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Original article

Design, synthesis and biological evaluation of **WC-9** analogs as antiparasitic agents

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ABSTRACT

As a part of our project pointed at the search of new safe chemotherapeutic and chemoprophylactic agents against parasitic diseases, several compounds structurally related to 4-phenoxyphenoxyethyl thiocyanate (**WC-9**), which were modified at the terminal aromatic ring, were designed, synthesized and evaluated as antiproliferative agents against *Trypanosoma cruzi*, the parasite responsible of American trypanosomiasis (Chagas disease) and *Toxoplasma gondii*, the etiological agent of toxoplasmosis. Most of the synthetic analogs exhibited similar antiparasitic activity being slightly more potent than the reference compound **WC-9**. For example, the nitro derivative **13** showed an ED_{50} value of 5.2 μM. Interestingly, the regioisomer of **WC-9**, compound **36** showed similar inhibitory action than **WC-9** indicating that *para*-phenyl substitution pattern is not necessarily required for biological activity. The biological evaluation against *T. gondii* was also very promising. The ED_{50} values corresponding for **13**, **36** and **37** were at the very low micromolar level against tachyzoites of *T. gondii*.

1. Introduction

American trypanosomiasis (Chagas disease) and toxoplasmosis [1] are major parasitic diseases that affect millions of individuals according to the World Health Organization [2,3]. The etiologic agent of American trypanosomiasis is the hemoflagellated protozoan *Trypanosoma cruzi*, which has a complex life cycle involving blood-sucking Reduviid insects and mammals [4]. The occurrence of American trypanosomiasis in countries where this disease is not endemic has been attributed to transfusion of infected blood or congenital transmission [5,6]. On the other hand, the opportunistic pathogen *Toxoplasma gondii* causes a broad spectrum of disease but most infections are asymptomatic [7]. This Apicomplexan parasite has adopted an essential intracellular life style. The parasite actively penetrates host cells, sets up a privileged compartment in which it replicates and finally lyses the cell [8,9]. Chemotherapy for these parasitic diseases is still deficient and mainly based on old or empirically discovered drugs [10,11]. Therefore, there is an urgent requirement of having at hand new safe drugs based on the knowledge of the biochemistry and physiology of the responsible agents of these diseases.

Isoprenoids are essential compounds of the cellular machinery of all organisms due to their roles in a variety of biological processes. Several enzymes of this pathway in *T. cruzi*, involved in the synthesis of sterols [12] and farnesyl diphosphate [13], and in protein prenylation [14], have been reported to be excellent drug targets against pathogenic parasites. Despite their structural and functional variety, all isoprenoids derive from a common precursor: isopentenyl diphosphate (IPP), and its isomer, dimethylallyl diphosphate (DMAPP). In *T. cruzi*, IPP is synthesized only via the so-called mevalonate pathway, which has the 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase as the key regulatory enzyme [15,16]. *T. gondii* lacks the mevalonate pathway and uses instead a prokaryotic-type 1-deoxy-D-xylulose-5-phosphate (DOXP) pathway to make IPP and DMAPP [7]. The DOXP pathway localizes to the apicoplast and is essential [17].

Trypanosomatids have a strict requirement for specific endogenous sterols for survival and cannot use the abundant supply of cholesterol present in their mammalian hosts [18–20]. It has been reported that ergosterol biosynthesis inhibitors with potent *in vitro*

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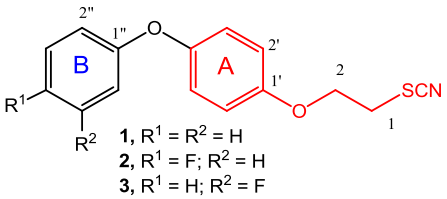


Fig. 1. Chemical structure of **WC-9** (compound **1**) and closely related analogs.

activity and special pharmacokinetic properties in mammals can induce radical parasitological cure in animal models of both acute and chronic experimental Chagas disease [20]. 4-Phenoxyphenoxyethyl thiocyanate (**1**) known as **WC-9** is an interesting compound that presents IC_{50} values at the low nanomolar range against the clinically more relevant form of *T. cruzi* (amastigotes) (Fig. 1) [21–23]. **WC-9** induces a dose dependent effect of growth of the epimastigotes (EP strain) with a minimal inhibitory concentration of 1 μM. The growth inhibitory effect of **WC-9** is associated with a depletion of the parasite endogenous sterols, ergosterol and its 24-ethyl analog. An almost complete disappearance of the parasite sterols is observed, with no accumulation of sterol intermediates or precursors indicating a blockade of the biosynthetic pathway at a pre-squalene level [24].

Squalene synthase (SQS) catalyzes the first committed step in sterol biosynthesis, where a reductive dimerization of two molecules of farnesyl pyrophosphate yields squalene. This enzyme is the target of **WC-9**. Highly purified glycosomes and mitochondrial membrane vesicles obtained from *T. cruzi* epimastigotes have been used as enzyme source [25]. **WC-9** is a potent inhibitor of both glycosomal and mitochondrial *T. cruzi* SQS, with IC_{50} values of 88 nM and 129 nM. The dose–response curves for the activity of **WC-9** against *Tc*SQS were consistent with non-competitive inhibition with $K_i = IC_{50}$; these K_i values are two to three orders of magnitude lower that the K_m of the substrates [24].

As **WC-9** targets *Tc*SQS, this fact suggested that **WC-9**, with its thiocyanate group bearing an electrophilic carbon atom linked to the hydrophobic 4-phenoxyphenoxyethyl moiety, could act by mimicking the carbocationic transition state of the reaction leading to the formation of the cyclopropylcarbinyl intermediate presqualene-diphosphate **5** [26,27]. A similar rationale has been advanced to explain the potent anti-SQS activity of arylquinuclidine derivatives against both mammalian and *T. cruzi* SQS [28,29]. Based on this hypothesis, it should be possible to design new and more potent SQS inhibitors, using **WC-9** as lead drug. The above results justify the mechanism of action of 4-phenoxyphenoxy derivatives at a molecular level, suggesting that these drugs represent promising SQS inhibitors, with potential antiparasitic and cholesterol-lowering activity in humans (Scheme 1).

It has been demonstrated that *T. gondii* does not synthesize cholesterol and imports it from the host suggesting that inhibitors of the host SQS could potentially inhibit *T. gondii* growth [30].

2. Rationale

We have established a rigorous SAR studies on **WC-9** chemical structure finding that the phenoxyethyl thiocyanate moiety, which is colored in red in Fig. 1, can be considered as the structure of the pharmacophore [21–23,31,32]. However, pharmacokinetic properties of this representative drug still should be improved. Although **WC-9** is able to impair parasitemia in murine models of Chagas disease the level of protection is not as efficient as ketoconazole, used as a positive control [33]. This lack of efficacy may be attributed to poor pharmacokinetic properties. In this context, we have demonstrated that structural variations at the B ring have influenced biological activity. For example, the introduction of a fluorine atom at the B ring of **WC-9** gives rise to compounds **2** and **3**, which have estimated log *P* values of 4.71 versus log *P* of 4.51 for **WC-9**, indicating a better distribution between water/octanol. In fact, these two drugs, **2** and **3**, are significantly more potent than **WC-9** [32].

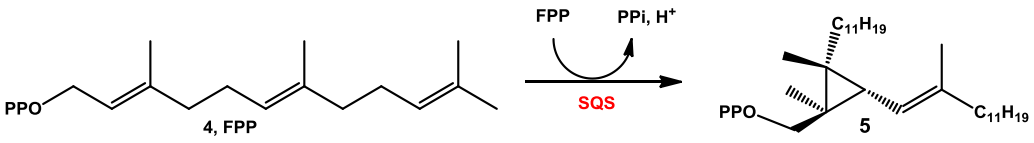
The key step for preparing either **2** or **3** is a coupling reaction between a suitable substituted phenylboronic acid and a conveniently functionalized phenol [34,35]. However, this method suffers from some disadvantages such as low reaction yields, the cost of the phenylboronic acids, and the availability of diverse functionalized products, which are impractical to access from the synthetic point of view. The approach developed by Buchwald results an interesting alternative to the expensive and commercially not available phenylboronic acids. Therefore, it is possible to obtain straightforwardly either *O*-arylation products of formula 6 or the *N*-arylation compounds of formula 7 (Scheme 2) [36].

To date crystal structures of *Tc*SQS are not available. However, the X-ray crystallographic structure of **WC-9** bound to dehydrosqualene synthase from *Staphylococcus aureus* has been published [37]. This enzyme catalyzes dehydrosqualene formation, a metabolite that is further transformed into staphyloxanthin. It has been postulated that **WC-9** might bind into the same hydrophobic S2 pocket in *Tc*SQS as it does in dehydrosqualene synthase keeping the same polar interactions with the thiocyanate group [37].

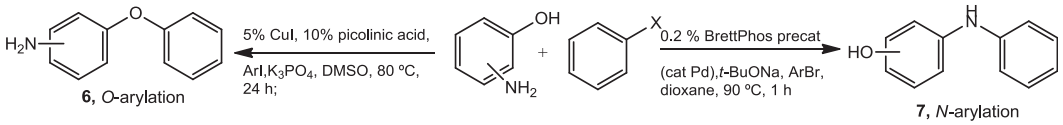
3. Results and discussion

For the above reasons, attempts to improve the biological activity of **WC-9** would be conducted through a classical approach. The preparation of the title compounds was performed employing a Buchwald coupling reaction as a key step in all cases [36]. Structural variations at the B ring as well as the relative position of the B ring to the main chain were contemplated. The nitro derivative **13** was synthesized starting from the already depicted tetrahydropyranyl derivative **8** [23], which on treatment with hydrogen at 3 atm, in the presence of palladium on charcoal, afforded the free phenol **9** in 48% yield. **9** was coupled with 1-iodo-3-nitrobenzene in the presence of 5% cuprous iodide, 10% picolinic acid and potassium phosphate to give **10** in 52% yield. This compound was deprotected by treatment with pyridinium *p*-toluenesulfonate in methanol to afford free alcohol **11**, which was treated with tosyl chloride in pyridine to afford the expected tosylate **12** in 88% yield. This compound was further transformed into the thiocyanate derivative **13** by treatment with potassium thiocyanate in *N*,*N*-dimethylformamide at 100 °C in 61% yield (Scheme 3).

