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Article

# Identification of Substituted 4-Aminocinnolines as Broad-Spectrum Antiparasitic Agents

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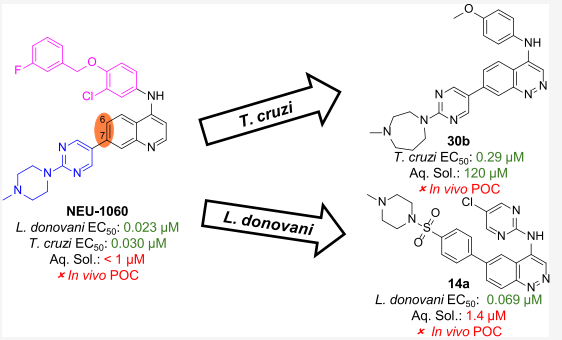
**ABSTRACT:** Neglected tropical diseases such as Chagas disease, human African trypanosomiasis, leishmaniasis, and schistosomiasis have a significant global health impact in predominantly developing countries, although these diseases are spreading due to increased international travel and population migration. Drug repurposing with a focus on increasing antiparasitic potency and drug-like properties is a cost-effective and efficient route to the development of new therapies. Here we identify compounds that have potent activity against *Trypanosoma cruzi* and *Leishmania donovani*, and the latter were progressed into the murine model of infection. Despite the potent *in vitro* activity, there was no effect on parasitemia, necessitating further work to improve the pharmacokinetic properties of this series. Nonetheless, valuable insights have been obtained into the structure−activity and structure−property relationships of this compound series.




**KEYWORDS:** *neglected tropical diseases, Chagas disease, drug repurposing, leishmaniasis, human African trypanosomiasis, schistosomiasis*

Neglected tropical diseases are a group of 20 communicable diseases as described by the World Health Organization (WHO).[1] They are a leading cause of morbidity and mortality in developing countries. Chagas disease, human African trypanosomiasis, visceral and cutaneous leishmaniasis, and schistosomiasis represent a significant public health problem, being responsible for 376,188,010 disability-adjusted life years (DALYs) annually.[2] Over 1 billion people are living at risk in endemic countries, and population migration is spreading these diseases to previously unaffected areas. Current therapeutics show increasing numbers of treatment failure[3−5] and have low efficacy[4] or severe side effects associated with their use,[5,6] highlighting the urgent need for new treatments for these diseases.

We have previously shown that derivatives of lapatinib, an approved human epidermal growth factor receptor (EGFR) inhibitor, show promise against the causative organisms of Chagas disease (*Trypanosoma cruzi*), human African trypanosomiasis (*T. brucei*), leishmaniasis (*Leishmania donovani* and *L. major*), schistosomiasis (*Schistosoma mansoni*), and malaria (*Plasmodium falciparum*).[7−11]

We previously reported the identification of **NEU-1060** (Figure 1), which was derived from lapatinib following multiparasite screening. A scaffold hop to the 4-aminocinnoline led to the identification of **NEU-1017**.[7] This compound demonstrated submicromolar inhibition against *T. brucei*, *L. major*, and *P. falciparum*; however, it was poorly water-soluble, had high plasma protein binding, and was metabolically labile.

Here, we sought to explore the structure−activity (SAR), and structure−property relationships (SPR) for this chemotype versus kinetoplastid parasites *T. brucei*, *T. cruzi*, and *Leishmania*. The discussion has been separated into sections for each parasite. We note that we also screened these compounds versus *S. mansoni* given that we have identified

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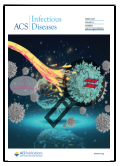

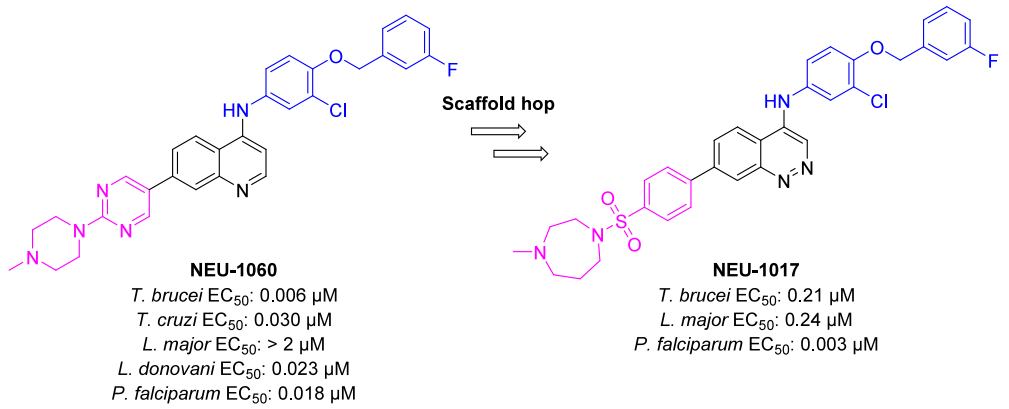


**Figure 1.** The starting point for our optimization, **NEU-1017**, was identified following cross-parasite screening and scaffold hopping from lapatinib.

**Table 1. Targeted Properties for Hit and Lead Compounds**[a]

| assay | | hit criteria | lead criteria |
|---|---|---|---|
| potency | *T. brucei* $EC_{50}$ | 0.1 μM | 0.05 μM |
| | *T. brucei* LLE | ≥4 | |
| | *T. cruzi* $EC_{50}$ | 5.0 μM | 1 μM |
| | *T. cruzi* LLE | ≥4 | |
| | *Leishmania* spp. $EC_{50}$ | <10 μM | 1 μM |
| | *Leishmania* spp. LLE | ≥4 | |
| Tox | HepG2 $CC_{50}$ | 10 × $EC_{50}$ | 10 × $EC_{50}$ |
| | host cell line $CC_{50}$ | 10 × $EC_{50}$ | 10 × $EC_{50}$ |
| ADME/phys properties | MW | ≤360 | ≤360 |
| | LogD | 2-4 | 2-4 |
| | RH $Cl_{int}$ | ≤27 | ≤5 |
| | HLM $Cl_{int}$ | ≤47 | ≤9 |
| | aq sol (μM) | >10 | >100 |
| | PPB (%) | measured | measured |
| PK | | free plasma concentration > 10 × $EC_{50}$ | free plasma concentration > $EC_{99}$ |
| efficacy | | reduction, control, or elimination of parasitemia following PO or IM dosing at levels that provide sufficient exposure (above) | *human African trypanosomiasis:* parasitological cure after ≤10 day dosing, PO, or IM[16]<br>*Chagas disease:* parasitological cure (not inferior to benznidazole)[17]<br>*visceral leishmaniasis:* > 70% reduction in liver parasite burden after <5 doses at 50 mg/kg, PO, QD, or BID[18]<br>*cutaneous leishmaniasis:* 60−70% flattening of lesion following PO, IP, IM, or TOP administration[19] |

[a]RH = rat hepatocyte (μL/min/$10^6$ cells); HLM = human liver microsome (μL/min/mg of protein); PPB = plasma protein binding; LLE (lipophilic ligand efficiency) = $pEC_{50}$ − cLogP.

related compounds to be active. There is a brief summary of the outcomes included in the discussion.

The target candidate (TCP) and target product profiles (TPP) are well-established for human African trypanosomiasis, Chagas disease, and leishmaniasis, enabling focused drug design and synthesis of candidates that meet the requisite criteria. For HAT, the first priority is to develop a safe and effective treatment that can be used for both the hemolymphatic and central nervous system stages.[12] Given the lack of new treatments for Chagas disease and the difficulty in identifying novel chemical matter, the hit requirement is set at $EC_{50}$ < 5 μM. However, the ultimate goal is the identification of a treatment that is safer and better tolerated than the currently available therapeutics, preferring a once-per-day, inexpensive oral drug.[13,14] For visceral leishmaniasis, the minimum requirement is a safe, oral drug with >90% efficacy within 10 days. Relevant for early compound triage is that a drug must be active against all resistant strains. The rate of hit discovery for leishmaniasis is low, and thus, $EC_{50}$ < 10 μM is considered a hit against intracellular *L. donovani*.[15] For all these diseases, it is important that any product is adapted to tropical climates and, for Chagas disease and leishmaniasis, that there is demonstrated efficacy against a broad spectrum of parasitic strains/species.

The TPPs/TCPs have been used to establish guidelines for targeted properties considered during drug discovery programs. The guidelines used in our optimization campaign are presented in Table 1 and are based on potency goals stated by the WHO and DNDi, as well as established *in vitro* ADME guidelines that are pointed toward good pharmacokinetic exposure in animals.


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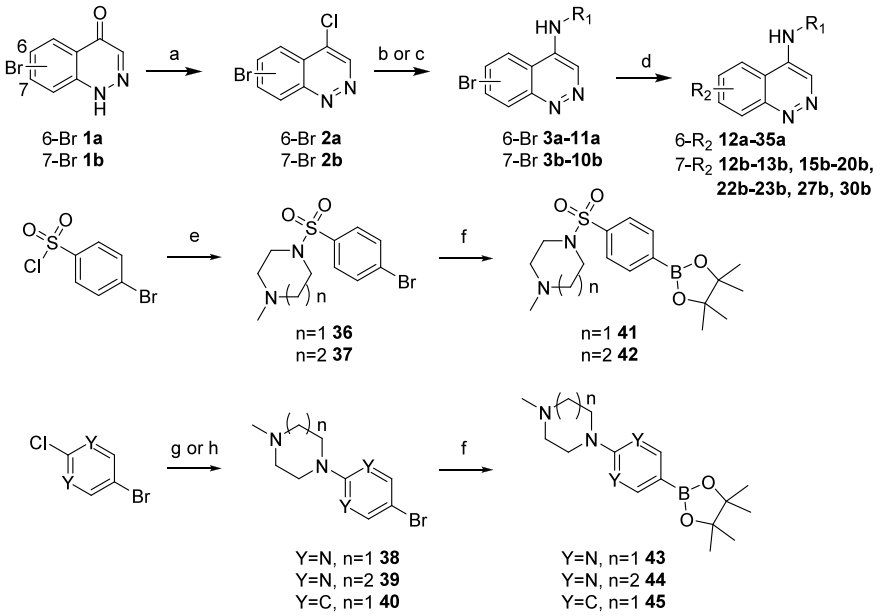
**Scheme 1. Synthesis of Substituted Cinnoline Analogs**[a]




[a]Reagents and conditions:(a) $POCl_3$, 120 °C, 1.5 h; (b) Ar-$NH_2$, NaOtBu, DMF, r.t., 8 h, 17−75%; (c) Ar-$NH_2$, NaH, DMF, r.t., 8 h, 31−88%; (d) Ar-Bpin, Pd(dppf)$Cl_2$·$CH_2Cl_2$, $K_2CO_3$, 4:1 dioxane/$H_2O$, 130 °C, 10 min, 6−83%; (e) $R^1R^2NH$, TEA, THF, r.t., 3 h, 61%; (f) $(BPin)_2$, Pd(dppf)$Cl_2$·$CH_2Cl_2$, dioxane, 160 °C, 1 h; (g) $R^1R^2NH$, DIPEA, *tert*-butanol, 150 °C, 0.5 h, 96−98%; and (h) NaO*t*Bu, $Pd(OAc)_2$, RuPhos, dioxane, 100 °C 18 h, 45%.

## RESULTS AND DISCUSSION

The synthesis of the cinnoline analogs was performed as previously described and is briefly outlined in Scheme 1.[7] From the appropriately substituted cinnolone, chlorination could be achieved with $POCl_3$. It was necessary to add THF as a cosolvent to reduce the formation of the undesired 4,6-dichlorocinnoline byproduct. From here, displacement of the chlorine with the appropriate aniline and a final Suzuki coupling led to the isolation of the desired product.

Our synthetic efforts sought to assess the SAR trends for a series of truncations around the substituted aniline (blue in Figure 1) where we concurrently compared the attachment of the phenylsulfonamide (pink in Figure 1) at the 6- and 7-positions of the cinnoline scaffold. We designed analogs with the aim to reduce cLogP (Supporting Information, Table S4), which has been demonstrated to improve aqueous solubility.[8,20] Compounds **12a** and **12b** had cLogP values of 6.9 and 6.8, respectively, and the substituted aniline was recognized as a large, flat, lipophilic area of the molecule suitable for truncation; thus, we targeted analogs with smaller, aromatic $R_1$ groups to reduce the lipophilicity with the goal of improving the ADME profile, particularly the aqueous solubility.

