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Article

Discovery and Optimization of a Compound Series Active against *Trypanosoma cruzi*, the Causative Agent of Chagas Disease

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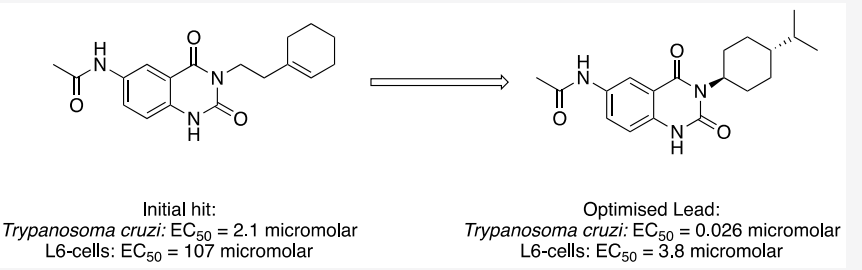

ABSTRACT: Chagas disease is caused by the protozoan parasite *Trypanosoma cruzi*. It is endemic in South and Central America and recently has been found in other parts of the world, due to migration of chronically infected patients. The current treatment for Chagas disease is not satisfactory, and there is a need for new treatments. In this work, we describe the optimization of a hit compound resulting from the phenotypic screen of a library of compounds against *T. cruzi*. The compound series was optimized to the level where it had satisfactory pharmacokinetics to allow an efficacy study in a mouse model of Chagas disease. We were able to demonstrate efficacy in this model, although further work is required to improve the potency and selectivity of this series.

INTRODUCTION

Chagas disease is caused by the parasite *Trypanosoma cruzi* and is endemic in South and Central America, although the disease has spread across the globe, through migration of infected individuals.[1] Chagas disease is defined as a neglected tropical disease. It is estimated that about 8 million people are currently infected, and it gives rise to approximately 8000 deaths per year.[2,3]

The parasite has a complex life cycle. Transmission from the vector (triatomine or reduvvid bug) occurs when the extracellular metacyclic trypomastigotes, which are found in faeces or urine from the vector, penetrate human skin, typically through damaged skin where the insect has taken a blood meal. However, penetration can occur through mucosal membranes such as the conjunctiva.[4] The vector is particularly associated with poorer quality housing. Vector control through the use of insecticides and improvements in housing are key methods to reduce transmission.[5] Transmission can also occur from mother to unborn child and through blood transfusion. Infection can also occur orally; there have been reported incidences of transmission through drinking fruit juice, thought to be due to contamination of the fruit by triatomine faeces or insects contaminating the fruit during preparation.[6,7]

In the human host, the parasites can multiply in different cell types, and there is evidence that they invade many different organs within the body. Within the cells, the parasites are predominantly found as rounded intracellular amastigotes. The intracellular amastigotes divide. Eventually, they transform into trypomastigotes, which burst out of the cells, and can then invade other cells within the human host. The life cycle is explained in detail on the Centers for Disease Control and Progression Web site.[4]

The disease has an acute stage and a chronic indeterminate stage. The acute stage is typified by the presence of parasites in the blood. Most people do not have symptoms, but it has a high fatality rate among children. However, as many of the symptoms are relatively nonspecific, the disease is often not diagnosed. The parasite then enters a chronic stage, where the level of parasitemia is very low and essentially becomes undetectable. The chronic stage is not well understood. However, it is becoming apparent that there are slow-growing

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or dormant forms.[8] It is thought about 30% of patients with chronic Chagas disease go on to develop heart disease (failure) or megacolon, and this is only found when the patient is critically ill or even dead.

Unfortunately, the current treatment options are very limited and are confined to the nitro-aromatics benznidazole and nifurtimox (Figure 1).[3,9,10] These drugs are reasonably

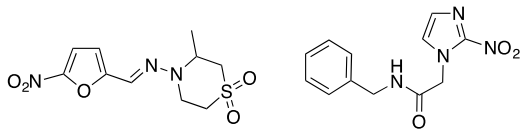

Figure 1. Structures of compounds used to treat Chagas disease.

effective for acute Chagas disease, but evidence suggests limited efficacy against chronic disease. The efficacy of benznidazole on patients with chronic Chagas disease was recently studied in detail with the BENEFIT trial,[11] in which patients with Chagas disease cardiomyothapy were treated with either a placebo or benznidazole and then followed up for about 5 years. While those in the benznidazole arm had reduced the levels of parasites detected in the blood, there was essentially no difference in cardiac clinical outcomes between the two groups. Benznidazole and nifurtimox have significant side effects, which means that many patients cannot finish the treatment. A recent study, the BENDITA trial,[12,13] has looked at reducing the length of treatment or dose levels with benznidazole. There are some promising indications from this trial.

Recently, there have been clinical trials of two azoles, posaconazole (NCT01377480)[14] and fosravuconazole (E1224) (NCT01489228),[15] which inhibit an enzyme involved in ergosterol biosynthesis, called CYP51. Despite initially causing a significant drop in parasitemia, there were very high levels of relapse, meaning that these sorts of drugs are not suitable for further development.[3,16] The BENDITA trial has looked at combinations of fosravuconazole with benznidazole. The analysis of this data is ongoing. A phase II clinical study using fexinidazole (NCT03587766),[17] a drug developed for human African trypanosomiasis, was carried out for Chagas disease but was stopped due to safety concerns at high doses. A new study (NCT02498782) at lower doses and shorter regimens is underway.

The pipeline for new chemical entities (NCEs) for Chagas disease is very sparse. DNDi has no NCEs in regulatory preclinical studies or Phase 1.[18]

In this work, we report the results of a screen against *T. cruzi* and subsequent optimization of the hits, to the stage of activity in a mouse model of infection, in an attempt to discover new starting points for drug discovery projects.

RESULTS AND DISCUSSION

Screening and Initial Hit Expansion. The project was initiated by screening a small, diverse chemical library of about 7000 compounds from Medivir against the parasite. This library was a general diversity set, not specifically targeted toward *T. cruzi*. The screening was carried out against the clinically relevant intracellular stage; in this case, the parasites were cultured in L6 cells, which are rat skeletal muscle myoblasts (ATCC CRL1458). Compounds were also screened against L6 to deprioritize compounds, which are generally cytotoxic.

From the screen, quinazolinedione (**1**) was identified as a selective hit, where the compound is more active against *T. cruzi* than the mammalian L6-cells (Figure 2 and Table 1). In

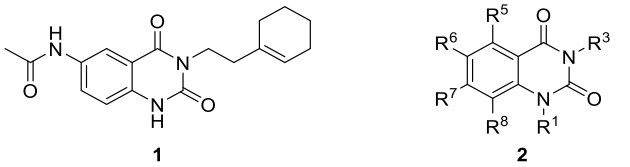

Figure 2. Hit molecule and generic structure.

the early stages of a medicinal chemistry project, the selectivity should be >10-fold, and this value should be increased to >100-fold during the hits to lead the process. Compound **1** is chemically tractable and multiple analogues can be readily prepared.

In the first phase of hit expansion, SAR was investigated for substituents R^1–R^6 of the core quinazolinedione (Figure 2). Variations of R^3 and R^6 are reported in Table 1 and Table 2, respectively. These two substituent positions are central for SAR efficacy development.

The activity of the hit compound **1** was confirmed with close analogues, such as compounds **3**, **4**, **5**, **6**, **12**, **14**, **15**, **16**, **17**, and **18**, all of which are lipophilic alkyl or cycloalkyl substituents. At most, the potency was increased about 5-fold among these (compounds **4** and **6**). Joining the cycloalkyl directly to the quinazolinedione caused some loss of activity (compounds **16**, **17**, and **18**) relative to the cycloalkyl compounds **4** and **6**. The loss activity was more pronounced with the *tert*-butyl group directly attached to the core (compound **13**). The cycloalkyl group could be replaced to some extent with phenyl as in compounds **9**, **10**, and **11**, whereas more polar functions such as ether (compound **7**) or amide (compound **8**) resulted in significantly diminished or loss of activity. Given the lipophilic character of some of the substituents, it is important to keep an eye on the logP values and, in particular, the lipophilic ligand efficiency (LLE).[19] The latter is defined as LLE = pEC_{50} − clogP and is a suitable measure to ensure that activity is not being driven by lipophilicity. For drugs, LLE is generally >5.

A breakthrough came with further increasing the lipophilic character by adding a *tert*-butyl to the cyclohexyl substituent (compound **19**), which caused a near 100-fold increase in potency relative to the hit compound **1**. This compound had a good selectivity (180-fold), and although there was a small increase in the lipophilicity (clogP = 3.3), the LLE of 4.4 was the highest in Table 1.

Several synthetic routes were developed to the quinazolinediones. Heating isatoic anhydride **20** with amines gave intermediate amides, which were cyclized by treatment with carbonyldiimidazole in refluxing THF (Scheme 1). This route allowed the rapid exploration of the R^3 substituent. An alternative route that allowed the installation of the R^6 amide as the final step commenced with acid **22** (Scheme 2). Amide formation with the appropriate R^3 amine and propylphosphonic anhydride, followed by cyclization with either carbonyldiimidazole or ethyl chloroformate, gave the 6-nitroquinazolinediones **24**. Hydrogenation of the nitro group

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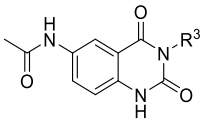
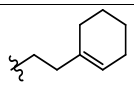
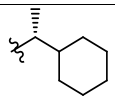
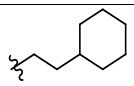
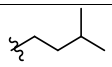
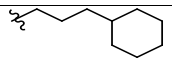
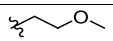
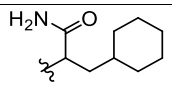
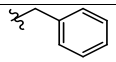
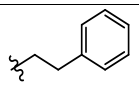
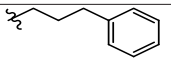
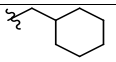
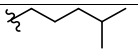
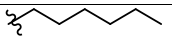
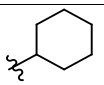
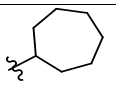
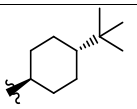
Table 1. Variation around the R^3 Position[a]



	R^3	Tc μM	L6 μM (selectivity)	clogP	LLE
1		2.1±0.52	107± 41 (51)	1.9	3.8
3		2.2±0.13	88± 6.8 (40)	3.0	2.7
4		0.73±0.14	52± 16 (110)	3.0	3.3
5		9.7±0.18	163± 18 (17)	1.7	3.3
6		0.48±0.06	87± 24 (180)	3.4	2.9
7		35±11	0.1± 0.04 (0.003)	0.3	4.2
8		170±6.3	222± 1 (1.3)	1.9	1.9
9		17±2.2	148± 20 (9)	1.8	3.0
10		1.1±0.16	139±17 (130)	2.0	3.8

	R^3	Tc μM	L6 μM (selectivity)	clogP	LLE
11		7.5±0.1	134±36 (18)	2.4	2.7
12		2.3±0.19	54± 12 (23)	2.8	2.8
13		61±1	37±7.3 (0.6)	1.5	2.7
14		4.0±0.34	173± (43)	2.1	3.3
15		2.9±0.42	154±11 (53)	2.6	2.9
16		12±2.7	184±14 (15)	1.7	3.2
17		4.2±0.8	70±26 (17)	2.4	3.0
18		2.1±0.12	112±13 (53)	3.0	2.7
19		0.022±0.004	3.9±0.04 (180)	3.3	4.4

[a]The EC_{50} values are means of two independent determinations ± deviation from the mean. Control compounds: benznidazole for the *T. cruzi* assay EC_{50} ± SD 1.94 ± 0.49 μM, podophyllotoxin for the cytotoxicity assay EC_{50} ± SD 0.014 ± 0.005 μM. clogP was calculated using StarDrop (www.optibrium.com)

then gave anilines **25**, which were then coupled with carboxylic acids to give amides **26**.

The synthesis of 4-substituted cyclohexylamines started from the corresponding cyclohexanones **27** (Scheme 3). Reductive amination with benzylamine and sodium borohydride gave a ca. 2:1 ratio of *trans* to *cis* isomers of **28**, which were separated by column chromatography on silica. Hydrogenolysis of the benzyl protecting group gave the amine intermediate **29**, which was incorporated into the quinazolinedione scaffold using our standard methods described above.

Simultaneous to the SAR R^3 investigations, hit expansion was also carried out to investigate the R^6 position (Table 2). This was initially carried out using the ethylcyclohexyl substituent on the *N*-3-position (**4**). Replacing the acetamide with amino (**30**) gave a compound with similar activity, although with about 10-fold less selectivity compared to L6 cells. The amino substituent at R^6 was subsequently employed (Tables 5 and 7) in addition to the acetamide substituent (Tables 3, 4, and 6).

Replacing the acetamide with more bulky substituents (**31**, **32**) led to a drop in activity and selectivity. Interestingly the acetamide could be replaced by either a 3- or 4-pyridine (**35**, **37**) with relatively little loss in activity, but a loss in selectivity. In contrast, the pyrimidine analogue (**36**) and the isoxazole (**34**) both lost activity. The methylene nitrile **33** gave a similar activity as **4** with a smaller loss in selectivity. This latter compound has a different pattern of H-bond donors at the R^6 position. All of these modifications led to a loss in selectivity and LLE.

The heteroaryl groups were introduced at the R^6 position by Suzuki coupling of 6-iodo intermediate **40** with the appropriate boronic acid (Scheme 4). When isozazol-4-yl boronic acid was reacted under these conditions, the 6-acetonitrile product **33** was obtained as the product of a tandem Suzuki coupling−isoxazole fragmentation.[20]

Hits to Lead Chemistry. Given the promising activity of compound **19**, and on the basis of the SAR data in Tables 1 and 2, we set about optimizing this further. A particular concern with this molecule is the hydrophobic nature of the substituent on R^3. This gave a compound with lower solubility than desired, and while the stability of compound **19** in mouse liver microsomes was <5 mL/min/g, we ideally wanted this to be <1 mL/min/g. A series of compounds was made to understand the SAR at the R^3 position and to address the issues raised (Tables 3−7). Compounds were prepared with *trans*- or *cis*-relationship at the cyclohexyl ring. The *tert*-butyl group could be replaced by an *iso*-propyl group (**43**, **44**), a propyl group (**47**, **48**), or an ethyl group (**45**, **46**) with similar or marginally lower activity. In the case of the

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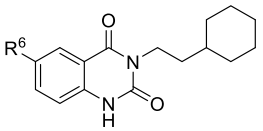
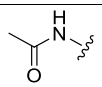
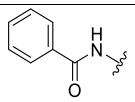
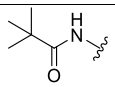
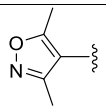
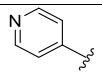
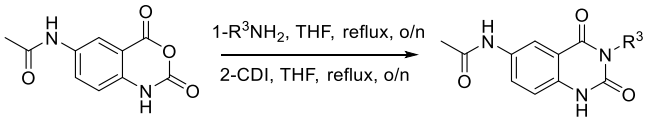
Table 2. SAR at the R^6 Position[a]



	R^6	Tc μM	L6 μM (selectivity)	clogP	LLE
4		0.73±0.14	52±16 (120)	3.0	3.3
30	NH_2	1.9±0.66	19±0.6 (10)	3.1	2.6
31		15±0.7	16± 4.3 (1.1)	4.2	0.6
32		7.1±0.22	13± 1.2 (1.8)	3.9	1.2
33		2.1±0.6	70± 3 (33)	3.3	2.4
34		120±34	88±39 (2.8)	4.6	-0.1
35		1.9±0.15	14±1.4 (7.4)	3.8	1.9
36		14±0.7	126±3.3 (9)	4.2	0.7
37		1.7±0.7	14±0.35 (8)	3.8	2.0

[a]The EC_{50} values are means of two independent determinations ± deviation from the mean. Control compounds: benznidazole for the *T. cruzi* assay EC_{50} ± SD 1.94 ± 0.49 μM, podophyllotoxin for the cytotoxicity assay EC_{50} ± SD 0.014 ± 0.005 μM. clogP was calculated using StarDrop (www.optibrium.com)

Scheme 1. General Procedure B for the Synthesis of 6-Acetamide-R3-Substituted Quinazolinediones



20 **21**

iso-propyl group, the *cis* isomer (**44**) was slightly less active, but this was not the case with the other substituents. *cis*-Cyclohexyl derivative **42** showed a particularly good level of stability. The methyl substituent (**49**) gave a 60-fold loss of activity, while the dimethyl compound (**50**), however, showed only 8-fold reduced activity compared to **19**. This suggests some lipophilic substituents should be present on the 4-position of the cyclohexyl group. Replacing the cyclohexyl with a cyclopentyl (**51**) retained activity, while replacing the cyclohexyl with a phenyl (**52**) led to a loss in activity. All of the compounds in the table retained good selectivity (>100-fold) and good LLE, except compounds **49**, **50**, and **52**.

All of the compounds tested had very similar levels of poor solubility. Our aim in terms of microsomal stability was to have a compound with sufficient stability for an *in vivo* proof of concept (Cli <5 mL/min/g, although a candidate would probably require <1 mL/min/g). Interestingly the mouse microsomal stability varied significantly from compound to compound in a way that was not directly linked to lipophilicity. The different diastereoisomers had different levels of microsomal stability.

3-(*tert*-Butyl)cyclopentanamine **56**, required for the synthesis of **51**, was prepared in four steps (Scheme 5). Oxidative cleavage of 4-(*tert*-butyl)cyclohexan-1-ol **53** with sodium nitrite in trifluoroacetic acid by the method of Matsumura et al. gave the diacid **54**.[21] Ketonic decarboxylation with sodium carbonate at 240 °C gave cyclopentanone **55**.[22] Our standard reductive amination/hydrogenolysis procedure then gave **56** as a racemic mixture of all diastereomers.

In order to reduce the lipophilicity, with the aim of increasing the solubility and in the cases of metabolic stability of the cyclohexyl moiety, heteroatoms were introduced into the cycloalkyl ring (Table 4). In summary, all such modifications resulted in less activity or a loss of activity, similar to the findings for polar substituents reported in Table 1, and also a loss in selectivity.

Replacement of the *tert*-butyl with trifluoromethyl (**57**, **58**) led to a loss in activity. Even replacement of one of the methyls of the *tert*-butyl with a fluoro (**59**) led to a 100-fold loss in activity. Replacing one of the methyls with a hydroxyl (**60**) led to a much larger loss in activity. Similarly, adding an ether oxygen between the cyclohexyl and the substituent (**63**, **64**) led to a loss in activity,

We also put two oxygen atoms in the cyclohexyl ring (**61**, **62**), although both lost activity; the *trans*-isomer was 10-fold more active than the *cis*-isomer. This compound (**61**) also had improved solubility and LLE. Putting just one oxygen led to reduced activity (**65**), albeit the *tert*-butyl substituent was at the 3-position rather than the 4-position, which introduced an additional chiral center. Similarly, nitrogen was not tolerated in the ring (**66**). Various analogues of **66** were made with the aim of reducing the basicity, in case charge was not tolerated at this position: the carbamates (**67**, **71**), the amide (**68**), and the sulphonamide (**69**). All of these lost more than 1000-fold in activity compared to **19**. Finally, when an amine was not in the cyclohexyl ring (**70**), the compound was also inactive.

Synthesis of compounds is as follows. Prins-cyclization of 3-buten-1-ol with pivaldehyde gave tetrahydropyranol **73**, which was oxidized with Dess–Martin Periodinane to the tetrahydropyranone **74**. Our standard methodology then converted this into the racemic *cis* and *trans* amines **75** (Scheme 6), which were separated by chromatography of the benzyl intermediates.

The 2-fluoropropanyl intermediate **80** was obtained via deoxyfluorination of 2-propylalcohol **78** with XtalFluorE followed by hydrogenolysis of the Cbz protecting group (Scheme 7). Compound **78** was prepared, in turn, by the addition of methylmagnesium bromide to ester **77** (Scheme 7).

The reaction of benzyl (1,3-dihydroxypropan-2-yl)-carbamate **81** with pivaldehyde gave the 1,3-dioxane **82** as

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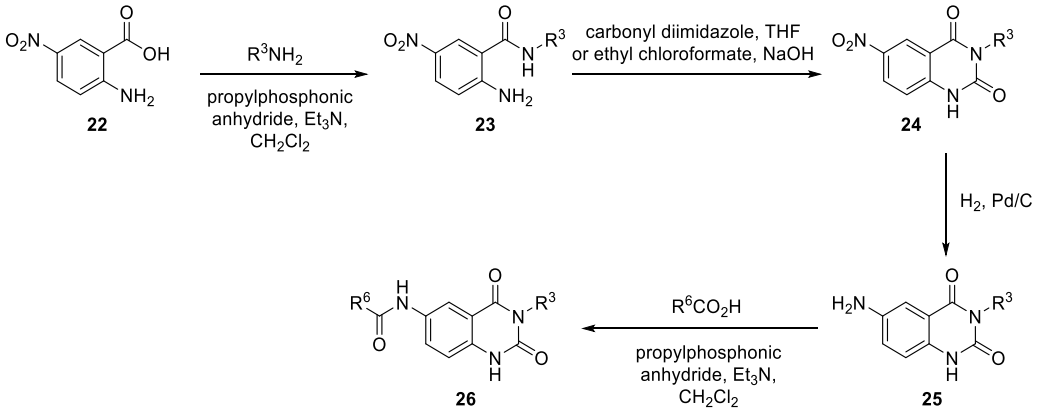
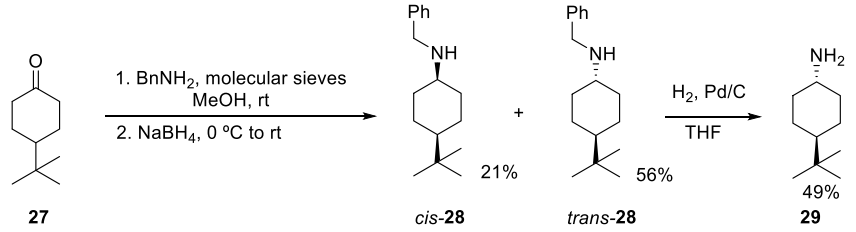
J. Med. Chem. XXXX, XXX, XXX−XXX

Scheme 2. Alternative Quinazolinedione Synthesis



Scheme 3. General Procedure C for the Synthesis of 4-Substituted Cyclohexylamines



a mixture of *cis* and *trans* diastereomers. Hydrogenolysis of the Cbz protecting group gave the amines **83**, which were separated by chromatography (Scheme 8).

Alcohol **84** was alkylated by heating with Boc anhydride in the presence of catalytic zinc perchlorate hexahydrate according to the method of Bartoli et al.[23] to give *tert*-butyl ether **85** (Scheme 9). The isopropyl ether **87** was obtained from the same alcohol by reaction with isopropyltrichloroacetimidate and catalytic trifluoroacetic acid (Scheme 10).

When there was an amine at the 6-position instead of an acetamide, the compounds were also active (**30**, Table 2). Therefore crossover compounds were made with the **19** R^1 substituents (Table 5). These compounds were also very potent and had activities similar to the acetamide derivatives (**89** and **19**; **90** and **42**; **91** and **43**; **92** and **45**; **93** and **46**). Where tested, these compounds appeared to show a small increase in solubility (compare **45** with **92** and **46** with **93**). They also had good LLE values.

It was also discovered that the pyrido version of the compound, with the pyridine in the 8-position, was also tolerated, although there was a slightly different SAR with these compounds (Table 6). The pyridine analogue of **19** was much less active. However, the *cis*-isomer (**95**) was only slightly less active than **19**. With the *iso*-propyl analogues (**96**, **97**), the *trans*-isomer was more active than the *cis*-isomer. These compounds, in general, retained the LLE. However, despite the extra heteroatom, these compounds had poorer solubility.

Examples were also made in which the pyrido-analogues were prepared with the amino group on the 6-position, rather than the amide (Table 7). These compounds were also active, but in general, slightly less active than **19**. Solubility was, in general, poor.

The pyrido-analogues were prepared by a synthetic sequence starting from methyl 2-aminonicotinate **101** (Scheme 11). Thus, nitration with gave methyl 2-amino-5-nitronicotinate **102**. Ester hydrolysis and coupling with the appropriate cyclohexyl amines gave amides **103**, which were cyclized with carbonyl diimidazole to give pyrido[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-diones **104**. Hydrogenation of the nitro group gave anilines **105**, which were acylated with acetic anhydride to give the target compounds **106**.

Pharmacokinetics and Efficacy Studies. Two compounds were selected for a more detailed evaluation to establish the proof of concept in a rodent model of Chagas disease, **43** (Table 3) and **89** (Table 5). Compound **19** had a very similar profile to compound **43**, but the latter has a slightly lower lipophilicity. These compounds had the following profiles shown in Table 8.

Compound **43** was dosed intraperitoneally (IP) and orally to female NMRI mice at 10 mg/kg (n = 3 mice/dose route). The dose formulation chosen was 10% DMSO; 60% PEG400; 30% distilled water (to maintain the dose as a solution; aqueous solubility is poor). Blood concentration−time profiles following IP and oral dosing are shown in Figures 3 and 4, respectively. Higher exposure is seen when the compound was dosed IP, compared to oral dosing. Interestingly when dosed IP, the compound took longer to reach the maximum concentration compared to when dosed orally. This may be due to a depot effect, due to a relatively slow dissolution.

Compound **43** has an EC_{90} of 38 ng/mL. Considering the fraction unbound in mouse plasma (Fu = 0.028), 30 mg/kg IP once a day is predicted to maintain C_{trough} free concentration at approximately EC_{90}. A twice-daily dose at 30 mg/kg orally is predicted to deliver C_{trough} free concentration at approximately EC_{50}. These doses were used for 10 days in the mouse model of acute Chagas disease as the best options, considering all of the above-mentioned constraints to maximize the probability of success.