The preparation of analogs of **WC-9** bearing methoxy groups at the B ring was outlined in Scheme 4 employing 4-iodophenol as starting material. This compound was transformed into the tetrahydropyranyl ether derivative **14** by treatment with 2-bromoethyl



Scheme 1. First step of the reductive dimerization of FPP.



Scheme 2. Buchwald coupling reaction to prepare either phenyl amines or phenyl ethers.

tetrahydro-2*H*-pyran-2-yl ether in a suspension of potassium hydroxide in dimethyl sulfoxide, according to a modified Williamson procedure [38]. Buchwald coupling reaction of iodo derivative **14** either with 4-methoxyphenol or 3-methoxyphenol afforded **15** and **16** in moderately yields, respectively. Following the general strategy each tetrahydropyranyl derivative **15** and **16** was deprotected by treatment with pyridinium *p*-toluenesulfonate affording alcohols **17** and **18** in good yields, which were tosylated to give **19** and **20**. On treatment with potassium thiocyanate, in separate experiments, these compounds were converted into the title compounds **21** and **22**, respectively.

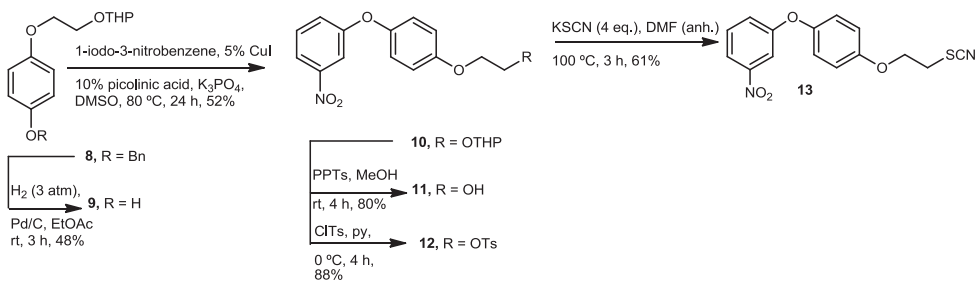
An interesting structural variation was the replacement of the terminal phenyl group by a pyridyl group where the nitrogen atom occupied the 2″ position. The key step in the preparation of this title compound was the incorporation of the pyridyl unit. Therefore, Buchwald coupling of 4-benzyloxyphenol with 3-bromopyridine afforded **23** in a low but reproducible yield. The benzyl group of **23** was cleaved by treatment with hydrogen at 3 atm in the presence of palladium on charcoal to give the substituted phenol **24**. Similarly to the preparation of **21** and **22**, incorporation of the terminal ethoxy group through a Williamson procedure, followed by tetrahydropyranyl cleavage and further tosylation and substitution with the thiocyanate ion gave rise to the title compound **28** (Scheme 5).

In order to study whether the relative position of terminal phenyl group might have influence on biological activity, two **WC-9** analogs were envisioned where the phenyl groups were at the C-3′ position rather than at C-4′ one. The strategy to get these compounds is presented in Scheme 4. Then, 3-iodophenol was reacted with 2-bromoethyl tetrahydro-2*H*-pyran-2-yl ether according to the already depicted Williamson procedure affording the committed intermediate **29**, which was coupled either with phenol or 4-methoxyphenol gave rise to **30** and **31**, respectively. Then, following the general strategy, each of these compounds experienced tetrahydropyranyl cleavage, tosylation and nucleophilic displacement of the tosylate group by treatment with potassium thiocyanate affording the title compounds **36** and **37**, respectively (Scheme 6).

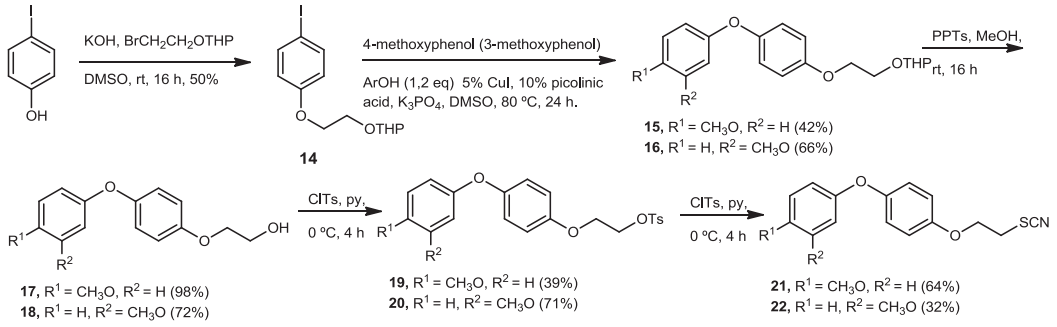
Biological activity of these **WC-9** analogs was very promising. The title compound **13** was a potent growth inhibitor of the intracellular form of *T. cruzi*, which is the clinically more relevant replicative form of the parasite. Certainly, this compound bearing an electron withdrawing group at the C-3″ position exhibited an ED_{50} value of 5.2 μM, being two-fold more potent than our lead drug **WC-9**, used as a positive control, under the same assay conditions. The tetrahydropyranyl precursor **10** of **13** was devoid of biological activity against *T. cruzi* (amastigotes) proliferation. This observation was not in agreement with our previous results in other closely related compounds, where the biological activity of **WC-9** analogs correlated quite well with the activity exhibited by their synthetic tetrahydropyranyl ether precursors when bonded to the same aromatic skeleton [22,23,32]. Compound **13** was also a potent inhibitor of *T. gondii* (tachyzoites) growth possessing an ED_{50} values at the very low micromolar level (2.0 μM). Compounds **21** and **22** bearing electron donor groups proved to be effective growth inhibitors of *T. cruzi* (amastigotes) exhibiting ED_{50} values of 6.1 μM and 4.6 μM, respectively. **21** and **22** were also potent inhibitors of *T. gondii* growth but to a slight lesser extent than **13** showing ED_{50} values of 3.8 μM and 6.7 μM, respectively. Pyridyl analog **28** showed potent antiparasitic action having ED_{50} values of 7.4 μM and 7.5 μM against *T. cruzi* and *T. gondii*, respectively. Its synthetic precursor **25** exhibited vanishing antiparasitic activity. Compounds **36** and **37** are regioisomers of **WC-9** and **21**, respectively where the terminal ring is at the C-3″ in lieu of C-4″. Both of these compounds practically hold the antiparasitic activity of **WC-9** (ED_{50}s of 11.2 μM and 10.1 μM against *T. cruzi* and 4.0 μM and 2.9 μM against *T. gondii*, respectively). These data indicated that the *para*-aryl substitution pattern would not be necessarily required for biological activity. The results are presented in Table 1.

It can be concluded that, from the chemical point of view, the Buchwald coupling reaction is a powerful tool to access to diverse **WC-9** derivatives not only those modified at the B ring as it is the case of the present study, but also at the A ring in future works. In addition, structural variations at the B ring, regardless whether electron withdrawing groups or electron donor groups was present, gave rise to potent inhibitors of *T. cruzi* proliferation exhibiting an efficacy superior to our lead drug **WC-9**. These **WC-9** analogs also exhibited excellent prospective as anti-*Toxoplasma* agents because they also exhibited potent inhibitory action against *T. gondii* growth. The fact that **WC-9** analogs have reasonable drug-like character overcomes the approach to established a rigorous structure/activity relationship.

Our results are in agreement with previous reports indicating that mevalonate pathway inhibitors are active against different Apicomplexan parasites such as *Babesia divergens* [39], *Plasmodium falciparum* [39,40], *Cryptosporidium parvum* [41], and *T. gondii* [42], suggesting that these parasites, which lack a mevalonate pathway, are dependent on host synthesis of precursors of the isoprenoid pathway.



Scheme 3. Synthesis of the 2″-nitro derivative of **WC-9** at the B ring.



Scheme 4. Synthesis of methoxy derivatives of **WC-9** at the B ring.

4. Experimental section

The glassware used in air and/or moisture sensitive reactions was flame-dried and carried out under a dry argon atmosphere. Unless otherwise noted, chemicals were commercially available and were used without further purification. Solvents were distilled before use. Tetrahydrofuran and ethyl ether were distilled from sodium/benzophenone ketyl. Chloroform and acetonitrile was distilled from phosphorus pentoxide. Anhydrous *N*,*N*-dimethylformamide was used as supplied from Aldrich.

Nuclear magnetic resonance spectra were recorded using a Bruker AM-500 MHz spectrometer. Chemical shifts are reported in parts per million (δ) relative to tetramethylsilane. Coupling constants are reported in Hertz. ^{13}C NMR spectra were fully decoupled. Splitting patterns are designated as s, singlet; d, doublet; t, triplet; q, quartet.

High-resolution mass spectra were obtained 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 using a Fisher–Johns apparatus and are uncorrected.

Column chromatography was performed with E. Merck silica gel plates (Kieselgel 60, 230–400 mesh). Analytical thin layer chromatography was performed employing 0.2 mm coated commercial silica gel plates (E. Merck, DC-Aluminum sheets, Kieselgel 60 F_{254}).

