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Activity of Fluorine-Containing Analogues of WC-9 and Structurally Related Analogues against Two Intracellular Parasites: *Trypanosoma cruzi* and *Toxoplasma gondii*

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Two obligate intracellular parasites, *Trypanosoma cruzi*, the agent of Chagas disease, and *Toxoplasma gondii*, an agent of toxoplasmosis, upregulate the mevalonate pathway of their host cells upon infection, which suggests that this host pathway could be a potential drug target. In this work, a number of compounds structurally related to **WC-9** (4-phenoxyphenoxyethyl thiocyanate), a known squalene synthase inhibitor, were designed, synthesized, and evaluated for their effect on *T. cruzi* and *T. gondii* growth in tissue culture cells. Two fluorine-containing derivatives, the 3-(3-fluorophenoxy)- and 3-(4-fluorophenoxy)phenoxyethyl thiocyanates, exhibited half-maximal effective concentration (EC_{50}) values of 1.6 and 4.9 μM, respectively, against tachyzoites of *T. gondii*, whereas they showed similar potency to **WC-9** against intracellular *T. cruzi* (EC_{50} values of 5.4 and 5.7 μM, respectively). In addition, 2-[3-(phenoxy)phenoxyethylthio]ethyl-1,1-bisphosphonate, which is a hybrid inhibitor containing 3-phenoxyphenoxy and bisphosphonate groups, has activity against *T. gondii* proliferation at sub-micromolar levels (EC_{50} = 0.7 μM), which suggests a combined inhibitory effect of the two functional groups.

Introduction

Obligate intracellular parasites depend on the integrity of their host cell to survive. They have evolved sophisticated strategies to manipulate their host and establish with them a close metabolic link in order to complete their development. A case in point is that of parasites like *Trypanosoma cruzi*, a trypanosomatid, and *Toxoplasma gondii*, an Apicomplexan parasite, which upregulate host genes for the mevalonate pathway upon infection.[1–3] This up-regulation is probably performed to acquire cholesterol and other isoprenoids needed to accommodate an increasing intracellular parasite load and/or to provide these lipids to be scavenged by the intracellular parasites.[3] *T. cruzi* is the agent of Chagas disease (American trypanosomiasis), the largest parasitic disease burden of the Americas.[4] Treatment of *T. cruzi* infection is with nifurtimox or benznidazole. Neither of these compounds are drugs approved by the US Food and Drug Administration (FDA), and they are only available in the United States from the Centers for Disease Control and Prevention (CDC) under investigational protocols. On the other hand, *T. gondii* is the agent of toxoplasmosis,[5] which affects a wide range of hosts, particularly humans and other warm-blooded animals.[6] Toxoplasmosis can be considered as one of the most prevalent parasitic diseases: it affects almost one billion people worldwide.[7] This parasite can cause mortality among immune-compromised individuals, such as AIDS patients and organ-transplant recipients, as well as in congenitally infected children.[8] Toxoplasmosis may also lead to severe ocular disease in immune-competent patients.[9] The current chemotherapy for toxoplasmosis is also deficient, because the available drugs may cause toxic side effects and they are not able to properly access the central nervous system. Another drawback of the present chemotherapy is its high cost.[10]

The up-regulation of the mevalonate pathway of the host by these intracellular parasites provides an additional potential drug target, because its inhibition could affect the parasite and the host cell in which the parasite resides. *T. gondii* does not synthesize cholesterol and imports it from the host;[11] it is also able to take up isoprenoids like farnesyl diphosphate (FPP) and geranylgeranyl diphosphate synthesized by the host. As with other trypanosomatids, *T. cruzi* has a strict requirement for specific endogenous sterols for survival, although it can take up cholesterol from its mammalian host.[12,13] Appropriate ergosterol biosynthesis inhibitors can induce a parasitological cure in both acute and chronic experimental models of Chagas disease.[14] 4-Phenoxyphenoxyethyl thiocyanate (compound **1**; **WC-9**) is a potent inhibitor of the intracellular amastigote forms of *T. cruzi*.[15] **WC-9** is a noncompetitive inhibitor of *T. cruzi* squalene synthase (*Tc*SQS) that acts at low nanomolar

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concentration.[16] This enzyme catalyzes the first step in sterol biosynthesis, which consists of reductive dimerization of two molecules of farnesyl diphosphate to yield squalene. Additional synthetic derivatives of **WC-9** (**2**–**6**) are shown in Figure 1.[15,17–21]

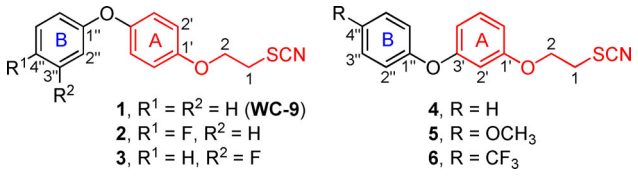

Figure 1. Structure of **WC-9** and other closely related inhibitors of *T. cruzi* proliferation.

It is interesting to note that *T. gondii* lacks the mevalonate pathway and uses the essential 1-deoxy-D-xylulose-5-phosphate pathway to make isopentenyl diphosphate and dimethylallyl diphosphate.[22] As *T. gondii* does not synthesize cholesterol and imports it from the host,[11] it is reasonable to consider that inhibitors of the host SQS could eventually control *T. gondii* growth. Certainly, other mevalonate pathway inhibitors modulate the growth of various intracellular Apicomplexan parasites that are devoid of this pathway, such as *Babesia divergens*,[23] *Plasmodium falciparum*,[23,24] *Cryptosporidium parvum*,[25] and *T. gondii*,[26] which indicates that parasites lacking the mevalonate pathway are reliant on host precursors of isoprenoid biosynthesis. Interestingly, there is a synergistic effect if the mevalonate pathway of the host and the isoprenoid pathway of the parasite are targeted independently.[27] For example, zoledronic acid, a bisphosphonate that inhibits *T. gondii* farnesyl diphosphate synthase (*Tg*FPPS), and atorvastatin, a statin that inhibits the host 3-hydroxymethyl-glutaryl coenzyme A (3-HMG-CoA)-reductase, exhibit a marked synergism in the inhibition of *T. gondii* growth.[27]

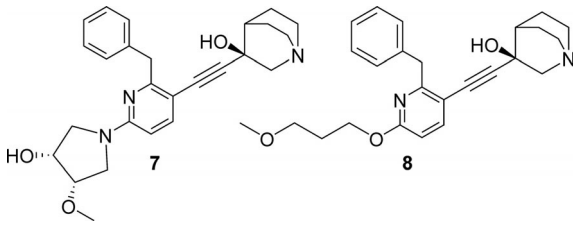

Figure 2. Structures of quinuclidine derivatives E5700 (**7**) and ER-119884 (**8**).

Rationale

WC-9 is one of the few examples of a pharmacologically important lead compound possessing a thiocyanate group covalently bound to its main skeleton.[28] At the present time, there is no crystal structure available for the complex **WC-9**–*Tc*SQS. However, an X-ray crystal structure of **WC-9** with human SQS has been recently reported (Protein Data Bank (PDB) ID: 3WCD).[29] On the other hand, the X-ray crystal structures of the complexes E5700–*Tc*SQS and ER-119884–*Tc*SQS are available.[29] The quinuclidine derivatives E5700 (**7**) and ER-119884 (**8**) are potent inhibitors of *T. cruzi* growth that act as *Tc*SQS inhibitors (Figure 2).[30,31] Both of these compounds are extremely potent inhibitors of the enzymatic activity of *Tc*SQS: they exhibit half-maximal inhibitory concentration (IC_{50}) values of 0.84 and 3.5 nM, respectively.[31]

Figure 3 shows a superimposition of the E5700–*Tc*SQS and ER-119884–*Tc*SQS complexes with the crystal structure of the **WC-9**–human SQS complex. A high degree of similarity is observed between the *T. cruzi* and human protein structures. Furthermore, the quinoclidine inhibitors occupy the same binding site as **WC-9**. Given that these inhibitors were found to be mixed type (**7**) and noncompetitive (**8**),[30] they provide evidence that **WC-9** may, in fact, occupy the same binding site in *Tc*SQS.

Interestingly, *Tc*SQS activity is also inhibited by bisphosphonates.[32] Bisphosphonates are the main modulators of FPPS, a key enzyme of isoprenoid biosynthesis.[33] Moreover, several X-ray crystal structures of *Tc*SQS are available with a number of bisphosphonates, such as BPH1344 (**9**; PDB ID: 3WCG; Figure 4).[29]

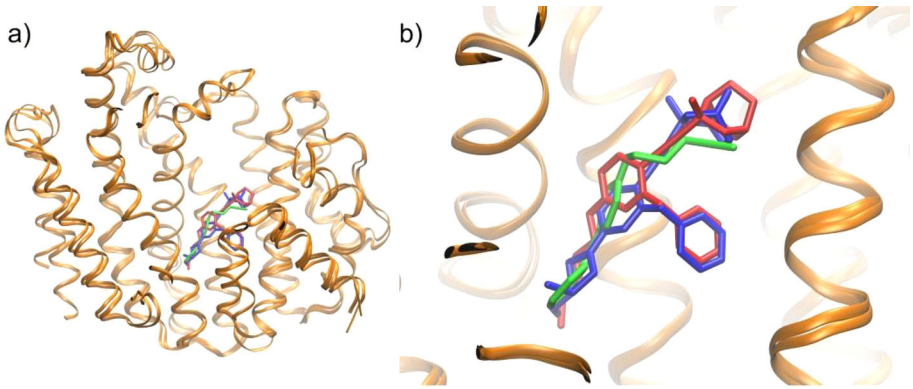

Figure 3. a) Superposition of the crystal structures of human SQS with **WC-9** and *T. cruzi* SQS with **7** and **8**. A high degree of similarity is observed between the protein chains. b) Expansion of the structures showing that the quinuclidine derivatives **7** (red) and **8** (blue) occupy the same S2 site (homoallylic site) as **WC-9** (green). The mechanisms of action of these compounds (**7** mixed-type and **8** non-competitive) provide further evidence that **WC-9** may indeed bind to the S2 site in *T. cruzi* SQS.

