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Chagas Disease Drug Discovery: Multiparametric Lead Optimization against *Trypanosoma cruzi* in Acylaminobenzothiazole Series

Charlotte Fleau,[†] Angel Padilla,[§] Juan Miguel-Siles,[‡] Maria T. Quesada-Campos,[‡] Isabel Saiz-Nicolas,[‡] Ignacio Cotillo,[‡] Juan Cantizani Perez,[‡] Rick L. Tarleton,*[,§] Maria Marco,*[,‡] and Gilles Courtemanche*[,†]

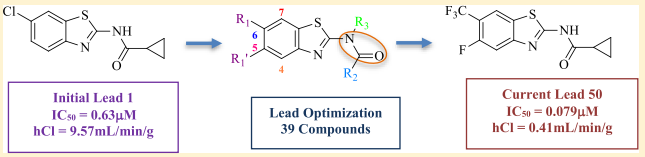
[†]BIOASTER, Microbiology Technology Institute, 28 rue du Docteur Roux, 75015 Paris, Ile-de-France, France

[‡]Global Health R&D, GlaxoSmithKline, Calle Severo Ochoa 2, 28760 Tres Cantos, Madrid, Spain

[§]Center for Tropical and Emerging Global Infectious Diseases and Department of Cellular Biology, University of Georgia, 30602 Athens, Georgia, United States

Supporting Information

ABSTRACT: Acylaminobenzothiazole hits were identified as potential inhibitors of *Trypanosoma cruzi* replication, a parasite responsible for Chagas disease. We selected compound **1** for lead optimization, aiming to improve in parallel its anti-*T. cruzi* activity (IC_{50} = 0.63 μM) and its human metabolic stability (human clearance = 9.57 mL/min/g). A total of 39 analogues of **1** were synthesized and tested in vitro. We established a multiparametric structure−activity relationship, allowing optimization of antiparasite activity, physicochemical parameters, and ADME properties. We identified compound **50** as an advanced lead with an improved anti-*T. cruzi* activity in vitro (IC_{50} = 0.079 μM) and an enhanced metabolic stability (human clearance = 0.41 mL/min/g) and opportunity for the oral route of administration. After tolerability assessment, **50** demonstrated a promising in vivo efficacy.



INTRODUCTION

Chagas disease is a neglected tropical disease caused by a protozoan kinetoplastid parasite, *Trypanosoma cruzi*. A total of 70 million people live in areas at risk of contracting the disease, and approximately 6−7 million are actually infected. Chagas disease kills more than 10 000 people a year,[1] but is also responsible for substantial disabilities with significant social and economic impacts (unemployment, loss of resources, and a vicious circle of poverty).[2] *T. cruzi* is transmitted to humans by an insect of the family Reduviidae, trivially known as the "kissing bug", during his blood meal.[3] More precisely, *T. cruzi* is frequently transmitted when the vector insect bites the host and defecates near its bite.[4] The trypomastigote, a non-replicative but mobile and infectious form can then infect host cells. Inside the cells, the parasite transforms to an amastigote form and replicates by binary fission. Once the host cell is saturated with amastigotes (usually 4−5 days postinfection), the parasites convert back into trypomastigotes and leave the cell, spreading into the bloodstream and other host tissues.[5,6] Blood transfusion, organ transplants, congenital transmission, and ingestion of contaminated food are other means of infection.[7]

The acute phase of *T. cruzi* infection, during which parasites can often be detected in the blood, is often asymptomatic and resolved by immune recognition and control of the parasite load within a few months.[5] The majority of the infected subjects will continue their life unaffected. However, some, estimated at approximately 30%, will progress to chronic disease some 10−25 years after the initial infection.[8,9] Such individuals might experience severe cardiac and/or digestive changes that, if left unmanaged, can end in death. Chagas disease is today the leading cause of cardiomyopathy in the world.

There is no vaccine, and the currently available drugs, benznidazole and nifurtimox, were discovered more than 40 years ago. Their effectiveness has been demonstrated consistently in acute infections but efficacy in chronically infected subjects is less dependable.[10] Treatment duration is frequently long (60−90 days), and treated subjects may suffer from side effects which could be quite serious.[11,12] In the 2010s, a new class of molecules, CYP51 inhibitors (such as posaconazole and E1224, the prodrug of ravuconazole) reached clinical development. Unfortunately, they proved to be ineffective in humans[13] with treatment failure in patients reaching at least 70%, as opposed to maximum 30% failure for benznidazole-treated patients,[14] and thus, they were all abandoned, leaving the R&D pipeline nearly completely empty today.

Endemic in 21 Latin American countries, Chagas disease is now spreading beyond the American continent to Europe, Japan, Australia, and North America because of the globalization of movements of people carrying the parasite without

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knowing.[8] At the same time, global warming widens the potential housing area of insect vectors.[15]

That is why, it is now urgent to revive research to fight Chagas disease, with the ambitious goal of discovering an effective and safe molecule, active in both phases, and compatible with the field.

RESULTS AND DISCUSSION

From Hit Identification to Lead Selection. We selected the acylaminobenzothiazole (AAB) series from an HTS campaign performed in 2009 by the Broad Institute on the NIH compound collection. 303 286 compounds were tested in a luminescence cell-based/microorganism bioassay to identify 4065 inhibitors of *T. cruzi* replication (AID 1885).[16] Our selection criteria was *T. cruzi* activity < 1.2 μM, selectivity index (SI) versus host cell > 100,[17] following the Lipinski rule of 5,[18] novel chemotypes (i.e., not already described in the literature or in patents, by SCIfinder search) and amenable to further optimization (structure−activity relationship with three points of diversity), resulting in 300 compounds which were progressed to rapid in vivo assay. Only 171 compounds out of the total 300 resulting selection were available in an amount compatible with 2 days dosing in vivo activity (IVA) assessment and seven of these demonstrated an efficacy similar to benznidazole. Three of them were from the same AAB series (CID 673490, CID 16194665 and CID 12006028) (Figure 1).

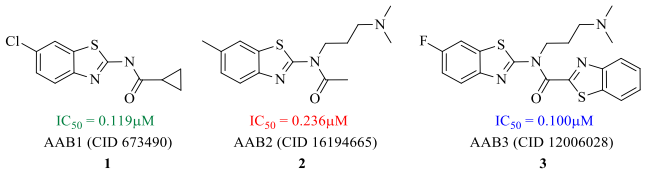

Figure 1. Structure of the AAB compounds selected from HTS to in vivo screening.

Because of its in vitro and in vivo antiparasitic activities (Table 1, Figure 2), compound **1** was selected to be further profiled. Compound **1** is readily synthesized in one step from cheap raw materials (Scheme 1).

Table 1. In Vitro Parasitology Determined by GSK for Compound 1

test	results
T. cruzi growth inhibition IC_{50} (μM)	0.63
cytotoxicity (μM)	>50 (H9C2)
SI = TC_{50}/IC_{50}	>80

In vitro metabolite identification[19] demonstrated a different behavior of compound **1** when subjected to human and mice hepatocytes. While mouse hepatocytes induced a hydrolysis of the amide bond, human hepatocytes induced a cascade of hydroxylation, epoxidation, and dichlorination reactions on the benzo ring (Figure 3).

With these data in hand, began the optimization of the AAB series, with the objective of improving their anti-*T. cruzi* potency and metabolic stability.

General Strategy. SAR exploration was carried out, varying substituents at R1, R2, and R3 positions. Initially, varying substitution at R1 with different electron withdrawing and electron donating groups and displacing chlorine atoms were investigated. Second, substitution of the cyclopropane by diverse groups R2 was evaluated. Third, the amide bond between the thiazole and the R2 group was replaced by different linkers, and the importance of the hydrogen was evaluated by its substitution by alkyl groups (Figure 4). Finally, after determining the best substituents, the disubstitution of the aromatic core was explored in order to increase metabolic stability by blocking position 5.

Chemistry. SAR exploration was focussed on finding the best position for the chlorine atom in the benzo group in terms of the antiparasite activities of the AAB series. Hence, AAB analogues substituted at positions 4, 5, and 7 were synthesized in one to four steps (Scheme 2). Precursor **8** was prepared in three steps using 3-chloro-2-iodophenylamine **5** as the starting material. Compound **5** was converted into 1-benzoyl 3-(3′-chloro-2′-iodo phenyl)thiourea **6** which was treated by K_2CO_3 to obtain 1-(3-chloro-2-iodophenyl)thiourea **7** followed by a copper-catalyzed cyclization reaction to furnish the desired aminobenzothiazole **8**.[20,21] Aminobenzothiazole **10** was obtained from 3-chloroaniline **9** and potassium thiocyanate using a process commonly described in the literature.[22,23] Then, AAB **12**, **13**, and **14** were obtained by reacting precursor **8**, **10**, and **11** with cyclopropanecarbonyl chloride, respectively.

The electronic effect of the aromatic substituent on potency was studied by synthesizing several AABs with different electron withdrawing and donating groups at position 6. This synthesis was accomplished from the corresponding aniline following a well-known literature procedure which yielded compounds **17** to **21** in only two steps (Scheme 3).[24,25] Compounds **22** to **26** were obtained in one step from the corresponding aminobenzothiazole.

Exploration of the cyclopropyl group replacement was also considered in order to improve the potency of **1**. Several R2 groups were envisaged as heterocycle, linear, ramified, and alicyclic carbon chains. Two procedures were used depending on the availability of starting materials (Scheme 4). Aminobenzothiazole **4** was converted into acylaminobenzo-thiazole **27** to **30** in the presence of the corresponding acyl chloride reagent, as described previously. N acylation of **4** was accomplished by activation of the corresponding carboxylic acid with 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo-[4,5-*b*]pyridinium 3-oxide hexafluorophosphate (HATU) in the presence of Hunig's base to furnish final compounds **31** to **34**.[26]

To determine the importance of the hydrogen of the amide bond, it was replaced by different alkyl groups as methyl, ethyl, and butyl (Scheme 5). Alkylaminobenzothiazole **37a−c** were obtained in four steps starting from the commercially available 6-chloroaminobenzothiazole **4** which was first protected using di-*tert*-butyl dicarbonate (Boc anhydride) to get **35**. Protected intermediate **35** was then subjected to N-alkylation to get compounds **36a** to **36c** which were then deprotected with trifluoracetic acid (TFA) to get N-alkylated aminobenzothiazoles **37a−c**. Additionally, the introduction of a more polar ether alkyl chain was considered. As such, **37d** was obtained in one step by reacting **4** with 2-bromoethanol. Then, N-alkylated aminobenzothiazole was engaged in the amide bond formation reaction using conditions described in Scheme 2.

The effect of the amide bond replacement on the anti-*T. cruzi* activity of AAB derivatives was investigated by preparing compounds **42**, **44**, and **45** (Scheme 6). Reduction of the amide bond of **1** using lithium aluminum hydride yielded secondary amine **42**.[27] Compound **44** was synthesized in two

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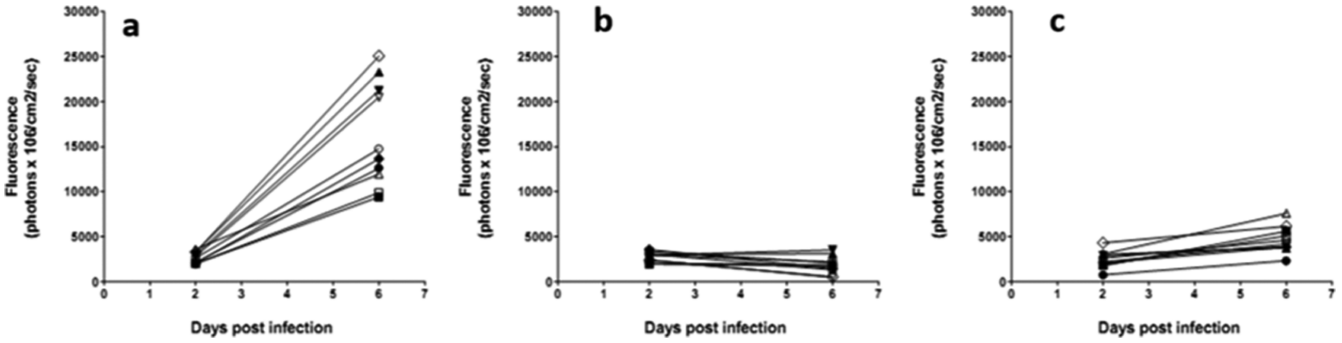
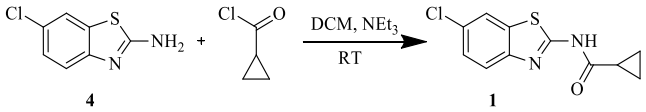


Figure 2. Rapid in vivo test. (a) Untreated mice. (b) Mice treated with benznidazole. (c) Mice treated with CID 673490.

Scheme 1. Synthesis Route for CID 673490 (1)



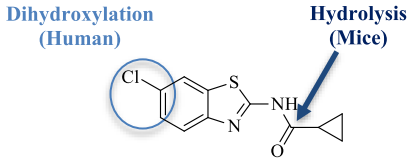


Figure 3. Metabolite identification of **1** in mice and in human.

