead molecule 2.25b----------------------------------------------------------60

Chapter 5: Experimental section---------------------------------------------------------------------------------------------------------------------------62

References---------------------------------------------------------------------------------------------------------------------------97

Appendix: Representative NMR spectra for final compounds from Chapter 2 and 3---------102
**List of abbreviations**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADME</td>
<td>absorption, distributions, metabolism, and excretion</td>
</tr>
<tr>
<td>AUC</td>
<td>area under the curve</td>
</tr>
<tr>
<td>BF</td>
<td>bloodstream form</td>
</tr>
<tr>
<td>CD</td>
<td>Chagas disease</td>
</tr>
<tr>
<td>ClogD</td>
<td>calculated distribution coefficient at pH = 7.4</td>
</tr>
<tr>
<td>ClogP</td>
<td>calculated partition coefficient, lipophilicity</td>
</tr>
<tr>
<td>CNS</td>
<td>central nervous system</td>
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<tr>
<td>d</td>
<td>doublet</td>
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<tr>
<td>DALY</td>
<td>disability-adjusted life year</td>
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<tr>
<td>DCM</td>
<td>dichloromethane</td>
</tr>
<tr>
<td>DIEA</td>
<td>diisopropylethyl amine</td>
</tr>
<tr>
<td>DME</td>
<td>dimethoxyethane</td>
</tr>
<tr>
<td>DMF</td>
<td>N-N'-Dimethylformamide</td>
</tr>
<tr>
<td>DMF-DMA</td>
<td>dimethylformamide-dimethacetal</td>
</tr>
<tr>
<td>DMSO</td>
<td>dimethylsulfoxide</td>
</tr>
<tr>
<td>DNDi</td>
<td>Drug for Neglected Diseases <em>initiative</em></td>
</tr>
<tr>
<td>DTU</td>
<td>discrete types of unit</td>
</tr>
<tr>
<td>EC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>half maximal effective concentration</td>
</tr>
<tr>
<td>EGFR</td>
<td>epidermal growth factor receptor</td>
</tr>
<tr>
<td>EtOAc</td>
<td>ethyl acetate</td>
</tr>
<tr>
<td>ESI</td>
<td>electrospray ionization</td>
</tr>
<tr>
<td>Et&lt;sub&gt;2&lt;/sub&gt;O</td>
<td>diethyl ether</td>
</tr>
<tr>
<td>GBD</td>
<td>global burden of disease</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>GSK</td>
<td>GlaxoSmithKline</td>
</tr>
<tr>
<td>h</td>
<td>hours</td>
</tr>
<tr>
<td>HA</td>
<td>hydrogen acceptor</td>
</tr>
<tr>
<td>HAT</td>
<td>human African trypanosomiasis</td>
</tr>
<tr>
<td>HD</td>
<td>hydrogen donor</td>
</tr>
<tr>
<td>¹H-NMR</td>
<td>proton Nuclear Magnetic Resonance</td>
</tr>
<tr>
<td>HPLC</td>
<td>high-performance Liquid Chromatography</td>
</tr>
<tr>
<td>HBD</td>
<td>hydrogen bond donors</td>
</tr>
<tr>
<td>HepG2</td>
<td>human liver carcinoma cell line</td>
</tr>
<tr>
<td>HTS</td>
<td>high-throughput screening</td>
</tr>
<tr>
<td>IC₅₀</td>
<td>half maximal inhibitory concentration</td>
</tr>
<tr>
<td>IP</td>
<td>intraperitoneal</td>
</tr>
<tr>
<td>IPA</td>
<td>Isopropanol</td>
</tr>
<tr>
<td>J</td>
<td>coupling constant</td>
</tr>
<tr>
<td>LC-MS</td>
<td>liquid chromatography-mass spectrometry</td>
</tr>
<tr>
<td>LE</td>
<td>ligand efficiency</td>
</tr>
<tr>
<td>LLE</td>
<td>lipophilic ligand efficiency</td>
</tr>
<tr>
<td>Lmj</td>
<td>Leishmania major</td>
</tr>
<tr>
<td>m</td>
<td>multiplet</td>
</tr>
<tr>
<td>MAPK</td>
<td>mitogen-activated protein kinase</td>
</tr>
<tr>
<td>MeCN</td>
<td>acetonitrile</td>
</tr>
<tr>
<td>MeOH</td>
<td>methanol</td>
</tr>
<tr>
<td>MPO</td>
<td>multiparameter optimization</td>
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</tbody>
</table>
MRT mean retention time
MW molecular weight
NCE new chemical entities
NTDs neglected tropical diseases
NMR nuclear magnetic resonance
P. fal *Plasmodium falciparum*
PD pharmacodynamics
POCl₃ phosphorous oxytrichloride
PK pharmacokinetics
ppm parts-per-million
PTK protein tyrosine kinase
q quartet
Ro5 rule of five
rt Retention time
RT Room Temperature
S singlet
SAR structure-activity relationship
Ser serine
SM Starting Material
t triplet
Tbr *T. brucei*
T. b. b *Trypanosoma brucei brucei*
TC₅₀ half-maximal response in the cytotoxicity assay
TFA Trifluoroacetic acid
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>TEA</td>
<td>Triethylamine</td>
</tr>
<tr>
<td>THF</td>
<td>Tetrahydrofuran</td>
</tr>
<tr>
<td>Thr</td>
<td>Threonine</td>
</tr>
<tr>
<td>TLC</td>
<td>Thin-layer chromatography</td>
</tr>
<tr>
<td>TPSA</td>
<td>Topological polar surface area</td>
</tr>
<tr>
<td>TPP</td>
<td>Target product profile</td>
</tr>
<tr>
<td>UV</td>
<td>Ultraviolet</td>
</tr>
</tbody>
</table>
List of figures

Chapter 1: Introduction and background

Figure 1.1 The geographical distribution of the Neglected Tropical Diseases---------------------------------------------2
Figure 1.2 Worldwide distribution of Chagas disease, 2006-2009---------------------------------------------------4
Figure 1.3 Life cycle of the Trypanosoma cruzi parasite inside the human host--------------------------------------5
Figure 1.4 Chemical structures of front-line drugs available for Chagas disease--------------------------------------6
Figure 1.5 Chemical structures of drugs studied for Chagas Diseases-----------------------------------------------7
Figure 1.6 Chemical structure of thalidomide---------------------------------------------------------------------10
Figure 1.7 Chemical structure of dasatinib, drug recommended for treating CML disease------------------------------11
Figure 1.8 Chemical structures of Human PTK inhibitors developed as cancer drugs screened for trypanosomal properties-------------------------------------------------------------------------------------------------------------------14
Figure 1.9 Discovery of 1.25, potent inhibitor of Trypanosoma brucei growth-----------------------------------------16
Figure 1.10 Chemical structures of most potent lapatinib analogs with varying tail groups-------------------------------17

Chapter 2: Scaffold replacements

Figure 2.1 Chemical structure of lapatinib----------------------------------------------------------------------------19
Figure 2.2 Summary of library templates proposed based on known kinase scaffolds----------------------------------20
Figure 2.3 Some examples of potent 3-cyanoquinoline analogs with different kinase inhibitory activity--------------------------------------------------21
Figure 2.4 Similar charge distribution observed for substituted quinazoline and 3-cyanoquinolines-----------------22
Figure 2.5 Chemical structures of boronates synthesized-------------------------------------------------------------26
Figure 2.6 Chemical structures of library of eight analogs synthesized---------------------------------------------27
Figure 2.7 Mean plasma concentration-time profile of 2.25b following a single IP administration to female BALB mice-------------------------------------------------------------------------------------------------------------------31
Figure 2.8 In vivo animal study results for 2.25b---------------------------------------------------------------------33
Chapter 3: Head group replacements for NEU-924

Figure 3.1  Screening of hPPB % free vs MW for 250 conformers, highlighting 16 amines chosen for synthesis---------------------------------------------------------------37

Figure 3.2  List of monomers chosen from Frontier Scientific-----------------------------------------------39

Figure 3.3  Plot of lipophilicity and molecular weight for 94 conformers, highlighting the 16 library compounds chosen for synthesis---------------------------------------------------------------------------------39

Figure 3.4  Some of the library amines (final product) decomposed during lyophilization----------------------53

Chapter 4: Future directions

Figure 4.1  Plot showing the library amines chosen based on rank and increased lipophilicity-------------------62

Figure 4.2  Summary of planned future work for 2.25b----------------------------------------------------------63

Figure 4.3  Summary of SAR at C-7 of 3-cyanoquinoline template--------------------------------------------------64
List of Schemes

Chapter 2: Scaffold replacements

Scheme 2.1  Synthetic route of 3-cyanoquinoline core molecule 2.13-------------------------------22
Scheme 2.2  Alternate route proposed for the synthesis of 2.13---------------------------------23
Scheme 2.3  Synthesis of head group analogues 2.21 followed by Suzuki coupling reactions-------24
Scheme 2.4  Synthesis of head group analogues 2.20---------------------------------------------25
Scheme 2.5  Synthesis of boronic ester analogues 2.23-------------------------------------------25

Chapter 3: Head group replacements for NEU-924

Scheme 3.1  Proposed route for library synthesis with improved physicochemical properties-------36
Scheme 3.2  Head group substitution to the core molecule 2.14 followed by Suzuki coupling--------42
Scheme 3.3  General route proposed for library synthesis-------------------------------------------46
Scheme 3.4  Alternate route to the synthesis of 3.3-----------------------------------------------47
Scheme 3.5  Modified approach for the synthesis of 3.3-------------------------------------------48
Scheme 3.6  Synthesis of the intermediate 2.23b------------------------------------------------50
Scheme 3.7  Optimized route for synthesis of 3.3-----------------------------------------------50
Scheme 3.8  Optimized route for library synthesis-----------------------------------------------52
List of Tables

Chapter 1: Introduction and background

Table 1.1 Different PTK inhibitor drugs displays varied growth inhibition concentration (50%) on bloodstream *T. brucei*---------------------------------------------------------------13

Table 1.2 Inhibitory concentrations of *T. brucei brucei* Lister 427 cells---------------------------------------------------------------15

Table 1.3 Analogs of lapatinib showing varied potency against different parasites-----------------------------------------------17

Chapter 2: Scaffold replacements

Table 2.1 Screening results for the eight analogs built upon 3-cyanoquinoline template------------------------29

Table 2.2 Computed physicochemical properties for the eight analogs---------------------------------------------------------------30

Table 2.3 Pharmacokinetic parameters of 2.25b in plasma following a single intraperitoneal administration in female BALB/c mice---------------------------------------------------------------------32

Chapter 3: Head group replacements for 2.25b

Table 3.1 Computed properties of 16 conformers chosen for synthesis from virtual screening------------------41

Table 3.2 Screening results for head truncation group series using manually-selected amines against *T. cruzi*------------------------------------------------------------------44

Table 3.3 Cross screening results against other trypanosomal parasites for head group truncation series---------------------------------------------------------------44

Table 3.4 Physicochemical properties of the head group truncation series-----------------------------------------------45

Table 3.5 Reaction conditions screened for the synthesis of 3.3---------------------------------------------------------------46

Table 3.6 Reaction conditions screened for optimizing 3.5---------------------------------------------------------------47

Table 3.7 Reaction conditions for optimizing 3.6---------------------------------------------------------------48

Table 3.8 Reaction conditions attempted for the monomers coupling to the intermediate 2.14-----------------40

Table 3.9 Reaction conditions screened for library synthesis---------------------------------------------------------------51

Table 3.10 Screening results against *T. cruzi*---------------------------------------------------------------------56

Table 3.11 Cross-screening results of related parasites---------------------------------------------------------------57
Table 3.12  Calculated properties for library series

| Calculated properties for library series | |
Chapter 1: Introduction and background

1.1 Neglected tropical diseases (NTDs)

1.1.1 Background of NTDs

NTDs are a group of infectious communicable diseases caused by a diverse group of bacteria, parasitic worms and vector-borne protozoa infecting more than 1 billion people worldwide [1, 2]. NTDs are broadly categorized by The World Health Organization (WHO) into 17 different types which affect the poorest and marginalized population in low-resource setting across 149 countries and territories. Among them there are 13 core NTDs that are particularly targeted due to their global prevalence and neglect by the public and private sector [3]. They are ascariasis, buruli ulcer, Chagas’ disease, dracunculiasis, hookworm infection, human African trypanosomiasis, leishmaniasis, leprosy, lymphatic filariasis, onchocerciasis, schistosomiasis, trachoma, and trichuriasis [1]. Annually, the estimated impact of NTDs is over 534,000 deaths and they are also a major cause of disease burden in lower income countries resulting in 57 million disability adjusted life years (DALY), which are estimated to be higher than malaria and tuberclosis [2, 4, 5]. DALY is calculated as the sum of the Years of Life Lost (YLL) due to premature mortality in the population and the Years Lost due to Disability (YLD) for people living with the health conditions [6].

Some of the NTDs are termed “tool-deficient” as they are (1) difficult and costly to manage (2) limited availability of appropriate control tools (3) lower investment in research and development and (4) relatively unknown to general public [1, 7]. NTDs account for a major burden of misery, disability and poverty for the people living in remote rural areas with limited access to diagnosis and treatment [4]. Some cases have also been reported in the United States, disproportionately affecting impoverished populations in states like Florida and Louisiana. Hence, cost-effective treatments are imperative to combat these diseases [8].

The successes achieved so far in combating these illnesses prove the emerging collaborations by several global pharmaceutical companies and the international community to provide inexpensive multi-
drug therapy which resulted in the elimination of several of the major NTDs [6, 7]. However this advancement has not changed the fact that more than half the world’s population is at risk from one or more of the other NTDs [7] (Figure 1.1). Drugs that are currently used to treat them are generally toxic, expensive and some are lethal. Most of them have reduced efficacy due to increased drug resistance among strains of several parasites [1, 9]. Research and development to provide safe and effective drugs is impeded by the high cost of “traditional” drug discovery approach and lack of financial incentives for drug discovery effort against NTDs [4, 10].


The main objective of this thesis is focused on a particular group of NTDs belonging to the trypanosomatid family of the single-flagellar Kinetoplastida order which are concentrated exclusively in the destitute populations of underdeveloped tropical countries [11]. The parasites of this trypanosomatid family cause several major diseases in humans which include African trypanosomiasis (sleeping sickness) caused by *Trypanosoma brucei*, American trypanosomiasis (Chagas Disease) caused by *T. cruzi* and leishmaniasis caused by various species of *Leishmania* [12]. According to DNDi (Drug for Neglected Diseases initiative), the number of new therapeutic drugs developed for NTDs account for only
37 (4%) of the 850 new therapeutic products registered in the period of 2000-11 and only 4 new chemical entities (NCE) approved for NTD accounting for 1% of the total 336 NCE approved.

Globally, there is no universal methodology to control these illnesses and the current focus is on early detection, combination therapies using existing drugs, and vector control/animal reservoir control [1]. In recent times, there have been no major breakthroughs reported in terms of developing safe, cheap and effective drugs and this is largely attributed to underinvestment by the majority of research-based pharmaceutical companies.

1.2 Chagas disease (CD)

1.2.1 Introduction to Chagas disease

Chagas disease or American trypanosomiasis is a major neglected tropical infectious disease endemic in 21 countries (Figure 1.2) across South and Central America with an increasing number of cases reported in non-endemic countries like the US, Europe, Australia and Japan [13]. In the US alone, there are now more than 300,000 cases reported, transforming Chagas into a global medical challenge [14-16]. According to the WHO, nearly 8 million people living in rural, impoverished areas are affected by this disease and 100 million people are at risk mainly in Latin America [17].

Chagas disease is caused by the protozoan hemoflagellate parasite Trypanosoma cruzi commonly transmitted by the blood-feeding triatomine bug (kissing bug) insect vector followed by autoinoculation [18]. Of more than 150 species of triatomines that are responsible for spreading this disease, two species, Triatoma infestans and Rhodnius prolixus are the major vectors as they are well adapted to the human conditions. When a triatomine bug infected with T. cruzi feeds on host blood, it releases the parasite by defecating into the fresh bite wounds. It can also be transmitted orally through food, from mother to child during pregnancy and also by blood transfusion or organ transplantation [15, 19]. According to a report from the DNDi, Chagas is the leading cause of cardiomyopathy, resulting in more death in Latin America than any other NTDs and malaria. The anti-parasitic drugs that are currently used can prevent progression of the infection and are more successful during the acute phase of the disease.
However, their efficacy diminishes during the chronic phase of the condition. If left untreated, the infection tends to damage the heart and digestive system potentially leading to death [20].


Chagas disease in human occurs in two stages: an acute stage, which occurs shortly after initial infection, and a chronic stage which develops over a long period of time. Treatment of Chagas disease is complicated due to the complex nature of the life cycle of the parasite (Figure 1.3). During the acute phase of infection the parasite cycles between the non-replicative trypanastigotes found in vector gut and replicative intracellular amastigotes found in host cell [13, 21]. The intracellular amastigotes divides by binary fission and transform into trypanastigotes to be released into the bloodstream, and can then infect other new cells. The acute stage is evident by mild symptoms like fever, swollen lymph nodes and rash lasting for few weeks which can be treated with medications. The survival rate for the acute stage is higher and rare cases of death are reported in children and pregnant women. If not treated in the acute stage, these diseases can develop into a chronic stage which can be symptomatic or asymptomatic [20]. Even with treatment, infections persist to develop into an asymptomatic phase for a longer time inside the human host to result in the chronic form of the disease. The chronic stage of Chagas disease may take 15-20 years to develop inside the human body, causing considerable damage to heart and gastrointestinal tract [13]. Among individuals with chronic phase of the disease, 60-80 % of people may
never develop symptoms (indeterminate chronic Chagas) and 30-40% may develop symptoms (determinate chronic Chagas) such as cardiac damage, dementia and chronic encephalopathy, eventually leading to death [20].

Figure 1.3 Life cycle of the *Trypanosoma cruzi* parasite inside the human host, cited from DPDx-Laboratory Identification of Parasitic Diseases of Public health Concern, CDC.

1.2.2 Current treatment for Chagas disease

Current treatment to treat the acute phase of infection depends solely on high doses of benznidazole (1.1) and nifurtimox (1.2) (Figure 1.4) for 30-60 days resulting in undesirable side effects including toxicity, drug resistance, plus ineffectiveness against chronic condition [17, 22]. These two medications are not widely available in non-endemic countries. For example, they are not FDA approved drugs in USA and require special request from the Centers for Disease Control (CDC). The clinical evaluation of this disease is further complicated by the fact that this parasite presents high genetic
variability, classified into six discrete types of units. Such diversity allows the parasite to survive and elude the different form of drug treatment [13, 19].

The current chemotherapy for Chagas disease depends only on the above mentioned registered drugs for parasitic treatment, namely benznidazole and nifurtimox, that act by generating free radicals and/or by electrophilic metabolites in T. cruzi cells [23, 24]. Both of the drugs are orally administered nitroheterocyclic compounds that require prolonged treatment during the acute phase. Their limitations include severe side effects, poor patient tolerance, contraindication in pregnancy, low efficiency for the chronic phase, peripheral neuropathy and bone marrow suppression [19]. Despite these extensive limitations, benznidazole is the first line of drug choice for Chagas disease over nifurtimox for the acute phase with 80% of therapeutic success reported [18, 20]. The drug usage dates back to the late 1960s but the mechanism of action has not been not fully elucidated. The drug fails in the parasitological cure (complete elimination of the parasite) of chronically infected patients and, in addition, drug resistance by several strains of T. cruzi parasite is also an emerging problem. In particular, some of the Trypanosoma (Schizotrypanum) cruzi strains like Y (DTU II), VL-10 (DTU II), and Colombian (DTU III), which are capable of causing high parasitemia and 100% mortality, show complete resistance to benznidazole during the chronic phase of the infection [19].

![Figure 1.4 Chemical structures of front-line drugs available for Chagas disease](image-url)

**Figure 1.4** Chemical structures of front-line drugs available for Chagas disease

Other drugs that have been in research studies for Chagas disease include repurposed antifungal CYP51 inhibitor drugs, such as theazole derivatives shown in **Figure 1.5**. Earlier, the first generationazole drugs like imidazoles (such as ketoconazole, miconazole and clotrimazole) and triazoles (such as Itraconazole (1.3) and fluconazole (1.4)) were found to be potent against the parasite but failed in the
animal model studies. An experimental azole drug D0870 that was developed as anti-fungal cured chronically infected mice with T. cruzi but was withdrawn due to undesirable side effects [20].

![Chemical structures of drugs studied for Chagas Disease](image)

**Figure 1.5** Chemical structures of drugs studied for Chagas Disease

The second-generation azole drugs like voriconazole (1.5) and posaconazole (1.7) were advanced into clinical studies and showed promising efficacy against the parasite, with the latter being more potent than the former [13]. Further studies show that posaconazole, which is also active against T. cruzi resistant strains in addition to their favorable pharmacokinetic properties, can be successfully repurposed for Chagas disease. But the high cost of the drug is a setback for it to be used widely in the low income countries [20]. Another azole drug, ravuconazole (1.6), having a 1 nM EC$_{50}$ against intracellular amastigotes were initially taken as a promising lead candidate for Chagas disease. However, the long half-life and large volume of distribution makes it as a less favorable lead drug [20, 25].