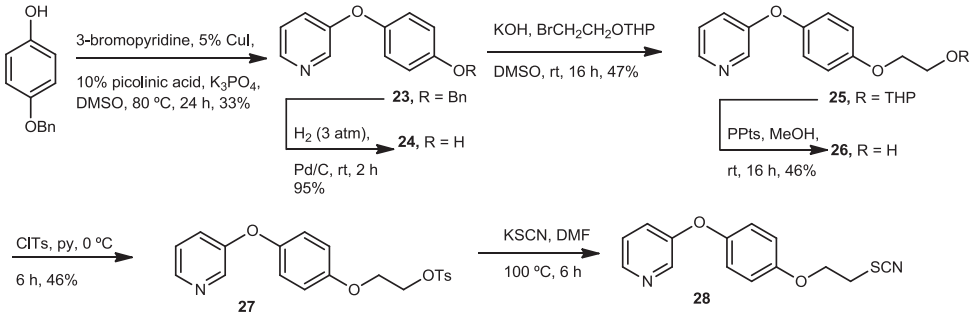
As judged from the homogeneity of the ^{1}H, ^{13}C, spectra of the title compounds and HPLC analyses employing an IMC-Pack ODS-2 column 5 μM, 250 × 10 mm eluting with methanol–water (9:1) at 3.00 mL/min with a variable wavelength detector (275 nM) indicated a purity >97%.

4.1. 4-Hydroxyphenoxyethyl tetrahydro-2H-pyran-2-yl ether (**9**)

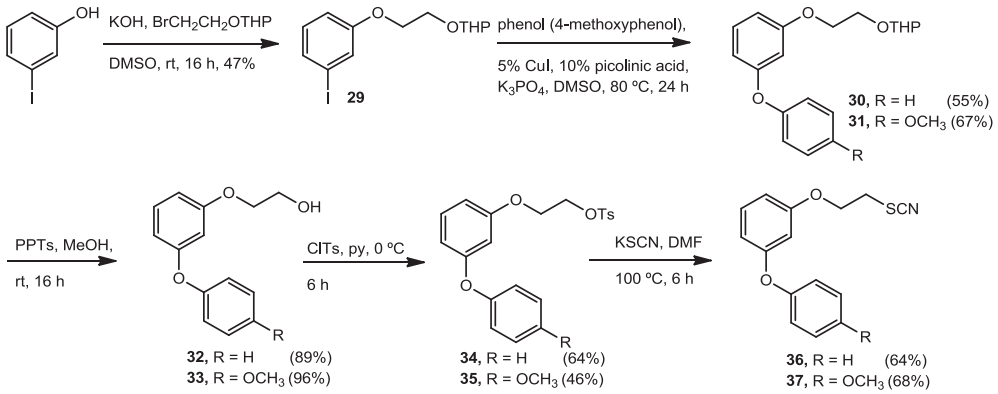
A solution of **8** (3.032 g, 9.2 mmol) in ethyl acetate (40 mL) in the presence of 5% palladium on charcoal (40 mg) was treated with hydrogen at 3 atm. The reaction was stirred at room temperature for 4 h. The mixture was filtered off and the solvent was evaporated. The residue was purified by column chromatography (silica gel) employing hexane–EtOAc (9:1) as eluant to produce 1.056 g (48% yield) of pure **9** as a colorless oil: R_f 0.41 (hexane–EtOAc; 3:2); ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.55–1.74 (m, 6H, H-3‴, H-4‴, H-5‴), 3.54 (m, 1H, H-6‴$_a$), 3.80 (m, 1H, H-6‴$_b$), 3.90 (m, 1H, H-1$_a$), 4.01 (m, 1H, H-1$_b$), 4.11 (m, 2H, H-2), 4.72 (t, J = 3.4 Hz, 1H, H-2″), 6.77 (m*AB*, 4H, aromatic protons); ^{13}C NMR (50 MHz, $CDCl_3$) δ 19.3 (C-4″), 25.4 (C-5″), 30.5 (C-3″), 62.3 (C-6″), 66.0 (C-1), 68.1 (C-2), 99.0 (C-2″), 115.9 (C-2′), 116.0 (C-3′), 149.8 (C-4′), 152.7 (C-1′). HRMS (ESI) calcd for $C_{13}H_{18}O_4Na$ $[M + Na]^+$ 261.1103; found 261.1102.

4.2. 4-(3-Nitrophenoxy)phenoxyethyl tetrahydro-2H-pyran-2-yl ether (***10***)

A mixture of compound **9** (960 mg, 4.0 mmol), 1-iodo-3-nitrobencene (836 mg, 3.4 mmol), copper(I) iodide (31.9 mg, 0.17 mmol), 2-picolinic acid, (41.3 mg, 0.33 mmol), and potassium phosphate tribasic (1.425 g, 6.7 mmol) under anhydrous conditions was evacuated and back-filled with argon. This sequence was repeated twice. Then, dimethyl sulfoxide was added (15.0 mL) and the reaction mixture was stirred vigorously at 80 °C for 24 h. The mixture was cooled to room temperature and partitioned between ethyl acetate (20 mL) and water (20 mL). The aqueous layer was extracted with ethyl acetate (2 × 20 mL). The combined organic phases were washed with brine (5 × 50 mL), dried ($MgSO_4$), and the solvent was evaporated. The product was purified by column chromatography (silica gel) employing hexane–EtOAc (19:1) as eluent to afford 635 mg (52% yield) of pure compound **10** as a colorless oil: R_f 0.39 (hexane–EtOAc; 7:3); ^{1}H NMR (500.13 MHz, $CDCl_3$) δ 1.52–1.68 (m, 4H, H-4‴, H-5‴), 1.73–1.79 (m, 1H, H-3‴$_a$), 1.82–1.87 (m, 1H, H-3‴$_b$), 3.55 (m, 1H, H-6‴$_a$), 3.84 (ddd, J = 11.0, 6.5, 4.2 Hz, 1H, H-6‴$_b$), 3.91 (ddd, J = 11.3, 8.2, 3.1 Hz, 1H, H-1$_a$), 4.08 (m, 1H, H-1$_b$), 4.18 (m, 2H, H-2), 4.73 (t, J = 3.5 Hz, 1H, H-2‴), 6.97 (d, J = 9.4 Hz, 2H, H-2′), 7.01 (d, J = 9.4 Hz, 2H, H-3′), 7.27 (ddd, J = 8.3, 2.5, 0.9 Hz, 1H, H-5″), 7.46 (t, J = 8.2 Hz, 1H, H-6″), 7.71 (t, J = 2.3 Hz, 1H, H-2″), 7.88 (ddd, J = 8.2, 2.2, 0.9 Hz, 1H, H-4″); ^{13}C NMR (125.77 MHz, $CDCl_3$) δ 19.4 (C-4‴), 25.4 (C-5‴), 30.5 (C-3‴),



Scheme 5. Preparation of nitrogen-containing thiocyanates at the B ring.



Scheme 6. Preparation of the regioisomers of **WC-9**.

62.2 (C-6‴), 65.8 (C-1), 67.9 (C-2), 99.0 (C-2‴), 111.7 (C-2″), 116.2 (C-2′), 117.0 (C-4″), 121.4 (C-3′), 123.2 (C-6″), 130.2 (C-5″), 148.3 (C-3″), 150.2 (C-4′), 156.2 (C-1′), 159.6 (C-1″). HRMS (ESI) calcd for $C_{19}H_{21}O_6NNa$ $[M + Na]^+$ 382.1267; found 382.1274.

4.3. 4-(3-Nitrophenoxy)phenoxyethanol (*11*)

A solution of compound **10** (420 mg, 1.17 mmol) in methanol (20 mL) was treated with pyridinium 4-toluensulfonate (30 mg). The reaction mixture was stirred at room temperature overnight. Then, water (50 mL) and the mixture was extracted with methylene chloride (3 × 50 mL). The combined organic layers were washed with brine (3 × 50 mL), dried ($MgSO_4$), and the solvent was evaporated. The residue was purified by column chromatography eluting with hexane–EtOAc (7:3) to give 257.1 mg (80% yield) of pure alcohol **11** as a white solid: R_f 0.13; ^{1}H NMR (500.13 MHz, $CDCl_3$) δ 2.03 (t, J = 6.0 Hz, 1H, O*H*), 4.00 (q, J = 4.9 Hz, 2H, H-1), 4.11 (t, J = 4.8 Hz, 1H, H-2), 6.97 (d, J = 9.1 Hz, 2H, H-2′), 7.02 (d, J = 9.4 Hz, 2H, H-3′), 7.27 (ddd, J = 8.3, 2.5, 0.9 Hz, 1H, H-5″), 7.45 (t, J = 8.3 Hz, 1H, H-6″), 7.71 (t, J = 2.3 Hz, 1H, H-2″), 7.89 (ddd, J = 8.2, 2.1, 0.9 Hz, 1H, H-4″); ^{13}C NMR (125.77 MHz, $CDCl_3$) δ 61.5 (C-1), 69.7 (C-2), 111.7 (C-2″), 116.0 (C-2′), 117.1 (C-4″), 121.5 (C-3′), 123.3 (C-6″), 130.2 (C-5″), 148.9 (C-4′), 155.8 (C-1′), 159.6 (C-1″).

4.4. 4-(3-Nitrophenoxy)phenoxyethyl 4-Toluenesulfonate (*12*)

A solution of alcohol **11** (128 mg, 0.47 mmol) in pyridine (5 mL) was treated with *p*-toluenesulfonyl chloride (266 mg, 1.4 mmol) and the mixture was stirred at room temperature for 4 h. Then, 5% HCl (50 mL) was added and the reaction mixture was stirred for an additional hour. The mixture was extracted with methylene chloride (50 mL) and the organic layer was washed with 5% HCl (3 × 50 mL) and H_2O (3 × 50 mL). The organic phase was dried ($MgSO_4$) and the solvent was evaporated. The product was purified by column chromatography (silica gel) employing a mixture of hexane–EtOAc (19:1) as eluent to afford 171 mg of tosylate **12** (86% yield) as a colorless oil. R_f 0.32 (hexane–EtOAc, 7:3); ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.46 (s, 3H, C*H*$_3$), 4.01 (distorted t, J = 4.0 Hz, 2H, H-2), 4.10 (distorted t, J = 4.0 Hz, 2 H, H-1), 7.00 (m, 4H, aromatic protons), 7.30 (m, 1H), 7.46 (t, J = 8.2 Hz, 1H), 7.72 (t, J = 2.3 Hz, 1H, H-2″), 7.82–7.92 (m, 5H, aromatic protons).