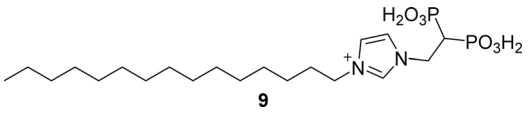

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Figure 4. Structure of the bisphosphonate compound known as BPH1344 (**9**).

Figure 5 shows a superposition of the **WC-9**–human SQS complex and the BPH1344–*Tc*SQS complex, with the bisphosphonate group deleted, to show the putative binding site that **WC-9** could occupy in *Tc*SQS.[29] As can be observed, the two structures show a high degree of similarity, except for the α helix 284–294 in *T. cruzi*, which acquires a loop organization in the corresponding human SQS structure.[29] The X-ray crystal structure of **WC-9** with dehydrosqualene synthase from *Staphylococcus aureus*, an enzyme very similar to SQS that catalyzes dehydrosqualene formation, is also available.[34]

At the present time, there is no computer-assisted protocol to predict binding of **WC-9** analogues to *Tc*SQS. However, there are abundant structure–activity relationship (SAR) data available on *T. cruzi* and *T. gondii* cells that can be used to facilitate drug design.[17–21,35] In addition, there is strong evidence to state that the phenoxyethyl thiocyanate moiety of **WC-9** (Figure 1) is the pharmacophore of this family of molecules. A question that emerges, the answer to which is still pending, is whether the optimum substitution pattern will be at the C4′ or C3′ position. The availability of the Buchwald coupling reaction allowing us to access a variety of **WC-9** analogues encouraged us to go further in searching for better inhibitors against either *T. cruzi* or *T. gondii* cells.[36–39]

Results and Discussion

The introduction of a fluorine atom into the **WC-9** structure to give rise to compounds **2** and **3** was a significant structural change, which was quite beneficial for the inhibitory action.[19] Therefore, it seemed of interest to study the biological activity of the fluorine-containing analogues of the regioisomer of **WC-9**, compound **4**, and we envisioned compounds **20**, **21**, and **22** as target molecules. Compound **10**[20] was treated with 2-fluoro-, 3-fluoro-, and 4-fluorophenol under typical Buchwald coupling reaction conditions to generate tetrahydropyranyl derivatives **11**, **12**, and **13** in 53, 33, and 71% yields, respectively. Each tetrahydropyranyl protecting group present in these compounds was cleaved by treatment with pyridinium *p*-toluenesulfonate to produce the corresponding free alcohols **14**, **15**, and **16** in very good yields. The alcohols were tosylated to give **17**, **18**, and **19** in 66, 73, and 84% yields, respectively. On treatment with potassium thiocyanate, in separate experiments, these compounds were converted into the target molecules **20**, **21**, and **22**, respectively, as illustrated in Scheme 1.

The fluoro derivatives of **WC-9** at C4″ and C3″, namely **2** and **3**, were more potent than the lead drug **WC-9**.[19] It seemed reasonable to also produce the fluorinated analogue at C2″ (compound **27**), which had not been prepared before. The Buchwald coupling reaction of **23**[20] with 2-fluorophenol generated the tetrahydropyranyl derivative **24** in a low but reproducible yield, which produced the free alcohol **25** in 62% yield on treatment with pyridinium 4-toluenesulfonate. Tosylation of this compound to produce **26** followed by treatment with potassium thiocyanate resulted in the desired compound **27** in 36% yield (Scheme 2).

An interesting structural variation was the replacement of the terminal phenyl group by a pyridyl group, in which the nitrogen atom occupied the 4″ position (compound **31**). We have previously synthesized and evaluated the corresponding 2-pyridyl[21] and 3-pyridyl[20] derivatives, so the availability of **31** would complete the corresponding SAR analysis. A straightforward synthesis of **31** was accomplished by starting from **23**,[20] which was treated with 4-hydroxypyridine under typical coupling reaction conditions to generate **28**. On treatment with pyridinium 4-toluenesulfonate, **28** was converted into alcohol **29**, which was further transformed into **30** by reaction with *N*-bromosuccinimide and triphenylphosphine[40] in 50% yield. On reaction with potassium thiocyanate, **30** was transformed into the desired compound **31** in 34% yield (Scheme 2).

Although a significant number of structural variations have been made on the structure of **WC-9**, only a few of them cor-

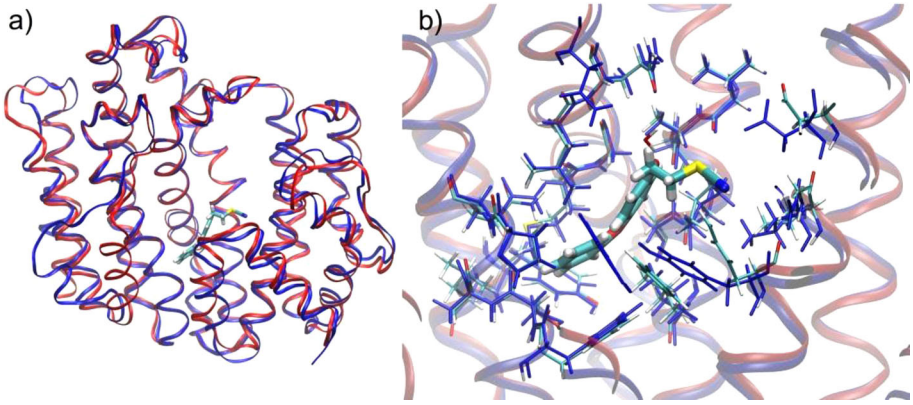
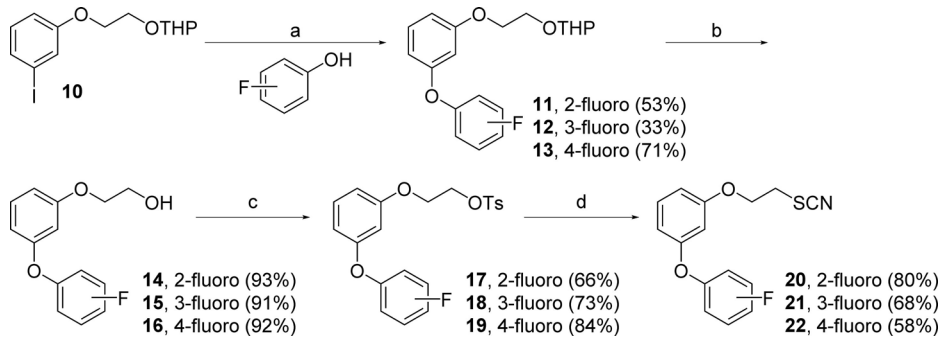
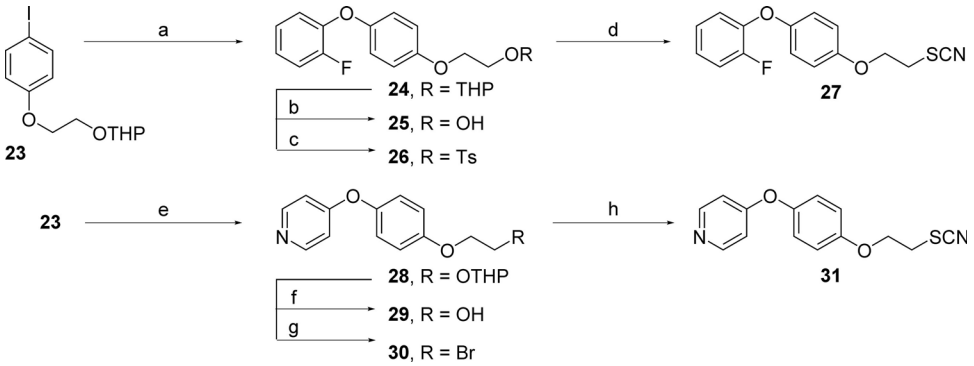


Figure 5. a) The binding site (amino acids within 4 Å of the ligand) of **WC-9** in the human SQS structure (amino acids shown in blue licorice representation) and b) the putative site that **WC-9** would occupy in the *T. cruzi* SQS (amino acids in red licorice representation). A high degree of similarity is observed between the two binding sites. In terms of sequence, all amino acids in the binding sites are the same, except for Ser 256 in *T. cruzi* instead of Cys 254 in the human enzyme.



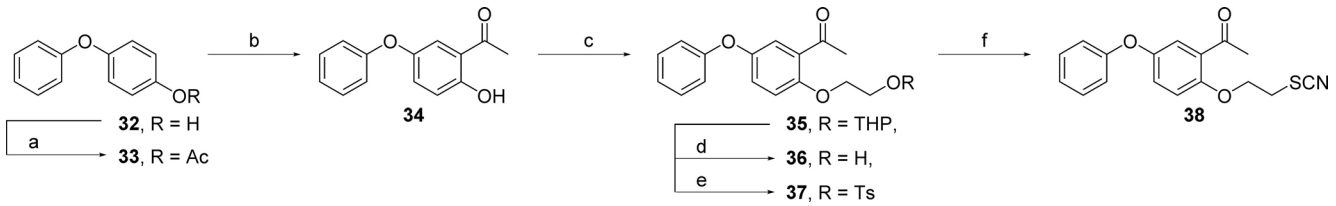
Scheme 1. *Reagents and conditions:* a) 5% CuI, 10% picolinic acid, (2-fluoro-, 3-fluoro-, 4-fluorophenol), K_3PO_4, DMSO, 90 °C (4 d for **11**, 14 d for **12**, 4 d for **13**); b) PPTs, CH_3OH, RT, 16 h; c) CITs, py, 0 °C, 6 h; d) KSCN, DMF, 100 °C, 6 h. PPTs: pyridinium *p*-toluenesulfonate; py: pyridine; THP: tetrahydropyranyl; Ts: toluene-4-sulfonyl.