Compound **89** demonstrated improved solubility and could be formulated in 10% DMSO; 60% PEG400; 30% distilled

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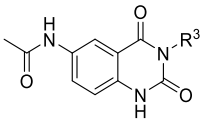
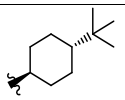
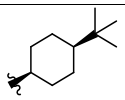
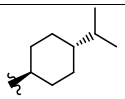
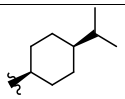
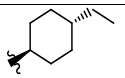
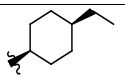
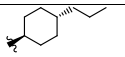
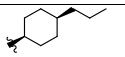
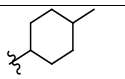
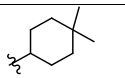
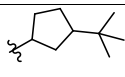
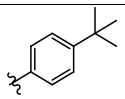
J. Med. Chem. XXXX, XXX, XXX−XXX

Table 3. SAR at the R^3 Position Based on 19[a]



		Tc μM	L6 μM (selectivity)	clogP	LLE	Aq. Solubility μM	Mouse Cli mL/min/g
19		0.022±0.004	3.9±0.04 (180)	3.3	4.4	131	3.2
42		0.017±0.006	5.0± 0.3(290)	3.3	4.5	95	<0.5
43		0.026±0.002	3.8±0.3 (150)	2.9	4.7	73	3.2
44		0.20±0.08	41±0.24 (210)	2.9	3.8	nd	26
45		0.058±0.026	14±0.12 (240)	2.7	4.5	115	2.4
46		0.085±0.029	39±3 (460)	2.7	4.4	73	12
47		0.015±0.007	11±1.8 (730)	3.2	4.6	47	1.5
48		0.020±0.007	14±1.8 (700)	3.2	4.5	102	19
49	 Mixture of diastereomers	1.4±0.37	54±6.2 (39)	2.3	3.6	nd	Nd
50		0.18±0.001	12±0.7 (67)	2.8	3.9	128	3.7
51	 Racemic mixture of diastereomers	0.020±0.003	41±2 (2,100)	3.0	4.7	nd	31
52		4.3±1.1	17±5 (4.0)	2.9	2.5	nd	nd

[a]The EC_{50} values are means of two independent determinations ± deviation from the mean. Control compounds: benznidazole for the *T. cruzi* assay EC_{50} ± SD 1.94 ± 0.49 μM, podophyllotoxin for the cytotoxicity assay EC_{50} ± SD 0.014 ± 0.005 μM. clogP was calculated using StarDrop (www.optibrium.com)

water or as a fine suspension in 0.5% w/v HPMC with 0.4% v/v Tween 80 and 0.5% v/v benzyl alcohol (when nanomilled). Intraperitoneal pharmacokinetics were assessed in mice using both formulations to optimize exposure for the efficacy study (Figure 5 and Figure 6). Oral pharmacokinetics were also assessed (Figure 7) but offered no improvement in the exposure. When dosed orally, the compound took a long time to reach the maximum concentration. The slow rate of absorption could be due to slow solubilization in the gastrointestinal tract

The EC_{90} for compound **89** is high at 1200 ng/mL, and the compound has a low fraction unbound in mouse plasma (Fu = 0.011). It was, therefore, decided to progress this compound to the mouse model of acute Chagas disease at the maximum tolerated dose (MTD), which was 30 mg/kg intraperitoneally once daily using the DMSO/PEG400/water formulation. Additionally, it was also decided to explore a 50 mg/kg twice-daily oral regimen in the better-tolerated vehicle (0.5% HPMC with 0.4% v/v Tween 80 and 0.5% v/v benzyl alcohol), which would offer an improved duration of exposure. This would provide the best opportunity for proof of concept, although acknowledging likelihood was low because of the low free exposure versus potency both ip and orally.

Efficacy Studies. A murine model for acute Chagas disease was used. In brief, groups of 5 Balb/c female mice were infected with 10^5 trypomastigote forms of *T. cruzi* Brazil strain expressing luciferase, and the infection was quantified 3 days later by an imaging device sensitive to luminescence (IVIS Lumina imager). The signal is proportional to the load of parasites. The treatment began on the fourth day of infection. The results were expressed as the ratio of infection at the end of treatment versus the base infection before treatment for each animal.

In vivo efficacy studies were carried out with both **89** and **43** in this mouse model of acute Chagas disease at the dose levels defined above with the aim to determine *in vivo* proof of concept for this series of chemistry. Dosing was continued for 10 days. Animals were imaged at 5 days and 10 days.

Compound **43** shows a significant reduction in parasite levels compared to the vehicle control when dosed both IP (30 mg/kg qd) or PO (30 mg/kg bid) for 5 or 10 days (Figure 8). Compound **89** showed no significant reduction in parasite levels at these doses, likely due to the fact that it did not maintain C_{trough} levels above the EC_{50}, via either dosing regimen.

CONCLUSION

An *in vivo* proof of concept has been achieved with **43** demonstrating efficacy orally in a mouse model of infection. However, the compounds need further optimization in terms of both solubility and selectivity, for progression, as dosing was limited by solubility, and the efficacious dose was close to the maximum tolerated dose (MTD). Mode of action studies, published separately, have revealed that the target of compound **43** is cytochrome *b*.[27]

Furthermore, in light of more recent advances in the development of better translational mouse models of Chagas disease, it will be important to investigate future analogues of these compounds in these improved models, which can differentiate between benznidazole-like and posaconazole-like compounds.[24] Subsequently to this work being carried out, new *in vitro* assays have been introduced to help select compounds for further development.[25] These include the rate of kill assays, activity against a range of different strains, and studies in washout experiments.

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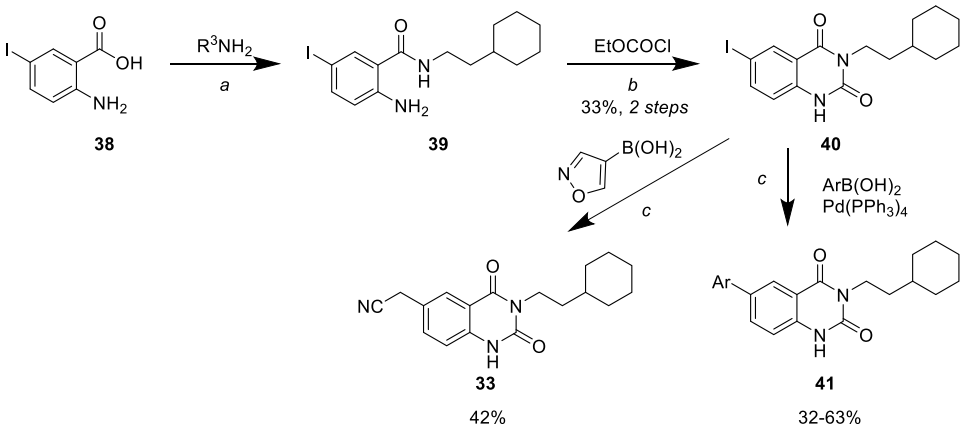
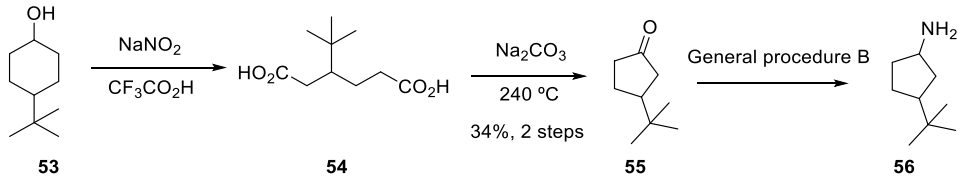
J. Med. Chem. XXXX, XXX, XXX−XXX

Scheme 4. Aryl Substituent at R[6][a]



[a]Reagents and conditions: (a) cyclohexylethylamine, HATU, NMM, DMF, rt, 16 h; (b) ethyl chloroformate, 90 °C, 1.5 h, then KOH, EtOH, 85 °C, 3.5 h; (c) $ArB(OH)_2$, $Pd(PPh_3)_4$, Na_2CO_3 or Cs_2CO_3, water, dioxane, 90 °C, 16 h.

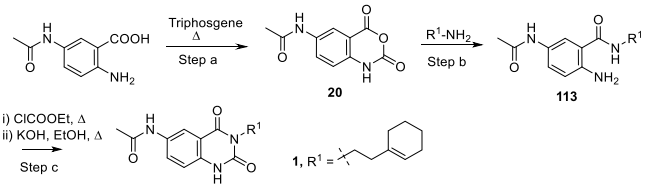
Scheme 5. Synthesis of 3-(*tert*-Butyl)cyclopentanamine



EXPERIMENTAL SECTION

Chemistry. *General Chemistry Methods.* Chemicals and solvents were purchased from commercial vendors and were used as received, unless otherwise stated. Dry solvents were purchased in Sure Seal bottles stored over molecular sieves. Analytical thin-layer chromatography (TLC) was performed on precoated TLC plates (Kieselgel 60 F254, BDH). Developed plates were air-dried and analyzed under a UV lamp (UV 254/365 nm), and/or $KMnO_4$ was used for visualization. Flash chromatography was performed using Combiflash Companion or Combiflash Rf machines (Teledyne ISCO), and prepacked silica gel columns were purchased from Grace Davison Discovery Science or SiliCycle. Mass-directed preparative HPLC separations were performed using a Waters HPLC (2545 binary gradient pumps, 515 HPLC makeup pump, 2767 sample manager) connected to a Waters 2998 photodiode array and a Waters 3100 mass detector. HPLC chromatographic separations were conducted using Waters XBridge C18 columns, 19 mm × 100 mm, 5 μm particle size, using 0.1% ammonia in water (solvent A) and acetonitrile (solvent B) as a mobile phase. Chiral HPLC separations were performed using CHIRAL Phenomenox Lux Cellulose-4 (250 × 4.6) mm and CHIRAL PAK IC (250 × 4.6) mm, 5 μm particle size columns. ^{1}H NMR spectra were recorded on a Bruker Advance II 500 or 400 spectrometer operating at 500 and 400 MHz using $CDCl_3$, methanol-d_4, or DMSO-d_6 solutions. Chemical shifts (δ) are expressed in ppm recorded using the residual solvent as the internal reference in all cases. Signal splitting patterns are described as singlet (s), doublet (d), triplet (t), multiplet (m), broadened (b), or a combination thereof. Coupling constants (J) are quoted to the nearest 0.1 Hz (hertz). High-resolution mass spectroscopy (HRMS) was performed using a Bruker Daltonics MicrOTOF mass spectrometer. LC−MS analysis and chromatographic separation were conducted with a Bruker Daltonics MicrOTOF mass spectrometer or an Agilent Technologies 1200 series HPLC connected to an Agilent Technologies 6130 quadrupole LC/MS, where both instruments were connected to an Agilent diode array detector. All assay compounds had a measured purity of ≥95% as determined using these analytical LC−MS systems. The column used was a Waters XBridge column (50 mm × 2.1 mm, 3.5 μm particle size), and the compounds were eluted with a gradient of 5−95% acetonitrile/water + 0.1% ammonia.

General Procedure A for the Synthesis of 5-Acetamide-R^1-Substituted Quinazolinediones Using Ethyl Chloroformate.



To exemplify General Procedure A the preparation of compound **1** is described: *N*-[3-(2-cyclohex-1-enyl-ethyl)-2,4-dioxo-1,2,3,4-tetrahydro-quinazolin-6-yl]-acetamide (**1**).

*Step a: N-(2,4-Dioxo-1,4-dihydro-2H-benzo[d][1,3]oxazin-6-yl)-acetamide (**20**).* Triphosgene (3.8 g, 1.28 mmol) was added to a solution of 5-acetamido-2-amino-benzoic-acid (5 g, 25.7 mmol) in 1,4-dioxane (100 mL), and the solution was heated at 110 °C for 6 h. The solution was then cooled to room temperature; then, a saturated aqueous solution of $NaHCO_3$ (20 mL) was added. The mixture was filtered, and the filtered solid was washed with water, followed by hexanes. The solid was dried at 60 °C under a vacuum to afford the title compound as a solid (5 g, 89%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.66 (s, 1H), 10.17 (s, 1H), 8.24 (d, J = 2.4 Hz, 1H), 7.80 (dd, J = 8.8, 2.4 Hz, 1H), 7.10 (d, J = 8.8 Hz, 1H), 2.04 (3H, s). MS: m/z 221 $[M + 1]^+$.

*Step b: 5-Acetamido-2-amino-N-(2-(cyclohex-1-en-1-yl)ethyl)-benzamide (**113**).* DMAP (0.5 mmol) was added to a solution of cyclohexenyl ethylamine (0.087 g, 0.7 mmol), and isatoic anhydride **20** (0.1 g, 0.45 mmol) was dissolved in DMF (10 mL). The solution was stirred at room temperature for 3 h. After removal of the solvent under reduced pressure, water was added to the crude and extracted with EtOAc (3 × 10 mL). The combined organics were dried (Na_2SO_4), filtered, and concentrated. The crude material was taken to the next step without further purification.

*Step c: N-[3-(2-Cyclohex-1-enyl-ethyl)-2,4-dioxo-1,2,3,4-tetrahydro-quinazolin-6-yl]-acetamide (**1**).* The crudeamide from step b (3.1 mmol) and ethyl chloroformate (4 mL) was heated at 90 °C for 1.5 h. The solvent was removed under reduced pressure, and the

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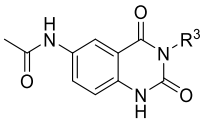
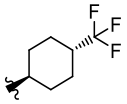
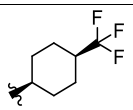
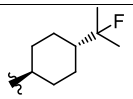
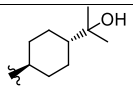
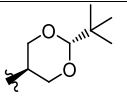
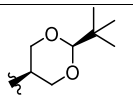
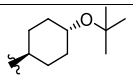
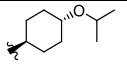
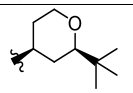
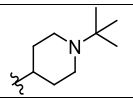
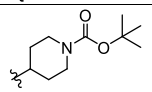
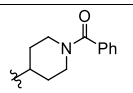
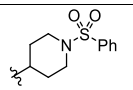
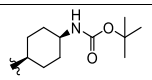
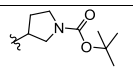
J. Med. Chem. XXXX, XXX, XXX−XXX

Table 4. Introduction of Heteroatoms into R^3[a]

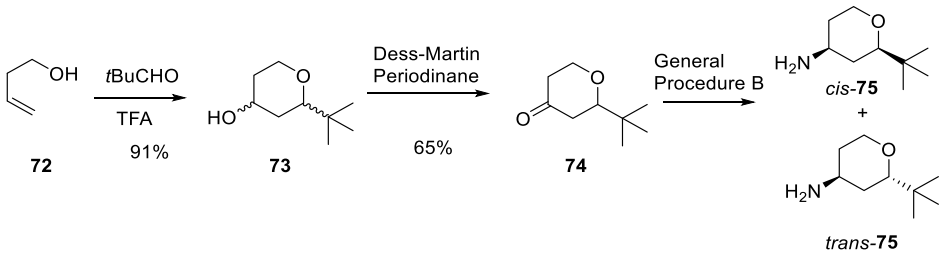


		Tc μM	L6 μM (selectivity)	clogP	LLE	Aq. Solubility μM	Mouse Cli mL/min/g
57		0.35±0.1	9.5±0.74 (27)	2.8	3.7	180	<0.5
58		4.3±0.7	18±2.5 (4.2)	2.8	2.6	nd	2.7
59		2.6±0.05	17±2.1 (6.5)	2.8	2.8	130	Nd
60		139±4	136±13 (0.98)	2.3	1.6	Nd	nd
61		0.58±0.06	27±4.8 (47)	1.4	4.8	>270	<0.5
62		6.4±0.58	183±34 (29)	1.4	3.8	nd	7.2
63		3.5±1.6	123±21 (35)	2.7	2.8	nd	<0.5

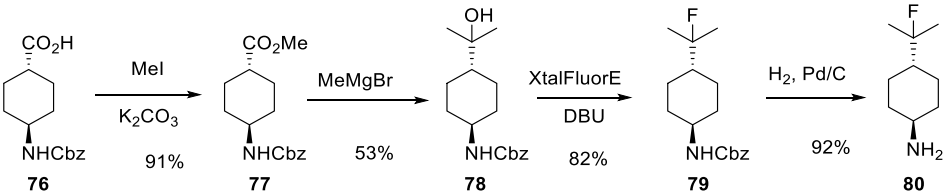
		Tc μM	L6 μM (selectivity)	clogP	LLE	Aq. Solubility μM	Mouse Cli mL/min/g
64		3.1±0.04	136±18 (44)	2.5	3.0	nd	<0.5
65		2.1±0.12	125±4.4 (60)	2.3	3.4	nd	16
66		220±13	>280 (>1)	1.8	1.9	nd	nd
67		30±3	127±3.1 (4.2)	2.0	2.5	nd	nd
68		140±2	135±0.4 (~1)	1.5	2.4	nd	nd
69		52±12	90±9.7 (17)	1.7	2.6	nd	nd
70		2.6±0.1	118±6.7 (45)	2.5	3.1	nd	nd
71	 racemic	54±0.7	170±0.1 (3.1)	3.0	1.3	nd	nd

[a]The EC_{50} values are means of two independent determinations ± deviation from the mean. Control compounds: benznidazole for the *T. cruzi* assay EC_{50} ± SD 1.94 ± 0.49 μM, podophyllotoxin for the cytotoxicity assay EC_{50} ± SD 0.014 ± 0.005 μM. clogP was calculated using StarDrop (www.optibrium.com)

Scheme 6. Synthesis of 2-(*tert*-Butyl)tetrahydro-2*H*-pyran-4-amines



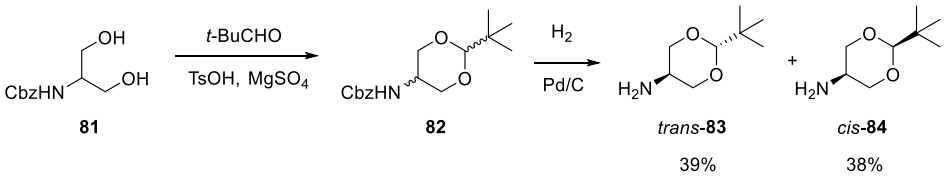
Scheme 7. Synthesis of (1*R*,4*R*)-4-(2-Fluoropropan-2-yl)cyclohexanamine



crude was dissolved in EtOH (40 mL), followed by the addition of KOH (0.35 g, 6.3 mmol). The mixture was heated at 85 °C for 2 h. The solvent was removed under reduced pressure, followed by the addition of water (10 mL) and extraction with EtOAc (3 × 20 mL). The combined organics were washed with a 10% aqueous solution of acetic acid until pH 6. This was followed by extraction with EtOAc (3 × 20 mL); the combined organics were dried over sodium sulfate, and the solvent was concentrated. The crude was purified by flash column chromatography on silica gel eluted with MeOH in $CHCl_3$, which gave the title compound (0.045 g, 30%)

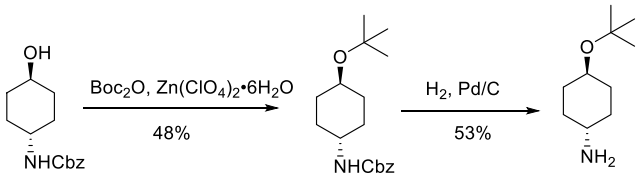
https://dx.doi.org/10.1021/acs.jmedchem.9b01852
J. Med. Chem. XXXX, XXX, XXX−XXX

Scheme 8. Synthesis of 2-(*tert*-Butyl)-1,3-dioxan-5-amines



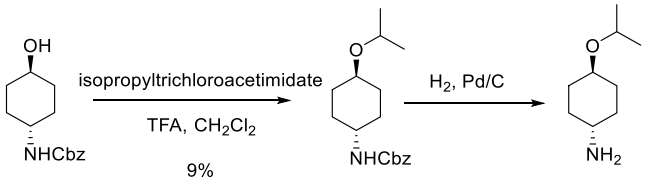
81 82 *trans*-83 39% *cis*-84 38%

Scheme 9. Synthesis of (1*R*,4*R*)-4-(*tert*-Butoxy)cyclohexanamine



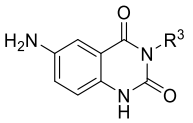
84 85 86

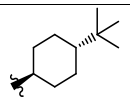
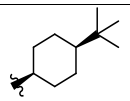
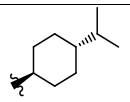
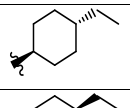
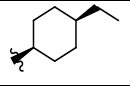
Scheme 10. Synthesis of (1*R*,4*R*)-4-Isopropoxycyclohexanamine



84 87 88

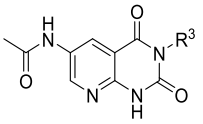
Table 5. Key R^3 Substituents with $R^6 = NH_2$[a]

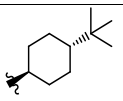
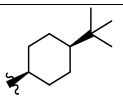
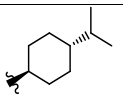
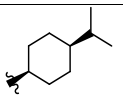
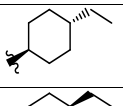
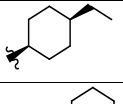
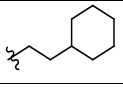


		Tc μM	L6 μM (selectivity)	clogP	LLE	Aq. Solubility μM	Mouse Cli mL/min/g
89		0.063±0.02	21±0.7 (330)	3.4	3.8	240	2.0
90		0.022±0.01	4.1± 1.9(1,900)	3.4	4.3	Nd	3.9
91		0.027±0.009	22±0.13 (810)	3.0	4.6	nd	nd
92		0.12±0.03	32±12 (270)	2.8	4.1	>350	4.2
93		0.33±0.06	52±16 (160)	2.8	3.7	310	5.2

[a]The EC_{50} values are means of two independent determinations ± deviation from the mean. Control compounds: benznidazole for the *T. cruzi* assay EC_{50} ± SD 1.94 ± 0.49 μM, podophyllotoxin for the cytotoxicity assay EC_{50} ± SD 0.014 ± 0.005 μM. clogP was calculated using StarDrop (www.optibrium.com)

Table 6. Key R^3 Substituent Pyrido System with R^5 = NHAc[a]

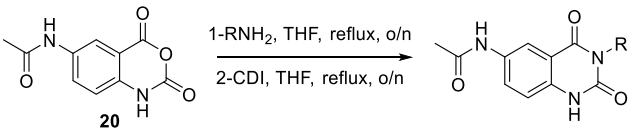


		Tc μM	L6 μM (selectivity)	clogP	LLE	Aq. Solubility μM	Mouse Cli mL/min/g
94		0.84±0.13	67±4.6 (80)	2.5	3.6	<28	1.4
95		0.10±0.05	15±0.1 (150)	2.5	4.5	nd	15.0
96		0.061±0.022	32±3 (520)	2.1	5.1	15	1.4
97		0.38±0.021	16 ±0.6 (42)	2.1	4.3	22	10.0
98		0.30±0.03	215±0.7 (720)	1.9	4.6	48	0.5
99		0.33±0.005	48±2.9 (150)	1.9	4.6	97	2.7
100		2.3±0.35	130±2.7 (57)	2.1	3.5	nd	nd

[a]The EC_{50} values are means of two independent determinations ± deviation from the mean. Control compounds: benznidazole for the *T. cruzi* assay EC_{50} ± SD 1.94 ± 0.49 μM, podophyllotoxin for the cytotoxicity assay EC_{50} ± SD 0.014 ± 0.005 μM. clogP was calculated using StarDrop (www.optibrium.com)

over two steps. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.33 (s, 1H), 10.09 (s, 1H), 8.21 (d, *J* = 2.4 Hz, 1H), 7.78 (dd, *J* = 8.8 Hz, 2.4 Hz, 1H), 7.11 (d, *J* = 8.8 Hz, 1H), 5.29 (m, 1H), 3.97−3.93 (m, 2H), 1.19−2.14 (m, 2H), 2.03 (s, 3H), 1.98 (m, 2H), 1.85 (m, 2H), 1.58−1.52 (m, 4H). MS: *m*/*z* 326 $[M - 1]^-$.

General Procedure B for the Synthesis of 5-Acetamide-R^1-Substituted Quinazolinediones Using Carbonyldiimidazole.

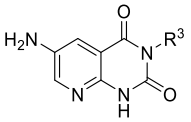


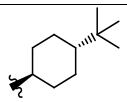
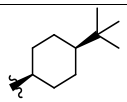
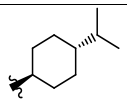
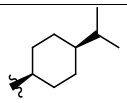
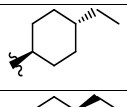
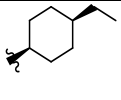
20

N-(2,4-Dioxo-2,4-dihydro-1*H*-benzo[*d*][1,3]oxazin-6-yl)acetamide **20** (150 mg, 0.682 mmol) was combined with the relevant amine (0.675 mmol, 0.99 equiv) in THF (5 mL) and heated under reflux overnight. Upon cooling, carbonyldiimidazole (162.2 mg, 1.02 mmol, 1.5 equiv) was added, and the mixture was heated under

https://dx.doi.org/10.1021/acs.jmedchem.9b01852
J. Med. Chem. XXXX, XXX, XXX−XXX

Table 7. Key R^3 Substituent Pyrido System with R^6 = NH_2[a]

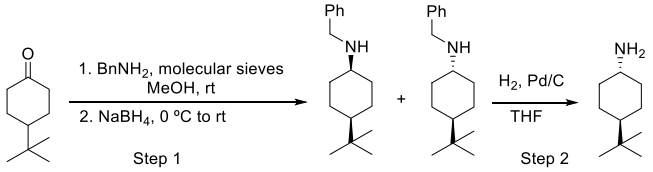


		Tc μM	L6 μM	clogP	LLE	Aq. Solubility μM	Mouse Cli mL/min/g
107		0.66±0.19	63±0.2 (95)	2.6	3.6	16	0.5
108		0.24±0.01	44±3.4 (183)	2.6	4.0	Nd	1.6
109		0.12±0.025	46±0.2 (380)	2.2	4.7	25	1.9
110		0.57±0.08	46±1.8 (81)	2.2	4.0	17	4.8
111		0.34±0.01	51±2.3 (150)	1.9	4.6	55	0.9
112		1.2±0.04	250±45 (210)	1.9	4.0	<17	1.7

[a]The EC_{50} values are means of two independent determinations ± deviation from the mean. Control compounds benznidazole for the *T. cruzi* assay EC_{50} ± SD 1.94 ± 0.49 μM, podophyllotoxin for the cytotoxicity assay EC_{50} ± SD 0.014 ± 0.005 μM. clogP was calculated using StarDrop (www.optibrium.com)

reflux for 24 h. Upon reaction completion, the reaction mixture was cooled, and the resulting precipitate was collected by filtration, washed with water, CH_2Cl_2, and Et_2O, and dried *in vacuo*. If a precipitate did not form, then the reaction mixture was concentrated *in vacuo*. The resultant residue was diluted with water and extracted with EtOAc. The organic layer was then dried ($MgSO_4$) and concentrated. Purification by flash chromatography gave the desired compound.