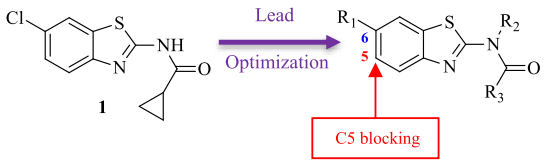
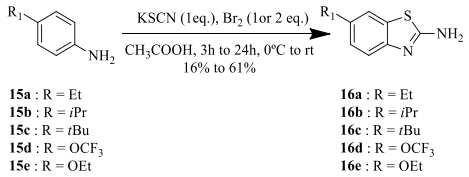
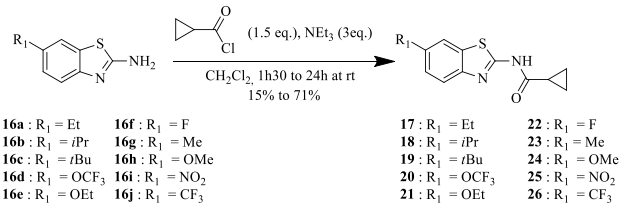


Figure 4. General strategy for lead optimization.

steps by conversion of **16j** to benzothiazol-2-hydrazine **43** followed by hydrazone formation in the presence of cyclopropanecarboxaldehyde. Sulfonamide **45** was obtained in one step by reacting **16j** with cyclopropanesulfonyl chloride.

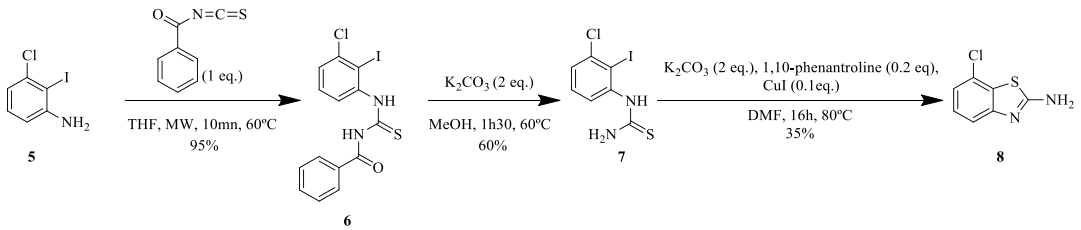
Scheme 3. Synthesis Route 17−26

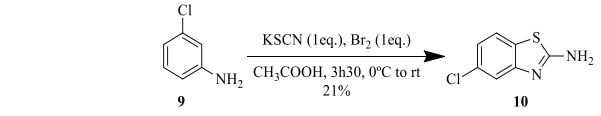


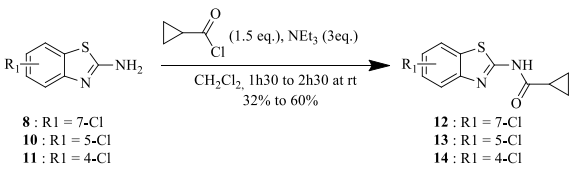


Another issue for this series was the observed low metabolic stability of **1**. In order to improve it, synthesis of disubstituted acylaminobenzolthiazole was accomplished (Scheme 7). Using the classical route with potassium thiocyanate and bromine, only traces of the disubstituted aminobenzothiazole were obtained in the case of **50** because these conditions are in favor of bromination at position 6 of the starting aniline **46a**. Hence, a new synthetic approach for disubstituted aminobenzothiazole was necessary. We based our strategy on the synthesis of **8**. Aniline precursors **46a−c** were commercially available and

Scheme 2. Synthesis Route 12−14







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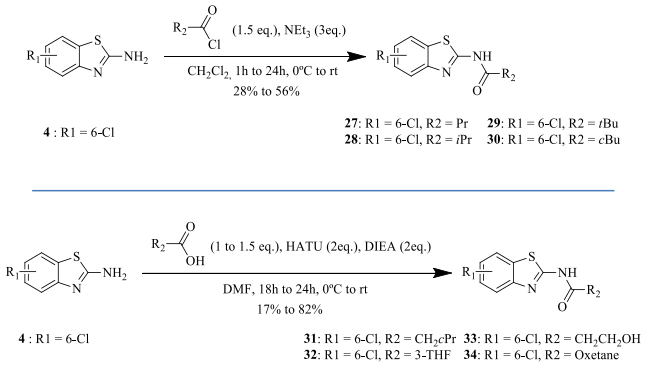
Scheme 4. Synthesis Route 27−34



anilines **46d−h** were prepared using the previously described procedures (see Experimental Section).[28−31] First, anilines were submitted to an iodination reaction catalyzed by *p*-toluene sulfonic acid or by boron trifluoride etherate to control the regioselectivity at position 6. Intermediates **47a−h** were converted to the corresponding 1-benzoyl 3-(3′-R1-4′-R1′-6′-iodophenyl) derivatives using benzoyl isothiocyanate under microwave irradiation and were treated by K_2CO_3 to yield disubstituted aminobenzothiazole **49a−h** (Scheme 7). Final compounds **50−57** were obtained by reacting the corresponding aminobenzothiazole with cyclopropanecarbonyl chloride.

Finally, after improving anti-*T. cruzi* activity and metabolic stability by changing R1, R2, and R3 compounds, **58**, **59,** and **60** were designed using a mix and match approach. Compounds **58**, **59,** and **60** were synthesized using conditions, as described in Scheme 4 (Scheme 8).

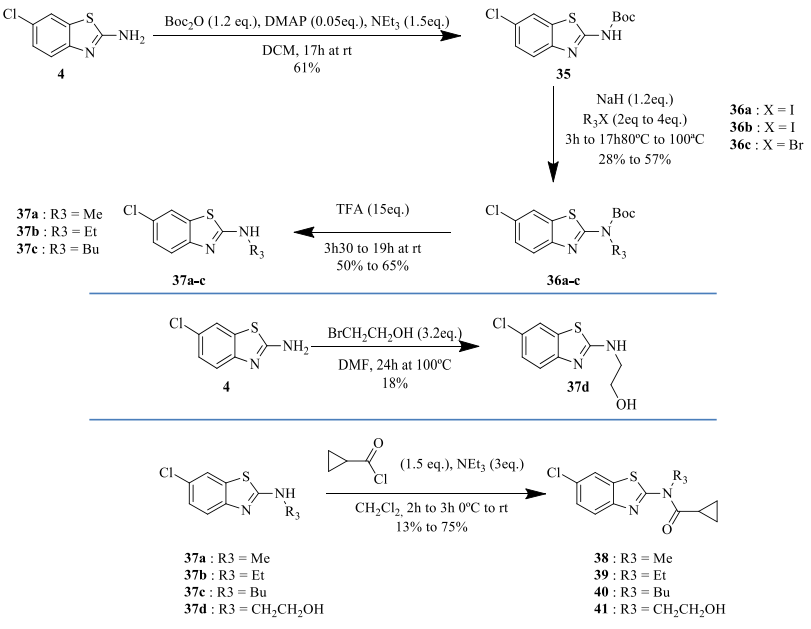
Biology. Compounds were tested against *T. cruzi* using an intracellular assay with cardiomyocytes H9c2 as the host cell, permitting, in the same time, the determination of the cytotoxicity. Initial frontrunner **1** was used as the reference to evaluate the effect of different substituents at R1, R2, and R3 positions.

In Vitro Anti-T. cruzi Activity and Aromatic Chlorine Position. The first SAR exploration was focussed on the investigation of the best position for the aromatic substituent around the final group. When chlorine was moved from position 6 to position 5, higher anti-*T. cruzi* activity and SI (**13** IC_{50} = 0.32 μM vs **1** IC_{50} = 0.63 μM) was observed while substitution at positions 7 and 4 led to the loss of antiparasite activity (**12** IC_{50} = 24 μM and **14** IC_{50} > 50 μM) suggesting the importance for R1 substitution to be placed at positions 5 or 6 (Table 2).

In Vitro Anti-T. cruzi Activity and 6-Substitution. After determining the importance of the position in the phenyl ring for antiparasite activity, investigation around the effect of varying the nature of the substituent was carried out (Table 3). Replacing the chlorine by alkylic carbon chains such as isopropyl **18**, tertbutyl **19,** or methyl **23** decreased the activity; however, substitution by an ethyl group **17** increased the anti-*T. cruzi* activity. Mesomere-donating groups such as a fluorine **22**, ethoxy **21,** methoxy substituents **24** led to lower anti-*T. cruzi* activity. Replacement of the chlorine by a trifluoromethoxy substituent **20** yielded a higher potency (**20** IC_{50} = 0.19 μM), however, with some cytotoxicity against the host cell observed (**20** SI = 28). These results suggest that mesomere-donating groups do not improve antiparasite activity of the series. Derivatives **25** and **26**, that hold nitro and trifluoromethyl groups, respectively, presented higher activity and good SIs pointing toward a beneficial effect of electron withdrawing groups the anti-*T. cruzi* activity of AAB derivatives (**25** IC_{50} = 0.04 μM, **26** IC_{50} = 0.1 μM).

In Vitro Anti-T. cruzi Activity and Cyclopropyl Replacement. In parallel of the determination of the best substituents on the aromatic core, the acyl group R2 was explored (Table 4). The cyclopropyl moiety was first replaced by an alkylic carbon chain (propyl) leading to a significative decrease of activity (**27** IC_{50} = 4.73 μM), whereas a ramified such *tert*-butyl slightly decreased potency (**29** IC_{50} = 0.95 μM) and isopropyl marginally increased antiparasite potency (**28** IC_{50} = 0.41 μM). Substitution of the cyclopropyl moiety by cyclobutyl

Scheme 5. Synthesis Route of 38−41



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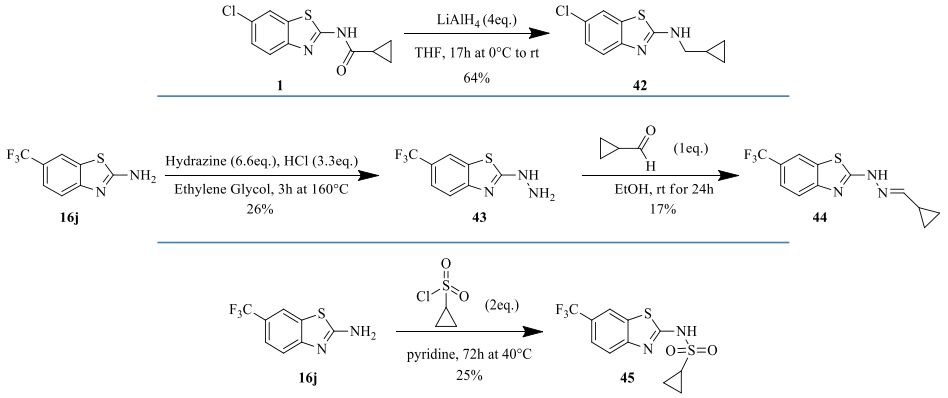
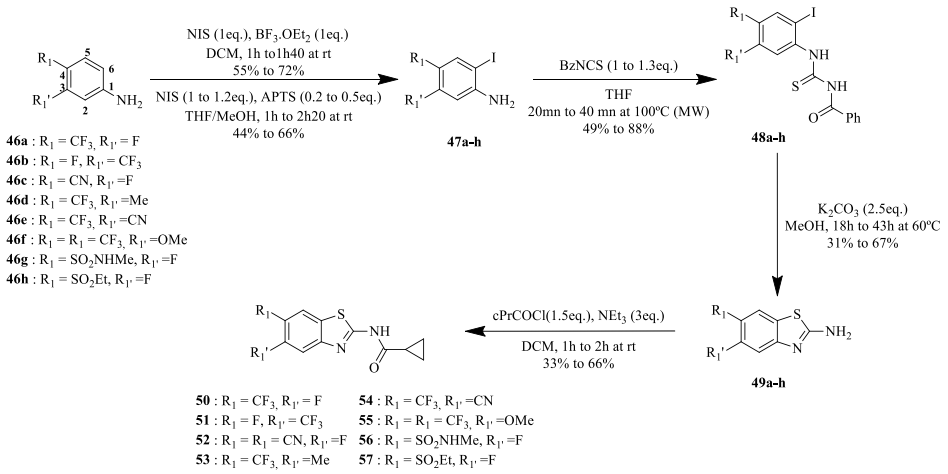
Scheme 6. Synthesis Route of 42, 44, and 45



Scheme 7. Synthesis Route of 50 to 61



Scheme 8. Synthesis Route of 58 to 60

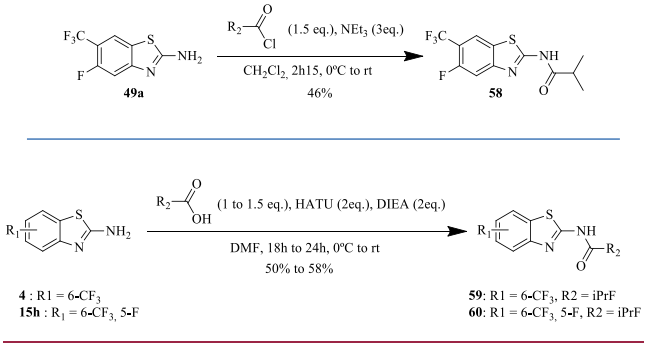


Table 2. Effect of the Chlorine Position on Anti-*T. cruzi* Activity and on Cytotoxicity

compound	R1	IC_{50} (μM) vs *T. cruzi*	IC_{50} (μM) vs host cell (H9C2)	SI
1	6-Cl	0.63	>50	>80
12	7-Cl	24	>50	>2
13	5-Cl	0.32	>50	>158
14	4-Cl	>50	>50	

decreased the activity by twofold (**1** IC_{50} = 0.63 μM and **30** IC_{50} = 1.32 μM). Increasing the flexibility of the R2 group by introducing methylene linkers between the amide bond and cyclopropyl led to lower antiparasite potency (**31** IC_{50} = 5.4 μM). Introducing polarity at R2 through heterocyclic group

Table 3. Effect of 6-Substituent on Anti-*T. cruzi* Activity and on Cytotoxicity

compound	R1	IC_{50} (μM) vs *T. cruzi*	IC_{50} (μM) vs host cell (H9C2)	SI
17	6-Et	0.14	>50	>357
18	6-*i*Pr	1.19	>50	>50
19	6-*t*Bu	7.85	32	4
20	6-OCF_3	0.19	5	28
21	6-OEt	2.5	>50	>20
22	6-F	4.22	>50	>12
23	6-Me	4.52	>50	>11
24	6-OMe	13	>50	>4
25	6-NO_2	0.04	>50	>1200
26	6-CF_3	0.1	>50	>500

substitutions such as tetrahydrofuran (THF) **32,** oxetane **34,** and an ethanol chain **33** had a detrimental effect on anti-*T. cruzi* activity suggesting that ramified alkyls such as cyclopropyl or isopropyl are better tolerated in terms of anti-*T. cruzi* activity of AAB compounds.