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

Compound	*Trypanosoma cruzi* (amastigotes) ED_{50} (μM)	*Toxoplasma gondii* (tachyzoites) ED_{50} (μM)	Cytotoxicity on Vero cells ED_{50} (μM)
10	>10	3.28 ± 1.36	>50
13	5.2	2.03 ± 0.34	>50
21	6.1	3.81 ± 1.07	>100
22	4.5	6.7 ± 3.1	>50
25	>10	3.25% at 10 μM	>50
28	7.4 ± 0.45	7.5 ± 0.8	>50
36	11.2	3.95 ± 0.99	>50
37	10.1	2.93 ± 0.93	>50
WC-9	5.0 ± 1.1	4.8 ± 0.41	>50
Benznidazole	1.75 ± 0.48		–
Atovaquone		0.032 ± 0.019	–

[a] Data are from one experiment in triplicate, or expressed as means ± S.D. of two (*T. cruzi*, **28**) or three experiments (*T. cruzi*, benznidazole; *T. gondii*), each one in triplicate.

4.5. 4-(3-Nitrophenoxy)phenoxyethyl thiocyanate (*13*)

A solution of tosylate **12** (171 mg, 0.40 mmol) in anhydrous dimethylformamide (5 mL) was treated with potassium thiocyanate (155 mg, 1.6 mmol). The reaction mixture was heated at 100 °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 methylene chloride (2 × 30 mL) and the combined organic layers were washed with brine (5 × 30 mL) and water (2 × 30 mL). The solvent was dried ($MgSO_4$) and evaporated. The residue was purified by column chromatography (silica gel) eluting with hexane–EtOAc (9:1) to give 77 mg (61% yield) of pure compound **13** as a colorless oil; R_f 0.31 (hexane–EtOAc, 7:3); ^{1}H NMR (500.13 MHz, $CDCl_3$) δ 3.36 (t, J = 5.8 Hz, 2H, H-1), 4.34 (t, J = 5.8 Hz, 2H, H-2), 6.97 (d, J = 9.2 Hz, 2H, H-2′), 7.04 (d, J = 9.2 Hz, 2H, H-3′), 7.29 (ddd, J = 8.3, 2.5, 0.9 Hz, 1H, H-5″), 7.46 (t, J = 8.2 Hz, 1H, H-6″), 7.72 (t, J = 2.3 Hz, 1H, H-2″), 7.90 (ddd, J = 8.2, 2.2, 0.9 Hz, 1H, H-4″); ^{13}C NMR (125.77 MHz, $CDCl_3$) δ 33.3 (C-1), 66.4 (C-2), 111.7 (S*C*N), 111.8 (C-2″), 116.2 (C-2′), 117.2 (C-4″), 121.5 (C-3′), 123.4 (C-6″), 130.3 (C-5″), 149.2 (C-3″), 149.4 (C-4′), 155.0 (C-1′), 159.2 (C-1″). HRMS (ESI) calcd for $C_{15}H_{12}O_4N_2SNa$ $[M + Na]^+$ 339.0415; found 339.0419.

4.6. 4-Iodophenoxyethyl tetrahydro-2H-pyran-2-yl ether (*14*)

A solution of iodophenol (5.00 g, 22.7 mmol) in dimethyl sulfoxide (5 mL) was treated with potassium hydroxide (2.82 g, 50.3 mmol). The suspension was stirred for 30 min at room temperature. Then, bromoethyl tetrahydropyranyl ether (5.70 g, 27.3 mmol) was added; the reaction mixture was stirred at room temperature overnight. The mixture was partitioned between methylene chloride (30 mL) and water (30 mL). The aqueous phase was extracted with methylene chloride (2 × 70 mL). The combined organic layers were washed with a saturated solution of sodium chloride (2 × 100 mL) and dried ($MgSO_4$), and the solvent was evaporated. The product was purified by column chromatography (silica gel) eluting with hexane to afford 4.10 g (50% yield) of pure

compound **14** as a colorless oil: R_f 0.40 (hexane–EtOAc; 4:1); ^{1}H NMR (500.13 MHz, $CDCl_3$) δ 1.51–1.86 (m, 6H, H-3″, H-4″, H-5″), 3.53 (m, 1H, H-6‴$_a$), 3.80 (ddd, J = 11.2, 6.4, 4.1 Hz, 1H, H-6‴$_b$), 3.89 (ddd, J = 11.2, 8.1, 3.2 Hz, 1H, H-1$_a$), 4.04 (m, 1H, H-1$_b$), 4.11 (t, J = 3.8 Hz, 1H, H-2$_a$), 4.12 (t, J = 4.4 Hz, 1H, H-2$_b$), 4.69 (t, J = 3.7 Hz, 1H, H-2″), 6.71 (t, J = 9.2 Hz, 2H, H-2′), 7.54 (t, J = 9.2 Hz, 2H, H-3′); ^{13}C NMR (125.77 MHz, $CDCl_3$) δ 19.3 (C-4″), 25.4 (C-5″), 30.5 (C-3″), 62.2 (C-6″), 65.7 (C-1), 67.5 (C-2), 82.9 (C-4′), 99.0 (C-2″), 117.1 (C-2′), 138.1 (C-3′), 158.8 (C-1′).

4.7. 4-(4-Methoxyphenoxy)phenoxyethyl tetrahydro-2H-pyran-2-yl ether (**15**)

A mixture of *p*-methoxyphenol (217.3 mg, 1.75 mmol), compound **14** (508.0 mg, 1.46 mmol), copper(I) iodide (13.9 mg, 0.073 mmol), 2-picolinic acid, (18.0 mg, 0.15 mmol), and potassium phosphate tribasic (619.4 mg, 2.9 mmol) was treated as depicted for the preparation of **10**. Purification of the product by column chromatography (silica gel) eluting with hexane–EtOAc (19:1) afforded 211 mg (42% yield) of pure **15** as a colorless oil: R_f 0.54 (hexane–EtOAc); ^{1}H NMR (500.13 MHz, $CDCl_3$) δ 1.51–1.86 (m, 6H, H-3‴, H-4‴, H-5‴), 3.53 (m, 1H, H-6‴$_a$), 3.78 (s, 3H, OCH_3), 3.80 (ddd, J = 11.2, 6.5, 4.4 Hz, 1H, H-6‴$_b$), 3.90 (ddd, J = 11.4, 8.3, 3.1 Hz, 1H, H-1$_a$), 4.04 (m, 1H, H-1$_b$), 4.12 (m, 2H, H-2), 4.71 (t, J = 3.7 Hz, 1H, H-2″), 6.84 (t, J = 9.2 Hz, 2H, aromatic protons), 6.89 (m*AB*, 4H, aromatic protons), 6.91 (t, J = 9.2 Hz, 2H, aromatic protons); ^{13}C NMR (125.77 MHz, $CDCl_3$) δ 19.3 (C-4‴), 25.4 (C-5‴), 30.5 (C-3‴), 55.6 (OCH_3), 62.2 (C-6‴), 65.9 (C-1), 68.0 (C-2), 99.0 (C-2‴), 114.7 (C-3″), 115.7 (C-2′), 119.4 (C-3′), 119.5 (C-2″), 151.5 (C-4′), 151.7 (C-1″), 154.6 (C-1′), 155.3 (C-4″).

4.8. 4-(3-Methoxyphenoxy)phenoxyethyl tetrahydro-2H-pyran-2-yl ether (**16**)

A mixture of *m*-methoxyphenol (287.5 mg, 2.3 mmol), compound **14** (672.0 mg, 1.9 mmol), copper(I) iodide (18.4 mg, 0.010 mmol), 2-picolinic acid, (23.7 mg, 0.19 mmol), and potassium phosphate tribasic (819.4 mg, 3.9 mmol) was treated as depicted for the preparation of **10**. The crude was purified by column chromatography (silica gel) eluting with hexane–EtOAc to give 437 mg (66% yield) of pure compound **16** as a colorless oil: R_f 0.55 (hexane–EtOAc, 7:3); ^{1}H NMR (500.13 MHz, $CDCl_3$) δ 1.51–1.86 (m, 6H, H-3‴, H-4‴, H-5‴), 3.53 (m, 1H, H-6‴$_a$), 3.76 (s, 3H, OCH_3), 3.82 (ddd, J = 11.1, 6.3, 4.2 Hz, 1H, H-6‴$_b$), 3.91 (ddd, J = 11.2, 8.2, 3.1 Hz, 1H, H-1$_a$), 4.06 (m, 1H, H-1$_b$), 4.15 (m, 2H, H-2), 4.72 (t, J = 3.6 Hz, 1H, H-2″), 6.51 (m, 2H, aromatic protons), 6.59 (ddd, J = 8.2, 2.3, 1.0 Hz, 1H, aromatic proton), 6.91 (t, J = 9.2 Hz, 2H, aromatic protons), 6.98 (t, J = 9.2 Hz, 2H, aromatic protons), 7.18 (t, J = 8.5 Hz, 1H, aromatic proton); ^{13}C NMR (125.77 MHz, $CDCl_3$) δ 19.4 (C-4‴), 25.4 (C-5‴), 30.5 (C-3‴), 55.3 (OCH_3), 62.2 (C-6‴), 65.9 (C-1), 68.0 (C-2), 99.0 (C-2‴), 103.7 (C-2″), 108.0 (C-4″), 109.7 (C-6″), 115.8 (C-2′), 120.9 (C-3′), 130.0 (C-5″), 150.0 (C-4′), 155.0 (C-1′), 159.8 (C-1″), 160.9 (C-3″).