Scheme 2. *Reagents and conditions:* a) 5% CuI, 10% picolinic acid, 2-fluorophenol, K_3PO_4, DMSO, 90 °C, 12 d, 37%; b) PPTs, CH_3OH, RT, 24 h, 62%; c) CITs, py, RT, 24 h, 92%; d) KSCN, DMF, 80 °C, 6 h, 36%; e) CuI, 10% picolinic acid, 4-hydroxypyridine, K_3PO_4, DMSO, 80 °C, 24 h, 29%; f) PPTs, CH_3OH, RT, 24 h, 59%; g) NBS, Ph_3P, 0 °C, 6 h, 50%; h) KSCN, DMF, 80 °C, 6 h, 34%. NBS: *N*-bromosuccinimide.

respond to substitutions on the A ring.[17] An interesting method to incorporate an acetyl group at the C2′ is the photo-Fries rearrangement reaction.[41] Therefore, 4-phenoxyphenol (**32**) was acetylated to give **33** in excellent yield. Compound **33** was then irradiated at 254 nm to produce the corresponding photo-Fries rearranged product **34** in 39% yield. Compound **34** was treated with 2-bromoethyl tetrahydro-2*H*-pyran-2-yl ether in a Williamson etherification reaction to give **35** in 62% yield. This compound was deprotected by treatment with pyridinium 4-toluenesulfonate in methanol to generate free alcohol **36** (92% yield), which was treated with tosyl chloride in pyridine to give the expected tosylate **37** in 95% yield. This compound was further transformed into the thiocyanate derivative **38** in 71% yield by treatment with potassium thiocyanate in *N*,*N*-dimethylformamide at 80 °C (Scheme 3).

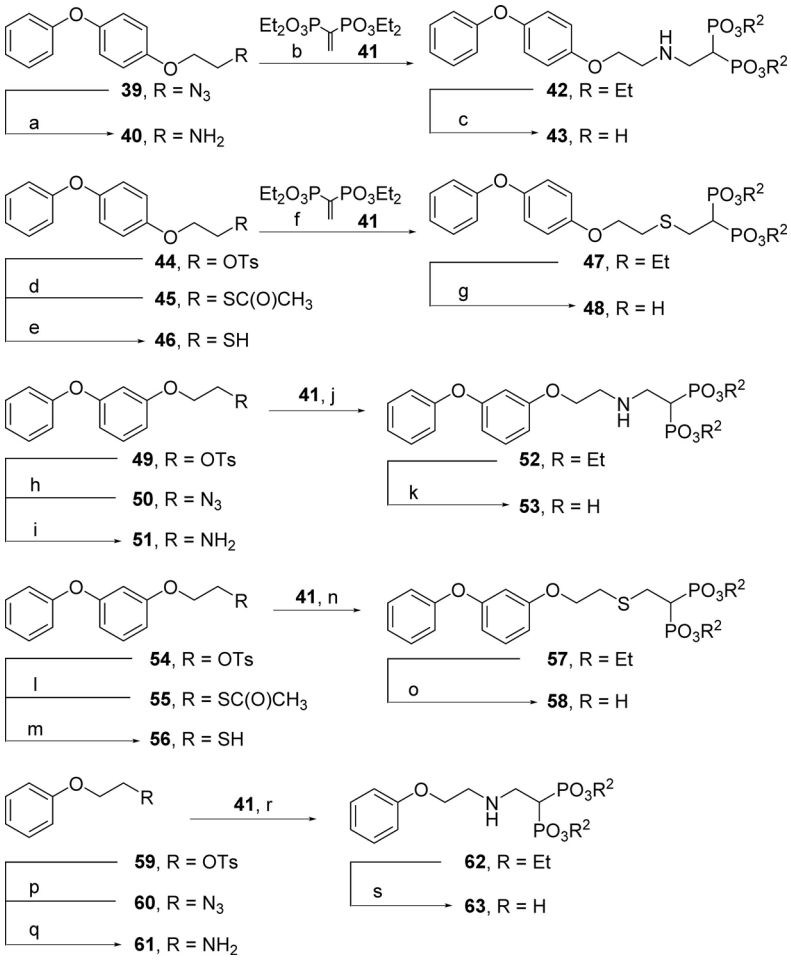
The concept of a hybrid drug, in that one compound possesses a dual mode of action, is fascinating.[42–44] For this reason, hybrid molecules, such as **43** and **48**, were envisioned with a phenoxy unit to target SQS and a bisphosphonate moiety to act against FPPS.[33] The production of **42** was straightforward by employing the already-described azide **39**.[45] This compound was reduced to the corresponding amine **40** in 77% yield through a Staudinger reaction by treatment with triphenylphosphine in dichloromethane.[46] A Michael-type addition of the resulting amine **40** with the Michael acceptor **41**[47] yielded **42** in 82% yield. Hydrolysis of **42** by treatment with hydrochloric acid at reflux produced the desired compound **43**. Hybrid compound **48** was straightforwardly prepared by starting from the previously described tosylate **44**.[15] Upon treatment of **44** with potassium thioacetate



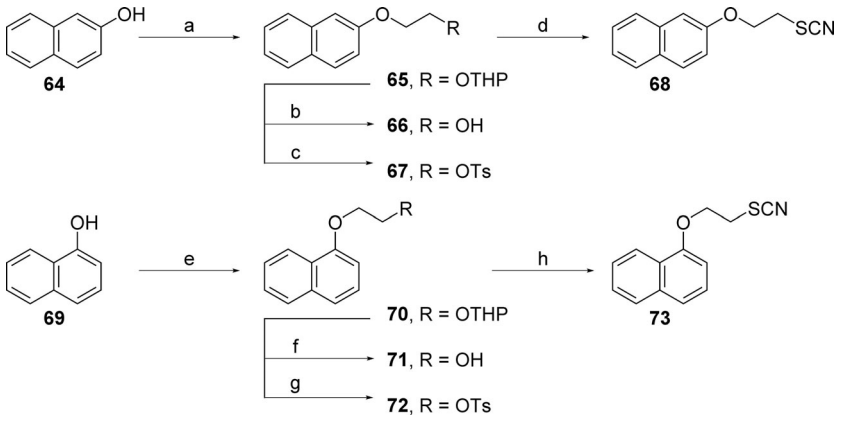
Scheme 3. *Reagents and conditions:* a) Ac_2O, py, RT, 16 h, 97%; b) *hν*, C_6H_{12}, RT, 12 h, 39%; c) KOH, DMSO, $BrCH_2CH_2OTHP$, RT, 12 h, 62%; d) PPTs, CH_3OH, RT, 16 h, 92%; e) CITs, py, RT, 6 h, 95%; f) KSCN, DMF, 80 °C, 6 h, 71%.

in *N*,*N*-dimethylformamide at 90 °C, **45** was produced. When **45** was treated with lithium aluminum hydride in anhydrous tetrahydrofuran, it yielded the corresponding thiol **46** in good yield. The Michael addition reaction of this resultant compound with **41** followed by hydrolysis of the phosphonate esters by treatment with bromotrimethylsilane and digestion with methanol produced the desired compound **48**. By following a quite similar approach, **53** was prepared from the already-described tosylate **49**,[20] which was transformed into azide **50** by treatment with sodium azide in *N*,*N*-dimethylformamide. Under Staudinger-type conditions, **50** was converted into amine **51**, which was then treated with **41** to produce the Michael adduct **52**. Hydrolysis of this compound by treatment with concentrated hydrochloric acid at reflux produced the desired compound **53**. The synthesis of **58** was also straightforward. The known tosylate **54**[20] was treated with potassium thioacetate in an S_N2 reaction in *N*,*N*-dimethylformamide at 80 °C to give rise to **55** in 83% yield. Hydrolysis of the acetyl group by treatment with potassium carbonate followed by reduction with zinc in glacial acetic acid generated the corresponding thiol **56** in 75% yield. A Michael addition with this thiol followed by hydrolysis by treatment with bromotrimethylsilane and methanol digestion produced the desired compound **58** in 65% yield. Similarly, **63** was obtained from the already-described tosylate **59**,[18] which was transformed into **60** by treatment with sodium azide. This compound was transformed into **61**, which was then treated with **41** to yield **62**. Treatment with concentrated hydrochloric acid at reflux resulted in the desired compound **63** (Scheme 4).