In Vitro Anti-T. cruzi Activity and Hydrogen Amide Bond Replacement. Substitution of the amide hydrogen by different R3 groups represented another site of SAR investigation. Hydrogen replacement by the alkyl group such as methyl **38,** ethyl **39,** and butyl **40** or an ethanol chain **41** turned compounds completely inactive, suggesting this hydrogen is required to maintain activity (Table 5).

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Table 4. Effect of the Acyl Group on Anti-*T. cruzi* Activity and on Cytotoxicity

compound	R1	R2	IC_{50} (μM) vs *T. cruzi*	IC_{50} (μM) vs host cell (H9C2)	SI
27	6-Cl	Pr	4.73	>50	>11
28	6-Cl	*i*Pr	0.41	>50	>123
29	6-Cl	*t*Bu	0.95	>50	>52
30	6-Cl	*c*Bu	1.32	>50	>38
31	6-Cl	CH_2cPr	5.4	>50	>9
32	6-Cl	3-THF	4.4	>50	>11
33	6-Cl	CH_2CH_2OH	14	>50	>3
34	6-Cl	oxetane	4.4	>50	>11

Table 5. Effect of Amide Bond Hydrogen Replacement on Anti-*T. cruzi* Activity and on Cytotoxicity

compound	R3	IC_{50} (μM) vs *T. cruzi*	IC_{50} (μM) vs host cell (H9C2)	SI
38	Me	25	>50	>2
39	Et	>50	>50	1
40	Bu	>50	>50	1
41	CH_2CH_2OH	>50	>50	1

SAR Conclusion. It has been demonstrated that an electron withdrawing group as trifluoromethyl at position 6 of the aromatic core led to a potent antiparasite compound. Furthermore, ramified alkyls as cyclopropyl or isopropyl as the R2 group maintained anti-*T. cruzi* activity. Finally, hydrogen of the amide bond is needed to observe antiparasite activity. Using those preliminary results, additional compounds were synthesized and tested to improve the anti-*T. cruzi* activity and the metabolic stability.

In Vitro Anti-T. cruzi Activity and Amide Bond Replacement. The importance of the amide bond for anti-*T. cruzi* activity was also investigated (Table 6). Reduction of the amide bond to get the corresponding secondary alkylic amine resulted in the loss of antiparasite activity (**42** IC_{50} = 27 μM). When the amide was transformed into sulfonamide **45**, the compound was completely inactive. Substitution of the amide bond by hydrazone maintained an activity but increased drastically cytotoxicity (**44** IC_{50} = 2.5 μM and SI = 5). In summary, the amide bond seems to be important for antiparasite activity.

Table 6. Effect of Amide Bond Replacement on Anti-*T. cruzi* Activity and on Cytotoxicity

compound	IC_{50} (μM) vs *T. cruzi*	IC_{50} (μM) vs host cell (H9C2)	SI
42	27	>50	>2
44	2.4	13	5
45	>50	6.6	<0.13

Metabolic Stability. Because compound **1** had already been identified as metabolically unstable in vitro versus human microsomes, the most promising compounds in terms of their antiparasite activity were assessed against mouse and human liver microsomes (Table 7). Replacement of chlorine by ethyl **17** drastically increases in vitro mouse clearance and had no effect on human microsomal stability, but when it was replaced by an electron withdrawing group as nitro **25** or trifluoromethyl **26**, improved metabolic stability was observed although compounds were still not stable (Table 7).

Table 7. Metabolic Stability of Lead Compounds

compound	mouse clearance (mL/min/g)	human clearance (mL/min/g)	mouse % LBF (%)	human % LBF (%)
1	2.51	9.57	50	93
17	125	10.4	98	93
25	3.06	1.57	55	68
26	2.71	0.94	52	56

In Vitro Anti-T. cruzi Activity/Metabolic Stability and 5-6-Disubstitution. Having evaluated the effect of substituents at position 6 on the anti-*T. cruzi* activity and on microsomal stability, we decided to explore disubstitution of the aromatic core at positions 5 and 6 looking for a further improvement in metabolic stability of these derivatives. Position 5 was chosen because it was suggested that the first hydroxylation of **1** occurred at this position during the metabolic degradation process. A trifluoromethyl group was selected in the first place because it is an electron withdrawing group with the potential for development (as opposed to a nitro substituent, **25**, which can induce formation of free radical species during its reduction and lead to toxicity).[32] This risk together with an unsatisfactory improvement of human metabolic stability and a very low solubility pushed us to discard compound **25** despite its very good in vitro activity. Then, the effect of several other substituents at 6-position was explored. Introduction of a fluorine atom as in **50** slightly increased the activity of **26** (**26** IC_{50} = 0.1 μM, **50** IC_{50} = 0.079 μM), but a contrary effect was observed when methyl **53**, nitrile **54**, or methoxy **55** substituents were introduced, resulting compounds being less potent or inactive. Interestingly, reversing the substituent position of **50** completely inhibited the anti-*T. cruzi* activity (**51** IC_{50} = 31 μM). Then, it was suggested that an electron withdrawing group at position 6 with a fluorine at position 5 was the best option in term of potency. Hence, other electron withdrawing groups to replace trifluoromethyl were investigated such as nitrile **52**, sulfonamide **56**, and sulfone **57**, but they yielded a loss in antiparasite activity (Table 8). Intrinsic clearance of **50** was then determined in mouse and in human microsomes, being higher for mouse (5.26 mL/min/g) than for human (0.41 mL/min/g), however, representing a marked improvement in human microsomal stability. Using this improved scaffold, additional exploration of the R2 position was carried out (Table 9). Following the previous observations, an isopropyl group was introduced; however, in this case a slight decrease in antiparasite activity was observed instead (**50** IC_{50} = 0.079 μM and **58** IC_{50} = 0.25 μM), an effect that

Table 8. Effect of disubstitution of the aromatic core on Anti-*T. cruzi* Activity and on Cytotoxicity

compound	R1, R1′	IC_{50} (μM) vs *T. cruzi*	IC_{50} (μM) vs host cell (H9C2)	SI
50	6-CF_3, 5-F	0.079	>50	>630
51	6-F, 5-CF_3	35	46	1
52	6-CN, 5-F	2.3	>50	>22
53	6-CF_3, 5-Me	26	36	1
54	6-CF_3, 5-CN	>50	15.5	
55	6-CF_3, 5-OMe	20	>50	>2.5
56	6-SO_2NHMe, 5-F	26	>50	>2
57	6-SO_2Et, 5-F	42	>50	>1

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Table 9. Effect of R1′ and R2 Replacements on Anti-*T. cruzi* Activity and on Cytotoxicity

compound	R1′	R2	IC_{50} (μM) vs *T. cruzi*	IC_{50} (μM) vs host cell (H9C2)	SI
58	F	*i*Pr	0.25	>50	>200
59	H	*i*PrF	0.13	>50	>384
60	F	*i*PrF	1	>50	>50

was more pronounced when a 2-fluoroisopropyl substituent was introduced, (**50** IC_{50} = 0.079 μM and **60** IC_{50} = 1 μM). In conclusion, the cyclopropyl moiety gave the best results in terms of antiparasite activity and metabolic stability, compound **50** having the best overall profile and was selected for further profiling.

*In Vitro ADME Properties of **50**.* The physicochemical, and in vitro and in vivo DMPK profiles for compound **50** were evaluated in order to evaluate its progression. Compound **50** displayed low-moderate kinetic solubility [charged aerosol detection (CAD)] and low-moderate aqueous solubility in fasted simulated intestinal fluid (FaSSIF) media. Permeability values measured with MDCKII-MDR1 were high in both directions with no evidence for significant efflux. Plasma protein binding measured in both human and mouse species was high, and blood to plasma partitioning in mouse resulted was moderate (Table 10).

Table 10. 50 in Vitro ADME Profile

solubility CAD (μM)/FaSSIF (μg/mL)	36/51
MDCK-permeability (with inhibitor) (P_{app} nm/s)	174 (ER = 1.5)
B/P (mouse/human)	1.2/0.8

*In Vivo Pharmacokinetics of **50**.* Compound **50** in vivo pharmacokinetics was investigated after intravenous and oral administration in mice. The compound exhibited moderate blood clearance, which correlates with the observed clearance in mouse microsomes (less than two-fold difference when comparing in vitro and in vivo results), moderate half-life, and high volume of distribution. Metabolite **49a** was detected (percentage of metabolite = 32%), and its C_{max} (634 ng/mL) and AUC (10 065.32 ng h/mL) were estimated. The blood concentration versus time data profile shows that metabolism is saturated at 50 mg/kg after oral gavage administration, and compound is well absorbed. T_{max}, C_{max}, and AUC parameters were estimated. Bioavailability was not estimated as different mouse strains were used for intravenous and oral administration.

*In Vivo Anti-T. cruzi Activity of **50**.* The IVA of compound **50** was assessed in an acute model of *T. cruzi* infection in mice (Figure 5). A single oral dose of 50 mg/kg showed efficacy in controlling the parasite load at the site of infection compared to untreated animals. The specific IVA for compound **50** (78.5) approached that for the positive control benznidazole (101.6).

CONCLUSIONS

Synthesis and evaluation of anti-*T. cruzi* activity of several AAB derivatives was accomplished starting from **1**. Modifications at R1 introducing electron withdrawing groups such as trifluoromethyl or nitro substituents at position 6 improved antiparasite activity of these derivatives, whereas blocking position 5 of the benzothiazole scaffold with a fluorine had a positive effect on lowering human microsomal clearance. Only small ramified or small cyclic carbon groups are tolerated at position R2, but the initial cyclopropyl remained the best substituent. Finally, the amide bond with an unsubstituted hydrogen is required for anti-*T. cruzi* activity. As a result of this optimization, a new lead compound **50** was identified. Compound **50** showed a favorable ADME and pharmacokinetic profile. Furthermore, **50** showed an interesting in vivo antiparasite activity confirming the potential of AAB series to furnish progressable compounds. The target of this series, which is not CYP51 (result not presented here), is still to be identified.

ETHICAL STATEMENT

"All studies were conducted in accordance with the GSK Policy on the Care, Welfare, and Treatment of Laboratory Animals and were reviewed the Institutional Animal Care and Use Committee either at GSK or by the ethical review process at the institution, where the work was performed."

EXPERIMENTAL SECTION

Chemistry. *General.* All starting materials were purchased from commercial suppliers (Sigma-Aldrich, Fluorochem, Enamine and combi-blocks). ^{1}H NMR spectra were recorded on a Bruker AVANCE 400 MHz. The following abbreviations were used: s (singlet), d (doublet), dd (doublet of doublet), t (triplet), q (quartet),

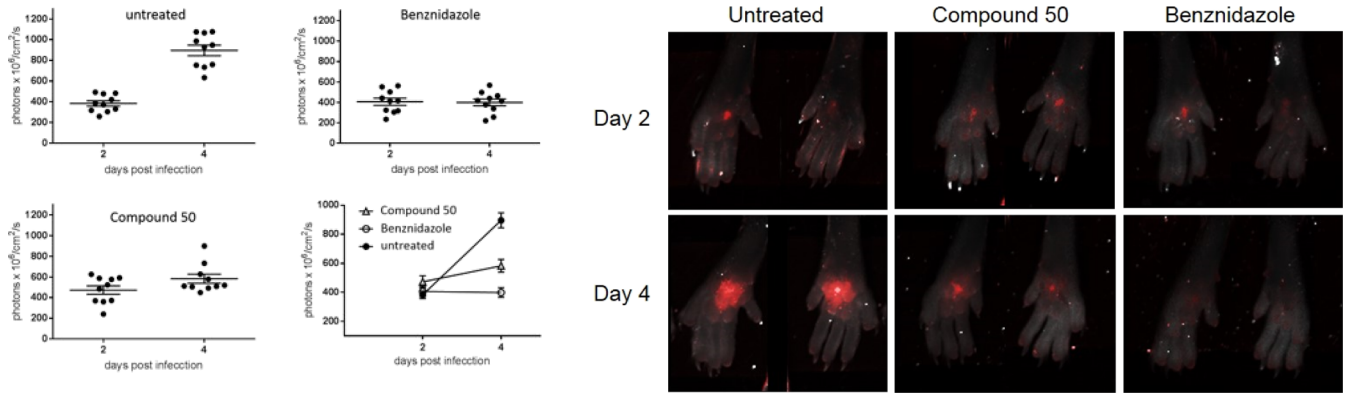

Figure 5. Left: IVA of **50**. Parasite load in C57BL/6 mice was determined after a single dose of **50**, benznidazole, or mice receiving no treatment. Right: Growth of *T. cruzi* in footpads of representative mice at 2 and 4 days postinfection. Single oral dose of compound **50** or benznidazole was administered on day 2.