4.9. 4-(4-Methoxyphenoxy)phenoxyethanol (**17**)

A solution of **15** (234.9 mg, 0.68 mmol) in methanol (20 mL) was treated with pyridinium 4-toluensulfonate (30 mg). The reaction mixture was stirred at room temperature overnight and was quenched as described for the preparation of **11**. Evaporation of the solvent afforded 174.0 mg (98% yield) of alcohol **17** as white solid that was used in the next step without further purification: mp = 104–105 °C; R_f 0.19 (hexane–EtOAc); ^{1}H NMR (500.13 MHz, $CDCl_3$) δ 3.79 (s, 3H, OCH_3), 3.96 (m, 2H, H-1), 4.06 (m, 2H, H-2), 6.85–6.93 (m, 8H, aromatic protons); ^{13}C NMR (125.77 MHz, $CDCl_3$) δ 55.7 (OCH_3), 61.6 (C-1), 69.7 (C-2), 114.8 (C-3″), 115.6 (C-2′), 119.5 (C-3′), 119.7 (C-2″).

4.10. 4-(3-Methoxyphenoxy)phenoxyethanol (**18**)

To a solution of **16** (430.1 mg, 0.8 mmol) in methanol (20 mL) was treated with pyridinium 4-toluensulfonate (30 mg). The reaction mixture was stirred at room temperature overnight and was treated as depicted for the preparation of **11**. The product was purified by column chromatography (silica gel) eluting with hexane–EtOAc (17:3) to afford 240 mg (72% yield) of pure **18** as a colorless oil: R_f 0.18 (hexane–EtOAc, 7:3); ^{1}H NMR (500.13 MHz, $CDCl_3$) δ 3.77 (s, 3H, OCH_3), 3.97 (dist. t, J = 4.5 Hz, 2H, H-1), 4.08 (dist. t, J = 4.5 Hz, 2H, H-2), 6.51 (t, J = 2.1 Hz, 1H, H-2″), 6.52 (ddd, J = 8.3, 2.3, 0.9 Hz, 1H, H-6″), 6.60 (ddd, J = 8.3, 2.3, 0.9 Hz, 1H, H-4″), 6.90 (d, J = 9.1 Hz, 2H, H-2′), 6.99 (d, J = 9.2 Hz, 2H, H-3′), 7.19 (t, J = 8.5 Hz, 1H, H-5″); ^{13}C NMR (125.77 MHz, $CDCl_3$) δ 55.3 (OCH_3), 61.5 (C-1), 69.7 (C-2), 103.8 (C-2″), 108.1 (C-4″), 109.8 (C-6″), 115.6 (C-2′), 121.0 (C-3′), 130.0 (C-5″), 150.3 (C-4′), 155.0 (C-1′), 159.6 (C-1″), 160.9 (C-3″).

4.11. 4-(4-Methoxyphenoxy)phenoxyethyl 4-Toluenesulfonate (**19**)

To a solution of **17** (170 mg, 0.65 mmol) in pyridine (5 mL) was added *p*-toluenesulfonyl chloride (373.4 mg, 1.96 mmol) following the method of the preparation described for **12**. The product was purified by column chromatography (silica gel) eluting with hexane–EtOAc (9:1) to give 104 mg (39% yield) of pure **19** as a colorless oil: R_f 0.44 (hexane–EtOAc, 7:3); ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.45 (s, 3H, CH_3), 3.79 (s, 3H, OCH_3), 4.11 (m, 2H, H-1), 4.34 (m, 2H, H-2), 6.73 (d, J = 8.9 Hz, 2H), 6.89 (m, 7H, aromatic protons), 7.32 (d, J = 8.3 Hz, 2H, H-3‴), 7.82 (d, J = 8.3 Hz, 2H, H-2‴); HRMS (ESI) calcd for $C_{22}H_{22}O_6Na$ $[M + Na]^+$ 437.1035; found 437.1014.

4.12. 4-(3-Methoxyphenoxy)phenoxyethyl 4-Toluenesulfonate (**20**)

A solution of alcohol **18** (240 mg, 0.92 mmol) in pyridine (5 mL) was treated with *p*-toluenesulfonyl chloride (527.4 mg, 2.77 mmol) following a similar method as depicted for the preparation of **12**. Evaporation of the solvent afforded 269 mg (71% yield) of **20** as a white solid: mp = 77 °C; R_f 0.40 (hexane–EtOAc, 7:3); ^{1}H NMR (500.13 MHz, $CDCl_3$) δ 2.45 (s, 3H, CH_3), 3.77 (s, 3H, OCH_3), 4.14 (m, 2H, H-1), 4.36 (m, 2H, H-2), 6.50 (m, 2H, aromatic protons), 6.60 (m, 1H, aromatic proton), 6.77 (d, J = 9.1 Hz, 2H, H-3′), 6.94 (d, J = 9.0 Hz, 2H, H-2′), 7.18 (t, J = 8.5 Hz, 1H, aromatic proton), 7.85 (d, J = 8.3 Hz, 2H, H-3‴), 7.83 (d, J = 8.3 Hz, 2H, H-2‴); ^{13}C NMR (125.77 MHz, $CDCl_3$) δ 21.6 (CH_3), 55.3 (OCH_3), 66.0 (C-1), 68.1 (C-2), 103.8 (C-2″), 108.2 (C-4″), 109.9 (C-6″), 115.7 (C-2′), 120.9 (C-3′), 128.0 (C-2‴), 129.9(C-5″), 130.0 (C-3‴), 132.9 (C-4‴), 145.0 (C-1‴), 150.6 (C-4′), 154.3 (C-1′), 159.5 (C-1″), 160.9 (C-3″).

4.13. 4-(4-Methoxyphenoxy)phenoxyethyl thiocyanate (**21**)

A solution of tosylate **19** (50 mg, 0.12 mmol) in anhydrous dimethylformamide (5 mL) was treated with potassium thiocyanate (47 mg, 0.48 mmol). The product was purified by column chromatography (silica gel) eluting hexane–EtOAc (9:1) as eluent to afford 23 mg (64% yield) of pure **21** as a white solid: mp = 60–62 °C; ^{1}H NMR (500.13 MHz, $CDCl_3$) δ 3.32 (t, J = 5.8 Hz, 2H, H-1), 3.79 (s, 3H, OCH_3), 4.28 (t, J = 5.8 Hz, 2H, H-1), 6.87 (m, 4H, aromatic protons), 6.92 87 (m, 4H, aromatic protons); ^{13}C NMR (125.77 MHz, $CDCl_3$) δ 33.3 (C-1), 55.6 (OCH_3), 66.5 (C-2), 111.7 (S*C*N), 114.8 (C-3″), 115.4 (C-2′), 115.9 (C-3′), 119.4 (C-2″), 151.1 (C-4′), 152.7 (C-1″), 153.4

(C-1′), 155.5 (C-4″). HRMS (ESI) calcd for $C_{16}H_{15}O_3NSNa$ $[M + Na]^+$ 324.0670; found 324.0673.

4.14. 4-(3-Methoxyphenoxy)phenoxyethyl thiocyanate (**22**)

To a solution of tosylate **20** (269 mg, 0.65 mmol) in anhydrous dimethylformamide (5 mL) was added potassium thiocyanate (252.3 mg, 2.6 mmol) according to the general procedure. The crude was purified by column chromatography (silica gel) employing a mixture of hexane–EtOAc (7:3) as eluent to give 63.1 mg (32% yield) as a colorless oil: ^{1}H NMR (500.13 MHz, $CDCl_3$) δ 3.34 (t, J = 5.8 Hz, 2H, H-1), 3.77 (s, 3H, OCH_3), 4.30 (t, J = 5.7 Hz, 2H, H-2), 6.52 (m, 2H, aromatic protons), 6.61 (m, 1H, aromatic proton), 6.90 (d, J = 9.1 Hz, 2H, H-3′), 7.00 (d, J = 9.2 Hz, 2H, H-2′), 7.19 (t, J = 8.5 Hz, 1H, aromatic proton); ^{13}C NMR (125.77 MHz, $CDCl_3$) δ 33.3 (C-1), 55.3 (OCH_3), 66.4 (C-2), 103.9 (C-2″), 108.3 (C-4″), 109.9 (C-6″), 111.7 (SCN), 115.9 (C-2′), 120.9 (C-3′), 130.1 (C-5″), 150.9 (C-4′), 154.1 (C-1′), 159.4 (C-1″), 160.9 (C-3″). HRMS (ESI) calcd for $C_{16}H_{16}O_3NS$ $[M + H]^+$ 302.0845; found 302.0856.

4.15. 4-(Pyridin-3-yloxy)phenoxyethyl benzyl ether (**23**)

A mixture of 4-benzyloxyphenol (2.00 g, 10.0 mmol), 3-bromopyridine (1.58 g, 10.0 mmol), copper(I) iodide (95.2 mg, 0.5 mmol), 2-picolinic acid, (123 mg, 1.0 mmol), and potassium phosphate tribasic (4.25 g, 20 mmol). The mixture was reacted similarly as described for the preparation of **10**. The product was purified by column chromatography (silica gel) eluting with hexane–EtOAc (9:1) to afford 912 mg (33% yield) of pure **23** as a yellowish solid: mp = 64–65 °C; R_f 0.18 (hexane–EtOAc, 7:3); ^{1}H NMR (500.13 MHz, $CDCl_3$) δ 5.06 (s, 2H, H-1), 6.99 (m*AB*, 4H, aromatic protons), 7.27 (m, 2H, aromatic protons), 7.32–7.45 (m, 5H, aromatic protons), 8.32 (m, 1H, aromatic proton), 8.36 (m, 1H, aromatic proton); ^{13}C NMR (125.77 MHz, $CDCl_3$) δ 70.5 (C-1), 116.2 (C-2′), 120.8 (C-3′), 124.2 (C-6″), 124.8 (C-5″), 127.5 (C-2‴), 128.1 (C-4‴), 128.6 (C-3‴), 136.8 (C-1‴), 139.7 (C-2″), 142.9 (C-4″), 149.2 (C-4′), 155.2 (C-1′), 155.7 (C-1″).