A new anti-fungal triazole derivative E1224, a prodrug of ravuconazole with improved solubility and oral bioavailability, has been shown to have potent activity against the T. cruzi parasite. With its benign safety profile, favorable pharmacokinetics and affordability, E1224 was considered a priority candidate for
clinical development and alternate treatment for CD [20, 26]. But recent studies showed that the drug failed in the phase 2 studies for efficacy and safety profile owing to the relapse of parasitemia levels. Further studies for combination with benznidazole were in progress recently, though the clinical trial results have not yet been released publically. There are currently several other CYP51 inhibitor drugs being studied for their anti-trypanosomal property including albaconazole, TAK-187, tipifarnib and its analogues [20].

1.2.3 Target Product Profile for Chagas disease

A Target Product Profile (TPP) describes the critical attributes required for the drug candidates to become a suitable end product better than the existing drug. TPP is an essential tool that ensures the right product is developed to meet all the requirements for a new therapy [27, 28].

According to the CDC, the ideal TPP requirements for Chagas Disease are outlined as below

- Active in both acute and chronic stage of diseases
- Active against all strains of T. cruzi in particular Tcl, TcII, TcV and TcVI strains
- Safe and effective for all age groups
- Better safety profile than existing drugs
- Clinical efficacy superior to benznidazole with little or no side effects
- Ideal oral bioavailability of the candidate
- Length of duration of treatment better than the current treatments
- Affordable, easy-to-use and safe pediatric formulations
- Stable in tropical climates

1.3 Target repurposing approach

1.3.1 Target repurposing approach for NTDs

NTDs are poverty-promoting and stigmatizing conditions affecting rural and economically disadvantaged populations with highest rates of infections. Development of drugs based on novel
chemical matter is a risky undertaking as it takes more than a decade for a new drug to be launched into the market, following a high failure rate in clinical trials [29]. Several important contributions to combat these diseases have been made by many World Health Assembly resolutions, the UN Millennium Development Goals and other public-private organizations. Only low financial return for pharmaceutical and biotechnologies was observed and is mainly attributed to the poor funding allocations for NTD in the drug discovery program in R&D. Hence a novel approach to accelerate the discovery of new therapeutic leads for NTDs is needed.

One such approach is the Target Repurposing or repositioning strategy which is used in our lab for finding new drugs for NTDs. The project described in this thesis is based on such an approach, which relies on identifying homologous biological targets in pathogenic parasites to that of known human targets that have been already pursued for other drug discovery program to treat other medical conditions. The wealth of knowledge available (medicinal chemistry and biochemistry, for example) for these human targets can be repurposed to find an alternate therapeutic effects for parasite target. Target Repurposing is based on the concept that drug like molecules are known to bind specific protein and these druggable protein are the target for drug discovery in industrial settings [29]. This is further supported by the elucidation of genomes of several pathogens showing striking similarities between proteins of various organisms including humans. This will help to find the parasitic targets that are similar to the human target with established drug discovery programs. The next step is to find the importance of such homologs target required for the survival or replication of the trypanosomal parasites. Thus, a drug developed for particular human protein target can be repurposed for similar parasite target.

In our work, “drugs” are small (MW < 500) organic molecules that bind to biomolecular targets to activate or inhibit their functions. Finding new applications for approved drugs saves the time and costs associated with it. One approach is to identify a secondary or “off-target” for a drug that will provide desirable new drug-target interactions, termed as a "known compound-new target” approach. For example, the drug thalidomide (1.8) which was initially used to treat nausea and insomnia in pregnant women, was later approved as a treatment for leprosy and multiple myeloma owing to its anti-angiogenic
and immunomodulatory effects [30] (Figure 1.6). Another approach is to establish a relevance of “known drug target to new diseases” as these targets can be involved in several biological processes. In addition, repurposed compound can enter the clinical trials more rapidly at a less cost than the new chemical entity.

![Chemical structure of thalidomide](image)

**Figure 1.6 Chemical structure of thalidomide**

Target repurposing is a logical process to speed up the drug discovery for the NTDs by identifying the similarity between the human druggable targets to that of the pathogens. This is based on the fact that the approved drugs whose pharmacokinetics and toxicity profiles that are well-established for the human targets can be utilized to inhibit similar biological targets in the pathogenic parasites. The wealth of knowledge gathered in research areas such as medicinal chemistry, biochemistry and structural biology for these targets can be utilized for repurposing them for any anti-parasitic activity [29].

### 1.3.2 Discovery and development of EGFR kinase inhibitors

Kinases are an important class of drug targets that are directly involved in cell proliferation and growth. To date, there have been over 30 distinct kinase targets studied for several diseases like cancer, inflammation, neurological and infectious diseases [31, 32]. Small molecule kinase inhibitors have been developed to understand the function of specific protein kinases and they are emerging class of targeted therapeutic agents [33]. The human genome encodes 518 different kinases and they are the largest group of druggable targets [31, 33]. In addition, inhibition of selective kinase activity in normal cells is well tolerated, for example, the drug dasatinib (1.9, Figure 1.7), approved for treating chronic myelogenous leukemia (CML), was shown to have a fewer side effects in spite of having several off-target mechanism (inhibits all nine members of Src family of kinases and other TKs) [31]. Numerous protein tyrosine kinase (PTKs) inhibitor drugs like lapatinib (inhibits EGFR/HER2, 1.10), canertinib (pan-inhibitor of EGFR, 1.11)
and AEE788 (inhibits EGFR and VEGFR, 1.12) were developed for clinical use to treat different form of cancers (Figure 1.8) [34, 35].

![Chemical structure of dasatinib](image)

**Dasatinib (1.9)**

**Figure 1.7** Chemical structure of dasatinib, drug recommended for treating CML disease

These small molecule kinase inhibitors are aromatic heterocyclic molecules that bind to the hinge region of the targeted kinases [31, 33, 34]. These molecules can be modified using medicinal chemistry approaches to inhibit a specific kinase with high selectivity. Many of the parasite kinases are homologous to those of human kinases that have been pursued as druggable targets. Hence FDA-approved specific kinase inhibitor drugs for human disease with acceptable physicochemical properties and known toxicity profiles can be used as a lead for new parasitic infectious diseases treatments [29]. The trypanosomatid genome codes for a wide range of protein kinases with studies, suggesting 176, 190 and 199 protein kinases encoded in *T. brucei*, *T. cruzi* and *Leishmania major* genomes respectively [12]. Some of the kinases expressed in *T. brucei* (glycogen synthase kinase-3, phosphoinositol-3-kinases/TOR and Aurora kinase 1) have been pursued for drug discovery research and identified as druggable targets [36-38]. Moreover, kinase inhibitor drugs were shown to have multiple off-target kinetic interactions which allow exploring alternative therapeutic effects for these approved drugs [31].

These human tyrosine kinase inhibitor drugs were not studied widely as an anti-parasitic compounds since they primarily target receptor tyrosine kinases, which are not expressed in the trypanosomatids [12, 36]. Indeed, research has determined the absence of EFGR/VEGFR kinases in the genome of the parasite [34]. The presence of Tyr-phosphorylated proteins suggests that Tyr-phosphorylation must be performed by dual-specificity enzymes that act on Ser/Thr as well as Tyr residues [34, 36]. Further studies suggest that the enzymes with “EGFR-like” kinase domains are present
in these parasites and the inhibitors of EGFR/HER2 drugs (eg. canertinib, lapatinib, AEE788) show low micromolar activity against *T. brucei* [36, 39]. Also, it has been reported that trypanosome protein kinases bind some PTK drugs like lapatinib (1.10), C1-1033 (1.11) and AEE788 (1.12) used for the treatment of cancer [31]. In addition, tyrosine kinase inhibitor Tryphostin A47 inhibits transferrin protein which is required by the parasite for the uptake of iron for its growth [36]. These results show that drugs targeted against PTKs could be a good lead for anti-trypanosome drug discovery.

Based on the above findings, a focused screening of several PTK inhibitors was performed in order to assess for anti-trypanosomal activity (Figure 1.8) [40]. Lapatinib inhibited bloodstream *T. brucei* with EC$_{50}$ (half-maximal effective concentration) of 1.5 µM, and Imatinib was relatively inactive with EC$_{50}$ of over 10 µM [40] (Table 1.1). From these results, three PTK inhibitors (1.10, 1.11, 1.12) were chosen for further studies owing to their predictable plasma concentration levels after oral administration in mice [36, 40]. In addition, bioinformatics and chemical proteomics prediction were made for the three PTK inhibitors to find the novel trypanosomal target for these drugs [34]. Based on the results of these analyses, lapatinib was chosen as promising lead candidate as it can kill trypanosomes after a short period of incubation (3 h) and also cured 25% of infected mice as demonstrated in a mouse model of HAT better than other drugs like C1-1033 and AEE788 [40]. In addition, lapatinib binds to four trypanosome protein kinases (TbLBPK1, TbLBPK2, TbLBPK3, and TbLBPK4) that are essential for parasite replication [34]. These drugs are orally bioavailable and have established safety and toxicity profiles. These data suggest that the tyrosine kinase inhibitor drugs that are approved for mammalian diseases can also be pursued as anti-trypanosomal agents [40].
Table 1.1 Different PTK inhibitor drugs displays varied Growth Inhibition concentration (50%) on Bloodstream *T. brucei* [40]

<table>
<thead>
<tr>
<th>Drugs</th>
<th><em>T. brucei</em> EC&lt;sub&gt;50&lt;/sub&gt; (µM)</th>
<th>Target Kinase in Humans</th>
<th>HepG2 TC&lt;sub&gt;50&lt;/sub&gt; (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1-1033</td>
<td>1.4</td>
<td>EGFR</td>
<td>5.4</td>
</tr>
<tr>
<td>Erlotinib</td>
<td>1.9</td>
<td>EGFR</td>
<td>-</td>
</tr>
<tr>
<td>PK1-166</td>
<td>1.3</td>
<td>EGFR</td>
<td>-</td>
</tr>
<tr>
<td>Lapatinib</td>
<td>1.5</td>
<td>EGFR/HER2</td>
<td>&gt;20</td>
</tr>
<tr>
<td>AEE788</td>
<td>2.5</td>
<td>EGFR, VEGFR</td>
<td>13.8</td>
</tr>
<tr>
<td>Axitinib</td>
<td>2.0</td>
<td>VEGFR, PDGFR</td>
<td>-</td>
</tr>
<tr>
<td>Sunitinib</td>
<td>1.3</td>
<td>VEGFR, PDGFR</td>
<td>-</td>
</tr>
<tr>
<td>Imatinib</td>
<td>&gt; 10</td>
<td>c-Abl, PDGFR, c-Kit, c-Src</td>
<td>-</td>
</tr>
</tbody>
</table>

TC<sub>50</sub>: half-maximal response in cytotoxicity assay
1.3.3 Lapatinib as anti-trypanosomal drug and discovery of 1.25

Lapatinib (4-aniloquinazoline scaffold) is one of the most selective among 38 kinase inhibitors tested against 317 human enzymes [39]. A set of nine quinazoline based EGFR inhibitors donated by GSK were examined in growth inhibition assays for trypanocidal activities. These panels of compounds were screened against culture of *T. brucei brucei* Lister 427 cells and they were found to show different range of potency against the parasite [36] (Table. 1.2).
Table 1.2 Inhibitory concentrations of *T. brucei brucei* Lister 427 cells [36]

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
<th>R&lt;sub&gt;1&lt;/sub&gt;</th>
<th>GSK #</th>
<th>Tbb EC&lt;sub&gt;50&lt;/sub&gt; (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.16</td>
<td>H</td>
<td></td>
<td>GW58337A</td>
<td>0.41</td>
</tr>
<tr>
<td>1.17</td>
<td>F</td>
<td>Pr&lt;sub&gt;2&lt;/sub&gt;O&lt;sub&gt;2&lt;/sub&gt;S—NH—</td>
<td>GW601906A</td>
<td>0.43</td>
</tr>
<tr>
<td>1.18</td>
<td>F</td>
<td>-iPr&lt;sub&gt;2&lt;/sub&gt;O&lt;sub&gt;2&lt;/sub&gt;S—NH—</td>
<td>GW633460A</td>
<td>0.48</td>
</tr>
<tr>
<td>1.19</td>
<td>F</td>
<td>MeO&lt;sub&gt;2&lt;/sub&gt;S—N—CN—</td>
<td>GW616030X</td>
<td>0.52</td>
</tr>
<tr>
<td>1.20</td>
<td>F</td>
<td>PhO&lt;sub&gt;2&lt;/sub&gt;S—O—</td>
<td>GW615311X</td>
<td>0.55</td>
</tr>
<tr>
<td>1.21</td>
<td>H</td>
<td>MeO&lt;sub&gt;2&lt;/sub&gt;S—NH—</td>
<td>GW580496A</td>
<td>0.56</td>
</tr>
<tr>
<td>1.22</td>
<td>F</td>
<td></td>
<td>GW576924A</td>
<td>0.60</td>
</tr>
<tr>
<td>1.23</td>
<td>F</td>
<td>MeO&lt;sub&gt;2&lt;/sub&gt;S—B&lt;sub&gt;n&lt;/sub&gt;—N—</td>
<td>GW616907X</td>
<td>1.51</td>
</tr>
<tr>
<td>1.10</td>
<td>F</td>
<td>MeO&lt;sub&gt;2&lt;/sub&gt;S—NH—</td>
<td>Lapatinib</td>
<td>1.54</td>
</tr>
</tbody>
</table>

The screening data in Table 1.2 shows that the derivatives of 4-anilinoquinazolines drugs were able to inhibit the parasite growth at low- and sub-micromolar concentrations. Of all, lapatinib had a EC<sub>50</sub> (drug concentration to inhibit *T. brucei* replication by 50%) of 1.5 µM. In addition, it was more selective...
towards the parasite cell to that of human as measured on a human HeLa cell line for toxicity [40]. Lapatinib was selected for further broad SAR studies to create a virtual library of analogs for trypanosomal properties.

From the initial set of 10 compounds synthesized in our lab by Dr. Caitlin Karver, an equipotent compound 1.24 (NEU-369) (EC$_{50}$ = 1.39 µM) to that of lapatinib with a better HepG2 cell growth (TC0 > 15 µM) was identified. Retaining the newly identified tail group, further SAR studies were performed to explore the effect of substituents on aniline head group and that of the linker in the tail group. This focused library of analogs (Dr. Gautam Patel) led to a most potent and selective compound 1.25 (NEU-617) (T. brucei EC$_{50}$ = 0.042 µM and HepG2 TC$_{50}$ > 20 µM) (Figure 1.9). Though the subsequent study on this compound in a mouse model of HAT had resulted in a modest efficiency in reducing parasitemia, it showed high plasma protein binding (99.6%) and poor CNS exposure of the compound [36]. The compound’s high molecular weight and high lipophilicity were attributed towards the poor brain penetration for 1.25, as well as the lack of efficacy.

Figure 1.9 Discovery of 1.25, potent inhibitor of Trypanosoma brucei growth [36].

Thus, a new anti-trypanosomal lead compound was identified by a focused screening of established human EGFR inhibitor drugs against bloodstream of T. brucei cell cultures, followed by a short optimization campaign. The approach avoided the need for extensive screening campaign in order to arrive at a lead compound. Future work against T. brucei has been focused on further optimization of 1.25 to reduce its high molecular weight, clogP, plasma protein binding and to increase CNS exposure.
1.3.4 Cross screening of analogs of lapatinib, discovery of 1.26

Since the analogs of lapatinib show a considerable anti-*Trypanosoma brucei* activity, this library of compounds were tested against other kinetoplastid parasites like *T. cruzi*, *and Leishmania major* and also against cultures of *Plasmodium falciparum* (the causative agent of malaria) assuming that these pathogens are related with a distinct similarity in their kinomes [12]. This cross screening led to the discovery of analog 1.26 (NEU-628) having 0.5 µM (Figure 1.10) activity against the *T. cruzi* parasite, the most potent compound against this parasite in the library tested (Table 1.3). The difference between molecules 1.25 and 1.26 is that the tail group at C-6 of the quinazoline has been replaced with bulky basic and lipophilic N-methyl homopiperazine phenylsulfonamide substituent, retaining the same lapatinib head group.

![Chemical structures of most potent lapatinib analogs with varying tail groups](image)

**Figure 1.10** Chemical structures of most potent lapatinib analogs with varying tail groups

This confirms the hypothesis that the various trypanosomatid parasites, though having distinct genomic differences, show general sensitivity against similar kinase-directed inhibitors, albeit with subtle differences in SAR.

<table>
<thead>
<tr>
<th>Compound</th>
<th><em>T. brucei</em> EC$_{50}$ (µM)</th>
<th><em>T. cruzi</em> EC$_{50}$ (µM)</th>
<th>HePG2 TC$_{50}$ (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.25 (NEU-617)</td>
<td>0.042</td>
<td>1.79</td>
<td>&gt;25</td>
</tr>
<tr>
<td>1.26 (NEU-628)</td>
<td>0.81</td>
<td>0.51</td>
<td>1.81</td>
</tr>
</tbody>
</table>
1.4 Summary

Chagas disease is a major neglected parasitic endemic disease in Latin America, spreading across non-endemic countries rapidly. Current treatment relies only on two drugs developed during 1960s that are not effective in the late chronic phase of the disease and that pose severe side effects and resistant complications. In a search to find a new therapeutics for several NTDs, we have applied a target repurposing approach to find novel trypanosomal targets for several EGFR/HER2 kinase inhibitor drugs. Among them, lapatinib has showed modest activity against T. brucei cell line which led to further optimization of 4-anilinoquinazoline scaffold as a lead for anti-trypanosome drugs. From a broad SAR study of focused library of these analogs, a most potent compound 1.25 against T. brucei was identified. These libraries of compounds were cross screened against related parasites like T. cruzi and Leishmaniasis spp., since they were shown to have highly orthologous enzymes and assumed they will have similar kinetic pathways. This led to the discovery of most potent compound 1.26 against the parasite T. cruzi.

With this in mind, our focus shifted to the replacement of the 4-anilinoquinazoline scaffold for several other EGFR/HER2/Src kinase inhibitors scaffolds in order to determine the importance of the core molecule for trypanosomal activity. Using the knowledge of isosteric replacements and analogue synthesis approach, we envisioned to initiate a lead scaffold replacement campaign. This study, described in subsequent chapters of this thesis, includes design strategy; SAR studies, synthesis and optimization to identify a potent lead molecule against the T. cruzi parasite, causative agent of Chagas Disease.
Chapter 2: Scaffold Replacements

2.1 Introduction

From the previous work done in our lab as described in Chapter 1 of this thesis, a novel analog (1.25) of dual tyrosine kinase inhibitor drug lapatinib was designed and synthesized as a lead for antitrypanosomal drug discovery [36]. We envisioned that these libraries of analogs screened against T. brucei cell cultures would have efficacy against other related protozoan parasites. The subsequent screening results led to the discovery of several potent analogs against T. cruzi, Leishmania and Plasmodium falciparum. These libraries of compounds obtained were based on a broad SAR studies focusing on the head and tail group of the lapatinib molecule retaining the core quinazoline moiety (Figure 2.1) [34, 36].

Figure 2.1 Chemical structure of lapatinib

Hence, the next step was to determine the importance of this quinazoline scaffold for trypanosomal activity and further optimization for increased effectiveness against all other trypanosomal parasites. This can be achieved by a technique called “scaffold hopping” to identify potent and selective core templates with similar inhibitory properties [41, 42]. The plan was to design the library of analogs based upon the kinase inhibitor templates that were well established in the literature as inhibitors of tyrosine kinases related to EGFR [31, 39]. Substituted quinazolines are known dual tyrosine kinase inhibitor (epidermal growth factor receptor (EGFR) and ErbB-2 (HER-2)) chemotype [41, 43]. These belong to the family of Type 1 receptor tyrosine kinases (RTKs)[44]. Some of the other scaffolds were chosen by the team based on this hypothesis are summarized in Figure 2.2 [42, 44, 45]
Figure 2.2 Summary of library templates proposed based on known kinase scaffolds

For each core template, an initial virtual library was generated based on the best possible head and tail group for each parasite and all the analogs were cross-screened against all four different parasites.

2.1.1 Rationale for 3-cyanoquinoline template as EGFR/ErbB kinase inhibitor

Several small molecule kinase inhibitors that were specifically designed to inhibit both EGFR and Her2 kinases were shown to have predominantly quinazoline or other fused bicyclic aromatic compounds as core structure (Figure 2.2) [43, 45]. The substituted templates shown above were also well-known inhibitors of several other kinases and some of these inhibitors were shown to have inhibitory activities against certain kinases that have mutated and developed resistance to conventional inhibitors molecules [46, 47]. For example Kwak and coworkers have shown that compounds 2.1 (EKB-569) and 2.2 (HKI-272), both 3-cyanoquiniline-based irreversible tyrosine kinase inhibitors to have activity against erlotinib and gefitinib-resistant cell lines in addition to having potency against some of the other erbB kinase family receptors (Figure 2.3). They were also selective inhibitor of EGFR (2.1) and HER2 (2.2) kinases and are currently in phase 3 clinical trials for advanced stage solid tumors overexpressing EGFR or HER2 kinases.
Based on the medicinal chemistry literature, we identified that the 3-cyanoquinoline core has broad utility as inhibitors of Ser/Thr kinases (e.g., the CDKs and CHKs), receptor tyrosine kinases (e.g., EGFR/HER subfamily), non-receptor tyrosine kinases (e.g., Src family), and MEK (mitogen-activated protein kinase, MAPK) (Figure 2.3) [48-50]. The compounds’ differential selectivity for the kinases depends on the nature of the substituent at varied positions on the molecule [51, 52].