General Procedure C for the Synthesis of 4-Substituted Cyclohexylamines.



Step 1: Reductive Amination. A solution of the ketone (32 mmol, 1 equiv) in anhydrous methanol (160 mL) was treated with benzylamine (4.9 mL, 38 mmol, 1.2 equiv) and 4 Å molecular sieves (6 g). The mixture was stirred at room temperature overnight. The mixture was then cooled to 0 °C and treated with sodium borohydride (6.0 g, 160 mmol, 5 equiv) in small portions over 30 min (caution: exothermic, effervescence). The mixture was allowed to warm slowly to room temperature and stirred at room temperature for ca. 4 h. The reaction was quenched by the addition of water and then filtered through a plug of Celite. The filtrate was concentrated, treated with water (150 mL), and extracted with dichloromethane (4 × 150 mL). The combined organic phases were filtered through a hydrophobic frit and concentrated.

The diastereomers were separated by flash chromatography on silica using a gradient elution of 0−10% methanol/ammonia in dichloromethane, with the eluent held at 2% methanol/ammonia until the first diastereomer had eluted. In the cases investigated, the *cis*-product eluted before the *trans*-product.

Step 2: Debenzylation. The reaction vessel containing a solution of the benzylamine (8.5 mmol) in THF (20 mL) was purged with three vacuum/argon cycles. Palladium on carbon (0.41 g) was added, and the vessel was purged three times with vacuum/argon cycles and then three times with vacuum/hydrogen cycles. The mixture was stirred at room temperature for 2 days and then filtered through a plug of Celite. The filter cake was washed with ethyl acetate, and the combined organic phases were concentrated to give the product, which was used without further purification.

General Procedure D: Amidations. 5-Acetamido-2-aminobenzoic acid (100 mg, 0.515 mmol, 1 equiv) and the relevant amine (0.566 mmol, 1.1 equiv) in DCM (5 mL) were combined at room temperature and stirred. A 50% solution of propylphosphonic anhydride in EtOAc (196.6 mg, 0.618 mmol, 1.2 equiv) was added dropwise, and the reaction was stirred at room temperature overnight. The mixture was treated with aq $NaHCO_3$ and vigorously stirred for approximately 30 min. The phases were separated, and the aqueous layer was extracted with CH_2Cl_2 (×2). The combined organic layers were washed with water, dried ($MgSO_4$), and concentrated to give the crude product. Purification was carried out by flash chromatography.

General Procedure E: Preparation of 6-Aminopyrido[2,3-d]-pyrimidine-2,4(1H,3H)-diones. To exemplify General Procedure E, the preparation of compound **107** is described: 6-amino-3-(*transtrans*-4-*tert*-butyl-cyclohexyl)-1*H*-pyrido[2,3-*d*]pyrimidine-2,4-dione (**107**)

*Step a: 2-Amino-5-nitronicotinic Acid Methyl Ester (**102**).* A solution of 2-amino-3-nicotinic acid methyl ester (4 g, 26 mmol) in a mixture of concentrated HNO_3 (2.8 mL) and H_2SO_4 (10 mL) was stirred for 45 min at 0 °C, followed by room temperature for 19 h, and at 70 °C for 4 h. The reaction mixture was cooled to 0 °C, and a saturated aqueous solution of $NaHCO_3$ (40 mL) was added until basic (pH 8). Extraction with EtOAc (3 × 40 mL), filteration, and concentration of the combined organics afforded the title compound (3.5 g, 68%), which was used in the next step without further purification. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.05 (d, J = 2.8 Hz, 1H), 8.69 (d, J = 2.8 Hz, 1H), 8.64 (br s, 1H), 8.15 (br s, 1H), 3.88 (s, 3H). MS: m/z 198 $[M + 1]^+$.

Step b: Lithium Salt of 2-Amino-5-nitronicotinic Acid. LiOH (0.12 g, 5 mmol) was added to a solution of the methyl ester **102** (1 g, 5 mmol) in a mixture of 1% MeOH in THF (10 mL). The solution was stirred at room temperature for 17 h and then concentrated under reduced pressure. The solid obtained (0.8 g) was used in the next step without further purification.

*Step c: 2-Amino-N-(transtrans-4-tert-butyl-cyclohexyl)-5-nitro-nicotinamide (**103**).* BOP (2.7 g, 6 mmol) was added to a suspension of the above lithium salt (0.8 g, 4 mmol), *trans*-4-*tert*-butylcyclohexylamine hydrochloride salt (1.16 g, 6 mmol), and triethylamine (1.2 g, 12 mmol) in DMF. The suspension was stirred at room temperature for 6 h and then concentrated under reduced pressure. Water (15 mL) was added, and the mixture was extracted with EtOAc (3 × 20 mL). The combined organics were dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The crude obtained was purified by flash column chromatography on silica gel, which gave the title compound (0.8 g, 62%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.57 (s, 1H), 7.93 (d, J = 8 Hz, 1H), 7.38 (d, J = 2.4 Hz, 1H), 5.91 (2H, s), 3.65−3.60 (m, 1H), 1.86−1.84 (m, 2H), 1.77−1.73 (m, 2H), 1.35−1.20 (m, 2), 1.09−0.95 (m, 3H), 0.84 (s, 9H). MS: m/z 321 $[M + 1]^+$.

*Step d: 3-(transtrans-4-tert-Butyl-cyclohexyl)-6-nitro-1H-pyrido-[2,3-d]pyrimidine-2,4-dione (**104**).* 1,1′-Carbonyldiimidazole (1.06 g, 6.5 mmol) was added to a solution of compound **103** (0.7 g, 2.1 mmol) in THF (7 mL), and the solution was heated at 90 °C for 48

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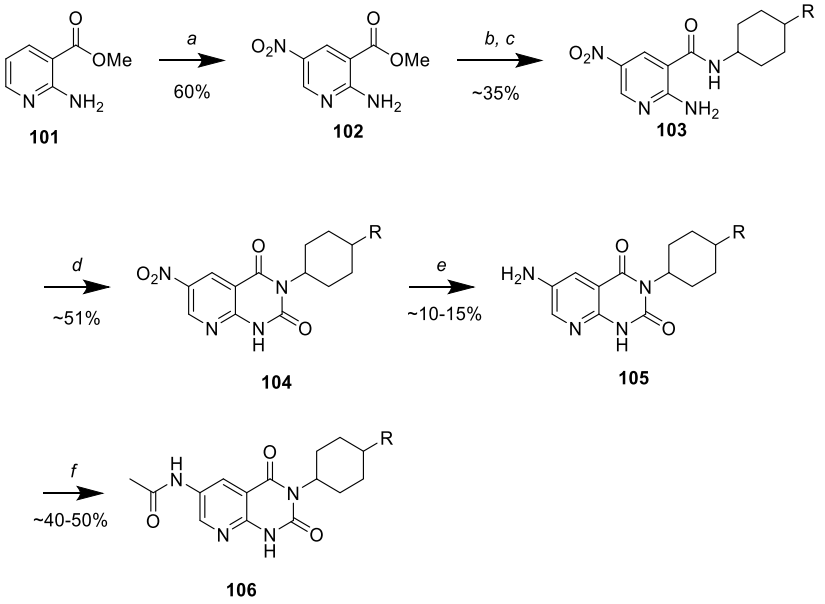
J. Med. Chem. XXXX, XXX, XXX−XXX

Scheme 11. Synthesis of Pyrido-Analogues[a]



[a]Reagents and conditions: (a) concentrated HNO_3, concentrated H_2SO_4, 0 °C to rt, 16 h then 70 °C, 4 h; (b) LiOH, THF, MeOH, water, rt, 17 h; (c) amine, BOP, Et_3N, DMF, rt, 24 h; (d) carbonyl diimidazole, THF, 85 °C, 48 h; (e) Pd/C, H_2, CH_2Cl_2, MeOH, rt, 2 h; (f) Ac_2O, Et_3N, CH_2Cl_2, rt, 3 h.

Table 8. Profiles of Compounds 43 and 89

	43	89
T. cruzi (EC_{50} μM)	0.026	0.063
L6 cells (EC_{50} μM)	3.8	21
kinetic aqueous solubility (μM)	73	240
mouse Cli (mL/min/g)	3.2	2.0
mouse PPB (Fu)	0.028	0.011

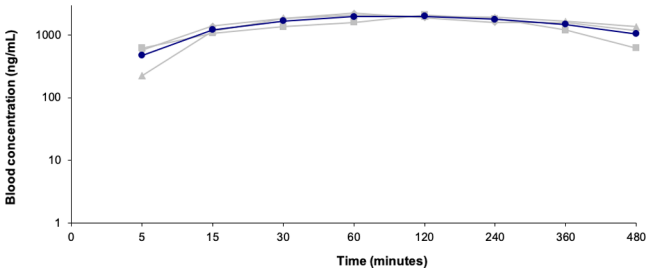


Figure 3. Mean blood concentration−time profile of **43** following single IP administration at 10 mg/kg to the female NMRI mouse. Gray lines are individual animal profiles. The dark blue line is mean data.

h. The solvent was removed under reduced pressure, water (10 mL) was added, and the mixture was extracted with EtOAc (3 × 20 mL). The combined organic phases were concentrated under reduced pressure, and the afforded crude was purified by flash column chromatography on silica gel, which gave the title compound (0.6 g, 82%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.89 (s, 1H), 7.07 (d, J = 2.4 Hz, 1H), 6.89 (d, J = 2.4 Hz, 1H), 4.72−4.65 (m, 1H), 2.50−2.38 (m, 2H), 1.83−1.80 (m, 2H), 1.60−1.57 (m, 2H), 1.08−1.04 (m, 3H), 0.85 (m, 9H). MS: m/z 345 $[M - 1]^-$.

*Step e: 6-Amino-3-(trans-4-tert-butyl-cyclohexyl)-1H-pyrido[2,3-d]pyrimidine-2,4-dione (**107**).* A solution of the nitro compound **104** (0.6 g, 1.7 mmol) in 30% CH_2Cl_2 in MeOH (9 mL) was purged with N_2 (g), followed by the addition of 10% Pd in C (0.12 g). The reaction was hydrogenated at atmospheric pressure for 3 h and then filtered through a Celite bed, and the filtrate was

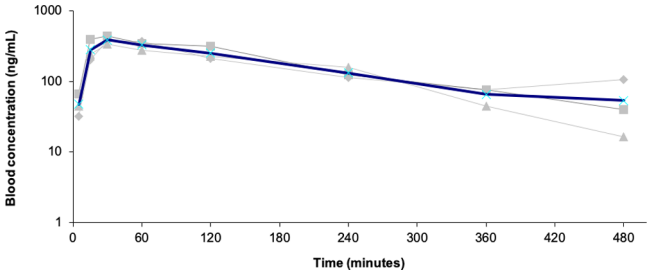


Figure 4. Mean blood concentration−time profile of **43** following single oral administration at 10 mg/kg to the female NMRI mouse. Gray lines are individual animal profiles. The dark blue line is mean data.

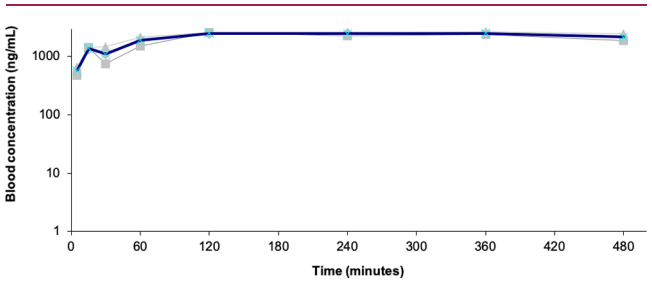


Figure 5. Mean blood concentration−time profile of **89** following single intraperitoneal administration at 30 mg/kg to the female NMRI mouse. Gray lines are individual animal profiles. The dark blue line is mean data. Dose Formulation: 10% DMSO; 60% PEG400; 30% Milli-Q water.

concentrated under reduced pressure. The crude was purified by flash column chromatography on silica gel, which gave the title compound (0.11 g, 21%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.36 (s, 1H), 8.00 (s, 1H), 7.44 (s, 1H), 5.36 (s, 2H), 4.68−4.62 (m, 1H), 2.42−2.32 (m, 2H), 1.83−1.81 (m, 2H), 1.62−1.59 (m, 2H), 1.10−1.04 (m, 3H), 0.86 (s, 9H). MS: m/z 317 $[M + 1]^+$

General Procedure F: Acetylation of 6-Aminopyrido[2,3-d]-pyrimidine-2,4(1H,3H)-dione. To exemplify General Procedure F,

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J. Med. Chem. XXXX, XXX, XXX−XXX

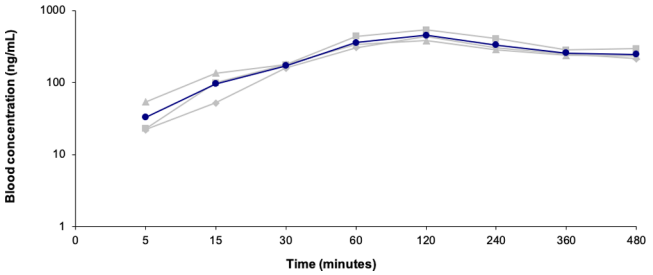

Figure 6. Mean blood concentration−time profile of **89** following single intraperitoneal administration at 10 mg/kg to the female NMRI mouse. Gray lines are individual animal profiles. The dark blue line is mean data. Dose formulation: 0.5% w/v HPMC with 0.4% v/v Tween 80 and 0.5% v/v benzyl alcohol.

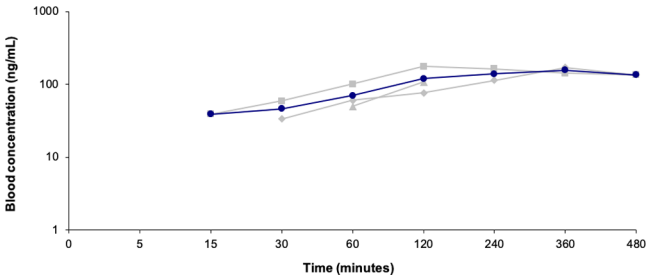

Figure 7. Mean blood concentration−time profile of **89** following single oral administration at 10 mg/kg to the female NMRI mouse. Gray lines are individual animal profiles. The dark blue line is mean data. Dose formulation: 0.5% w/v HPMC with 0.4% v/v Tween 80 and 0.5% v/v benzyl alcohol.

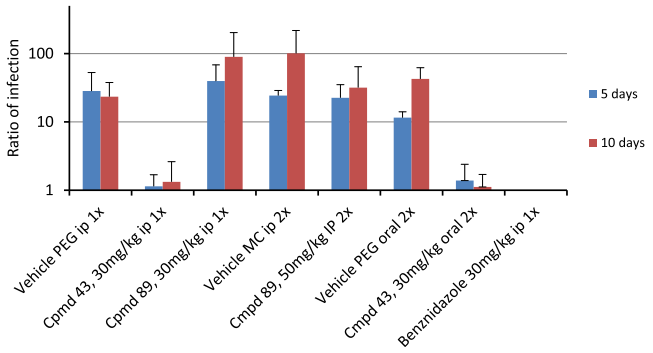

Figure 8. Ratio of parasitemia levels for mice infected with *T. cruzi* after treatment with compounds for 5 days and 10 days compared to starting levels of parasitemia. Data is based on 5 mice in each group, except for vehicles with MC (4 mice) and oral **43** (3 mice). The error bars show the standard deviation. PEG is 10% DMSO; 60% PEG400; 30% water; MC is 0.5% MC, 0.4% Tween 80, and 0.5% benzyl alcohol.

the preparation of compound **94** is described: *N*-[3-(*trans*-4-*tert*-butyl-cyclohexyl)-2,4-dioxo-1,2,3,4-tetrahydro-pyrido[2,3-*d*]-pyrimidin-6-yl]-acetamide (**94**). Triethylamine (0.076 g, 0.75 mmol) was added at 0 °C to a solution of 6-amino-3-(*trans*-4-*tert*-butyl-cyclohexyl)-1*H*-pyrido[2,3-*d*]pyrimidine-2,4-dione **107** (0.08 g, 0.25 mmol) in CH_2Cl_2 (2 mL). The solution was stirred for 5 min; then Ac_2O (0.10 g, 1 mmol) was added, and the stirring was continued at room temperature for 3 h. A saturated aqueous solution of $NaHCO_3$ (15 mL) was added, and the mixture was extracted with EtOAc (3 × 20 mL). The combined organic phases were concentrated, and the afforded crude was purified by flash column chromatography on silica gel, which gave the title compound (0.042 g, 47%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.70 (br s, 1H), 10.29 (s, 1H), 8.68 (d, J = 2 Hz, 1H), 8.53 (d, J = 2 Hz, 1H), 4.69−4.62 (m, 1H), 2.41−2.32 (m, 2H), 2.07 (s, 3H), 1.84−1.81 (m, 2H), 1.65−1.62 (m, 2H), 1.10−1.04 (m, 3H), 0.86 (s, 9H). MS: m/z 359 $[M + 1]^+$.

Synthesis of Compounds. *(R)-N-(3-(1-Cyclohexylethyl)-2,4-dioxo-1,2,3,4-tetrahydroquinazolin-6-yl)acetamide (**3**).* Isatoic anhydride **20** (0.2 g, 0.9 mmol) and (*R*)-1-cyclohexylethan-1-amine (0.11 g, 0.9 mmol) were reacted according to the procedure described in General Procedure A, steps b and c, which gave the title compound (0.1 g, 34%) over two steps. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.50−11.10 (br m, 1H), 10.08 (s, 1H), 8.21 (d, J = 2.4 Hz, 1H), 7.77−7.75 (m, 1H, m), 7.09 (d, J = 8.4 Hz, 1H), 4.75−4.55 (m, 1H), 2.18−2.09 (m, 1H), 2.04 (s, 3H), 1.89−1.86 (m, 1H), 1.73−1.70 (m, 1H), 1.59−1.57 (m, 2H), 1.40−1.34 (m, 4H), 1.35−1.20 (m, 2H), 1.17−1.11 (m, 2H), 0.91−0.88 (m, 2H). MS: m/z 328 $[M - 1]^-$.

*N-[3-(2-Cyclohexylethyl)-2,4-dioxo-1,2,3,4-tetrahydroquinazolin-6-yl]acetamide (**4**).* Isatoic anhydride **20** (1.0 g, 4.5 mmol) and cyclohexyl ethylamine (0.89 g, 7 mmol) were reacted according to the procedure described in General Procedure A, steps b and c, which gave the title compound (0.8 g, 54%) over two steps. ^{1}H NMR (400 MHz DMSO-d_6,): δ 11.35 (s, 1H), 10.09 (s, 1H), 8.21 (d, J = 2 Hz, 1H), 7.78 (dd, J = 8.8, 2 Hz, 1H), 7.11 (d, J = 8.8 Hz, 1H), 3.92−3.88 (m, 2H), 2.04 (s, 3H), 1.76−1.72 (m, 3H), 1.66−1.64 (m, 3H), 1.45−1.42 (m, 2H), 1.33−1.23 (m, 3H), 1.22−1.18 (m, 2H). MS m/z 328 $[M - 1]^-$.

*N-(3-Isopentyl-2,4-dioxo-1,2,3,4-tetrahydroquinazolin-6-yl)-acetamide (**5**).* Isatoic anhydride **20** (0.3 g, 1.3 mmol) and isopentyl amine (0.11 g, 1.3 mmol) were reacted according to the procedure described in General Procedure A steps b and c, which gave the title compound (0.15 g, 38%) over two steps. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.34 (s, 1H), 10.09 (s, 1H), 8.21 (d, J = 2.4 Hz, 1H), 7.77 (dd, J = 8.8, 2.4 Hz, 1H), 7.10 (d, J = 8.8 Hz, 1H), 3.90−3.86 (m, 1H), 2.03 (s, 3H), 1.60−1.53 (m, 1H), 1.45−1.40 (m, 2H), 0.91−0.90 (d, J = 6.4 Hz, 6H). MS m/z 290 $[M + 1]^+$.

*N-[3-(3-Cyclohexyl-propyl)-2,4-dioxo-1,2,3,4-tetrahydro-quinazolin-6-yl]-acetamide (**6**).* Isatoic anhydride **20** (0.1 g, 0.45 mmol) and cyclohexyl ethylamine (0.098 g, 0.7 mmol) were reacted according to the procedure described in General Procedure A, steps b and c, which gave the title compound (0.045 g, 29%) over two steps. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.34 (s, 1H), 10.10 (s, 1H), 8.22 (d, J = 2 Hz, 1H), 7.78 (dd, J = 8.8, 2 Hz, 1H), 7.12 (d, J = 8.8 Hz, 1H), 3.86−3.82 (m, 1H), 2.04 (s, 3H), 1.70−1.5 (m, 7H), 1.22−1.13 (m, 6H), 0.9−0.8 (m, 2H). MS m/z 342 $[M - 1]^-$.

*N-(3-(2-Methoxyethyl)-2,4-dioxo-1,2,3,4-tetrahydroquinazolin-6-yl)acetamide (**7**).* Isatoic anhydride **20** (0.8 g, 3.6 mmol) and 2-methoxyethyl amine (0.41 g, 5.4 mmol) were reacted according to the procedure described in General Procedure A, steps b and c, which gave the title compound (0.28 g, 28%) over two steps. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.35 (s, 1H), 10.09 (s, 1H), 8.22 (d, J = 2.4 Hz, 1H), 7.78 (dd, J = 8.8, 2.4 Hz, 1H), 7.12 (d, J = 8.8 Hz, 1H), 4.07 (t, J = 6 Hz, 2H), 3.51 (t, J = 6 Hz, 2H), 3.24 (s, 3H), 2.04 (s, 3H). MS m/z 278 $[M + 1]^+$.

*2-(6-Acetamido-2,4-dioxo-1,4-dihydroquinazolin-3(2H)-yl)-3-cyclohexylpropanamide (**8**).* Isatoic anhydride **20** (0.1 g, 0.45 mmol) and 2-amino-3-cyclohexylpropanamide (0.13 g, 0.67 mmol) were reacted according to the procedure described in General Procedure A, steps b and c, which gave the title compound (0.025 g, 15%) over two steps. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.33 (br s, 1H), 10.10 (s, 1H), 8.21 (d, J = 2.4 Hz, 1H), 7.78 (dd, J = 8.8, 2.4 Hz, 1H), 7.31 (s, 1H), 7.12 (d, J = 8.8 Hz, 1H), 6.90 (s, 1H), 5.28−5.25 (m, 1H), 2.04 (s, 3H), 2.0−1.80 (m, 3H), 1.62−1.49 (m, 4H), 1.19−0.09 (m, 4H), 0.89−0.79 (m, 2H). MS m/z 371 $[M - 1]^-$.

*N-(3-Benzyl-2,4-dioxo-1,2,3,4-tetrahydroquinazolin-6-yl)-acetamide (**9**).* The compound was prepared following General Procedure B, using *N*-(2,4-dioxo-2,4-dihydro-1*H*-benzo[*d*][1,3]-oxazin-6-yl)acetamide (100 mg, 0.45 mmol), benzylamine (0.050 mL, 0.49 mmol), THF (4 mL), and carbonyldiimidazole (105 mg, 0.65 mmol). Purification by flash chromatography (0−20% MeOH/

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J. Med. Chem. XXXX, XXX, XXX−XXX

CH_2Cl_2) gave the title compound as an off-white powder (78.3 mg, 0.25 mmol, 56%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.49 (s, 1H), 10.12 (s, 1H), 8.26 (d, J = 2.4 Hz, 1H), 7.81 (dd, J = 8.8, 2.4 Hz, 1H), 7.31 (m, 4H), 7.25 (m, 1H), 7.16 (d, J = 8.8 Hz, 1H), 5.09 (s, 2H), 2.05 (s, 3H). LC−MS m/z: 310 $[M + H]^+$, t_R = 3.3 min.

*N-(2,4-Dioxo-3-phenethyl-1,2,3,4-tetrahydroquinazolin-6-yl)-acetamide (**10**).* The compound was prepared following General Procedure B, using 2-phenylethylamine (85 μL). Purification by flash chromatography (0−5% MeOH/CH_2Cl_2 + 0.1% NH_3) gave the title compound as an off-white powder (90 mg, 0.278 mmol, 41%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.39 (s, 1H), 10.11 (s, 1H), 8.24 (d, J = 2.3 Hz, 1H), 7.80 (dd, J = 8.8, 2.4 Hz, 1H), 7.32−7.20 (m, 5H), 7.13 (d, J = 8.8 Hz, 1H), 4.12−4.09 (m, 2H), 2.89−2.86 (m, 2H), 2.15 (s, 3H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 168.2, 161.6, 149.7, 138.6, 134.9, 134.3, 128.5, 128.4, 126.5, 126.3, 114.6, 115.5, 113.7, 41.2, 33.3, 23.8. LC−MS m/z: 324 $[M + H]^+$, t_R = 3.6 min.