4.16. 4-(Pyridin-3-yloxy)phenol (**24**)

A solution of **23** (907.6 g, 3.3 mmol) in ethyl acetate (30vmL) in the presence of 5% palladium on charcoal (30 mg) was treated with hydrogen at 3 atm. The reaction was stirred at room temperature for 2 h. The mixture was worked-up as described for the preparation of compound **9** to afford 582 mg (95% yield) of pure **24** as a white solid. This compound was used as such in the next step: mp = 134–136 °C; R_f 0.18 (hexane–EtOAc, 3:2).

4.17. 4-(Pyridin-3-yloxy)phenoxyethyl tetrahydro-2H-pyran-2-yl ether (**25**)

A solution of **24** (582 mg, 3.1 mmol) in dimethyl sulfoxide (5 mL) was treated with potassium hydroxide (698 mg, 12.4 mmol). The suspension was stirred for 30 min at room temperature. Then, bromoethyl tetrahydropyranyl ether (975 mg, 4.7 mmol) was added. The reaction mixture was treated as depicted for the preparation of **14**. The product was purified by column chromatography (silica gel) employing a mixture of hexane–EtOAc (4:1) as eluent to afford 457 mg (47% yield) of pure **25** as a colorless oil: R_f 0.33 (hexane–EtOAc, 1:1); ^{1}H NMR (500.13 MHz, $CDCl_3$) δ 1.50–1.88 (m, 6H, H-3‴, H-4‴, H-5‴), 3.54 (m, 1H, H-6‴$_a$), 3.82 (ddd, J = 11.2, 6.4, 4.2 Hz, 1H, H-6‴$_b$), 3.91 (ddd, J = 11.3, 8.4, 3.0 Hz, 1H, H-1$_a$), 4.06 (m, 1H, H-1$_b$), 4.15 (m, 2H, H-2), 4.72 (t, J = 3.6 Hz, 1H, H-2″), 6.94 (d, J = 9.5 Hz, 2H, H-3′), 6.98 (d, J = 9.2 Hz, 2H, H-2′), 7.21 (m, 2H, aromatic protons), 8.31 (m, 1H, aromatic proton), 8.36 (m, 1H, aromatic proton); ^{13}C NMR (50 MHz, $CDCl_3$) δ 19.4 (C-4‴), 25.4 (C-5‴), 30.5 (C-3‴), 62.2 (C-6‴), 65.8 (C-1), 68.0 (C-2), 99.0 (C-2‴), 116.1 (C-2′), 120.7 (C-3′), 124.2 (C-6″, C-5″), 140.3 (C-2″), 143.4 (C-4″), 149.3 (C-4′).

4.18. 4-(Pyridin-3-yloxy)phenoxyethanol (**26**)

A solution of compound **25** (457 mg, 1.45 mmol) in methanol (20 mL) was treated with 4-toluensulfonic acid (50 mg). The reaction mixture was stirred at room temperature overnight. After the usual treatment, the product was purified by column chromatography (silica gel) eluting with hexane–EtOAc (7:3) to afford 160 mg (47% yield) of pure **26** as a white solid: mp = 73–75 °C; R_f 0.09 (hexane–EtOAc, 1:1); ^{1}H NMR (500.13 MHz, $CDCl_3$) δ 3.98 (dist. t, J = 4.6 Hz, 2H, H-1), 4.09 (dist. t, J = 4.5 Hz, 2H, H-2), 6.94 (d, J = 9.2 Hz, 2H, H-3′), 7.00 (d, J = 9.2 Hz, 2H, H-2′), 7.24 (m, 2H, aromatic protons), 8.32 (m, 1H, aromatic proton), 8.36 (m, 1H, aromatic proton); ^{13}C NMR (125.77 MHz, $CDCl_3$) δ 61.5 (C-1), 69.7 (C-2), 115.9 (C-2′), 120.9 (C-3′), 124.1 (C-6″), 124.5 (C-5″), 140.1 (C-2″), 143.4 (C-4″), 149.5 (C-4′), 155.5 (C-1′, C-1″).

4.19. 4-(Pyridin-3-yloxy)phenoxyethyl 4-Toluenesulfonate (**27**)

A solution of alcohol **26** (154 mg, 0.67 mmol) in pyridine (5 mL) was treated with *p*-toluenesulfonyl chloride (380.9 mg, 2.0 mmol) and the mixture was stirred at room temperature for 6 h according to the method of preparation depicted for **12**. The product was purified by column chromatography eluting with a mixture of hexane–EtOAc (3:1) to give 118 mg (46% yield) of pure **27** as a colorless oil: R_f 0.25 (hexane–EtOAc, 2:3); ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.45 (s, 3H, CH_3), 4.15 (m, 2H, H-1), 4.37 (m, 2H, H-2), 6.80 (d, J = 9.0 Hz, 2H, H-2′) 6.96 (d, J = 9.2 Hz, 2H, H-3′), 7.23 (m, 2H, aromatic protons), 7.35 (d, J = 8.0 Hz, 2H, H-3‴), 7.83 (d, J = 8.0 Hz, 2H, H-2‴), 8.34 (m, 2H, aromatic protons).

4.20. 4-(Pyridin-3-yloxy)phenoxyethyl thiocyanate (**28**)

To a solution of **27** (118 mg, 0.31 mmol) in *N*,*N*-dimethylformamide (5 mL) was added potassium thiocyanate (118 mg, 1.2 mmol). The reaction mixture was treated according to the general procedure. The product was purified by column chromatography (silica gel) eluting with hexane–EtOAc (3:1) to afford 53.3 mg (63% yield) of **28** as a colorless oil: R_f 0.30 (hexane–EtOAc; 2:3); ^{1}H NMR (500.13 MHz, $CDCl_3$) δ 3.35 (t, J = 5.8 Hz, 2H, H-1), 4.32 (t, J = 5.8 Hz, 2H, H-2), 6.94 (d, J = 9.2 Hz, 2H, H-3′), 7.01 (d, J = 9.2 Hz, 2H, H-2′), 7.23 (m, 2H, aromatic protons), 8.24 (m, 1H, aromatic proton), 8.37 (m, 1H, aromatic proton); ^{13}C NMR (125.77 MHz, $CDCl_3$) δ 33.3 (C-1), 66.4 (C-2), 111.7 (SCN), 116.1 (C-2′), 120.8 (C-3′), 124.0 (C-6″), 124.4 (C-5″), 140.5 (C-2″), 143.8 (C-4″), 150.2 (C- 4′), 154.5 (C-1′), 154.7 (C-1″). HRMS (ESI) calcd for $C_{14}H_{13}O_2N_2S$ $[M + H]^+$ 273.0698; found 273.0702.

4.21. 2-(3-Iodophenoxy)ethyl tetrahydro-2H-pyran-2-yl ether (**29**)

A solution of 3-iodophenol (5 g, 227 mmol) in dimethyl sulfoxide (5 mL) was treated with potassium hydroxide (2.54 g, 454 mmol). The suspension was stirred for 30 min at room temperature. Then, bromoethyl tetrahydropyranyl ether (4.75 g, 227 mmol) was added; the reaction mixture was stirred at room temperature overnight. The mixture was partitioned between methylene chloride (30 mL) and water (30 mL). The aqueous phase was extracted with methylene chloride (2 × 70 mL). The combined organic layers were washed with a saturated solution

of sodium chloride (2 × 100 mL) and dried ($MgSO_4$), and the solvent was evaporated. The residue was purified by column chromatography (silica gel) eluting with hexane to afford 3.12 g (38% yield) of pure compound **29** as a colorless oil: R_f 0.54 (hexane–EtOAc, 4:1); ^{1}H NMR (500.13 MHz, $CDCl_3$) δ 1.54–1.68 (m, 4H, H-4″, H-5″), 1.76 (m, 1H, H-3″$_a$), 1.86 (m, 1H, H-3″$_b$), 3.53 (m, 1H, H-6″$_a$), 3.80 (ddd, J = 11.1, 6.5, 4.3 Hz, 1H, H-6″$_b$), 3.88 (ddd, J = 11.2, 8.2, 3.1 Hz, 1H, H-1$_a$), 4.06 (m, 1H, H-1$_b$), 4.15 (m, 1H, H-2), 4.70 (t, J = 3.6 Hz, 1H, H-2″), 6.90 (ddd, J = 8.3, 2.4, 0.7 Hz, 1H, H-6″), 6.99 (t, J = 8.0 Hz, 1H, H-5″), 7.28 (dt, J = 7.8, 1.0 Hz, 1H, H-4″), 7.30 (t, J = 1.9 Hz, 1H, H-2″); ^{13}C NMR (125.77 MHz, $CDCl_3$) δ 19.3 (C-4″), 25.4 (C-5″), 30.5 (C-3″), 62.2 (C-6″), 65.7 (C-1), 67.6 (C-2), 94.2 (C-3′), 99.0 (C-2″), 114.4 (C-6′), 123.9 (C-2′), 130.0 (C-4′), 130.9 (C-5′), 159.5 (C-1′).