![Chemical structures of potent 3-cyanoquinoline analogs with different kinase inhibitory activity](image)

**Figure 2.3** Some examples of potent 3-cyanoquinoline analogs with different kinase inhibitory activity
Figure 2.4 Similar charge distribution observed for substituted quinazolines and 3-cyanoquinolines [43].

Also several research studies have shown that 3-cyanoquinoline analogs were far more potent inhibitor of several kinases than the corresponding quinazolines. In addition, based on homology modeling of the catalytic domain of EGFR kinase previously reported, 3-cyanoquinoline was postulated to be a better replacement for quinazoline moiety [44, 53]. From the docking studies, it has been postulated that the N3 nitrogen of quinazoline were able to indirectly bind to the receptor through a bridging water molecule, whereas the C-3 cyano nitrogen directly interacts with a hydroxyl of a threonine residue (Thr 830) displacing the water molecule (Figure 2.4) [43, 44]. Based on the above facts, we have chosen 3-cyanoquinoline as a better scaffold for replacing the quinazolines for further optimization. The preparation of the 3-cyanoquinoline template and corresponding analogs are summarized in Schemes 2.1-2.4.

2.2 Design and synthesis of 3-cyanoquinoline analogs

2.2.1 Optimization of 3-cyanoquinoline core (2.13) synthesis

\[ 2.10 \quad 2.11 \quad a \quad 2.12 \]

\[ b \quad 2.13 \quad c \quad 2.14 \]

Scheme 2.1 Synthetic route of 3-cyanoquinoline core molecule 2.13. Reagents and conditions: (a) Dry toluene, 125 °C, N2, 6 h, 77%; (b) Diphenyl ether, 300 °C, N2, 15 h, 87%; (c) POCl3, 115 °C, N2, 4 h, 89%.
The initial strategy to prepare 2.13 to enable parallel synthesis began with the synthesis of 2.12 as previously reported in the literature [49] (Scheme 2.1). Condensation reaction of commercially available 4-iodo aniline 2.11 with ethyl-2-cyano-3-ethoxyacrylate 2.10 afforded cyanoacrylate 2.12. Thermal cyclization of 2.12 in diphenyl ether at a high temperature (>300 °C) for several days gave 6-iodo-4-oxo-1,4-dihydroquinoline-3-carbonitrile 2.13 in 87% yield. Chlorination using POCl₃ provided 2.14. Since the synthesis of 2.13 required prolonged heating at a high temperature coupled with the inconsistency in the complete conversion of cyanoacrylate intermediate in a larger scale led to the alternate synthesis of oxo-cyanoquinoline core molecule as depicted in Scheme 2.2 [49].

![Scheme 2.2 Alternate route proposed for the synthesis of 2.13.](image)

**Scheme 2.2** Alternate route proposed for the synthesis of 2.13.
Reagents and conditions: (a) Dry DMF, 120 °C N₂, 4 h, 96%; (b) i. n-Buli/DIPA/THF, -78 °C, N₂; ii. Acetonitrile, -78 °C; iii. 2.16, THF -78 °C, 1 h, RT, 86%.

The reaction sequence showed in Scheme 2.2 involves the condensation reaction of commercially available 5-iodoanthranilic acid 2.15 in refluxing N, N’-dimethylformamide dimethyl acetal (DMF-DMA) with a co-solvent dimethylformamide (DMF) to yield the corresponding intermediate amidine 2.16 which was directly used in the next step without further purification. The amidine was cyclized to 2.13 with lithiated acetonitrile (CH₃CN) using lithium diisopropylamide (LDA) which was freshly prepared from n-butyllithium and diisopropylamine in THF at -78 °C. The resulting oxo compound 2.13 was chlorinated to 4-chloro-6-iodoquiniline 2.14 using POCl₃ as shown in Scheme 2.1. Addition of appropriate amines to the core template 2.14 to substitute for the chloro group (Scheme 2.3) yielded 2.21 in good yield.
enabling parallel synthesis. Suzuki coupling reaction of 2.21 at the C-6 position of cyanoquinoline template with the corresponding boronic esters provided the desired analogs 2.24-2.27 [49]

2.2.2 Optimization of head group chemistry and Suzuki coupling reaction

![Scheme 2.3 Synthesis of head group analogues 2.21 followed by Suzuki coupling reactions](image)

The requisite anilines 2.20 were synthesized by the alkylation of 2-chloro-4-nitrophenol 2.17 with the corresponding benzyl bromides 2.18 to give the intermediate 2.19, which was then taken for the reduction of the nitro group using zinc or iron as the reducing agent (Scheme 2.4) [36]. These corresponding anilines were coupled to the template 2.14 to give the intermediates 2.21 (Scheme 2.3). The next step of the synthesis was to introduce the desired functionalities at C-6 of 2.21 to create a library of analogs with different boronic esters 2.23 which was prepared by parallel Suzuki coupling reaction with varied bromo derivative of 2.22 with bis(pinacolato)diboron as shown in Scheme 2.5.
Scheme 2.4 Synthesis of head group derivatives 2.20
Reagents and conditions: (a) K$_2$CO$_3$, Acetonitrile, 85°C, 8 h, 87-99%; (b) Zn, NH$_4$Cl, MeOH, H$_2$O, 55 °C, 6 h, 64-69%.

The design and synthesis of analogs 2.24-2.27 were performed to explore possible SAR cross-over between the lapatinib scaffold quinazoline and 3-cyanoquinoline moiety. These analogs were designed with the best head and tail group match for each parasite with 3-cyanoquinoline scaffold. Both quinazoline and cyanoquinoline are well established tyrosine kinase inhibitors and the quinazoline analogs were also shown to have trypanosomal properties as reported in the previous work from our lab. Biological screening of the above synthesized analogs with cyanoquinoline core group against different parasites (T. cruzi, T. brucei, L. major and P. falciparum) will determine the importance of specific scaffolds for anti-parasitic activity.

Scheme 2.5. Synthesis of boronic ester analogues 2.23
Reagents and conditions: (a) Bis(pinacolato)diboron, PdCl$_2$(dpf)-CH$_2$Cl$_2$, Potassium Acetate, 1,4-dioxane, 80 °C, N$_2$, 12 h, 34–72%.
2.3 Biological screening results and discussions

A key design goal of swapping the core template quinazoline with that of other equipotent EGFR scaffolds is to access their respective utility for anti-parasitic properties. The 3-cyanoquinoline was chosen based on its role as an inhibitor of other human tyrosine kinases, similar to the quinazoline core of lapatinib. The aim was to retain the best combinations of substituents at C4 and C6 of the quinazolines for each parasite resulting in a small focused library of analogs. The synthesized compounds were tested for their ability to inhibit parasitic growth. The results of the in vitro assessment of this inhibitor collection are summarized in Table 2.1. The compound potency was measured as EC$_{50}$ (the effective concentration giving 50% inhibition of parasite growth). The in vitro potencies of analogs maintaining the lipophilic C4 amino substitution and varying substituents at C6 were not greatly different for parasites like T. brucei and Leishmania. Whereas, T. cruzi was refractory to all the inhibitors tested, except for 2.25b (NEU-924, EC$_{50}$ = 0.09 µM). However, this library of analogs designed to study the simple replacement of cyanoquinoline template shows significant changes in the activities for the analogs against the parasite P. falciparum. In particular, analog 2.25b (NEU-924) with the lapatinib head group and homopiperizine sulfonamide moiety at the tail was identified to be the most potent analog against T. cruzi parasite in the library of scaffold replacements. The corresponding quinazoline analogs showed a modest activity of EC$_{50}$ = 0.5 µM against...
the same parasite. Analog 2.25b is also shown to have modest-to-excellent activity against *P. falciparum* (0.03 µM) and *T. brucei* (0.34 µM). Other analogs of 3-cyanoquinolines with different tails are essentially inactive against other parasites (2.24, 2.26, and 2.27).

The presence of halogens on the head group has subtle effects on the activities of these analogs except in the case of 2.25a (EC₅₀ = 0.95 µM), where the replacement of the fluorine atom with hydrogen results in decreased potency against *T. cruzi*. The role of fluorine is not well established for other analogs in the library as replacing the fluoro for hydrogen has no significant change in the activity. Both 2.25b and 2.25a shows less than 3 µM potency against *Leishmania* (promastigotes and amastigotes). Whereas, replacing the tail end with morpholine sulfonamide, 4-methylpiperazine sulfonamide or phenyl morpholine shows complete loss of activity against *T. cruzi* and *Leishmania major*, such analogs display varied potency against *P. falciparum* D6 strain. Analogs with the morpholine sulfonamide tail have less than 0.2 uM potency against *P. falciparum* (2.24a and 2.24b). These data suggests that the cyanoquinoline scaffold is an appropriate quinazoline replacement for some parasites (though not all).

![Chemical structures of library of eight analogs synthesized](image)

**Figure 2.6** Chemical structures of library of eight analogs synthesized
The calculated log P (cLogP) for the library members along with other physicochemical properties is summarized in Table 2.2. All the eight analogs have a high molecular weight (> 500) and lipophilicity (logP) ranging from 5 to 6.5. As a prediction of the partitioning of a drug between octanol and water, cLog P can also be suggestive of a compound’s ability to permeate through the cell membrane. It is thus an important physical property of a drug with respect to absorption, distribution, potency and elimination. The lack of efficacy for many of the analogs may be partly attributed to the high lipophilicity of the molecules, which reduces the compounds aqueous solubility. Hence the free drug concentration availability will be too low to produce desired efficacy. The drug-like properties of a lead molecule is most often characterized by Lipinski’s rule of five (Ro5), which suggests that a good, orally-bioavailable candidate molecule will have MW < 500 Da; clogP < 5; HBD < 5; HBA < 10; TPSA < 140 Å². Compounds that violate Lipinski’s Ro5 often require further optimization to improve their physicochemical properties. The lead molecule for *T. cruzi* from this library (2.25b) shows excellent potency and hence can be taken for further study to improve its properties and anti-parasitic activity.
### Table 2.1 Screening results for the eight analogs built upon 3-cyanoquinoline template

<table>
<thead>
<tr>
<th>Compound</th>
<th>X</th>
<th>R</th>
<th><strong>T. brucei</strong></th>
<th><strong>T. cruzi</strong></th>
<th><strong>Leishmania major</strong></th>
<th><strong>P. falciparum</strong></th>
</tr>
</thead>
</table>
|            |     |       | **EC$_{50}$
(µM)** | **EC$_{50}$
(µM)** | **Host cell Tox
TC$_{50}$
(µM)** | **Pro EC$_{50}$
(µM)** | **Ama EC$_{50}$
(µM)** | **D$_6$
EC$_{50}$ (µM)** |
| NEU-914 2.24a | H   |       | > 50 | > 50 | > 20 | > 15 | 0.176 |
| NEU-926 2.24b | F   |       | > 50 | > 50 | > 20 | > 15 | 0.105 |
| NEU-995 2.25a | H   |       | 0.427 | 0.95 | 11.14 | 1.56 | 2.311 | 0.62 |
| NEU-924 2.25b | F   |       | 0.34 | 0.09 | 23.6 | 0.92 | 1.596 | 0.033 |
| NEU-996 2.26a | H   |       | 0.753 | > 50 | > 100 | > 20 | > 15 | 15.263 |
| NEU-925 2.26b | F   |       | > 50 | > 50 | > 20 | > 15 | 0.395 |
| NEU-994 2.27a | H   |       | 1.288 | > 50 | > 100 | 4.06 | > 15 | 2.517 |
| NEU-993 2.27b | F   |       | 1.202 | > 50 | > 100 | > 20 | > 15 | 1.128 |
Table 2.2 Computed physicochemical properties for the eight analogs

<table>
<thead>
<tr>
<th>Compound</th>
<th>Mol Wt</th>
<th>logP</th>
<th>pKa</th>
<th>PSA</th>
<th>HD/HA</th>
<th>HAC*</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.24a</td>
<td>611.11</td>
<td>6.08</td>
<td>3.65</td>
<td>104.55</td>
<td>1/7</td>
<td>43</td>
</tr>
<tr>
<td>2.24b</td>
<td>629.1</td>
<td>6.22</td>
<td>3.65</td>
<td>104.55</td>
<td>1/7</td>
<td>46</td>
</tr>
<tr>
<td>2.25a</td>
<td>638.18</td>
<td>6.21</td>
<td>7.07</td>
<td>98.56</td>
<td>1/7</td>
<td>41</td>
</tr>
<tr>
<td>2.25b</td>
<td>656.17</td>
<td>6.35</td>
<td>7.07</td>
<td>98.56</td>
<td>1/7</td>
<td>44</td>
</tr>
<tr>
<td>2.26a</td>
<td>547.05</td>
<td>7.13</td>
<td>3.66</td>
<td>70.41</td>
<td>1/6</td>
<td>45</td>
</tr>
<tr>
<td>2.26b</td>
<td>565.04</td>
<td>7.28</td>
<td>3.66</td>
<td>70.41</td>
<td>1/6</td>
<td>44</td>
</tr>
<tr>
<td>2.27a</td>
<td>624.15</td>
<td>6.15</td>
<td>6.02</td>
<td>98.56</td>
<td>1/7</td>
<td>45</td>
</tr>
<tr>
<td>2.27b</td>
<td>642.14</td>
<td>6.29</td>
<td>6.02</td>
<td>98.56</td>
<td>1/7</td>
<td>40</td>
</tr>
</tbody>
</table>

*HAC: heavy atom count

2.4 Pharmacokinetic and animal efficacy studies in Chagas infection model

The analog 2.25b was identified to be the only potent inhibitor against T. cruzi in the library prepared. Encouraged by the cellular activity observed, this lead compound was further evaluated in a mouse pharmacokinetics study. Pharmacokinetic (PK) studies evaluate the behavior of drugs in living organisms by measuring the concentration of the drugs in blood/tissue after drugs administration. An intraperitoneal injection (IP) of 2.25b in female BALB/c mice was conducted at two different dosages (2 and 10 mg/kg). Blood samples were collected at different time intervals (pre-dose, 0.08, 0.25, 0.5, 1, 2, 4, 8 and 24 hr.) and PK parameters were measured by LC-MS/MS method using a non-compartmental analysis tool. Following a single IP administration, plasma concentrations were observed up to 4 hrs. $T_{\text{max}}$ (time taken to reach the maximum concentration) in plasma was observed to be 0.50 hr for 2mg/kg dosage and 1hr for the 10mg/kg dosage, which indicates slower drug uptake from the peritoneal cavity at the higher dose. Interestingly, the increased dosage (10 mg/kg) resulted in a less-than proportional $C_{\text{max}}$ (maximum concentration of the drug after a dose), compared to the 2 mg/kg dose; yet the area under the curve (AUC), which describes the total drug concentration over time, is dose proportional (Figure 2.7 and Table 2.3).
Figure 2.7 Mean plasma concentration-time profile of 2.25b following a single intraperitoneal administration to female BALB mice. (a) Dose: 2 mg/kg and (b) 10 mg/kg
Table 2.3 Pharmacokinetic parameters of 2.25b in plasma following a single Intraperitoneal (Dose: 2mg/kg and 10mg/kg) administration in female BALB/c mice

<table>
<thead>
<tr>
<th>Compound</th>
<th>Route</th>
<th>Matrix</th>
<th>Dose (mg/kg)</th>
<th>max (hr)</th>
<th>Cmax (ng/mL)</th>
<th>AUC_{last} (hr*ng/mL)</th>
<th>AUC_{inf} (hr*ng/mL)</th>
<th>MRT_{last}</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.25b (NEU-924)</td>
<td>i.p.</td>
<td>Plasma</td>
<td>2</td>
<td>0.50</td>
<td>714.35</td>
<td>1351.21</td>
<td>1572.24</td>
<td>1.35</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>10</td>
<td>1.00</td>
<td>342.91</td>
<td>3026.17</td>
<td>3283.93</td>
<td>6.39</td>
</tr>
</tbody>
</table>

The results suggest that at the higher dose the drug is precipitating in the peritoneal cavity and is more gradually absorbed. In any event, even at 2 mg/kg, drug exposure was in excess of the EC_{50} for ~16 hours.

Since the drug exposure was high following IP dosing, the analog 2.25b was tested in a mouse model of Chagas disease. Dosing at 10 mg/kg produced no discernable reduction in parasitemia in mice as shown in Figure 2.8, despite having sub-micromolar efficacy in the \textit{in vitro} tests. This contradiction can be surprising, given that, in the \textit{in vitro} studies, the compound was directly incubated with the intracellular amastigote form of the parasite and was able to penetrate both host and parasite cells. We suspect that the observed disconnect between good drug exposure and poor efficacy suggest that the poor physicochemical properties of 2.25b, such as its high clogp (6.3) and MW, most likely contributed to a high volume of distribution and subsequent lack of efficacy. Hence it is necessary to design analogs of 2.25b to have reduced lipophilicity and size without losing the \textit{in vitro} parasitic properties.
In summary, as a part of a continuing effort to develop potent and selective compounds for anti-trypanosomal activity, we have identified a series of 4-aminosubstituted-6-aryl-3-cyanoquinoline analogs. Cross-screening of the analogs against other related parasites led to the identification of molecules from this series with sub-micromolar potencies against different parasites. We found that 3-cyanoquinoline is far superior in potency against *T. cruzi* than the corresponding quinazolines and the lead molecule shows nanomolar EC<sub>50</sub> against *T. cruzi*. The best cell activity was observed with analogs having N-methylhomopiperazine group. However removal of small substituent like fluorine group results in a surprising 10 fold loss of activity from the most potent lead. The lead compound was advanced into *in vivo* studies. While the pharmacokinetic study showed good exposure (though non-linear pharmacokinetics), we observed little efficacy in the animal model. We hypothesize this to be due to sub-optimal physicochemical properties. Nevertheless the 3-cyanoquinoline core exhibits anti-parasitic activity, and these compounds represent a novel structure as anti-trypanosomal agents.

### 2.5 Future directions

The initial lead was obtained from a selective evaluation of compounds prepared with 3-cyanoquinoline template as putative EGFR/HER2 kinase inhibitor. Despite excellent potency against *T. cruzi* and good PK profile it also has unfavorable physicochemical properties like high molecular weight,
logP and high plasma protein binding which led to the lack of efficacy in the animal model. Future work is underway to identify the other structural features of 2.25b required for T. cruzi activity and also to improve the overall physicochemical properties by adhering to the following criteria.

- Reduced molecular weight, MW < 400 for better oral bioavailability
- Reduced logP < 4 for minimizing high plasma binding
- Increase more polar substituent for better solubility
- Enable parallel chemistry for library synthesis
- Increased cellular activity against the parasite and selectivity over host cells

One way to achieve the above desired properties is to design a library of analogs replacing the lipophilic lapatinib head group with small heterocyclic molecules having basic nitrogen at C-4 position. Introducing more polar group and water solubilizing groups will also enhance the solubility.
Chapter 3: Head group truncation and replacement for 2.25b

3.1 Introduction

The results obtained from the work presented thus far in this thesis have demonstrated that repurposing human kinase inhibitor drugs for trypanosomal inhibitory property is indeed a fruitful path to speed lead discovery [36]. This work was further advanced to find several potent analogs against different trypanosomal parasites using a cross- parasite screening approach. Initial SAR developed on the lapatinib analogs led to the most potent anti-trypanosomal leads. Cross screening and core replacement tactics led to a nanomolar potent compound against *T. cruzi* parasite. The results obtained from cyanoquinoline analogs indicate that this core template is indeed has anti-tyrpanosomal properties and can be pursued for further optimization. These arsenals of small molecules designed to inhibit the intracellular tyrosine kinase activity showed promising future for identifying a new lead for Chagas disease.

An important finding from the core replacement strategy (chapter 2) was that all the analogs had high logP and molecular weight and this in turn may have led to some of the compounds poor efficacy *in vivo*. The high lipophilicity is partly due to the bulky and lipophilic head group of lapatinib, an aspect that will be optimized in head group replacement strategy. Even the compounds that had considerable efficacy were marked with poor physicochemical properties. Hence, the aim is to design a new library of analogs for 2.25b with reduced size and lipophilicity to deduce possible SAR from the series. This will also give an idea of whether the lapatinib head group is indeed an essential pharmacophore for *T. cruzi* inhibition. The initial plan was to focus on the head group region of 2.25b, with a key design goal to introduce small polar heterocyclic amines to replace bulky lipophilic head groups. A suitable lead compound should exert molecular properties in accordance with Lipinski’s Ro5, with good potency (<1 uM) and cell permeability. Since the lead compound 2.25b meets the criteria only for potency, the rest of these properties need to be optimized to identify better leads.
3.1.1 Design strategy

The first step was the enumeration of a virtual library using commercially available primary amines available in pre-weighed quantities from Frontier Scientific Services. This library was then filtered to retain molecules that had reduced molecular weight (< 555), low logP (< 5) and high polarity to improve aqueous solubility (Scheme 3.1). A diverse subset of the filtered 94 analogs were then screened in a shape/electrostatics similarity search against 2.25b and based on the combo score and tractability for synthesis, a list of 16 compounds was prioritized for first generation library synthesis.

Scheme 3.1 Proposed route for library series with improved physicochemical properties.

Drug molecules can be bound to proteins and lipids in plasma (PPB) or in tissue or are free (unbound). Mostly the free drug interacts with the therapeutic target. This free drug concentration determines the in vivo efficacy and in vivo pharmacokinetics. We hypothesize that the lead compound 2.25b has a high volume of distribution in vivo, it was not delivered effectively to infected host cells, leading to failure in the in vivo animal model. With this in mind, besides considering Ro5 compliance, these newly designed conformers were then subjected to predictions (at AstraZeneca) of aqueous solubility, human plasma protein binding, passive permeability, Caco2 permeability, Caco2 efflux, and blood-brain barrier partitioning. Some of these results are shown in Figure 3.1.