As another structural variation, hydrophobic analogues of **WC-9**, such as **68** and **73**, were also produced. Both of these compounds were easily prepared from β-naphthol (**64**) and α-naphthol (**69**), respectively. Therefore, each compound, in separate experiments, was treated with 2-bromoethyl tetrahydro-2*H*-pyran-2-yl ether under Williamson-type conditions to produce tetrahydropyranyl derivatives **65** and **70**, respectively, which were easily deprotected by treatment with pyridinium 4-toluenesulfonate to yield the corresponding free alcohols **66** and **71**, respectively. On treatment with an excess of tosyl chloride, **66** and **71** were converted into tosylates **67** and **72**, re-



Scheme 4. *Reagents and conditions:* a) PPh_3, CH_2Cl_2, RT, 4 h, 77%; b) **41**, CH_2Cl_2, NEt_3, RT, 5 h, 59%; c) HCl (concd), reflux, 7 h, 81%; d) $CH_3C(O)SK$, DMF, 2 h, 90 °C, 83%; e) $LiAlH_4$, THF, RT, 3 h, 92%; f) **41**, CH_2Cl_2, NEt_3, RT, 5 h, 59%; g) 1) $BrSi(CH_3)_3$, CH_2Cl_2, RT, 48 h, 2) CH_3OH, RT, 24 h, 69%; h) NaN_3, DMF, 80 °C, 2 h, 90%; i) PPh_3, THF, RT, 2 h, 90%; j) **41**, CH_2Cl_2, NEt_3, RT, 24 h, 97%; k) HCl (concd), reflux, 7 h, 35%; l) $CH_3C(O)SK$, DMF, 3 h, 80 °C, 98%; m) 1) K_2CO_3, CH_3OH/H_2O, 40 min, RT, 2) Zn/AcOH, 1 h, 110 °C, 75%; n) **41**, CH_2Cl_2, NEt_3, RT, 16 h, 75%; o) 1) $BrSi(CH_3)_3$, CH_2Cl_2, RT, 48 h, 2) CH_3OH, RT, 24 h, 65%; p) NaN_3, DMF, 80 °C, 2 h, 81%; q) PPh_3, THF, RT, 2 h, 67%; r) **41**, CH_2Cl_2, NEt_3, RT, 16 h, 88%; s) HCl (concd), reflux, 7 h, 11%.



Scheme 5. *Reagents and conditions:* a) KOH, DMSO, $BrCH_2CH_2OTHP$, RT, 5 d, 48%; b) PPTs, CH_3OH, RT, 24 h, 85%; c) ClTs, py, RT, 4 h, 83%; d) KSCN, DMF, 80 °C, 6 h, 55%; f) KOH, DMSO, $BrCH_2CH_2OTHP$, RT, 5 d, 65%; g) PPTs, CH_3OH, RT, 24 h, 81%; e) ClTs, py, RT, 4 h, 90%; f) KSCN, DMF, 80 °C, 6 h, 58%.

spectively. On reaction with potassium thiocyanate in *N*,*N*-dimethylformamide, **67** and **72** were converted into the desired molecules **68** and **73**, respectively, as shown in Scheme 5.

Biological evaluation of these new compounds structurally related to **WC-9** gave promising results. All fluorine-containing derivatives of compound **4**, that is, compounds **20**, **21**, and **22**, were effective inhibitors of tachyzoites of *T. gondii* and exhibited half-maximal effective concentration (EC_{50}) values of 3.1, 1.6, and 4.9 μM, respectively, with **20** and **21** being slightly more potent than **4** (EC_{50} = 4.0 μM).[20] The fluorinated compounds were also effective growth inhibitors of the intracellular form of *T. cruzi* (amastigotes), which is the clinically more relevant replicative form of the parasite. In fact, all of the compounds bearing a fluorine atom bound to the C2′′, C3′′, and C4′′ positions exhibited relatively similar EC_{50} values (7.0, 5.4, and 5.7 μM, respectively) to that of **WC-9**, used as a positive control, under the same assay conditions with *T. cruzi*.[20] The fluorine analogue of **WC-9** at C2′′, compound **27**, which is the regioisomer of **2** and **3**, was also a potent inhibitor of *T. gondii* tachyzoite growth, with an EC_{50} value of 4.5 μM. Compound **27** was more potent than **2** (EC_{50} = 15.8 μM)[19] but exhibited quite similar efficacy to that of **3** (EC_{50} = 3.9 μM).[19] Quite unexpectedly, in spite of the regioisomers **2** and **3** being potent growth inhibitors of amastigotes of *T. cruzi*,[19] **27** was devoid of antiparasitic activity against this parasite. Pyridyl derivative **31** was free of antiparasitic activity against *T. cruzi*; however, **31** behaved as a growth inhibitor of *T. gondii* and showed an EC_{50} value of 7.9 μM. The introduction of an acetyl group at the C2′ position in **38** proved to be quite beneficial for the biological activity, because this compound showed potent inhibitory action against *T. gondii*. In fact, its EC_{50} value was 2.4 μM. Compound **38** was less effective against *T. cruzi* with an EC_{50} value of 11.3 μM. The hybrid molecules **53** and **58** were both devoid of activity against *T. cruzi*, but they showed a strong inhibitory action against tachyzoites of *T. gondii*. The sulfur-containing analogue **58** showed a sub-micromolar activity against *T. gondii* with an EC_{50} value of 0.7 μM. In fact, the 3′-phenoxy substitution pattern found in **53** and **58** was more beneficial for biological activity against *T. gondii* than the 4′-phenoxy substitution present in **43** and **48**. Compounds **53** and **58** were also recognized as potent inhibitors of the target enzyme *Tg*FPPS and exhibited IC_{50} values of 0.040 and 0.076 μM, respectively. The simplified naphthyl derivatives **68** and **73** turned out to be potent inhibitors of *T. gondii* with EC_{50} values of 3.6 and 3.1 μM, respectively, but these compounds exhibited no inhibitory action against *T. cruzi*. The results are presented in Table 1.

Given the high in vitro activity of hybrid derivatives of **WC-9**, we performed molecular-dynamics-based computational studies to gain further insight into the interactions of this kind of compound with the active site of FPPS. By taking **58** as a representative example and because no crystal structure of *Tg*FPPS is available, we constructed the structure using homology modeling based on the crystal structure of *Plasmodium vivax* FPPS (PDB ID: 3MAV), the enzyme showing the highest degree of similarity with *Tg*FPPS. We inserted **58**, three Mg^{2+} ions, and isopentenyl pyrophosphate as a coligand by alignment with the *Tc*FPPS alendronate complex structure (PDB ID: 1YHM). We carried out a 10 ns molecular dynamics simulation in explicit water, and performed a clustering analysis to obtain the most populated structure. Based on an implicit solvent energy decomposition calculation, we identified the key interactions present with the binding site. Tyr 303 forms a hydrogen bond with the sulfur atom at position 3 of **58**, Gln 204 forms a hydrogen bond with the first bridging oxygen atom, and Thr 200 forms a hydrogen bond with the second bridging oxygen atom. π–π interactions are present between Tyr 303 and ring A and between Phe 131 and ring B. Hydrophobic interactions take place between ring B and Leu 307 and Asn 196. The high degree of specificity of these interactions is an indication of why *meta*-substituted **58** shows a much higher potency than *para*-substituted **48** (Figure 6). Further investigation of the ability of different isoprenoids to rescue the growth inhibition could help to pin down the actual target of the bisphosphonate.

We cannot rule out that this inhibitory effect of **WC-9** analogues is, in part, due to inhibition of the host squalene synthase and of other host enzymes of the mevalonate pathway. In this regard, microarray experiments have shown that 3-

Table 1. Biological activity of **WC-9** analogues against *T. gondii* (tachyzoites), *T. cruzi* (amastigotes), and Vero cells.[a]

Compound	*T. gondii* growth EC_{50} [μM]	*Tg*FPPS IC_{50} [μM]	*T. cruzi* growth EC_{50} [μM]	*Tc*FPPS IC_{50} [μM]	*Hh*FPPS IC_{50} [μM]	Cytotoxicity EC_{50} [μM]
20	3.12 ± 0.37	ND	7.01 ± 0.51	ND	ND	> 60
21	1.63 ± 0.36	ND	5.38 ± 0.82	ND	ND	> 50
22	4.92 ± 1.94	ND	5.69 ± 0.47	ND	ND	> 55
27	4.54 ± 0.57	ND	> 10	ND	ND	> 55
31	7.86 ± 0.78	ND	> 10	ND	ND	> 200
38	2.42 ± 0.81	ND	11.3 ± 2.5	ND	ND	75.7 ± 7.3
43	5.90 ± 0.07	0.094 ± 0.020	> 10	ND	> 10	> 100
48	> 10	0.362 ± 0.002	> 20	0.8 ± 0.12	> 15	> 200
53	2.40 ± 0.70	0.040 ± 0.002	> 10	> 10	> 10	> 50
58	0.67 ± 0.23	0.076 ± 0.019	> 10	ND	> 10	> 100
63	> 10	0.180 ± 0.015	> 10	0.32 ± 0.04	> 10	ND
68	3.62 ± 0.38	ND	> 10	ND	ND	ND
73	3.13 ± 0.58	ND	> 10	ND	ND	7.5
WC-9	4.8 ± 0.41[20]	ND	5.0 ± 1.1[20]	ND	ND	82.6 ± 7.3
benznidazole	ND	ND	1.52 ± 0.10	ND	ND	ND

[a] Values are the mean ± SD from at least three independent experiments, $n = 3$. ND: not determined.

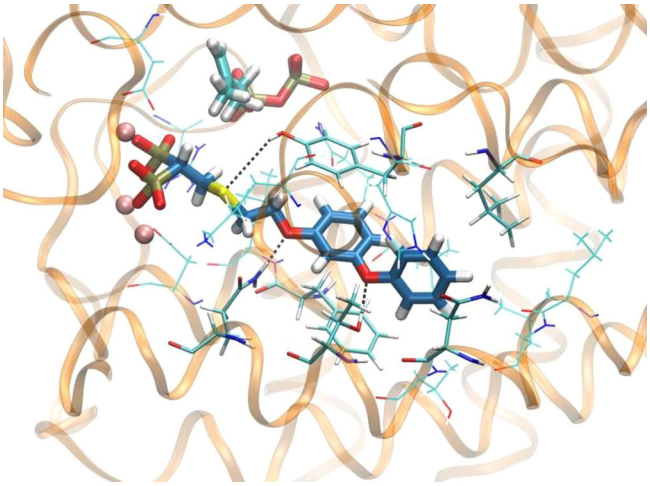


Figure 6. Interaction of **58** with *Tg*FPPS. The structure was formed by homology modeling from *P. vivax* FPPS, and **58** (blue), three Mg ions (pink), and isopentenyl pyrophosphate (cyan) were inserted by alignment with the *Tc*FPPS alendronate crystal structure (PDB ID: 1YHM). Residues at a distance of 4 Å from the ligand are shown in thick line representation, with those contributing significantly to the binding energy in thin licorice representation.