4.22. 3-Phenoxyphenoxyethyl tetrahydro-2H-pyran-2-yl ether (**30**)

To a round bottom flask was added copper(I) iodide (21.9 mg, 0.11 mmol), 2-picolinic acid, (28.3 mg, 0.23 mmol), compound **29** (400 mg, 1.15 mmol), phenol (129 mg, 1.38 mmol) and potassium phosphate tribasic (487 mg, 2.3 mmol). The flask was evacuated and back-filled with argon. The evacuation/backfill sequence was repeated twice. Then, dimethyl sulfoxide was added (2.0 mL) and the reaction mixture was stirred vigorously at 80 °C for 24 h. The reaction mixture was cooled to room temperature and was partitioned between ethyl acetate (20 mL) and water (20 mL). The aqueous layer was extracted with ethyl acetate (2 × 20 mL). The combined organic layers were washed with brine (5 × 50 mL), dried ($MgSO_4$), and the solvent was evaporated. The residue was purified by column chromatography (silica gel) eluting with a mixture of hexane–EtOAc (97:3) to afford 200 mg (55% yield) of pure compound **30** as a colorless oil: ^{1}H NMR (500.13 MHz, $CDCl_3$) δ 1.49–1.84 (m, 6H, H-3‴, H-4‴, H-5‴), 3.52 (m, 1H, H-6‴$_a$), 3.79 (ddd, J = 11.1, 6.4, 4.2 Hz, 1H, H-6‴$_b$), 3.88 (ddd, J = 11.2, 8.3, 2.9 Hz, 1H, H-1$_a$), 4.03 (m, 1H, H-1$_b$), 4.12 (m, 2H, H-2), 4.69 (t, J = 3.6 Hz, 1H, H-2‴), 6.60 (m, 2H, Ph), 6.68 (m, 1H, Ph), 7.02 (d, J = 8.4 Hz, 1H, Ph), 7.10 (t, J = 7.5 Hz, 1H, Ph), 7.21 (t, J = 8.5 Hz, 1H, Ph), 7.33 (t, J = 8.0 Hz, 2H, Ph); ^{13}C NMR (125.77 MHz, $CDCl_3$) δ 19.3 (C-4‴), 25.4 (C-5‴), 30.5 (C-3‴), 62.2 (C-6‴), 65.7 (C-1), 67.5 (C-2), 99.0 (C-2‴), 105.6 (C-2′), 109.5 (C-6′), 111.2 (C-4′), 119.1 (C-2″), 123.5 (C-4″), 129.7 (C-3″), 130.0 (C-5′), 157.0 (C-1″), 158.4 (C-1′), 160.2 (C-4′) 2 HRMS (ESI) calcd for $C_{19}H_{22}O_4Na$ $[M + Na]^+$ 337.1416; found 337.1421.

4.23. 3-(4-Methoxyphenoxy)phenoxyethyl tetrahydro-2H-pyran-2-yl ether (**31**)

A mixture of copper(I) iodide (16 mg, 0.09 mmol, 10 mol%), 2-picolinic acid, **1** (21.2 mg, 0.17 mmol, 20 mol%), compound **29** (300 mg, 0.86 mmol), 3-methoxyphenol (128 mg, 1.03 mmol) and potassium phosphate tribasic (365 mg, 1.72 mmol) was treated as depicted for the preparation of compound **30**. After the usual worked-up, the product was purified by column chromatography (silica gel) eluting with a mixture of hexane–EtOAc (97:3) to afford 197 mg (67% yield) of pure compound **31** as a colorless oil: ^{1}H NMR (500.13 MHz, $CDCl_3$) δ 1.50–1.84 (m, 6H, H-3‴, H-4‴, H-5‴), 3.52 (m, 1H, H-6‴$_a$), 3.80 (m, 1H, H-6‴$_b$), 3.88 (m, 1H, H-1$_a$), 4.03 (m, 1H, H-1$_b$), 4.10 (m, 2H, H-2), 4.69 (t, J = 3.4 Hz, 1H, H-2‴), 6.51 (t, J = 2.4 Hz, 1H, H-2′), 6.55 (ddd, J = 8.4, 2.5, 0.5 Hz, 1H, H-6′), 6.61 (m, 1H, H-4′), 6.89 (d, J = 9.1 Hz, 2H, H-3″), 6.99 (d, J = 9.1 Hz, 2H, H-2″), 7.19 (t, J = 8.2 Hz, 1H, H-5′); ^{13}C NMR (125.77 MHz, $CDCl_3$) δ 19.4 (C-4‴), 25.4 (C-5‴), 30.5 (C-3‴), 55.6 (OCH_3), 61.4 (C-1), 62.2 (C-6‴), 69.2 (C-2), 99.0 (C-2‴), 104.2 (C-2′), 108.4 (C-6′), 110.2 (C-4′), 114.9 (C-3″), 121.0 (C-2″), 130.1 (C-5′), 149.7 (C-1″), 156.1 (C-4″), 159.7 (C-1′), 159.9 (C-3′). HRMS (ESI) calcd for $C_{20}H_{24}O_5Na$ $[M + Na]^+$ 367.1521; found 367.1527.

4.24. 3-Phenoxyphenoxyethanol (**32**)

To a solution of compound **30** (130 mg, 0.41 mmol) in methanol (20 mL) was added pyridinium *p*-toluenesulfonate (10 mg). The reaction mixture was stirred at room temperature for 14 h. The mixture was partitioned between water (50 mL) and methylene chloride (50 mL). The aqueous phase was extracted with methylene chloride (2 × 30 mL) and the combined organic layers were washed with brine (2 × 50 mL), dried ($MgSO_4$), and the solvent was evaporated to give 92 mg (89% yield) of pure alcohol **32** as a colorless oil that was use as such in the next step without further purification: R_f 0.18 (hexane–EtOAc, 4:1); ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.06 (br s, 1H, O*H*), 3.93 (dist. t, J = 3.9 Hz, 2H, H-1), 6.58 (t, J = 2.2 Hz, 1H, Ph), 4.04 (t, J = 7.9 Hz, 2H, H-2), 6.58 (t, J = 2.2 Hz, 1H, Ph), 6.61 (dd, J = 8.2, 2.0 Hz, 1H, Ph), 6.66 (dd, J = 8.3, 2.2 Hz, 1H, Ph), 7.02 (d, J = 8.3 Hz, 2H, Ph), 7.11 (m, 1H, Ph), 7.22 (t, J = 7.2 Hz, 1H, Ph), 7.34 (t, J = 7.9 Hz, 1H, Ph); ^{13}C NMR (125.77 MHz, $CDCl_3$) δ 61.4 (C-1), 69.2 (C-2), 105.4 (C-2′), 109.2 (C-6′), 111.3 (C-4′), 119.2 (C-2″), 123.5 (C-4″), 129.7 (C-3″), 130.2 (C-5′), 156.8 (C-1″), 158.6 (C-1′), 159.9 (C-4′). HRMS (ESI) calcd for $C_{14}H_{14}O_3Na$ $[M + Na]^+$ 253.0835; found 253.0844.

4.25. 3-(4-Methoxyphenoxy)phenoxyethanol (**33**)

To a solution of **31** (199 mg, 0.58 mmol) in methanol (30 mL) was added pyridinium *p*-toluenesulfonate (10 mg). The reaction mixture was stirred at room temperature for 14 h. The mixture was partitioned between water (70 mL) and methylene chloride (70 mL). The aqueous phase was extracted with methylene chloride (2 × 30 mL) and the combined organic layers were washed with brine (2 × 50 mL), dried ($MgSO_4$), and the solvent was evaporated to afford 145 mg (96% yield) of pure alcohol **33** as a colorless oil that was used as such in the next step: R_f 0.06 (hexane–EtOAc, 4:1); ^{1}H NMR (500.13 MHz, $CDCl_3$) δ 3.80 (s, 3H, OCH_3), 3.95 (dist. t, J = 4.2 Hz, 2H, H-1) 4.03 (dist. t, J = 4.6 Hz, 2H, H-2), 6.51 (t, J = 2.3 Hz, 1H, H-2′), 6.56 (ddd, J = 8.1, 2.2, 0.6 Hz, 1H, H-6′), 6.60 (ddd, J = 8.1, 2.2, 0.6 Hz, 1H, H-4′), 6.88 (d, J = 9.1 Hz, 2H, H-3″), 6.99 (d, J = 9.1 Hz, 2H, H-2″), 7.18 (t, J = 8.2 Hz, 1H, H-5′); ^{13}C NMR (125.77 MHz, $CDCl_3$) δ 55.6 (OCH_3), 61.3 (C-1), 69.2 (C-2), 104.2 (C-2′), 108.4 (C-6′), 110.1 (C-4′), 114.8 (C-3″), 121.0 (C-2″), 130.1 (C-5′), 149.7 (C-1″), 156.0 (C-4″), 159.83 (C-1′), 159.84 (C-3′). HRMS (ESI) calcd for $C_{15}H_{16}O_4Na$ $[M + Na]^+$ 283.0946; found 283.0948.