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m (multiplet), and J (coupling constant). High-resolution mass spectra were recorded on a Hybrid Quadrupole-TOF LC−MS/MS (QSTAR Elite System). Mass spectra of intermediate compounds were recorded on an Agilent Technology 1290 Affinity LC−MS (column: XBridge C18 3.5 μM 4.6 × 60 mm). Purities of final compounds were determined by using an LC−MS instrument (column: UPLC-BEH C18 1.7u 3 × 50 mm) with a UV detection at 299 nm. Purities were ≥95% for 37 final compounds and >90% for two final compounds. Characterization of intermediates issued from aminobenzothiazole synthesis are described in the Supporting Information. IUPAC compound names were determined using chemdraw.

General Synthesis Protocol of N-(Benzothiazol-2-yl)-cyclopropanecarboxamide. To an ice-cold solution of aminobenzothiazole (1 equiv) in DCM (0.13 M) were successively added triethylamine (3 equiv) and cyclopropanecarbonyl chloride (1.5 equiv). After stirring from 1 to 24 h, the mixture was diluted in 10 mL of brine and extracted with DCM. The organic layers were combined, washed with a saturated solution of $NaHCO_3$, dried over Na_2SO_4, and filtered and concentrated under reduced pressure. The crude was purified by chromatography eluting with cyclohexane/EtOAc.

*N-(6-Chlorobenzo[d]thiazol-2-yl)cyclopropanecarboxamide (**1**).* It was obtained in 51% yield. Purity (LC−MS) > 95%. NMR: ^{1}H (DMSO, 400 MHz): δ 12.72 (br s, 1H), 8.10 (d, J = 2.0 Hz, 1H), 7.71 (d, J = 8.4 Hz, 1H), 7.43 (dd, J = 2.0 and 8.8 Hz, 1H), 1.96−2.01 (m, 1H) and 0.94−0.97 (m, 4H). HRMS: mass calculated for $C_{11}H_{10}ClN_2OS$, 253.0197 and mass found, 253.0185.

*7-Chlorobenzothiazol-2-amine (**8**).* Benzoyl isothiocyanate (0.080 mL, 0.591 mmol) was added to a solution of 3-chloro-2-iodophenylamine (0.150 g, 0.591 mmol) in THF (0.6 mL). The mixture was stirred under microwave activation during 10 min at 60 °C and a solid was obtained. A solution of acidified water (pH = 5) was added to the mixture, and the product was extracted with EtOAc (3 × 10 mL). The organic layers were combined, dried over Na_2SO_4, and filtered and concentrated to furnish **6** as a yellow solid (0.234 g, 95%). K_2CO_3 (0.155 g, 1.12 mmol) was added to a solution of **6** (0.234 g, 0.561 mmol) in MeOH (13 mL). The solution was warmed up to 60 °C and stirred for about 1.5 h. The mixture was then diluted in EtOAc (15 mL) and washed with water (3 × 10 mL). The organic layer was dried over Na_2SO_4, and filtered and concentrated under vacuum. The crude was purified by chromatography eluting with cyclohexane/EtOAc (95/5 to 50/50) to furnish 7 as a white solid (0.122 g, 70%). To a mixture of K_2CO_3 (0.108 g, 0.780 mmol), phenanthroline (0.00131 g, 0.008 mmol), and CuI (0000.7 g, 0.004 mmol) were added a solution of 7 (0.122 g, 0.390 mmol) in DMF (2 mL). The solution was warmed up to 80 °C and stirred overnight. The mixture was quenched by the addition of water (15 mL), and the product was extracted using EtOAc (3 × 15 mL). Organic layers were combined, dried over Na_2SO_4, and filtered and concentrated under vacuum. The crude was purified by chromatography eluting with cyclohexane/EtOAc (95/5 to 50/50) to furnish **8** as a yellow solid (0.025 g, 35%).

NMR: ^{1}H (DMSO, 400 MHz): δ 7.73 (NH_2, 2H, br), 7.37 (H4, 1H, dd, J_{3-2} = 7.6 Hz and J_{3-1} = 1 Hz), 7.24 (H5, 1H, t, J_{2-3} = 7.6 Hz) and 7.09 (H6, 1H, dd, J_{1-2} = 7.6 Hz and J_{1-3} = 1 Hz). MS: M + H^+, 185.0 (exact mass of $C_7H_5ClN_2S$: 183.99).

General Synthesis Protocol for 6-Substituted Aminobenzothiazole. To an ice-cold solution of aniline (1 equiv) in AcOH (C = 0.1 M) were added successively KSCN (1−2 equiv) and bromine (1 equiv). The mixture was warmed up to room temperature and stirred for over 3−24 h. The solution was then diluted in water, and pH was increased to 10 with ammonia. Organic products were extracted with EtOAc, organic layers were combined, dried over Na_2SO_4, and filtered and concentrated under vacuum. Crudes were purified by chromatography eluting with cyclohexane/EtOAc.

*5-Chlorobenzothiazol-2-amine (**10**).* It was obtained in 21% yield. NMR: ^{1}H (DMSO, 400 MHz): δ 7.45 (H7, 1H, d, J_{7-6} = 8.6 Hz), 6.81 (H4, 1H, d, J_{4-6} = 2.5 Hz), 6.58 (H6, 1H, dd, J_{6-7} = 8.6 Hz and J_{6-4} = 2.5 Hz) and 6.11 (NH_2, 2H, br). MS: M + H^+, 185.00 (exact mass of $C_7H_5ClN_2S$: 183.99).

*Characterization of N-(Benzothiazol-2-yl)-cyclopropanecarboxamide **12** to **14**. N-(7-Chlorobenzothiazol-2-yl)cyclopropanecarboxamide (**12**).* It was obtained in 41% yield. Purity (LC−MS) > 95%. NMR: ^{1}H (DMSO, 400 MHz): δ 12.85 (NH, 1H, br), 7.73 (H4, 1H, dd, J_{3-2} = 8.1 Hz and J_{3-1} = 0.8 Hz), 7.48 (H5, 1H, t, J_{2-3} = 8.1 Hz), 7.40 (H6, 1H, dd, J_{1-2} = 8.1 Hz and J_{1-3} = 0.8 Hz), 2.02 (CHcPr, 1H, m) and 0.98 (CH_2cPr, 4H, m). HRMS: mass calculated for $C_{11}H_{10}ClN_2OS$, 253.0197 and mass found, 253.0202.

*N-(5-Chlorobenzothiazol-2-yl)cyclopropanecarboxamide (**13**).* It was obtained in 32% yield. Purity (LC−MS) > 95%. NMR: ^{1}H (DMSO, 400 MHz): δ 10.66 (NH, 1H, br), 8.06 (H4, 1H, d, J_{4-6} = 2.3 Hz), 7.77 (H7, 1H, d, J_{7-6} = 8.8 Hz), 7.59 (H6, 1H, dd, J_{6-7} = 8.8 Hz and J_{6-4} = 2.3 Hz), 1.78 (CHcPr, 1H, m) and 0.86 (CH_2cPr, 4H, m). HRMS: mass calculated for $C_{11}H_{10}ClN_2OS$, 253.0197 and mass found, 253.0188.

*N-(4-Chlorobenzothiazol-2-yl)cyclopropanecarboxamide (**14**).* It was obtained in 60% yield. Purity (LC−MS) > 95%. NMR: ^{1}H (DMSO, 400 MHz): δ 13.04 (NH, 1H, br), 7.95 (H7, 1H, dd, J_{7-6} = 8 Hz and J_{7-5} = 0.9 Hz), 7.53 (H5, 1H, dd, J_{5-6} = 7.8 Hz and J_{5-7} = 0.9 Hz), 7.29 (H6, 1H, t, J_{4-5} = 7.8 Hz), 2.01 (CHcPr, 1H, m) and 0.97 (CH_2, 4H, m). HRMS: mass calculated for $C_{11}H_{10}ClN_2OS$, 253.0197 and mass found, 253.0200. mp (°C) 146.2 & 181.1 & 233.3 (three crystalline forms present).

*Characterization of 6-Substituted Aminobenzothiazole **16a** to **16e**. 6-Ethylbenzothiazol-2-amine (**16a**).* It was obtained in 26% yield. NMR: ^{1}H (DMSO, 400 MHz): δ 7.47 (H7, 1H, d, J_{7-5} = 1.5 Hz), 7.31 (NH_2, 2H, br), 7.23 (H4, 1H, d, J_{4-5} = 8.3 Hz), 7.04 (H5, 1H, dd, J_{5-4} = 8.3 Hz and J_{5-7} = 1.5 Hz), 2.58 (CH_2, 2H, q, $J_{CH_2-CH_3}$ = 7.5 Hz) and 1.18 (CH_3, 3H, t, $J_{CH_3-CH_2}$ = 7.5 Hz). MS: M + H^+, 179.0 (exact mass of $C_9H_{10}N_2S$: 178.06).

*6-Isopropylbenzothiazol-2-amine (**16b**).* It was obtained in 20% yield . NMR: ^{1}H (DMSO, 400 MHz): δ 7.51 (H7, 1H, d, J_{7-5} = 1.8 Hz), 7.31 (NH_2, 2H, br), 7.23 (H4, 1H, d, J_{4-5} = 8.1 Hz), 7.07 (H5, 1H, dd, J_{5-4} = 8.1 Hz and J_{5-7} = 1.8 Hz), 2.89 (CH, 1H, sx, J_{CH-CH_3} = 6.9 Hz) and 1.18 (CH_3, 6H, d, $J_{CH_3-CH_2}$ = 6.9 Hz). MS: M + H^+, 193.1 (exact mass of $C_{10}H_{12}N_2S$: 192.07).

*6-(tert-Butyl)benzothiazol-2-amine (**16c**).* It was obtained in 16% yield. NMR: ^{1}H (DMSO, 400 MHz): δ 7.65 (H7, 1H, m), 7.31 (NH_2, 2H, br), 7.24 (H4 and H5, 2H, m), 1.29 (CH_3, 9H, s). MS: M + H^+, 207.0 (exact mass of $C_{11}H_{14}N_2S$: 206.09).

*6-(Trifluoromethoxy)benzothiazol-2-amine (**16d**).* It was obtained in 61% yield. NMR: ^{1}H (DMSO, 400 MHz): δ 7.78 (H7, 1H, d, J_{7-5} = 1.8 Hz), 7.68 (NH_2, 1H, br), 7.36 (H4, 1H, d, J_{4-5} = 8.8 Hz), and 7.18 (H5, 1H, dd, J_{5-4} = 8.8 Hz and J_{5-7} = 1.8 Hz). MS: M − H^+, 233.00 (exact mass of $C_8H_5F_3N_2OS$: 234.01).

*6-Ethoxybenzothiazol-2-amine (**16e**).* It was obtained in 45% yield. NMR: ^{1}H (DMSO, 400 MHz): δ 7.26 (H7, 1H, d, J_{7-5} = 2.5 Hz), 7.21 (H4, 1H, d, J_{4-5} = 8.8 Hz), 7.19 (NH_2, 1H, br), 6.79 (H5, 1H, dd, J_{5-4} = 8.8 Hz and J_{5-7} = 2.5 Hz), 3.98 (CH_2, 2H, q, $J_{CH_2-CH_3}$ = 7.1 Hz) and 1.31 (CH_3, 3H, t, $J_{CH_3-CH_2}$ = 7.1 Hz). MS: M + H^+, 195.0 (exact mass of $C_9H_{10}N_2OS$: 194.05).

*Characterization of N-(Benzothiazol-2-yl)-cyclopropanecarboxamide **17** to **26**. N-(6-Ethylbenzothiazol-2-yl)cyclopropanecarboxamide (**17**).* It was obtained in 52% yield. Purity (LC−MS) > 95%. NMR: ^{1}H (DMSO, 400 MHz): δ 12.54 (NH, 1H, br), 7.77 (H7, 1H, d, J_{7-5} = 1.6 Hz), 7.63 (H4, 1H, d, J_{4-5} = 8.3 Hz), 7.27 (H5, 1H, dd, J_{5-4} = 8.3 Hz and J_{5-7} = 1.6 Hz), 2.69 (CH_2, 2H, q, $J_{CH_2-CH_3}$ = 7.6 Hz), 1.99 (CHcPr, 1H, m), 1.22 (CH_3, 3H, t, $J_{CH_3-CH_2}$ = 7.6 Hz) and 0.95 (CH_2cPr, 4H, m). HRMS: mass calculated for $C_{13}H_{15}N_2OS$, 247.0900 and mass found, 247.0905.

*N-(6-Isopropylbenzothiazol-2-yl)cyclopropanecarboxamide (**18**).* It was obtained in 61% yield. Purity (LC−MS) > 95%. NMR: ^{1}H (DMSO, 400 MHz): δ 12.54 (NH, 1H, br), 7.81 (H7, 1H, d, J_{7-5} = 1.8 Hz), 7.64 (H4, 1H, d, J_{4-5} = 8.3 Hz), 7.31 (H5, 1H, dd, J_{5-4} = 8.3 Hz and J_{5-7} = 1.8 Hz), 2.99 (CH^{i}Pr, 1H, sx, $J_{CHiPr-CH_3}$ = 7.1 Hz), 1.98 (CHcPr, 1H, m) 1.25 (CH_3, 6H, d, $J_{CH_3-CHiPr}$ = 7.1 Hz) and 0.95

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(CH_2cPr, 4H, m). HRMS: mass calculated for $C_{14}H_{17}N_2OS$, 261.1056 and mass found, 261.1051. mp (°C) 168.8.

N-(6-(tert-Butyl)benzothiazol-2-yl)cyclopropanecarboxamide ***(19)***. It was obtained in 71% yield. Purity (LC−MS) > 95%. NMR: ^{1}H (DMSO, 400 MHz): δ 12.53 (NH, 1H, br), 7.94 (H7, 1H, d, J_{7-5} = 1.8 Hz), 7.64 (H4, 1H, d, J_{4-5} = 8.6 Hz), 7.48 (H5, 1H, dd, J_{5-4} = 8.6 Hz and J_{5-7} = 1.8 Hz), 1.99 (CHcPr, 1H, m), 1.33 (CH_3, 9H, s) and 0.95 (CH_2cPr, 4H, m). HRMS: mass calculated for $C_{15}H_{19}N_2OS$, 275.1213 and mass found, 275.1220.