*N-(2,4-Dioxo-3-(3-phenylpropyl)-1,2,3,4-tetrahydroquinazolin-6-yl)acetamide (**11**).* The compound was prepared following General Procedure B, using *N*-(2,4-dioxo-2,4-dihydro-1*H*-benzo[*d*]-[1,3]oxazin-6-yl)acetamide (100 mg, 0.45 mmol), benzenepropanamine (0.065 mL, 0.46 mmol), THF (4 mL), and carbonyldiimidazole (105 mg, 0.65 mmol) to give the title compound as a white solid (110.2 mg, 0.33 mmol, 72%), which needed no further purification. ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.35 (s, 1H), 10.10 (s, 1H), 8.23 (d, J = 1.8 Hz, 1H), 7.79 (dd, J = 8.7, 1.9 Hz, 1H), 7.27 (t, J = 7.4 Hz, 2H), 7.23 (d, J = 7.1 Hz, 2H), 7.17 (d, J = 7.5 Hz, 1H), 7.12 (d, J = 8.7 Hz, 1H), 3.93 (t, J = 7.3 Hz, 2H), 2.64 (t, J = 7.6 Hz, 2H), 2.05 (s, 3H), 1.88 (m, 2H). LC−MS m/z: 338 $[M + H]^+$, t_R = 3.8 min.

*N-(3-(Cyclohexylmethyl)-2,4-dioxo-1,2,3,4-tetrahydroquinazolin-6-yl)acetamide (**12**).* The compound was prepared following General Procedure B, using cyclohexylmethylamine (76.41 mg). Purification by flash chromatography (0−100% EtOAc/hexane) gave the title compound as a pale pink off-white powder (167 mg, 0.530 mmol, 78%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.33 (s, 1H), 10.08 (s, 1H), 8.22 (d, J = 2.4 Hz, 1H), 7.79 (dd, J = 8.8, 2.4 Hz, 1H), 7.12 (d, J = 8.8 Hz, 1H), 3.77 (d, J = 7.3 Hz, 2H), 2.05 (s, 3H), 1.76−1.73 (m, 1H), 1.67−1.65 (m, 2H), 1.59−1.56 (m, 3H), 1.19−1.10 (m, 3H), 1.02−0.96 (m, 2H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 168.2, 162.0, 150.1, 134.9, 134.2, 126.5, 116.6, 115.4, 113.6, 45.5, 35.8, 30.2, 25.8, 25.2, 23.8. LC−MS m/z: 316 $[M + H]^+$, 631 $[2M + H]^+$, t_R = 3.8 min.

*N-(3-(tert-Butyl)-2,4-dioxo-1,2,3,4-tetrahydroquinazolin-6-yl)-acetamide (**13**).* The compound was prepared following General Procedure B, using *tert*-butylamine (49.37 mg). Purification by flash chromatography (0−60% EtOAc/hexane) gave the title compound as an off-white powder (36 mg, 0.131 mmol, 19%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.15 (s, 1H), 8.29 (d, J = 2.5 Hz, 1H), 7.82 (dd, J = 8.8, 2.6 Hz, 1H), 7.68 (s, 1H), 7.23 (d, J = 8.8, 1H), 2.06 (s, 3H), 1.47 (s, 9H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 168.2, 159.7, 145.9, 134.6, 128.2, 124.4, 116.4, 112.5, 50.8, 28.3, 23.8. LC−MS m/z: 276 $[M + H]^+$, 551 $[2M + H]^+$, t_R = 4.8 min. HRMS (ES^+): found 276.1335 $[M + H]^+$; $C_{14}H_{18}N_3O_3^+$ $[M + H]^+$, requires 276.1343.

*N-(3-(4-Methylpentyl)-2,4-dioxo-1,2,3,4-tetrahydroquinazolin-6-yl)acetamide (**14**).* The compound was prepared following General Procedure B, using 4-methylpentan-1-amine (69 mg, 0.68 mmol), and gave the title compound as a tan-colored powder (132.3 mg, 0.44 mmol, 64%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.35 (s, 1H), 10.10 (s, 1H), 8.23 (d, J = 2.3 Hz, 1H), 7.79 (dd, J = 8.8, 2.3 Hz, 1H), 7.12 (d, J = 8.8 Hz, 1H), 3.86 (t, J = 7.5 Hz, 2H), 2.05 (s, 3H), 1.54 (m, 3H), 1.18 (m, 2H), 0.86 (d, J = 6.6 Hz, 6H). LC−MS m/z: 304 $[M + H]^+$, t_R = 3.9 min.

*N-(3-Hexyl-2,4-dioxo-1,2,3,4-tetrahydroquinazolin-6-yl)-acetamide (**15**).* The compound was prepared following General Procedure B, using 1-hexylamine (0.090 mL, 0.68 mmol). Purification by flash chromatography (0−10% MeOH/CH_2Cl_2) gave the title compound as a white powder (58.3 mg, 0.21 mmol, 31%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.35 (s, 1H), 10.10 (s, 1H), 8.23 (d, J = 2.4 Hz, 1H), 7.79 (dd, J = 8.8, 2.4 Hz, 1H), 7.12 (d, J = 8.8 Hz, 1H), 3.87 (t, J = 7.5 Hz, 2H), 2.05 (s, 3H), 1.56 (m, 2H), 1.28 (m, 6H), 0.87 (t, J = 7.8 Hz, 3H). LC−MS m/z: 304 $[M + H]^+$, t_R = 3.9 min.

*N-(3-Cyclopentyl)-2,4-dioxo-1,2,3,4-tetrahydroquinazolin-6-yl)-acetamide (**16**).* The compound was prepared following General Procedure B, using cyclopentylamine (57.48 mg). Purification by flash chromatography (0−80% EtOAc/hexane) gave the title compound as a white powder (127 mg, 0.442 mmol, 65%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.28 (s, 1H), 10.09 (s, 1H), 8.22 (d, J = 2.4 Hz, 1H), 7.77 (dd, J = 8.8, 2.4 Hz, 1H), 7.11 (d, J = 8.8, 1H), 5.30 (quintet, J = 8.7 Hz, 1H), 2.10−2.05 (m, 5H), 1.94−1.86 (m, 2H), 1.80−1.73 (m, 2H), 1.60−1.52 (m, 2H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 168.2, 162.0, 149.8, 134.8, 134.1, 126.4, 116.5, 115.2, 114.0, 51.9, 28.0, 25.3, 23.8. LC−MS m/z: 288 $[M + H]^+$, 305 $[M + NH_4]^+$, 575 $[2M + H]^+$, t_R = 4.3 min. HRMS (ES^+): found 288.1337 $[M + H]^+$; $C_{15}H_{18}N_3O_3^+$ $[M + H]^+$, requires 288.1343.

*N-(3-Cyclohexyl-2,4-dioxo-1,2,3,4-tetrahydroquinazolin-6-yl)-acetamide (**17**).* The compound was prepared by following General Procedure B, using cyclohexylamine (66.95 mg). Purification by flash chromatography (0−60% EtOAc/hexane) gave the title compound as a white powder (101 mg, 0.335 mmol, 49%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.25 (s, 1H), 10.09 (s, 1H), 8.19(d, J = 2.4 Hz, 1H), 7.79 (dd, J = 8.8, 2.4 Hz, 1H), 7.09 (d, J = 8.8 Hz, 1H), 4.76−4.71 (m, 1H), 2.43−2.35 (dq, J = 15.8, 3.3 Hz, 2H), 2.04 (s, 3H), 1.80 (d, J = 12.9 Hz, 2H), 1.64 (d, J = 12.6 Hz, 1H), 1.58 (d, J = 10.5 Hz, 2H), 1.35−1.27 (m, 2H), 1.19−1.14 (m, 1H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 168.2, 162.1, 150.0, 135.0, 134.1, 126.4, 116.6, 115.1, 114.1, 52.7, 28.3, 25.9, 25.0, 23.8. LC−MS m/z: 302 $[M + H]^+$, 319 $[M + NH_4]^+$, t_R = 4.5 min. HRMS (ES^+): found 302.1499 $[M + H]^+$; $C_{16}H_{20}N_3O_3^+$ $[M + H]^+$, requires 302.1499.

*N-(3-Cycloheptyl-2,4-dioxo-1,2,3,4-tetrahydroquinazolin-6-yl)-acetamide (**18**).* The compound was prepared by following General Procedure B, using cycloheptylamine (76.41 mg). Purification by flash chromatography (0−60% EtOAc/hexane) gave the title compound as a beige powder (137 mg, 0.434 mmol, 64%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.25 (s, 1H), 10.08 (s, 1H), 8.20(d, J = 2.4 Hz, 1H), 7.78 (dd, J = 8.8, 2.3 Hz, 1H), 7.09 (d, J = 8.8, 1H), 4.89 (bs, 1H), 2.35−2.27(m, 2H), 2.04 (s, 3H), 1.76−1.66 (m, 4H), 1.61−1.54 (m, 4H), 1.52−1.41 (m, 2H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 168.2, 161.7, 149.8, 135.0, 134.1, 126.4, 116.7, 115.1, 54.0, 31.3, 27.5, 25.9, 23.8. LC−MS m/z: 316 $[M + H]^+$, 648 $[M + NH_3]^+$, t_R = 3.8 min.

*N-(3-(trans-4-tert-Butyl)cyclohexyl)-2,4-dioxo-1,2,3,4-tetrahydroquinazolin-6-yl)acetamide (**19**).* The compound was prepared following General Procedure B, using *N*-(2,4-dioxo-2,4-dihydro-1*H*-benzo[*d*][1,3]oxazin-6-yl)acetamide (110 mg, 0.50 mmol), *trans*-4-(*tert*-butyl)cyclohexanamine (78 mg, 0.50 mmol), THF (5 mL), and carbonyldiimidazole (122 mg, 0.75 mmol). Purification by flash chromatography (0−10% MeOH/CH_2Cl_2) gave the title compound as a white powder (60 mg, 0.17 mmol, 33%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.23 (s, 1H), 10.06 (s, 1H), 8.18 (d, J = 2.4 Hz, 1H), 7.79 (dd, J = 8.8, 2.4 Hz, 1H), 7.08 (d, J = 8.8 Hz, 1H), 4.71 (m, 1H), 2.42 (m, 2H), 2.04 (s, 3H), 1.84 (d, J = 10.6 Hz, 1H), 1.64 (d, J = 9.9 Hz, 2H), 1.14−1.04 (m, 3H), 0.88 (s, 9H). LC−MS m/z: 356 $[M + H]^+$, t_R = 4.4 min. *trans*-4-(*tert*-Butyl)-cyclohexanamine was prepared from 4-(*tert*-butyl)cyclohexanone following General Procedure C and used without purification. ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.58 (m, 1H), 1.91 (m, 2H), 1.77 (m, 2H), 1.61 (s, 2H), 1.11−0.94 (m, 5H), 0.86 (s, 9H).

*N-(2,4-Dioxo-1,4-dihydro-2H-benzo[d][1,3]oxazin-6-yl)-acetamide (**20**).* See General Procedure A.

*6-Amino-3-(2-cyclohexylethyl)quinazoline-2,4(1H,3H)-dione (**30**).* 5-Acetylamino-2-amino-*N*-(2-cyclohexyl-ethyl)-benzamide (0.1 g, 0.33 mmol), was taken in ethyl chloroformate (0.4 mL) and heated to 90 °C for 1.5 h. The solvent was removed under reduced pressure, and the crude was dissolved in EtOH (2 mL), followed by

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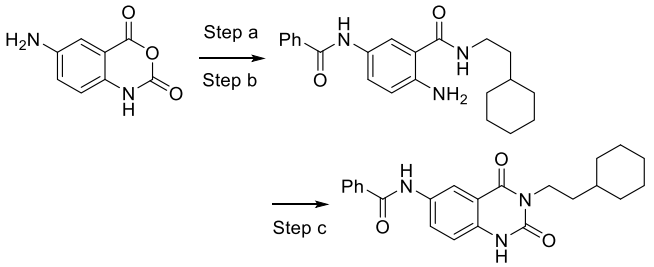
J. Med. Chem. XXXX, XXX, XXX−XXX

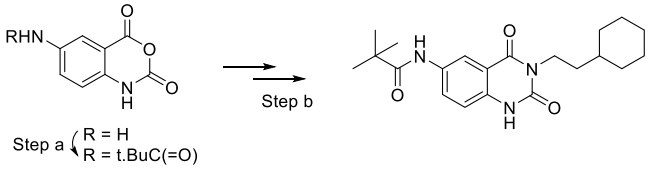
the addition of KOH (0.15 g, 2.6 mmol) and heating at 85 °C for 16 h. The solvent was removed under reduced pressure, followed by the addition of water (10 mL) and extraction with EtOAc (3 × 10 mL). The combined organics were dried (Na_2SO_4), filtered, and concentrated. The crude was purified by flash column chromatography on silica gel, which gave the title compound as a solid (0.035 g, 36%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.98 (s, 1H), 7.08 (d, J = 2 Hz, 1H), 6.95−6.88 (m, 2H), 5.16 (s, 2H), 3.89−3.85 (m, 2H), 1.74−1.58 (m, 7H), 1.44−1.25 (m, 4H), 0.9−0.8 (m, 2H). MS: m/z 288 $[M + 1]^+$.



*N-(3-(2-Cyclohexylethyl)-2,4-dioxo-1,2,3,4-tetrahydroquinazolin-6-yl)benzamide (**31**). Step a: N-(2,4-Dioxo-1,4-dihydro-2HH-benzo[d][1,3]oxazin-6-yl)benzamide.* HATU (0.93 g, 2.44 mmol) was added to a solution at 0 °C of benzoic acid (0.25 g, 2.04 mmol) in DMF (5 mL). The mixture was stirred for 10 min; then, 5-aminoisatoic anhydride (0.64 g, 2.04 mmol) and NMM (0.61 g, 6.12 mmol) were added, and the stirring was continued at room temperature for 16 h. The solvent was removed under reduced pressure, water (10 mL) was added, and the mixture was extracted with EtOAc (3 × 10 mL). The combined organics were dried (Na_2SO_4), filtered, and concentrated. The afforded crude compound was used in the next step without further purification. MS: m/z 283 $[M + 1]^+$.

Step b: 2-Amino-5-benzamido-N-(2-cyclohexylethyl)benzamide. The crude material (0.18 g) from the previous step was added to a solution of cyclohexylethyl amine (0.12 g, 0.9 mmol) and DMAP (16 mg) in DMF (0.8 mL). The reaction mixture was stirred at room temperature for 3 h and then concentrated under reduced pressure. Water (10 mL) was added, and the mixture was extracted with EtOAc (3 × 10 mL). The combined organics were dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The afforded crude compound was taken to the next step without further purification. MS: m/z 366 $[M + 1]^+$.

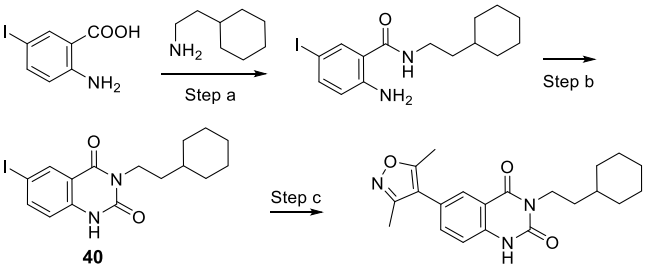
*Step c: N-(3-(2-Cyclohexylethyl)-2,4-dioxo-1,2,3,4-tetrahydroquinazolin-6-yl)benzamide (**31**).* The crude material from the previous step (0.09 g) was taken in ethyl chloroformate (0.35 mL) and heated to 90 °C for 1.5 h. The solvent was removed under reduced pressure, and the crude was dissolved in EtOH (3.36 mL), followed by the addition of KOH (0.06 g, 1.06 mmol) and heating at 85 °C for 5 h. The solvent was removed under reduced pressure, water (5 mL) was added, and the mixture was extracted with EtOAc (3 × 5 mL). The combined organics were dried (Na_2SO_4), filtered, and concentrated. The crude was purified by flash column chromatography on silica gel, which gave the title compound (0.055 g, 57%). MS: m/z 390 $[M - 1]^-$.



*N-(3-(2-Cyclohexylethyl)-2,4-dioxo-1,2,3,4-tetrahydroquinazolin-6-yl)pivalamide (**32**). Step a: N-(2,4-Dioxo-1,4-dihydro-2HH-benzo[d][1,3]oxazin-6-yl)-3,3-dimethylbutanamide.* EDC·HCl (0.8 g, 4.2 mmol) was added to a solution of *tert*-butyl acetic acid (0.97 g, 8.4 mmol) in DMF (11 mL). The mixture was stirred at room temperature for 30 min; then, 5-aminoisatoic anhydride (0.5 g, 2.8 mmol) was added, and the stirring was continued at room temperature. After 16 h, additional *tert*-butyl acetic acid (0.65 g, 5.6 mmol) and EDC × HCl (0.53 g, 2.8 mmol) were added, and the reaction mixture was stirred for an additional 4 h. The solvent was removed under reduced pressure, followed by the addition of water (10 mL) and extraction with EtOAc (3 × 10 mL). The combined organics were dried (Na_2SO_4), filtered, and concentrated. The afforded crude was used in the next step without further purification. MS: m/z 277 $[M + 1]^+$.

*Step b: N-(3-(2-Cyclohexylethyl)-2,4-dioxo-1,2,3,4-tetrahydroquinazolin-6-yl)pivalamide (**32**).* The crude compound from the previous step was reacted with cyclohexylethylamine followed by carbonylation and ring closure according to the method described in General Procedure A, steps b and c, which gave the title compound (0.075 g, 20%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.35 (s, 1H), 9.97 (s, 1H), 8.24 (d, J = 2.4 Hz, 1H), 7.79 (dd, J = 8.8, 2.4 Hz, 1H), 7.11 (d, J = 8.8 Hz, 1H), 3.92−3.88 (m, 2H), 2.18 (s, 2H), 1.80−1.70 (m, 2H), 1.70−1.60 (m, 3H), 1.47−1.43 (m, 2H), 1.25−1.22 (m, 4H), 1.0 (m, 9H), 0.90−0.80 (m, 2H). MS: m/z 385 $[M - 1]^-$.

*2-(3-(2-Cyclohexylethyl)-2,4-dioxo-1,2,3,4-tetrahydroquinazolin-6-yl)acetonitrile (**33**).* $Pd(PPh_3)_4$ (0.06 g, 0.005 mmol) was added under nitrogen to a degassed solution of iodo derivative **40** (0.1 g, 0.25 mmol) in 1,4-dioxane (1 mL), water (0.5 mL), Na_2CO_3 (0.08 g, 0.7 mmol), and isoxazol-4-yl-boronic-acid (0.04 g, 0.4 mmol) in a screw-capped reaction vessel. The vessel was tightly sealed, and the reaction mixture was heated at 90 °C for 16 h. The vessel was opened at room temperature, then water (10 mL) was added, and the mixture was extracted with EtOAc (3 × 10 mL). The combined organics were dried (Na_2SO_4), filtered, and concentrated. The crude was purified by flash column chromatography on silica gel, which gave the title compound (0.03 g, yield 42%) as a solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.46 (s, 1H), 7.91 (s, 1H), 7.60 (m, 1H), 7.18 (d, J = 8.8 Hz, 1H), 4.08 (s, 2H), 3.95−3.88 (m, 2H), 1.75−1.73 (m, 2H), 1.67−1.59 (m, 3H), 1.47−1.44 (m, 2H), 1.27−1.11 (m, 4H), 0.97−0.90 (m, 2H). MS: m/z 310 $[M - 1]^-$.



*3-(2-Cyclohexylethyl)-6-(3,5-dimethylisoxazol-4-yl)quinazoline-2,4(1HH,3HH)-dione (**34**). Step a: 2-Amino-N-(2-cyclohexylethyl)-5-iodobenzamide.* HATU (6.84 g, 18 mmol) was added to a cold (0 °C) solution of 2-amino-5-iodo benzoic acid (4 g, 15 mmol) in DMF (80 mL). The solution was stirred for 5 min; then cyclohexyl ethylamine (1.93 g, 15 mmol) and NMM (4.5 g, 45 mmol) were added. The reaction mixture was stirred at room temperature for 16 h and concentrated under reduced pressure. Water (50 mL) was added, and the mixture was extracted with EtOAc (3 × 50 mL). The combined organics were dried (Na_2SO_4), filtered, and concentrated. The afforded crude was taken to the next step without further purification. MS: m/z 373 $[M + 1]^+$.

*Step b: 3-(2-Cyclohexylethyl)-6-iodoquinazoline-2,4(1HH,3HH)-dione (**40**).* The crude material from the previous step (2 g) was taken in ethyl chloroformate (7.6 mL) and heated to 90 °C for 1.5 h. The solvent was removed under reduced pressure, and the crude was dissolved in EtOH (60 mL), followed by the addition of KOH (0.45 g, 8.06 mmol) and heating at 85 °C for 3 h. The solvent was removed under reduced pressure followed by the addition of water (100 mL). The solution was acidified with glacial acetic acid until pH 7 and extracted with EtOAc (3 × 50 mL). The combined organics were dried (Na_2SO_4), filtered, and concentrated. The obtained solid (2 g, 33% after two steps) was used in the next step. MS: m/z 397 $[M - 1]^-$.

*Step c: 3-(2-Cyclohexylethyl)-6-(3,5-dimethylisoxazol-4-yl)-quinazoline-2,4(1HH,3HH)-dione (**34**).* Water (1 mL), Na_2CO_3

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J. Med. Chem. XXXX, XXX, XXX−XXX

(0.16 g, 1.5 mmol), and 3,5-dimethylisoxazol-4-yl-boronic-acid (0.14 g, 1 mmol) were added to a solution of iodo derivative **40** (0.2 g, 0.50 mmol) in 1,4-dioxane (2 mL). The solution was degasified, and then, $PdP(Ph_3)_4$ (0.12 g, 0.01 mmol) was added under nitrogen. The reaction mixture was heated at 90 °C for 16 h, then water (20 mL) was added, and the mixture was extracted with EtOAc (3 × 20 mL). The combined organic phases were dried (Na_2SO_4), filtered, and concentrated. The crude was purified by flash column chromatography on silica gel, which gave the title compound (0.03 g, yield 32% based on the recovered starting material) as a solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.53 (s, 1H,), 7.85 (d, J = 2 Hz, 1H), 7.68 (dd, J = 8.4, 2 Hz, 1H), 7.27 (d, J = 8.4 Hz, 1H), 3.94−3.90 (m, 2H), 2.49 (s, 3H), 2.39 (s, 3H), 1.80−1.70 (m, 2H), 1.70−1.59 (m, 3H), 1.55−1.40 (m, 2H), 1.29−1.11 (m, 4H), 0.90−0.70 (m, 2H). MS: m/z 366 $[M - 1]^-$.

*3-(2-Cyclohexylethyl)-6-(pyridin-3-yl)quinazoline-2,4(1H,3H)-dione (**35**).* Pyridine-3-boronic acid (0.04 g, 0.37 mmol) was reacted with the iodo derivative **40** (0.1 g, 0.25 mmol) according to the method described above for **34**, step c, but using Cs_2CO_3 instead of Na_2CO_3, which gave the title compound (0.05 g, 57%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.56 (s, 1H), 8.90 (d, J = 2 Hz, 1H), 8.57 (dd, J = 8, 2 Hz, 1H), 8.19 (d, J = 2 Hz, 1H), 8.10 (d, J = 8 Hz, 1H), 8.05 (dd, J = 8.4, 2.4 Hz, 1H), 7.62−7.48 (m, 1H), 7.29 (d, J = 8.4 Hz, 1H), 3.95−3.91 (m, 2H), 1.77−1.74 (m, 2H), 1.67−1.59 (m, 3H), 1.49−1.44 (m, 2H), 1.28−1.22 (m, 1H), 1.19−1.11 (m, 3H), 0.97−0.90 (m, 2H). MS: m/z 348 $[M - 1]^-$.

*3-(2-Cyclohexylethyl)-6-(pyrimidin-5-yl)quinazoline-2,4(1H,3H)-dione (**36**).* Pyrimidine-5-boronic acid (0.045 g, 0.37 mmol) was reacted with the iodo derivative **40** (0.1 g, 0.25 mmol) according to the method described above for **34**, step c, but using Cs_2CO_3 instead of Na_2CO_3, which gave the title compound (0.035 g, 40%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.60 (s, 1H), 9.18 (s, 1H), 9.16 (s, 2H), 8.29 (d, J = 2 Hz, 1H), 8.10 (dd, J = 8.4, 2 Hz, 1H), 7.31 (d, J = 8.4 Hz, 1H), 3.95−3.91 (m, 2H), 1.77−1.73 (m, 2H), 1.65−1.59 (m, 3H), 1.49−1.44 (m, 2H), 1.33−1.11 (m, 4H), 0.97−0.88 (m, 2H). MS: m/z 349 $[M - 1]^-$.