Our SAR exploration efforts also explored the $R_2$ region (pink in Figure 1), maintaining a variety of $R_1$ heterocycles that demonstrated favorable potency against one or more of the kinetoplastids (Table 3 and Supporting Information, Table S1). Specifically, 2-amino-5-chloropyrimidine (Table 2, group B; preferred for *Leishmania* spp. and *T. brucei*), 4-methoxyaniline (Table 2, group F; preferred for *T. brucei*), and 4-trifluoromethoxyaniline (Table 2, group H; preferred for *Leishmania* spp. and *T. brucei*) were combined with various $R_2$ group replacements. Again, we focused our efforts on incorporating groups that have been successful at improving the aqueous solubility in other optimization programs (such as addition of the homopiperazine that is more basic than the piperazine[8]) and increasing the polarity by incorporating additional heteroatoms in the phenyl ring (such as **32a**; $LogD_{7.4}$: 1.8).[21] ADME data for all of the compounds can be found in the Supporting Information, Table S4.

***Leishmania* spp.** Leishmaniasis has received attention from pharmaceutical companies, such as GlaxoSmithKline, and public−private consortia like the Drugs for Neglected Diseases initiative over the last several years. This has translated to several compounds currently under clinical investigation for visceral leishmaniasis, including LXE408[22] and DNDI-6174[23] that target the parasitic proteasome and cyctochrome $bc_1$ complex, respectively. However, there has been recent attrition with programs failing in early clinical trials due to toxicity.[24] Additionally, there are no investigational drugs currently in the pipeline for cutaneous leishmaniasis.

When looking at the SAR for the cinnolines with respect to *L. donovani*, some trends begin to emerge: *para*-substituted anilines (cf. **17a**, **13b**, **18a**, and **19a**) were generally well tolerated, and incorporation of the strongly electron-withdrawing trifluoromethoxy in place of the methoxy proved valuable in terms of potency with both **28a** and **24a** being more potent than their matched pairs (**27a** and **23a**, respectively). 2-Amino-5-chloropyrimidine (cf. **14a**) and 4-trifluoromethoxyaniline (cf. **20a**) yielded the most promising results in terms of both activity and selectivity. While the relationship between 6- and 7-regioisomers proved difficult to deconvolute with activity being $R_1$ and $R_2$ combination specific, there was a general preference for substitution at the 7-position (compare **15a** and **15b**, Table 2; and **S5a** and **S5b**, Supporting Information, Table S1).

In general, the pyrimidine−homopiperazine $R_2$ group afforded the desired boost in aqueous solubility and was tolerated in terms of potency against *L. donovani* in combination with specific aniline groups (cf. **32a**, *L. donovani* $EC_{50}$: 8.0 $\mu$M, aq sol: 130 $\mu$M; and **29a**, *L donovani* $EC_{50}$: 0.22


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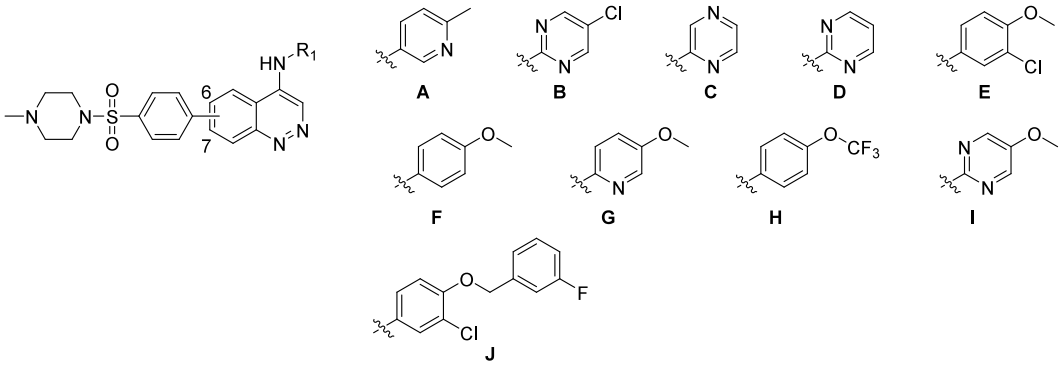
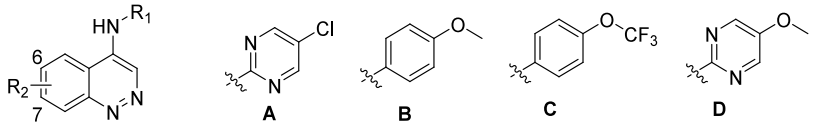
**Table 2. Effect of Aniline Replacement and Position of Phenylsulfonamide on Antiparasitic Activity[g]**

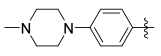
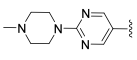
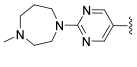
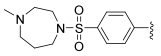



| ID | position | $R_1$ | *L. maj* $EC_{50}$ ($\mu$M); ($r^2$) | *L. don* $EC_{50}$ ($\mu$M)[e] | *T. brucei* $EC_{50}$ ($\mu$M) ± S.D. | *T. cruzi* $EC_{50}$ ($\mu$M)[d] |
|---|---|---|---|---|---|---|
| **12a**[7] | 6 | J | 1.9 (0.94) | *n.t.* | 0.98[c] | >50[b] |
| **12b**[7] | 7 | J | 2.0 (0.95) | *n.t.* | 1.0[c] | 3.1[b] |
| **13a** | 6 | A | >21 | >10 | 1.3 ± 0.027 | 8.2 |
| **13b** | 7 | A | >21 | 6.9 | 12 ± 1.9 | 6.9 |
| **14a** | 6 | B | 0.005 (0.80) | 0.069 | 0.82 ± 0.19 | 4.1 |
| **15a** | 6 | C | 7.8 | >10 | 1.2 ± 0.18 | >10[f] |
| **15b** | 7 | C | >22 | 5.52 | 9.7 ± 2.1 | 6.5 ± 2.3 |
| **16a** | 6 | D | >22 | 9.0 | >20 | >10[f] |
| **16b** | 7 | D | >11 | >10 | 11 ± 1.5 | >10[f] |
| **17a** | 6 | E | >19 | 9.3 | 1.6 ± 0.36 | >10 |
| **17b** | 7 | E | >1.9 | *n.t.* | 8.3 ± 0.35 | >10 |
| **18a** | 6 | F | >20 | 3.4 | 0.39 ± 0.20 | 8.0 |
| **18b** | 7 | F | >20 | *n.t.* | 12 ± 0.63 | 3.2 |
| **19a** | 6 | G | >20 | 4.9 | 1.9 ± 0.073 | 3.8 |
| **19b** | 7 | G | >20 | >10 | *n.t.*[a] | >10 |
| **20a** | 6 | H | 4.8 (0.88) | 0.11 | 0.35 ± 0.26 | 6.8 |
| **20b** | 7 | H | >3.6 | *n.t.* | 0.58 ± 0.25 | >10 |
| **21a** | 6 | I | >4.1 | 5.77 | 6.6 ± 1.7 | 1.3 |

[a]Not soluble at 5 mM. [b]Different host cell line (assay run at NYU). [c]Assay run at NYU [d]Benznidazole $EC_{50}$: 3.77 $\mu$M. [e]Amphotericin B $EC_{50}$: 0.07 $\mu$M. [f]$n$ = 1 biological replicate, with three technical replicates. [g]*n.t.*: not tested; S.D.: standard deviation.

**Table 3. Antiparasitic Activity of the Aryl Piperazine and Homopiperazine Analogs[d]**




| ID | $R_2$ | Pos | $R_1$ | *L. maj* $EC_{50}$ (μM); ($r^2$) | *L. don* $EC_{50}$ (μM)[c] | *T. brucei* $EC_{50}$ (μM) ± S.D. | *T. cruzi* $EC_{50}$ (μM)[b] |
|---|---|---|---|---|---|---|---|
| **22a** |  | 6 | A | > 4.6 | > 10 | 0.15 ± 0.068 | 0.31 |
| **22b** | | 7 | A | 4.2 (0.86) | > 10 | 0.72 ± 0.47 | 2.3 |
| **23a** | | 6 | B | 4.7 (0.79) | 2.3 | 0.31 ± 0.18 | 1.7 |
| **23b** | | 7 | B | > 24 | *n.t.* | 0.078 ± 0.031 | 1.1 |
| **24a** | | 6 | C | > 21 | 0.52 | 0.41 ± 0.024 | 0.87 |
| **25a** | | 6 | D | > 4.7 | 9.4 | 1.2 ± 0.38 | 6.1 |
| **26a** |  | 6 | A | > 4.6 | > 10 | *n.t.*[a] | 2.7 |
| **27a** | | 6 | B | > 23 | > 10 | 1.3 ± 0.27 | 4.4 |
| **27b** | | 7 | B | > 4.7 | *n.t.* | 0.36 ± 0.076 | 3.3 |
| **28a** | | 6 | C | > 21 | 1.5 | 1.0 ± 0.12 | 4.2 |
| **29a** |  | 6 | A | > 22 | 0.22 | 1.7 ± 0.50 | 6.7 |
| **30a** | | 6 | B | > 23 | > 10 | 2.1 ± 0.64 | 5.1 |
| **30b** | | 7 | B | > 23 | *n.t.* | 0.24 ± 0.088 | 0.29 |
| **31a** | | 6 | C | > 20 | > 10 | 0.25 ± 0.11 | > 10 |
| **32a** | | 6 | D | > 23 | 8.0 | 2.9 ± 0.8 | > 10 |
| **33a** |  | 6 | A | 17 | > 10 | 0.99 ± 0.29 | 5.3 |
| **34a** | | 6 | C | > 18 | 1.8 | 0.65 ± 0.021 | > 10 |
| **35a** | | 6 | D | > 20 | *n.t.* | 0.27 ± 0.008 | > 10 |

[a]Not soluble at 5 mM. [b]Benznidazole $EC_{50}$: 3.77 $\mu$M. [c]Amphotericin B $EC_{50}$: 0.07 $\mu$M. [d]*n.t.*: not tested.



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μM, aq sol: 64 μM). Note that, for these compounds, the matched 7-position analogue was not synthesized but could be imagined as a future analogue given the minor improvement in potency that was observed with other analogs. Figure 2 presents a summary of the SAR as currently understood.

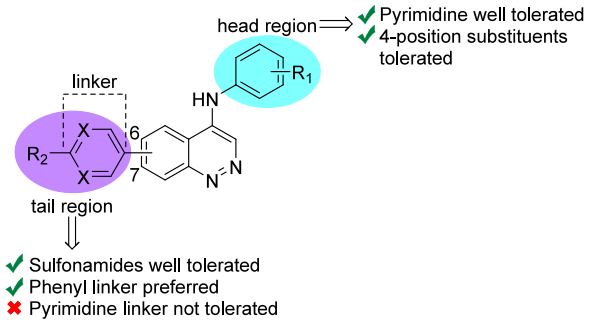


**Figure 2.** SAR summary of cinnoline scaffold for *L. donovani*.

Given the challenge to identify potent inhibitors of *Leishmania* spp. and the requirement for cross-species activity in the target candidate profile for new antileishmanial treatments,[18] we opted to progress **14a** into an *in vivo* pharmacokinetic (PK) study. Because **14a** demonstrated potent activity against *L. donovani* (0.069 μM) and *L. major* (0.005 μM) and had good host cell selectivity (B10R $CC_{50}$: 5.0 μM; Supporting Information,Table S5), we felt that this would provide an opportunity to obtain proof-of-concept activity for the series, and the optimization of the ADME profile (aq sol: 1.4 μM; HLM $CL_{int}$: 300 μL/min/mg protein; RH $CL_{int}$: 68 μL/min/$10^6$ cells; Supporting Information, Table S4) would then become a focus for future work. This compound was tested in a dose escalation PK study administered P.O. at 2, 10, and 25 mg/kg in female BALB/c mice. The compound was well-tolerated and demonstrated plasma levels 10 × $EC_{50}$ for >24 h when dosed at 10 mg/kg (Supporting Information, Figures S1), and we opted to progress to a proof-of-concept *in vivo* efficacy study.