N-(6-(Trifluoromethoxy)benzothiazol-2-yl)-cyclopropanecarboxamide ***(20)***. It was obtained in 59% yield. Purity (LC−MS) > 95%. NMR: ^{1}H (DMSO, 400 MHz): δ 12.75 (NH, 1H, br), 8.10 (H7, 1H, d, J_{7-5} = 1.8 Hz), 7.81 (H4, 1H, d, J_{4-5} = 8.8 Hz), 7.42 (H5, 1H, dd, J_{5-4} = 8.8 Hz and J_{5-7} = 1.8 Hz), 2.01 (CHcPr, 1H, m) and 0.97 (CH_2cPr, 4H, m). HRMS: mass calculated for $C_{12}H_{10}F_3N_2O_2S$, 303.0410 and mass found, 303.0414.

N-(6-Ethoxybenzothiazol-2-yl)cyclopropanecarboxamide ***(21)***. It was obtained in 34% yield. Purity (LC−MS) > 95%. NMR: ^{1}H (DMSO, 400 MHz): δ 12.47 (NH, 1H, br), 7.61 (H4, 1H, d, J_{4-5} = 8.8 Hz), 7.52 (H7, 1H, d, J_{7-5} = 2.5 Hz), 7.00 (H5, 1H, dd, J_{5-4} = 8.8 Hz and J_{5-7} = 2.5 Hz), 4.06 (CH_2, 2H, q, $J_{CH_2-CH_3}$ = 7.1 Hz), 1.97 (CHcPr, 1H, m), 1.34 (CH_3, 3H, t, $J_{CH_3-CH_2}$ = 7.1 Hz) and 0.94 (CH_2cPr, 4H, m). HRMS: mass calculated for $C_{13}H_{15}N_2O_2S$, 263.0849 and mass found, 263.0857.

N-(6-Fluorobenzothiazol-2-yl)cyclopropanecarboxamide ***(22)***. It was obtained in 51% yield. Purity (LC−MS) > 95%. NMR: ^{1}H (DMSO, 400 MHz): δ 12.65 (NH, 1H, br), 7.87 (H7, 1H, dd, J_{7-F} = 8.7 Hz and J_{7-5} = 2.7 Hz), 7.74 (H4, 1H, dd, J_{4-5} = 8.8 Hz and J_{4-5} = 4.8 Hz), 7.27 (H5, 1H, td, $J_{5-4\text{ and }5-F}$ = 8.7 Hz and J_{5-7} = 2.7 Hz), 1.99 (CHcPr, 1H, m) and 0.96 (CH_2cPr, 4H, m). HRMS: mass calculated for $C_{11}H_{10}FN_2OS$, 237.0492 and mass found, 237.0498.

N-(6-Methylbenzothiazol-2-yl)cyclopropanecarboxamide ***(23)***. It was obtained in 47% yield. Purity (LC−MS) > 95%. NMR: ^{1}H (DMSO, 400 MHz): δ 12.55 (NH, 1H, br), 7.74 (H7, 1H, d, J_{7-5} = 1.3 Hz), 7.61 (H4, 1H, d, J_{4-5} = 8.1 Hz), 7.24 (H5, 1H, dd, J_{5-4} = 8.1 Hz and J_{5-7} = 1.3 Hz), 2.40 (CH_3, 3H, s), 1.98 (CHcPr, 1H, m) and 0.94 (CH_2cPr, 4H, m). HRMS: mass calculated for $C_{12}H_{13}N_2OS$, 233.0743 and mass found, 233.0742.

N-(6-Methoxybenzothiazol-2-yl)cyclopropanecarboxamide ***(24)***. It was obtained in 54% yield. Purity (LC−MS) > 95%. NMR: ^{1}H (DMSO, 400 MHz): δ 12.46 (NH, 1H, br), 7.62 (H4, 1H, d, J_{4-5} = 8.8 Hz), 7.55 (H7, 1H, d, J_{7-5} = 2.5 Hz), 7.02 (H5, 1H, dd, J_{5-4} = 8.8 Hz and J_{5-7} = 2.5 Hz), 3.80 (OMe, 3H, s), 1.97 (CHcPr, 1H, m) and 0.93 (CH_2cPr, 4H, m). HRMS: mass calculated for $C_{12}H_{13}N_2O_2S$, 249.0701 and mass found, 249.0692. mp (°C) 205.3.

N-(6-Nitrobenzothiazol-2-yl)cyclopropanecarboxamide ***(25)***. It was obtained in 15% yield. Purity (LC−MS) > 95%. NMR: ^{1}H (DMSO, 400 MHz): δ 13.05 (NH, 1H, br), 9.03 (H7, 1H, d, J_{7-5} = 1.6 Hz), 8.27 (H5, 1H, dd, J_{5-4} = 9.2 Hz and J_{5-7} = 1.6 Hz), 7.88 (H4, 1H, d, J_{4-5} = 9.2 Hz), 2.04 (CHcPr, 1H, m) and 0.99 (CH_2cPr, 4H, m). HRMS: mass calculated for $C_{11}H_{10}N_3O_3S$, 264.0437 and mass found, 264.0444.

N-(6-(Trifluoromethyl)benzothiazol-2-yl)-cyclopropanecarboxamide ***(26)***. It was obtained in 59% yield. Purity (LC−MS) > 95%. NMR: ^{1}H (DMSO, 400 MHz): δ 12.88 (NH, 1H, br), 8.47 (H7, 1H, d, J_{7-5} = 1.7 Hz), 7.89 (H4, 1H, d, J_{4-5} = 8.6 Hz), 7.73 (H5, 1H, dd, J_{5-4} = 8.6 Hz and J_{5-7} = 1.7 Hz), 2.02 (CHcPr, 1H, m) and 0.99 (CH_2cPr, 4H, m). HRMS: mass calculated for $C_{12}H_{10}F_3N_2OS$, 287.0460 and mass found, 287.0464.

General Synthesis Protocol of N-Substituted(benzothiazol-2-yl) ***27 to 30***. To an ice-cold solution of aminobenzothiazole (1 equiv) in DCM (0.13 M) were successively added triethylamine (3 equiv) and acyl chloride (1.5 equiv). After stirring from 1 to 24 h, the mixture was diluted in 10 mL of brine and extracted with DCM. The organic layers were combined, washed with a saturated solution of $NaHCO_3$, dried over Na_2SO_4, and filtered and concentrated under reduced pressure. The crude was purified by chromatography eluting with cyclohexane/EtOAc.

N-(6-Chlorobenzothiazol-2-yl)butyramide ***(27)***. It was obtained in 28% yield. Purity (LC−MS) > 95%. NMR: ^{1}H (DMSO, 400 MHz): δ 12.40 (NH, 1H, br), 8.11 (H7, 1H, d, J_{7-5} = 2.2 Hz), 7.72 (H4, 1H, d, J_{4-5} = 8.8 Hz), 7.44 (H5, 1H, dd, J_{5-4} = 8.8 Hz and J_{5-7} = 2.2 Hz), 2.47 (CH_2CO, 1H, t, $J_{CH_2CO-CH_2CH_3}$ = 7.2 Hz), 2.03 (CH_2CH_3, 2H, sx) and 0.92 (CH_3, 3H, d, $J_{CH_3-CH_2}$ = 7.5 Hz). HRMS: mass calculated for $C_{11}H_{11}ClN_2OS$, 255.0353 and mass found, 255.0352.

N-(6-Chlorobenzothiazol-2-yl)isobutyramide ***(28)***. It was obtained in 56% yield. Purity (LC−MS) > 95%. NMR: ^{1}H (DMSO, 400 MHz): δ 12.40 (NH, 1H, br), 8.12 (H7, 1H, d, J_{7-5} = 2 Hz), 7.72 (H4, 1H, d, J_{4-5} = 8.6 Hz), 7.45 (H5, 1H, dd, J_{5-4} = 8.6 Hz and J_{5-7} = 2 Hz), 2.79 (CH*i*Pr, 1H, q, $J_{CH-iPr-CH_3}$ = 6.9 Hz) and 1.15 (CH_3*i*Pr, 6H, d, $J_{CH_3-CH-iPr}$ = 6.9 Hz). HRMS: mass calculated for $C_{11}H_{12}ClN_2OS$, 255.0353 and mass found, 255.0345. mp (°C) 181.

N-(6-Chlorobenzothiazol-2-yl)pivalamide ***(29)***. It was obtained in 46% yield. Purity (LC−MS) > 95%. NMR: ^{1}H (DMSO, 400 MHz): δ 12.14 (NH, 1H, br), 8.12 (H7, 1H, d, J_{7-5} = 2 Hz), 7.72 (H4, 1H, d, J_{4-5} = 8.6 Hz), 7.46 (H5, 1H, dd, J_{5-4} = 8.6 Hz and J_{5-7} = 2 Hz) and 1.27 (CH_3*t*Bu, 9H, m). HRMS: mass calculated for $C_{12}H_{14}ClN_2OS$, 269.0510 and mass found, 269.0510. mp (°C) 142.8.

N-(6-Chlorobenzothiazol-2-yl)cyclobutanecarboxamide ***(30)***. It was obtained in 47% yield. Purity (LC−MS) > 95%. NMR: ^{1}H (DMSO, 400 MHz): δ 12.30 (NH, 1H, br), 8.12 (H7, 1H, d, J_{7-5} = 2.3 Hz), 7.71 (H4, 1H, d, J_{4-7} = 8.6 Hz), 7.44 (H5, 1H, dd, J_{5-4} = 8.6 Hz and J_{5-7} = 2.3 Hz), 3.41 (CHcBu, 1H, q, $J_{CH-CBu-CH_2}$ = 8.3 Hz) and 2.33−1.81 (CH_2cBu, 6H, m). HRMS: mass calculated for $C_{12}H_{12}ClN_2OS$, 267.0353 and mass found, 267.0352. mp (°C) 167 & 189 (two crystalline forms present).

General Synthesis Protocol of N-Substituted(benzothiazol-2-yl) ***31 to 34***. To an ice-cold solution of 2-amino-subtituted-benzothiazole (1 equiv) in DMF (0.1 mL) were added successively HATU (2 equiv), DIEA (2 equiv), and corresponding carboxylic acid (1 to 1.5 equiv). The mixture was warmed up to room temperature and stirred from 18 to 24 h. The reaction was quenched by the addition of water, and the product was extracted using EtOAc. The organic layers were combined, washed with brine, dried over Na_2SO_4, and filtered and concentrated under vacuum. The crude was purified by chromatography eluting with cyclohexane/EtOAc.

N-(6-Chlorobenzothiazol-2-yl)-2-cyclopropylacetamide ***(31)***. It was obtained in 82% yield. Purity (LC−MS) > 95%. NMR: ^{1}H (DMSO, 400 MHz): δ 12.36 (NH, 1H, br), 8.12 (H7, 1H, d, J_{7-5} = 2.2 Hz), 7.72 (H4, 1H, d, J_{4-5} = 8.6 Hz), 7.44 (H5, 1H, dd, J_{5-4} = 8.6 Hz and J_{5-7} = 2.2 Hz), 3.39 (CH_2, 2H, d, $J_{CH_2-CHcPr}$ = 7.1 Hz), 1.07 (CHcPr, 1H, m), 0.5 (CH_2cPr, 2H, m) and 0.21 (CH_2cPr, 2H, m). HRMS: mass calculated for $C_{12}H_{12}ClN_2OS$, 267.0353 and mass found, 267.0346.

N-(6-Chlorobenzothiazol-2-yl)tetrahydrofuran-3-carboxamide ***(32)***. It was obtained in 85% yield. Purity (LC−MS) > 95%. NMR: ^{1}H (DMSO, 400 MHz): δ 12.57 (NH, 1H, br), 8.13 (H7, 1H, d, J_{7-5} = 2.3 Hz), 7.73 (H4, 1H, d, J_{4-5} = 8.6 Hz), 7.46 (H5, 1H, dd, J_{5-4} = 8.6 Hz and J_{5-7} = 2.3 Hz), 3.94−3.71 (CH_2, 4H, m), 3.33 (CH, 1H, m) and 2.13 (CH_2, 2H, m). HRMS: mass calculated for $C_{12}H_{12}ClN_2O_2S$, 283.0303 and mass found, 283.0306.

N-(6-Chlorobenzothiazol-2-yl)-3-hydroxypropanamide ***(33)***. It was obtained in 17% yield. Purity (LC−MS) > 95%. NMR: ^{1}H (DMSO, 400 MHz): δ 12.40 (NH, 1H, br), 8.12 (H7, 1H, d, J_{7-5} = 2.8 Hz), 7.72 (H4, 1H, d, J_{4-5} = 8.8 Hz), 7.45 (H5, 1H, dd, J_{5-4} = 8.8 Hz and J_{5-7} = 2.8 Hz), 4.78 (OH, 1H, br), 3.74 (CH_2, 2H, t, $J_{CH_2-CH_2}$ = 6 Hz) and 2.65 (CH_2, 2H, t, $J_{CH_2-CH_2}$ = 6 Hz). HRMS: mass calculated for $C_{10}H_{10}ClN_2O_2S$, 257.0146 and mass found, 257.0153.