*3-(2-Cyclohexylethyl)-6-(pyridin-4-yl)quinazoline-2,4(1H,3H)-dione (**37**).* Pyridine-4-boronic acid (0.045 g, 0.37 mmol) was reacted with the iodo derivative **40** (0.1 g, 0.25 mmol) according to the method described above for **34**, step c, but using Cs_2CO_3 instead of Na_2CO_3, which gave the title compound (0.055 g, 63%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.61 (s, 1H), 8.64 (d, J = 5.2 Hz, 2H), 8.28 (d, J = 2 Hz, 1H), 8.12 (dd, J = 8.4, 2 Hz, 1H), 7.73 (d, J = 5.2 Hz, 2H), 7.30 (d, J = 8.4 Hz, 1H), 3.95−3.91 (m, 2H), 1.77−1.74 (m, 2H), 1.67−1.59 (m, 3H), 1.49−1.44 (m, 2H), 1.28−1.22 (m, 1H), 1.19−1.11 (m, 3H), 0.97−0.90 (m, 2H). MS: m/z 348 $[M - 1]^-$.

*3-(2-Cyclohexylethyl)-6-iodoquinazoline-2,4(1H,3H)-dione (**40**).* See the synthesis of compound **34**.

*N-(3-(cis-4-(tert-Butyl)cyclohexyl)-2,4-dioxo-1,2,3,4-tetrahydroquinazolin-6-yl)acetamide (**42**).* The compound was prepared following General Procedure B, using *N*-(2,4-dioxo-2,4-dihydro-1*H*-benzo[*d*][1,3]oxazin-6-yl)acetamide (110 mg, 0.50 mmol), *cis*-4-(*tert*-butyl)cyclohexanamine (78 mg, 0.50 mmol), THF (5 mL), and carbonyldiimidazole (122 mg, 0.75 mmol). Purification by flash chromatography (0−10% MeOH/CH_2Cl_2) gave the title compound as a white powder (27.5 mg, 0.077 mmol, 15%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.21 (s, 1H), 10.06 (s, 1H), 8.21 (d, J = 2.4 Hz, 1H), 7.77 (dd, J = 8.8, 2.4 Hz, 1H), 7.09 (d, J = 8.8 Hz, 1H), 4.92 (m, 1H), 2.33 (m, 2H), 2.05 (s, 3H), 1.73 (m, 2H), 1.50 (m, 4H), 1.33 (m, 1H), 0.92 (s, 9H). LC−MS m/z: 356 $[M + H]^+$, t_R = 4.4 min. *cis*-4-(*tert*-Butyl)cyclohexanamine was prepared from 4-(*tert*-butyl)cyclohexanone following procedure C and used without purification. ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.08 (m, 1H), 1.87 (m, 1H), 1.69 (m, 2H), 1.55 (m, 6H), 1.30 (m, 1H), 1.00 (m, 1H), 0.88 (s, 9H).

*N-(3-(transtrans-4-Isopropylcyclohexyl)-2,4-dioxo-1,2,3,4-tetrahydroquinazolin-6-yl)acetamide (**43**).* The compound was prepared following General Procedure B using *N*-(2,4-dioxo-2,4-dihydro-1*H*-benzo[*d*][1,3]oxazin-6-yl)acetamide (1.167 g, 5.30 mmol), *trans*-4-isopropylcyclohexanamine (0.823 g, 5.83 mmol), THF (50 mL), and carbonyldiimidazole (1.3 g, 8 mmol). Purification by flash chromatography (0−10% MeOH/CH_2Cl_2) gave the title compound as a white powder (0.742 g, 2.11 mmol, 40%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.26 (s, 1H), 10.09 (s, 1H), 8.19 (d, J = 2.4 Hz, 1H), 7.79 (dd, J = 8.8, 2.4 Hz, 1H), 7.08 (d, J = 8.8 Hz, 1H), 4.71 (m, 1H), 2.42 (m, 2H), 2.04 (s, 3H), 1.77 (m, 2H), 1.61 (d, J = 11.4 Hz, 2H), 1.46 (m, 1H), 1.08 (m, 4H), 0.89 (d, J = 6.8 Hz, 6H). LC−MS m/z: 344 $[M + H]^+$, t_R = 4.2 min. *trans*-4-Isopropylcyclohexanamine was prepared from 4-isopropylcyclohexanone, following procedure C, and used without purification. ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.82 (br s, 2H), 2.68 (m, 1H), 1.94 (m, 2H), 1.74 (m, 2H), 1.44−1.02 (m, 6H), 0.87 (d, J = 6.8 Hz, 6H).

*N-(3-(cis-4-Isopropylcyclohexyl)-2,4-dioxo-1,2,3,4-tetrahydroquinazolin-6-yl)acetamide (**44**).* The compound was prepared following General Procedure B, using *N*-(2,4-dioxo-2,4-dihydro-1*H*-benzo[*d*][1,3]oxazin-6-yl)acetamide (110 mg, 0.50 mmol), *cis*-4-isopropylcyclohexanamine (71 mg, 0.50 mmol), THF (5 mL), and carbonyldiimidazole (122 mg, 0.75 mmol). Purification by flash chromatography (0−10% MeOH/CH_2Cl_2) gave the title compound as a white powder (47 mg, 0.14 mmol, 27%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.06 (s, 1H), 8.21 (d, J = 2.4 Hz, 1H), 7.76 (dd, J = 8.8, 2.4 Hz, 1H), 7.10 (d, J = 8.8 Hz, 1H), 4.77 (m, 1H), 2.53−2.46 (m, 2H), 2.04 (s, 3H), 1.97 (m, 1H), 1.90 (d, J = 13.6 Hz, 2H), 1.44−1.34 (m, 4H), 1.17 (m, 1H), 0.92 (d, J = 6.5 Hz, 6H). LC−MS m/z: 344 $[M + H]^+$, t_R = 4.3 min. *cis*-4-Isopropylcyclohexanamine was prepared from 4-isopropylcyclohexanone, following procedure C, and used without purification. ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.06 (m, 1H), 1.88 (br s, 2H), 1.57−1.38 (m, 9H), 1.06 (m, 1H), 0.87 (d, J = 6.9 Hz, 6H).

*N-(3-(trans-4-Ethylcyclohexyl)-2,4-dioxo-1,2,3,4-tetrahydroquinazolin-6-yl)acetamide (**45**).* The compound was prepared following General Procedure B, using *N*-(2,4-dioxo-2,4-dihydro-1*H*-benzo[*d*][1,3]oxazin-6-yl)acetamide (220 mg, 1.00 mmol), *trans*-4-ethylcyclohexanamine (140 mg, 1.00 mmol), THF (8 mL), and carbonyldiimidazole (243 mg, 1.50 mmol). Purification by flash chromatography (0−10% MeOH/CH_2Cl_2) gave the title compound as a white powder (51.8 mg, 0.157 mmol, 16%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.26 (s, 1H), 10.09 (s, 1H), 8.19 (d, J = 2.4 Hz, 1H), 7.78 (dd, J = 8.8, 2.4 Hz, 1H), 7.08 (d, J = 8.8 Hz, 1H), 4.73 (m, 1H), 2.42 (qd, J = 12.6 and 3.2 Hz, 2H), 2.04 (s, 3H), 1.83 (d, J = 12.0 Hz, 2H), 1.58 (d, J = 9.6 Hz, 2H) 1.23 (m, 2H), 1.16 (m, 1H), 0.98 (qd, J = 12.8, 3.1 Hz, 2H), 0.89 (t, J = 7.4 Hz, 3H). LC−MS m/z: 330 $[M + H]^+$, t_R = 4.1 min. *trans*-Ethylcyclohexanamine was prepared from 4-ethylcyclohexanone, following procedure C, and used without purification. ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.58 (m, 1H), 1.84 (m, 2H), 1.74 (m, 2H), 1.33 (br s, 2H), 1.27−1.17 (m, 3H), 1.05 (m, 2H), 0.91 (m, 2H), 0.86 (t, J = 7.4 Hz, 3H).

*N-(3-(cis-4-Ethylcyclohexyl)-2,4-dioxo-1,2,3,4-tetrahydroquinazolin-6-yl)acetamide (**46**).* The compound was prepared following General Procedure B, using *N*-(2,4-dioxo-2,4-dihydro-1*H*-benzo[*d*][1,3]oxazin-6-yl)acetamide (132 mg, 0.60 mmol), *cis*-4-ethylcyclohexanamine (84 mg, 0.60 mmol), THF (5 mL), and carbonyldiimidazole (146 mg, 0.90 mmol). Purification by flash chromatography (0−10% MeOH/CH_2Cl_2) gave the title compound as a white powder (34.7 mg, 0.088 mmol, 18%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.24 (s, 1H), 10.09 (s, 1H), 8.21 (d, J = 2.4 Hz, 1H), 7.77 (dd, J = 8.8, 2.4 Hz, 1H), 7.09 (d, J = 8.8 Hz, 1H), 4.72 (m, 1H), 2.53 (m, 2H), 2.05 (s, 3H), 1.70 (d, J = 12.6 Hz), 1.50 (m, 5H), 1.33 (d, J = 9.7 Hz, 2H), 0.89 (t, J = 7.3 Hz, 3H). LC−MS m/z: 330 $[M + H]^+$, t_R = 3.8 min. *cis*-4-Ethylcyclohexanamine was prepared from 4-ethylcyclohexanone, following procedure C, and used without purification. ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.99 (m, 1H), 1.61−1.25 (m, 13H), 0.90 (t, J = 7.2 Hz, 3H).

*N-(2,4-Dioxo-3-(trans-4-propylcyclohexyl)-1,2,3,4-tetrahydroquinazolin-6-yl)acetamide (**47**).* The compound was prepared following General Procedure B, using *N*-(2,4-dioxo-2,4-dihydro-1*H*-benzo[*d*][1,3]oxazin-6-yl)acetamide (220 mg, 1.00 mmol), *trans*-4-

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J. Med. Chem. XXXX, XXX, XXX−XXX

propylcyclohexanamine (155 mg, 1.00 mmol), THF (8 mL), and carbonyldiimidazole (243 mg, 1.50 mmol). Purification by flash chromatography (0−10% MeOH/CH_2Cl_2) gave the title compound as a white powder (64.1 mg, 0.187 mmol, 19%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.26 (s, 1H), 10.09 (s, 1H), 8.19 (d, J = 2.4 Hz, 1H), 7.78 (dd, J = 8.8, 2.4 Hz, 1H), 7.08 (d, J = 8.8 Hz, 1H), 4.72 (m, 1H), 2.42 (qd, J = 8.5, 3.0 Hz, 2H), 2.04 (s, 3H), 1.81 (d, J = 12.0 Hz, 2H), 1.58 (d, J = 9.8 Hz), 1.36−1.16 (m, 5H), 0.99 (qd, J = 12.5, 3.0 Hz, 2H), 0.88 (t, J = 7.3 Hz, 3H). LC−MS m/z: 344 $[M + H]^+$, t_R = 4.1 min. *trans*-4-Propylcyclohexanamine was prepared from 4-propylcyclohexanone, following procedure C, and used without purification. ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.60 (m, 1H), 1.85 (m, 2H), 1.74 (m, 2H), 1.38 (br s, 2H), 1.31 (m, 2H), 1.17 (m, 3H), 1.07 (m, 2H), 0.94 (m, 2H), 0.88 (t, J = 7.3 Hz, 3H).

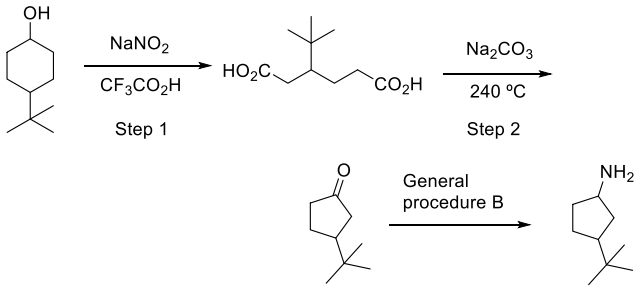
*N-(2,4-Dioxo-3-(cis-4-propylcyclohexyl)-1,2,3,4-tetrahydroquinazolin-6-yl)acetamide (**48**).* The compound was prepared following General Procedure B, using *N*-(2,4-dioxo-2,4-dihydro-1*H*-benzo[*d*][1,3]oxazin-6-yl)acetamide (220 mg, 1.00 mmol), *cis*-4-propylcyclohexanamine (155 mg, 1.00 mmol), THF (8 mL), and carbonyldiimidazole (243 mg, 1.50 mmol). Purification by flash chromatography (0−10% MeOH/CH_2Cl_2) gave the title compound as a white powder (51.3 mg, 0.149 mmol, 15%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.23 (s, 1H), 10.09 (s, 1H), 8.22 (d, J = 2.4 Hz, 1H), 7.76 (dd, J = 8.8, 2.4 Hz, 1H), 7.10 (d, J = 8.8 Hz, 1H), 4.73 (m, 1H), 2.53 (m, 2H), 2.05 (s, 3H), 1.67 (m, 3H), 1.55−1.42 (m, 4H), 1.30 (m, 4H), 0.93 (t, J = 7.3 Hz, 3H). LC−MS m/z: 344 $[M + H]^+$, t_R = 4.2 min. *cis*-4-Propylcyclohexanamine was prepared from 4-propylcyclohexanone, following procedure C, and used without purification. ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.94 (m, 1H), 1.55 (m, 2H), 1.50−1.21 (m, 13H), 0.88 (t, J = 7.2 Hz, 3H).

*N-(3-(4-Methylcyclohexyl)-2,4-dioxo-1,2,3,4-tetrahydroquinazolin-6-yl)acetamide (**49**).* The compound was prepared per General Procedure B, using methylcyclohexyl amine (112.1 mg, 0.990 mmol). Purification by flash chromatography (0−80% EtOAc/hexane) gave the title compound as a white/pale pink powder (189 mg, 0.599 mmol, 88%) as a mixture of isomers. ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.23 (s, 1H), 10.07 (s, 1H), 8.26−8.24(m, 1H), 8.20 (dd, J = 11.7, 2.4 Hz, 1H), 7.80−7.76 (m, 1H), 7.71−7.68 (m, 1H), 7.10 (dd, J = 8.8, 5.2 Hz, 1H), 7.04−7.01 (m, 1H), 4.74−4.69 (m, 1H), 2.66−2.58 (m, 1H), 2.46 (dd, J = 12.6, 3.1 Hz, 1H), 2.05 (s, 3H), 1.95−1.87 (m, 1H), 1.77−1.66 (m, 2H), 1.63−1.48 (m, 4H), 1.45−1.33 (m, 3H), 1.08−1.00 (m, 3H), 0.98−0.91 (d, J = 6.9 Hz, 1H), 0.90−0.87 (m, 2H). LC−MS m/z: 316 $[M + H]^+$, 631 $[2M + H]^+$, t_R = 5.0 min.

*N-(3-(4,4-Dimethylcyclohexyl)-2,4-dioxo-1,2,3,4-tetrahydroquinazolin-6-yl)acetamide (**50**).* The compound was prepared following General Procedure B, using *N*-(2,4-dioxo-2,4-dihydro-1*H*-benzo[*d*][1,3]oxazin-6-yl)acetamide (110 mg, 0.50 mmol), 4,4-dimethylcyclohexanamine (64 mg, 0.50 mmol), THF (5 mL), and carbonyldiimidazole (122 mg, 0.75 mmol). Purification by flash chromatography (0−10% MeOH/CH_2Cl_2) gave the title compound as a white powder (16.3 mg, 0.050 mmol, 10%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.09 (s, 1H), 8.21 (d, J = 2.4 Hz, 1H), 7.77 (dd, J = 8.8, 2.4 Hz, 1H), 7.09 (d, J = 8.8 Hz, 1H), 4.69 (m, 1H), 2.60 (qd, J = 13.0, 3.2 Hz, 2H), 2.05 (s, 3H), 1.44 (d, J = 12.4 Hz, 2H), 1.38 (m, 2H), 1.28 (td, J = 13.3, 3.6 Hz, 2H), 1.01 (s, 3H), 0.94 (s, 9H). LC−MS m/z: 328 $[M + H]^+$, t_R = 4.1 min. 4,4-Dimethylcyclohexanamine was prepared from 4,4-dimethylcyclohexanone, following procedure C, and used without purification. ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.58 (m, 1H), 1.64 (m, 2H), 1.39−1.18 (m, 8H), 0.90 (s, 6H).

*N-(3-(3-(tert-Butyl)cyclopentyl)-2,4-dioxo-1,2,3,4-tetrahydroquinazolin-6-yl)acetamide (**51**).* The compound was prepared following General Procedure B, using *N*-(2,4-dioxo-2,4-dihydro-1*H*-benzo[*d*][1,3]oxazin-6-yl)acetamide (165 mg, 0.75 mmol), 3-(*tert*-butyl)cyclopentanamine (117 mg, 0.83 mmol), THF (6 mL), and carbonyldiimidazole (182 mg, 1.12 mmol). Purification by flash chromatography (0−10% MeOH/CH_2Cl_2) gave the title compound, a mixture of diastereomers, as a white powder (139.8 mg, 0.407 mmol, 54%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.26 (s, 1H), 10.09 and 10.08 (2*s*, 1H), 8.23 (m, 1H), 7.76 (m, 1H), 7.10 (m, 1H), 5.25 (m, 1H), 2.36−1.23 (m, 7H), 2.05 (s, 3H), 0.89 and 0.86 (2s, 9H). LC−MS m/z: 342 $[M - H]^-$, t_R = 4.6 min.

3-(*tert*-Butyl)cyclopentanamine was made as follows:



3-(tert-Butyl)cyclopentanamine. Step 1. The procedure of Matsumura and Yoshihiro et al.[26] was used. A solution of 4-*tert*-butylcyclohexanol (10.0 g, 64 mmol, a mixture of *cis*- and *trans*-isomers) in trifluoroacetic acid (100 mL) at 0 °C was treated with sodium nitrite (17.0 g, 250 mmol) in small portions over 90 min. The thick suspension was stirred at room temperature overnight and then concentrated. The residue was poured onto ice, treated with aqueous $NaHCO_3$ (500 mL), and then made basic by the addition of solid NaOH. The aqueous solution was washed with dichloromethane (3 × 200 mL) and then acidified to pH < 1 with concentrated hydrochloric acid, extracted with ethyl acetate (3 × 300 mL), dried ($MgSO_4$), and concentrated to give crude 3-(*tert*-butyl)hexanedioic acid as a brown oil (13.05 g, ca. 100%), which was used without further purification. ^{1}H NMR (500 MHz, $CDCl_3$): δ 9.63 (br s, 2H), 2.56 (dd, J = 16.3 and 3.9 Hz, 1H), 2.50−2.39 (m, 2H), 2.12 (dd, J = 16.3 and 8.0 Hz, 1H), 1.98 (m, 1H), 1.74 (m, 1H), 1.44 (m, 1H), 0.93 (s, 9H).

Step 2. Crude 3-(*tert*-butyl)hexanedioic acid (8.33 g, 41 mmol) and solid sodium carbonate (0.216 g, 2 mmol) were heated together to 240 °C in a Kugelrohr distillation apparatus for 1 h. 3-(*tert*-Butyl)cyclopentanone was distilled from the reaction mixture and was collected as a green oil (1.983 g, 14 mmol, 34%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.38 (dd, J = 8.1, 1.1 Hz), 2.29−2.17 (m, 2H), 2.07−1.94 (m, 2H), 1.61 (m, 1H), 0.93 (s, 9H).

3-(*tert*-Butyl)cyclopentanone was converted into 3-(*tert*-butyl)-cyclopentanamine as a mixture of *cis*- and *trans*-isomers following General Procedure B, which was used without purification. ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.56−3.24 (m, 1H), 2.00−1.84 (m, 2H), 1.73 (m, 1H), 1.59 (m, 1H), 1.51−1.44 (m, 3H), 1.37−1.22 (m, 2H), 0.86 (s, 5.5H), 0.85 (s, 3.5H).

*N-(3-(4-(tert-Butyl)phenyl)-2,4-dioxo-1,2,3,4-tetrahydroquinazolin-6-yl)acetamide (**52**).* Following General Procedure D, 5-acetamido-2-aminobenzoic acid (200 mg, 1.03 mmol) and *tert*-butylaniline (152.2 mg, 1.02 mmol) were combined in CH_2Cl_2 (10 mL) at room temperature, followed by the addition of DIPEA (15.79 mg, 0.156 mmol), and the mixture was stirred. A 50% solution of propylphosphonic anhydride in EtOAc (399.1 mg, 1.25 mmol) was added dropwise, and the reaction was stirred at room temperature overnight. The resultant mixture was washed with aq $NaHCO_3$ and water, dried ($MgSO_4$), and concentrated. Purification by flash chromatography (0−100% EtOAc/hexane) gave the intermediate amide (147 mg, 0.452 mmol). This was suspended in THF (15 mL), CDI (251 mg, 1.55 mmol) was added, and the reaction was heated to 100 °C overnight. Upon cooling, the mixture was concentrated and purified by flash chromatography (0−100% EtOAc/hexane) to the title compound as a pale orange powder (81 mg, 0.231 mmol, 22%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.48 (s, 1H), 10.11 (s, 1H), 8.21 (d, J = 2.4 Hz, 1H), 8.89 (dd, J = 8.8, 2.4 Hz, 1H), 7.50 (d, J = 8.6 Hz, 2H), 7.23 (d, J = 8.5, 2H), 7.18 (d, J = 8.8 Hz, 2H), 2.05 (s, 3H), 1.35 (s, 9H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 168.2, 162.1, 150.3, 150.0, 135.3, 134.3, 133.1, 128.4, 126.7, 125.5, 116.7, 115.5, 114.2, 34.3, 31.1, 23.8. LC−MS m/z: 352 $[M + H]^+$, t_R = 3.9 min.

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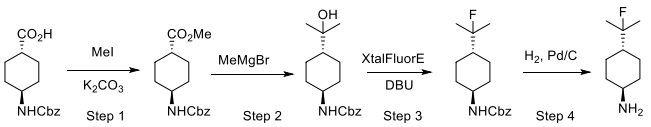
J. Med. Chem. XXXX, XXX, XXX−XXX

*N-(2,4-Dioxo-3-(trans-4-(trifluoromethyl)cyclohexyl)-1,2,3,4-tetrahydroquinazolin-6-yl)acetamide (**57**).* The compound was prepared following General Procedure B, using *N*-(2,4-dioxo-2,4-dihydro-1*H*-benzo[*d*][1,3]oxazin-6-yl)acetamide (165 mg, 0.75 mmol), *trans*-4-(trifluoromethyl)cyclohexanamine (125 mg, 0.75 mmol), THF (6 mL), and carbonyldiimidazole (122 mg, 0.75 mmol). Purification by flash chromatography (0−10% MeOH/CH_2Cl_2) gave the title compound as a white powder (59.6 mg, 0.16 mmol, 22%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.30 (s, 1H), 10.10 (s, 1H), 8.20 (d, J = 2.4 Hz, 1H), 7.80 (dd, J = 8.8, 2.4 Hz, 1H), 7.09 (d, J = 8.8 Hz, 1H), 4.76 (m, 1H), 2.49 (m, 2H), 2.30 (m, 1H), 2.04 (s, 3H), 1.98 (d, J = 11.8 Hz, 2H), 1.71 (d, J = 10.2 Hz, 2H), 1.39 (qd, J = 12.8, 3.4 Hz, 2H). LC−MS m/z: 368 $[M + H]^+$, t_R = 4.0 min. *trans*-4-(Trifluoromethyl)cyclohexanamine was prepared from 4-(trifluoromethyl)cyclohexan-1-one, following procedure C, and used without purification. ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.65 (m, 1H), 1.94 (m, 5H), 1.40 (br s, 2H), 1.36 (m, 2H), 1.09 (m, 2H).

*N-(2,4-Dioxo-3-(cis-4-(trifluoromethyl)cyclohexyl)-1,2,3,4-tetrahydroquinazolin-6-yl)acetamide (**58**).* The compound was prepared following General Procedure B, using *N*-(2,4-dioxo-2,4-dihydro-1*H*-benzo[*d*][1,3]oxazin-6-yl)acetamide (165 mg, 0.75 mmol), *cis*-4-(trifluoromethyl)cyclohexanamine (125 mg, 0.75 mmol), THF (6 mL), and carbonyldiimidazole (122 mg, 0.75 mmol). Purification by flash chromatography (0−10% MeOH/CH_2Cl_2) gave the title compound as a white powder (40.2 mg, 0.11 mmol, 15%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.25 (s, 1H), 10.09 (s, 1H), 8.21 (d, J = 2.4 Hz, 1H), 7.77 (dd, J = 8.8, 2.4 Hz, 1H), 7.09 (d, J = 8.8 Hz, 1H), 4.80 (m, 1H), 2.53 (m, 3H), 2.05 (m, 5H), 1.73 (m, 2H), 1.50 (m, 2H). LC−MS m/z: 368 $[M + H]^+$, t_R = 3.8 min. *cis*-4-(Trifluoromethyl)cyclohexanamine was prepared from 4-(trifluoromethyl)cyclohexan-1-one, following procedure C, and used without purification. ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.18 (m, 1H), 2.05 (m, 1H), 1.68 (m, 8H), 1.34 (br s, 2H).