BALB/c mice were infected with *L. donovani*, and the infection was allowed to progress for 2 weeks. The mice were dosed with **14a** once daily for 2 weeks (dose: 10 mg/kg). One week after the last dose, the mice were sacrificed, and the liver and spleen were collected for measurement of parasite burden. Despite its high potency, **14a** failed to have any effect on the parasite burden in either the liver or spleen (Supporting Information,Figures S2 and S3). The compound was highly bound to human plasma protein (98% bound), and it is not unreasonable to expect that this would also be observed in mice, which would impact the actual exposure of drug, accounting for the failed outcome. Further, the $EC_{99}$ of **14a** is 2.1 μM (1040 ng/mL), and it is possible that the exposure was not sufficient to impact parasite burden. No data are available as to the distribution of **14a** in tissue, which is also an important consideration in *Leishmania* infection and could account for the lack of effect on liver and spleen parasite burden.

Given the promise of this series against *L. donovani*, we cross-screened our analogs against *L. major* (causative agent of cutaneous leishmaniasis). Although the majority of the analogs were inactive against *L. major*, there were two low micromolar inhibitors (**15a** and **20a**) and one submicromolar inhibitior (**14a**), although none of these warranted further progression due to the high intrinsic clearance, high plasma protein binding, and low aqueous solubility (Supporting Information,Table S4).

***T. brucei.*** Truncation of the substituted aryl ether ($R_1$) to the 3-chloro-4-methoxyaniline was well tolerated, although this was associated with some toxicity versus HepG2 cells (Supporting Information,Table S5). However, this truncation also highlighted a clear preference for the 6-position (**17a** $EC_{50}$: 1.6 μM) over the 7-position (**17b** $EC_{50}$: 8.3 μM). Compared to **17a**, potency could be recovered upon removal of the 3-chloro (**18a** $EC_{50}$: 0.39 μM), although the 7-position matched pair did not follow this trend (**18b** $EC_{50}$: 12 μM). Replacement with the strongly electron-withdrawing 4-trifluoromethoxyphenyl was well tolerated for the 6- and 7-position regioisomers (**20a** $EC_{50}$: 0.35 μM; **20b** $EC_{50}$: 0.58 μM). 5-Methoxypyridine led to a loss in potency for the 6-position regioisomer (**19a** $EC_{50}$: 1.9 μM). Moving to 5-methoxypyrimidine saw a further decrease in potency for the 6-position regiosiomer (**21a** $EC_{50}$: 6.6 μM). Replacement of the methoxy with the chloro reversed this trend (**14a** $EC_{50}$: 0.82 μM) and demonstrated improved selectivity (HepG2 $CC_{50}$: >20 μM), although removal of the substituent (**16a**) led to a complete loss of potency ($EC_{50}$: 110 μM).

The *T. brucei* activity could be positively modulated by excising the sulfonamide from the $R_2$ group (cf. **18b** $EC_{50}$: 12 μM and **23b** $EC_{50}$: 0.078 μM). Further modification of phenyl to pyrimidine was well tolerated (cf. **27b** $EC_{50}$: 0.36 μM and **23b** $EC_{50}$: 0.078 μM), suggesting that polarity is favorable in this region but does not impart any benefit to the solubility. However, when replacing *N*-methylpiperazine (**27b**) with *N*-methylhomopiperazine (**30b**), we see that potency is unaffected, but a significant boost in the aqueous solubility of the compound is achieved (cf. **27b** aq sol: 3.2 μM and **30b** aq sol: 120 μM), although it still suffers from high intrinsic clearance (Supporting Information,Table S4).

Although several analogs displayed submicromolar inhibition versus *T. brucei*, we were unable to combine this with an improved ADME profile and opted not to progress these compounds further. Figure 3 presents a summary of the SAR and SPR as currently understood.

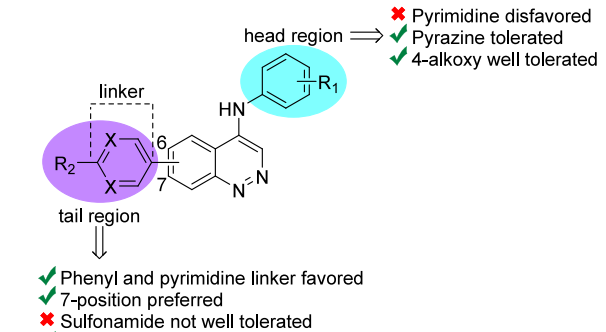


**Figure 3.** SAR summary of cinnoline scaffold for *T. brucei*.

***T. cruzi.*** A key criterion for the identification of a hit compound against *T. cruzi* has been established as $EC_{50}$ < 5 μM.[15] Those compounds that showed low micromolar inhibition of *T. cruzi* all possessed a substituent *para* to the aniline nitrogen in $R_1$ (cf. **18b** $EC_{50}$: 3.2 μM and **21a** $EC_{50}$: 1.3 μM). The exception to this is 3-chloro-4-methoxyaniline,


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which resulted in a complete loss in potency for both the 6- and 7-position analogs (**17a** and **17b**, respectively).

Across this series of analogs, there is no clear preference for $R_2$ at the 6- vs 7-position. Similar to the *T. brucei* SAR, the potency could be positively modulated by excising the sulfonamide (cf. **14a** $EC_{50}$: 4.1 $\mu$M and **22a** $EC_{50}$: 0.31 $\mu$M), although in this instance replacement of phenyl with pyrimidine negatively impacted the potency of the analogs versus *T. cruzi* (cf. **26a** $EC_{50}$: 2.7 $\mu$M, and **22a** $EC_{50}$: 0.31 $\mu$M). Replacement of *N*-methylpiperazine with *N*-methylhomopiperazine was generally well tolerated, although it was associated with only moderate potency, with one notable exception, **30b** ($EC_{50}$: 0.29 $\mu$M), which was one of the most potent compounds identified for *T. cruzi*. Figure 4 presents a summary of the SAR as it is currently understood.

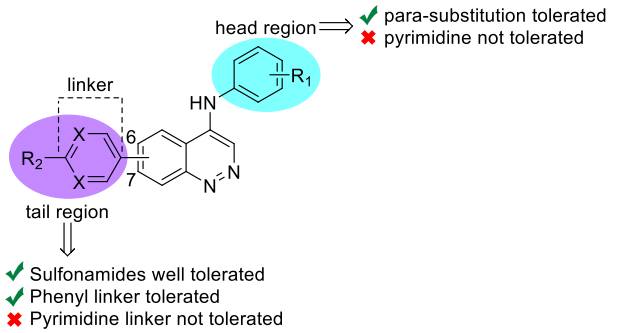


**Figure 4.** SAR summary of the cinnoline scaffold for *T. cruzi*.

We simultaneously progressed **30b** (which had demonstrated improved aqueous solubility: 120 $\mu$M) and our starting compound **NEU-1060** (Figure 1) into a PK study in mice due to their superior potency and the added advantage of exploring multiple scaffolds. We first assessed the maximum tolerated dose. Compound **30b** was dosed i.p. at 100, 50, 20, and 4 mg/kg ($N$ = 2 mice per dose group). However, acute toxicity was observed at even the lowest of doses, and we opted not to move the compound forward. In contrast, 50 mg/kg of **NEU-1060** was tolerated when administered by oral gavage twice daily (b.i.d.). The infection level of the mice was measured just before the initiation of treatment in the efficacy study and was found to be comparable for all three mice. While we did not observe signs of acute toxicity, there was no significant antiparasitic effect after 4 days of treatment except for one female mouse that had noticeable parasite clearance (Supporting Information,Figures S4 and S5), which corresponded with a reduction of the luminescence signal.

***S. mansoni.*** We took the opportunity to screen these compounds against the parasitic blood fluke, *S. mansoni*, which is a causative agent of schistosomiasis, in the hope of expanding their antiparasitic potential and because protein kinases are present in this parasite.[25,26] We have previously identified structurally related analogs to those presented here as active against *S. mansoni*.[11] The schistosome can demonstrate multivariate responses, *i.e.*, relating to motility, density, and shape, as a function of time and concentration to chemical insult. Using a constrained nomenclature of descriptors to describe these effects, each descriptor is given a value of typically 1. These values are added up to generate a severity score whereby a score of 0 is similar to DMSO control and the maximum score of 4 represents the severest responses (Table S2, Supporting Information).[27−30] A severity score of 2 or more is considered significant.

Of the 31 compounds assessed at 10 $\mu$M, four generated the maximum severity score of 4 after 24 h: **17b** was the most potent, reaching that score 5 h after exposure. However, because this compound has significant solubility issues in DMSO, no ADME data could be obtained, and limited cell toxicity measurements were possible (Table S4,Supporting Information). Given this, we did not seek to advance any compounds further for schistosomiasis.

## CONCLUSIONS

Multiparasite screening has proven invaluable in navigating the challenges of resource constraints, enabling the exploration of novel avenues in drug discovery. By identifying activity among related kinetoplastids,[31,32] such as *T. brucei*, *T. cruzi*, and *Leishmania* spp., which share common cellular features[33] and highly similar proteomes,[34−36] we gained insights into differential SAR. Our investigation has identified variations in SAR between these parasites, facilitating an enhanced understanding of how to modulate the SPR within this series. In pursuit of additional value, we extended our screening efforts to the blood fluke *S. mansoni*, but no compounds of interest were identified.

Advancing our findings, three compounds were selected for *in vivo* assessments. Compounds **30b** and **NEU-1060** were progressed to proof-of-concept activity in Chagas disease, but a lack of antiparasitic activity led to the decision to cease further development. Meanwhile, compound **14a** progressed to the murine efficacy model for visceral leishmaniasis. Unfortunately, no discernible impact on parasitemia was observed, prompting a comprehensive analysis. Factors such as time over $EC_{99}$, the static vs cidal nature of the compound, tissue distribution, and differential exposure in the diseased animal model are being investigated to understand the reasons behind the observed ineffectiveness.

## METHODS

The protocols for the biological assays of *Leishmania major* amastigotes and HepG2 cell toxicity were performed as previously described.[37−39] Compounds **12a** and **12b** were evaluated against *T. cruzi* using the NIH3T3 host cell as previously reported,[37,40] and all other compounds were tested against *T. cruzi* using the C2C12 host cell as described here.

***T. brucei In Vitro* Assay.** The high-throughput trypanosome proliferation inhibition assay was performed and analyzed as previously described.[41]

***T. cruzi In Vitro* Assay.** The protocols for the biological assays of *T. cruzi* CA-I/72 and C2C12 cells (ATCC CRL-1772) were performed as previously described.[42,43] All compounds were tested in technical triplicates, and most compounds were tested in three biological replicates. The standard deviation for all $EC_{50}$s were within 1.5-fold of the reported $EC_{50}$ values.

***L. donovani In Vitro* Assay.** To examine the antiparasitic activity of these compounds against *L. donovani* parasites, we followed the same protocol previously published.[44] All compounds were tested with technical triplicates or quadruplicates and three biological replicates. The standard deviation for all $EC_{50}$s were within 1.5-fold of the reported $EC_{50}$ values.


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***S. mansoni In Vitro* Assay.** Maintenance of the *Schistosoma mansoni* life cycle (NMRI isolate), preparation of adult worms (≥42 days old), and their coincubation with test compounds were as described.[28,29,45] Phenotypic responses were visually recorded using a constrained nomenclature and converted to severity scores on a scale from 0 (no activity) to 4 (maximal activity).[27−30]

**Host Cell Toxicity *In Vitro* Assay.** To assess the host cell cytotoxicity for both *T. cruzi* and *L. donovani* screening assays, we first determined the average number of host cells in the negative control wells that are infected and untreated with any compound. To assess the cell viability from each well with a tested compound, we divided the number of host cells from that well by the average number of cells from the negative controls from that same plate. The result informed the percentage of cells relative to an average without any toxicity. Calculating the average number of cells from negative controls minus 3 standard deviations from that average is always ca. 50% cell toxicity. We therefore considered <50% cell viability as a sign of observed toxicity caused by the tested compound.