For the shape and electrostatics similarity studies we used Omega, ROCS and EON (OpenEye Scientific Software). This approach was based on the principle that chemical interactions are regulated by shape and electrostatics of molecules [54]. A three dimensional conformer library consisting of all
accessible conformers of members of the virtual library described above was compared to a conformation of 2.25b [55]. This helps to identify potential lead molecules that are similar in shapes and electrostatics with the lead compound.

![Figure 3.1 Scatter plot of hPPB % free vs Molecular weight of 250 conformers, highlighting the 16 amines chosen for synthesis](image)

**Figure 3.1** Scatter plot of hPPB % free vs Molecular weight of 250 conformers, highlighting the 16 amines chosen for synthesis

### 3.1.2 Conformational model generators: shape/electrostatic similarity search

OMEGA is a high-throughput generator of 3D conformers to generate accessible conformations of the chosen query. The goal is to produce structurally diverse low energy conformational ensembles of small molecules and the molecular database containing these conformers will be screened for shape/electrostatic similarities by ROCS/EON [56, 57]. ROCS (Rapid Overlay of Chemical Structures) shape-based virtual screening was employed using this conformer library.

ROCS identify potentially active leads based on three dimensional shape similarities. It determines the Tanimoto measure of the shape similarity as it's metric and the Shape Tanimoto values (ST) ranging from 0-1 was used in this study to find the similarity between query and database. The multiconformer files generated by OMEGA were used as input database for performing ROCS similarity search. The queries generated from the hit molecules were saved as output files and then ranked according to their combo score [55].
EON uses the electrostatic similarity of molecules to identify lead molecules with that of pre-aligned potent molecules. ROCS hits structures were used as input for EON analysis. EON calculates the Electrostatic (similarity) Tanimoto between each database molecule (ROCS overlay hits) and the query. It compares through-space electrostatics near molecules and determines rigorous Tanimoto measures between the fields. Poisson-Boltzmann function is used to calculate the electrostatic similarity and expressed as Electrostatic Tanimoto (ET) values. The results from EON were clustered and scored based on shapes and electrostatics EON (ETCombo) score [54].

3.1.3 Results of in silico virtual screening models

The virtual screen included three stages, in the first stage OMEGA was used to generate 3D conformer ensembles for the newly designed 250 molecules. This database was then screened by ROCS for 3D shape similarity which resulted in 94 overlays of conformers in the second stage. Finally EON was used to refine the ROCS hits by electrostatic similarity yielding 94 overlays with different ET combo score ranging from 0-2. From this list we have manually selected 16 conformers (Figure 3.2) based on the molecular properties of the fragments as a first generation library series.

Lead optimization approach usually involves the analysis of different regions of the lead molecule independently to discover the pharmacophores responsible for activity. We hypothesized that our approach would identify smaller, more polar compounds that matched the large and lipophilic headgroup pharmacophore. The monomer precursors for the predicted optimal headgroup replacements are shown in Figure 3.2 and their improved physicochemical properties like MW and logP were shown in Figure 3.3.
Figure 3.2 List of monomers chosen from Frontier Scientific.

Figure 3.3 Plot of lipophilicity vs molecular weight for 94 compounds, highlighting the 16 library members chosen for synthesis.

3.1.4. Library shaping based on properties

The success of drug discovery research relies on the optimization of various fragments of lead candidate to improve not only their physicochemical parameters but also to modify more complex parameters like acceptable toxicity, solubility, selectivity and bioavailability. Structure-based methodologies may not yield all the desired properties in a molecule resulting in the increased attrition rate of drug-like properties for the compounds. Hence the concept of ligand efficiency (LE) which
normalizes biological activity for molecular size, providing a means to assess the quality of hits [58]. This metric is defined as the free energy of binding divided by the number of heavy atoms (n) in a molecule [59, 60].

\[
(\text{LE} = \frac{-\log(\text{IC}_{50})}{\text{HAC}})
\]

For a compound to evolve from a lead-like to drug-like molecule, LE of 0.30 kcal/mol/heavy atom would be ideal [61].

Another property that can be taken into consideration for assessing druggability and library shaping includes lipophilic ligand efficiency (LLE) defined as the difference between in vitro potency and lipophilicity of a compound [60]. LLE is defined as:

\[
\text{LLE} = \text{pIC}_{50} - c\text{LogP}
\]

LLE or LiPE estimate the specificity of a molecule in binding to the target and it combines both potency and lipophilicity to define the drug-likeliness a compound. A drug-like candidate is recommended to have LLE > 4 [60]. Hence optimizing both size and lipophilicity is an essential criterion to identify leads with promising physicochemical and ADMET profiles. This can be achieved by choosing fragments with better LE and LLE for future optimization. Based on this hypothesis, the first generation headgroup replacement library we designed has better physicochemical properties (Table 3.1).

### 3.1.5. Enumeration of virtual library of headgroup replacement analogs and computed properties

The initial sets of 16 conformers chosen from the 94 hits results from ROCS/EON modulation based on their ET combo score are listed in Table 3.1. This library has good range of physicochemical properties, predicted aqueous solubility, high polarity, molecular diversity, drug-likeliness and chemical tractability. Reducing the molecular size may also reduce the steric interactions between the binding ligands and the target protein, increasing the chance of finding hits. Also, smaller fragments can have efficient interactions as seen from their improved ligand efficiency.
Table 3.1 Computed properties of 16 conformers chosen for synthesis from virtual screening

<table>
<thead>
<tr>
<th>R</th>
<th>MolWt</th>
<th>LogP</th>
<th>ET_Combo</th>
<th>Rank</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1" alt="Structure1" /></td>
<td>502.588</td>
<td>2.62</td>
<td>1.74</td>
<td>11</td>
</tr>
<tr>
<td><img src="image2" alt="Structure2" /></td>
<td>517.19</td>
<td>3.65</td>
<td>1.72</td>
<td>28</td>
</tr>
<tr>
<td><img src="image3" alt="Structure3" /></td>
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<td>1.84</td>
<td>1.713</td>
<td>39</td>
</tr>
<tr>
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<td>2.82</td>
<td>1.711</td>
<td>40</td>
</tr>
<tr>
<td><img src="image5" alt="Structure5" /></td>
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<td>2.61</td>
<td>1.674</td>
<td>80</td>
</tr>
</tbody>
</table>
3.2. Library pilot chemistry using manually-selected headgroup replacements

3.2.1. Design and synthesis

The synthesis of small focused library of analogs using manually-selected amines to reduce the size of head group was pursued as shown in Scheme 3.2 (Structures shown in Table 3.2). The initial strategy was to adapt the standard conditions developed in the lab for the synthesis of previous 3-cyanoquinoline analogs as shown in Chapter 2 of this thesis. The aim was to make 2.14 in larger quantity using the synthetic route shown in Scheme 2.2 and introduce different R groups at the beginning of the library synthesis followed by Suzuki coupling reactions with N-methyl homopiperazineboronate (2.23b). Using these generalized procedures, analogs 3.2a to 3.2d were synthesized. The initial route of coupling the amines with the core 2.14 using IPA under refluxing conditions worked well for 3.1a to 3.1d analogs. For analogs 3.1e-3.1f, direct heating of the intermediate 2.14 with methanolic ammonia without any co-solvent provided the required product in good yield. All the intermediates were isolated greater than 90% purity and taken forward to the Suzuki coupling reaction with boronates without any further purification. The homopiperazineboronate (2.23b) used in the synthesis was purchased from Frontier Scientific.

Scheme 3.2 Head group substitution to the core molecule 2.14 followed by Suzuki coupling
Reagents and conditions: (a) R= Manually-selected primary amines, (i) R, IPA, 80 °C, 72-80%; (ii) R, 65 C, 6h, 75-97%; (b) Pd(PPh3)4, 2 M Na2CO3, DME, Ethanol, 85 °C, 6 h

The design and synthesis of these analogs were primarily aimed at optimizing the parallel chemistry methodology in addition to explore possible SAR for the reduction of compound size and...
lipophilicity in comparison to 2.25b. These analogs were then tested against *T. cruzi* and also cross-screened against other protozoan parasites as before. The computed properties and screening results were summarized in Table 3.2-3.4

### 3.2.2 Results and discussion

Interesting biological results were obtained from these small focused library series. The screening data for the analogs against *T. cruzi* shows no improved potency over 2.25b but improved selectivity over host cell (expressed as TC\textsubscript{50}) was observed. All the analogs show considerable efficacy with increased selectivity except for 3.2a. Compound 3.2d is the most potent in the series (EC\textsubscript{50} = 0.445 µM) and compound 3.2b showed a 4-fold increase in selectivity. The complete loss of activity for 3.2f shows the importance of having C-4 aniline substitutions. Placing larger substituents at C-4 seems essential for activity.

Cross-screening of these analogs displays a range of improved potencies against other parasites with 3.2d being more potent against *T. brucei* (EC\textsubscript{50} = 0.216 µM) and *Leishmania* (EC\textsubscript{50} = 0.84 µM) than 2.25b. Thus, an initial SAR was developed for the head group truncation series against different parasites with improved selectivity profile ranging from 29.1 to > 100 for *T. cruzi*. Further exploration of the head group region will provide us a better understanding of size and lipophilicity reduction correlations.

In summary, the process of lead optimization usually results in increased size and lipophilicity of the original hit as observed with 2.25b but that resulted in poor PK properties. Hence starting with smaller less lipophilic molecule will be a better strategy to avoid chasing after the potency at the expense of physicochemical properties despite having weaker binding with the target as seen from the potency of the small focused library. But an improved predicted physicochemical property profile was achieved for these analogs shown in Table 3.4 in comparison to the most potent analog for *T. cruzi*. 

43
Table 3.2 Screening results for head truncation group series using manually selected amines against *T. cruzi*

![Chemical structure](image)

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
<th>NEU #</th>
<th><em>T. cruzi</em> EC&lt;sub&gt;50&lt;/sub&gt; (µM)</th>
<th>Host cell Tox TC&lt;sub&gt;50&lt;/sub&gt; (µM)</th>
<th>LE</th>
<th>LLE</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.2a</td>
<td></td>
<td>NEU-1054</td>
<td>10.965</td>
<td>4.7</td>
<td>0.13</td>
<td>0.94</td>
</tr>
<tr>
<td>3.2b</td>
<td></td>
<td>NEU-1055</td>
<td>4.438</td>
<td>&gt; 100</td>
<td>0.14</td>
<td>0.87</td>
</tr>
<tr>
<td>3.2c</td>
<td></td>
<td>NEU-1056</td>
<td>1.406</td>
<td>82.3</td>
<td>0.13</td>
<td>0.11</td>
</tr>
<tr>
<td>3.2d</td>
<td></td>
<td>NEU-1057</td>
<td>0.445</td>
<td>29.1</td>
<td>0.14</td>
<td>0.75</td>
</tr>
<tr>
<td>3.2e</td>
<td></td>
<td>NEU-1059</td>
<td>19.367</td>
<td>84.1</td>
<td>0.15</td>
<td>2.01</td>
</tr>
<tr>
<td>3.2f</td>
<td></td>
<td>NEU-1058</td>
<td>&gt; 50.0</td>
<td>72.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.25b</td>
<td></td>
<td>NEU-924</td>
<td>0.09</td>
<td>23.6</td>
<td>0.15</td>
<td>0.70</td>
</tr>
</tbody>
</table>

TC<sub>50</sub> toxic concentration, 24hr concentration resulting in 50% cell death

Table 3.3 Cross-Screening results against other trypanosomal parasites for head group truncation series

<table>
<thead>
<tr>
<th>Compound</th>
<th>Tbr EC&lt;sub&gt;50&lt;/sub&gt; (µM)</th>
<th>P. fal D6 EC&lt;sub&gt;50&lt;/sub&gt; (µM)</th>
<th>P. fal W2 EC&lt;sub&gt;50&lt;/sub&gt; (µM)</th>
<th>P. fal C235 EC&lt;sub&gt;50&lt;/sub&gt; (µM)</th>
<th>Lmj pro EC&lt;sub&gt;50&lt;/sub&gt; (µM)</th>
<th>Lmj Ama EC&lt;sub&gt;50&lt;/sub&gt; (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.2a</td>
<td>0.477</td>
<td>0.181</td>
<td>1.03</td>
<td>0.293</td>
<td>&gt;18.8</td>
<td>&gt; 7.0</td>
</tr>
<tr>
<td>3.2b</td>
<td>0.311</td>
<td>0.134</td>
<td>0.135</td>
<td>0.166</td>
<td>1.66</td>
<td>&gt; 7.0</td>
</tr>
<tr>
<td>3.2c</td>
<td>0.463</td>
<td>0.291</td>
<td>0.558</td>
<td>0.357</td>
<td>2.63</td>
<td>3.96</td>
</tr>
<tr>
<td>3.2d</td>
<td>0.216</td>
<td>0.065</td>
<td>0.267</td>
<td>0.127</td>
<td>0.19</td>
<td>0.84</td>
</tr>
<tr>
<td>3.2e</td>
<td>1.965</td>
<td>3.001</td>
<td>3.459</td>
<td>3.519</td>
<td>0.16</td>
<td>4.06</td>
</tr>
<tr>
<td>3.2f</td>
<td>3.778</td>
<td>0.573</td>
<td>2.057</td>
<td>1.659</td>
<td>1.44</td>
<td>&gt; 7.0</td>
</tr>
<tr>
<td>2.25b</td>
<td>0.34</td>
<td>0.033</td>
<td>0.097</td>
<td>0.039</td>
<td>0.92</td>
<td>1.596</td>
</tr>
</tbody>
</table>
Table 3.4 Physicochemical properties of the head group truncation series

<table>
<thead>
<tr>
<th>Compound</th>
<th>MW</th>
<th>clogP</th>
<th>HD/HA</th>
<th>PSA</th>
<th>HA</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.2a</td>
<td>533.044</td>
<td>4.02</td>
<td>1/7</td>
<td>102.22</td>
<td>37</td>
</tr>
<tr>
<td>3.2b</td>
<td>562.082</td>
<td>4.48</td>
<td>1/7</td>
<td>98.56</td>
<td>39</td>
</tr>
<tr>
<td>3.2c</td>
<td>621.724</td>
<td>5.74</td>
<td>1/7</td>
<td>98.56</td>
<td>45</td>
</tr>
<tr>
<td>3.2d</td>
<td>603.733</td>
<td>5.6</td>
<td>1/7</td>
<td>98.56</td>
<td>44</td>
</tr>
<tr>
<td>3.2e</td>
<td>449.568</td>
<td>2.7</td>
<td>0/6</td>
<td>80.54</td>
<td>32</td>
</tr>
<tr>
<td>3.2f</td>
<td>421.515</td>
<td>1.77</td>
<td>1/6</td>
<td>103.32</td>
<td>30</td>
</tr>
<tr>
<td>2.25b</td>
<td>656.17</td>
<td>6.35</td>
<td>1/7</td>
<td>98.56</td>
<td>46</td>
</tr>
</tbody>
</table>

3.3. Headgroup truncation library synthesis

3.3.1. Optimization of library synthesis

Encouraged by the results obtained from our pilot study using manually-selected amines, we envisioned that the head group SAR studies will result in improved potency/selectivity for trypanosomal parasites.

Initially, to enable parallel chemistry synthesis the following Scheme 3.3 was proposed for the library synthesis. The first synthetic effort to synthesize 3.3 was to make the intermediate 2.14 using the general route as described in the second chapter [49] (Scheme 2.2) of this thesis followed by Suzuki coupling reaction with the 2.23b to give 3.3 in larger quantities. Having 3.3 in gram quantities will enable us to proceed with the coupling of various amines to create the library of interest. To achieve our desired goals we needed to optimize the reaction conditions for Suzuki coupling reactions. A few milligrams of 2.23b were purchased from Frontier Scientific to test the reaction conditions. The reaction conditions tried for optimizing this step is summarized in Table 3.5. All reaction conditions utilized microwave heating. Attempts to get 3.3 in single step resulted in either di-substituted product or partial conversion of the starting material. Failure to synthesize 3.3 in good yield coupled with the high cost of 2.23b led to the proposal of alternate route as shown in Scheme 3.4
**Scheme 3.3**: General route proposed for library synthesis

**Table 3.5** Reaction conditions screened for the synthesis of 3.3

<table>
<thead>
<tr>
<th>2.14 (equiv)</th>
<th>2.23b (Equiv)</th>
<th>Reaction Conditions</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>TEA/Pd(OAc)$_2$/EtOH/120 °C</td>
<td>Disubstituted product</td>
</tr>
<tr>
<td>1</td>
<td>0.5</td>
<td>TEA/Pd(OAc)$_2$/EtOH/120 °C</td>
<td>Several spots</td>
</tr>
<tr>
<td>1</td>
<td>0.5</td>
<td>TEA/Pd(Oac)$_2$/H$_2$O/EtOH/50 °C</td>
<td>40% conversion</td>
</tr>
<tr>
<td>1</td>
<td>0.5</td>
<td>Tetrakis/Na$_2$CO$_3$/DME/EtOH/85°C</td>
<td>Disubstituted product</td>
</tr>
<tr>
<td>1</td>
<td>0.5</td>
<td>1M Cs$_2$CO$_3$/PdCl$_2$(dppf)/THF/65 °C</td>
<td>20% conversion</td>
</tr>
<tr>
<td>1</td>
<td>0.5</td>
<td>1M Cs$_2$CO$_3$/PdCl$_2$(dppf)/THF/90 °C</td>
<td>Disubstituted product</td>
</tr>
</tbody>
</table>

3 equiv. of base used was reduced to 1.5 equiv to avoid disubstituted product
0.025 Equiv. of catalyst used, increase in the equiv of catalyst gave multiple spots by TLC

A second approach (**Scheme 3.4**) was proposed to convert the intermediate 2.13 to the boronate derivative of 3.5 followed by reverse Suzuki coupling with 2.22b. Two different solvent conditions were tried for the coupling reaction to synthesize 3.5 under both conventional and microwave irradiation (**Table 3.6**). The amount of base used was gradually increased in several attempts to get maximal conversion.
Use of a relatively non-polar aprotic solvent like 1,4-dioxane or toluene resulted in no conversion whereas a high boiling polar aprotic solvent like DMF gave the required product along with major side product 3.5a (Scheme 3.5). Due to high solubility of 3.5 in water and purification difficulties, this route was not further pursued. A modified approach to synthesize 3.3 is summarized in Scheme 3.5 and Table 3.7, which also did not give the desired results.

![Scheme 3.4 Alternate route to the synthesis of 3.3](image)

**Table: 3.6 Reaction conditions screened for optimizing 3.5**

<table>
<thead>
<tr>
<th>Reaction Conditions</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>PdCl$_2$(dppe)CH$_2$Cl$_2$/KOAc/1,4-dioxane or toluene/100 °C</td>
<td>No reaction</td>
</tr>
<tr>
<td>$^a$PdCl$_2$(dppe)CH$_2$Cl$_2$/KOAc/DMF/95°C</td>
<td>38-77% product with major side product</td>
</tr>
<tr>
<td>TEA/Pd(OAc)$_2$/EtOH/H$_2$O/85°C</td>
<td>45 % product</td>
</tr>
</tbody>
</table>

$^a$0.0241 equiv. gave highest yield than 0.05 and 0.04
Scheme 3.5 Modified approach for the synthesis of 3.3

Table 3.7 Reaction conditions for optimizing 3.6

<table>
<thead>
<tr>
<th>Reaction Conditions</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>bPdCl₂(dppf)CH₂Cl₂/KOAc/1,4-dioxane/100 °C</td>
<td>No reaction</td>
</tr>
<tr>
<td>PdCl₂(dppf)CH₂Cl₂/KOAc/DMF/100-145 °C</td>
<td>No reaction</td>
</tr>
</tbody>
</table>

a Base and the b catalyst equiv was increase by 0.5 and 0.0015 equiv for each attempt

Several attempts were made for each of the reaction conditions shown in the above Table 3.5-3.7 by varying the equivalence of base and the catalyst used. Meanwhile, attempts were made to introduce amines to the core template 2.14 at the beginning of the synthesis followed by Suzuki coupling at the C-6 positions to give the desired products. With only a few milligrams (<6 mg) of library amines available for synthesis, we decided to optimize the reaction conditions using substituted amine reagents available in our inventory. The various reaction conditions that were attempted with different amines are summarized in Table 3.8. Although this method worked for some of the amines, this transformation was difficult from a practical standpoint as it involves one more additional step to get the final product which was not optimized for the library amines.
Table 3.8 Reaction conditions attempted for the monomers coupling to the intermediate 2.14

![Diagram]

<table>
<thead>
<tr>
<th>Reaction conditions</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>IPA/85 °C</td>
<td>NR</td>
<td>P</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>P</td>
</tr>
<tr>
<td>HCl/IPA/85 °C</td>
<td>NR</td>
<td>-</td>
<td>NR</td>
<td>NR</td>
<td>-</td>
<td>NR</td>
</tr>
<tr>
<td>NaH/DMF/RT80 °C</td>
<td>NR</td>
<td>-</td>
<td>NR</td>
<td>NR</td>
<td>-</td>
<td>NR</td>
</tr>
</tbody>
</table>

NR = No Reaction; P = Product

This methodology could not be extended in a general sense to date. Hence further research is required to find an optimized reaction condition to carry out these transformations.

