Design and Synthesis of Aryloxyethyl Thiocyanate Derivatives as Potent Inhibitors of Trypanosoma cruzi Proliferation

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As a part of our project directed at the search of new chemotherapeutic agents against American trypanosomiasis (Chagas’ disease), several drugs possessing the 4-phenoxyphenoxy skeleton and other closely related structures employing the thiocyanate moiety as polar end group were designed, synthesized, and evaluated as antiproliferative agents against Trypanosoma cruzi, the parasite responsible for this disease. These thiocyanate analogues were envisioned bearing in mind the potent activity shown by 4-phenoxyphenoxyethyl thiocyanate (compound 8) taken as lead drug. This compound had previously proved to be an extremely active growth inhibitor against T. cruzi with IC₅₀ values ranging from the very low micromolar level in epimastigotes to the low nanomolar level in the intracellular form of the parasite. Of the designed compounds, the ethyl thiocyanate drugs connected to nonpolar skeletons, namely, arylthio, 2,4-dichlorophenoxy, ortho-substituted aryloxy, and 2-methyl-4-phenoxyphenoxy (compounds 15, 34, 47, 52, 72, respectively), were shown to be very potent antireplicative agents against T. cruzi. On the other hand, conformationally restricted analogues as well as branched derivatives at the aliphatic side chain were shown to be moderately active against T. cruzi growth. The biological activity of drugs bearing the thiocyanate group correlated quite well with the activity exhibited by their normal precursors, the tetrahydropyranyl ether derivatives, when bonded to the same nonpolar skeleton. Compounds having the tetrahydropyranyl moiety as polar end were proportionally much less active than sulfur-containing derivatives in all cases. Drugs 47 and 72 also resulted to be very active against the amastigote form of the parasite growing in myoblasts; however, they were slightly less active than the lead drug 8. On the other hand, compounds 34 and 52 were almost devoid of activity against myoblasts. Surprisingly, the dithio derivative 15 was toxic for myoblasts.

Introduction

American trypanosomiasis (Chagas’ disease), caused by the kinetoplastid protozoon Trypanosoma cruzi, is considered by the World Health Organization to be one of the major tropical parasitic diseases worldwide together with malaria and schistosomiasis. This major health problem afflicts 16–18 million persons in Latin America, who are infected with T. cruzi.1 At the present time, it was calculated that around of 2–3 million people present the typical symptoms that characterize the chronic stage of American trypanosomiasis producing 45 000 deaths yearly.2 This illness is transmitted in rural areas to humans and other mammals by Reduviid bugs, for instance Rhodnius prolixus and Triatoma infestans,3,4 as a consequence of the blood-sucking activity of Chagas’ disease vectors on mammals when feeding in a cyclic process. The parasite occurs in three main morphological forms in a complex life cycle. It multiplies within the crop and midgut of Chagas’ disease vectors as the epimastigote form, and it is released with the insect excrements as the nondividing highly infective metacyclic trypomastigotes that invade mammalian tissues via wounds provoked by blood-sucking action. The parasite proliferates intracellularly as the amastigote, the clinically more important form of the parasite, which is released as the nondividing bloodstream trypomastigote forms that invade other tissues.3,4

In the past few years a tremendous impetus in the study of T. cruzi biochemistry and physiology has been observed.5 As a result of these studies, several crucial enzymes for parasite survival and not present in the host have been identified as potential targets for the design of new drugs.6–11 However, the existing chemotherapy to control this parasitic infection is based on old and quite unspecific drugs associated with long-term treatments and severe side effects. Certainly, the only two drugs currently in use for clinical treatment of this disease, nifurtimox (4-(5-nitrofurfurylidene)amino)-3-methylthiomorpholine 1,1-dioxide) and benznidazole (N-benzyl-2-nitro-1-imidazolacetamide), are able to cause negativization of parasitemia and serology in most of the cases.12,13 However, they are not specific enough to all T. cruzi strains to warrant complete cure and hence the divergence of efficacy observed for both drugs in different endemic areas.14–17 Due to the chemotherapy

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drawbacks, there is significant interest in developing novel chemotherapeutic approaches against Chagas’ disease based on unique aspects of T. cruzi structure and metabolism. Moreover, the chronic stage of this disease leads to irreversible cardiac and digestive disorders and is the leading cause of heart disease in Latin America with close to 90 million people at risk. Many chagasic patients die from heart failure associated with cardiomyopathy during the chronic phase of the disease, while in the acute phase myocarditis occurs in near 60% of patients with an estimated 9% of mortality occurring in endemic areas. In addition, the main way of transmission in large urban centers takes place by transfusion of infected blood or through the placenta.

This kind of mechanism is responsible for the occurrence of Chagas’ disease in countries where this illness is not endemic. In the past few years, this illness has been encountered, in the United States, as a consequence of vector treated with this compound was less susceptible to natural infection with T. cruzi than untreated controls. These interesting results led to evaluate several modified structures, which had previously shown to be active on the Chagas’ disease vector T. cruzi epimastigotes are treated with the thiocyanate derivative 8, an accumulation of low molecular weight metabolites from mevalonate to squaleone is observed (Chart 3).