**Ethics Statement.** PK studies and *in vivo* efficacy studies in mice were performed under approved protocols S16064 (pharmacokinetic profiling) and S14187 (efficacy studies) from the Institutional Animal Care and Use Committee (IACUC), University of California San Diego (AAALAC Accreditation Number 000503), and in compliance with the Animal Welfare Act and adheres to the principles stated in the Guide for the Care and Use of Laboratory Animals, National Research Council, 2011. Likewise, the use of hamsters for maintaining the *S. mansoni* life cycle was approved under protocol S17117 by the IACUC of the University of California San Diego.

***T. cruzi In Vivo* Efficacy Study.** Six week old female and male BALB/c mice (The Jackson Laboratory, Bar Harbor, ME) were anesthetized by isoflurane inhalation and infected by i.p. injection with $10^3$ *T. cruzi* CL-luc.[46] Dosing began 3 days after infection, after groups of three males and three females were randomly separated, and continued for 4 consecutive days with groups of three males and three females treated with **NEU-1060** 50 mg/kg bid by oral gavage, **16a** 20 mg/kg bid by oral gavage, control groups with benznidazole 50 mg/kg (in 20% Solutol, Sigma cat. no. Kolliphor HS15) bid by oral gavage, and control groups not treated (vehicle only). The vehicle used for the test compounds and vehicle control groups was 10% NMP/90% PEG300. On the seventh day post infection, mice were injected with 150 mg/kg D-luciferin (GoldBio, cat. no. eLUCK-1G) and imaged with an IVIS *in vivo* imaging system. The total flux, measured in photons per second (p/s), represents the parasite burden in the mice. The GraphPad Prism software was used for ANOVA statistics with Tukey's multiple comparisons post hoc analysis.

***L. donovani In Vivo* Efficacy Study.** Six week old female and male BALB/c mice (The Jackson Laboratory, Bar Harbor, ME) were anesthetized by isoflurane inhalation and infected by i.v. injection (lateral tail vein) with $5 \times 10^7$ *L. donovani* stationary phase promastigotes in 100 $\mu$L PBS per mouse.[47] Two weeks after infection, groups of six mice (three female and three male) were treated with test compound, vehicle, or amphotericin B in 100 $\mu$L total volume per mouse per dose for 2 weeks. A group of three infected untreated male mice and a group of six (three female and three male) naïve untreated mice were also included in the study. The test compounds and vehicle were dosed b.i.d., and the amphotericin B was dosed q.a.d. **14a** was dosed at 25 mg/kg, and amphotericin B was dosed at 8 mg/kg. The vehicle used for the test compounds and vehicle control groups was 40% polyethylene glycol (PEG) 300, 5% Kolliphor HS 15 (Sigma-Aldrich, St. Louis, MO), and 55% D5W (5% dextrose in water). Ten percent dimethylsulfoxide (10%) (DMSO) was used as the vehicle for the amphotericin B control group. Mice were euthanized 5 weeks post infection by exposure to $CO_2$ in a chamber with a flow rate that displaces 10 to 30% of the chamber volume/min followed by cervical dislocation. The whole spleens and whole livers were then weighed and collected in 3 mL of media per tissue for a parasite-limiting dilution assay. A limiting dilution was performed to assess parasite burden per tissue as described before.[48]

**Statistical Analysis.** One-way ANOVA with Dunnett's multiple comparisons post hoc test was used for evaluation of differences in experimental data between groups. Values were considered statistically significant when $p \leq 0.05$. Statistics were analyzed by the GraphPad Prism Software, version 6.05 (GraphPad Software, San Diego, CA).

**Chemistry Experiments.** All reagents and starting materials were procured commercially from Sigma-Aldrich Inc., Fisher Scientific, or Combi-blocks and used as received. Melting points were recorded by using a Thermo Scientific MEL-TEMP apparatus. NMR spectra were obtained on a Varian NMR system operating at 400 and 500 MHz. Chemical data for protons are reported in parts per million (ppm) and are referenced to the residual proton in the NMR solvent [$(CD_3)_2SO$, 2.50; $CD_3OD$, 3.31; $CDCl_3$, 7.26; and $(CD_3)_2CO$, 2.05; ppm]. Liquid chromatography−mass spectrometry (LCMS) analysis was performed using a Waters e2795 Alliance, Waters e2695 Alliance, or Agilent 1100 reverse-phase high-performance liquid chromatography−mass spectrometry (HPLC−MS) and a 3.5 $\mu$m Waters SunFire C18 4.6 × 50 $mm^2$ column, with a multiwavelength photodiode array detector ($\lambda$ = 200−600 nm) and a MicroMass ZQ single quadrupole mass spectrometer (electrospray ionization). Gradients for the LCMS analysis were water or acetonitrile, both with 0.1% v/v formic acid. Microwave reactions were performed in a Biotage Initiator+ or CEM Discovery SP instruments. Purification of intermediates and final compounds was performed by silica gel chromatography using the Biotage Isolera One flash purification system, unless otherwise noted. All newly synthesized compounds were deemed >95% pure by LCMS (PDA, $\lambda$ = 200−600 nm).

*General Procedure 1: Attachment of Aniline with NaOtBu.* A solution of the desired chlorocinnoline (1.77 mmol), the desired aniline (3.55 mmol), and NaOtBu (1.95 mmol) in dry DMF (20 mL) was stirred at room temperature overnight. Upon completion of the reaction, the mixture was quenched with saturated $NaHCO_3$, and the precipitate was filtered and washed heavily with water to remove the aniline.

*General Procedure 2: Attachment of the Aniline Group with NaH.* A solution of the desired chlorocinnoline (1.87 mmol), the desired aniline (2.06 mmol), and 60% NaH in mineral oil (5.62 mmol) in dry DMF (20 mL) was stirred at room temperature overnight. Upon completion of the reaction, the mixture was quenched with saturated $NaHCO_3$, and the precipitate was filtered and washed with water to remove aniline.

*General Procedure 3: Suzuki Coupling.* The desired cinnoline (0.16 mmol), $PdCl_2(dppf)\cdot CH_2Cl_2$ (6.48 mg, 0.01 mmol), the desired boronic ester (0.16 mmol), and potassium


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carbonate (66 mg, 0.46 mmol) were added to a degassed microwave vial and heated at 130 °C for 5 min. The resulting mixture was then filtered through Celite, and the filtrate was concentrated under reduced pressure and purified by flash column chromatography to afford the pure product.

*6-Bromo-4-chlorocinnoline (**2a**).* This compound was synthesized according to Devine et al.[7] Briefly, phosphorus oxychloride (2.67 mmol) was added to a solution of 6-bromocinnolin-4(1*H*)-one (0.89 mmol) in anhydrous THF (5 mL), and the resulting solution was heated to 70 °C for 1.5 h. Once no starting material remained by TLC, the solution was cooled to 0 °C and was quenched by dropwise addition of saturated $NaHCO_3$. The mixture was allowed to warm to ambient temperature with stirring for an additional hour. DCM and water were added to the solution, and the layers were separated. The aqueous layer was extracted with DCM, and the combined organic layers were washed with saturated $NaHCO_3$, washed with brine, dried over $Na_2SO_4$, filtered, and concentrated under reduced pressure to obtain the desired product as a dark gray solid that was carried forward without further purification.

*Note that the reaction can be run without THF, in which case 5.34 mmol of $POCl_3$ is used; however, this leads to the formation of 4,6-dichlorocinnoline.

*7-Bromo-4-chlorocinnoline (**2b**).* Phosphorus oxychloride (5.34 mmol) was added to 7-bromocinnolin-4(1*H*)-one (0.89 mmol), and the resulting solution was heated to 70 °C for 1.5 h. Once no starting material remained by TLC, the solution was cooled to 0 °C and was quenched by dropwise addition of saturated $NaHCO_3$. The mixture was allowed to warm to ambient temperature while being stirred for an additional hour. DCM and water were added to the solution, and the layers were separated. The aqueous layer was extracted with DCM, and the combined organic layers were washed with saturated $NaHCO_3$, washed with brine, dried over $Na_2SO_4$, filtered, and concentrated under reduced pressure to obtain the desired product as a dark gray solid that was carried forward without further purification.

*6-Bromo-N-(6-methylpyridin-3-yl)cinnolin-4-amine (**3a**).* Using general procedure 2, 6-methylpyridin-3-amine was employed to get the product as a light red solid; 44% yield. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ ppm 9.35 (br. S., 1 H) 8.79 (br. S., 1 H) 8.70 (br S, 1 H) 8.48 (br. S., 1 H) 8.13 (d, $J$ = 7.8 Hz, 1 H) 7.93−7.97 (m, 1 H) 7.32 (d, $J$ = 6.3 Hz, 1 H) 3.12 (s, 3 H). LCMS $[M + H]^+$ = 316.3.

*7-Bromo-N-(6-methylpyridin-3-yl)cinnolin-4-amine (**3b**).* Using general procedure 2, 6-methylpyridin-3-amine was employed to get the product as a dark red solid; 49% yield. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ ppm 9.52 (s, 1 H) 8.70 (s, 1 H) 8.48 (s, 1 H) 8.30 (d, $J$ = 8.0 Hz, 1 H) 7.99 (s, 1 H) 7.52 (br. s., 1 H) 7.38 (br. s., 1 H) 7.22 (d, $J$ = 8.7 Hz, 1 H) 3.90 (s, 3 H). LCMS $[M + H]^+$ 316.9.

*6-Bromo-N-(5-chloropyrimidin-2-yl)cinnolin-4-amine (**4a**).* Using general procedure 2, 5-chloropyrimidin-2-amine was employed to get the product as a light brown solid; 72% yield. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ ppm 10.61 (br. s., 1 H) 10.16 (br. s., 1 H) 8.89 (br. s., 1 H) 8.73−8.82 (m, 2 H) 8.24 (d, $J$ = 8.7 Hz, 1 H) 7.98 (d, $J$ = 9.2 Hz, 1 H). LCMS $[M + H]^+$ 337.3.

*7-Bromo-N-(5-chloropyrimidin-2-yl)cinnolin-4-amine (**4b**).* Using general procedure 1, 5-chloropyrimidin-2-amine was employed to get the product as a brown solid; 75% yield. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ ppm 10.04−10.14 (m, 1 H) 8.74−8.79 (m, 2 H) 8.56−8.62 (m, 1 H) 8.48−8.54 (m, 1 H) 7.77−7.92 (m, 1 H). LCMS $[M + H]^+$ 337.3.

*6-Bromo-N-(pyrazin-2-yl)cinnolin-4-amine (**5a**).* Using general procedure 1, pyrazin-2-amine was employed to get the product as a light yellow solid; 35% yield. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ ppm 8.88 (br S, 1 H) 8.71 (br. S., 1 H) 8.43 (d, $J$ = 1.4 Hz, 1 H) 8.30 (d, $J$ = 2.4 Hz, 1 H) 8.05 (d, $J$ = 8.7 Hz, 1 H) 7.83−7.87 (m, 1 H) 7.65 (t, $J$ = 2.4 Hz, 1 H). LCMS $[M + H]^+$ 303.3.

*7-Bromo-N-(pyrazin-2-yl)cinnolin-4-amine (**5b**).* Using general procedure 1, pyrazin-2-amine was employed to get the product as a yellow solid; 54% yield. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ ppm 8.88 (br. s., 3 H) 8.71 (br. s., 4 H) 8.43 (d, $J$ = 1.4 Hz, 4 H) 8.30 (d, $J$ = 2.4 Hz, 4 H) 8.05 (d, $J$ = 8.7 Hz, 1 H) 7.83−7.87 (m, 1 H) 7.65 (t, $J$ = 2.4 Hz, 2 H). LCMS $[M + H]^+$ 303.5.

*6-Bromo-N-(pyrimidin-2-yl)cinnolin-4-amine (**6a**).* Using general procedure 1, pyrimidin-2-amine was employed to get the product as a light yellow solid; 17% yield. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ ppm 10.45 (s, 1 H) 10.30−10.35 (m, 1 H) 8.98 (d, $J$ = 1.9 Hz, 1 H) 8.72 (d, $J$ = 4.8 Hz, 1 H) 8.26 (d, $J$ = 8.7 Hz, 1 H) 8.00−8.03 (m, 1 H) 7.16 (t, $J$ = 4.8 Hz, 1 H). LCMS $[M + H]^+$ 303.4.