3.3.2 Execution of library

In an attempt to devise a more concise approach to enable parallel synthesis with the monomers we first produced 2.23b (Scheme 3.6) in larger quantities. This can be taken for Suzuki coupling with 2.13 to get 3.8 which can be converted to the penultimate chloro derivative using POCl₃ as chlorinating agent (Scheme 3.7). Though 2.23b is commercially available, it is expensive, and thus a manually-selected synthesis was warranted. The first step was to synthesize 3.7 from commercially available 4-bromobenzen-1-sulfonyl chloride and N-methylhomopiperazine using triethylamine as a base in anhydrous DCM.
Scheme 3.6 Synthesis of the intermediate 2.23b.
Reagents and conditions: (a) TEA, DCM, 0 °C, 95%, (b) PdCl$_2$(dppf)CH$_2$Cl$_2$/KOAc/1,4-dioxane/90 °C

The compound 3.7 was obtained in 95% yield and was taken for the Suzuki-Miyaura reaction using bis (pinacolato)diboron as a boron source. This cross-coupling reaction was catalyzed by PdCl$_2$(dppf)CH$_2$Cl$_2$ adduct in the presence of potassium acetate in 1,4-dioxane as co-solvent at 90 °C. Work up and purification of this material proved challenging because of the solubility issue. Many attempts of purification based on the procedures of isolation and extraction were failed due to the high solubility of the crude mixture in water. Finally the product was purified by elaborate work up methodology that included charcoal treatment to remove any colored impurities followed by trituration afforded the desired product in good yield. This was then utilized in a palladium catalyzed Suzuki coupling reaction followed by chlorination using POCl$_3$ to get 3.3 in a moderate yield.

Scheme 3.7 Optimized route for synthesis of 3.3.
Reagents and conditions: (a) Pd(PPh$_3$)$_4$, 2 M Na$_2$CO$_3$, DME, Ethanol, 85 °C, 16h, 90%; (b) POCl$_3$, 115 °C, 60%
With 3.3 available in hand, optimized conditions for introducing the library amines were attempted (Table. 3.9)

**Table 3.9** Reaction conditions screened for library synthesis

<table>
<thead>
<tr>
<th>Reaction conditions</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>IPA/80-125 °C</td>
<td>No reaction</td>
</tr>
<tr>
<td>Hcl/dioxane/IPA/90 °C</td>
<td>No reaction/Hydrolyzed product</td>
</tr>
<tr>
<td>AlCl₃/ BuOH/90 °C</td>
<td>No reaction/Hydrolyzed product</td>
</tr>
<tr>
<td>BINAP/Pd(Oac)₂/KotBu/Tol/120 °C</td>
<td>No reaction/Hydrolyzed product/product</td>
</tr>
<tr>
<td>Cs₂CO₃/Xantphos/Pd(Oac)₂/DMF/150°C</td>
<td>No product</td>
</tr>
<tr>
<td>KotBu/Xantphos/ Pd(Oac)₂/DMF/120 °C</td>
<td>No reaction/ No product</td>
</tr>
<tr>
<td>Pd₂dba₂/xpos/ Cs₂CO₃/DMF/160 °C</td>
<td>Hydrolyzed product</td>
</tr>
<tr>
<td>Pd₂dba₂/xpos/ Cs₂CO₃/Dioxane/140 °C</td>
<td>No reaction/product</td>
</tr>
<tr>
<td>Pd₂dba₂/xpos/ NaOtBu/tol/140 °C</td>
<td>No reaction</td>
</tr>
<tr>
<td>NaH/DMF/RT</td>
<td>product</td>
</tr>
</tbody>
</table>

After establishing a procedure for the preparation of the penultimate compound 3.3, we envisioned introducing the library amines via parallel synthesis. The first approach towards this synthesis was to use the manually-selected available amines along with a slightly bulkier amine purchased from ASDI in larger quantities for optimizing the reaction conditions. Conventional and microwave heating was utilized. Inspired by our earlier success of introducing the head group amines in refluxing IPA condition, we
attempted a few reactions using these similar conditions. This protocol proved to be inefficient for all the amines attempted resulting in no reaction. Hence we changed the solvents and introduced catalytic amounts of acids to facilitate the reaction. The results were the same with no improvement in the progress of the reaction to get the desired product resulting in unwanted side product formation. By LC-MS characterization, the side product was found to be the hydrolyzed product 3.9

![Image of compound 3.9]

Hence we changed our protocol to introduce palladium-catalyzed conditions which were initially avoided to opt for economically and environmentally benign methods with simpler reagents. Buchwald-Hartwig amination reaction is extensively used in the cross-coupling reactions of amines with aromatic halides using strong base. Initially the 1,2,4-triazin-3-amine was used for optimization of Buchwald conditions. The different reaction conditions used are outlined in Table 3.9. Surprisingly all the reactions conditions failed to produce any isolable products as expected and the side product 3.9 was found to be the major byproduct for most of the reactions. It was unclear why the reaction failed but we assumed that the side product could have resulted from moisture present in the solvent or reaction mixtures. Hence to prevent the formation of byproduct, all the reaction was performed under carefully controlled conditions using newly purchased dry solvents and performing degassing for a longer time. This also resulted in the same outcome as described before.

![Image of Scheme 3.8]

**Scheme 3.8** Optimized route for library synthesis.
Reagents and conditions: (a) R= Primary amines, NaH, dry DMF, RT, N₂, 47-88%
Finally the amination reaction was performed by using NaH in the presence of DMF at room temperature. The reaction conversion was 100% in 30 min when performed with 5 mg of the starting material 3.3. The formation of side product was also limited to 15-20% for most of the amines. Based on the optimization studies, DMF was the best solvent for the reaction compared to THF and toluene. The reaction was carried out under rigorously inert atmosphere to reduce the side product formation and the reaction was successful for all library amines proposed. The advantage of this new methodology is that the reaction is simple, no expensive catalyst required, fast (< 3h) and can be performed at RT. The drawbacks of the synthesis involve the removal of high boiling DMF (which required Genevac evaporation). Most of the amines survived this step except for 4 amines which decomposed under these harsh conditions to remove the solvents (Figure 3.4). Nevertheless, we were able to successfully synthesize 12 of the 16 prioritized library compounds using a novel method for the first generation head group truncation library series. All the final compounds were purified by injecting the reaction mixture on prep HPLC C18 analytical column at 40 mL/min with 40% of acetonitrile in water as gradient while monitoring the chromatogram by absorbance at 214 nM and by ESI-MS. Major peaks with purity > 95% were collected and pooled from multiple runs, lyophilized and characterized by ESI-MS in positive mode. All the analogs including the intermediates were screened against all four protozoan parasites.

Figure 3.4 Some of the library amines (final product) decomposed during lyophilization
3.4 Results and discussions

So far the SAR performed on the 3-cyanoquinoline scaffold was focused on the head group region to reduce the size and lipophilicity of the lead molecule. The design and synthesis of head truncation analogs was performed to explore a possible SAR cross-over with the lead 2.25b. The screening results of the library against T. cruzi and the cross-screening data against other related parasites are summarized in Table 3.10-3.12. It was evident from the in vitro screening results of library analogs, that swapping small polar molecules resulted in loss of activity against T. cruzi. No improved potency over the lead molecule was observed and most of them were totally inactive with EC₅₀ > 50 µM. We can also infer from the SAR study of all the analogs so far synthesized with cyanoquinoline, the C-4 aniline substitution is essential for activity (3.2a-3.2d and 3.4f, 3.4j). Small five-membered heterocyclic rings like thiazoles, oxazoles, isoxazoles and triazoles are totally inactive. Swapping the phenyl group for its bio-isostere pyridine or pyrimidine also resulted in reduced activity but not complete loss of activity.

The degree of selectivity over host cell has been increased tremendously for most of the analogs. Only two analogs with pyridine-3-amine 3.4f (EC₅₀ = 15.13 µM) and pyrazine-2-amine 3.4j (EC₅₀ = 12.49 µM) shows modest degree of activity with an improved LLE.

The goal of our optimization campaign at this point was to increase potency without increasing lipophilicity at the same time and the results shows the impact of lipophilicity on the activity. Reducing the logP has resulted in reduced host cell toxicity, though, unfortunately, with loss of potency for these library analogs against T. cruzi. Overall, the data in Table 3.10 shows decrease in potency with increasing amine basicity. By reducing MW and logP, we can improve LE and LLE to determine the optimal fragment size required for activity. But a clear correlation between LE/LLE to potency could not be established since most of the analogs were inactive for T. cruzi. An advantage of having reduced logP and MW for compounds with moderate activity is that it can be taken for further optimization. The results also suggest the importance of retaining C-4 aniline substitutions, or its bioisosteres.
For other parasites, we were able to observe a wide range of cellular activity with improved potencies. The most potent compound with a 3-cyanoquinoline core against the parasite *T. brucei* was identified to be **3.4f** with 0.095 µM activity better than the lead compound.

We observed several active compounds against the D6 strain of *P. falciparum* with **3.2g** having 70 nM potency. None of the compounds were active against *Leishmania*, except for **3.2g** (EC$_{50}$ = 4.46), which was better than the lead **2.25b**. Despite having a flat SAR for this head group truncation library series, we were able to improve the overall physicochemical properties along with ligand efficiency and lipophilic efficiency.

### 3.5. Summary

The ultimate goal of this library synthesis was to create analogs that are closer to compliance with Lipinski’s Ro5 for oral bioavailability without the loss of activity. In this case, despite achieving improved predicted physicochemical properties, the screening results did not validate our hypothesis for swapping the bulky lipophilic head group with small polar heterocyclic monomers. But an initial SAR was developed for the most potent Chagas compound which shows considerable potencies against other trypanosomal parasites. The first library has given us some insight about the head group substitution required for activity. A more detailed study of a larger set of chemically diverse analogs is needed to deduce the relationship of potency and lipophilic ligand efficiency. Future work will focus on addressing the need for increased anti-parasitic potency. Optimizing both head and tail region of the 3-cyanoquinoline template will decide if this chemotype holds any strong anti-trypanosomal inhibitory properties as hoped initially based on lead compound **2.25b**.
Table 3.10 Screening results against *T. cruzi*

<table>
<thead>
<tr>
<th>Compound</th>
<th>Head group</th>
<th>NEU #</th>
<th>EC&lt;sub&gt;50&lt;/sub&gt; (µM)</th>
<th>Host cell Toxicity TC&lt;sub&gt;50&lt;/sub&gt; (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.4a</td>
<td></td>
<td>NEU-1886</td>
<td>&gt; 50.0</td>
<td>&gt; 100</td>
</tr>
<tr>
<td>3.4b</td>
<td></td>
<td>NEU-1887</td>
<td>&gt; 50.0</td>
<td>&gt; 100</td>
</tr>
<tr>
<td>3.4c</td>
<td></td>
<td>NEU-1888</td>
<td>&gt; 50.0</td>
<td>&gt; 100</td>
</tr>
<tr>
<td>3.4d</td>
<td></td>
<td>NEU-1889</td>
<td>&gt; 50.0</td>
<td>&gt; 100</td>
</tr>
<tr>
<td>3.4e</td>
<td></td>
<td>NEU-1890</td>
<td>&gt; 50.0</td>
<td>81.9</td>
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Table 3.1 Cross-screening results of related parasites

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Table 3.12 Calculated properties for library series

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Chapter 4: Future directions

4.1 Status and success achieved with lapatinib analogs for anti-trypanosomal drug discovery

The work described so far in this thesis has shown that repurposing human tyrosine kinase inhibitor for anti-trypanosomal properties is indeed possible. The broad SAR research studies conducted in our lab on the human EGFR/Her2 kinase inhibitor drug lapatinib have yielded several potent analogs for *T. brucei* growth inhibition. We advanced our work by cross-screening these analogs against other protozoan parasites. Thus we were able to identify new leads with improved activity and selectivity against all three trypanosomal parasites responsible for HAT, Chagas disease, Leishmaniasis and also against a highly resistant strain of *P. falciparum* causing Malaria.

The most potent compound identified for Chagas disease (2.25b) was advanced to PK studies and *in vivo* animal efficacy studies. Despite having a good *in vitro* potency and PK profile, the lead molecule failed in the *in vivo* animal studies due to poor physicochemical properties. Hence we hypothesized that optimizing physicochemical properties will result in improved lead candidates with better activity profile. Using 3D shape and electrostatics similarity searching, we attempted to design and synthesize analogs to address these issues, which has resulted in reduced host cell toxicity and predicted physicochemical properties for Chagas disease. Unfortunately the first generation of head group truncation library series created for *T. cruzi* inhibition did not give the anticipated retention of potency. These results suggest that more extensive SAR studies need to be performed to address the above issues without compromising the parasitic activity.

4.2 Next steps for the development of SAR on 3-cyanoquinoline template for Chagas disease

The eventual goal of this project is to design smaller molecule that shows stronger anti-parasitic activity along with acceptable physicochemical properties for success in the *in vivo* animal studies. Also we need to identify the pharmacophores responsible for potency by performing a detailed SAR studies on different part of the molecule separately. The optimization strategy should include both head and tail end of the molecule and identify the best possible combination for lead discovery. This can be achieved in two stages as discussed below.
4.2.1 More focused library of head group truncations for better efficacy and physicochemical properties

So far the SAR performed on 3-cyanoquinoline molecule was focused on the head group region to reduce the size and lipophilicity of the lead molecule. It was evident from the in vitro screening results for these library analogs, that swapping small polar molecules resulted in complete loss of activity. We can also infer from the preliminary SAR studies of all the analogs so far synthesized with 3-cyanoquinoline, that C-4 aniline substitution is one of the essential criteria for activity (3.4f, 3.4j).

Hence the second generation of library analogs can be designed for optimizing the C-4 aniline substituents using a fragment based approach. This will result in the increased lipophilicity and size of the analogs, but the library design can be made using the virtual screening tools to maintain the molecular property within the limit of Lipinski’s rule as described in Chapter 2 of this thesis. This set of analogs would provide detailed knowledge of the preferred substitution (meta, ortho, para) and polarity profile for activity. (Figure 4.2)

Third generation of library analogs can be made by choosing the conformers from the ROCS/EON list generated previously for optimizing the head group (Chapter 2). More focused library based on rankings, choosing conformers within the top 20 and maintaining the logP in the range of 3.5 to 4.5 would increase the chance of better activity (Figure 4.1) Also, we can choose slightly bulkier molecule with polar substituent to compromise for the increased lipophilicity and TPSA.

![Figure 4.1](image.png)

Figure 4.1 Plot showing the library amines chosen based on Rank and increased lipophilicity
4.2.2 Optimization of the tail end of the lead molecule 2.25b

The tail end optimization work done so far was focused on small library of analogs with different boronates at the C-6 position of 3-cyanoquinoline template (Chapter 2). Except for N-methyl homopiperazine phenyl sulfonamide, the rest of the boronates resulted in loss of potency. Hence a more focused library can be generated by methodical reduction of the size followed by addition of water solubilizing groups to explore the pharmacophore responsible for activity of the lead molecule. For initial SAR, the C-4 substitution of the lead molecule 2.25b can be retained as none of the other truncated analogs were more active.

![Diagram showing the optimization process]

**Figure 4.2** Summary of planned future work for 2.25b
Interestingly, several literature studies have identified the 3-cyanoquinoline core possessing a C-7 substitution to be a potent EGFR/Src-kinase inhibitor and other type of kinase inhibitors [49, 62]. Also the presence of certain functional groups at C-7 was shown to be more active than they were at C-6 [49]. Based on these findings, we can design library with similar C-6 substitution at C-7 of 3-cyanoquinoline. A few of the suggested analogs are listed in Figure 4.3

![Chemical Structure](image)

**Figure 4.3** Summary of SAR at C-7 of 3-cyanoquinoline template
Chapter 5: Experimental Section

General Experimental

Chemical Synthesis: All reagents were purchased from commercial suppliers such as Sigma-Aldrich, Inc. (St. Louis, MO), Fisher Scientific, Frontier Scientific Services, Inc. (Newark, DE), Matrix Scientific (Columbia, SC) were used as received unless otherwise noted. Reaction solvents were dried and purified by passage through activated alumina columns on a purification system manufactured by Innovative Technology (Newburyport, MA). All the reactions were performed under nitrogen (N$_2$) atmosphere. Microwave reactions were performed using a Biotage Initiator-8 instrument. Proton (¹H) Nuclear Magnetic Resonance (NMR) spectra were obtained with Varian NMR systems, operating at 400 or 500 MHz. Chemical shifts (δ) were reported in parts per million (ppm) relative to deuterated NMR solvents such as CDCl$_3$, DMSO$_6$, CD$_3$OD and (CD$_3$)$_2$CO as internal standard. LC-MS analysis was performed on a Waters Alliance reverse-phase HPLC using single-wavelength UV-visible detector and LCT Premier time-of-flight mass spectrometer (electrospray ionization). All the final compounds synthesized were purified to ≥ 95% (AUC) by LC-MS and characterized by NMR spectra prior to biological testing. Preparative LC-MS was performed on a Waters FractionLynx system with a Waters Micro Mass ZQ mass spectrometer (electrospray ionization) and a single-wavelength UV-visible detector, using acetonitrile/H$_2$O gradients with 0.1% formic acid as a modifier. Fractions were collected based on UV absorbance and mass detection. The pure product fractions were finally dried by lyophilization using Genevac. Percentage yields reported for all the final products represent the amount of pure material isolated by preparative HPLC and the impure fractions were not re-purified. Data for ¹H NMR spectra are reported as follows: chemical shift (ppm), multiplicity (s=singlet, d = doublet, t = triplet, q = quartet, dd = doublet of doublets, m = multiplet), coupling constant (Hz) integration.
Experimental procedures and characterization

Synthesis of 3-cyanoquinoline core template (2.14)

Reagents and conditions: (a) Dry toluene, 125 °C, N₂, 6 h, 77%; (b) Diphenyl ether, 300 °C, N₂, 15 h, 87%; (c) POCl₃, 115 °C, N₂, 4 h, 89%.

Procedures

Ethyl 2-cyano-3-((4-iodophenyl) amino) acrylate (2.12, white solid, 77% isolated yield): A mixture of ethyl 2-cyano-3-ethoxyacrylate (2.10) (0.386 g, 2.283 mmol, 1 equiv) and 4-iodoaniline (2.11) (0.500 g, 2.283 mmol, 1 equiv) in dry toluene (1.2 mL) was refluxed at 125 °C for 6 h and the reaction was monitored by LC-MS. The reaction mixture was cooled to 20 °C at which it became a brown solid. To this, diethyl ether (50 mL) was added in portions, stirred and the solids were filtered. Solvents were further removed under reduced pressure at 45 °C to afford 0.6 g of 2.12 (17.54 mmol, 77 %) as white solid. ¹H NMR (399 MHz, CDCl₃) δ (ppm) 10.74 (d, J = 12.48 Hz, 1H), 7.81 (d, J = 13.96 Hz, 1H), 7.70 (d, J = 8.79 Hz, 2H), 6.86 (d, J = 8.79 Hz, 1H), 4.27 - 4.33 (m, 2H), 1.37 (t, J = 6.98 Hz, 3H). ESI-MS m/z C₁₂H₁₁I₂N₂O₂ [M+H]⁺, calcd 342.99; obsd 343.0
6-iodo-4-oxo-1, 4-dihydroquinoline-3-carbonitrile (2.13, pale brown solid, 87% isolated yield): A mixture of ethyl 2-cyano-3-((4-iodophenyl) amino) acrylate (2.12) (0.400 g, 1.169 mmol, 1 equiv) and diphenyl ether (4.00 mL, 25.1 mmol, 21.5 equiv) were combined in a flask. Heating (40 °C) was required to melt solid diphenyl ether prior to the addition. The flask was immersed in a pre-heated sand bath and the temperature of the sand bath was maintained above 300 °C under N₂ and refluxed for 28 h. The reaction was monitored by LC-MS. Upon completion of the reaction, the reaction mixture was cooled to ambient temperature. Diethyl ether (10 mL) was added to the reaction mixture and stirred at RT temperature for 10 min. The brown solids were filtered under vacuum and washed with diethyl ether (3×5 mL). It was then air-dried to afford 0.3 g of 2.13 (1.013 mmol, 87%) as pale brown solid. ¹H NMR (500 MHz, (CD₃)₂SO) δ (ppm) 12.93 (br, s, 1H), 8.78 (s, 1H), 8.39 (d, J = 2.5 Hz, 1H), 8.07 (dd, J = 2, 8.5 Hz, 1H), 7.45 (d, J = 9 Hz, 1H). ESI-MS m/z C₁₀H₉IN₂O [M+H]⁺, calcd 296.94; obsd 296.93

4-chloro-6-iodoquinoline-3-carbonitrile (2.14, pale yellow solid, 89% isolated yield): A solution of phosphoryltrichloride (5.73 ml, 61.5 mmol, 26 equiv) was slowly added to 0.7 g of 6-iodo-4-oxo-1, 4-dihydroquinoline-3-carbonitrile (2.13) (0.700 g, 2.364 mmol, 1 equiv) in a flask and refluxed at 115 °C under N₂ for 4 h. The reaction progress was monitored by LC-MS. Upon completion of the reaction, the crude mass was cooled to 20 °C and quenched carefully with saturated sodium bicarbonate solution. Solids collected by filtration were washed with cold water and dried under vacuum overnight. Collected solids were washed with diethyl ether (50 mL) in portions and the combined ether layer was evaporated
to obtain 0.66 g of 2.14 (2.099 mmol, 89%) as pale yellow solid. $^1$H NMR (400 MHz, (CD$_3$)$_2$SO) $\delta$ (ppm) 9.23 (s, 1H), 8.65 (d, J = 2 Hz, 1H), 8.33 (dd, J = 2.4, 8.8 Hz, 1H), 7.96 (d, J = 8.8 Hz, 1H). ESI-MS m/z C$_{10}$H$_4$ClIN$_2$ [M+H]$^+$, calcd 314.91; obsd 314.97.