N-(6-Chlorobenzothiazol-2-yl)oxetane-3-carboxamide ***(34)***. It was obtained in 22% yield. Purity (LC−MS) > 95%. NMR: ^{1}H (DMSO, 400 MHz): δ 12.46 (NH, 1H, br), 8.15 (H7, 1H, d, J_{7-5} = 2 Hz), 7.73 (H4, 1H, d, J_{4-5} = 8.8 Hz), 7.46 (H5, 1H, dd, J_{5-4} = 8.8 Hz and J_{5-7} = 2 Hz), 4.72 (CH_2, 4H, m) and 4.13 (CH, 1H, m). HRMS:

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mass calculated for $C_{11}H_{10}ClN_2O_2S$, 269.0146 and mass found, 269.0151.

*General Synthesis Protocol of N-Alkylated Aminobenzothizole **37a–c**.* To an ice-cold solution of 2-amino-6-chlorobenzothiazole (0.200 g, 1.08 mmol) in DCM (10 mL) were added successively triethylamine (0.30 mL, 1.30 mmol), DMAP (0.0066 g, 0.054 mmol), and Boc anhydride (0.164 g, 1.62 mmol). The mixture was warmed up to room temperature and stirred 17 h. The solution was diluted in brine (20 mL), and the product was extracted with DCM (3 × 15 mL). The organic layers were combined, dried over Na_2SO_4, and filtered and concentrated under vacuum. The crude was purified with chromatography eluting with cyclohexane/EtOAc (95/5 to 85/15) to furnish **35** as a white solid (0.187 g, 61%).

To an ice-cold solution of **35** (1 equiv) in THF were added successively NaH (1.2 equiv) and iodomethane, iodoethane, or bromobutane (2–4 equiv). The solution was warmed up to 80–100 °C. The reaction was stirred during 3–17 h and quenched by the addition of 20 mL of water. The product was extracted with EtOAc (3 × 10 mL), and the organic phases were combined, dried over Na_2SO_4, and filtered and reduced under vacuum. Crudes were purified by chromatography eluting with cyclohexane/EtOAc to furnish **36a** to **36c**.

To a solution of **36a**, **36b**, or **36c** (1 equiv) in DCM (0.1 M) was added TFA (15 equiv). The solution was stirred at room temperature 3.5–17 h, and the reaction was quenched by the addition of saturated $NaHCO_3$ (pH = 9). The product was extracted with EtOAc (3 × 10 mL), the organic layers were combined, dried over Na_2SO_4, and filtered and concentrated under vacuum. Crudes were purified by chromatography eluting with cyclohexane/EtOAc to furnish **37a** to **37c** (yields are given for the last step, see Supporting Information for the other steps).

*6-Chloro-N-methylbenzothiazol-2-amine (**37a**).* It was obtained in 50% yield. NMR: ^{1}H (DMSO, 400 MHz): δ 8.05 (NH, 1H, m), 7.79 (H7, 1H, d, J_{7-5} = 2 Hz), 7.36 (H4, 1H, d, J_{4-5} = 8.4 Hz), 7.23 (H5, 1H, dd, J_{5-4} = 8.4 Hz and J_{5-7} = 2 Hz) and 2.93 (Me, 3H, d, J_{Me-NH} = 4.8 Hz). MS: M + H$^+$ = 198.9 (exact mass of $C_8H_7ClN_2S$: 198.00).

*6-Chloro-N-ethylbenzothiazol-2-amine (**37b**).* It was obtained in 59% yield. NMR: ^{1}H (DMSO, 400 MHz): δ 8.10 (NH, 1H, t, J_{NH-CH_2} = 5.2 Hz), 7.78 (H7, 1H, d, J_{7-5} = 2 Hz), 7.34 (H4, 1H, d, J_{4-5} = 8.6 Hz), 7.22 (H5, 1H, dd, J_{5-4} = 8.6 Hz and J_{5-7} = 2 Hz), 3.37 (CH_2, 2H, m), 1.18 (CH_3, 3H, t, $J_{CH_3-CH_2}$ = 7.2 Hz). MS: M − H$^+$ 211.0 (exact mass of $C_9H_9ClN_2S$: 212.02).

*6N-Butyl-6-chlorobenzothiazol-2-amine (**37c**).* It was obtained in 65% yield. NMR: ^{1}H (DMSO, 400 MHz): δ 8.09 (NH, 1H, t, J_{NH-CH_2} = 5.3 Hz), 7.72 (H7, 1H, d, J_{7-5} = 2 Hz), 7.33 (H4, 1H, d, J_{4-5} = 8.6 Hz), 7.21 (H5, 1H, dd, J_{5-4} = 8.6 Hz and J_{5-7} = 2 Hz), 3.34 (CH_2, 2H, m), 1.55 (CH_2, 2H, qt), 1.36 (CH_2, 2H, m) and 0.90 (HCH_3, 3H, t, $J_{H_{11}-H_{10}}$ = 7.3 Hz). MS: M + H$^+$ 241.0 (exact mass of $C_{11}H_{13}ClN_2S$: 240.05).

*Synthesis Protocol of 2-((6-Chlorobenzothiazol-2-yl)amino)-ethanol (**37d**).* 2-Bromoethanol (0.042 mL, 0.630 mmol) was added to a solution of 2-amino-6-chlorobenzothiazole (50 mg, 0.271 mmol) in DMF (0.5 mL). The solution was stirred at 100 °C overnight, before quenching by the addition of water and of a solution of NaOH 1 M (2 mL). Then, the product was extracted with EtOAc (3 × 10 mL), and the organic layers were combined, washed with brine, dried over Na_2SO_4, and filtered and concentrated under vacuum. The crude was purified by chromatography eluting with cyclohexane/EtOAc (95/5 to 0/100) to furnish **37d** as a white solid (11 mg, 18% yield).

NMR: ^{1}H (DMSO, 400 MHz): δ 8.30 (NH, 1H, s), 7.53 (H7, 1H, d, J_{7-5} = 2 Hz), 7.23 (H5, 1H, dd, J_{5-4} = 8.6 Hz and J_{5-7} = 2 Hz), 7.08 (H4, 1H, d, J_{4-5} = 8.6 Hz), 4.86 (OH, 1H, br), 3.93 (CH_2, 2H, t, $J_{CH_2-CH_2}$ = 5.9 Hz), and 3.62 (CH_2, 2H, m). MS: M + H$^+$ 229.0 (exact mass of $C_9H_9ClN_2SO$: 228.01).

*Characterization of N-Alkylated(6-chlorobenzothiazol-2-yl)-cyclopropanecarboxamide **38** to **41**. N-(6-Chlorobenzothiazol-2-yl)-N-methylcyclopropanecarboxamide (**38**).* It was obtained in 75% yield. Purity (LC–MS) > 95%. NMR: ^{1}H (DMSO, 400 MHz): δ 8.09 (H7, 1H, d, J_{7-5} = 2.3 Hz), 7.79 (H4, 1H, d, J_{4-5} = 8.6 Hz), 7.45 (H5, 1H, dd, J_{5-4} = 8.6 Hz and J_{5-7} = 2.3 Hz), 3.92 (CH_3, 3H, s), 2.37 (CHcPr, 1H, m) and 1.05 (CH_2cPr, 4H, m). HRMS: mass calculated for $C_{12}H_{12}ClN_2OS$, 267.0353 and mass found, 267.0358.

*N-(6-Chlorobenzothiazol-2-yl)-N-ethylcyclopropanecarboxamide (**39**).* It was obtained in 30% yield. Purity (LC–MS) > 90%. NMR: ^{1}H (DMSO, 400 MHz): δ 8.10 (H7, 1H, d, J_{7-5} = 2 Hz), 7.8 (H4, 1H, d, J_{4-5} = 8.8 Hz), 7.45 (H5, 1H, dd, J_{5-4} = 8.8 Hz and J_{5-7} = 2 Hz), 4.35 (CH_2, 2H, q, $J_{CH_2-CH_3}$ = 7 Hz), 2.36 (CHcPr, 1H, m), 1.4 (CH_3, 3H, t, $J_{CH_3-CH_2}$ = 7 Hz) and 1.07 (CH2cPr, 4H, m). HRMS: mass calculated for $C_{13}H_{14}ClN_2OS$, 281.0510 and mass found, 281.0514.

*N-(6-Chlorobenzothiazol-2-yl)-N-butylcyclopropanecarboxamide (**40**).* It was obtained in 13% yield. Purity (LC–MS) > 90%. NMR: ^{1}H (DMSO, 400 MHz): δ 8.09 (H7, 1H, d, J_{7-5} = 2.3 Hz), 7.79 (H4, 1H, d, J_{4-5} = 8.6 Hz), 7.45 (H5, 1H, dd, J_{5-4} = 8.6 Hz and J_{5-7} = 2 Hz), 4.48 (CH_2, 2H, m), 2.33 (CHcPr, 1H, m), 1.80 (CH_2, 2H, m), 1.41 (CH_2, 2H, m), 1.06 (CH2cPr, 4H, m) and 0.97 (CH_3, 3H, t, $J_{H_{11}-H_{10}}$ = 7.6 Hz). HRMS: mass calculated for $C_{15}H_{18}ClN_2OS$, 309.0823 and mass found, 309.0820.

*N-(6-Chlorobenzothiazol-2-yl)-N-(2-hydroxyethyl)-cyclopropanecarboxamide (**41**).* It was obtained in 34% yield. Purity (LC–MS) > 95%. NMR: ^{1}H (DMSO, 400 MHz): δ 7.99 (H7, 1H, d, J_{7-5} = 2.3 Hz), 7.67 (H4, 1H, d, J_{4-5} = 8.8 Hz), 7.52 (H5, 1H, dd, J_{5-4} = 8.8 Hz and J_{5-7} = 2.3 Hz), 4.91 (OH, 1H, t, J_{OH-CH_2} = 5.7 Hz), 4.41 (CH_2, 2H, t, $J_{CH_2-CH_2}$ = 5.6 Hz), 3.78 (CH_2, H, q, J = 5.6 Hz) 1.83 (CHcPr, 1H, m) and 0.92 (CH_2cPr, 4H, m). HRMS: mass calculated for $C_{13}H_{14}ClN_2O_2S$, 297.0459 and mass found, 297.0469.

*Synthesis of 6-Chloro-N-(cyclopropylmethyl)benzothiazol-2-amine (**42**).* To an ice-cold solution of **1** (0.030 g, 0.118 mmol) in THF (0.7 mL) was added dropwise a solution of $LiAlH_4$ in THF (0.47 mL, 0.47 mmol). The solution was warmed up to room temperature and stirred for 17 h. The mixture was diluted in water (4 mL). The product was extracted with EtOAc (3 × 10 mL), and the organic layers were combined, dried over Na_2SO_4, and filtered and reduced under vacuum. The crude was purified on preparative TCL eluting with cyclohexane/EtOAc (70/30) to furnish **42** as a beige solid (0.018 g, 64%).

Purity (LC–MS) > 95%. NMR: ^{1}H (DMSO, 400 MHz): δ 8.23 (NH, 1H, t, J_{NH-CH_2} = 5.4 Hz) 7.7 (H7, 1H, d, J_{7-5} = 2.3 Hz), 7.33 (H4, 1H, d, J_{4-5} = 8.6 Hz), 7.21 (H5, 1H, dd, J_{5-4} = 8.6 Hz and J_{5-7} = 2.3 Hz), 3.23 (CH_2, 2H, m), 1.09 (CHcPr, 1H, m), 0.48 (CH_2cPr, 2H, m) and 0.25 (CH_2cPr, 2H, m). HRMS: mass calculated for $C_{11}H_{12}ClN_2S$, 239.0404 and mass found, 239.0413.

*Synthesis of 2-Hydrazinyl-6-(trifluoromethyl)benzothiazole (**43**).* To an ice-cold solution of hydrazine monohydrate (0.066 mL, 1.37 mmol) were successively added hydrochloric acid (0.062 mL, 0.756 mmol), ethylene glycol (0.5 mL), and 6-trifluoromethylbenzothiazol (0.050 g, 0.229 mmol). The solution was warmed up to reflux (160 °C) and stirred for 3 h. Then, the mixture was cooled down to room temperature and dissolved in 10 mL of EtOAc. The organic phase was washed with brine, dried over Na_2SO_4, and filtered and concentrated under vacuum. The crude was purified by chromatography eluting with cyclohexane/EtOAc (95/5 to 30/70) to furnish **43** as a brown solid (0.014 g, 26%).

NMR: ^{1}H (DMSO, 400 MHz): δ 9.44 (NH, 1H, br), 8.12 (H7, 1H, d, J_{7-5} = 1.5 Hz), 7.50 (H5, 1H, dd, J_{4-5} = 8.6 Hz and J_{4-7} = 1.5 Hz), 7.42 (H4, 1H, d, J_{4-5} = 8.6 Hz) and 5.19 (NH_2, 2H, br). MS: M + H$^+$ 234.0 (exact mass of $C_8H_6F_3N_3S$: 233.00).

*Synthesis of (2-(Cyclopropylmethylene)hydrazinyl)-6-(trifluoromethyl)benzothiazole (**44**).* To a solution **43** (0.014 g, 0.06 mmol) in ethanol (0.3 mL) was added cyclopropanecarboxaldehyde (4.5 μL, 0.06 mmol), and the solution was stirred at room temperature for 24 h. The mixture was quenched by the addition of water, and the product was extracted with EtOAc (3 × 10 mL). The organic layers were combined, dried over Na_2SO_4, and filtered and concentrated under vacuum. The crude was purified by chromatog-

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raphy eluting with cyclohexane/EtOAc (95/5 to 30/70) to furnish **44** as a yellow solid (0.003 g, 17%).