*7-Bromo-N-(pyrimidin-2-yl)cinnolin-4-amine (**6b**).* Using general procedure 1, pyrimidin-2-amine was employed to get the product as a yellow solid; 57% yield. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ ppm 10.52 (s, 1 H) 10.30 (s, 1 H) 8.71 (d, $J$ = 4.8 Hz, 2 H) 8.52−8.62 (m, 2 H) 7.89−7.98 (m, 1 H) 7.16 (t, $J$ = 4.8 Hz, 1 H) 6.52 (t, $J$ = 4.8 Hz, 1 H). LCMS $[M + H]^+$ 303.6.

*6-Bromo-N-(3-chloro-4-methoxyphenyl)cinnolin-4-amine (**7a**).* Using general procedure 1, 3-chloro-4-methoxyaniline was employed to get the product as a light brown solid; 68% yield. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ ppm 9.42 (s, 2 H) 8.72 (s, 2 H) 8.38 (s, 1 H) 8.33 (d, $J$ = 8.0 Hz, 2 H) 7.89 (s, 2 H) 7.50 (br S, 2 H) 7.38 (br. S., 3 H) 7.24 (d, $J$ = 8.7 Hz, 2 H) 3.88 (s, 3 H). LCMS $[M + H]^+$ = 365.4.

*7-Bromo-N-(3-chloro-4-methoxyphenyl)cinnolin-4-amine (**7b**).* Using general procedure 1, 3-chloro-4-methoxyaniline was employed to get the product as a light brown solid; 44% yield. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ ppm 9.42 (s, 2 H) 8.72 (s, 2 H) 8.38 (s, 1 H) 8.33 (d, $J$ = 8.0 Hz, 2 H) 7.89 (s, 2 H) 7.50 (br. s., 2 H) 7.38 (br. s., 3 H) 7.24 (d, $J$ = 8.7 Hz, 2 H) 3.88 (s, 3 H). LCMS $[M + H]^+$ 365.8.

*6-Bromo-N-(4-methoxyphenyl)cinnolin-4-amine (**8a**).* Using general procedure 1, 4-methoxyaniline was employed to get the product as a light red solid; 41% yield. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ ppm 9.24 (s, 1 H) 8.69 (s, 1 H) 8.07 (d, $J$ = 9.2 Hz, 1 H) 7.91 (dd, $J$ = 9.0, 1.71 Hz, 1 H) 7.30 (d, $J$ = 8.7 Hz, 2 H) 7.02 (d, $J$ = 8.7 Hz, 2 H) 3.76 (s, 3 H). LCMS $[M + H]^+$ = 331.4.

*7-Bromo-N-(4-methoxyphenyl)cinnolin-4-amine (**8b**).* Using general procedure 1, 4-methoxyaniline was employed to get the product as a light brown solid; 43% yield. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ ppm 9.39 (s, 2 H) 8.63 (s, 2 H) 8.37 (s, 1 H) 8.35 (s, 3 H) 7.85 (d, $J$ = 10.2 Hz, 2 H) 7.33 (d, $J$ = 8.7 Hz, 5 H) 7.04 (d, $J$ = 8.7 Hz, 5 H) 3.79 (s, 3 H). LCMS $[M + H]^+$ = 331.3.

*6-Bromo-N-(5-methoxypyridin-2-yl)cinnolin-4-amine (**9a**).* Using general procedure 2, 5-methoxypyridin-2-amine was employed to get the product as a brown solid; 44% yield. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ ppm 10.16−10.29 (m, 1 H) 9.50−9.60 (m, 1 H) 8.73−8.86 (m, 1 H) 8.08−8.17 (m, 1 H)


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7.87−7.99 (m, 1 H) 7.46−7.57 (m, 1 H) 7.25−7.39 (m, 1 H) 3.76−3.84 (m, 3 H). LCMS $[M + H]^+$ 332.3.

*7-Bromo-N-(5-methoxypyridin-2-yl)cinnolin-4-amine (**9b**).* Using general procedure 2, 5-methoxypyridin-2-amine was employed to get the product as a brown solid; 58% yield. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ ppm 10.19−10.24 (m, 1 H) 9.68−9.71 (m, 1 H) 8.48−8.53 (m, 1 H) 8.43−8.46 (m, 1 H) 8.12−8.18 (m, 1 H) 7.87−7.95 (m, 1 H) 7.32−7.38 (m, 1 H) 3.84 (s, 3 H). LCMS $[M + H]^+$ 332.3.

*6-Bromo-N-(4-(trifluoromethoxy)phenyl)cinnolin-4-amine (**10a**).* Using general procedure 2, 4-(trifluoromethoxy)aniline was employed to get the product as a light red solid; 42% yield. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ ppm 9.43 (br. s., 1 H) 8.95 (s, 1 H) 8.69 (s, 1 H) 8.13 (d, $J$ = 9.2 Hz, 1 H) 7.95 (d, $J$ = 8.7 Hz, 1 H) 7.50 (d, $J$ = 8.3 Hz, 2 H) 7.42 (d, $J$ = 8.3 Hz, 2 H). LCMS $[M + H]^+$ 385.3.

*7-Bromo-N-(4-(trifluoromethoxy)phenyl)cinnolin-4-amine (**10b**).* Using general procedure 1, 4-(trifluoromethoxy)aniline was employed to get the product as a brown solid; 57% yield. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ ppm 8.53−8.70 (m, 1 H) 8.31−8.40 (m, 1 H) 8.00−8.07 (m, 1 H) 7.60−7.69 (m, 1 H) 7.39 (br. s., 4 H). LCMS $[M + H]^+$ 385.6.

*6-Bromo-N-(5-methoxypyrimidin-2-yl)cinnolin-4-amine (**11a**).* Using general procedure 2, 5-methoxypyrimidin-2-amine was employed to get the product as a brown solid; 31% yield. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ ppm 10.10−10.19 (m, 1 H) 9.61−9.68 (m, 1 H) 8.48−8.53 (m, 1 H) 8.42−8.45 (m, 2 H) 7.42−7.48 (m, 1 H) 3.82 (s, 3 H). LCMS $[M + H]^+$ 333.4.

*N-(3-Chloro-4-((3-fluorobenzyl)oxy)phenyl)-6-(4-((4-methylpiperazin-1-yl)sulfonyl)phenyl)cinnolin-4-amine (**12a**).* This compound was synthesized according to Devine et al.[7]

*N-(3-Chloro-4-((3-fluorobenzyl)oxy)phenyl)-7-(4-((4-methylpiperazin-1-yl)sulfonyl)phenyl)cinnolin-4-amine (**12b**).* This compound was synthesized according to Devine et al.[7]

*6-(4-((4-Methylpiperazin-1-yl)sulfonyl)phenyl)-N-(6-methylpyridin-3-yl)cinnolin-4-amine (**13a**).* Using general procedure 3, 6-bromo-*N*-(6-methylpyridin-3-yl)cinnolin-4-amine (**3a**) and 1-methyl-4-((4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)sulfonyl)piperazine (**40**) were employed, and the product was purified by flash column chromatography over a gradient of 1−6% methanol in dichloromethane to get the product as a dark yellow solid; 8% yield. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ ppm 9.47 (s, 1 H) 8.81 (s, 1 H) 8.51 (s, 1 H) 8.29−8.32 (m, 1 H) 8.24 (s, 1 H) 8.14 (d, $J$ = 8.3 Hz, 2 H) 7.87 (d, $J$ = 8.3 Hz, 2 H) 7.78 (s, 1 H) 2.91 (br s, 4 H) 2.48 (s, 3 H) 2.33 (br s, 4 H) 2.10 (s, 3 H). LCMS $[M + H]^+$ 490.8.

*7-(4-((4-Methylpiperazin-1-yl)sulfonyl)phenyl)-N-(6-methylpyridin-2-yl)cinnolin-4-amine (**13b**).* Using general procedure 3, 7-bromo-*N*-(5-methylpyridin-2-yl)cinnolin-4-amine (**3b**) and 1-methyl-4-((4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)sulfonyl)piperazine (**40**) were employed, and the product was purified by flash column chromatography over a gradient of 3−6% methanol in dichloromethane to get the product as a dark orange solid; 24% yield. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ ppm 9.44 (s, 1 H) 8.82 (s, 1 H) 8.60 (d, $J$ = 1.4 Hz, 1 H) 8.57 (s, 1 H) 8.53−8.56 (m, 1 H) 8.20−8.25 (m, 2 H) 7.86−7.90 (m, 2 H) 7.81 (dd, $J$ = 8.3, 2.4 Hz, 1 H) 7.36 (d, $J$ = 8.3 Hz, 1 H) 2.95 (br s, 4 H) 2.51 (s, 3 H) 2.38 (br s, 4 H) 2.14 (s, 3 H). LCMS $[M + H]^+$ = 475.8.

*N-(5-Chloropyrimidin-2-yl)-6-(4-((4-methylpiperazin-1-yl)sulfonyl)phenyl)cinnolin-4-amine (**14a**).* Using general procedure 3, 6-bromo-*N*-(5-chloropyrimidin-2-yl)cinnolin-4-amine (**4a**) and 1-methyl-4-((4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)sulfonyl)piperazine (**40**) were employed, and the product was purified by flash column chromatography over a gradient of 2−5% methanol in dichloromethane to get the product as a dark yellow solid; 11% yield. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ ppm 10.83 (s, 1 H) 10.23 (s, 1 H) 9.01 (s, 1 H) 8.79 (s, 2 H) 8.41 (d, $J$ = 8.7 Hz, 1 H) 8.29 (d, $J$ = 8.7 Hz, 1 H) 8.22 (d, $J$ = 7.8 Hz, 2 H) 7.86 (d, $J$ = 7.8 Hz, 2 H) 2.91 (br s, 4 H) 2.33 (br s, 4 H) 2.09 (s, 3 H). LCMS $[M + H]^+$ = 496.8.

*6-(4-((4-Methylpiperazin-1-yl)sulfonyl)phenyl)-N-(pyrazin-2-yl)cinnolin-4-amine (**15a**).* Using general procedure 3, 6-bromo-*N*-(pyrazin-2-yl)cinnolin-4-amine (**5a**) and 1-methyl-4-((4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)-sulfonyl)piperazine (**40**) were employed, and the product was purified by flash column chromatography over a gradient of 4−9% methanol in dichloromethane to get the product as a dark yellow solid; 25% yield. $^1$H NMR (500 MHz, methanol- $d_4$) $\delta$ ppm 10.46−10.56 (m, 1 H) 8.87 (s, 1 H) 8.69 (s, 1 H) 8.45 (dd, $J$ = 2.4, 1.4 Hz, 1 H) 8.30 (d, $J$ = 8.7 Hz, 1 H) 8.26 (d, $J$ = 2.4 Hz, 1 H) 8.17 (d, $J$ = 8.3 Hz, 2 H) 7.97 (d, $J$ = 8.3 Hz, 2 H) 3.07−3.17 (m, 4 H) 2.55 (t, $J$ = 4.6 Hz, 4 H) 2.29 (s, 3 H). LCMS $[M + H]^+$ 461.8.

*7-(4-((4-Methylpiperazin-1-yl)sulfonyl)phenyl)-N-(pyrazin-2-yl)cinnolin-4-amine (**15b**).* Using general procedure 3, 7-bromo-*N*-(pyrazin-2-yl)cinnolin-4-amine (**5b**) and 1-methyl-4-((4-(4,4,5,5-/tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)-sulfonyl)piperazine (**40**) were employed, and the product was purified by flash column chromatography over a gradient of 0−4% methanol in dichloromethane to get the product as a dark yellow solid; 6% yield. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ ppm 10.36 (s, 1 H) 10.06−10.10 (m, 1 H) 8.76 (s, 1 H) 8.70 (br. s., 2 H) 8.41 (s, 1 H) 8.22−8.27 (m, 4 H) 7.89 (d, $J$ = 8.3 Hz, 3 H) 2.95 (br. s., 5 H) 2.34−2.39 (m, 6 H) 2.08−2.18 (m, 3 H). LCMS $[M + H]^+$ 461.9.