HMG-CoA reductase, diphosphomevalonate decarboxylase, and farnesyl diphosphate synthase are induced 24 h after *T. gondii* infection of human foreskin fibroblasts (HFF).[1] Squalene epoxidase, the second enzyme in the cholesterol biosynthesis pathway and one of the rate-limiting enzymes, is upregulated fivefold following infection of either HFF[1] or the porcine kidney epithelial cell line PK13,[2] which suggests that induction of mevalonate biosynthetic enzymes is necessary to increase cellular levels of squalene that could be scavenged by the parasite.[1] Similarly, 3-HMG-CoA reductase, diphosphomevalonate decarboxylase, and farnesyl diphosphate synthase are induced several-fold 24–72 h post-infection of *Macaca mulatta* LLcMK2 kidney cells with *T. cruzi*.[3]

Conclusions

It can be concluded that most of the target compounds exhibited potent action against *T. gondii* proliferation and, to a lesser extent, some of them were also growth inhibitors of *T. cruzi*. The druglike characters of our lead compound (for instance, it follows all Lipinki rules:[48] no hydrogen-bond donors, three hydrogen-bond acceptors, molecular weight is 271.3, and log P is 4.20) and other closely related analogues offer good chances for optimizing their chemical structures. The availability of the crystal structure of **WC-9** with dehydrosqualene synthase from *S. aureus*, together with crystallographic structure of this compound bound to human SQS and proper docking studies in homology models, will allow the production of additional more active **WC-9** analogues. A more detailed computational study is under way to further understand the interactions of the current inhibitors and guide the development of new compounds; the results will be published elsewhere.

Experimental Section

General methods

The glassware used in air- and/or moisture-sensitive reactions was flame dried, and the reactions were performed under a dry argon atmosphere. Unless otherwise noted, chemicals were commercially available and were used without further purification. Anhydrous *N*,*N*-dimethylformamide and anhydrous dimethyl sulfoxide were used as supplied from Aldrich. Nuclear magnetic resonance spectra were performed by using a Bruker AM-500 MHz apparatus. Chemical shifts (δ) are reported in parts per million relative to tetramethylsilane. ^{13}C NMR spectra were fully decoupled. High-resolution mass spectra were carried out by using a Bruker micrOTOF-Q II spectrometer, which is a hybrid quadrupole time-of-flight mass spectrometer with MS–MS capability. Melting points were determined by using a Fisher–Johns apparatus. Column chromatography was performed with Merck silica gel plates (Kieselgel 60, 230–400 mesh). Analytical thin-layer chromatography was performed by employing 0.2 mm coated commercial silica gel plates (Merck, DC

Aluminum sheets, Kieselgel 60 F254). As judged from the homogeneity of the ^{1}H, ^{13}C, ^{19}F, and ^{31}P NMR spectra and HPLC analyses by employing a Beckmann Ultrasphere ODS-2 column (5 μM, 250× 10 mm, elution with acetonitrile/water (9:1) at a rate of 3.00 mL min^{-1} with a refractive index detector), the title compounds had purities of >97%.

Syntheses

3-(2-Fluorophenoxy)phenoxyethyl tetrahydro-2*H*-pyran-2-yl ether (11): A mixture of compound **10** (900 mg, 2.6 mmol), 2-fluorophenol (580 mg, 5.2 mmol), copper(I) iodide (49.2 mg, 0.26 mmol), 2-picolinic acid (63.6 mg, 0.52 mmol), and potassium phosphate tribasic (1.1 g, 5.2 mmol) under anhydrous conditions was placed in a flask, which was evacuated and back-filled with argon. This sequence was repeated twice. Dimethyl sulfoxide was then added (3.0 mL), and the reaction mixture was stirred at 90 °C for 4 d. The mixture was cooled to room temperature and partitioned between dichloromethane (20 mL) and water (20 mL). The aqueous layer was extracted with dichloromethane (2×20 mL), the combined organic phases were washed with brine (5×50 mL) and dried ($MgSO_4$), and the solvent was evaporated. The product was purified by column chromatography (silica gel) with hexane/EtOAc (24:1) as the eluent to produce 453 mg (53% yield) of pure compound **11** as a colorless oil.

3-(3-Fluorophenoxy)phenoxyethyl tetrahydro-2*H*-pyran-2-yl ether (12): Dimethyl sulfoxide (3.0 mL) was added to a mixture of **10** (893 mg, 2.6 mmol), 3-fluorophenol (575 mg, 5.1 mmol), copper(I) iodide (48.9 mg, 0.26 mmol), 2-picolinic acid, (63.2 mg, 0.51 mmol), and potassium phosphate tribasic (1.092 g, 5.1 mmol) as described for the preparation of **11**. The reaction mixture was stirred at 90 °C for 14 d. After the usual workup, the product was purified by column chromatography (silica gel) with hexane/EtOAc (49:1) as the eluent to produce 280 mg (33% yield) of **12** as a colorless oil.

3-(4-Fluorophenoxy)phenoxyethyl tetrahydro-2*H*-pyran-2-yl ether (13): A mixture of **10** (877 mg, 2.5 mmol), 4-fluorophenol (565 mg, 5.0 mmol), copper(I) iodide (48.0 mg, 0.25 mmol), 2-picolinic acid, (62.0 mg, 0.50 mmol), and potassium phosphate tribasic (1.072 g, 5.0 mmol) was treated with dimethyl sulfoxide (3.0 mL) as described for the preparation of **11**. The reaction mixture was stirred at 90 °C for 4 d. The reaction was quenched as described for the workup of **11**, and the product was purified by column chromatography (silica gel) with hexane/EtOAc (24:1) as the eluent to produce 594 mg (71% yield) of **13** as a colorless oil.

3-(2-Fluorophenoxy)phenoxyethanol (14): A solution of **11** (426 mg, 1.3 mmol) in methanol (10 mL) was treated with pyridinium 4-toluenesulfonate (30 mg). The reaction mixture was stirred at room temperature overnight. Water (50 mL) was then added, and the mixture was extracted with dichloromethane (3×50 mL). The combined organic layers were washed with brine (3×50 mL) and dried ($MgSO_4$), and the solvent was evaporated. The residue was purified by column chromatography with hexane/EtOAc (85:15) as the eluent to produce 297 mg (93% yield) of pure alcohol **14** as a colorless oil.

3-(3-Fluorophenoxy)phenoxyethanol (15): A solution of **12** (269 mg, 0.81 mmol) in methanol (10 mL) was treated with pyridinium 4-toluenesulfonate (30 mg) as described for the preparation of **14**. The residue was purified by column chromatography with hexane/EtOAc (83:17) as the eluent to give 182 mg (91% yield) of alcohol **15** as a colorless oil.

3-(4-Fluorophenoxy)phenoxyethanol (16): A solution of **13** (581 mg, 1.7 mmol) in methanol (10 mL) was treated with pyridinium 4-toluenesulfonate (30 mg) as described for the preparation of **14**. The crude product was purified by column chromatography with hexane/EtOAc (41:9) as the eluent to produce 399 mg (92% yield) of alcohol **16** as a colorless oil.

3-(2-Fluorophenoxy)phenoxyethyl 4-toluenesulfonate (17): A solution of alcohol **14** (253 mg, 0.95 mmol) in pyridine (3 mL) was treated with 4-toluenesulfonyl chloride (546 mg, 2.9 mmol), and the mixture was stirred at 0 °C for 6 h. Then, 5% HCl (50 mL) was added, and the reaction mixture was stirred for an additional hour. The mixture was partitioned between dichloromethane (50 mL) and water (50 mL). The organic layer was washed with 5% HCl (3× 50 mL) and water (3×50 mL). The organic phase was dried ($MgSO_4$), and the solvent was evaporated. The product was purified by column chromatography with hexane/EtOAc (9:1) as the eluent to produce 226 mg of **17** (66% yield) as a colorless oil.

3-(3-Fluorophenoxy)phenoxyethyl 4-toluenesulfonate (18): A solution of alcohol **15** (175 mg, 0.70 mmol) in pyridine (3 mL) was treated with 4-toluenesulfonyl chloride (402 mg, 2.1 mmol), and the mixture was stirred at 0 °C for 6 h. The reaction was quenched as described for the preparation of **17**. The product was purified by column chromatography with hexane/EtOAc (91:9) as the eluent to produce 207 mg of **18** (73% yield) as a colorless oil.

3-(4-Fluorophenoxy)phenoxyethyl 4-toluenesulfonate (19). A solution of alcohol **16** (388 mg, 1.6 mmol) in pyridine (3 mL) was treated with 4-toluenesulfonyl chloride (894 mg, 4.7 mmol) as described for the preparation of **17**. The product was purified by column chromatography with hexane/EtOAc (91:9) as the eluent to produce 226 mg of **19** (84% yield) as a colorless oil.

3-(2-Fluorophenoxy)phenoxyethyl thiocyanate (20): A solution of tosylate **17** (381 mg, 0.98 mmol) in anhydrous *N*,*N*-dimethylformamide (3.0 mL) was treated with potassium thiocyanate (460 mg, 4.7 mmol). The reaction mixture was heated at 100 °C for 6 h. The mixture was allowed to cool to room temperature, and water (20 mL) was added. The aqueous phase was extracted with dichloromethane (2×30 mL), the combined organic layers were washed with brine (5×30 mL) and water (2×30 mL), then dried ($MgSO_4$), and the solvent was evaporated. The residue was purified by column chromatography with hexane/EtOAc (23:2) as the eluent to produce 226 mg (80% yield) of **20** as a colorless oil.