Alternate route for the synthesis of 2.13

Reagents and conditions: (a) DMF-DMA, Dry DMF, 120 °C N$_2$, 4 h, 96%; (b) i. n-Buli/DIPA/THF -78 °C, N$_2$; ii. Acetonitrile -78 °C; iii. 2.16, THF -78 °C, 1 h, RT, 86%.

(Z)-Methyl 2-(((dimethylamino)methylene)amino)-5-iodobenzoate (2.16, pale brown solid, 96% isolated yield): To a solution of 2-amino-5-iodobenzoic acid (2.15) (6.00 g, 22.81 mmol, 1 equiv) in DMF (30 mL) was added DMF-DMA (10.08 mL, 75 mmol, 3.76 equiv). The reaction mixture was heated to 120 °C for 3 h at which the reaction turned purple to brown. The progress of the reaction was monitored by LC-MS and TLC. Upon completion of the reaction, the solvents were concentrated in high vacuum and the crude sample was characterized by LC-MS and NMR. The product 2.16 was isolated as brown solid, (21.98 mmol, 7.3g, 96%). $^1$H NMR (399 MHz, CDCl$_3$) $\delta$ (ppm) 8.01 (d, J = 2.20 Hz, 1H), 7.60 (d, J = 2.20 Hz, 1H), 7.33 (s, 1H), 6.62 (d, J = 8.79 Hz, 1H), 3.84 (s, 3H), 3.02 (s, 6H). ESI-MS m/z C$_{11}$H$_9$IN$_2$O$_2$ [M+H]$^+$, calcd 333.0 obsd 333.4.
6-iodo-4-oxo-1,4-dihydroquinoline-3-carbonitrile (2.13, pale yellow solid, yield 86%): To a solution of diisopropylamine (6.26 ml, 44.0 mmol, 2 equiv) in 50 mL of THF at -78 °C, n-butyl lithium (21.98 ml, 44.0 mmol, 2 equiv) was added slowly under a steady stream of N₂ atmosphere. After stirring the reaction mixture for 30 min at -78 °C acetonitrile (2.311 ml, 44.0 mmol, 2 equiv) was added slowly and the reaction was stirred for an additional one hour while maintaining the reaction temperature at -78 °C.

A solution of (Z)-methyl 2-(((dimethylamino)methylene)amino)-5-iodobenzoate (2.16) (7.30 g, 21.98 mmol, 1 equiv) in dry THF (175 mL) was added slowly to the reaction mixture at -78 °C which immediately turned yellow colored ppt. The reaction mass was slowly warmed to ambient temperature and stirred for 1 h. The reaction progress was monitored by LC-MS and TLC. After confirming the reaction completion, the reaction mixture was cooled to -50 °C and the pH was adjusted to 1 by the addition of 1M HCl solution. The solids precipitated out were then filtered and the filtrate was further concentrated in vacuo. The combined residues were dried under vacuum. The solids were washed with of DCM (100 mL) followed by of hexane (100 mL) in portions to remove the non-polar impurities and were dried under vacuum to obtain 2.13 (5.57g, 9.36 mmol, 86%) of as pale yellow solid. ¹H NMR (399 MHz, (CD₃)$_₂$SO) δ ppm 8.79 (s, 1H), 8.39 (d, $J=2.20$ Hz, 1H), 8.07 (dd, $J=1.83$, 8.43 Hz, 1H), 7.45 (d, $J=8.79$ Hz, 1H).

ESI-MS m/z C₁₀H₅IN₂O [M+H]$^+$, calcd 296.94; obsd 296.94
Synthesis of head group derivatives (2.20)

Reagents and conditions: (a) K$_2$CO$_3$, Acetonitrile, 85 °C, 8 h, 87-99%; (b) Zn, NH$_4$Cl, MeOH, H$_2$O, 55 °C, 6 h, 64-69%.

1-(benzyloxy)-2-chloro-4-nitrobenzene (2.19a, pale yellow solid, 87%): To a mixture of 2-chloro-4-nitrophenol 2.17 (0.500 g, 2.88 mmol, 1 equiv) and potassium carbonate (0.597 g, 4.32 mmol, 1.5 equiv) in acetonitrile was added (bromomethyl)benzene 2.18a (0.377 mL, 3.17 mmol, 1.1 equiv) slowly through syringe. The reaction mass was stirred at 85 °C under N$_2$ for 8 h at which the reaction mixture (orange) became yellow. After confirming the reaction completion by TLC, the reaction mixture was filtered and the inorganic solids were washed with DCM (10 mL). The filtrate was concentrated in vacuo to yield 0.66g of 2.19a (2.503 mmol, 87%) as pale yellow solid. \textsuperscript{1}H NMR (399 MHz, CDCl$_3$) δ (ppm) 8.16 (d, $J = 2.93$ Hz, 1H), 7.97 (dd, $J = 2.56$, 9.16 Hz, 1H), 7.19 - 7.31 (m, 4H), 6.87 (d, $J = 9.53$ Hz, 1H), 5.12 (s, 2H). ESI-MS m/z C$_{13}$H$_{10}$ClNO$_3$ [M+H]$^+$, calcd 264.03; obsd 264.13.

4-(benzyloxy)-3-chloroaniline (2.20a, pale brown solid, 69%): To a solution of 1-(benzyloxy)-2-chloro-4-nitrobenzene 2.19a (0.650 g, 2.465 mmol, 1 equiv) and Zinc (0.484 g, 7.40 mmol, 3 equiv) in methanol (10 mL) was added ammonium chloride (0.791 g, 14.79 mmol, 6 equiv) in water (5 mL). The resulting
suspension was stirred at 55 °C for 6 h. The reaction progress was monitored by TLC and LC-MS. Upon completion of the reaction, the contents of the reaction mass was cooled to ambient temperature and diluted with DCM: methanol (10 mL). It was then filtered through a celite bed to remove inorganic residues and the filtrate was concentrated. The crude mass was dissolved in ethyl acetate, washed with water (2×5), saturated brine solution (5 mL) and concentrated. The brown solid was triturated with a mixture of ether/hexane (10 mL) to yield the desired product **2.20a** as pale brown solid (0.4g, 1.712 mmol, 70%). $^1$H NMR (399 MHz, CDCl$_3$) δ (ppm) 7.43 - 7.47 (m, 2H), 7.37 (t, $J$ = 7.33 Hz, 2H), 7.32 (d, $J$ = 7.33 Hz, 1H), 6.79 (d, $J$ = 8.06 Hz, 1H), 6.76 (d, $J$ = 2.20 Hz, 1H), 6.50 (dd, $J$ = 2.93, 8.79 Hz, 1H), 5.05 (s, 2H). ESI-MS m/z C$_{13}$H$_{12}$ClNO [M+H]$^+$, calcd 234.06; obsd 234.03.

The above procedure for **2.19a** and **2.20a** were used for the synthesis of **2.19b** and **2.20b**

![2.19b](image)

**2-chloro-1-((3-fluorobenzyl)oxy)-4-nitrobenzene (2.19b, yellow solid, yield 99%)** $^1$H NMR (399 MHz, CDCl$_3$) δ (ppm) 8.16 (d, $J$ = 2.93 Hz, 1H), 7.97 (dd, $J$ = 2.56, 9.16 Hz, 1H), 7.19 - 7.31 (m, 4H), 6.87 (d, $J$ = 9.53 Hz, 1H), 5.16 (s, 2H). ESI-MS m/z C$_{13}$H$_9$ClFNO$_3$ [M+H]$^+$, calcd 282.03; obsd 282.03.

![2.20b](image)

**3-chloro-4-((3-fluorobenzyl)oxy)aniline (2.20b, brown solid, yield 70%)**: $^1$H NMR (399 MHz, CDCl$_3$) δ (ppm) 7.37 - 7.40 (m, 1H), 7.26 (t, $J$ = 7.33 Hz, 2H), 7.04 - 7.08 (m, 1H), 6.82 - 6.85 (m, 2H), 6.55 - 6.58 (m, 1H), 5.09 (s, 2H), 3.56 (br. s., 2H). ESI-MS m/z C$_{13}$H$_{11}$ClFNO [M+H]$^+$, calcd 252.05; obsd 252.03.
Synthesis of head group analogues (2.21)

Reagents and conditions: (a) Isopropanol, 85 °C, N₂, 4 h, 77-80%.

4-((4-(benzyloxy)-3-chlorophenyl)amino)-6-iodoquinoline-3-carbonitrile (2.21a, yellow solid, yield 53%): A mixture of 4-chloro-6-iodoquinoline-3-carbonitrile 2.14 (0.070 g, 0.223 mmol, 1 equiv) and 4-(benzyloxy)-3-chloroaniline 2.20a (0.063 g, 0.270 mmol, 1.1 equiv) in 2-propanol (3 mL) was refluxed at 85 °C under nitrogen for 4 h. The reaction mixture turned yellow and formed a precipitate upon heating. Progress of the reaction was monitored by LC-MS. Upon completion of the reaction the crude mass was cooled to 25 °C. Yellow solid settled were filtered and washed with 2-propanol (5 mL) followed by diethyl ether (5 mL) to afford 2.21a (0.06 g, 0.117 mmol, 53%) as yellow solid. ¹H NMR (500 MHz, (CD₃)₂SO) δ (ppm) 9.02 (s, 1H), 8.82 (br, s, 1H), 8.22 (d, J = 9 Hz, 1H), 7.72 (d, J = 8.5 Hz, 1H), 7.57 (s, 1H), 7.5 (d, J = 7 Hz, 2H), 7.43 (t, J = 7.5 Hz, 2H), 7.35 (m, 3H), 5.26 (s, 2H). ESI-MS m/z C₂₃H₁₅ClIN₃O [M+H]⁺, calcd 511.99; obsd 511.99.
4-((3-chloro-4-((3-fluorobenzyl)oxy) phenyl) amino)-6-iodoquinoline-3-carbonitrile (2.21b, yellow solid, 80% yield): A mixture of reactants 4-chloro-6-iodoquinoline-3-carbonitrile 2.14 (0.130 g, 0.413 mmol, 1 equiv) and 3-chloro-4-((3-fluorobenzyl)oxy)aniline 2.20b (0.114 g, 0.455 mmol, 1 equiv) in 2-propanol (6 mL) were refluxed at 85 °C under N₂ for 4 h. The reaction mixture turned yellow precipitate upon heating. Progress of the reaction was monitored by LC-MS. Upon completion of the reaction, the reaction mass was cooled to 25 °C. Yellow solid settled were filtered and washed with 5 mL of 2-propanol followed by 5 mL of diethyl ether to afford 0.175 g 2.21b (0.175 g, 0.330 mmol, 80%) of as yellow solid.

¹H NMR (400 MHz, (CD₃)₂SO) δ (ppm) 9.03 (s, 1H), 8.85 (br, s, 1H), 8.23 (d, J = 8.8 Hz, 1H), 7.73 (d, J = 8.8, 1H), 7.59 (d, J = 2.4, 1H), 7.45-7.50 (m, 1H), 7.30-7.39 (m, 4H), 7.18-7.22 (m, 1H), 5.30 (s, 2H). ESI-M) m/z C₂₃H₁₄ClIFN₃O [M+H]⁺, calcd 529.99; obsd 529.99.

Synthesis of Boronic ester Analogues (2.23)

Reagents and conditions: (a) Bis(pinacolato)diboron, PdCl₂(dppf)-CH₂Cl₂, Potassium Acetate, 1,4-dioxane, 80 °C, N₂, 12 h, 34–72%.
1-methyl-4-((4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)sulfonyl)piperazine (2.23a, pale brown solid, yield 35%): A mixture of 1-((4-bromophenyl)sulfonyl)-4-methylpiperazine 2.22a (0.500 g, 1.566 mmol, 1 equiv), 4,4,4',4',5,5,5',5'-octamethyl-2,2'-bis(1,3,2-dioxaborolane) (0.597 g, 2.349 mmol, 1.5 equiv) in 1,4-dioxane (8 mL) were added potassium acetate (0.461 g, 4.70 mmol, 3 equiv) followed by PdCl$_2$(dpdpf)-CH$_2$Cl$_2$Adduct (0.064 g, 0.078 mmol, 0.05 equiv). The contents were stirred at 80 °C for 9 h under N$_2$ and the reaction was monitored by TLC and LC-MS. The solvents were evaporated in vacuo and the crude mass was dissolved in ethyl acetate (10 mL), washed with water (5 mL) followed by saturated brine solution (5 mL) and concentrated in vacuo at 40 °C. The crude product (black solid) was re-dissolved in DCM (15 mL) and stirred with 0.5 g of charcoal for 20 min. It was then filtered through celite bed and washed with a mixture of 1:1 ratio of DCM:MeOH (15 mL). The combined organic layers were then evaporated to afford the desired product 2.23a as a pale brown solid (0.2 g, 0.546 mmol, 35%). $^1$H NMR (399 MHz, CDCl$_3$) δ (ppm) 7.94 (d, $J = 8.1$ Hz, 1H), 7.72 (d, $J = 8.1$ Hz, 1H), 3.02 (br. s., 2H), 2.46 (t, $J = 4.8$ Hz, 3H), 2.25 (s, 2H), 1.36 (s, 12H). ESI-MS m/z C$_{17}$H$_{27}$BN$_2$O$_4$S [M+H]$^+$, calcd 367.18; obsd 367.23.
1-methyl-4-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenylsulfonyl)-1,4-diazepane (2.23b, tan solid, 153%): To a mixture of 1-(4-bromophenylsulfonyl)-4-methyl-1,4-diazepane 2.22b (4.00 g, 12.00 mmol, 1 equiv), and 4,4,4',4',5,5,5',5'-octamethyl-2,2'-bi(1,3,2-dioxaborolane) (3.96 g, 15.60 mmol, 1.3 equiv) in 1,4-dioxane (36 mL) were added potassium acetate (5.89 g, 60.0 mmol, 5 equiv) followed by PdCl$_2$(dppf)-CH$_2$Cl$_2$Adduct (0.490 g, 0.600 mmol, 0.05 equiv) was added. The contents were stirred at 90 °C for 12 h and the reaction was monitored by TLC and LC-MS. Upon completion of the reaction, the solvents were evaporated and the crude mass was dissolved in DCM / EtOAc (50 mL) mixture. To this charcoal (16 g) was added and stirred at ambient temperature for 30 min. The black colored suspension was then filtered through celite bed and the washed with methanol (25 mL). The combined organic layers were concentrated to get reddish brown viscous liquid. This was triturated with diethyl ether (50 mL) in portions. The solids obtained were filtered and the filtrate was concentrated to get brown solid. These crude mixture were given hexane (200 mL ) wash in portions and filtered to yield the desired product 2.23b as a tan solid (7 g, 18.41 mmol, 153%). $^1$H NMR (399 MHz, CDCl$_3$) δ ppm 7.93 (d, $J = 7.2$ Hz, 2H), 7.75 (d, $J = 7.2$ Hz, 2H), 3.32 - 3.46 (m, 4H), 2.70 (br. s., 4H), 2.39 (br. s., 3H), 1.91 (br. s., 2H), 1.35 (s, 12H). (ESI-MS) m/z C$_{18}$H$_{26}$BN$_2$O$_4$S $[M+H]^+$, calcd 381.19; obsd 381.18.
4-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)morpholine (2.23c, pale orange liquid, yield 100%): To a mixture of 4-(3-bromophenyl)morpholine 2.22c (1.000 g, 4.13 mmol, 1 equiv), and 4,4,4',4',5,5,5',5'-octamethyl-2,2'-bi(1,3,2-dioxaborolane) (1.573 g, 6.20 mmol, 1.5 equiv) in 1,4-dioxane (16 mL) in a reaction vial, potassium acetate (1.216 g, 12.39 mmol, 3 equiv) and PdCl$_2$ (dppf)-CH$_2$Cl$_2$ Adduct (0.169 g, 0.207 mmol, 0.05 equiv) were added. The reaction mixture was stirred at 80 °C for 6 h and the reaction was monitored by TLC and LC-MS. Upon completion of the reaction, the solvents were evaporated and the crude mass was dissolved in ethyl acetate (25 mL), washed with water (5×2), saturated brine solution (10 mL) and concentrated in vacuo at 40 °C to afford the desired product 2.23c quantitatively as a pale orange liquid (1.2 g, 4.15 mmol). $^1$H NMR (399 MHz, CDCl$_3$) δ (ppm) 7.26 - 7.37 (m, 3 H), 6.98 - 7.06 (m, 1 H), 3.86 (br. s., 4 H), 3.20 (br. s., 4 H), 1.34 (s, 12 H). ESI-MS m/z C$_{16}$H$_{24}$BNO$_3$ [M+H]$^+$, calcd 290.18; obsd 290.18.

Synthesis of Analogues via Suzuki coupling (2.24-2.27)

Libraries of 2.24-2.27 were synthesized by Suzuki coupling of 2.21a-b with respective boronic acid/esters (2.23a-c) following the below general procedures.
To a mixture of 4-((3-chloro-4-((3-fluorobenzyl)oxy)phenyl)amino)-6-iodoquinoline-3-carbonitrile 2.21 (0.050 g, 0.094 mmol, 1 equiv), and the corresponding boronic esters 2.23 (0.104 mmol, 1.1 equiv) in DME (2 mL): EtOH (1.3 mL) were added tetrakis (triphenyl phosphine) palladium (0) (5.45 mg, 4.72 µmol, 0.05 equiv) followed by 2M solution of sodium carbonate (0.283 ml, 0.566 mmol, 6 equiv) under N₂. The reaction mass was heated to 85 °C in a sealed reaction vial and monitored by TLC and LC-MS. Upon completion of the reaction, solvents were removed in vacuo and the crude mass was dissolved in ethyl acetate. The organic layers were washed with water followed by saturated brine solution. Dried over anhydrous sodium sulfate and concentrated to get the crude product which was then purified using flash column chromatography (FCC) using 5-30% of methanol in DCM as eluents. The combined pure fractions were concentrated and were further trititated with diethyl ether/DCM mixture to yield compounds 2.24-2.27 (95% purity, 25%-77% yield).
4-{4-(benzyl oxy)-3-chlorophenyl}amino)-6-{4-(morpholinosulfonyl)phenyl}quinoline-3-carbonitrile- (2.24a, NEU-914, yellow solid, yield 50%): FCC: 0-20% of ethyl acetate in hexane, isolated as yellow solid. $^1$H NMR (399 MHz, CDCl$_3$) $\delta$ (ppm) 8.70 (s, 1H), 8.11 - 8.17 (m, 2H), 7.98 (dd, $J = 1.47$, 8.79 Hz, 1H), 7.74 - 7.78 (m, 2H), 7.68 - 7.72 (m, 2H), 7.63 (s, 1H), 7.44 - 7.50 (m, 2H), 7.38 - 7.44 (m, 3H), 7.32 - 7.37 (m, 1H), 7.17 (dd, $J = 2.93$, 8.79 Hz, 1H), 7.03 (d, $J = 8.79$ Hz, 1H), 5.20 (s, 2H), 3.66 - 3.72 (m, 4H), 2.95 - 3.01 (m, 4H). ESI-MS m/z $C_{33}H_{27}ClN_4O_4S [M+H]^+$, calcd 611.14; obsd 611.14.

4-{3-chloro-4-((3-fluorobenzyl)oxy)phenyl}amino)-6-{4-(morpholinosulfonyl)phenyl}quinoline-3-carbonitrile (2.24b, NEU-926, yellow solid, yield 77%): FCC: 0-30% of ethyl acetate in hexane, isolated as yellow solid. $^1$H NMR (400 MHz, (CD$_3$)$_2$SO) $\delta$ (ppm) 10.04 (br, s, 1H), 8.91 (s, 1H), 8.60 (s, 1H), 8.28 (d, $J = 8.8$ Hz, 1H), 8.18 (d, $J = 8$ Hz, 2H), 8.05 (d, $J=8$ Hz, 1H), 7.89 (d, $J = 8$ Hz, 2H), 7.56 (d, $J = 2.4$ Hz, 1H), 7.45-7.50 (m, 1H), 7.29-7.38 (m, 4H), 7.20 (t, $J = 7.4$ Hz, 1H), 5.30 (s, 2H), 3.65 (t, $J = 4.4$ Hz, 4H), 2.92 (t, $J = 4.4$ Hz, 4H). ESI-MS m/z $C_{33}H_{26}ClF_{1}N_4O_4S [M+H]^+$, calcd 629.13; obsd 629.13.
2.25a (NEU-995)

4-((4-(benzyloxy)-3-chlorophenyl)amino)-6-(4-((4-methyl-1,4-diazepan-1-yl)sulfonyl)phenyl)quinoline-3-carbonitrile (2.25a, NEU-995, tan solid, yield 27%) FCC: 0-30% of Methanol in Dichloromethane, isolated as tan solid. $^1$H NMR (399 MHz, Acetone) δ (ppm) 9.24 (s, 1H), 8.78 (s, 1H), 8.61 (s, 1H), 8.21 - 8.28 (m, 1H), 8.03 - 8.12 (m, 3H), 7.94 (d, $J = 8.06$ Hz, 2H), 7.51 - 7.58 (m, 2H), 7.40 - 7.46 (m, 2H), 7.35 - 7.39 (m, 2H), 7.26 - 7.30 (m, 1H), 5.29 (s, 2H), 3.30 - 3.46 (m, 4H), 2.51 - 2.67 (m, 4H), 2.29 (s, 3H), 1.76 - 1.86 (m, 2H). ESI-MS m/z $C_{35}H_{32}ClN_5O_3S$ [M+H]$^+$, calcd 638.19; obsd 638.13.