*N-(3-(trans-4-(2-Fluoropropan-2-yl)cyclohexyl)-2,4-dioxo-1,2,3,4-tetrahydroquinazolin-6-yl)acetamide (**59**).* The compound was prepared following General Procedure B, using *N*-(2,4-dioxo-2,4-dihydro-1*H*-benzo[*d*][1,3]oxazin-6-yl)acetamide (165 mg, 0.75 mmol), *trans*-4-(2-fluoropropan-2-yl)cyclohexanamine (119 mg, 0.75 mmol), THF (6 mL), and carbonyldiimidazole (182 mg, 1.12 mmol). Purification by flash chromatography (0−10% MeOH/CH_2Cl_2) gave the title compound as a brown solid (145 mg, 0.40 mmol, 54%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.24 (s, 1H), 10.06 (s, 1H), 8.19 (d, J = 2.4 Hz, 1H), 7.79 (dd, J = 8.8, 2.4 Hz, 1H), 7.09 (d, J = 8.8 Hz, 1H), 4.73 (m, 1H), 2.45 (qd, J = 2.9, 12.4 Hz, 2H), 2.05 (s, 3H), 1.86 (d, J = 12.1 Hz, 2H) 1.66 (d, J = 10.1 Hz, 2H), 1.54 (q, J = 12.0 Hz, 1H), 1.30 (d, J = 22.2 Hz, 6H), 1.19 (qd, J = 2.9, 12.6 Hz, 2H). LC−MS m/z: 362 $[M + H]^+$, t_R = 3.9 min.



trans-4-(2-Fluoropropan-2-yl)cyclohexanamine. Step 1. A suspension of *trans*-4-(carbobenzoxyamino)cyclohexanecarboxylic acid (8.093 g, 29.2 mmol) and freshly ground potassium carbonate (4.24 g, 30.7 mmol) in DMF (140 mL) was stirred at room temperature for 40 min then cooled to 0 °C. Iodomethane (1.90 mL, 30.5 mmol) was added dropwise. The mixture was stirred at room temperature overnight and then concentrated and partitioned between water (100 mL) and EtOAc (3 × 100 mL). The combined organic phases were dried ($MgSO_4$) and concentrated to give methyl *trans*-4-(carbobenzoxyamino)cyclohexanecarboxylate as an off-white solid (7.722 g, 26.5 mmol, 91%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.35 (m, 5H), 5.11 (s, 2H), 4.64 (br s, 1H), 3.69 (s, 3H), 3.52 (m, 1H), 2.25 (tt, J = 12.2, 3.6 Hz, 1H), 2.11 (br d, J = 10.8 Hz, 2H), 2.04 (br d, J = 13.2 Hz, 2H), 1.56 (br q, J = 12.1 Hz, 2H), 1.16 (qd, J = 12.8, 3.4 Hz, 2H).

Step 2. A solution of methyl *trans*-4-(carbobenzoxyamino)-cyclohexanecarboxylate (3.00 g, 10.3 mmol) in Et_2O (180 mL) at 0 °C was treated dropwise with MeMgBr, 3 M in Et_2O (10.5 mL, 31.5 mmol). The white suspension was stirred at 0 °C for 90 min and then at room temperature for 21 h. The reaction mixture was treated with saturated aqueous NH_4Cl (100 mL), and the phases were separated. The aqueous phase was extracted with Et_2O (2 × 100 mL), and the combined organic phases were washed with brine, dried ($MgSO_4$), and concentrated. Purification by flash chromatography (0−100% EtOAc/hexane) gave benzyl ((1*R*,4*R*)-4-(2-hydroxypropan-2-yl)cyclohexyl)carbamate as a white solid (1.599 g, 5.49 mmol, 53%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.35 (m, 5H), 5.11 (s, 2H), 4.62 (br s, 1H), 3.46 (m, 1H), 2.12 (br d, J = 11.0 Hz, 2H), 1.89 (br d, J = 12.0 Hz, 2H), 1.31−1.09 (m, 11H).

Step 3. A suspension of benzyl (*trans*-4-(2-hydroxypropan-2-yl)cyclohexyl)carbamate (0.585 g, 2.01 mmol) in CH_2Cl_2 at −78 °C was treated with DBU (0.45 mL, 3.0 mmol) and XtalFluor-E (0.684 g, 2.99 mmol). The resulting pale yellow solution was stirred at −78 °C for 1 h and then at room temperature overnight. The resulting brown solution was treated with saturated aqueous $NaHCO_3$ (12 mL) and vigorously stirred for 15 min. The phases were separated, the aqueous phase was further extracted with CH_2Cl_2, and the organic phases were filtered through plugs of $MgSO_4$ and silica and concentrated. Purification by flash chromatography (0−100% EtOAc/petroleum ether 40−60) gave benzyl ((1*R*,4*R*)-4-(2-fluoropropan-2-yl)cyclohexyl)carbamate as a yellow solid (0.486 g, 1.66 mmol, 82%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.35 (m, 5H), 5.11 (s, 2H), 4.61 (br s, 1H), 3.47 (m, 1H), 2.12 (br d, J = 11.5 Hz, 2H), 1.87 (br d, J = 12.7 Hz, 2H), 1.51 (m, 1H), 1.32 (d, J = 22.1 Hz, 6H), 1.28−1.09 (m, 4H).

Step 4. A vessel containing a solution of (*trans*-4-(2-fluoropropan-2-yl)cyclohexyl)carbamate (0.360 g, 1.23 mmol) in ethanol (5 mL) was purged with three vacuum/argon cycles. Palladium on carbon (60 mg) was added, and the vessel was purged three times with vacuum/argon cycles and then three times with vacuum/hydrogen cycles. The mixture was stirred at room temperature for 3 days, then filtered through a plug of Celite, and concentrated to give (1*R*,4*R*)-4-(2-fluoropropan-2-yl)cyclohexanamine as a cloudy oil (0.180 g, 1.13 mmol, 92%), which was used without purification. ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.54 (m, 1H), 1.85 (m, 2H), 1.74 (m, 2H), 1.43 (m, 3H), 1.22 (d, J = 22.1 Hz, 6H), 1.10−0.98 (m, 4H).

*N-(3-(trans-4-(2-Hydroxypropan-2-yl)cyclohexyl)-2,4-dioxo-1,2,3,4-tetrahydroquinazolin-6-yl)acetamide (**60**).* The compound was prepared following General Procedure B, using *N*-(2,4-dioxo-2,4-dihydro-1*H*-benzo[*d*][1,3]oxazin-6-yl)acetamide (165 mg, 0.75 mmol), 2-(*trans*-4-aminocyclohexyl)propan-2-ol (130 mg, 0.83 mmol), THF (6 mL), and carbonyldiimidazole (182 mg, 1.12 mmol). Purification by flash chromatography (0−10% MeOH/CH_2Cl_2) gave the title compound as an off-white solid (54.9 mg, 0.153 mmol, 20%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.18 (s, 1H), 10.06 (s, 1H), 8.19 (d, J = 2.4 Hz, 1H), 7.79 (dd, J = 8.8, 2.4 Hz, 1H), 7.09 (d, J = 8.8 Hz, 1H), 4.71 (m, 1H), 4.03 (s, 1H), 2.41 (qd, J = 12.4, 3.0 Hz, 2H), 2.04 (s, 3H), 1.90 (d, J = 13.1 Hz, 2H) 1.63 (d, J = 9.5 Hz, 2H), 1.25 (m, 2H), 1.15−1.01 (m, 9H). LC−MS m/z: 358 $[M - H]^-$, t_R = 3.4 min.

*N-(3-(trans-2-(tert-Butyl)-1,3-dioxan-5-yl)-2,4-dioxo-1,2,3,4-tetrahydroquinazolin-6-yl)acetamide (**61**).* The compound was prepared following General Procedure B, using *N*-(2,4-dioxo-2,4-dihydro-1*H*-benzo[*d*][1,3]oxazin-6-yl)acetamide (110 mg, 0.50 mmol), *trans*-2-(*tert*-butyl)-1,3-dioxan-5-amine (see below) (80 mg, 0.50 mmol), THF (4 mL), and carbonyldiimidazole (122 mg, 0.75 mmol). Purification by flash chromatography (0−10% MeOH/CH_2Cl_2) gave the title compound as an off-white powder (96 mg, 0.29 mmol, 57%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.39 (s, 1H), 10.11 (s, 1H), 8.22 (d, J = 2.4 Hz, 1H), 7.79 (dd, J = 8.8, 2.4 Hz, 1H), 7.10 (d, J = 8.8 Hz, 1H), 5.01 (m, 1H), 4.52 (dd appearing as t, J = 10.7 Hz, 2H), 4.22 (s, 1H), 4.12 (dd appearing as q, J = 5.3 Hz, 2H), 2.05 (s, 3H), 0.91 (s, 9H). LC−MS m/z: 276 $[M - C_5H_{11}O + H]^+$, t_R = 4.0 min.

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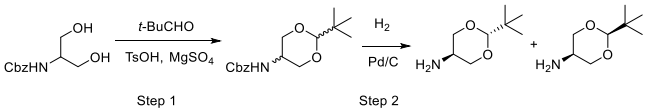
J. Med. Chem. XXXX, XXX, XXX−XXX

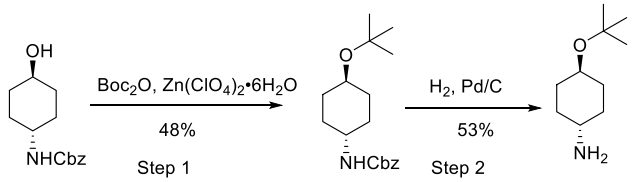
*N-(3-(cis-2-(tert-Butyl)-1,3-dioxan-5-yl)-2,4-dioxo-1,2,3,4-tetrahydroquinazolin-6-yl)acetamide (**62**).* The compound was prepared following General Procedure B, using *N*-(2,4-dioxo-2,4-dihydro-1*H*-benzo[*d*][1,3]oxazin-6-yl)acetamide (110 mg, 0.50 mmol), *cis*-2-(*tert*-butyl)-1,3-dioxan-5-amine (see below) (80 mg, 0.50 mmol), THF (4 mL), and carbonyldiimidazole (122 mg, 0.75 mmol). Purification by flash chromatography (0−10% MeOH/CH_2Cl_2) gave the title compound as a white powder (53.4 mg, 0.15 mmol, 36%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.29 (s, 1H), 10.09 (s, 1H), 8.21 (s, 1H), 7.77 (d, J = 8.8 Hz, 1H), 7.09 (d, J = 8.8 Hz, 1H), 5.09 (m, 1H), 4.32 (m, 3H), 4.12 (m, 2H), 2.04 (s, 3H), 0.88 (s, 9H). LC−MS m/z: 276 [M − $C_5H_{11}O$ + H]$^+$, t_R = 3.7 min.



trans-2-(tert-Butyl)-1,3-dioxan-5-amine and cis-2-(tert-Butyl)-1,3-dioxan-5-amine. Step 1. A mixture of benzyl (1,3-dihydroxypropan-2-yl)carbamate (1.122 g, 4.98 mmol), trimethylacetaldehyde (1.03 mL, 9.48 mmol), toluenesulfonic acid monohydrate (52 mg, 0.27 mmol), and anhydrous magnesium sulfate (2.4 g, 20 mmol) in anhydrous THF (15 mL) was heated under reflux overnight. The mixture was cooled, treated with aqueous $NaHCO_3$ (20 mL), and stirred until effervescence ceased. The phases were separated, and the aqueous phase was extracted with diethyl ether (3 × 20 mL). The combined organic phases were washed with water and brine, dried ($MgSO_4$), and concentrated to give a colorless oil. The oil was triturated with petroleum ether 40−60, and the resulting suspension was extracted with petroleum ether (3 × 50 mL) and filtered to remove the solid. The filtrate was concentrated to give a colorless waxy solid, benzyl (2-(*tert*-butyl)-1,3-dioxan-5-yl)carbamate (1.068 g, 3.64 mmol, 73%), as a ca. 1:1 mixture of *cis*- and *trans*-isomers, which was used in the next step without further purification. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.37 (m, 5H), 5.66 (d, J = 9.2 Hz, 0.5H), 5.17−5.10 (m, 2.5H), 4.56 (m, 0.5H), 4.22 (m, 1H), 4.16 (s, 0.5H), 4.00 (m, 1.5H), 3.89 (m, 1H), 3.64 (m, 0.5H), 3.30 (m, 1H), 0.93 (s, 9H).

Step 2. A vessel containing a solution of benzyl (2-(*tert*-butyl)-1,3-dioxan-5-yl)carbamate (1.027 g, 3.50 mmol, a mixture of diastereomers) in methanol (10 mL) was purged with three vacuum/argon cycles. Palladium on carbon (0.120 g) was added, and the vessel was purged three times with vacuum/argon cycles and then three times with vacuum/hydrogen cycles. The mixture was stirred at room temperature overnight, then filtered through a plug of Celite, and concentrated. The diastereomers were separated by flash chromatography on silica using a gradient elution of 0−10% methanol/ammonia in dichloromethane to give first the *trans*-product, *trans*-2-(*tert*-butyl)-1,3-dioxan-5-amine (0.218 g, 1.37 mmol, 39%) and then the *cis*-product, *cis*-2-(*tert*-butyl)-1,3-dioxan-5-amine (0.211 g, 1.33 mmol, 38%). *trans*-2-(*tert*-Butyl)-1,3-dioxan-5-amine ^{1}H NMR (500 MHz, $CDCl_3$): δ 4.13 (ddd, J = 9.8, 4.9, 1.4 Hz, 2H), 3.99 (s, 1H), 3.19 (ddd appearing as td, J = 10.3, 1.3 Hz), 3.01 (m, 1H), 1.00 (br s, 2H), 0.91 (s, 9H). *cis*-2-(*tert*-Butyl)-1,3-dioxan-5-amine ^{1}H NMR (500 MHz, $CDCl_3$): δ 4.12 (s, 1H), 3.90 (dd, J = 10.5, 1.7 Hz, 2H), 3.87 (dd, J = 10.5, 1.7 Hz, 2H), 2.65 (m, 1H), 1.83 (br s, 2H), 0.92 (s, 9H).

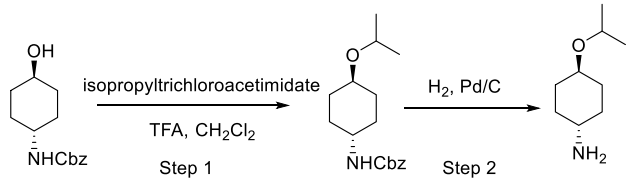
*N-(3-(trans-4-(tert-Butoxy)cyclohexyl)-2,4-dioxo-1,2,3,4-tetrahydroquinazolin-6-yl)acetamide (**63**).* The compound was prepared following General Procedure B, using *N*-(2,4-dioxo-2,4-dihydro-1*H*-benzo[*d*][1,3]oxazin-6-yl)acetamide (165 mg, 0.75 mmol), *trans*-4-(*tert*-butoxy)cyclohexanamine (129 mg, 0.75 mmol), THF (6 mL), and carbonyldiimidazole (122 mg, 0.75 mmol). Purification by flash chromatography (0−10% MeOH/CH_2Cl_2) gave the title compound as a white powder (76.2 mg, 0.20 mmol, 27%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.22 (s, 1H), 10.09 (s, 1H), 8.17 (d, J = 2.4 Hz, 1H), 7.80 (dd, J = 8.8, 2.4 Hz, 1H), 7.08 (d, J = 8.8 Hz, 1H), 4.71 (m, 1H), 3.43 (m, 1H), 2.08 (s, 3H), 1.82 (d, J = 10.5 Hz, 1H), 1.56 (d, J = 11.2 Hz, 2H), 1.28 (m, 2H), 1.15 (m, 11H). LC−MS m/z: 372 [M − H]$^-$, t_R = 3.8 min.



trans-4-(tert-Butoxy)cyclohexanamine. Step 1. A suspension of *trans*-4-(benzyloxycarbonylamino)cyclohexan-1-ol (2.50 g, 10.0 mmol) and zinc perchlorate hexahydrate (0.50 g, 1.34 mmol) in 1,2-dichloroethane (24 mL) was heated to 80 °C. A solution of Boc_2O (6.0 g, 27.5 mmol) in 1,2-dichloroethane (6 mL) was added dropwise. The mixture was heated at 80 °C for 5.5 h and then cooled and treated with water (40 mL) and brine (20 mL). The mixture was extracted with EtOAc (3 × 50 mL). The combined organic extracts were washed with brine, dried ($MgSO_4$), and concentrated to give a white solid. The solid residue was extracted with petroleum ether 40−60 (100 mL), and this extract was concentrated to give benzyl ((1*R*,4*R*)-4-(*tert*-butoxy)cyclohexyl)-carbamate as a white solid (1.465 g, 4.80 mmol, 48%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.35 (m, 5H), 5.11 (s, 2H), 3.48 (m, 1H), 3.37 (m, 1H), 2.04 (m, 2H), 1.83 (m, 2H), 1.43 (m, 2H), 1.22 (m, 2H), 1.20 (s, 9H).

Step 2. A vessel containing a solution of the benzyl ((*trans*-4-(*tert*-butoxy)cyclohexyl)carbamate (0.506 g, 1.66 mmol) in methanol (5 mL) was purged with three vacuum/argon cycles. Palladium on carbon (70 mg) was added, and the vessel was purged three times with vacuum/argon cycles and then three times with vacuum/hydrogen cycles. The mixture was stirred at room temperature for 3 days, then filtered through a plug of Celite, and concentrated to give (1*R*,4*R*)-4-(*tert*-butoxy)cyclohexanamine as a waxy white solid (0.152 g, 0.887 mmol, 53%), which was used without further purification. ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.36 (m, 1H), 2.70 (m, 1H), 1.99 (s, 2H), 1.87 (m, 2H), 1.81 (m, 2H), 1.35 (m, 2H), 1.21 (m, 2H), 1.20 (s, 9H).

*N-(3-(trans-4-Isopropoxycyclohexyl)-2,4-dioxo-1,2,3,4-tetrahydroquinazolin-6-yl)acetamide (**64**).* The compound was prepared following General Procedure B, using *N*-(2,4-dioxo-2,4-dihydro-1*H*-benzo[*d*][1,3]oxazin-6-yl)acetamide (154 mg, 0.70 mmol), (1*R*,4*R*)-4-isopropoxycyclohexanamine (110 mg, 0.70 mmol), THF (5.6 mL), and carbonyldiimidazole (170 mg, 1.05 mmol) to give the title compound as a brown powder (39.3 mg, 0.11 mmol, 16%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.28 (s, 1H), 10.10 (s, 1H), 8.18 (d, J = 2.4 Hz, 1H), 7.79 (dd, J = 8.8, 2.4 Hz, 1H), 7.08 (d, J = 8.8 Hz, 1H), 4.73 (m, 1H), 3.71 (septet, J = 6.0 Hz, 1H), 3.32 (m, 1H), 2.49 (m, 2H), 2.04 (s, 3H), 1.98 (d, J = 11.2 Hz, 2H), 1.58 (d, J = 10.6 Hz, 2H), 1.22 (m, 2H), 1.08 (d, J = 6.0 Hz, 9H). LC−MS m/z: 358 [M − H]$^-$, t_R = 4.0 min.



trans-4-Isopropoxycyclohexanamine. Step 1. A suspension of *trans*-4-(benzyloxycarbonylamino)cyclohexan-1-ol (1.91 g, 7.66 mmol) and isopropyl trichloroacetimidate (3.22 g, 15.3 mmol) in CH_2Cl_2 (30 mL) was treated with trifluoroacetic acid (0.090 mL, 1 mmol) and stirred at room temperature for 3 days. Further isopropyl trichloroacetimidate (1 g, 5 mmol) and trifluoroacetic acid (8 drops) were added, and the reaction was stirred for a further day and then treated with aqueous $NaHCO_3$ (50 mL) and extracted with CH_2Cl_2 (3 × 50 mL). The organic phases were concentrated to give a yellow solid, which was extracted with Et_2O (3 × 50 mL). The combined ethereal extracts were concentrated and purified by flash chromatography (0−20% EtOAc/CH_2Cl_2) to give benzyl (*trans*-4-isopropoxycyclohexyl)carbamate as a yellow solid (0.206 g, 0.71 mmol, 9%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.34 (m, 5H), 5.11 (s, 2H), 3.70 (septet, J = 6.1 Hz, 1H), 3.52 (m, 1H), 3.29 (m,

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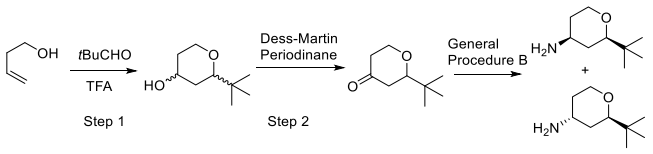
J. Med. Chem. XXXX, XXX, XXX−XXX

1H), 2.06 (m, 2H), 1.97 (m, 2H), 1.39 (m, 2H), 1.19 (m, 2H), 1.15 (d, J = 6.1 Hz, 6H).

Step 2. A vessel containing a solution of the benzyl (*trans*-4-isopropoxycyclohexyl)carbamate (0.186 g, 0.64 mmol) in ethanol (2 mL) was purged with three vacuum/argon cycles. Palladium on carbon (32 mg) was added, and the vessel was purged three times with vacuum/argon cycles and then three times with vacuum/hydrogen cycles. The mixture was stirred at room temperature overnight, then filtered through a plug of Celite, and concentrated to give *trans*-4-isopropoxycyclohexanamine as a waxy solid (0.124 g, 0.79 mmol, >100% crude yield), which was used without further purification. ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.61 (septet, J = 6.1 Hz, 1H), 3.22 (m, 1H), 2.81 (m, 1H), 1.91 (m, 4H), 1.24 (m, 4H), 1.06 (d, J = 6.1 Hz, 6H).

*(±)N-(3-(cis-2-(tert-Butyl)tetrahydro-2H-pyran-4-yl)-2,4-dioxo-1,2,3,4-tetrahydroquinazolin-6-yl)acetamide (**65**).* The compound was prepared following General Procedure B, using *N*-(2,4-dioxo-2,4-dihydro-1*H*-benzo[*d*][1,3]oxazin-6-yl)acetamide (166 mg, 0.75 mmol), (±)*cis*-2-(*tert*-butyl)tetrahydro-2*H*-pyran-4-amine (117 mg, 0.74 mmol), THF (6 mL), and carbonyldiimidazole (183 mg, 1.13 mmol). Purification by flash chromatography (0−10% MeOH/CH_2Cl_2) gave the racemic title compound as a yellow solid (143 mg, 0.40 mmol, 53%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.30 (s, 1H), 10.10 (s, 1H), 8.21 (d, J = 2.4 Hz, 1H), 7.79 (dd, J = 8.8, 2.4 Hz, 1H), 7.10 (d, J = 8.8 Hz, 1H), 5.00 (m, 1H), 4.04 (dd, J = 11.3, 3.8 Hz, 1H), 3.40 (m, 1H), 2.96 (dd, J = 11.2, 1.4 Hz, 1H), 2.59 (dq, J = 4.8, 12.2 Hz, 1H), 2.36 (q, J = 12.2 Hz, 1H), 2.04 (s, 3H), 1.57 (m, 1H), 1.48 (m, 1H), 0.88 (s, 9H). LC−MS m/z: 360 $[M + H]^+$, t_R = 3.8 min.



(±)cis-2-(tert-Butyl)tetrahydro-2H-pyran-4-amine. Step 1. A solution of trimethylacetaldehyde (2.2 mL, 20 mmol) and 3-buten-1-ol (1.7 mL, 20 mmol) in CH_2Cl_2 (15 mL) was cooled to 0 °C and treated with trifluoroacetic acid (5 mL). The mixture was allowed to warm to room temperature and stirred overnight. The mixture was concentrated, redissolved in MeOH (20 mL), and cooled to 0 °C. Solid potassium carbonate (3.6 g) was added portionwise, and the mixture was stirred at room temperature for 5 h before being concentrated. The residue was extracted with Et_2O (100 mL) and concentrated to give 2-(*tert*-butyl)tetrahydro-2*H*-pyran-4-ol as a colorless oil, which was used without further purification (2.868 g, 18.1 mmol, 91%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 4.04 (ddd, J = 11.7, 5.0, 1.6 Hz, 1H), 3.77 (m, 1H), 3.36 (td, J = 12.2, 2.1 Hz, 1H), 2.88 (dd, J = 11.4, 1.7 Hz, 1H), 1.97 (m, 1H), 1.88 (m, 1H), 1.76 (br s, 1H), 1.48 (m, 1H), 1.22 (m, 1H), 0.92 (s, 9H).

Step 2. A solution of 2-(*tert*-butyl)tetrahydro-2*H*-pyran-4-ol (1.007 g, 6.37 mmol) in CH_2Cl_2 (125 mL) was treated with Dess−Martin Periodinane (4.04 g, 9.53 mmol) and stirred at room temperature for 3 days. Saturated aqueous $NaHCO_3$ (100 mL) and 20% aqueous sodium thiosulfate (100 mL) were then added, and the mixture was vigorously stirred for 1 h. The phases were separated, and the aqueous phase was extracted with further CH_2Cl_2 (2 × 100 mL). The combined organic extracts were concentrated to give a yellow oil, which was purified by flash chromatography (0−100% Et_2O/hexane) to give 2-(*tert*-butyl)dihydro-2*H*-pyran-4(3*H*)-one as a colorless oil (0.648 g, 4.15 mmol, 65%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 4.32 (ddd, J = 11.4, 7.6, 1.0 Hz, 1H), 3.61 (ddd, J = 14.1, 11.4, 2.7 Hz, 1H), 3.20 (dd, J = 11.5, 2.7 Hz, 1H), 2.58 (m, 1H), 2.42−2.29 (m, 3H), 0.95 (s, 9H).

2-(*tert*-Butyl)dihydro-2*H*-pyran-4(3*H*)-one was converted into (±)*cis*-2-(*tert*-butyl)tetrahydro-2*H*-pyran-4-amine and (±)*trans*-2-(*tert*-butyl)tetrahydro-2*H*-pyran-4-amine, following General Procedure C, which were used without purification. (±)*cis*-2-(*tert*-Butyl)tetrahydro-2*H*-pyran-4-amine ^{1}H NMR (500 MHz, $CDCl_3$): δ 4.04 (m, 1H), 3.39 (t, J = 12.0 Hz, 1H), 2.89 (m, 1H), 1.84 (d, J = 12.4 Hz, 1H), 1.75 (d, J = 12.7 Hz, 1H), 1.49 (br s, 2H), 1.21 (m, 1H), 0.96 (m, 1H), 0.83 (s, 9H).