Purity (LC−MS) > 95%. NMR: ^{1}H (DMSO, 400 MHz): δ 12.11 (NH, 1H, br), 8.18 (H7, 1H, br), 7.55 (H5 and NCH, 2H, m), 7.03 (H4, 1H, d, J_{4-5} = 7.6 Hz), 1.68 (CHcPr, 1H, m), 0.91 (CH_2cPr, 2H, m) and 0.71 (CH_2cPr, 2H, m). HRMS: mass calculated for $C_{12}H_{11}F_3N_3S$, 286.0620 and mass found, 286.0633.

*Synthesis of N-(6-(Trifluoromethyl)benzothiazol-2-yl)-cyclopropanesulfonamide (**45**).* To a solution of 6-trifuoromethyl-benzothiazole (0.030 g, 0.137 mmol) in pyridine (0.3 mL) was added cyclopropanesulfonyl chloride (0.028 mL, 0.274 mmol). The reaction was warmed up to 40 °C and stirred for 3 days. The reaction was quenched by the addition of water (5 mL), and the product was extracted with EtOAc (3 × 5 mL). The organic layers were combined, dried over Na_2SO_4, and filtered and concentrated under vacuum. The crude was purified by chromatography eluting with cyclohexane/EtOAc (95/5 to 0/100) to furnish **45** as a white solid (0.011 g, 25% yield).

Purity (LC−MS) > 95%. NMR: ^{1}H (DMSO, 400 MHz): δ 13.35 (NH, 1H, br), 8.25 (H7, 1H, s), 7.71 (H5, 1H, dd, J_{5-4} = 8.3 Hz and J_{5-7} = 1.5 Hz) 7.44 (H4, 1H, d, J_{4-5} = 8.3 Hz), 2.73 (CHcPr, 1H, m) and 0.96 (CH_2cPr, 4H, m). HRMS: mass calculated for $C_{11}H_{10}F_3N_2O_2S_2$, 323.0130 and mass found, 323.0126.

*General Synthesis Route for 5,6-Disubstituted Aminobenzothiazole **49a−h**.* Iodination using boron trifluoride diethyletherate: to a solution of disubstituted aniline (1 equiv) in DCM (0.28 M) were successively added NIS (1 equiv) and BF_3OEt_2 (1 equiv). The mixture was stirred 60−100 min at rt, and the reaction was quenched by the addition of sodium thiosulfate. The product was extracted with DCM, and the organic layers were combined, dried over Na_2SO_4, and filtered and concentrated under vacuum. The crude was purified by chromatography eluting with cyclohexane/EtOAc. Iodination using pTSA: to a solution of disubstituted aniline (1 equiv) in THF/MeOH (0.25 M) were successively added NIS (1−1.2 equiv) and pTSA (0.2−0.5 equiv). The mixture was stirred 1 h to 2h20 at rt, and the reaction was quenched by the addition of brine (10 mL). The product was extracted with EtAOc, and the organic layers were combined, washed with $NaHCO_3$, dried over Na_2SO_4, and filtered and concentrated under vacuum. The crude was purified by chromatography eluting with cyclohexane/EtOAc to furnish 5-iodinated anilines. Iodinated intermediate (1 equiv) was dissolved in THF (1 M) and benzoyl isothiocyanate (1−3 equiv) was added to the solution. The mixture was stirred under microwave activation during 20−40 min at 100 °C. The solvent was concentrated under vacuum, and the crude was purified by chromatography eluting with cyclohexane/EtOAc (95/5 to 90/10) to furnish 1-benzoyl 3-(3′-R1-4′-R1′-6′-iodophenyl) intermediates **48a−h**. To a solution of 1-benzoyl 3-(3′-R1-4′-R1′-6′-iodophenyl) (1 equiv) in MeOH (0.03 M) was added K_2CO_3 (1 equiv). The solution was warmed up to 60 °C and stirred for 18−43 h. The mixture was then diluted in AcOEt and in water. The product was extracted with EtOAc, the organic layer was dried over Na_2SO_4, and filtered and concentrated under vacuum. The crude was purified by chromatography eluting with cyclohexane/EtOAc to furnish disubstituted aminobenzothiazole (yields are given for the last step, see Supporting Information for the other steps).

*5-Fluoro-6-(trifluoromethyl)benzothiazol-2-amine (**49a**).* It was obtained in 67% yield. NMR: ^{1}H (DMSO, 400 MHz): δ 8.1 (H7, 1H, d, J_{7-F} = 7.6 Hz), 8.06 (NH_2, 2H, br) and 7.34 (H4, 1H, d, J_{4-F} = 12 Hz). MS: M + H$^+$ 237.0 (exact mass of $C_8H_4F_4N_2S$: 236.00).

*6-Fluoro-5-(trifluoromethyl)benzothiazol-2-amine (**49b**).* It was obtained in 50% yield. NMR: ^{1}H (DMSO, 400 MHz): δ 7.92 (H7, 1H, d, J_{7-F} = 10.8 Hz), 7.79 (NH_2, 2H, br) and 7.57 (H4, 1H, d, J_{4-F} = 6.3 Hz). MS: M + H$^+$ 237.0 (exact mass of $C_8H_4F_4N_2S$: 236.00).

*2-Amino-5-fluorobenzothiazole-6-carbonitrile (**49c**).* It was obtained in 31% yield. NMR: ^{1}H (DMSO, 400 MHz): δ 8.25 (NH_2, 2H, br), 8.19 (H7, 1H, d, J_{5-F} = 6.8 Hz), and 7.36 (H4, 1H, d, J_{2-F} = 11.1 Hz). MS: M + H$^+$ 194.30 (exact mass of $C_8H_4FN_3S$: 193.01).

*5-Methyl-6-(trifluoromethyl)benzothiazol-2-amine (**49d**).* It was obtained in 38% yield. NMR: ^{1}H (DMSO, 400 MHz): δ 8.01 (H7, 1H, s), 7.81 (NH_2, 2H, br), 7.32 (H4, 1H, s) and 3.86 (CH_3, 3H q, $J_{CH_3-CF_3}$ = 1.4 Hz). MS: M + H$^+$ 233.10 (exact mass of $C_9H_7F_3N_2S$: 232.03).

*2-Amino-6-(trifluoromethyl)benzothiazole-5-carbonitrile (**49e**).* It was obtained in 53% yield. NMR: ^{1}H (DMSO, 400 MHz): δ 8.42 (H7, 1H, s), 8.24 (NH_2, 2H, br) and 7.98 (H4, 1H, s). MS: M + H$^+$ 244.00 (exact mass of $C_9H_4F_3N_3S$: 243.01).

*5-Methoxy-6-(trifluoromethyl)benzothiazol-2-amine (**49f**).* It was obtained in 32% yield. NMR: ^{1}H (DMSO, 400 MHz): δ 7.92 (H7, 1H, s), 7.82 (NH_2, 2H, br), 7.13 (H4, 1H, s) and 3.86 (CH_3, s, 3H). MS: M + H$^+$ 249.0 (exact mass of $C_9H_7F_3N_2OS$: 248.02).

*2-Amino-5-fluoro-N-methylbenzothiazole-6-sulfonamide (**49g**).* It was obtained in 48% yield. NMR: ^{1}H (DMSO, 400 MHz): δ 8.08 (H7, 1H, d, J_{7-F} = 7.6 Hz), 8.06 (NH_2, 2H, br), 7.47 (NH, 1H, q, J_{NH-CH_3} = 4.8 Hz), 7.29 (H4, 1H, d, J_{4-F} = 11.6 Hz) and 2.46 (CH_3, 3H, J_{CH_3-NH} = 4.8 Hz). MS: M + H$^+$ 262.1 (exact mass of $C_8H_8FN_3S_2O_2$: 261.00).

*6-(Ethylsulfonyl)-5-fluorobenzothiazol-2-amine (**49h**).* It was obtained in 34% yield. NMR: ^{1}H (DMSO, 400 MHz): δ 8.17 (NH_2, 2H, br), 8.08 (H7, 1H, d, J_{7-F} = 7.4 Hz), 7.34 (H4, 1H, d, J_{4-F} = 12 Hz), 3.32 (CH_2, 2H, q, $J_{CH_2-CH_3}$ = 7.3 Hz) and 1.13 (CH_3, 3H, $J_{CH_3-CH_2}$ = 7.3 Hz). MS: M + H$^+$ 261.1 (exact mass of $C_9H_9FN_2S_2O_2$: 260.01).

*Characterization of N-(5-6-Disubsituted-benzothiazol-2-yl)-cyclopropanecarboxamide **50** to **57**. N-(5-Fluoro-6-(trifluoromethyl)benzothiazol-2-yl)cyclopropanecarboxamide (**50**).* It was obtained in 66% yield. Purity (LC−MS) > 95%. NMR: ^{1}H (DMSO, 400 MHz): δ 12.98 (NH, 1H, br), 8.52 (H7, 1H, d, J_{7-F} = 7.3 Hz), 7.84 (H4, 1H, d, J_{4-F} = 12 Hz), 2.03 (CHcPr, 1H, m) and 0.98 (CH_2cPr, 6H, m). HRMS: mass calculated for $C_{12}H_9F_4N_2OS$, 305.0366 and mass found, 305.0378. mp (°C) 222.1.

*N-(6-Fluoro-5-(trifluoromethyl)benzothiazol-2-yl)-cyclopropanecarboxamide (**51**).* It was obtained in 33% yield. Purity (LC−MS) > 95%. NMR: ^{1}H (DMSO, 400 MHz): δ 12.91 (NH, 1H, br), 8.22 (H7, 1H, d, J_{7-F} = 10.4 Hz), 8.07 (H4, 1H, d, J_{4-F} = 6.1 Hz), 2.03 (CHcPr, 1H, m) and 0.98 (CH_2cPr, 6H, m).HRMS: mass calculated for $C_{12}H_9F_4N_2OS$, 305.0366 and mass found, 305.0370.

*N-(6-Cyano-5-fluorobenzothiazol-2-yl)-cyclopropanecarboxamide (**52**).* It was obtained in 49 % yield. Purity (LC−MS) > 95%. NMR: ^{1}H (DMSO, 400 MHz): δ 13.07 (NH, 1H, br), 8.59 (H7, 1H, d, J_{7-F} = 6.8 Hz), 7.86 (H4, 1H, d, J_{4-F} = 10.8 Hz), 2.03 (CHcPr, 1H, m) and 0.99 (CH_2cPr, 6H, m). HRMS: mass calculated for $C_{12}H_9FN_3OS$, 262.0445 and mass found, 262.0447.

*N-(5-Methyl-6-(trifluoromethyl)benzothiazol-2-yl)-cyclopropanecarboxamide (**53**).* It was obtained in 39% yield. Purity (LC−MS) > 95%. NMR: ^{1}H (DMSO, 400 MHz): δ 12.85 (NH, 1H, br), 8.37 (H7, 1H, s), 7.76 (H4, 1H, s), 2.53 (CH_3, 3H, s), 2.01 (CHcPr, 1H, m) and 0.97 (CH_2cPr, 6H, m). HRMS: mass calculated for $C_{13}H_{12}F_3N_2OS$, 301.0617 and mass found, 301.0612.

*N-(5-Cyano-6-(trifluoromethyl)benzothiazol-2-yl)-cyclopropanecarboxamide (**54**).* It was obtained in 65% yield. Purity (LC−MS) > 95%. NMR: ^{1}H (DMSO, 400 MHz): δ 13.16 (NH, 1H, br), 8.78 (H7, 1H, s), 8.55 (H4, 1H, s), 2.04 (CHcPr, 1H, m) and 1.00 (CH_2cPr, 6H, m). HRMS: mass calculated for $C_{13}H_9F_3N_3OS$, 312.0413 and mass found, 312.0401.

*N-(5-Methoxy-6-(trifluoromethyl)benzothiazol-2-yl)-cyclopropanecarboxamide (**55**).* It was obtained in 36% yield. Purity (LC−MS) > 95%. NMR: ^{1}H (DMSO, 400 MHz): δ 12.81 (NH, 1H, br), 8.30 (H7, 1H, s), 7.50 (H4, 1H, s), 3.95 (CH_3, 3H, s), 2.03 (CHcPr, 1H, m) and 0.97 (CH_2cPr, 6H, m). HRMS: mass calculated for $C_{13}H_{12}F_3N_2O_2S$, 317.0566 and mass found, 317.0559.

*N-(5-Fluoro-6-(N-methylsulfamoyl)benzothiazol-2-yl)-cyclopropanecarboxamide (**56**).* It was obtained in 9% yield. Purity (LC−MS) > 95%. NMR: ^{1}H (DMSO, 400 MHz): δ 12.95 (NH, 1H, br), 8.46 (H7, 1H, d, J_{7-F} = 8 Hz), 7.77 (H4, 1H, d, J_{4-F} = 12 Hz), 7.65 (NH, 1H, q, J_{NH-CH_3} = 5.2 Hz), 2.52 (CH_3, 3H, d, J_{CH_3-NH} = 5.2 Hz), 2.03 (CHcPr, 1H, m) and 0.98 (CH_2cPr, 6H, m). MS: M + H$^+$ 330.20 (exact mass of $C_{12}H_{12}F_3N_3S_2O_3$: 329.03).

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*N-(6-(Ethylsulfonyl)-5-fluorobenzothiazol-2-yl)-cyclopropanecarboxamide (**57**).* It was obtained in 47% yield. Purity (LC−MS) > 95%. NMR: ^{1}H (DMSO, 400 MHz): δ 13.03 (NH, 1H, br), 8.55 (H7, 1H, d, J_{7-F} = 7.2 Hz), 7.84 (H4, 1H, d, J_{4-F} = 10.8 Hz), 3.41 (CH_2, 2H, q, $J_{CH_2-CH_2}$ = 7.6 Hz), 2.03 (CHcPr, 1H, m), 1.16 (CH_3, 3H, $J_{CH_3-CH_2}$ = 7.6 Hz) and 0.99 (CH_2cPr, 6H, m). HRMS: mass calculated for $C_{13}H_{14}FN_2O_3S_2$, 329.0424 and mass found, 329.0410.