2.25b (NEU-924)

4-((3-chloro-4-((3-fluorobenzyl)oxy)phenyl)amino)-6-(4-((4-methyl-1,4-diazepan-1-yl)sulfonyl)phenyl)quinoline-3-carbonitrile (2.25b, NEU-924, pale yellow solid, yield 32%) FCC: 0-30% of Methanol in Dichloromethane, isolated as pale yellow solid. $^1$H NMR (400 MHz, CDCl$_3$) δ (ppm) 8.71 (s, 1H), 8.14 (d, $J = 8.8$ Hz, 1H), 8.08 (d, $J = 2.4$ Hz, 1H), 7.99 (dd, $J = 8.8, 2.2$ Hz, 1H), 7.80-7.82 (m, 2H), 7.66 (d, $J = 8.8$ Hz, 2H), 7.47 (s, 1H), 7.41 (d, $J = 2$ Hz, 1H), 7.36-7.39 (m, 1H), 7.23 (s, 1H), 7.19-7.2 (m, 1H), 7.17 (d, $J = 2$ Hz, 1H), 7.05 (dd, $J = 8, 2.4$ Hz, 1H), 7.01 (d, $J = 8.8$ Hz, 1H), 5.19 (s, 2H), 3.36-3.40 (m, 4H), 2.56-2.62 (m, 4H), 2.33 (s, 3H), 1.81-1.85 (m, 2H). ESI-MS m/z $C_{35}H_{31}ClFNN_5O_3S$ [M+H]$^+$, calcd 656.18; obsd 656.19.
**2.26a (NEU-996)**

4-((4-(benzyloxy)-3-chlorophenyl)amino)-6-(3-morpholinophenyl)quinoline-3-carbonitrile (2.26a, NEU-996, yellow solid, yield 46%) FCC: 0-30% of Methanol in Dichloromethane, isolated as yellow solid. $^1$H NMR (400 MHz, (CD$_3$)$_2$SO) $\delta$ ppm 9.90 (br, s, 1 H), 8.72 (br, s, 1H), 8.55 (s, 1H), 8.18 (d, J=8.8 Hz, 1H), 7.97 (d, J=8.8 Hz, 1H), 7.49-7.52 (m, 3H), 7.37-7.44 (m, 4H), 7.30-7.34 (m, 4H), 7.02 (d, J=7.2 Hz, 1H), 5.26 (s, 2H), 3.77 (br, s, 4H), 3.21 (br, s, 4H). ESI-MS m/z C$_{35}$H$_{31}$ClFNO$_5$ [M+H]$^+$, calcd 547.18; obsd 547.20.

**2.26b (NEU-925)**

4-((3-chloro-4-((3-fluorobenzyl)oxy)phenyl)amino)-6-(3-morpholinophenyl)quinoline-3-carbonitrile (2.26b, NEU-925, yellow solid, yield 20%) FCC: 0-30% of Methanol in Dichloromethane, isolated as yellow solid. $^1$H NMR (400 MHz, (CD$_3$)$_2$SO) $\delta$ (ppm) 9.91 (br, s, 1H), 8.73 (s, 1H), 8.55 (s, 1H), 8.18 (d, J=8.8 Hz, 1H), 7.98 (d, J=8.8 Hz, 1H), 7.55 (d, J=2.4 Hz, 1H), 7.45-7.50 (m, 1H), 7.37-7.41 (m, 1H), 7.30-7.35 (m, 6H), 7.17-7.22 (m, 1H), 7.02 (d, J=8 Hz, 1H), 5.29 (s, 2H), 3.76-3.79 (m, 4H), 3.20-3.23 (m, 4H). ESI-MS m/z C$_{33}$H$_{26}$ClFNO$_2$ [M+H]$^+$, calcd 565.17; obsd 565.20.
2.27a (NEU-994)

4-((4-(benzyloxy)-3-chlorophenyl)amino)-6-((4-methylpiperazin-1-yl)sulfonyl)phenyl)quinoline-3-carbonitrile (2.27a, NEU-994, tan solid, yield 30%) FCC: 0-30% of Methanol in Dichloromethane, isolated as tan solid. $^1$H NMR (400 MHz, (CD$_3$)$_2$SO) δ ppm 10.01 (br, s, 1H), 8.90 (s, 1H), 8.59 (s, 1H), 8.27 (d, J=8.8 Hz, 1H), 8.16 (d, J=8 Hz, 2H), 8.04 (d, J=8.8 Hz, 1H), 7.88 (d, J=8 Hz, 2H), 7.50-7.54 (m, 3H), 7.43 (t, J=7.3 Hz, 2H), 7.31-7.38 (m, 3H), 5.26 (s, 2H), 2.95 (br, s, 4H), 2.38 (br, s, 4H), 2.14 (s, 3H). ESI-MS m/z C$_{34}$H$_{30}$ClN$_5$O$_3$S [M+H]$^+$, calcd 624.18; obsd 624.20.

2.27b (NEU-993)

4-((3-chloro-4-((3-fluorobenzyl)oxy)phenyl)amino)-6-((4-methylpiperazin-1-yl)sulfonyl)phenyl)quinoline-3-carbonitrile (2.27b, NEU-993, pale yellow solid, yield 17%) FCC: 0-30% of Methanol in Dichloromethane, isolated as pale yellow solid. $^1$H NMR (400 MHz, (CD$_3$)$_2$SO) δ (ppm) 10.01 (br, s, 1H), 8.89 (s, 1H), 8.59 (s, 1H), 8.27 (d, J=8.8 Hz, 1H), 8.16 (d, J=8 Hz, 2H), 8.05 (d, J=8.8 Hz, 1H), 7.88 (d, J=8 Hz, 2H), 7.55 (br, s, 1H), 7.45-7.70 (m, 1H), 7.31-7.35 (m, 4H), 7.17-7.21 (m, 1H), 5.30 (s, 2H), 2.95 (br, s, 4H), 2.38 (br, 4H), 2.14 (s, 3H). ESI-MS m/z C$_{34}$H$_{30}$ClN$_5$O$_3$S [M+H]$^+$, calcd 642.17; obsd 642.19.
Head group substitution to the intermediate (2.14) followed by Suzuki coupling reaction

\[
\begin{align*}
2.14 & \xrightarrow{\text{a}, \text{i}, \text{ii}} 3.1a-3.1f \\
3.1a-3.1f + 2.23b & \xrightarrow{\text{b}} 3.2a-3.2f
\end{align*}
\]

**Reagents and conditions:** (a) \( R = \) Manually-selected primary amines, (i) \( R, \) IPA, 80 °C, 72-80%; (ii) \( R, 65 \) °C, 6h, 75-97%; (b) \( \text{Pd(PPh}_3)_4, 2 \text{ M Na}_2\text{CO}_3, \) DME, Ethanol, 85 °C, 6 h

**Synthesis of analogues 3.1**

**General procedure A for the synthesis of 3.1a-3.1d:** A mixture of 4-chloro-6-iodoquinoline-3-carbonitrile 2.14 (40.0 mg, 0.127 mmol, 1 equiv) and 4-(benzyloxy)aniline (30.4 mg, 0.153 mmol, 1.2 equiv) in IPA (3 mL) was stirred at 80 °C for 12 h. The progress of the reaction was monitored by LC-MS. Upon completion of the reaction, solvents were concentrated *in vacuo* and the resulting mixture was filtered. The solids were washed with IPA (5 mL) followed by diethyl ether (5 mL) and dried under vacuum to afford 3.1a-3.1d (72-87% yield). These products were then directly used for Suzuki coupling reactions with the corresponding boronic esters without further purification.
3.1a

4-((5-chloropyridin-2-yl)amino)-6-iodoquinoline-3-carbonitrile (3.1a, pale yellow solid, 83%) \(^1^H\) NMR (399 MHz, (CD\(_3\))\(_2\)SO) \(\delta\) (ppm) 8.99 (br. s., 1H), 8.83 (br. s., 1H), 8.39 - 8.46 (m, 1H), 8.22 (d, \(J = 8.8\) Hz, 1H), 7.86 (d, \(J = 5.60\) Hz, 1H), 7.75 (d, \(J = 8.1\) Hz, 1H), 7.62 (d, \(J = 8.4\) Hz, 1H). ESI-MS m/z \(C_{15}H_8ClIN_4\) [M+H]\(^+\), calcd 406.95; obsd 406.97.

3.1b

4-((3-chloro-4-methoxyphenyl)amino)-6-iodoquinoline-3-carbonitrile (3.1b, brown solid, 87%) \(^1^H\) NMR (399 MHz, (CD\(_3\))\(_2\)SO) \(\delta\) (ppm) 9.04 (s, 1H), 8.82 (br. s., 1H), 8.22 (d, \(J = 8.79\) Hz, 1H), 7.73 (d, \(J = 8.79\) Hz, 1H), 7.55 (s, 1H), 7.35 - 7.41 (m, 1H), 7.24 (d, \(J = 8.79\) Hz, 1H), 7.19 (br. s., 1H), 3.91 (s, 3H). ESI-MS m/z \(C_{17}H_{11}ClIN_3O\) [M+H]\(^+\), calcd 435.96; obsd 435.97.

3.1c

4-((4-((3-fluorobenzyl)oxy)phenyl)amino)-6-iodoquinoline-3-carbonitrile (3.1c, yellow solid, 83%) \(^1^H\) NMR (500 MHz, (CD\(_3\))\(_2\)SO) \(\delta\) (ppm) 9.01 (s, 1H), 8.82 (br, s, 1H), 8.11 (d, \(J = 9\) Hz, 1H), 7.52 (d, \(J = 8.5\)

80
Hz, 1H), 7.45 (s, 1H), 7.6 (d, J = 7 Hz, 2H), 7.43 (t, J = 7.5 Hz, 2H), 7.35 (m, 3H), 5.16 (s, 2H). ESI-MS m/z C_{23}H_{15}F_{13}N_{3}O [M+H]^+, calcd 496.02; obsd 496.05.

4-(4-(benzyloxy)phenylamino)-6-iodoquinoline-3-carbonitrile (3.1d, yellow solid, yield 73%)

$^1$H NMR (500 MHz, (CD$_3$)$_2$SO) δ (ppm) 9.04 (s, 1H), 8.49 (br, s, 1H), 8.05 (d, J = 9 Hz, 1H), 7.63 (d, J = 8.5 Hz, 1H), 7.48 (s, 2H), 7.5 (d, J = 7 Hz, 2H), 7.40 (t, J = 7.5 Hz, 2H), 7.35 (m, 3H), 5.16 (s, 2H). ESI-MS m/z C_{23}H_{16}I_{13}N_{3}O [M+H]^+, calcd 478.03; obsd 478.05.

General procedure B for the synthesis of 3.1e-3.1fe: A mixture of 4-chloro-6-iodoquinoline-3-carbonitrile 2.14 (75 mg, 0.238 mmol, 1 equiv) and ammonia (5N in methanol) (5.96 mmol, 25 equiv) were stirred at 65 °C for 6 h. Upon reaction completion, the reaction mixture was filtered under vacuum, the solids was washed with DCM (10 mL) to yield the product as off-white solids of 3.1e-3.1fe (75-97%). The resulting compounds were taken into next step without further purification.
4-amino-6-iodoquinoline-3-carbonitrile (3.1e, off-white solid, yield 75%) $^1$H NMR (399 MHz, (CD$_3$)$_2$SO) δ (ppm) 8.83 (s, 1H), 8.52 (d, $J = 2.20$ Hz, 1H), 7.99 - 8.05 (m, 1H), 7.96 (br. s., 2H), 7.58 (dd, $J = 2.20, 8.79$ Hz, 1H). ESI-MS m/z C$_{10}$H$_6$IN$_3$ [M+H]$^+$, calcd 295.96; obsd 295.97.

4-(dimethylamino)-6-iodoquinoline-3-carbonitrile (3.1f, off-white solid, 97%) $^1$H NMR (399 MHz, CDCl$_3$) δ (ppm) 9.48 (br. s., 2H), 8.63 - 8.67 (m, 1H), 8.36 - 8.42 (m, 1H), 7.93 - 8.00 (m, 1H), 7.71 - 7.78 (m, 1H), 3.37 - 3.44 (m, 6H). ESI-M) m/z C$_{12}$H$_{10}$IN$_3$ [M+H]$^+$, calcd 323.99; obsd 323.97.

**General procedure C** for the synthesis of 3.1g-3.1h: To a mixture of 4-chloro-6-iodoquinoline-3-carbonitrile 2.14 (65.0 mg, 0.207 mmol, 1 equiv) and the corresponding primary amine (1.3 eq, 0.269 mmol) in a reaction vial was added 60% suspension of NaH (1.1eq, 0.227 mmol, 1.2 equiv) followed by Dry DMF under N$_2$ slowly. The resulting mixture was stirred at ambient temperature for 3 h. After
confirming the reaction completion by LC-MS, water (2 mL) was added and the solvents were removed by lyophilization. The dried product was slurried in diethyl ether for 5 min and the solids were filtered and air-dried under vacuum to obtain the desired product. The compounds \textit{3.1g-3.1h}(50-83\%) were taken for next step without further purification.

\textbf{3.1g}

6-iodo-4-(pyridin-3-ylamino)quinoline-3-carbonitrile (3.1g, yellow solid, yield 74\%)

$^1$H NMR (399 MHz, CDCl$_3$) δ (ppm) 8.74 (s, 1H), 8.58 (br. s., 2H), 8.22 (s, 1H), 8.05 (d, $J = 8.79$ Hz, 1H), 7.82 (d, $J = 8.79$ Hz, 1H), 7.35 - 7.57 (m, 2H), 7.06 (br. s., 1H). ESI-MS m/z C$_{15}$H$_9$IN$_4$ [M+H]$^+$, calcd 372.99; obsd 372.97.

\textbf{3.1h}

4-(3,5-dimethylisoxazol-4-ylamino)-6-iodoquinoline-3-carbonitrile (3.1h, off-white solid, yield 50\%).

$^1$H NMR (399 MHz, CDCl$_3$) δ (ppm) 8.64 (s, 1H), 8.46 (br. s., 1H), 8.07 (d, $J = 8.79$ Hz, 1H), 7.82 (d, $J = 8.79$ Hz, 1H), 2.39 (s, 3H), 2.29 (s, 3H). ESI-MS m/z C$_{15}$H$_{11}$IN$_4$O [M+H]$^+$, calcd 391.00; obsd 391.17.

**Synthesis of analogues of 3.2 by Suzuki coupling reaction:**
4-((5-chloropyridin-2-yl)amino)-6-((4-methyl-1,4-diazepan-1-yl)sulfonyl)phenyl)quinoline-3-carbonitrile (3.2a, NEU-1054, pale brown solid, 35.6%). FCC: 0-40% of methanol in DCM. $^1$H NMR (400 MHz, (CD$_3$)$_2$CO) δ (ppm) 9.39 (br. s., 1 H), 8.80 (br. s., 1 H), 8.73 (br. s., 1 H), 8.49 (br. s., 1 H), 8.29 (d, J=8.1 Hz, 1 H), 8.16 (d, J=9.5 Hz, 1 H), 8.07 (d, J=6.6 Hz, 2 H), 7.93 - 8.00 (m, 1 H), 7.89 (d, J=7.3 Hz, 1 H), 7.54 (d, J=10.3 Hz, 1 H), 3.33 - 3.51 (m, 4 H), 2.55 - 2.73 (m, 4 H), 2.34 (br. s., 3 H), 1.87 (br. s., 2 H). ESI-MS m/z C$_{27}$H$_{25}$ClN$_6$O$_2$S [M+H]$^+$, calcd 532.15; obsd 532.18.

4-((3-chloro-4-methoxyphenyl)amino)-6-((4-methyl-1,4-diazepan-1-yl)sulfonyl)phenyl)quinoline-3-carbonitrile (3.2b, NEU-1055, pale brown solid, 43%) $^1$H NMR (400 MHz, CDCl$_3$) δ (ppm) 8.67 (s, 1 H), 8.22 (br. s., 1 H), 8.05 - 8.14 (m, 1 H), 7.86 - 8.00 (m, 2 H), 7.63 - 7.81 (m, 4 H), 7.40 (d, J=2.9 Hz, 1 H), 7.20 - 7.25 (m, 1 H), 6.99 (d, J=8.8 Hz, 1 H), 3.85 - 3.98 (m, 3 H), 3.36 (dd, J=13.2, 6.6 Hz, 4 H), 2.61 (br. s., 4 H), 2.36 (br. s., 3 H), 1.84 (br. s., 2 H). ESI-M) m/z C$_{29}$H$_{25}$ClN$_6$O$_2$S [M+H]$^+$, calcd 562.16; obsd 562.19.
3.2c (NEU-1056)

4-(4-((3-fluorobenzyl)oxy)phenyl)amino)-6-(4-((4-methyl-1,4-diazepan-1-yl)sulfonyl)phenyl)quinoline-3-carbonitrile (3.2c, NEU-1056, pale brown solid, 29%). Reverse phase HPLC using 0-50% of ACN in water. \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) (ppm) 8.67 (s, 1 H), 8.07 - 8.13 (m, 2 H), 7.93 - 7.99 (m, 1 H), 7.74 - 7.81 (m, 2 H), 7.58 - 7.67 (m, 3 H), 7.31 - 7.42 (m, 1 H), 7.28 (s, 1 H), 7.22 (d, \(J=7.3\) Hz, 2 H), 6.98 - 7.07 (m, 3 H), 5.10 (s, 2 H), 3.27 - 3.46 (m, 4 H), 2.69 (br. s., 4 H), 2.40 (s, 3 H), 1.90 (br. s., 2 H). ESI-MS m/z C\(_{35}\)H\(_{32}\)FN\(_5\)O\(_3\)S \([\text{M+H}]^+\), calcd 622.22; obsd 622.24.

3.2d (NEU-1057)

4-((4-(benzyloxy)phenyl)amino)-6-(4-((4-methyl-1,4-diazepan-1-yl)sulfonyl)phenyl)quinoline-3-carbonitrile (3.2d, NEU-1057, tan solid, 40%). Reverse phase HPLC using 0-50% of ACN in water. \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) (ppm) 8.67 (s, 1 H), 8.03 - 8.13 (m, 2 H), 7.96 (d, \(J=8.8\) Hz, 1 H), 7.72 - 7.81 (m, 2 H), 7.55 - 7.66 (m, 3 H), 7.37 - 7.47 (m, 4 H), 7.24 (br. s., 2 H), 7.06 (d, \(J=8.8\) Hz, 2 H), 5.11 (s, 2 H), 3.28 - 3.52 (m, 4 H), 2.75 (br. s., 4 H), 2.44 (br. s., 3 H), 1.95 (br. s., 2 H). ESI-MS m/z C\(_{35}\)H\(_{33}\)N\(_5\)O\(_3\)S \([\text{M+H}]^+\), calcd 604.23; obsd 604.27.
4-amino-6-(4-(4-methyl-1,4-diazepan-1-yl)sulfonyl)phenyl)quinoline-3-carbonitrile (3.2e, NEU-1059, off-white, 20%). Reverse phase HPLC using 0-50% of ACN in water. $^1$H NMR (400 MHz, CD$_3$OD) δ ppm 8.62 (br. s., 1H), 8.51 (s, 1H), 8.15 (d, $J$ = 8.79 Hz, 1H), 8.03 (d, $J$ = 8.79 Hz, 2H), 7.95 (t, $J$ = 7.69 Hz, 3H), 3.39 - 3.50 (m, 4H), 2.63 - 2.76 (m, 4H), 2.37 (s, 3H), 1.86 - 1.94 (m, 2H). ESI-MS m/z C$_{22}$H$_{23}$N$_5$O$_2$S $[^{[M+H]^+}]$, calcd 422.15; obsd 422.15.

4-(dimethylamino)-6-(4-((4-methyl-1,4-diazepan-1-yl)sulfonyl)phenyl)quinoline-3-carbonitrile (3.2f, NEU-1058, tan solid, 28.8%). Reverse phase HPLC using 0-50% of ACN in water. $^1$H NMR (400 MHz, CDCl$_3$) δ (ppm) 8.72 (s, 1 H), 8.22 (s, 1 H), 8.12 (s, 1 H), 7.96 (dd, $J$=8.4, 1.8 Hz, 1 H), 7.86 - 7.90 (m, 2 H), 7.79 - 7.84 (m, 2 H), 3.70 (br. s., 2 H), 3.48 (s, 8 H), 3.19 - 3.31 (m, 4 H), 2.76 (s, 3 H). ESI-MS m/z C$_{24}$H$_{27}$N$_5$O$_2$S $[^{[M+H]^+}]$, calcd 450.18; obsd 450.19.
6-(4-((4-methyl-1,4-diazepan-1-yl)sulfonyl)phenyl)-4-((pyridin-3-ylamino)quinoline-3-carbonitrile (3.2g, NEU-1891, pale brown solid, 18.66%). FCC: 0-40% of methanol in DCM. $^1$H NMR (400 MHz, (CD$_3$)$_2$CO) δ ppm 9.59 (br. s., 1H), 8.92 (br. s., 1H), 8.63 - 8.78 (m, 1H), 8.45 (d, $J = 4.40$ Hz, 1H), 8.23 (d, $J = 8.79$ Hz, 1H), 8.15 (d, $J = 8.06$ Hz, 2H), 8.07 - 8.12 (m, 1H), 7.89 - 7.96 (m, 2H), 7.82 (d, $J = 7.33$ Hz, 1H), 7.39 - 7.48 (m, 1H), 3.36 - 3.50 (m, 5H), 2.66 - 2.78 (m, 4H), 2.33 - 2.41 (m, 3H), 1.85 - 1.96 (m, 2H). ESI-MS m/z C$_{27}$H$_{26}$N$_6$O$_2$S [M+H]$^+$, calcd 499.18; obsd 499.18.