*N-(3-(1-tert-Butyl)piperidin-4-yl)-2,4-dioxo-1,2,3,4-tetrahydroquinazolin-6-yl)acetamide (**66**).* The compound was prepared using General Procedure B, using (1-*tert*-butyl)piperidin-4-amine (104.8 mg). Upon reaction completion, the mixture was diluted with water and extracted with EtOAc. The desired product remained in the aqueous layer, which was concentrated. Upon the addition of MeOH, the title compound precipitated as a white powder (10 mg, 0.028 mmol, 4%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.41 (s, 1H), 10.21 (s, 1H), 8.26 (s, 1H), 7.80 (d, J = 8.2 Hz, 1H), 7.14 (d, J = 8.5 Hz, 1H), 5.10 (bs, 1H), 3.58 (bs, 2H), 3.11 (bs, 2H), 2.97−2.94 (m, 2H), 2.05 (s, 3H), 1.87 (d, J = 11.3 Hz, 2H), 1.38 (s, 9H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 168.3, 149.9, 116.7, 115.2, 62.8, 48.2, 46.3, 25.4, 24.1, 23.8; LC−MS m/z: 359 $[M + H]^+$, t_R = 4.3 min

*tert-Butyl 4-(6-Acetamido-2,4-dioxo-1,2-dihydroquinazolin-3(4H)-yl)piperidine-1-carboxylate (**67**).* The compound was prepared using General Procedure B; *tert*-butyl-4-aminopiperidine-1-carboxylate (1.80 g, 8.99 mmol) gave the title compound as an off-white powder (2.90 g, 7.21 mmol, 79%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.32 (s, 1H), 10.10 (s, 1H), 8.20 (d, J = 2.1 Hz, 1H), 7.80 (dd, J = 8.8, 2.3 Hz, 1H), 7.10 (d, J = 8.8 Hz, 1H), 4.39 (t, J = 11.9 Hz, 1H), 4.06 (bs, 2H), 2.80 (m, 2H), 2.46 (m, 2H), 2.04 (s, 3H), 1.57 (d, J = 10.4 Hz, 2H), 1.43 (s, 9H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 168.2, 162.1, 153.7, 149.7, 135.0, 134.2, 126.6, 116.6, 115.2, 114.0, 78.7, 50.8, 28.0, 27.4, 23.8. LC−MS m/z: 292 $[M + H]^+$, t_R = 0.6 min.

*N-(2,4-Dioxo-3-(piperidin-4-yl)-1,2,3,4-tetrahydroquinazolin-6-yl)acetamide, TFA Salt (**114**).* Carbamate **67** (2.87 g, 7.13 mmol) was suspended in 20% TFA/CH_2Cl_2 (60 mL) and stirred at room temperature overnight. The solvent was then removed *in vacuo*. The salt was resuspended in CH_2Cl_2/MeOH and reconcentrated. Finally CH_2Cl_2 was added, and the precipitate was filtered and dried to give the title compound as a white powder (2.56 g, 6.15 mmol, 86%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.40 (bs, 1H), 10.14 (s, 1H), 8.54 (bs, 1H), 8.25 (d, J = 2.4 Hz, 1H), 7.79 (dd, J = 8.8, 2.4 Hz, 1H), 7.11 (d, J = 8.8 Hz, 1H), 5.06 (m, 1H), 3.39−3.35 (m, 1H), 3.17 (s, 1H), 3.06 (m, 2H), 2.80 (dq, J = 16.8, 3.8 Hz, 2H), 2.05 (s, 3H), 1.80 (d, J = 12.3 Hz, 2H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 168.2, 162.2, 149.9, 135.0, 134.3, 126.6, 116.6, 115.2, 114.0, 48.5, 48.1, 43.4, 24.8, 23.8. ^{19}F NMR (470 MHz, DMSO-d_6): δ −73.5. LC−MS m/z: 303 $[M + H]^+$, 605 $[2M + H]^+$, t_R = 3.4 min; HRMS (ES^+): found 303.1442 $[M + H]^+$; $C_{15}H_{19}N_4O_3^+$ $[M + H]^+$, requires 303.1452.

*N-(3-(1-Benzoylpiperidin-4-yl)-2,4-dioxo-1,2,3,4-tetrahydroquinazolin-6-yl)acetamide (**68**).* TFA salt **114** (0.150 mg, 0.361 mmol) was suspended in pyridine (2 mL), followed by the addition of benzoyl chloride (60.87 mg, 0.433 mmol), and the mixture was stirred at room temperature overnight. Purification was carried out by semipreparative HPLC (Waters, basic mode, 5:95 MeCN/water + 0.1% NH_3) to give the title compound as an off-white powder (26 mg, 0.064 mmol, 18%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.30 (s, 1H), 10.08 (s, 1H), 8.22 (d, J = 2.4 Hz, 1H), 7.80 (dd, J = 8.8, 2.4 Hz, 1H), 7.50−7.47 (m, 3H), 7.41−7.39 (m, 2H), 7.11 (d, J = 8.8 Hz, 1H), 5.06 (m, 1H), 4.63 (bs, 1H), 3.67 (bs, 1H), 3.19−3.17 (m, 1H), 2.85−2.80 (m, 1H), 2.05 (s, 3H), 1.71−1.59 (m, 2H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 168.9, 168.2, 162.1, 155.3, 149.9, 136.1, 134.2, 132.8, 128.4, 126.5, 117.9, 116.6, 115.2, 114.3, 114.0, 50.63, 41.4, 27.3, 23.8. LC−MS m/z: 407 $[M + H]^+$, 813 $[2M + H]^+$, t_R = 4.4 min.

*N-(2,4-Dioxo-3-(1-(phenylsulphonyl)piperidin-4-yl)-1,2,3,4-tetrahydroquinazolin-6-yl)acetamide (**69**).* TFA salt **114** (0.150 mg, 0.361 mmol) was suspended in pyridine (2 mL), followed by the addition of benzene sulphonyl chloride (76.48 mg, 0.433 mmol), and the mixture was stirred at room temperature overnight. Purification was carried out by semipreparative HPLC (Waters, basic mode, 5:95 MeCN/water + 0.1% NH_3) to give the title compound as a white powder (6 mg, 0.014 mmol, 4%). ^{1}H NMR

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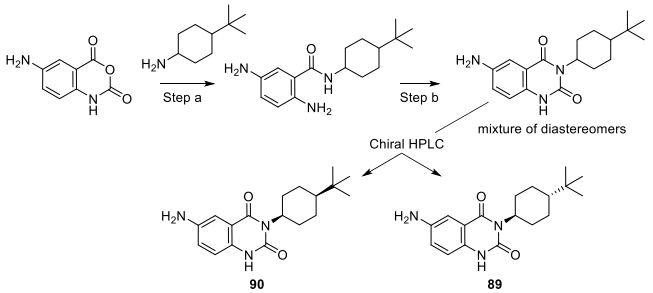
J. Med. Chem. XXXX, XXX, XXX−XXX

(500 MHz, DMSO-d_6): δ 11.29 (s, 1H), 10.06 (s, 1H), 8.17(d, J = 2.4 Hz, 1H), 7.81−7.74 (m, 4H), 7.69−7.66 (m, 2H), 7.08 (d, J = 8.8, 1H), 4.73−4.68 (m, 1H), 3.81−3.79 (m, 2H), 2.67 (dd, J = 12.6, 4.4 Hz, 1H), 2.63 (dd, J = 12.4, 4.0 Hz, 1H), 2.37−2.32 (m, 2H), 2.04 (s, 3H), 1.66−1.64 (m, 2H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 168.1, 162.1, 149.9, 135.7, 135.1, 134.2, 133.0, 129.3, 127.3, 126.5, 116.6, 115.3, 114.0, 49.8, 46.2, 26.8, 23.8. LC−MS m/z: 443 $[M + H]^+$, t_R = 3.8 min.

*tert-Butyl(cis-4-(6-acetamido-2,4-dihydroquinazolin-3(4H)-yl)-cyclohexyl)carbamate (**70**).* The compound was prepared following General Procedure B, using *cis-tert*-butyl-4-aminocyclohexyl)-carbamate (144.5 mg). Purification by flash chromatography (0−80% EtOAc/hexane) gave the title compound as a white/pale pink powder (176 mg, 0.423 mmol, 62%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.24 (s, 1H), 10.07 (s, 1H), 8.21 (d, J = 2.1 Hz, 1H), 7.78 (dd, J = 8.7, 2.2 Hz, 1H), 7.10 (d, J = 8.7, 1H), 6.50 (bs, 1H), 4.73 (tt, J = 12.2, 3.4 Hz, 1H), 3.58 (bs, 1H), 2.65−2.58 (m, 2H), 2.05 (s, 3H), 1.90 (d, J = 13.2 Hz, 2H), 1.55−1.47 (m, 2H), 1.43 (s, 9H), 1.39−1.35 (m, 2H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 168.1, 162.1, 155.0, 149.9, 135.0, 134.1, 126.5, 116.5, 115.1, 114.1, 77.5, 52.3, 44.3, 29.4, 28.2, 23.8, 22.9.

*tert-Butyl 3-(6-Acetamido-2,4-dioxo-1,2-dihydroquinazolin-3(4H)-yl)pyrrolidine-1-carboxylate (**71**).* Following General Procedure D, 5-acetamido-2-aminobenzoic acid (200 mg, 1.03 mmol), (*S*)-1-boc-3-aminopyrrolidine (59.00 μL, 0.338 mmol), and (*R*)-1-Boc-3-aminopyrrolidine (57.33 μL, 0.338 mmol) were combined in CH_2Cl_2 (10 mL) at room temperature, followed by the addition of DIPEA (15.79 mg, 0.156 mmol); the mixture was stirred. A 50% solution of propylphosphonic anhydride in EtOAc (399.1 mg, 1.25 mmol) was added dropwise, and the reaction was stirred at room temperature overnight. The resultant mixture was washed with aq $NaHCO_3$, water, dried ($MgSO_4$), and concentrated. This was suspended in THF (15 mL); CDI (251 mg, 1.55 mmol) was added, and the reaction was heated to 100 °C overnight. Upon cooling, the mixture was concentrated and purified by flash chromatography (0−5% MeOH/CH_2Cl_2) to give the racemic title compound as a light brown powder (16 mg, 0.041 mmol, 6%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.09 (s, 1H), 10.11 (s, 1H), 8.23 (d, J = 2.4 Hz, 1H), 7.80 (dd, J = 8.8, 2.4 Hz, 1H), 7.64 (s, 1H), 7.12 (d, J = 8.8 Hz, 1H), 5.56 (quintet, J = 8.3 Hz, 1H), 3.66−3.62 (m, 2H), 3.60−3.33 (m, 2H + MeOD), 2.05 (s, 3H), 1.43−1.40 (m, 11H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 168.2, 162.2, 153.4, 149.8, 134.9, 134.2, 126.6, 116.6, 115.3, 114.0, 78.25, 77.91, 49.9, 49.2, 46.3, 45.0, 44.6, 44.1, 43.9, 28.1, 27.5, 26.5, 23.8. LC−MS m/z: 378 $[M\text{-boc} + H]^+$, 755 $[2M\text{-boc} + H]^+$, t_R = 3.2 min.



Step a: 2,5-Diamino-N-(4-tert-butyl-cyclohexyl)-benzamide. DMAP (0.04 g, 0.3 mmol) was added to a cooled (0 °C) solution of a *cis*/*trans*-mix of 4-*tert*-butylcyclohexyl amine (1.2 g, 8.5 mmol) in DMF (10 mL), followed by the addition of 5-aminoisatoic anhydride (1g, 5.6 mmol). The solution was stirred for 3 h at room temperature and then concentrated under reduced pressure. Water (10 mL) was added, and the mixture was stirred and extracted with EtOAc (3 × 20 mL). The combined organics were concentrated under reduced pressure, and the afforded crude was taken to the next step without further purification. MS: m/z 288 $[M + 1]^+$.

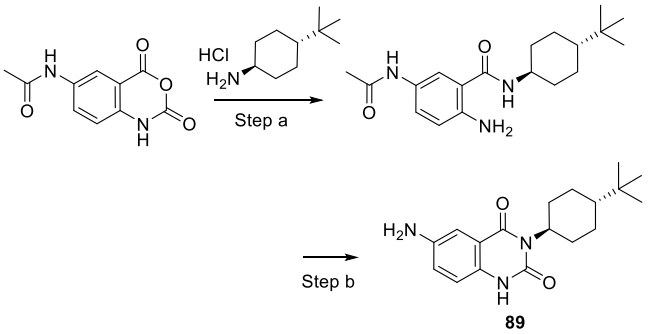
Step b: 6-Amino-3-(4-tert-butyl-cyclohexyl)-1H-quinazoline-2,4-dione (A Mixture of Diastereomers). The crudeamine from step a (0.9 g, 3.13 mmol) and ethyl chloroformate (3.4 mL) was heated at 90 °C for 1.5 h. The solvent was removed under reduced pressure, and the crude was dissolved in EtOH (33 mL). KOH (0.36 g, 6.26 mmol) was added, and the mixture was heated at 85 °C overnight. The solvent was removed under reduced pressure; water (10 mL) was added, and the mixture was extracted with EtOAc (3 × 20 mL). The combined organics were dried (Na_2SO_4), filtered, and concentrated. The afforded crude was purified by flash column chromatography on silica gel, which gave the title compound as a mixture of *cis*- and *trans*-isomers (0.3 g, 30%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.90 + 10.88 (s, 1H, each), 7.08 (d, J = 2 Hz, 1H), 6.92−6.85 (m, 2H), 5.15 (br s, 2H), 4.91 + 4.69 (m, 1H, each), 2.55−2.32 (m, 2H), 1.84−1.81 (m, 1H), 1.71−1.68 (m, 1H), 1.61−1.58 (m, 1H), 1.49−1.42 (m, 2H), 1.35−1.2 (m, 1H), 1.14−1.05 (m, 1H), 0.90 + 0.86 (s, 9H, each). MS: m/z 316 $[M + 1]^+$.

Separation of Diastereomers. The two diastereomers were separated by chiral prep. HPLC using a CHIRAL Phenomenox Lux Cellulose-4 (250 × 4.6) mm, 5 μm, flow = 1.0 mL/min, mobile phase A = hexanes/EtOH (70:30);

*6-Amino-3-(trans-4-tert-butyl-cyclohexyl)-1H-quinazoline-2,4-dione (**89**).* Chiral HPLC: t_R = 10.41 min. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.90 (s, 1H), 7.08 (s, 1H), 6.93−6.85 (m, 2H), 5.17 (br s, 2H), 4.73−4.65 (m, 1H), 2.50−2.40 (m, 2H), 1.84−1.80 (m, 2H), 1.62−1.58 (m, 2H), 1.10−1.04 (m, 3H), 0.86 (s, 9H).

*6-Amino-3-(cis-4-tert-butyl-cyclohexyl)-1H-quinazoline-2,4-dione (**90**).* Chiral HPLC: t_R = 8.19 min. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.88 (s, 1H), 7.08 (s, 1H), 6.93−6.85 (m, 2H), 5.14 (br s, 2H), 4.92−4.85 (m, 1H), 2.40−2.25 (m, 2H), 1.75−1.65 (m, 2H), 1.55−1.40 (m, 4H), 1.35−1.25 (m, 1H), 0.90 (s, 9H).

*Alternative Route for the Synthesis of 6-Amino-3-(trans-4-tert-butyl-cyclohexyl)-1H-quinazoline-2,4-dione (**89**).*



Step a: 5-Acetylamino-2-amino-N-(trans-4-tert-butyl-cyclohexyl)-benzamide. Et_3N (3.57 mL, 26 mmol) and *N*-(2,4-dioxo-1,4-dihydro-2*H*-benzo[*d*][1,3]oxazin-6-yl)acetamide (5 g, 22 mmol) were added to a solution of *trans*-4-*tert*-butyl-cyclohexylamine hydrochloride (6.5 g, 34 mmol) in DMF (40 mL) at 0 °C. The solution was stirred at room temperature for 3 h and then concentrated under reduced pressure, followed by addition of water and extraction with EtOAc (3 × 100 mL). The combined organics were washed with brine, dried (Na_2SO_4), filtered, and concentrated. The afforded crude was purified by flash column chromatography on silica gel, which gave the title compound (3.6 g, 49%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.57 (s, 1H), 7.93 (d, J = 2.4 Hz, 1H), 7.39 (d, J = 2.4 Hz, 1H), 7.33 (dd, J = 8.4, 2.4 Hz, 1H), 6.60 (d, J = 8.4 Hz, 1H), 5.91 (s, 2H), 3.64−3.60 (m, 1H), 1.95 (s, 3H), 1.86−1.84 (m, 2H), 1.76−1.73 (m, 2H), 1.32−1.21 (m, 2H), 1.09−0.9 (m, 3H), 0.84 (s, 9H). MS: m/z 332 $[M + 1]^+$.

*Step b: 6-Amino-3-(trans-4-tert-butyl-cyclohexyl)-1H-quinazoline-2,4-dione (**89**).* The intermediate above (3.2 g, 9.6 mmol) was taken up in ethyl chloroformate (11.84 mL) and heated to 90 °C for 1.5 h. The solvent was removed under reduced pressure, and the crude was dissolved in EtOH (64 mL), followed by the addition of KOH (4.4 g, 77 mmol) and heating at 85 °C for 16 h. The solvent was removed under reduced pressure, followed by the addition of water (100 mL) and extraction with EtOAc (3 × 100 mL). The combined organics were dried (Na_2SO_4), filtered, and concentrated. The crude was purified by flash column chromatography on silica gel, which gave the title compound (1.4 g, 46%) as a brown colored solid.

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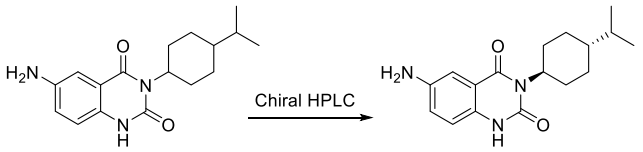
J. Med. Chem. XXXX, XXX, XXX−XXX



mixture of diastereomers **91**

6-Amino-3-(4-isopropylcyclohexyl)-1H-quinazoline-2,4-dione (Mixture of Diastereomers). The title compound was prepared from 5-aminoisatoic anhydride (1 g, 5.6 mmol) and 4-isopropylcyclohexyl amine (*cis*/*trans* mixture, 1.18 g, 8.5 mmol) according to the procedure described for **89** and **90** in the above steps a and b (0.4 g, 53%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.89 and 10.86 (s, 1H, each), 7.08 (s, 1H), 6.93−6.85 (m, 2H), 5.15 (s, 2H), 4.76−4.69 (m, 1H), 2.50−2.41 (m, 2H), 2.0−1.60 (m, 2H), 1.59−1.56 (m, 2H), 1.50−1.25 (m, 1H), 1.07−1.03 (m, 3H), 0.90 and 0.86 (d each, J = 6.8 Hz, 6H). MS: m/z 302 [M + 1]$^+$.

6-Amino-3-(trans-4-isopropylcyclohexyl)quinazoline-2,4(1H,3H)-dione (91). The *trans*-isomer was isolated by chiral prep HPLC using a CHIRAL PAK IC (250 × 4.6) mm, 5 μ, flow = 1.0 mL/min, mobile phase A = hexanes/IPA (90:10), t_R = 13.72 min. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.89 (s, 1H), 7.08 (d, J = 2.4 Hz, 1H), 6.93−6.8 (m, 2H), 5.15 (m, 2H), 4.73−4.65 (m, 1H), 2.50−2.40 (m, 2H), 1.77−1.75 (m, 2H), 1.59−1.56 (m, 2H), 1.45 (br m, 1H), 1.07−1.03 (m, 3H), 0.86 (d, J = 6.8 Hz, 6H). MS: m/z 302 [M + 1]$^+$.

6-Amino-3-(4-ethyl-cyclohexyl)-1H-quinazoline-2,4-dione, cis/trans Mixture (Mixture of Diastereomers) (92 and 93). The title compound was prepared from 5-aminoisatoic anhydride (1 g, 5.6 mmol) and 4-ethylcyclohexyl amine (*cis*, *trans* mix, 1.17 g, 8.5 mmol) according to the procedure described for **89** and **90** in the above steps a and b. Yield: 0.4 g, 51%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.89 and 10.88 (s, 1H, each), 7.08 (d, J = 2 Hz, 1H), 6.93−6.85 (m, 2H), 5.15 (s, 2H), 4.75−4.68 (m, 1H), 2.45−2.39 (m, 2H), 1.83−1.80 and 1.70−1.67 (m, 2H, each), 1.56−1.46 (m, 3H), 1.41−1.31 (m, 2H), 1.31−1.28 and 1.20 and 1.14 (m, 3H, each), 1.02−0.95 (m, 1H), 0.90−0.88 (m, 3H). MS: m/z 288 [M + 1]$^+$.

The two diastereomers were separated by chiral prep. HPLC using a CHIRAL Phenomenox Lux Cellulose-4 (250 × 4.6) mm, 5 μm, flow = 1.0 mL/min, mobile phase A = hexanes/EtOH (70:30).

6-Amino-3-(trans-4-ethyl-cyclohexyl)-1H-quinazoline-2,4-dione (92). Chiral HPLC: t_R = 8.32 min. ^{1}H NMR (400 MHz, MeOD,): δ 7.30 (d, J = 2.8 Hz, 1H), 7.07 (dd, J = 8.8, 2.8 Hz, 1H), 6.94 (d, J = 8.8 Hz, 1H), 4.85−4.81 (m, 1H), 2.60−2.50 (m, 2H), 1.93−1.90 (m, 2H), 1.67−1.64 (m, 2H), 1.32−1.26 (m, 3H), 1.12−1.04 (m, 2H), 0.94 (t, J = 8 Hz, 3H).

6-Amino-3-(cis-4-ethyl-cyclohexyl)-1H-quinazoline-2,4-dione (93). Chiral HPLC: t_R = 7.32 min. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.87 (s, 1H), 7.07 (d, J = 2 Hz, 1H), 6.94−6.85 (m, 2H), 5.15 (s, 2H), 4.76−4.69 (m, 1H), 2.60−2.50 (m, 2H), 1.71−1.67 (m, 2H), 1.53−1.45 (m, 5H), 1.32−1.25 (m, 2H), 0.88 (t, J = 7 Hz, 3H).

N-[3-(trans-4-tert-Butyl-cyclohexyl)-2,4-dioxo-1,2,3,4-tetrahydro-pyrido[2,3-d]pyrimidin-6-yl]-acetamide (94). See General Procedure F.

N-(3-(cis-4-(tert-Butyl)cyclohexyl)-2,4-dioxo-1,2,3,4-tetrahydropyrido[2,3-d]pyrimidin-6-yl)acetamide (95). The compound was prepared from **108** according to General Procedure F. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.79 (s, 1H), 10.29 (s, 1H), 8.67 (d, J = 2.4 Hz, 1H), 8.55 (d, J = 2.4 Hz, 1H), 4.89−4.86 (m, 1H), 2.20−2.32 (m, 2H), 2.08 (s, 3H), 1.76−1.70 (m, 2H), 1.52−1.46 (m, 4H), 1.33−1.30 (m, 1H), 0.86 (s, 9H). MS: m/z 359 [M + 1]$^+$.

N-(3-(trans-4-Isopropylcyclohexyl)-2,4-dioxo-1,2,3,4-tetrahydropyrido[2,3-d]pyrimidin-6-yl)acetamide (96). The compound was prepared from **109** according to General Procedure F. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.79 (s, 1H), 10.29 (s, 1H), 8.68 (d, J = 2.4 Hz, 1H), 8.54 (d, J = 2.4 Hz, 1H), 4.75−4.60 (m, 1H), 2.41−2.37 (m, 2H), 2.07 (s, 3H), 1.77 (m, 2H), 1.65−1.61 (m, 2H), 1.44 (m, 1H), 1.17−1.08 (m, 3H), 0.86 (d, J = 8 Hz, 6H). MS: m/z 345 [M + 1]$^+$.

N-(3-(cis-4-Isopropylcyclohexyl)-2,4-dioxo-1,2,3,4-tetrahydropyrido[2,3-d]pyrimidin-6-yl)acetamide (97). The compound was prepared from **110** according to General Procedure F. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.80 (s, 1H), 10.29 (s, 1H), 8.66 (d, J = 2.4 Hz, 1H), 8.54 (d, J = 2.4 Hz, 1H), 4.76−4.72 (m, 1H), 2.49−2.40 (m, 2H), 2.07 (s, 3H), 1.98−1.86 (m, 3H), 1.42−1.30 (m, 4H), 1.17−1.14 (m, 1H), 0.89 (d, J = 8 Hz, 6H). MS: m/z 345 [M + 1]$^+$.

N-(3-(trans-4-Ethylcyclohexyl)-2,4-dioxo-1,2,3,4-tetrahydropyrido[2,3-d]pyrimidin-6-yl)acetamide (98). The compound was prepared from **111** according to General Procedure F. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.79 (s, 1H), 10.30 (s, 1H), 8.68 (d, J = 2 Hz, 1H), 8.54 (d, J = 2 Hz, 1H), 4.72−4.65 (m, 1H), 2.41−2.37 (m, 2H), 2.08 (s, 3H), 1.83−1.80 (m, 2H), 1.58−1.55 (m, 2H), 1.25−1.10 (m, 3H), 1.0−0.9 (m, 2H), 0.87 (t, J = 7.2 Hz, 3H). MS: m/z 331 [M + 1]$^+$.

N-(3-(cis-4-Ethylcyclohexyl)-2,4-dioxo-1,2,3,4-tetrahydropyrido-[2,3-d]pyrimidin-6-yl)acetamide (99). The compound was prepared from **112** according to General Procedure F. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.80 (s, 1H), 10.29 (s, 1H), 8.68 (s, 1H), 8.55 (s, 1H), 4.67−4.70 (m, 1H), 2.51−2.49 (m, 2H), 2.08 (s, 3H), 1.71−1.67 (m, 2H), 1.54−1.48 (m, 5H), 1.37−1.30 (m, 2H), 0.88 (t, J = 6.8 Hz, 3H). MS: m/z 331 [M + 1]$^+$.