*6-(4-((4-Methylpiperazin-1-yl)sulfonyl)phenyl)-N-(pyrimidin-2-yl)cinnolin-4-amine (**16a**).* Using general procedure 3, 6-bromo-*N*-(pyrimidin-2-yl)cinnolin-4-amine (**6a**) and 1-methyl-4-((4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-phenyl)sulfonyl)piperazine (**40**) were employed, and the product was purified by flash column chromatography over a gradient of 1−4% methanol in dichloromethane to get the product as a dark yellow solid; 20% yield. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ ppm 8.32 (d, $J$ = 1.9 Hz, 1 H) 7.99−8.04 (m, 2 H) 7.88 (s, 1 H) 7.80 (d, $J$ = 8.7 Hz, 1 H) 7.75 (d, $J$ = 8.7 Hz, 1 H) 2.92 (br. s., 4 H) 2.35 (d, $J$ = 4.3 Hz, 4 H) 2.10−2.14 (m, 3 H). LCMS $[M + H]^+$ 462.8.

*7-(4-((4-Methylpiperazin-1-yl)sulfonyl)phenyl)-N-(pyrimidin-2-yl)cinnolin-4-amine (**16b**).* Using general procedure 3, 7-bromo-*N*-(pyrimidin-2-yl)cinnolin-4-amine (**6b**) and 1-methyl-4-((4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-phenyl)sulfonyl)piperazine (**40**) were employed, and the product was purified by flash column chromatography over a gradient of 1−5% methanol in dichloromethane to get the product as a dark yellow solid; 16% yield. $^1$H NMR (500 MHz, chloroform-*d*) $\delta$ ppm 10.69 (s, 1 H) 8.73 (s, 1 H) 8.65 (d, $J$ = 4.88 Hz, 1 H) 8.17 (d, $J$ = 8.7 Hz, 2 H) 7.93 (s, 2 H) 7.85−7.89 (m, 1 H) 7.71 (d, $J$ = 8.3 Hz, 1 H) 7.03 (s, 1 H) 3.11 (d, $J$ = 13.6 Hz, 4 H) 2.46−2.56 (m, 4 H) 2.28 (d, $J$ = 2.4 Hz, 4 H). LCMS $[M + H]^+$ 461.8.


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*N-(3-Chloro-4-methoxyphenyl)-6-(4-((4-methylpiperazin-1-yl)sulfonyl)phenyl)cinnolin-4-amine (**17a**).* Using general procedure 3, 6-bromo-*N*-(3-chloro-4-methoxyphenyl)cinnolin-4-amine (**7a**) and 1-methyl-4-((4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)sulfonyl)piperazine (**40**) were employed, and the product was purified by flash column chromatography over a gradient of 1−3% methanol in dichloromethane to get the product as a dark yellow solid; 18% yield. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ ppm 9.40 (s, 1 H) 8.79 (s, 1 H) 8.74 (s, 1 H) 8.26 (s, 1 H) 8.23 (s, 1 H) 8.13 (d, $J$ = 8.3 Hz, 2 H) 7.86 (d, $J$ = 8.3 Hz, 1 H) 7.49 (d, $J$ = 2.4 Hz, 1 H) 7.40 (d, $J$ = 8.3 Hz, 1 H) 7.24 (d, $J$ = 8.7 Hz, 1 H) 3.87 (s, 3 H) 2.90 (br s, 4 H) 2.33 (br s, 4 H) 2.09 (s, 3 H). LCMS [M + H]$^+$ 525.2.

*N-(3-Chloro-4-methoxyphenyl)-7-(4-((4-methylpiperazin-1-yl)sulfonyl)phenyl)cinnolin-4-amine (**17b**).* Using general procedure 3, 7-bromo-*N*-(3-chloro-4-methoxyphenyl)cinnolin-4-amine (**7b**) and 1-methyl-4-((4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)sulfonyl)piperazine (**40**) were employed, and the product was purified by product as a yellow solid; 16% yield. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ ppm 9.33 (s, 1 H) 8.72 (s, 1 H) 8.54 (s, 1 H) 8.50 (d, $J$ = 8.7 Hz, 1 H) 8.19 (d, $J$ = 8.3 Hz, 2 H) 7.85 (d, $J$ = 7.8 Hz, 2 H) 7.49 (d, $J$ = 1.9 Hz, 1 H) 7.39 (s, 1 H) 7.23 (d, $J$ = 8.7 Hz, 1 H) 6.49 (s, 1 H) 3.87 (s, 3 H) 2.92 (br. s., 4 H) 2.30−2.38 (m, 4 H) 2.11 (s, 4 H). LCMS [M + H]$^+$ 525.2.

*N-(4-Methoxyphenyl)-6-(4-((4-methylpiperazin-1-yl)sulfonyl)phenyl)cinnolin-4-amine (**18a**).* Using general procedure 3, 6-bromo-*N*-(4-methoxyphenyl)cinnolin-4-amine (**8a**) and 1-methyl-4-((4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)sulfonyl)piperazine (**40**) were employed, and the product was purified by flash column chromatography over a gradient of 2−6% methanol in dichloromethane to get the product as a dark yellow solid; 10% yield. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ ppm 9.38 (s, 1 H) 8.82 (s, 1 H) 8.66 (s, 1 H) 8.24 (s, 1 H) 8.14 (d, $J$ = 8.3 Hz, 2 H) 7.86 (d, $J$ = 8.3 Hz, 2 H) 7.34 (d, $J$ = 8.7 Hz, 2 H) 7.04 (d, $J$ = 8.7 Hz, 2 H) 3.77 (s, 3 H) 2.91 (br s, 4 H) 2.33 (br s, 4 H) 2.10 (s, 3 H). LCMS [M + H]$^+$ 490.7.

*N-(4-Methoxyphenyl)-7-(4-((4-methylpiperazin-1-yl)sulfonyl)phenyl)cinnolin-4-amine (**18b**).* Using general procedure 3, 7-bromo-*N*-(4-methoxyphenyl)cinnolin-4-amine (**8b**) and 1-methyl-4-((4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)sulfonyl)piperazine (**40**) were employed, and the product was purified by flash column chromatography over a gradient of 2−6% methanol in dichloromethane to get the product as a yellow solid; 14% yield. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ ppm 8.63 (s, 0 H) 8.51 (s, 1 H) 8.19 (br. s., 2 H) 7.86 (d, $J$ = 8.7 Hz, 2 H) 7.34 (br. s., 1 H) 7.03 (br. s., 2 H) 3.78 (s, 3 H) 2.91 (s, 2 H) 2.59 (br. s., 2 H) 2.32 (br. s., 2 H) 1.19 (s, 3 H). LCMS [M + H]$^+$ 490.2.

*N-(5-Methoxypyridin-2-yl)-6-(4-((4-methylpiperazin-1-yl)sulfonyl)phenyl)cinnolin-4-amine (**19a**).* Using general procedure 3, 6-bromo-*N*-(5-methoxypyridin-2-yl)cinnolin-4-amine (**9a**) and 1-methyl-4-((4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)sulfonyl)piperazine (**40**) were employed, and the product was purified by flash column chromatography over a gradient of 1−3% methanol in dichloromethane to get the product as a light brown solid; 8% yield. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ ppm 10.19 (s, 1 H) 9.72 (s, 1 H) 8.90 (s, 1 H) 8.35 (d, $J$ = 8.8 Hz, 1 H) 8.23−8.28 (m, 1 H) 8.17−8.22 (m, 2 H) 7.91 (d, $J$ = 8.3 Hz, 2 H) 7.54 (dd, $J$ = 9.0, 3.2 Hz, 1 H) 7.37 (d, $J$ = 9.3 Hz, 1 H) 6.52 (s, 1 H) 4.01 (q, $J$ = 7.2 Hz, 2 H) 3.85 (s, 3 H) 2.94 (br. s., 2 H) 2.37 (br. s., 2 H) 2.13 (s, 2 H) 1.95−1.99 (m, 3 H). LCMS [M + H]$^+$ 491.9.

*N-(5-Methoxypyridin-2-yl)-7-(4-((4-methylpiperazin-1-yl)sulfonyl)phenyl)cinnolin-4-amine (**19b**).* Using general procedure 3, 7-bromo-*N*-(5-methoxypyridin-2-yl)cinnolin-4-amine (**9b**) and 1-methyl-4-((4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)sulfonyl)piperazine (**40**) were employed, and the product was purified by flash column chromatography over a gradient of 2−6% methanol in dichloromethane to get the product as a brown solid; 21% yield. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ ppm 10.24 (s, 1 H) 9.66 (s, 1 H) 8.68 (d, $J$ = 9.3 Hz, 1 H) 8.61 (s, 1 H) 8.19−8.25 (m, 1 H) 8.16 (d, $J$ = 2.9 Hz, 1 H) 7.88 (d, $J$ = 8.3 Hz, 2 H) 7.52 (dd, $J$ = 8.8, 2.9 Hz, 1 H) 7.39 (d, $J$ = 8.8 Hz, 1 H) 3.85 (s, 3 H) 2.91−2.99 (m, 4 H) 2.32−2.41 (m, 4 H) 2.11−2.16 (m, 3 H). LCMS [M + H]$^+$ 491.8.

*6-(4-((4-Methylpiperazin-1-yl)sulfonyl)phenyl)-N-(4-(trifluoromethoxy)phenyl)cinnolin-4-amine (**20a**).* Using general procedure 3, 6-bromo-*N*-(4-(trifluoromethoxy)-phenyl)cinnolin-4-amine (**10a**) and 1-methyl-4-((4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)sulfonyl)-piperazine (**40**) were employed, and the product was purified by flash column chromatography over a gradient of 1−3% methanol in dichloromethane to get the product as a dark orange solid; 14% yield.$^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ ppm 9.54 (s, 1 H) 8.97 (s, 1 H) 8.79 (s, 1 H) 8.30−8.33 (m, 1 H) 8.22−8.27 (m, 1 H) 8.13 (d, $J$ = 8.3 Hz, 2 H) 7.99 (d, $J$ = 8.3 Hz, 1 H) 7.87 (d, $J$ = 8.3 Hz, 2 H) 7.81 (d, $J$ = 8.3 Hz, 1 H) 7.54 (d, $J$ = 8.7 Hz, 1 H) 7.44 (d, $J$ = 8.3 Hz, 1 H) 2.90 (br. s., 4 H) 2.33 (br. s., 4 H) 2.10 (s, 3 H). LCMS [M + H]$^+$ 544.8.

*7-(4-((4-Methyl-1,4-diazepan-1-yl)sulfonyl)phenyl)-N-(4-(trifluoromethoxy)phenyl)cinnolin-4-amine (**20b**).* Using general procedure 3, 7-bromo-*N*-(4-(trifluoromethoxy)-phenyl)cinnolin-4-amine (**10b**) and 1-methyl-4-((4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)sulfonyl)-1,4-diazepane (**41**) were employed, and the product was purified by flash column chromatography over a gradient of 1−3% methanol in dichloromethane to get the product as a light brown solid; 14% yield. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ ppm 9.50 (s, 1 H) 8.99 (s, 1 H) 8.59 (d, $J$ = 1.5 Hz, 1 H) 8.54 (d, $J$ = 8.8 Hz, 1 H) 8.18 (d, $J$ = 8.8 Hz, 2 H) 7.92 (d, $J$ = 8.3 Hz, 2 H) 7.57 (d, $J$ = 8.8 Hz, 2 H) 7.46 (d, $J$ = 8.3 Hz, 1 H) 3.34−3.38 (m, 2 H) 2.52−2.57 (m, 2 H) 2.46−2.47 (m, 2 H) 2.22 (s, 3 H) 1.70−1.77 (m, 2 H). LCMS [M + H]$^+$ = 588.24.

*N-(5-Methoxypyrimidin-2-yl)-6-(4-((4-methylpiperazin-1-yl)sulfonyl)phenyl)cinnolin-4-amine (**21a**).* Using general procedure 3, 6-bromo-*N*-(5-methoxypyrimidin-2-yl)cinnolin-4-amine (**11a**) and 1-methyl-4-((4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)sulfonyl)piperazine (**40**) were employed, and the product was purified by flash column chromatography over a gradient of 1−4% methanol in dichloromethane to get the product as a light brown solid; 42% yield. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ ppm 10.34 (s, 1 H) 10.28 (s, 1 H) 8.79 (d, $J$ = 8.8 Hz, 1 H) 8.67 (d, $J$ = 1.5 Hz, 1 H) 8.54 (s, 2 H) 8.23 (d, $J$ = 8.3 Hz, 2 H) 7.87 (d, $J$ = 8.3 Hz, 2 H) 3.91 (s, 3 H) 2.95 (br. S., 4 H) 2.38 (br. S., 4 H) 2.14 (s, 3 H). LCMS [M + H]$^+$ 492.4.