3-(3-Fluorophenoxy)phenoxyethyl thiocyanate (21): A solution of **18** (197 mg, 0.49 mmol) in anhydrous *N*,*N*-dimethylformamide (3.0 mL) was treated with potassium thiocyanate (237 mg, 2.4 mmol). The mixture was treated as described for the preparation of **20**. The residue was purified by HPLC with methanol/H_2O (9:1) as the eluent and by employing a Bechman Ultrasphere 5 μM column (250×10 mm) to give rise to 107 mg (68% yield) of **21** as a colorless oil.

3-(4-Fluorophenoxy)phenoxyethyl thiocyanate (22): A solution of **19** (514 mg, 1.3 mmol) in anhydrous *N*,*N*-dimethylformamide (3.0 mL) was treated with potassium thiocyanate (647 mg, 6.7 mmol). The reaction mixture was treated as described for the preparation of **20**. The residue was purified by column chromatography with hexane/EtOAc (47:3) as the eluent to produce 221 mg (58% yield) of **22** as a colorless oil.

4-(2-Fluorophenoxy)phenoxyethyl tetrahydro-2*H*-pyran-2-yl ether (24): Dimethyl sulfoxide (3.0 mL) was added to a mixture of **23** (858 mg, 2.5 mmol), 2-fluorophenol (549.5 mg, 4.9 mmol), copper(I) iodide (46.0 mg, 0.24 mmol), 2-picolinic acid, (59.4 mg,

0.51 mmol), and potassium phosphate tribasic (1.026 g, 4.9 mmol) as described for the preparation of **11**. The reaction mixture was stirred at 90 °C for 12 d. After the usual workup, the product was purified by column chromatography with hexane/EtOAc (19:1) as the eluent to produce 295 mg (37% yield) of **24** as a colorless oil.

4-(2-Fluorophenoxy)phenoxyethanol (25): A solution of **24** (280 mg, 0.84 mmol) in methanol (10 mL) was treated with pyridinium 4-toluenesulfonate (30 mg) as depicted for the preparation of **14**. The residue was purified by column chromatography with hexane/EtOAc (9:1) as the eluent to give 124 mg (62% yield) of alcohol **25** as a colorless oil.

4-(2-Fluorophenoxy)phenoxyethyl 4-toluenesulfonate (26). A solution of alcohol **25** (110 mg, 0.44 mmol) in pyridine (3.0 mL) was treated with 4-toluenesulfonyl chloride (250 mg, 1.3 mmol), and the mixture was stirred at room temperature for 24 h. The reaction was quenched as described for the preparation of **17**. The product was purified by column chromatography with hexane/EtOAc (19:1) as the eluent to produce 163 mg of **26** (92% yield) as a colorless oil.

4-(2-Fluorophenoxy)phenoxyethyl thiocyanate (27). A solution of **26** (151 mg, 0.38 mmol) in anhydrous *N*,*N*-dimethylformamide (3.0 mL) was treated with potassium thiocyanate (215.7 mg, 2.2 mmol). The reaction mixture was heated at 80 °C for 6 h and was quenched as described for the preparation of **20**. The residue was purified by column chromatography with hexane/EtOAc (19:1) as the eluent to produce 39.6 mg (36% yield) of **27** as a colorless oil.

4-(4-Pyridyl)oxyphenoxyethyl tetrahydro-2*H*-pyran-2-yl ether (28): Dimethyl sulfoxide (10.0 mL) was added to a mixture of **23** (2.145 g, 6.25 mmol), 4-hydroxypyridine (1.165 g, 12.3 mmol), copper(I) iodide (115.0 mg, 0.6 mmol), 2-picolinic acid, (149.2 mg, 1.28 mmol), and potassium phosphate tribasic (2.565 g, 12.3 mmol) as described for the preparation of **11**. The reaction mixture was stirred at 90 °C for 12 d. After the usual workup, the product was purified by column chromatography with hexane/EtOAc (1:1) as the eluent to produce 572 mg (29% yield) of **28** as a colorless oil.

4-(4-Pyridyl)oxyphenoxyethanol (29): A solution of **28** (556 mg, 1.76 mmol) in methanol (10 mL) was treated with pyridinium 4-toluenesulfonate (90 mg) as depicted for the preparation of **14**. The residue was purified by column chromatography with EtOAc/MeOH (49:1) as the eluent to give 240.1 mg (59% yield) of alcohol **29** as a colorless oil.

4-(4-Pyridyl)oxyphenoxyethyl bromide (30): A mixture of *N*-bromosuccinimide (187.0 mg, 1.05 mmol) and triphenylphosphine (275.4 mg, 1.05 mmol) in anhydrous dichloromethane (20 mL) cooled at 0 °C was treated with alcohol **29** (220.3 mg, 0.95 mmol), and the reaction mixture was stirred at 0 °C for 6 h. The solvent was evaporated, and the residue was purified by column chromatography with CH_2Cl_2/MeOH (99:1) as the eluent to give 154.2 mg (50% yield) of pure **30** as a colorless oil.

4-(4-Pyridyl)oxyphenoxyethyl thiocyanate (31): A solution of **30** (130 mg, 0.44 mmol) in anhydrous *N*,*N*-dimethylformamide (3.0 mL) was treated with potassium thiocyanate (172.6 mg, 1.8 mmol). The reaction mixture was heated at 80 °C for 6 h and was quenched as described for the preparation of **20**. The residue was purified by column chromatography with CH_2Cl_2/MeOH (19:1) as the eluent to produce 40.8 mg (34% yield) of **31** as a colorless oil.

4-Phenoxyphenyl acetate (33): A solution of 4-phenoxyphenol (**32**; 2.08 g, 11.2 mmol) in pyridine (5 mL) was treated with acetic anhydride (3 mL), and the reaction mixture was stirred at room temperature for 16 h. Water (10 mL) was then added, and the mixture was stirred for 1 h. The mixture was extracted with dichloromethane (2×30 mL). The combined organic layers were washed with an aqueous 1 N solution of hydrochloric acid (2×30 mL) and water (2×30 mL), then dried ($MgSO_4$), and the solvent was evaporated. The product was purified by column chromatography with hexane/EtOAc (19:1) as the eluent to give 2.478 g (97% yield) of **33** as a colorless oil.

2-Acetoxy-4-phenoxyphenol (34): A solution of acetate **33** (257.8 mg, 1.13 mmol) in cyclohexane (200 mL), previously degassed with dry nitrogen, in a septum-stopped quartz tube was irradiated with germicide lamps (4×20 W) centered at 254 nm at room temperature for 12 h. The solvent was evaporated, and the residue was purified by column chromatography with hexane/EtOAc (9:1) to produce 100.5 mg (39% yield) of pure **34** as a white solid.

2-Acetoxy-4-phenoxyphenoxyethyl tetrahydro-2*H*-pyran-2-yl ether (35): A solution of **34** (457.1 g, 2.0 mmol) in dimethyl sulfoxide (5.0 mL) was treated with potassium hydroxide (450 mg, 8.0 mmol). The mixture was stirred at room temperature for 5 min. Bromoethyl tetrahydropyranyl ether (418 mg, 2.0 mmol) was then added, and the reaction mixture was stirred at room temperature overnight. The mixture was partitioned between water (35 mL) and dichloromethane (35 mL). The aqueous phase was extracted with dichloromethane (2×30 mL). The combined organic layers were washed with a saturated solution of sodium chloride (5×30 mL), then dried ($MgSO_4$), and the solvent was evaporated. The product was purified by column chromatography with hexane/EtOAc (19:1) as the eluent to yield 442 mg (62% yield) of **35** as a colorless oil.

2-Acetoxy-4-phenoxyphenoxyethanol (36): A solution of **35** (420 mg, 1.2 mmol) in methanol (10 mL) was treated with pyridinium 4-toluenesulfonate (30 mg) as described for the preparation of **14**. The residue was purified by column chromatography with hexane/EtOAc (83:17) as the eluent to give 301 mg (92% yield) of alcohol **36** as a colorless oil.

2-Acetoxy-4-phenoxyphenoxyethyl 4-toluenesulfonate (37): A solution of alcohol **36** (280.2 mg, 1.03 mmol) in pyridine (3 mL) was treated with 4-toluenesulfonyl chloride (383 mg, 2.0 mmol), and the mixture was stirred at room temperature for 6 h. The reaction was quenched as described for the preparation of **17**. The product was purified by column chromatography with hexane/EtOAc (9:1) as the eluent to produce 417 mg of **37** (95% yield) as a colorless oil.

2-Acetoxy-4-phenoxyphenoxyethyl thiocyanate (38): A solution of **37** (348 mg, 0.82 mmol) in anhydrous *N*,*N*-dimethylformamide (3.0 mL) was treated with potassium thiocyanate (321.6 mg, 3.3 mmol). The reaction mixture was heated at 80 °C for 6 h and was quenched as described for the preparation of **20**. The residue was purified by column chromatography with hexane/EtOAc (9:1) as the eluent to produce 186.9 mg (71% yield) of **38** as a colorless oil.

4-Phenoxyphenoxyethyl amine (40): A solution of azide **39** (1.020 g, 4.0 mmol) in tetrahydrofuran (20 mL) was treated with triphenylphosphine (2.308 g, 8.8 mmol). The reaction mixture was stirred at room temperature for 4 h. Water (2.0 mL) was then added, and the mixture was stirred for an additional hour. The solvent was evaporated, and the residue was purified by column chromatography with CH_2Cl_2/MeOH (19:1) as the eluent to give 706 mg (77% yield) of **40** as a colorless oil.