N-(3-(2-Cyclohexylethyl)-2,4-dioxo-1,2,3,4-tetrahydropyrido[2,3-d]pyrimidin-6-yl)acetamide (100). This compound was prepared according to General Procedures E and F from cyclohexylethylamine. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.88 (s, 1H), 10.30 (s, 1H), 8.69 (d, J = 2.4 Hz, 1H), 8.57 (d, J = 2.4 Hz, 1H), 3.90−3.87 (m, 2H), 2.08 (s, 3H), 1.76−1.64 (m, 5H), 1.47−1.44 (m, 2H), 1.22−1.11 (m, 4H), 0.95−0.87 (m, 2H). MS: m/z 331 [M + 1]$^+$.

2-Amino-5-nitronicotinic Acid Methyl Ester (102). See General Procedure E.

2-Amino-N-(trans-4-tert-butyl-cyclohexyl)-5-nitro-nicotinamide (103). See General Procedure E.

3-(trans-4-tert-Butyl-cyclohexyl)-6-nitro-1H-pyrido[2,3-d]-pyrimidine-2,4-dione (104). See General Procedure E.

6-Amino-3-(trans-4-tert-butyl-cyclohexyl)-1H-pyrido[2,3-d]-pyrimidine-2,4-dione (107). See General Procedure E.

6-Amino-3-(cis-4-(tert-butyl)-cyclohexyl)pyrido[2,3-d]-pyrimidine-2,4(1H,3H)-dione (108). This compound was prepared according to General Procedure E using *cis*-4-(*tert*-butyl)-cyclohexanamine. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.37 (s, 1H), 8.01 (d, J = 2.8 Hz, 1H), 7.44 (d, J = 2.8 Hz, 1H), 5.37 (s, 2H), 4.92−4.84 (m, 1H), 2.33−2.30 (m, 2H), 1.72−1.68 (m, 4H), 1.32−1.31 (m, 1H), 1.33−1.30 (m, 1H), 0.90 (s, 9H). MS: m/z 317 [M + 1]$^+$.

6-Amino-3-(trans-4-isopropylcyclohexyl)pyrido[2,3-d]-pyrimidine-2,4(1H,3H)-dione (109). This compound was prepared according to General Procedure E using *trans*-4-isopropyl-cyclohexanamine. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.37 (s, 1H), 8.01 (d, J = 2.8 Hz, 1H), 7.44 (d, J = 2.8 Hz, 1H), 5.37 (s, 2H), 4.70−4.63 (m, 1H), 2.45−2.34 (m, 2H), 1.78−1.74 (m, 2H), 1.62−1.50 (m, 2H), 1.45−1.43 (m, 1H), 1.08−1.04 (m, 3H), 0.86 (d, J = 8 Hz, 6H). MS: m/z 303 [M + 1]$^+$.

6-Amino-3-(cis-4-isopropylcyclohexyl)pyrido[2,3-d]pyrimidine-2,4(1H,3H)-dione (110). This compound was prepared according to General Procedure E using *cis*-4-isopropyl-cyclohexanamine. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.37 (s, 1H) 8.01 (d, J = 2.8 Hz, 1H), 7.42 (d, J = 2.8 Hz, 1H), 5.37 (s, 2H), 4.75−4.69 (m, 1H), 2.49−2.40 (m, 2H), 1.97−1.85 (m, 3H), 1.42−1.30 (m, 4H), 1.15−1.13 (m, 1H), 0.86 (d, J = 8 Hz, 6H). MS: m/z 303 [M + 1]$^+$.

6-Amino-3-(trans-4-ethylcyclohexyl)pyrido[2,3-d]pyrimidine-2,4-(1H,3H)-dione (111). This compound was prepared according to General Procedure E using *trans*-4-ethyl-cyclohexanamine. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.38 (s, 1H), 8.02 (d, J = 2.8 Hz, 1H), 7.44 (d, J = 2.8 Hz, 1H), 5.37 (s, 2H), 4.72−4.65 (m, 1H), 2.41−2.37 (m, 2H), 1.83−1.80 (m, 2H), 1.58−1.55 (m, 2H), 1.25−1.10 (m, 3H), 1.0−0.9 (m, 2H), 0.87 (t, J = 7.2 Hz, 3H). MS: m/z 289 [M + 1]$^+$.

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J. Med. Chem. XXXX, XXX, XXX−XXX

*6-Amino-3-(cis-4-ethylcyclohexyl)pyrido[2,3-d]pyrimidine-2,4-(1H,3H)-dione (**112**).* This compound was prepared according to General Procedure E using *cis*-4-ethyl-cyclohexanamine. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.38 (s, 1H), 8.02 (d, J = 2.8 Hz, 1H), 7.44 (d, J = 2.8 Hz, 1H), 5.37 (s, 2H), 4.72−4.65 (m, 1H), 2.41−2.37 (m, 2H), 1.83−1.80 (m, 2H), 1.58−1.55 (m, 2H), 1.25−1.10 (m, 3H), 1.0−0.9 (m, 2H), 0.87 (t, J = 7.2 Hz, 3H). MS: m/z 289 $[M + 1]^+$.

Activity against *T. cruzi*. Rat skeletal myoblasts (L-6 cells, ATCC CRL1458) were seeded in 96-well microtiter plates at 2000 cells/well in 100 μL of the RPMI 1640 medium with 10% FBS and 2 mM L-glutamine. After 24 h, the medium was removed and replaced by 100 μL per well containing 5000 trypomastigote forms of the *T. cruzi* Tulahuen strain C2C4 containing the β-galactosidase (Lac Z) gene (Buckner et al. 1996). After 48 h, the medium was removed from the wells and replaced by 100 μL of fresh medium with or without a serial drug dilution of 11 3-fold dilution steps covering a range from 100 to 0.002 μg/mL. After 96 h of incubation, the plates were inspected under an inverted microscope to ensure the growth of the controls and sterility. Then, the substrate CPRG/Nonidet (50 μL) was added to all wells. A color reaction developed within 2−6 h and could be read photometrically at 540 nm. Data were analyzed with the graphic program Softmax Pro (Molecular Devices), which calculated EC_{50} values by linear regression and 4-parameter logistic regression from the sigmoidal dose inhibition curves. Benznidazole is used as a control.

***In Vitro* Cytotoxicity with L-6 Cells.** Assays were performed in 96-well microtiter plates, each well containing 100 μL of RPMI 1640 medium supplemented with 1% L-glutamine (200 mM) and 10% fetal bovine serum, and 4000 L-6 cells (rat skeletal muscle myoblasts; ATCC CRL1458) were seeded in 96-well plates. Serial drug dilutions of 11 3-fold dilution steps covering a range from 100 to 0.002 μg/mL were prepared. After 70 h of incubation, the plates were inspected under an inverted microscope to ensure the growth of the controls and sterile conditions. Ten μL of resazurin solution (12.5 mg of resazurin dissolved in 100 mL of distilled water) was then added to each well, and the plates were incubated for another 2 h. Then the plates were read with a Spectramax Gemini XS microplate fluorometer (Molecular Devices Cooperation, Sunnyvale, CA, USA) using an excitation wavelength of 536 nm and an emission wavelength of 588 nm. The IC_{50} values were calculated by linear regression and 4-parameter logistic regression from the sigmoidal dose inhibition curves using SoftmaxPro software (Molecular Devices Cooperation, Sunnyvale, CA, USA). Podophyllotoxin (Sigma P4405) was used as a control.

Aqueous Solubility by Nephelometry. The kinetic aqueous solubility of the test compounds was measured using laser nephelometry. Compounds were subject to serial dilution from 10 mg/mL to 0.5 mg/mL in DMSO. An aliquot was then mixed with Milli-Q water to obtain an aqueous dilution plate with a final concentration range of 100−5 ug/mL, with a final DMSO concentration of 1.0%. Triplicate aliquots were transferred to a flat-bottomed polystyrene plate, which was immediately read on the NEPHELOstar (BMG Lab Technologies). The amount of laser scatter caused by insoluble particulates (relative nephelometry units, RNU) was plotted against the compound concentration using a segmental regression fit, with the point of inflection being quoted as the compounds aqueous solubility (μg/mL).

The aqueous solubility of the test compounds was measured using laser nephelometry. Compounds were subject to serial dilution from 10 mM to 0.5 mM in DMSO. An aliquot was then mixed with Milli-Q water to obtain an aqueous dilution plate with a final concentration range of 250−12 uM, with a final DMSO concentration of 2.5%. Triplicate aliquots were transferred to a flat bottomed polystyrene plate, which was immediately read on the NEPHELOstar (BMG Lab Technologies). The amount of laser scatter caused by insoluble particulates (relative nephelometry units, RNU) was plotted against compound concentration using a segmental regression fit, with the point of inflection being quoted as the compound aqueous solubility (μM).

Intrinsic Clearance (Cli) Experiments. The test compound (0.5 μM) was incubated with female CD1 mouse liver microsomes (Xenotech LLC; 0.5 mg/mL 50 mM potassium phosphate buffer, pH 7.4), and the reaction was started with the addition of excess NADPH (8 mg/mL 50 mM potassium phosphate buffer, pH 7.4). Immediately, at time zero, then at 3, 6, 9, 15, and 30 min, an aliquot (50 μL) of the incubation mixture was removed and mixed with acetonitrile (100 μL) to stop the reaction. The internal standard was added to all samples, the samples centrifuged to sediment precipitated protein, and the plates were then sealed prior to UPLCMSMS analysis using a Quattro Premier XE (Waters Corporation, USA).

XLfit (IDBS, U.K.) was used to calculate the exponential decay and, consequently, the rate constant (k) from the ratio of peak area of the test compound to the internal standard at each time point. The rate of intrinsic clearance (CLi) of each test compound was then calculated using the following calculation:

$$\text{CLi (mL/min /g liver)} = k \times V \times \text{microsomal protein yield}$$

where V (mL/mg protein) is the incubation volume/mg protein added, and the microsomal protein yield is taken as 52.5 mg protein/g liver. Verapamil (0.5 μM) was used as a positive control to confirm acceptable assay performance.

***In Vivo* Pharmacokinetics.** *Female NMR1 Mouse.* Test compound **43** was dosed intraperitoneally or orally by gavage as a solution at 10 mg free base/kg (dose volume 10 mL/kg; dose vehicle, 10% (v/v) dimethyl sulfoxide (DMSO), 60% polyethylene glycol 400, and 30% deionized water); alternatively, compound **89** was dosed intraperitoneally as a solution or suspension at 10 mg free base/kg, respectively, or orally by gavage as a suspension at 10 mg free base/kg (dose volume 10 mL/kg; dose vehicle 10% (v/v) dimethyl sulfoxide (DMSO), 60% polyethylene glycol 400, and 30% deionized water or 0.5% w/v hydroxypropylmethylcellulose (HPMC) with 0.4% v/v Tween 80 and 0.5% v/v benzyl alcohol) to female Balb/c mice (n = 3). Blood samples (10 μL) were taken from each mouse at 0.08, 0.25, 0.5, 1, 2, 4, 6, and 8 h post dose and mixed with two volumes of distilled water (20 μL). After suitable sample preparation, the concentration of test compounds in blood was determined by UPLC−MS/MS using a Quattro Premier XE (Waters, USA). Pharmacokinetic parameters were derived from the mean blood concentration−time curve using PK solutions software v 2.0 (Summit Research Services, USA).

***In Vivo* Efficacy.** Trypomastigote forms from transgenic *T. cruzi* Brazil strain expressing firefly Luciferase were diluted in PBS and injected IP in Balb/c mice (10^5 trypomastigotes per mouse). Three days after infection, the mice were anesthetized by either IP injection of 300 mg/kg of xylazine and 3500 mg/kg of ketamine or by inhalation of isofluorane (controlled flow of 1.5% isofluorane in the air was administered through a nose cone via a gas anesthesia system). Mice were injected with 150 mg/kg of D-luciferin potassium-salt (Goldbio) dissolved in PBS. Mice were imaged 5−10 min after injection of luciferin with an IVIS 100 (Xenogen, Alameda, CA), and the data acquisition and analysis were performed with the software LivingImage (Xenogen). One day later (4 days after infection), treatment with compounds **43** and compound **89** or vehicle control (PEG) was started by IP injection in groups of 5 mice and continued daily for 10 days. On days 5 and 10 after treatment started, mice were imaged again after anesthesia and injection of luciferin as described above. The ratio of infection is calculated, dividing parasite levels (luciferase signal) in each mouse at a particular day of treatment by the level before treatment (day 3 of infection).

Ethical Statements. All regulated procedures on living animals in Dundee were carried out under the authority of a project license issued by the Home Office under the Animals (Scientific Procedures) Act 1986, as amended in 2012 (and in compliance with EU Directive EU/2010/63). License applications have been approved by the University's Ethical Review Committee (ERC) before submission to the Home Office. The ERC has a general remit to develop and oversee policy on all aspects of the use of

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J. Med. Chem. XXXX, XXX, XXX−XXX

animals on University premises and is a subcommittee of the University Court, its highest governing body.

This study was carried out in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health, U.S. The protocol was approved by the Institutional Animal Care and Use Committee of New York University School of Medicine, which is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jmedchem.9b01852.

HPLC traces of key compounds (PDF)

Molecular strings for all final compounds (CSV)

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Notes

The authors declare the following competing financial interest(s): Some of the authors were employees of companies while this work was undertaken. This is indicated in the address list.

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■ ABBREVIATIONS USED

HPMC, hydroxypropyl methylcellulose; LLE, lipophilic ligand efficiency; MC, methylcellulose; nd, not determined; PEG, polyethylene glycol

■ REFERENCES

(1) Lidani, K. C. F.; Andrade, F. A.; Bavia, L.; Damasceno, F. S.; Beltrame, M. H.; Messias-Reason, I. J.; Sandri, T. L. Chagas Disease: From Discovery to a Worldwide Health Problem. *Front. Public Health* **2019**, *7*, 166.

(2) Hollingsworth, T. D.; Adams, E. R.; Anderson, R. M.; Atkins, K.; Bartsch, S.; Basanez, M. G.; Behrend, M.; Blok, D. J.; Chapman, L. A.; Coffeng, L.; Courtenay, O.; Crump, R. E.; de Vlas, S. J.; Dobson, A.; Dyson, L.; Farkas, H.; Galvani, A. P.; Gambhir, M.; Gurarie, D.; Irvine, M. A.; Jervis, S.; Keeling, M. J.; Kelly-Hope, L.; King, C.; Lee, B. Y.; Le Rutte, E. A.; Lietman, T. M.; Ndeffo-Mbah, M.; Medley, G. F.; Michael, E.; Pandey, A.; Peterson, J. K.; Pinsent, A.; Porco, T. C.; Richardus, J. H.; Reimer, L.; Rock, K. S.; Singh, B. K.; Stolk, W.; Swaminathan, S.; Torr, S. J.; Townsend, J.; Truscott, J.; Walker, M.; Zoueva, A. Quantitative Analyses and Modelling to Support Achievement of the 2020 Goals for Nine Neglected Tropical Diseases. *Parasites Vectors* **2015**, *8*, 630.

(3) De Rycker, M.; Baragana, B.; Duce, S. L.; Gilbert, I. H. Challenges and Recent Progress in Drug Discovery for Tropical Diseases. *Nature* **2018**, *559*, 498−506.

(4) CDC. https://www.cdc.gov/parasites/chagas/biology.html.

(5) WHO. https://apps.who.int/iris/bitstream/handle/10665/259404/9789241513166-eng.pdf?sequence=1=y

(6) Silva-Dos-Santos, D.; Barreto-De-Albuquerque, J.; Guerra, B.; Moreira, O. C.; Berbert, L. R.; Ramos, M. T.; Mascarenhas, B. A. S.; Britto, C.; Morrot, A.; Serra Villa-Verde, D. M.; Garzoni, L. R.; Savino, W.; Cotta-de-Almeida, V.; de Meis, J. Unraveling Chagas Disease Transmission through the Oral Route: Gateways to *Trypanosoma cruzi* Infection and Target Tissues. *PLoS Neglected Trop. Dis.* **2017**, *11*, No. e0005507.

(7) Filigheddu, M. T.; Gorgolas, M.; Ramos, J. M. Orally-Transmitted Chagas Disease. *Med. Clin. (Barcelona)* **2017**, *148*, 125−131.

(8) Sanchez-Valdez, F. J.; Padilla, A.; Wang, W.; Orr, D.; Tarleton, R. L. Spontaneous Dormancy Protects *Trypanosoma Cruzi* During Extended Drug Exposure. *eLife* **2018**, *7*, No. e34039.

(9) Field, M. C.; Horn, D.; Fairlamb, A. H.; Ferguson, M. A.; Gray, D. W.; Read, K. D.; De Rycker, M.; Torrie, L. S.; Wyatt, P. G.; Wyllie, S.; Gilbert, I. H. Anti-Trypanosomatid Drug Discovery: An Ongoing Challenge and a Continuing Need. *Nat. Rev. Microbiol.* **2017**, *15*, 217−231.

(10) Barrett, M. P.; Croft, S. L. Management of Trypanosomiasis and Leishmaniasis. *Br. Med. Bull.* **2012**, *104*, 175−196.

(11) Morillo, C. A.; Marin-Neto, J. A.; Avezum, A.; Sosa-Estani, S.; Rassi, A.; Rosas, F.; Villena, E.; Quiroz, R.; Bonilla, R.; Britto, C.; Guhl, F.; Velazquez, E.; Bonilla, L.; Meeks, B.; Rao-Melacini, P.; Pogue, J.; Mattos, A.; Lazdins, J.; Rassi, A.; Connolly, S. J.; Yusuf, S. Randomized Trial of Benznidazole for Chronic Chagas' Cardiomyopathy. *N. Engl. J. Med.* **2015**, *373*, 1295−1306.

(12) DNDi. https://www.dndi.org/wp-content/uploads/2019/03/2page_BenditatStudyOverview_ENG.pdf.

https://dx.doi.org/10.1021/acs.jmedchem.9b01852
J. Med. Chem. XXXX, XXX, XXX−XXX

(13) DNDi. https://www.dndi.org/diseases-projects/portfolio/new-benz-regimens/.
(14) Morillo, C. A.; Waskin, H.; Sosa-Estani, S.; del Carmen Bangher, M.; Cuneo, C.; Milesi, R.; Mallagray, M.; Apt, W.; Beloscar, J.; Gascon, J.; Molina, I.; Echeverria, L. E.; Colombo, H.; Perez-Molina, J. A.; Wyss, F.; Meeks, B.; Bonilla, L. R.; Gao, P.; Wei, B.; McCarthy, M.; Yusuf, S.; Morillo, C.; Sosa-Estani, S.; Waskin, H.; Meeks, B.; Yusuf, S.; Diaz, R.; Acquatella, H.; Lazzari, J.; Roberts, R.; Traina, M.; Meeks, B.; Bonilla, L. R.; Gao, P.; Taylor, A.; Holadyk-Gris, I.; Whalen, L.; Bangher, M.C.; Romero, M.A.; Prado, N.; Hernandez, Y.; Fernandez, M.; Riarte, A.; Scollo, K.; Lopez-Albizu, C.; Cuneo, C.A.; Gutierrez, N.C.; Milesi, R.R.; Berli, M.A.; Mallagray, M.H.; Caceres, N.E.; Beloscar, J.S.; Petrucci, J.M.; Colombo, H.; Dellatorre, M.; Prado, A.; Apt, W.; Zulantay, I.; Echeverria, L.E.; Isaza, D.; Reyes, E.; Wyss, F.S.; Figueroa, A.; Guzman Melgar, I.; Rodriguez, E.; Gascon, J.; Aldasoro, E.; Posada, E.J.; Serret, N.; Molina, I.; Sanchez-Montalva, A.; Perez-Molina, J.A.; Lopez-Velez, R.; Reyes-Lopez, P.A. Benznidazole and Posaconazole in Eliminating Parasites in Asymptomatic *T. cruzi* Carriers: The Stop-Chagas Trial. *J. Am. Coll. Cardiol.* **2017**, *69*, 939−947.
(15) Torrico, F.; Gascon, J.; Ortiz, L.; Alonso-Vega, C.; Pinazo, M.-J.; Schijman, A.; Almeida, I. C; Alves, F.; Strub-Wourgaft, N.; Ribeiro, I.; Santina, G.; Blum, B.; Correia, E.; Garcia-Bournisen, F.; Vaillant, M.; Morales, J. R.; Pinto Rocha, J. J.; Rojas Delgadillo, G.; Magne Anzoleaga, H. R.; Mendoza, N.; Quechover, R. C.; Caballero, M. Y. E.; Lozano Beltran, D. F.; Zalabar, A. M.; Rojas Panozo, L.; Palacios Lopez, A.; Torrico Terceros, D.; Fernandez Galvez, V. A.; Cardozo, L.; Cuellar, G.; Vasco Arenas, R. N.; Gonzales, I.; Hoyos Delfin, C. F.; Garcia, L.; Parrado, R.; de la Barra, A.; Montano, N.; Villarroel, S.; Duffy, T.; Bisio, M.; Ramirez, J. C.; Duncanson, F.; Everson, M.; Daniels, A.; Asada, M.; Cox, E.; Wesche, D.; Diderichsen, P. M.; Marques, A. F; Izquierdo, L.; Sender, S. S.; Reverter, J. C.; Morales, M.; Jimenez, W. Treatment of Adult Chronic Indeterminate Chagas Disease with Benznidazole and Three E1224 Dosing Regimens: A Proof-of-Concept, Randomised, Placebo-Controlled Trial. *Lancet Infect. Dis.* **2018**, *18*, 419−430.
(16) Molina, I.; Gomez i Prat, J.; Salvador, F.; Trevino, B.; Sulleiro, E.; Serre, N.; Pou, D.; Roure, S.; Cabezos, J.; Valerio, L.; Blanco-Grau, A.; Sanchez-Montalva, A.; Vidal, X.; Pahissa, A. Randomized Trial of Posaconazole and Benznidazole for Chronic Chagas' Disease. *N. Engl. J. Med.* **2014**, *370*, 1899−1908.
(17) DNDi. Https://Www.Dndi.Org/Diseases-Projects/Portfolio/Fexinidazole-Chagas/.
(18) DNDi. Https://Www.Dndi.Org/Diseases-Projects/Portfolio/.
(19) Murray, C. W.; Erlanson, D. A.; Hopkins, A. L.; Keseru, G. M.; Leeson, P. D.; Rees, D. C.; Reynolds, C. H.; Richmond, N. J. Validity of Ligand Efficiency Metrics. *ACS Med. Chem. Lett.* **2014**, *5*, 616−618.
(20) Velcicky, J.; Soicke, A.; Steiner, R.; Schmalz, H. G. Palladium-Catalyzed Cyanomethylation of Aryl Halides through Domino Suzuki Coupling-Isoxazole Fragmentation. *J. Am. Chem. Soc.* **2011**, *133*, 6948−6951.
(21) Matsumura, Y.; Yamamoto, Y.; Moriyama, N.; Furukubo, S.; Iwasaki, F.; Onomura, O. Efficient Oxidation of Cycloalkanols by Sodium Nitrite with Molecular Oxygen in Trifluoroacetic Acid. *Tetrahedron Lett.* **2004**, *45*, 8221−8224.
(22) Renz, M. Ketonization of Carboxylic Acids by Decarboxylation: Mechanism and Scope. *Eur. J. Org. Chem.* **2005**, *2005*, 979−988.
(23) Bartoli, G.; Bosco, M.; Locatelli, M.; Marcantoni, E.; Melchiorre, P.; Sambri, L. Unusual and Unexpected Reactivity of T-Butyl Dicarbonate (Boc2o) with Alcohols in the Presence of Magnesium Perchlorate. A New and General Route to T-Butyl Ethers. *Org. Lett.* **2005**, *7*, 427−430.
(24) Lewis, M. D.; Fortes Francisco, A.; Taylor, M. C.; Burrell-Saward, H.; McLatchie, A. P.; Miles, M. A.; Kelly, J. M. Bioluminescence Imaging of Chronic *Trypanosoma cruzi* Infections Reveals Tissue-Specific Parasite Dynamics and Heart Disease in the Absence of Locally Persistent Infection. *Cell. Microbiol.* **2014**, *16*, 1285−1300.
(25) MacLean, L. M.; Thomas, J.; Lewis, M. D.; Cotillo, I.; Gray, D. W.; De Rycker, M. Development of *Trypanosoma cruzi in vitro* Assays to Identify Compounds Suitable for Progression in Chagas' Disease Drug Discovery. *PLoS Neglected Trop. Dis.* **2018**, *12*, No. e0006612.
(26) Matsumura, Y.; Yamamoto, Y.; Moriyama, N.; Furukubo, S.; Iwasaki, F.; Onomura, O. Efficient Oxidation of Cycloalkanols by Sodium Nitrite with Molecular Oxygen in Trifluoroacetic Acid. *Tetrahedron Lett.* **2004**, *45*, 8221−8224.
(27) Wall, R. J.; Carvalho, S.; Milne, R.; Bueren-Calabuig, J. A.; Moniz, S.; Cantizani-Perez, J.; MacLean, L.; Kessler, A.; Cotillo, I.; Sastry, L.; Manthri, S.; Patterson, S.; Zuccotto, F.; Thompson, S.; Martin, J.; Marco, M.; Miles, T. J.; De Rycker, M.; Thomas, M. G.; Fairlamb, A. H.; Gilbert, I. H.; Wyllie, S. The Qi Site of Cytochrome b is a Promiscuous Drug Target in *Trypanosoma cruzi* and *Leishmania donovani*. *ACS Infect. Dis.* **2020**, DOI: 10.1021/acsinfecdis.9b00426.

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