*N-(5-Chloropyrimidin-2-yl)-6-(4-(4-methylpiperazin-1-yl)-phenyl)cinnolin-4-amine (**22a**).* Using general procedure 3, 6-bromo-*N*-(5-chloropyrimidin-2-yl)cinnolin-4-amine (**4a**) and 1-methyl-4-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-phenyl)piperazine were employed, and the product was


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purified by flash column chromatography over a gradient of 1−3% methanol in dichloromethane to get the product as a yellow solid; 29% yield. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ ppm 10.71 (s, 1 H) 10.10 (s, 1 H) 8.77 (s, 2 H) 8.74 (s, 1 H) 8.29 (d, $J$ = 8.7 Hz, 1 H) 8.21 (s, 1 H) 7.87 (d, $J$ = 8.7 Hz, 2 H) 7.05 (d, $J$ = 8.7 Hz, 2 H) 3.21 (d, $J$ = 4.8 Hz, 4 H) 2.42 (d, $J$ = 4.3 Hz, 4 H) 2.19 (s, 3 H). LCMS [M + H]$^+$ 432.9.

*N-(5-Chloropyrimidin-2-yl)-7-(4-(4-methylpiperazin-1-yl)-phenyl)cinnolin-4-amine (**22b**).* Using general procedure 3, 7-bromo-*N*-(5-chloropyrimidin-2-yl)cinnolin-4-amine (**4b**) and 1-methyl-4-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-phenyl)piperazine were employed, and the product was purified by flash column chromatography over a gradient of 1−2% methanol in dichloromethane to get the product as a light brown solid; 4% yield. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ ppm 8.75 (s, 2 H) 8.52 (d, $J$ = 9.3 Hz, 1 H) 8.43−8.47 (m, 1 H) 8.10 (d, $J$ = 9.3 Hz, 1 H) 7.80 (d, $J$ = 8.3 Hz, 1 H) 7.64−7.72 (m, 1 H) 7.58 (d, $J$ = 8.3 Hz, 1 H) 7.05 (d, $J$ = 8.3 Hz, 1 H) 6.78−6.84 (m, 2 H) 6.71 (d, $J$ = 8.8 Hz, 1 H) 6.51−6.61 (m, 1 H) 3.18−3.22 (m, 2 H) 3.07−3.17 (m, 2 H) 2.37 (br. s., 2 H) 2.17−2.20 (m, 3 H) 2.14−2.17 (m, 2 H). LCMS [M + H]$^+$ 432.13.

*N-(4-Methoxyphenyl)-6-(4-(4-methylpiperazin-1-yl)-phenyl)cinnolin-4-amine (**23a**).* Using general procedure 3, 6-bromo-*N*-(4-methoxyphenyl)cinnolin-4-amine (**8a**) and 1-methyl-4-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-phenyl)piperazine were employed, and the product was purified by flash column chromatography over a gradient of 2−6% methanol in dichloromethane to get the product as a yellow solid; 14% yield. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ ppm 9.20 (s, 1 H) 8.60 (s, 1 H) 8.57 (s, 1 H) 8.12 (d, $J$ = 1.9 Hz, 2 H) 7.78 (d, $J$ = 8.7 Hz, 2 H) 7.32 (d, $J$ = 8.7 Hz, 2 H) 7.05 (d, $J$ = 8.7 Hz, 2 H) 7.03 (d, $J$ = 9.2 Hz, 2 H) 3.77 (s, 3 H) 3.21 (d, $J$ = 4.3 Hz, 4 H) 2.42 (d, $J$ = 4.8 Hz, 4 H) 2.19 (s, 3 H). LCMS [M + H]$^+$ 426.2.

*N-(4-Methoxyphenyl)-7-(4-(4-methylpiperazin-1-yl)-phenyl)cinnolin-4-amine (**23b**).* Using general procedure 3, 7-bromo-*N*-(4-methoxyphenyl)cinnolin-4-amine (**8b**) and 1-methyl-4-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-phenyl)piperazine were employed, and the product was purified by flash column chromatography over a gradient of 25−50% ethyl acetate in hexanes to get the product as a yellow solid; 83% yield. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ ppm 9.21 (br. S., 0 H) 8.55 (br. S., 1 H) 8.38 (br. S., 1 H) 8.27 (br S, 1 H) 7.99 (br. S., 1 H) 7.77 (d, $J$ = 8.3 Hz, 2 H) 7.30 (br. S., 1 H) 6.97−7.12 (m, 4 H) 3.76 (s, 3 H) 2.56−2.74 (m, 3 H) 2.33 (d, $J$ = 16.59 Hz, 4 H). LCMS [M + H]$^+$ = 426.7.

*6-(4-(4-Methylpiperazin-1-yl)phenyl)-N-(4-(trifluoromethoxy)phenyl)cinnolin-4-amine (**24a**).* Using general procedure 3, 6-bromo-*N*-(4-(trifluoromethoxy)-phenyl)cinnolin-4-amine (**10a**) and 1-methyl-4-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)piperazine were employed, and the product was purified by flash column chromatography over a gradient of 1−3% methanol in dichloromethane to get the product as a yellow solid; 19% yield. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ ppm 9.37 (s, 1 H) 8.89 (s, 1 H) 8.56 (s, 1 H) 8.20 (s, 1 H) 8.14−8.17 (m, 1 H) 7.78 (d, $J$ = 8.7 Hz, 2 H) 7.52 (d, $J$ = 9.2 Hz, 2 H) 7.42 (d, $J$ = 8.3 Hz, 2 H) 7.06 (d, $J$ = 8.7 Hz, 2 H) 3.19−3.23 (m, 4 H) 2.40−2.44 (m, 4 H) 2.19 (s, 3 H). LCMS [M + H]$^+$ 480.4.

*N-(5-Methoxypyrimidin-2-yl)-6-(4-(4-methylpiperazin-1-yl)phenyl)cinnolin-4-amine (**25a**).* Using general procedure 3, 6-bromo-*N*-(5-methoxypyrimidin-2-yl)cinnolin-4-amine (**11a**) and 1-methyl-4-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)piperazine were employed, and the product was purified by flash column chromatography over a gradient of 3−7% methanol in dichloromethane to get the product as a light brown solid; 18% yield. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ ppm 10.19−10.22 (m, 1 H) 10.16−10.19 (m, 1 H) 8.62−8.67 (m, 1 H) 8.51−8.54 (m, 1 H) 8.42−8.46 (m, 1 H) 8.05−8.11 (m, 1 H) 7.81−7.84 (m, 2 H) 7.06−7.11 (m, 2 H) 3.90−3.92 (m, 3 H) 3.22−3.26 (m, 4 H) 2.44−2.47 (m, 4 H) 2.21−2.23 (m, 3 H). LCMS [M + H]$^+$ 428.8.

*N-(5-Chloropyrimidin-2-yl)-6-(2-(4-methylpiperazin-1-yl)-pyrimidin-5-yl)cinnolin-4-amine (**26a**).* Using general procedure 3, 6-bromo-N-(5-chloropyrimidin-2-yl)cinnolin-4-amine (**4a**) and 2-(4-methylpiperazin-1-yl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyrimidine (**42**) were employed, and the product was purified by flash column chromatography over a gradient of 1−3% methanol in dichloromethane to get the product as a dark yellow solid; 9% yield. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ ppm 10.58 (br. S., 1 H) 10.18 (s, 1 H) 9.00 (s, 2 H) 8.84 (s, 1 H) 8.79 (s, 2 H) 8.33 (d, $J$ = 9.2 Hz, 1 H) 8.23 (d, $J$ = 8.7 Hz, 1 H) 3.79 (br. S., 4 H) 2.35 (t, $J$ = 4.6 Hz, 4 H) 2.18 (s, 3 H). LCMS [M + H]$^+$ 434.8.

*N-(4-Methoxyphenyl)-6-(2-(4-methylpiperazin-1-yl)-pyrimidin-5-yl)cinnolin-4-amine (**27a**).* Using general procedure 3, 6-bromo-*N*-(4-methoxyphenyl)cinnolin-4-amine (**8a**) and 2-(4-methylpiperazin-1-yl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyrimidine (**42**) were employed, and the product was purified by flash column chromatography over a gradient of 2−6% methanol in dichloromethane to get the product as a dark yellow solid; 9% yield. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ ppm 9.14 (s, 1 H) 8.93 (s, 1 H) 8.66 (s, 1 H) 8.61 (s, 1 H) 8.16 (d, $J$ = 3.9 Hz, 2 H) 7.33 (d, $J$ = 8.7 Hz, 2 H) 7.03 (d, $J$ = 8.7 Hz, 2 H) 3.78 (br. S., 4 H) 3.77 (s, 3 H) 2.35 (t, $J$ = 4.8 Hz, 4 H) 2.18 (s, 3 H). LCMS [M + H]$^+$ = 428.3.

*N-(4-Methoxyphenyl)-7-(2-(4-methylpiperazin-1-yl)-pyrimidin-5-yl)cinnolin-4-amine (**27b**).* Using general procedure 3, 7-bromo-*N*-(4-methoxyphenyl)cinnolin-4-amine (**8b**) and 2-(4-methylpiperazin-1-yl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyrimidine (**42**) were employed, and the product was purified by flash column chromatography over a gradient of 1−4% methanol in dichloromethane to obtain the product as a yellow solid; 23% yield. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ ppm 9.20 (s, 1 H) 8.94 (s, 2 H) 8.58 (s, 1 H) 8.36−8.49 (m, 2 H) 8.04 (d, $J$ = 8.3 Hz, 1 H) 7.32 (d, $J$ = 8.7 Hz, 2 H) 7.02 (d, $J$ = 8.7 Hz, 2 H) 3.71−3.85 (m, 8 H) 2.30−2.41 (m, 5 H) 2.21 (br. S., 3 H). LCMS [M + H]$^+$ 428.9.

*6-(2-(4-Methylpiperazin-1-yl)pyrimidin-5-yl)-N-(4-(trifluoromethoxy)phenyl)cinnolin-4-amine (**28a**).* Using general procedure 3, 6-bromo-*N*-(4-(trifluoromethoxy)-phenyl)cinnolin-4-amine (**10a**) and 2-(4-methylpiperazin-1-yl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyrimidine (**42**) were employed, and the product was purified by flash column chromatography over a gradient of 1−3% methanol in dichloromethane to get the product as a yellow solid; 6% yield. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ ppm 9.28−9.34 (m, 1 H) 8.91−8.96 (m, 2 H) 8.62−8.65 (m, 1 H) 8.22−8.26 (m, 1 H) 8.17−8.22 (m, 1 H) 7.51−7.55 (m, 2 H) 7.42−7.46 (m, 2 H) 6.47−6.51 (m, 1 H) 3.75−3.82 (m, 4 H) 2.33−2.40 (m, 4 H) 2.17−2.22 (m, 3 H). LCMS [M + H]$^+$ 481.6.

*N-(5-Chloropyrimidin-2-yl)-6-(2-(4-methyl-1,4-diazepan-1-yl)pyrimidin-5-yl)cinnolin-4-amine (**29a**).* Using general procedure 3, 6-bromo-*N*-(5-chloropyrimidin-2-yl)cinnolin-4-


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amine (**4a**) and 1-methyl-4-(5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyrimidin-2-yl)-1,4-diazepane (**43**) were employed, and the product was purified by flash column chromatography over a gradient of 1−3% methanol in dichloromethane to get the product as a dark yellow solid; 15%. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ ppm 10.53−10.59 (m, 1 H) 10.16−10.21 (m, 1 H) 8.96−9.00 (m, 2 H) 8.83−8.86 (m, 1 H) 8.77−8.82 (m, 2 H) 8.30−8.37 (m, 1 H) 8.20−8.26 (m, 1 H) 3.85−3.90 (m, 2 H) 3.77−3.82 (m, 2 H) 3.32−3.36 (m, 2 H) 3.10−3.14 (m, 2 H) 2.21−2.24 (m, 3 H) 1.83−1.90 (m, 2 H). LCMS [M + H]$^+$ = 448.5.