Tetraethyl 1-[(4-phenoxyphenoxyethylamino)ethyl]-1,1-bisphosphonate (42): Amine **40** (345.1 mg, 1.5 mmol) was added to a solution of **41** (300 mg, 1.5 mmol) in anhydrous dichloromethane (10 mL) under an argon atmosphere. The reaction mixture was stirred at room temperature for 4 h. The solvent was evaporated to produce 651 mg (82% yield) of **42**, which was used in next step without further purification.

1-[(4-Phenoxyphenoxyethylamino)ethyl]-1,1-bisphosphonic acid (43): Compound **42** (430.1 mg, 0.81 mmol) was treated with a concentrated aqueous solution of hydrochloric acid (3.0 mL). The resulting mixture was heated at reflux for 24 h. The solvent was evaporated, and the residue was crystallized from H_2O/ethanol (1:1) to give 274.1 mg (81% yield) of **52** as a white solid.

***S*-(2-(4-Phenoxyphenoxy)ethyl) ethanethioate (45)**: A solution of tosylate **44** (1.90 g, 4.9 mmol) in anhydrous *N*,*N*-dimethylformamide (10 mL) under an argon atmosphere was treated with potassium thioacetate (1.23 g, 10.8 mmol). The reaction mixture was stirred at 90 °C for 2 h. The solvent was evaporated, and the residue was partitioned between water (50 mL) and dichloromethane (50 mL). The aqueous phase was extracted with dichloromethane (2×50 mL). The combined organic layers were dried ($MgSO_4$), and the solvent was evaporated. The product was purified by column chromatography with hexane/EtOAc (19:1) as the eluent to produce 1.383 g (98% yield) of pure **45** as a colorless oil.

2-(4-Phenoxyphenoxy)ethanethiol (46): A solution of compound **45** (1.35 g, 4.7 mmol) in tetrahydrofuran (8.0 mL) was added to a mixture of lithium aluminum hydride (238 mg, 63 mmol) in anhydrous tetrahydrofuran (20.0 mL) cooled at 0 °C. The reaction mixture was allowed to reach room temperature and was stirred for 3 h. The reaction was quenched by addition of ethyl acetate (10 mL). The mixture was partitioned between an aqueous saturated solution of sodium potassium tartrate (50 mL) and dichloromethane (50 mL). The aqueous layer was extracted with dichloromethane (2×50 mL). The combined organic layers were dried ($MgSO_4$), and the solvent was evaporated. The residue was purified by column chromatography with hexane/EtOAc (99:1) as the eluent to give 1.064 g (92% yield) of **46** as a colorless oil.

Tetraethyl 1-[4-phenoxyphenoxyeth-1-ylthio)ethyl] 1,1-bisphosphonate (47): Triethylamine (139 μL, 101 mg, 1.0 mmol) and thiol **46** (246 mg, 1.0 mmol) were added to a solution of **41** (300 mg, 1 mmol) in anhydrous dichloromethane (10 mL). The reaction mixture was stirred at room temperature for 5 h. Water (20 mL) was added, and the mixture was extracted with dichloromethane (3×10 mL). The combined organic layers were washed with brine (20 mL) and dried ($MgSO_4$), and the solvent was evaporated to produce 336 mg (59% yield) of tetraethyl ester **47**. The product was used in the next step without further purification: colorless oil.

1-[4-Phenoxyphenoxyeth-1-ylthio)ethyl] 1,1-bisphosphonic acid (48): A solution of the tetraethyl ester **47** (290 mg, 0.53 mmol) in anhydrous dichloromethane (10 mL) was treated with bromotrimethylsilane (10 equiv) under an argon atmosphere. The reaction mixture was stirred at room temperature for 48 h. Methanol (1.0 mL) was then added, and the solvent was evaporated. The residue was dissolved in methanol (10.0 mL), and the mixture was stirred at room temperature for 24 h. The solvent was evaporated, and the residue was redissolved in methanol and evaporated four times, to complete the hydrolysis of remaining trimethylsilyl bromide and to remove the recently formed hydrobromic acid. The residue was purified by column chromatography (silica gel C18-reversed phase) with water/methanol (9:1) as the eluent to produce 159 mg (69% yield) of **48** as an amorphous solid.

3-Phenoxyphenoxyethylazide (50): A solution of tosylate **49** (999.6 mg, 2.60 mmol) in anhydrous *N*,*N*-dimethylformamide (5 mL) was treated with sodium azide (845.1 mg, 13.0 mmol). The reaction mixture was stirred at 80 °C for 2 h. The mixture was then allowed to cool to room temperature, and water (50 mL) was added. The mixture was extracted with dichloromethane (3×20 mL), and the combined organic layers were washed with an aqueous saturated solution of sodium chloride (5×20 mL) and water (2×20 mL), then dried ($MgSO_4$). The solvent was evaporated, and the product was purified by column chromatography with hexane/EtOAc (97:3) as the eluent to produce 590.1 mg (90% yield) of **50** as a colorless oil.

3-Phenoxyphenoxyethylamine (51): A solution of **50** (545.3 mg, 2.1 mmol) in tetrahydrofuran (10 mL) was treated with triphenylphosphine (616.3 mg, 2.4 mmol). The reaction mixture was stirred 2 h at room temperature. Water (20 mL) was then added, and the mixture was extracted with dichloromethane (3×20 mL). The combined organic phases were washed with water (2×20 mL) and dried ($MgSO_4$), and the solvent was evaporated. The product was purified by column chromatography with CH_2Cl_2/methanol (49:1) as the eluent to produce 431.1 mg (90% yield) of **51** as a colorless oil.

Tetraethyl 1-[(3-phenoxyphenoxyethylamino)ethyl]-1,1-bisphosphonate (52): Amine **51** (350.8 mg, 1.5 mmol) was added to a solution of **41** (451.0 mg, 1.5 mmol) in anhydrous dichloromethane (10 mL) under an argon atmosphere. The reaction mixture was stirred at room temperature overnight. The solvent was evaporated to give 770 mg (97% yield) of **52**, which was used in the next step without further purification.

1-[(3-Phenoxyphenoxyethylamino)ethyl]-1,1-bisphosphonic acid (53): Compound **52** (411.2 mg, 0.78 mmol) was treated with a concentrated aqueous solution of hydrochloric acid (2 mL). The resulting mixture was heated at reflux for 7 h. The solvent was evaporated, and the residue was crystallized from H_2O/ethanol (1:1) to give 114 mg (35% yield) of **53** as an amorphous solid.

***S*-[3-Phenoxy)phenoxyethyl] ethanethioate (55)**: A solution of **54** (660 mg, 1.7 mmol) in anhydrous *N*,*N*-dimethylformamide (3.0 mL) was treated with potassium thioacetate (392 mg, 3.4 mmol). The reaction mixture was stirred at 80 °C for 3 h. The mixture was allowed to cool to room temperature, and water (20 mL) was added. The aqueous phase was extracted with dichloromethane (2×30 mL), and the combined organic layers were washed with brine (5×30 mL) and water (2×30 mL), then dried ($MgSO_4$). The solvent was evaporated. The residue was purified by column chromatography with hexane/EtOAc (49:1) as the eluent to give 411 mg (83% yield) of **55** as a colorless oil.

2-(3-Phenoxyphenoxy)ethanethiol (56): Potassium carbonate (anhydrous powder) was added to a solution of **55** (957 mg, 3.3 mmol) in methanol (10 mL) with stirring, then water (3.0 mL) was added to obtain complete solution. The reaction mixture was stirred at room temperature for 40 min, and the solvent was evaporated. The residue was dissolved in glacial acetic acid, and zinc (1.8 g, 28 mmol) was added. The reaction mixture was heated at reflux for 1 h at 110 °C. The mixture was allowed to cool to room temperature and was partitioned between water (30 mL) and dichloromethane (30 mL). The organic phase was washed with water (2×30 mL), then dried ($MgSO_4$), and the solvent was evaporated to yield 616 mg (75% yield) of **56** as a colorless oil, which was used in the next step without further purification.

Tetraethyl 2-[3-(Phenoxy)phenoxyethylthio]ethyl-1,1-bisphosphonate (57): Thiol **56** (616 mg, 2.5 mmol) was added to a solution of **41** (751 mg, 2.5 mmol) in anhydrous dichloromethane (10 mL). The reaction mixture was stirred at room temperature for 16 h. Water (20 mL) was added, and the mixture was extracted with dichloromethane (3×10 mL). The combined organic layers were dried ($MgSO_4$), and the solvent was evaporated. The residue was purified by column chromatography with CH_2Cl_2/methanol (49:1) as the eluent to produce 1.1 g (81% yield) of **57** as a colorless oil.

2-[3-(Phenoxy)phenoxyethylthio]ethyl-1,1-bisphosphonic acid (58): A solution of **57** (1.1 g, 2.0 mmol) in anhydrous dichloromethane (10 mL) was treated with bromotrimethylsilane (3.1 g, 20.2 mmol) under an argon atmosphere. The reaction mixture was stirred at room temperature for 48 h. Methanol (1.0 mL) was then added, and the solvent was evaporated. The residue was dissolved in methanol (8 mL), and the mixture was stirred at room temperature for 24 h. The solvent was evaporated, and the residue was redissolved in methanol and evaporated four times. The residue was purified by column chromatography (silica gel C18-reversed phase) with water/methanol (1:1) as the eluent to produce 568 mg (65%) of **58** as a yellow pale solid after lyophilization.