4.26. 3-Phenoxyphenoxyethyl 4-Toluenesulfonate (**34**)

To a solution of alcohol **32** (92.7 mg, 0.40 mmol) in pyridine (3 mL) cooled at 0 °C was added *p*-toluenesulfonyl chloride (229 mg, 1.20 mmol) portion wise, and the mixture was stirred at room temperature for 3 h. Then, 5% HCl (10 mL) was added and the reaction mixture was stirred for an additional hour. The mixture was extracted with methylene chloride (20 mL) and the organic layer was washed with 5% HCl (3 × 20 mL) and water (3 × 20 mL). The organic phase was dried ($MgSO_4$) and the solvent was evaporated. The residue was purified by column chromatography (silica gel) eluting with hexane to afford 99.2 mg (64% yield) of pure compound **34** as a colorless oil: R_f 0.47 (hexane–EtOAc, 1:1); ^{1}H NMR (500.13 MHz, $CDCl_3$) δ 2.46 (s, 3H, CH_3), 4.10 (m, 2H, H-2), 4.34 (m, 2H, H-1), 6.41 (t, J = 2.2 Hz, 1H, H-), 6.52 (dd, J = 8.3, 2.2 Hz, 1H, H-2′), 6.62 (dd, J = 8.2, 2.0 Hz, 1H, H-1), 7.00 (d, J = 8.4 Hz, 2H, H-3‴), 7.12 (t, J = 7.4 Hz, 1H), 7.18 (t, J = 8.2 Hz, 1H), 7.32 (m, 2H, aromatic protons), 7.80 (d, J = 8.2 Hz, 2H, H-2‴); ^{13}C NMR (125.77 MHz, $CDCl_3$) δ 21.6 (CH_3), 65.5 (C-2), 68.0 (C-1), 105.5 (C-2′), 109.1 (C-6′), 111.6 (C-4′), 119.1 (C-2″), 123.5 (C-4″), 128.0 (C-2‴), 129.76 (C-3″), 129.82 (C-3‴), 130.2 (C-5′), 132.8 (C-4‴), 145.0 (C-

1‴), 156.8 (C-1″), 158.5 (C1′), 159.3 (C-4′). HRMS (ESI) calcd for $C_{21}H_{20}O_5SNa$ $[M + Na]^+$ 407.0924; found 407.0914.

4.27. 3-(4-Methoxyphenoxy)phenoxyethyl 4-Toluenenesulfonate (**35**)

To a solution of alcohol **33** (128 mg, 0.49 mmol) in pyridine (3 mL) cooled at 0 °C was added *p*-toluenesulfonyl chloride (281 mg, 1.47 mmol) portion wise, and the mixture was stirred at room temperature for 4 h. The reaction was worked up as depicted for the preparation of **12**. The residue was purified by column chromatography (silica gel) eluting with hexane to afford 94 mg (46% yield) of pure compound **35** as a colorless oil: R_f 0.10 (hexane–EtOAc, 4:1); ^{1}H NMR (500.13 Hz, $CDCl_3$) δ 2.44 (s, 3H, C*H*$_3$), 3.82 (s, 3H, OC*H*$_3$), 4.09 (m, 2H, H-1, H-1), 4.33 (m, 2H, H-2), 6.36 (t, J = 2.3 Hz, 1H, H-2′), 6.46 (dd, J = 8.3, 2.3 Hz, 1H, H-4′), 6.53 (dd, J = 8.2, 2.2 Hz, 1H, H-6′), 6.89 (d, J = 9.0 Hz, 2H, H-3″), 6.97 (d, J = 9.0 Hz, 2H, H-2″), 7.15 (t, J = 8.2 Hz, 1H, H-5′), 7.32 (d, J = 8.4 Hz, 2H, H-3‴), 7.80 (d, J = 8.2 Hz, 2H, H-2‴); ^{13}C NMR (125.77 MHz, $CDCl_3$) δ 21.6 (*C*H$_3$), 55.6 (O*C*H$_3$), 65.4 (C-2), 68.0 (C-1), 104.3 (C-2′), 108.3 (C-6′), 110.4 (C-4′), 114.9 (C-3″), 121.0 (C-2″), 128.0 (C-2‴), 129.8 (C-3‴), 130.1 (C-5′), 132.8 (C-4‴), 145.0 (C-4″), 149.7 (C-1″), 156.1 (C-4″), 159.2 (C-1′), 159.8 (C-3′). HRMS (ESI) calcd for $C_{22}H_{22}O_6SNa$ $[M + Na]^+$ 437.1035; found 437.1010.

4.28. 3-Phenoxyphenoxyethyl thiocyanate (**36**)

A solution of tosylate **34** (99 mg, 0.24 mmol) in anhydrous *N*,*N*-dimethylformamide (3 mL) was treated with potassium thiocyanate (125 mg, 1.29 mmol) according to the general procedure. The product was purified by column chromatography (silica gel) using a mixture of hexane–EtOAc (9:1) as eluent to give 45 mg (64% yield) of pure thiocyanate **36** as a colorless oil: ^{1}H NMR (500.13 MHz, $CDCl_3$) δ 3.32 (t, J = 5.9 Hz, 2H, H-1), 4.28 (t, J = 5.8 Hz, 2H, H-2), 6.58 (t, J = 2.3 Hz, 1H), 6.65 (m, 2H, Ph), 7.03 (dd, J = 8.7, 1.0 Hz, 2H, Ph), 7.13 (tt, J = 7.4, 1.0 Hz, 1H, Ph), 7.23 (d, J = 8.2 Hz, 2H, Ph), 7.35 (dd, J = 8.6, 7.4 Hz, 2H, Ph); ^{13}C NMR (125.77 MHz, $CDCl_3$) δ 33.2 (C-1), 65.8 (C-2), 105.5 (C-2′), 109.2 (C-6′), 111.7 (*S*C*N*), 111.9 (C-4′), 119.2 (C-2″), 123.6 (C-4″), 129.8 (C-3″), 130.4 (C-5′), 156.7 (C-1″), 158.7 (C-1′), 159.1 (C-4′). HRMS (ESI) calcd for $C_{15}H_{13}O_2NSNa$ $[M + Na]^+$ 294.0559; found 294.0553.

4.29. 3-(4-Methoxyphenoxy)phenoxyethyl thiocyanate (**37**)

To a solution of tosylate **35** (87 mg, 0.22 mmol) in anhydrous *N*,*N*-dimethylformamide (3 mL) was added potassium thiocyanate (105 mg, 1.08 mmol) according to the general procedure. The residue was purified by column chromatography (silica gel) using a mixture of hexane–EtOAc (9:1) as eluent to afford 43 mg (68% yield) of pure compound **37** as a colorless oil: R_f 0.58 (hexane–EtOAc, 4:1); ^{1}H NMR (500.13 MHz, $CDCl_3$) δ 3.31 (t, J = 5.8 Hz, 2H, H-1), 3.81 (s, 3H, OC*H*$_3$), 4.27 (t, J = 5.8 Hz, 2H, H-2), 6.51 (t, J = 2.4 Hz, 1H, H-2′), 6.57 (ddd, J = 8.2, 2.3, 0.8 Hz, 1H, H-4′), 6.60 (ddd, J = 8.2, 2.3, 0.8 Hz, 1H, H-6′), 6.89 (d, J = 9.1 Hz, 2H, H-3″), 6.99 (d, J = 9.2 Hz, 2H, H-2″), 7.20 (*t*, J = 8.2 Hz, 1H, H-5′); ^{13}C NMR (125.77 MHz, $CDCl_3$) δ 33.3 (C-1), 55.6 (O*C*H$_3$), 65.8 (C-2), 104.3 (C-2′), 108.4 (C-6′), 110.4 (C-4′), 114.9 (C-3″), 121.1 (C-2″), 130.3 (C-5′), 149.6 (C-1″), 156.2 (C-1′) 159.0 (C-4′). HRMS (ESI) calcd for $C_{16}H_{15}O_3NSNa$ $[M + Na]^+$ 324.0670; found 324.0674.

4.30. Drug screening

4.30.1. T. cruzi amastigote assays

Gamma-irradiated (2000 Rads) Vero cells (3.4×10^4 cells/well) were seeded in 96 well plates (black, clear bottom plates from Greiner Bio-One) in 100 μL RPMI media (Sigma) with 10% FBS. Plates were incubated overnight at 35 °C and 7% CO_2. After overnight incubation, Vero cells were challenged with 3.4×10^5 trypomastigotes/well (CL strain overexpressing a tdTomato red fluorescent protein) in 50 μL volume and incubated for 5 h at 35 °C and 7% CO_2. After infection, cells were washed once with Hanks solution (150 μL/well) to eliminate any extracellular parasites and compounds were added in serial dilutions in RPMI media in 150 μL volumes. Each dilution was tested in quadruplicate. Each plate also contained controls with host cells and no parasites (for background check), and controls with parasites and no drugs (positive control). Drugs were tested on *T. cruzi* at 5 different concentrations up to a maximum of 20 or 25 μM. For each set of experiments, benznidazole was also used as a positive control. After drug addition, plates were incubated at 35 °C and 7% CO_2. At day 3 post-infection, plates were assayed for fluorescence [43]. IC_{50} values were determined by non-linear regression analysis using SigmaPlot.

4.30.2. T. gondii tachyzoites assays

Experiments on *T. gondii* tachyzoites were carried out as described previously [44] using *T. gondii* tachyzoites expressing red fluorescent protein [45]. Cells were routinely maintained in hTERT cells grown in High Glucose Dulbecco's modified Eagle's medium (DMEM-HG) supplemented with 1% fetal bovine serum, 2 mM glutamine, 1 mM pyruvate, at 37 °C in a humid 5% CO_2 atmosphere. Confluent monolayers grown in 96-well black plates with optical bottoms (black, clear bottom plates from Greiner Bio-One) were used and drugs dissolved in the same medium and serially diluted in the plates. Freshly isolated tachyzoites were filtered through a 3 μm filter and passed through a 25 gauge needle, before use. The cultures were inoculated with 4×10^4 achyzoites/well in the same media. The plates were incubated at 37 °C and read daily in a Molecular Devices fluorescence plate reader. To preserve sterility the plates were read with covered lids, and both excitation (544 nm) and emission (590 nm) were read from the bottom [45]. For the calculation of the EC_{50}, the percent of growth inhibition was plotted as a function of drug concentration by fitting the values to the function: $I = I_{max}C/(EC_{50} + C)$, where I is the percent inhibition, I_{max} = 100% inhibition, C is the concentration of the inhibitor, and EC_{50} is the concentration for 50% growth inhibition. There was no evident cytotoxicity on the host cells with any of the drugs tested (visual assay).

4.31. Cytotoxicity for vero cells

The cytotoxicity was tested using the Alamar Blue™ assay as described by Recher et al., 2013 [46].

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2013.09.009.

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