*N-(4-Methoxyphenyl)-6-(2-(4-methyl-1,4-diazepan-1-yl)-pyrimidin-5-yl)cinnolin-4-amine (**30a**).* Using general procedure 3, 6-bromo-*N*-(4-methoxyphenyl)cinnolin-4-amine (**8a**) and 1-methyl-4-(5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyrimidin-2-yl)-1,4-diazepane (**43**) were employed, and the product was purified by flash column chromatography over a gradient of 2−5% methanol in dichloromethane to get the product as a dark yellow solid; 6% yield. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ ppm 9.12 (s, 1 H) 8.90 (s, 1 H) 8.63 (d, $J$ = 12.2 Hz, 1 H) 8.15 (d, $J$ = 7.8 Hz, 1 H) 7.33 (d, $J$ = 8.7 Hz, 2 H) 7.03 (d, $J$ = 8.7 Hz, 2 H) 3.86 (br. S., 3 H) 3.75−3.81 (m, 3 H) 2.59 (br. S., 2 H) 2.22 (s, 3 H) 2.20 (d, $J$ = 6.8 Hz, 2 H) 1.86 (br. S., 2 H). LCMS [M + H]$^+$ 442.7.

*N-(4-Methoxyphenyl)-7-(2-(4-methyl-1,4-diazepan-1-yl)-pyrimidin-5-yl)cinnolin-4-amine (**30b**).* Using general procedure 3, 7-bromo-*N*-(4-methoxyphenyl)cinnolin-4-amine (**8b**) and 1-methyl-4-(5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyrimidin-2-yl)-1,4-diazepane (**43**) were employed, and the product was purified by flash column chromatography over a gradient of 7−11% methanol in dichloromethane to get the product as a yellow solid; 18% yield. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ ppm 9.19 (s, 1 H) 8.93 (s, 1 H) 8.58 (s, 1 H) 8.41 (d, $J$ = 13.1 Hz, 2 H) 8.04 (d, $J$ = 8.7 Hz, 1 H) 7.32 (d, $J$ = 8.7 Hz, 2 H) 7.03 (d, $J$ = 8.7 Hz, 1 H) 3.85−3.89 (m, 2 H) 3.80 (d, $J$ = 6.3 Hz, 2 H) 3.77 (s, 3 H) 2.54−2.63 (m, 3 H) 2.23 (s, 3 H) 2.20 (d, $J$ = 4.3 Hz, 2 H) 1.86 (d, $J$ = 5.8 Hz, 2 H). LCMS [M + H]$^+$ 442.8.

*6-(2-(4-Methyl-1,4-diazepan-1-yl)pyrimidin-5-yl)-N-(4-(trifluoromethoxy)phenyl)cinnolin-4-amine (**31a**).* Using general procedure 3, 6-bromo-*N*-(4-(trifluoromethoxy)-phenyl)30innoline-4-amine (**10a**) and 1-methyl-4-(5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyrimidin-2-yl)-1,4-diazepane (**43**) were employed, and the product was purified by flash column chromatography over a gradient of 1−3% methanol in dichloromethane to get the product as a dark red solid; 18%. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ ppm 9.29 (s, 1 H) 8.94 (s, 1 H) 8.90 (s, 2 H) 8.62 (s, 1 H) 8.21−8.26 (m, 1 H) 8.16−8.21 (m, 1 H) 7.53 (d, $J$ = 8.7 Hz, 2 H) 7.43 (d, $J$ = 8.3 Hz, 2 H) 3.84−3.89 (m, 2 H) 3.78 (t, $J$ = 6.3 Hz, 2 H) 2.57−2.62 (m, 2 H) 2.22 (s, 3 H) 1.83−1.89 (m, 2 H). LCMS [M + H]$^+$ 496.5.

*N-(5-Methoxypyrimidin-2-yl)-6-(2-(4-methyl-1,4-diazepan-1-yl)pyrimidin-5-yl)cinnolin-4-amine (**32a**).* Using general procedure 3, 6-bromo-*N*-(5-methoxypyrimidin-2-yl)-cinnolin-4-amine (**11a**) and 1-methyl-4-(5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyrimidin-2-yl)-1,4-diazepane (**43**) were employed, and the product was purified by flash column chromatography over a gradient of 1−4% methanol in dichloromethane to get the product as an orange solid; 13%. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ ppm 10.25 (s, 1 H) 10.20 (s, 1 H) 8.98 (s, 1 H) 8.68 (d, $J$ = 8.8 Hz, 1 H) 8.55 (s, 1 H) 8.53 (s, 2 H) 8.11 (d, $J$ = 10.7 Hz, 1 H) 3.91 (s, 3 H) 3.81−3.86 (m, 2 H) 3.22 (s, 2 H) 2.62 (s, 2 H) 2.28−2.34 (m, 2 H) 1.93 (br. s., 2 H) 1.89 (s, 3 H). LCMS [M + H]$^+$ 444.8.

*N-(5-Chloropyrimidin-2-yl)-6-(4-((4-methyl-1,4-diazepan-1-yl)sulfonyl)phenyl)cinnolin-4-amine (**33a**).* Using general procedure 3, 6-bromo-*N*-(5-methoxypyridin-2-yl)cinnolin-4-amine (**4a**) and 1-methyl-4-((4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)sulfonyl)-1,4-diazepane (**41**) were employed, and the product was purified by flash column chromatography over a gradient of 1−4% methanol with 5% ammonium hydroxide in dichloromethane to get the product as a brown solid; 8% yield. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ ppm 10.85 (br. s., 1 H) 10.25 (br. s., 1 H) 9.02 (s, 1 H) 8.82 (s, 2 H) 8.42 (br. s., 1 H) 8.31 (d, $J$ = 7.8 Hz, 1 H) 8.21 (d, $J$ = 8.3 Hz, 2 H) 7.94 (d, $J$ = 8.3 Hz, 2 H) 3.34−3.37 (m, 2 H) 3.32 (d, $J$ = 6.3 Hz, 2 H) 2.52−2.55 (m, 2 H) 2.46 (br. s., 2 H) 2.21 (s, 3 H) 1.70−1.77 (m, 2 H). LCMS [M + H]$^+$ 511.1.

*6-(4-((4-Methyl-1,4-diazepan-1-yl)sulfonyl)phenyl)-N-(4-(trifluoromethoxy)phenyl)cinnolin-4-amine (**34a**).* Using general procedure 3, 6-bromo-*N*-(4-(trifluoromethoxy)-phenyl)cinnolin-4-amine (**10a**) and 1-methyl-4-((4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)sulfonyl)-1,4-diazepane (**41**) were employed, and the product was purified by flash column chromatography over a gradient of 1−4% methanol in dichloromethane to get the product as a red solid; 12% yield. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ ppm 9.57 (s, 1 H) 9.00 (s, 1 H) 8.81 (s, 1 H) 8.32−8.36 (m, 1 H) 8.27 (d, $J$ = 1.5 Hz, 1 H) 8.13 (d, $J$ = 8.3 Hz, 2 H) 7.95 (d, $J$ = 8.3 Hz, 2 H) 7.57 (d, $J$ = 8.8 Hz, 2 H) 7.48 (d, $J$ = 8.8 Hz, 2 H) 3.35 (d, $J$ = 4.9 Hz, 2 H) 2.53 (d, $J$ = 9.3 Hz, 2 H) 2.46 (d, $J$ = 5.4 Hz, 2 H) 2.21 (s, 3 H) 2.20 (br. s., 3 H) 1.69−1.75 (m, 4 H). LCMS [M + H]$^+$ 558.7.

*N-(5-Methoxypyrimidin-2-yl)-6-(4-((4-methyl-1,4-diazepan-1-yl)sulfonyl)phenyl)cinnolin-4-amine (**35a**).* Using general procedure 3, 6-bromo-*N*-(5-methoxypyrimidin-2-yl)-cinnolin-4-amine (**11a**) and 1-methyl-4-((4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)sulfonyl)-1,4-diazepane (**41**) were employed, and the product was purified by flash column chromatography over a gradient of 1−4% methanol in dichloromethane to get the product as a dark yellow solid; 27% yield. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ ppm 10.34 (s, 1 H) 10.27 (s, 1 H) 8.78 (d, $J$ = 9.3 Hz, 1 H) 8.65 (d, $J$ = 1.5 Hz, 1 H) 8.54 (s, 2 H) 8.19 (d, $J$ = 8.3 Hz, 2 H) 7.92 (d, $J$ = 8.3 Hz, 2 H) 3.91 (s, 3 H) 3.34−3.37 (m, 2 H) 2.52−2.56 (m, 2 H) 2.46 (br s, 2 H) 2.21 (s, 3 H) 1.74 (br s, 2 H). LCMS [M + H]$^+$ = 506.4.

*1-((4-Bromophenyl)sulfonyl)-4-methylpiperazine (**36**).* This compound was synthesized according to Devine et al.[7] To a solution of 4-bromobenzene-1-sulfonyl chloride (15.0 g, 58.7 mmol) in tetrahydrofuran (250 mL), 1-methylpiperazine (58.6 mL, 528 mmol) was added. The reaction mixture was stirred at room temperature overnight. The solvent was then removed in vacuo, and the crude was redissolved in dichloromethane (250 mL). The solution was washed with sat. $NaHCO_3$ (100 mL), washed with brine (50 mL), dried over $Na_2SO_4$, and concentrated to yield S31 as a cream solid in 93% yield. $^1$H NMR (500 MHz, chloroform-$d$) $\delta$ 7.63−7.68 (m, 2 H), 7.57−7.61 (m, 2 H), 3.02 (br. s., 4 H), 2.46 (t, J = 4.9 Hz, 4 H), 2.25 (s, 3 H). LCMS [M + H]$^+$ 318.9.

*1-((4-Bromophenyl)sulfonyl)-4-methyl-1,4-diazepane (**37**).* This compound was synthesized according to Devine et al.[7] Briefly, in a round-bottom flask, 4-bromobenzenesulfonyl chloride (2.00 g, 7.83 mmol), THF (40 mL), TEA (2.18 mL, 15.65 mmol), and 1-methylhomopiperazine (0.97 mL, 7.83


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mmol) were combined. The reaction mixture was allowed to stir at ambient temperature for 3 h. Once the reaction was complete, the solvent was removed under reduced pressure, and the residue was dissolved in DCM and washed with saturated sodium bicarbonate and then saturated sodium chloride. The organic phase was dried with $MgSO_4$, filtered, and concentrated under reduced pressure to afford the pure product as a light orange solid; 61% yield. LCMS $[M + H]^+$ 334.4.

*5-Bromo-2-(4-methylpiperazin-1-yl)pyrimidine (**38**).* This compound was synthesized according to Bachovchin et al.[8]

*1-(5-Bromopyrimidin-2-yl)-4-methyl-1,4-diazepane (**39**).* This compound was synthesized according to Bachovchin et al.[8]

*1-Methyl-4-((4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)sulfonyl)piperazine (**40**).* This compound was synthesized according to Devine et al.[7]

*1-Methyl-4-((4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)sulfonyl)-1,4-diazepane (**41**).* This compound was synthesized according to Devine et al.[7]

*2-(4-Methylpiperazin-1-yl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyrimidine (**42**).* Using general procedure 7, 5-bromo-2-(4-methylpiperazin-1-yl)pyrimidine (**38**) was employed to get the crude mixture. LCMS $[M + H]^+$ = 305.4.

*(2-(4-Methyl-1,4-diazepan-1-yl)pyrimidin-5-yl)boronic Acid, Pinacol Ester (**43**).* This compound was synthesized according to Bachovchin et al.[8]

## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsinfecdis.4c00666.

Molecular formula strings and all associated data (XLSX)

Antiparasitic activity of additional analogs against the kinetoplastids presented herein, activity of compounds against *Schistosoma mansoni*, physicochemical properties and host cell toxicity of compounds presented along with experiment protocols, plasma-concentration profile of NEU-5884, and liver and spleen parasite burden for NEU-5531 (PDF)

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### Notes

The authors declare no competing financial interest.

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## ■ ABBREVIATIONS USED

DALY: disability adjusted life year; EGFR: epidermal growth factor receptor; NMP: *N*-methylpyrrolidine.

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