Phenoxyethylazide (60): A solution of **59** (1.1054 g, 3.78 mmol) in anhydrous *N*,*N*-dimethylformamide (5 mL) was treated with sodium azide (1.2290 g, 18.9 mmol). The reaction mixture was stirred at 80 °C for 2 h. The mixture was then allowed to cool to room temperature, and water (50 mL) was added. The aqueous phase was extracted with dichloromethane (2×20 mL), and the combined organic layers were washed with an aqueous saturated solution of sodium chloride (5×20 mL) and water (2×20 mL), then dried ($MgSO_4$). The solvent was evaporated, and the residue was purified by column chromatography with hexane/EtOAc (97:3) as the eluent to give 498.4 mg (81% yield) of **60** as a colorless oil.

Phenoxyethylamine (61): A solution of azide **60** (463.6 mg, 2.84 mmol) in tetrahydrofuran (10 mL) was treated with triphenylphosphine (819.7 mg, 3.12 mmol) as described for the preparation of **40**. The residue was purified by column chromatography with hexane/EtOAc (9:1) as the eluent to afford 260.8 mg (67% yield) of **61** as a yellow pale oil.

Tetraethyl 1-[(Phenoxyethylamino)ethyl]-1,1-bisphosphonate (62): Amine **61** (194.6 mg, 1.5 mmol) was added to a solution of **41** (452.9 mg, 1.5 mmol) in anhydrous dichloromethane (10 mL) under an argon atmosphere. The reaction mixture was stirred at room temperature overnight. The solvent was evaporated to give 574.6 mg (88% yield) of **62**, which was used in the next step without further purification.

1-[(Phenoxyethylamino)ethyl]-1,1-bisphosphonic acid (63): Compound **62** (547.6 mg, 1.25 mmol) was treated with a concentrated aqueous solution of hydrochloric acid (4.0 mL). The mixture was heated at reflux for 7 h. The solvent was evaporated, and the residue was crystallized from H_2O/ethanol (1:1) to give 44.5 mg (11% yield) of **63** as a white solid.

2-(Naphthalen-2-yloxy)ethyl tetrahydro-2*H*-pyran-2-yl ether (65): A solution of **64** (1.0121 g, 7.02 mmol) in dimethyl sulfoxide (10.0 mL) was treated with potassium hydroxide (877 mg, 15.6 mmol) and bromoethyl tetrahydropyranyl ether (1.7957 g, 8.6 mmol) as described for the preparation of **35**. The product was purified by column chromatography with hexane/EtOAc (19:1) as the eluent to produce 917.6 mg (48% yield) of **65** as a colorless oil.

2-(Naphthalen-2-yloxy)ethanol (66): A solution of **65** (789.0 mg, 2.9 mmol) in methanol (10 mL) was treated with pyridinium 4-toluenesulfonate (30 mg) as described for the preparation of **14**. The residue was purified by column chromatography with hexane/EtOAc (9:1) as the eluent to give 463.5 mg (85% yield) of alcohol **66** as a colorless oil.

2-(Naphthalen-2-yloxy)ethyl 4-toluenesulfonate (67): A solution of alcohol **66** (450.3 mg, 2.45 mmol) in pyridine (5 mL) was treated with 4-toluenesulfonyl chloride (1.422 g, 7.5 mmol), and the mixture was stirred at room temperature for 4 h. The reaction was worked up as described for the preparation of **17**. The product was purified by column chromatography with hexane/EtOAc (91:9) as the eluent to produce 696.3 mg of **67** (83% yield) as a colorless oil.

2-(Naphthalen-2-yloxy)ethyl thiocyanate (68): A solution of **67** (464.1 mg, 1.35 mmol) in anhydrous *N*,*N*-dimethylformamide (3.0 mL) was treated with potassium thiocyanate (531 mg, 5.5 mmol). The reaction mixture was treated as described for the preparation of **20**. The residue was purified by column chromatography with hexane/EtOAc (19:1) as the eluent to produce 170.3 mg (55% yield) of **68** as a colorless oil.

2-(Naphthalen-1-yloxy)ethyl tetrahydro-2*H*-pyran-2-yl ether (70): A solution of **69** (537.1 g, 3.82 mmol) in dimethyl sulfoxide (5.0 mL) was treated with potassium hydroxide (503 mg, 8.9 mmol) and bromoethyl tetrahydropyranyl ether (1.1157 g, 5.3 mmol) as described for the preparation of **35**. The product was purified by column chromatography with hexane/EtOAc (19:1) as the eluent to produce 675.7 mg (65% yield) as a colorless oil.

2-(Naphthalen-1-yloxy)ethanol (71): A solution of **70** (562.8 mg, 2.07 mmol) in methanol (10 mL) was treated with pyridinium 4-toluenesulfonate (30 mg) as described for the preparation of **14**. The residue was purified by column chromatography with hexane/EtOAc (9:1) as the eluent to give 315.6 mg (81% yield) of alcohol **71** as a colorless oil.

2-(Naphthalen-1-yloxy)ethyl 4-toluenesulfonate (72): A solution of alcohol **71** (326.1 mg, 1.77 mmol) in pyridine (5 mL) was treated with 4-toluenesulfonyl chloride (1.027 g, 5.4 mmol), and the mixture was stirred at room temperature for 4 h. The reaction was worked up as described for the preparation of **17**. The product was purified by column chromatography with hexane/EtOAc (9:1) as the eluent to produce 545.5 mg of **72** (90% yield) as a colorless oil.

2-(Naphthalen-1-yloxy)ethyl thiocyanate (73): A solution of **67** (445.4 mg, 1.29 mmol) in anhydrous *N*,*N*-dimethylformamide (3.0 mL) was treated with potassium thiocyanate (550 mg, 5.7 mmol). The reaction mixture was treated as described for the preparation of **20**. The residue was purified by column chromatography with hexane/EtOAc (19:1) as the eluent to yield 171.6 mg (58% yield) of **73** as a colorless oil.

Drug screening

T. cruzi amastigote assays: These experiments were done as reported by using tdTomato-labeled trypomastigotes[49] with the modifications described by Recher et al.[50] EC_{50} values were determined by nonlinear regression analysis with SigmaPlot.

T. gondii tachyzoites assays: Experiments on *T. gondii* tachyzoites were carried out as described previously[51] by using *T. gondii* tachyzoites expressing red fluorescent protein[52] with the modifications described by Recher et al.[50] Plates were read with covered lids,

and both excitation (544 nm) and emission (590 nm) were read from the bottom.

Cytotoxicity for Vero cells: The cytotoxicity was tested by using the Alamar Blue™ assay as described by Recher et al.[50]

Computational methods

Model building: The structure of *Tg*FPPS was built with homology modeling by using SwissModel,[53] based on the structure of *P. vivax* FPPS (PDB ID: 3MAV), a protein showing the highest degree of similarity with *Tg*FPPS. The constructed *Tg*FPPS structure was aligned with the *Tc*FPPS alendronate complex (PDB ID: 1YHM),[54] the structure of **58** was built on that of the alendronate, and Mg^{2+} ions and the coligand isopentenyl pyrophosphate were added. Charges for **58** and isopentenyl pyrophosphate were obtained by using the RESP method at the Hartree Fock 6-31G* level, and the compounds were parameterized with the Generalized Amber force field. The complex was built by using the *tleap* module in AmberTools 15.[55] The Amber FF14SB force field was used to parameterize the protein structure, the charge was neutralized by addition of seven Na^+ ions, and the complex was solvated with a box of TIP3P waters extending 10 Å beyond the complex.

Molecular dynamics simulations: Simulations were run by using the *sander* module in AmberTools 15. The Mg and phosphate units of **58** were restrained during all simulations to their original coordinates by using a 100 Kcal mol^{-1} harmonic potential. The complex was minimized for 1000 steps of steepest descent, followed by 1000 steps of conjugate gradient. It was then heated from 0 to 300 K for 20 ps by using Langevin dynamics, followed by an 80 ps optimization with the NPT ensemble. The production run consisted of a 10 ns MD simulation with the NVT ensemble.

Energy calculations: Calculation of the binding energy was performed by using the MMPBSA.py module in AmberTools 15. The Generalized Born Surface Area model was used with igb = 5, the corresponding mbondi2 radii, and a salt concentration of 0.1 M. 100 frames at 100 ps intervals were taken from the MD simulation. Pairwise energy decomposition was carried out with the ligand decomposed fragment-wise by using an in-house modified version of MMPBSA.py.

Clustering analysis: Clustering analysis was performed with the *cpptraj* module in AmberTools 15 on the MD simulation stripped of solvent by using the dbscan algorithm, with an epsilon of 0.75 Å for the residues at 4 Å from the ligand. The MD snapshot corresponding to the most populated cluster was minimized for 1000 steps of steepest descent, followed by 1000 steps of conjugate gradient, with the explicit waters maintained for the minimization to assemble Figure 6.

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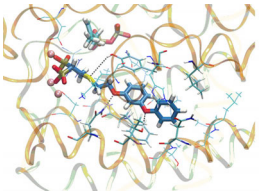
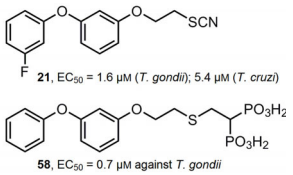
Activity of Fluorine-Containing Analogues of WC-9 and Structurally Related Analogues against Two Intracellular Parasites: *Trypanosoma cruzi* and *Toxoplasma gondii*





Potent against parasites: Derivatives of the squalene synthase inhibitor **WC-9** were designed, synthesized, and evaluated against the parasites *Trypanosoma cruzi* and *Toxoplasma gondii*. Derivative **21** exhibited good potency against *T. gondii* and similar potency to **WC-9** against *T. cruzi*. Compound **58**, a hybrid inhibitor, has sub-micromolar activity against *T. gondii*, which suggests a combined inhibitory effect of the two functional groups. Compound **58** was modelled in the active site of *T. gondii* farnesyl diphosphate synthase.