*Characterization of **58** to **60**. N-(5-Fluoro-6-(trifluoromethyl)-benzothiazol-2-yl)isobutyramide (**58**).* It was obtained in 46% yield. Purity (LC−MS) > 95%. NMR: ^{1}H (DMSO, 400 MHz): δ 12.66 (NH, 1H, br), 8.54 (H7, 1H, d, J_{7-F} = 8 Hz), 7.84 (H4, 1H, d, J_{4-F} = 12 Hz), 2.82 (CH*i*Pr, 1H, st, J_{CH-CH_3} = 6.8 Hz) and 1.16 (CH_3*i*Pr, 3H, t, $J_{CH_3-CH_2}$ = 6.8 Hz). HRMS: mass calculated for $C_{12}H_{11}F_4N_2OS$, 307.0523 and mass found, 307.0533.

*2-Fluoro-2-methyl-N-(6-(trifluoromethyl)benzothiazol-2-yl)-propenamide (**59**).* It was obtained in 58% yield. Purity (LC−MS) > 95%. NMR: ^{1}H (DMSO, 400 MHz): δ 12.76 (NH, 1H, br), 8.54 (H7, 1H, s), 7.95 (H4, 1H, d, J_{7-6} = 8.3 Hz), 7.77 (H5, 1H, d, J_{5-4} = 8.3 Hz and J_{5-7} = 1.8 Hz) and 1.64 (CH_3*i*PrF, 6H, d, J_{CH_3-F} = 22 Hz). HRMS: mass calculated for $C_{12}H_{11}F_4N_2OS$, 307.0523 and mass found, 307.0524.

*2-Fluoro-N-(5-fluoro-6-(trifluoromethyl)benzothiazol-2-yl)-2-methylpropanamide (**60**).* It was obtained in 50% yield. Purity (LC−MS) > 95%. NMR: ^{1}H (DMSO, 400 MHz): δ 12.89 (NH, 1H, br), 8.59 (H7, 1H, d, J_{7-F} = 6.6 Hz), 7.89 (H4, 1H, d, J_{4-F} = 12 Hz), 1.64 (CH_3*i*PrF, 6H, d, J_{CH_3-F} = 22 Hz). HRMS: mass calculated for $C_{12}H_{10}F_5N_2OS$, 325.0429 and mass found, 325.0431.

Biology. Compounds were tested in vitro in a *T. cruzi* high-content assay previously described.[33]

Intrinsic Clearance (Cl_{int}) in Microsomes. The test compound (0.5 μM) was incubated with male CD1 mouse, and human liver microsomes (0.5 mg/mL 50 mM potassium phosphate buffer, pH 7.4), and the reaction started with addition of excess NADPH. Immediately, at time zero, and serial times of up to 45 min, an aliquot of the incubation mixture was removed and mixed with acetonitrile to stop the reaction. Internal standard (IS) was added to all samples, the samples centrifuged to sediment precipitated protein, and the plates were then sealed prior to UPLC/MS/MS analysis. Midazolam (among others) was included as the positive control. Metabolic stability expressed as a percentage of parent remaining is calculated using the peak area ratio (compound peak area/IS peak area) of the parent remaining after each incubation time ($t = x$) compared to time zero ($t = 0$) of the incubation. The half-life ($t(1/2)$) was calculated using the following equation

$$t(1/2) = -\ln(2)/k$$

where k is the turn-over rate constant of the ln percent remaining versus time regression.

Intrinsic clearance (Cl_{int}) was calculated from the half-life ($t(1/2)$) using the following equations

$$Cl_{int}\ (\text{mL/min /g tissue}) = (0.693/(t(1/2)) \times (\text{mL of incubation /mg microsomal protein}) \times (\text{mg microsomal protein/g liver})$$

$$Cl_{int}\ (\text{mL/min /g tissue}) = (0.693/(t(1/2)) \times (\text{mL of incubation /number of cells per incubation}) \times (\text{number of cells/g liver})$$

Solubility of the Solid Compound in FaSSIF. This experiment determines the solubility of solid compounds in FaSSIF at pH 6.5, after 4 h equilibration at rt. Then, 1 mL of each buffers was added to manually weighed 1 mg of **50** in a 2 mL HPLC auto-sampler vial. The resulting suspension was shaken at 900 rpm for 4 h at RT and then transferred to a Multiscreen HTS, 96-well solubility filter plate. The residual solid was removed by filtration. The supernatant solution was quantified by HPLC-UV using single-point calibration of a known concentration of the compound in DMSO. The dynamic range of the assay was 1−1000 μg/mL.

Plasma Protein Binding. In vitro plasma protein binding of **50** was measured in mouse and human plasma using equilibrium dialysis at a nominal concentration of 2 μM. The RED inserts were placed in the 48 wells of the Teflon Plate (Pierce). Samples were prepared by mixing the test compound with plasma at the appropriate concentrations to yield a final drug concentration of 2 μM. Triplicate aliquots of plasma containing **50** at a concentration of 2 μM were pipetted to plasma side (red) of the insert, and PBS (phosphate buffered saline) pH 7.4 was placed into the receiver side (white) of the insert. The plate was covered with the sealing tape and incubated in a 37 °C orbital shaker water bath at approximately 150 rpm for 4 h. Following incubation, samples were prepared in a mixed matrix configuration. Aliquots of samples were pipetted into 96-well plates, and precipitation buffer was added to protein precipitate the samples. Samples were vortexed to mix, then centrifuged for 15 min at 3700 rpm and 4 °C. The supernatant was assayed directly by LC−MS/MS. The following equation was used to calculate the percentage bound drug fraction using this equilibrium dialysis method

$$\%\ \text{unbound} = ([\text{buffer chamber}]/[\text{plasma chamber}) \times 100$$

$$\%\ \text{PPB} = 100 - \%\ \text{unbound}$$

Blood to Plasma Ratio. The extent of association of **50** with blood cells was measured in vitro using mouse and human blood. Untreated whole blood was prewarmed on a rotary shaker at 37 °C (350 rpm). Samples were prepared by mixing the test compound with whole blood to yield a final drug concentration of 1 μM. Once mixing was complete, at $T(0)$ and $T(60\ \text{min})$, aliquots of incubated blood containing **50** were mixed with Milli-Q water. To generate a uniform mixed matrix, untreated plasma was added to each individual blood/water sample. In order to precipitate the samples for analysis, precipitation buffer containing IS was added to the individual samples. Samples were centrifuged for 10 min at 13 000 rpm. The supernatant was assayed directly by LC−MS/MS. The following equation was used to calculate the blood to plasma partitioning ratio

$$\text{blood/plasma ratio} = [\text{blood}]/[\text{plasma}]$$

Compound: IS peak area ratios are used as the representative of the relative compound concentrations in the blood and plasma samples.

In Vitro Investigation of the Passive Membrane Permeability in Human MDR1-MDCK Cells at pH 7.4. This assay was designed to determine passive cellular permeability (P_{app}) by using MDR1-MDCK cells. Test items transport was measured in two directions (apical-to-basolateral [AB] and basolateral-to-apical [BA]) in HBSS (Hank's balanced salt solution) transport medium at pH 7.4 (n = 2) at 3 μM at 60 min, with and without the presence of the P-gp inhibitor GF120918.

As reference compounds amprenavir (P-gp substrate), prazosin (low-moderate permeable compound), and propranolol (high permeable compound) were included. Samples were analyzed in an LC−MS/MS system to measure test items and reference compound concentrations: the compound concentrations were expressed as an area ratio determined by dividing the analyte peak area by the IS peak area.

TEER measurements were performed in each well at the beginning of the experiment. At the end of permeability experiments, the integrity of the cell monolayer was evaluated using the paracellular permeability marker Lucifer yellow (LY) in the apical to basolateral direction in each well.

In Vivo Pharmacokinetic Studies. The pharmacokinetics of **50** were investigated in mouse, following single intravenous (i.v.) and oral administration (p.o). In addition, pharmacokinetic profile of **49a** (metabolite) was evaluated after oral administration of **50** at a target dose of 50 mg/kg. Intravenous administration was performed in male CD1 mice, the same strain used for intrinsic clearance assay. Whereas

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oral pharmacokinetic study was performed in female mice C57BL/6, the same strain used for efficacy model, to support efficacy studies. In order to investigate in vivo clearance (Cl) and volume of distribution (V_{ss}), intravenous pharmacokinetic study was conducted in male CD-1 mice. Three animals were used. A dose of 5 mg/kg was administered intravenously, in a bolus form to mice. Compound **50** was dissolved in 5% DMSO/20% encapsine in saline. A dose of 50 mg/kg was orally administered by gavage to 3 mice C57BL/6. Compound **50** was formulated as a suspension of 1% methylcellulose to investigate oral pharmacokinetics. Peripheral blood samples were obtained at 5, 15, 30 min, 1, 2, 4, 8, and 24 h after intravenous administration and 15, 30, 45 min, 1, 2, 4, 8, and 24 h after oral administration. Blood was 1/2 diluted with Milli-Q water and immediately frozen on dry ice until analysis. Quantification was performed by means of LC−MS/MS (API4000), with a lower limit of quantification of 1 ng/mL.

Pharmacokinetic parameters, namely clearance (Cl), volume of distribution at steady state (V_{ss}), terminal half life ($t_{1/2}$), C_{max}, and area under the curve (AUC) were estimated using Phoenix 64 (Pharsight, Certara). Bioavailability (F %) was not estimated because i.v. and p.o. administrations were made in different mice strains. Metabolite levels in the whole blood were also evaluated in order to estimate C_{max}, T_{max}, and AUC (Table 11).

Table 11. Summary of Whole Blood Pharmacokinetic Parameters (Mean and Standard Deviation) Obtained after Intravenous and Oral Administration of 50 to CD-1 Mouse and C57BL/6 Mouse, Respectively[a]

parameters	mouse CD-1 male at 5 mg/kg i.v.	mouse C57BL/6 female at 50 mg/kg p.o.
Cl (mL/min/kg)	58.3 ± 7.4	
% LBF	46.2	
V_{ss} (L/kg)	5.73 ± 0.91	
$T_{1/2}$ (h)	0.97 ± 0.11	
AUC_{0-t} (ng h/mL)		31560.56 ± 2052
C_{max} (ng/mL)		3386.67 ± 272.09
T_{max} (h)		1.33 ± 0.58

[a]Clearance values are additionally expressed as the percentage of liver blood flow (% LBF).

In Vivo Anti-T. cruzi Activity Assay. The IVA of compound was assessed in a rapid acute in vivo assay measuring the parasite load using two doses of compound over 4 days (**1**) or a single dose over 2 days (compound **50**) as previously described.[34] In brief, C57BL/6 mice were infected subcutaneously in the footpads with 2.5×10^5 trypomastigotes of the *T. cruzi* CL strain expressing the fluorescent protein tdTomato. Two days after the infection, a basal level of fluorescence was measured using the Maestro in vivo imaging system (PerkinElmer, Waltham, MA) using the manufacturer's Green filter set (560/10/750); 2 × 2 bin; 180 ms exposure and 50 mg/kg of the compound suspended in 1% carboxymethyl cellulose, 0.1% Tween 80 was administered. The fluorescent intensity at the site of the infection was measured again at 4 or 6 days post-infection and the IVA of the compound (IVA) was determined by the formula:

$$100 - \left(\frac{\text{Tx fluor 4d} - \text{Tx fluor 2d}}{(\text{unTx fluor 4d} - \text{unTx fluor 2d})} \times 100 \right) = \text{IVA}$$

Tx: fluorescence intensity of the treated group.

unTx: fluorescence intensity of the untreated control group.

Groups of infected mice treated with 50 mg/kg of benznidazole or left untreated were used as positive and negative controls.

All animal use was performed in accordance with protocol A2014 09-017-R2 approved by the University of Georgia Institutional Animal Care and Use Committee. This protocol adhered to the animal welfare guidelines outlined in Guide for the Care and Use of Laboratory Animals, National Research Council USA.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jmedchem.9b01429.

Yields, NMR, and MS characterization of intermediates **6**, **7**, **35**, and **36a** to **36c** synthesis of oxetane-3-carboxylic acid, synthesis of anilines **46d** to **46h**, yields and characterization of intermediates **47b** to **47h** and **48b** to **48h**, and full characterization of **50** and of its intermediates (PDF)

Molecular formula strings for final compounds (CSV)

■ AUTHOR INFORMATION

Corresponding Authors

*E-mail: tarleton@uga.edu (R.L.T.).

*E-mail: maria.m.marco@gsk.com (M.M.).

*E-mail: gilles.courtemanche@bioaster.org (G.C.).

ORCID

Gilles Courtemanche: 0000-0002-4887-9961

Author Contributions

C.F., A.P., R.L.T., M.M. and G.C. contributed equally to this work.

Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

R&D, research and development; CYP51, lanosterol 14-alpha demethylase; NIH, mouse embryonic fibroblast cell line; AAB, acylaminobenzothiazole; SI, selectivity index; HATU, 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]-pyridinium 3-oxide hexafluorophosphate; EtOAc, ethyl acetate; NIS, *N*-iodosuccinimide; AcOH, acetic acid; DIEA, *N*,*N*-diisopropyethylamine; pTSA, ptoluenesulfonic acid; LBF, liver blood flow; Cl, clearance; CAD, charged aerosol detection; IVA, in vivo activity; TEER, transepithelial electrical resistance; MDR1-MDCK, Madin−Darby canine kidney cell, transfected with human MDR1 gene; P_{app}, passive cellular permeability; Cl_{int}, intrinsic clearance; IS, internal standard

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