4-((3,5-dimethylisoxazol-4-yl)amino)-6-(4-((4-methyl-1,4-diazepan-1-yl)sulfonyl)phenyl)quinoline-3-carbonitrile (3.2h, NEU-1933, yield 33%) Reverse phase HPLC using 0-50% of ACN in water. $^1$H NMR (400 MHz, CDCl$_3$) δ (ppm) 8.65 (s, 1H), 8.47 (s, 1H), 8.28 (s, 1H), 8.16 (d, $J = 8.06$ Hz, 1H), 8.03 (d, $J = 8.79$ Hz, 1H), 7.85 (q, $J = 8.79$ Hz, 4H), 3.61 (d, $J = 3.66$ Hz, 2H), 3.39 (t, $J = 6.23$ Hz, 2H), 3.13 - 3.25 (m, 4H), 2.73 (s, 3H), 2.38 (s, 3H), 2.21 (s, 5H). ESI-MS m/z C$_{27}$H$_{28}$N$_6$O$_3$S [M+H]$^+$, calcd 517.19; obsd 517.21.
Experimental procedure and characterization of Head group Truncation series

Reagents and conditions: (a) Pd(PPh$_3$)$_4$, 2 M Na$_2$CO$_3$, DME, Ethanol, 85 °C, 16h, 90%; (b) POCl$_3$, 115 °C, 60%

3.8 (NEU 1932)

6-(4-((4-methyl-1,4-diazepan-1-yl)sulfonyl)phenyl)-4-oxo-1,4-dihydroquinoline-3-carbonitrile (3.8, NEU 1932, tan solid, yield 90%) FCC: 5-80% DCM in methanol. A mixture of 1-methyl-4-((4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)sulfonyl)-1,4-diazepane 14.3 (1.927 g, 5.07 mmol, 1.2 equiv) and 6-iodo-4-oxo-1,4-dihydroquinoline-3-carbonitrile 4 (1.250 g, 4.22 mmol, 1 equiv) were taken in DME (45 mL) and Ethanol (35 mL) under N$_2$. Tetrakis (0.195 g, 0.169 mmol, 0.04 equiv) followed by 2M sodium carbonate (12.67 mL 25.3 mmol, 6 equiv) solution was added. The resulting mixture was stirred at 80 °C overnight. After confirming the reaction completion by LC-MS and TLC, the solvents were evaporated in rotovap. The resulting gummy mass was taken in methanol (20 mL) and added diethyl
ether (50 mL) to get brown colored solid. The crude product was purified using flash column chromatography with 5-80% of methanol in DCM to obtain 21 (1.6g, 3.79 mmol, 90%) of NEU 1932 as tan solid. $^1$H NMR (400 MHz, CD$_3$OD) δ (ppm) 8.53 - 8.60 (m, 2H), 8.13 - 8.19 (m, 1H), 7.91 - 8.02 (m, 4H), 7.79 (d, $J = 8.79$ Hz, 1H), 3.41 - 3.55 (m, 4H), 3.33 - 3.35 (m, 1H), 2.88 (dd, $J = 5.86$, 11.72 Hz, 4H), 2.52 (s, 3H), 1.91 - 2.01 (m, 2H). ESI-MS m/z C$_{22}$H$_{22}$N$_4$O$_3$S [M+H]$^+$, calcd 423.1; obsd 423.14.

![Image](image_url)

3.3 (NEU-1934)

6-(4-((4-methyl-1,4-diazepan-1-yl)sulfonyl)phenyl)-4-oxo-1,4-dihydroquinoline-3-carbonitrile (3.3, NEU-1934, pale yellow solid, yield 41-80%) FCC: 0-10% of methanol in DCM. A mixture of 6-(4-((4-methyl-1,4-diazepan-1-yl)sulfonyl)phenyl)-4-oxo-1,4-dihydroquinoline-3-carbonitrile (1.400 g, 3.31 mmol, 1 equiv) and phosphoryl trichloride (8.52 mL, 83 mmol, 25 equiv) were stirred at room temperature for 20 min and heated to 114 °C. After stirring for 7 h at 114 °C, the reaction was deemed complete by LC-MS. The reaction mixture was cooled to room temperature and basified using saturated NaHCO$_3$ solution. The solids obtained were filtered and dried under vacuum overnight. This crude product was then purified using FCC with 0-10% of methanol in DCM to afford 0.6 g of (22) as pale yellow solid. $^1$H NMR (400 MHz, CD$_3$OD) δ (ppm) 9.06 - 9.09 (m, 1H), 8.61 (s, 1H), 8.26 - 8.38 (m, 2H), 7.97 - 8.10 (m, 4H), 3.57 (d, $J = 3.66$ Hz, 2H), 3.41 - 3.48 (m, 2H), 3.12 (br. s., 4H), 2.69 (s, 3H), 1.99 - 2.09 (m, 2H). ESI-MS m/z C$_{22}$H$_{21}$ClN$_4$O$_2$S [M+H]$^+$, calcd 441.11; obsd 441.11.
Synthesis of Head group replacement analogues of 3.4

Reagents and conditions: (a) R= Primary amines, NaH, dry DMF, RT, N₂, 47-88%

General procedure: To a reaction mixture containing 4-chloro-6-(4-((4-methyl-1,4-diazepan-1-yl)sulfonyl)phenyl)quinoline-3-carbonitrile 3.3 (20.0 mg, 0.045 mmol, 1 equiv) and primary amine (1.5 equiv, 0.063 mmol) in dry DMF, sodium hydride (3.63 mg, 0.091 mmol, 2 equiv) was added under N₂. The reaction mixture was stirred at room temperature and after confirming the reaction completion by LC-MS, 2 mL of water was charged to quench. The solvent was removed by evaporation in a Genevac EZ 2.2 centrifugal evaporator on low-medium mixture setting at 35 °C. The crude product was purified by reverse phase column chromatography (water/MeCN with 0.1% TFA gradient from 10% MeCN to 50% MeCN) to give 3.4a-3.4i in 32-88% yield.

The above general procedure was used to synthesize the library of amines from 3.3

4-chloro-6-(4-((4-methyl-1,4-diazepan-1-yl)sulfonyl)phenyl)quinoline-3-carbonitrile (3.4a, NEU-1886, tan solid, yield 47.6%) Reverse phase HPLC 0-50% Acetonitrile in water. ¹H NMR (400 MHz, (CD₃)₂SO) δ (ppm) 10.42 (s, 1 H), 9.75 (s, 1 H), 8.54 (s, 1 H), 8.30 - 8.45 (m, 3 H), 8.17 (s, 1 H), 8.06 - 8.11 (m, 2
H), 7.97 - 8.02 (m, 2 H), 3.30 - 3.44 (m, 4 H), 2.60 (br. s., 2 H), 2.54 (s, 2 H), 2.26 (s, 3 H), 1.78 (d, J=5.9 Hz, 2 H). ESI-M) m/z C_{24}H_{24}N_{8}O_{2}S [M+H]^+; calcd 489.19; obsd 489.19.

3.4b (NEU-1887)

6-(4-((4-methyl-1,4-diazepan-1-yl)sulfonyl)phenyl)-4-(pyridazin-4-ylamino)quinoline-3-carbonitrile (3.4b, NEU-1887, yellow solid, yield 79%) Reverse phase HPLC using 0-50% Acetonitrile in water. $^1$H NMR (400 MHz, (CD$_3$)$_2$CO) δ (ppm) 8.86 (s, 1 H), 8.49 (s, 1 H), 8.31 - 8.38 (m, 1 H), 8.24 (d, J=8.8 Hz, 2 H), 8.11 - 8.18 (m, 2 H), 7.89 - 8.05 (m, 4 H), 3.34 - 3.46 (m, 4 H), 2.55 - 2.66 (m, 4 H), 2.30 (s, 3 H), 1.83 (br. s., 2 H). ESI-MS m/z C_{26}H_{25}N_{7}O_{2}S [M+H]^+; calcd 500.18; obsd 500.18.LCMS found 500 [M+H]^+.

3.4c (NEU-1888)

6-(4-((4-methyl-1,4-diazepan-1-yl)sulfonyl)phenyl)-4-((1-methyl-1H-pyrazol-3-yl)amino)quinoline-3-carbonitrile (3.4c, NEU-1888, pale yellow solid, yield 51.3%) Reverse phase HPLC using 0-50% Acetonitrile in water. $^1$H NMR (400 MHz, (CD$_3$)$_2$CO) δ (ppm) 8.84 (s, 1 H), 8.60 (s, 1 H), 8.19 - 8.26 (m, 1 H), 8.03 - 8.11 (m, 3 H), 7.95 (d, J=6.6 Hz, 2 H), 7.67 (s, 1 H), 3.89 (d, J=1.5 Hz, 3 H), 3.32 - 3.56 (m, 4 H), 2.61 - 2.81 (m, 4 H), 2.38 (s, 3 H), 1.82 - 1.96 (m, 2 H). ESI-MS m/z C_{26}H_{27}N_{7}O_{2}S [M+H]^+; calcd 502.19; obsd 502.25.
3.4d (NEU-1889)

6-((4-methyl-1,4-diazepan-1-yl)sulfonyl)phenyl)-4-(oxazol-2-ylamino)quinoline-3-carbonitrile
(3.4d, NEU-1889, pale yellow solid, yield 57.7%)
Reverse phase HPLC using 0-50% Acetonitrile in water. 

\[
\begin{align*}
\text{H NMR (400 MHz, (CD}_3\text{)}_2\text{CO) } &\delta \text{ (ppm) } \\
9.59 \text{ (s, 1 H), 9.18 (s, 1 H), 8.26 - 8.28 (m, 1 H), 8.13 - 8.19 (m, 3 H), 7.94 - 8.00 (m, 3 H), 7.55 (d, } J = 1.5 \text{ Hz, 1 H), 3.38 - 3.50 (m, 4 H), 2.61 - 2.70 (m, 4 H), 2.33 (s, 3 H), 2.31 - 2.35 (m, 1 H), 1.87 (dt, } J = 11.7, 5.9 \text{ Hz, 2 H). ESI-MS m/z C}_{25}\text{H}_{24}\text{N}_6\text{O}_3\text{S } [\text{M}+\text{H}]^+, \text{ calcd 489.16; obsd 489.16.} 
\end{align*}
\]

3.4e (NEU-1890)

6-(4-((4-methyl-1,4-diazepan-1-yl)sulfonyl)phenyl)-4-((3-methylisoxazol-5-yl)amino)quinoline-3-carbonitrile
(3.4e, NEU-1890, yellow solid, yield 52.6%)
FCC: 0-55% of methanol in DCM, isolated as yellow solid.

\[
\begin{align*}
\text{H NMR (400 MHz,CD}_3\text{OD) } &\delta \text{ (ppm) } \\
8.64 \text{ (d, } J = 3.7 \text{ Hz, 1 H), 8.55 (d, } J = 5.2 \text{ Hz, 1 H), 8.43 (d, } J = 5.2 \text{ Hz, 1 H), 8.14 - 8.23 (m, 1 H), 7.94 - 8.05 (m, 4 H), 7.91 (br. s., 1 H), 3.58 (br. s., 2 H), 3.45 (d, } J = 5.9 \text{ Hz, 2 H), 3.20 (br. s., 4 H), 2.75 (d, } J = 5.9 \text{ Hz, 3 H), 2.25 - 2.32 (m, 3 H), 2.06 (br. s., 2 H). ESI-MS m/z C}_{26}\text{H}_{26}\text{N}_6\text{O}_3\text{S } [\text{M}+\text{H}]^+, \text{ calcd 503.18; obsd 503.19} 
\end{align*}
\]
6-((4-methyl-1,4-diazepan-1-yl)sulfonyl)phenyl)-4-(pyridin-2-ylamino)quinoline-3-carbonitrile (3.4f, NEU-1892, yellow solid, yield 88%) FCC: 0-40% of methanol in DCM, isolated as yellow solid. $^1$H NMR (400 MHz, (CD$_3$)$_2$CO) δ (ppm) 9.62 (s, 1H), 9.40 (d, $J$ = 5.86 Hz, 1H), 9.22 (s, 1H), 8.13 - 8.25 (m, 2H), 7.98 - 8.12 (m, 4H), 7.88 - 7.95 (m, 1H), 7.61 (d, $J$ = 8.79 Hz, 1H), 7.16 (t, $J$ = 6.60 Hz, 1H), 3.50 (br. s., 2H), 3.42 - 3.47 (m, 2H), 2.69 (d, $J$ = 18.32 Hz, 4H), 2.52 (s, 3H), 1.90 (br. s., 2H). ESI-MS m/z C$_{27}$H$_{26}$N$_6$O$_2$S [M+H]$^+$, calcd 499.18; obsd 499.19

4-((1,3,4-thiadiazol-2-yl)amino)-6-((4-methyl-1,4-diazepan-1-yl)sulfonyl)phenyl)quinoline-3-carbonitrile (3.4g, NEU-1894, off-white solid yield 32%) FCC: 0-55% of methanol in DCM, isolated as off-white solid. $^1$H NMR (400 MHz, CD$_3$OD) δ (ppm) 9.47 (s, 1H), 8.11 - 8.22 (m, 1H), 7.92 - 8.04 (m, 3H), 7.90 (d, $J$ = 2.20 Hz, 1H), 7.60 - 7.68 (m, 3H), 3.40 - 3.53 (m, 4H), 2.82 (br. s., 4H), 2.46 (br. s., 3H), 1.95 (br. s., 2H). ESI-MS m/z C$_{24}$H$_{23}$N$_7$O$_2$S$_2$ [M+H]$^+$, calcd 506.14; obsd 506.19
6-(4-((4-methyl-1,4-diazepan-1-yl)sulfonyl)phenyl)-4-(thiazol-5-ylamino)quinoline-3-carbonitrile

(3.4h, NEU-1895, pale brown solid, yield 43.7%) FCC: 0-20% of methanol in DCM, isolated as pale brown solid. $^1$H NMR (400MHz, CD$_3$OD) $\delta$ (ppm) 8.99 - 9.05 (m, 1H), 8.71 (br. s., 1H), 8.51 - 8.57 (m, 1H), 8.16 - 8.25 (m, 1H), 7.99 - 8.08 (m, 3H), 7.90 - 7.97 (m, 2H), 7.85 - 7.89 (m, 1H), 3.38 - 3.52 (m, 4H), 3.33 - 3.37 (m, 1H), 2.70 - 2.82 (m, 4H), 2.40 - 2.44 (m, 3H), 1.92 (br. s., 2H). ESI-MS m/z C$_{25}$H$_{24}$N$_6$O$_2$S$_2$ \([\text{M+H}]^+\), calcd 505.14; obsd 505.19

6-(4-((4-methyl-1,4-diazepan-1-yl)sulfonyl)phenyl)-4-(thiazol-2-ylamino)quinoline-3-carbonitrile

(3.4i, NEU-1896, yellow solid, yield 77%) FCC: 0-50% of methanol in DCM, isolated as yellow solid. $^1$H NMR (400 MHz, CD$_3$OD) $\delta$ (ppm) 9.41 (s, 1H), 8.94 (s, 1H), 8.53 (s, 2H), 8.13-8.12 (m, 2H), 8.04 (d, $J = 9.53$Hz, 1H), 7.92 - 8.02 (m, 3H), 7.29 (d, $J = 4.40$Hz, 1H), 3.51 (d, $J = 4.40$Hz, 2H), 3.45 (t, $J = 5.86$Hz, 2H), 2.78 (dd, $J = 4.40$, 16.12 Hz, 4H), 2.43 (s, 3H), 1.90 - 1.98 (m, 2H). ESI-MS m/z C$_{25}$H$_{24}$N$_6$O$_2$S$_2$ \([\text{M+H}]^+\), calcd 505.14; obsd 505.19
6-(4-((4-methyl-1,4-diazepan-1-yl)sulfonyl)phenyl)-4-(pyrazin-2-ylamino)quinoline-3-carbonitrile

(3.4j, NEU-1897, yellow solid, yield 45.9%) A suspension of 4-chloro-6-(4-((4-methyl-1,4-diazepan-1-yl)sulfonyl)phenyl)quinoline-3-carbonitrile 3.3 (20.00 mg, 0.045 mmol, 1 equiv), pyrazin-2-amine (5.61 mg, 0.059 mmol, 1.3 equiv), xphos (1.297 mg, 2.72 µmol, 0.06 equiv), Pd2dba3 (4.15 mg, 4.54 µmol, 0.1 equiv), Cs2CO3 (36.9 mg, 0.113 mmol, 2.5 equiv) were taken in a 2 mL microwave vial and degasified with nitrogen. To this was added 1,4-dioxane (1 mL) and purged with nitrogen for additional 5 min. The contents of the vial were then irradiated at 100 °C for 50 min. Upon completion of the reaction by LC-MS, the solvents were evaporated under vacuum. The resulting crude mixtures were then directly purified using flash column chromatography with 0-45% of MeOH in DCM to obtain 3.4j as the desired product. 

1H NMR (400 MHz, CD3OD) δ (ppm) 9.49 (s, 1H), 9.20 (s, 1H), 9.02 (s, 1H), 8.91 (br. s., 1H), 8.16 - 8.22 (m, 2H), 7.91 - 8.05 (m, 4H), 7.61 - 7.63 (m, 2H), 3.42 - 3.49 (m, 4H), 2.70 (dt, J = 5.13, 10.26 Hz, 4H), 2.34 - 2.42 (m, 3H), 1.90 - 1.99 (m, 2H). ESI-MS m/z C26H25N7O2S [M+H]+, calcd 500.18; obsd 500.19.
3.4k (NEU-1893)

4-((1H-benzo[d][1,2,3]triazol-6-yl)amino)-6-((4-methyl-1,4-diazepan-1-yl)sulfonyl)phenyl)quinoline-3-carbonitrile (3.4k, NEU-1893, brown colored liquid, yield 42.6%)

To a 5 mL microwave vial containing a mixture of 4-chloro-6-((4-methyl-1,4-diazepan-1-yl)sulfonyl)phenyl)quinoline-3-carbonitrile 3.3 (25.00 mg, 0.057 mmol, 1 equiv), and 1H-benzo[d][1,2,3]triazol-5-amine (11.41 mg, 0.085 mmol, 1.5 equiv) in n-butanol, aluminum trichloride (18.90 mg, 0.142 mmol, 2.5 equiv) was added. The resultant reaction mixture was stirred at 60 °C for 30 min. After confirming the reaction completion by LC-MS, solvents were removed by rotary evaporator. The crude material was then dissolved in ethyl acetate (15 mL), washed with saturated NaHCO₃ solution (10 mL), water (5 mL) and saturated brine solution (5 mL). The organic layer was separated and dried over anhydrous sodium sulfate and concentrated. The crude product was finally purified by reverse phase HPLC using 0-40% of ACN in water as eluents. Pure fractions were lyophilized to afford 3.4k as brown liquid. "H NMR (400 MHz, CD₃OD) δ ppm 8.75 (s, 1H), 8.53 - 8.66 (m, 1H), 8.16 - 8.29 (m, 3H), 8.07 (dd, J = 2.93, 8.79 Hz, 3H), 7.92 - 8.02 (m, 3H), 7.85 (br. s., 1H), 7.40 - 7.58 (m, 1H), 3.63 (d, J = 3.66 Hz, 2H), 3.42 - 3.49 (m, 2H), 3.36 - 3.42 (m, 4H), 2.86 - 2.93 (m, 3H), 2.14 (d, J = 3.66 Hz, 2H). ESI-MS m/z C₂₈H₂₆N₆O₂S [M+H]⁺, calcd 539.19; obsd 539.21.
References


APPENDIX

Representative NMR spectra for final compounds from Chapter 2 and 3
3.2a, NEU-1054

$\text{(CD}_3\text{)}_2\text{CO}, \quad 400 \text{ MHz}$
3.2c, NEU-1056

CDCl₃, 400 MHz
3.2d, NEU-1057

\[ \text{CDCl}_3, \ 400 \text{ MHz} \]
3.2g, NEU-1891
3.2h, NEU-1933

CDC\textsubscript{3}, 400 MHz

\begin{center}
\includegraphics[width=\textwidth]{chemical_structure.png}
\end{center}

Normalized Intensity

Chemical Shift (ppm)

118
3.8, NEU-1932

CD$_2$OD, 400 MHz

![NMR Spectrum](image-url)
3.4b, NEU-1887

$(CD_3)_2CO, \ 400 \ MHz$
3.4c, NEU-1888

\[(\text{CD}_3)_2\text{CO}, 400 \text{ MHz}\]