We have reported that several compounds structurally related to the well-known insect growth regulator fenoxycarb (N-(2-{[4-(phenoxypyryl)ethyl]ethyl carbamate}) are very active trypanostatic agents. In fact, slight modifications on the fenoxycarb side chain give drugs 7 and 8 and other closely related compounds, which are shown to be potent inhibitors against T. cruzi growth. The molecule of fenoxycarb had been taken as lead structure on the basis that Chagas’ disease vector treated with this compound was less susceptible to natural infection with T. cruzi than untreated controls. These interesting results led to evaluate several modified structures, which had previously shown to be active on the Chagas’ disease vector Triatoma infestans, against the parasite establishing a good correlation between trypanostatic activity and juvenile hormone action and suggesting a mechanism of infection control. There is strong evidence that the target of 4-phenoxypyryl derivatives is the ergosterol biosynthetic pathway. When T. cruzi epimastigotes are treated with the thiocyanate derivative 8, an accumulation of low molecular weight metabolites from mevalonate to squaleone is observed (Chart 3).

Compounds 7 and 8 and other sulfur-containing derivatives were also effective agents against amastigotes, the clinically more relevant form of the parasite, and as observed for other sterol biosynthesis inhibitors, they were devoid of capacity to eradicate the nondividing highly infective trypanomastigotes.

Bearing in mind the ultrapotent activity exhibited by drug 8 (4 times more active than nifurtimox in epimastigotes and significantly more active in amastigotes, under the same assays conditions), a new set of related compounds having the aryloxyethyl thiocyanate moiety
were straightforwardly prepared from 4-bromodiphenyl ether with lithium aluminum hydride, produced the reagent that, on reaction with sulfur followed by reduction, exhibited marked effect in the biological action producing moderate active drugs. The introduction of a sulfur atom at the polar extreme in a series of 4-phenoxyphenoxyethyl derivatives and closely related compounds gives rise to extremely potent inhibitors against T. cruzi proliferation. The replacement of this sulfur atom by an oxygen atom brings about a dramatic impairing in the biological activity. On the other hand, the 4-phenoxyphenoxy unit is also very important to maintain high inhibitory values against T. cruzi growth, because replacement of the terminal phenoxy group by methoxy or benzoyl groups has given rise to the most active compounds related to drug 7 are the common intermediate for the preparation of thiocyanates, so it will be of interest to compare their biological activity with the final products, that is, the thiocyanate-containing drugs.

**Rationale**

The design of sulfur-containing derivatives and closely related compounds against T. cruzi has included the potential activity shown by the lead structure 8. The incorporation of the thiocyanate moiety at the terminal aliphatic chain of 4-phenoxyphenoxy derivatives and closely related compounds gives rise to potent growth inhibitors of T. cruzi. Therefore a new set of related compounds possessing the arylsulfonamido thiocyanate moieties was designed, prepared, and evaluated against the epimastigote forms of T. cruzi which is the preferred form to test new drugs.

Bearing in mind the unusual biological activity exhibited by sulfur-containing derivatives at C-1, it was likely that minor modifications in the vicinity of this position would have a strong effect on the biological activity. The replacement of the oxygen atom between the phenyl group and the aliphatic side chain (C-2) by other heteroatoms (sulfur or nitrogen) was the first modification considered. The designed drugs 11 and 15 were straightforwardly prepared from 4-bromodiphenyl ether (compound 9). This compound treated with metallic magnesium gave rise to the respective Grignard reagent that, on reaction with sulfur followed by reduction with lithium aluminum hydride, produced the respective thiophenol derivative 10 in theoretical yield. Compound 10 was transformed into the tetrahydro-2H-pyran-2-yl ether in a suspension of potassium hydroxide in dimethyl sulfoxide, via a modified Williamson procedure. Cleavage of the tetrahydro-2H-pyranyl group of 11 with pyridinium p-toluenesulfonate produced alcohol 12, which after treatment with tosyl chloride gave tosylate 13 and the undesired chloride derivative (compound 14) as a side product. Tosylate 13 reacted with potassium thiocyanate in N,N-dimethylformamide at 100 °C to yield the desired thiocyanate derivative 15. The nitrogen-containing derivatives (compounds 18, 24, and 25), replacing the oxygen atom at C-1 by a nitrogen atom, were prepared following a similar synthetic approach employing 4-phenoxyaniline (16) as starting material. Attempts to alkylate the nitrogen atom of 16 with 2-bromoethyl tetrahydro-2H-pyran-2-yl ether were unsuccessful; however, the acetylthio derivative 17, with a lower pKa, was easily N-alkylated by treatment with 2-bromoethyl tetrahydro-2H-pyran-2-yl ether to afford compound 18 in almost quantitative yield. Removal of tetrahydro-2H-pyran group with pyridinium p-toluenesulfonate gave alcohol 19. The normal precursor to obtain thiocyanate derivative was the corresponding tosylate because a good leaving group at C-1 has to be present; nonetheless an alkyl halide could be an alternate leaving group. Although 19 could not be tosylated under the usual conditions, this compound was transformed into the respective chloride (compound 20) by treatment with triphenylphosphine in carbon tetrachloride. Surprisingly, when 20 was treated with potassium thiocyanate in dimethylformamide it afforded acetate 21 instead of the expected thiocyanate derivative 25. The formation of compound 21 can be rationalized by the anionic mechanism of the neighbor acetate group. Hydrolysis of 21 with potassium carbonate led to 22 that reacted with triphenylphosphine in carbon tetrachloride to give 23 in good yield. Nucleophilic displacement with potassium thiocyanate afforded 24. As preliminary biological assays had indicated that 18 exhibited similar inhibitory action as the parent drug 8, it was of interest to prepare the acetylated analogue of 24. Therefore, compound 24 reacted with acetic anhydride in pyridine to yield 25 (Scheme 1).

As a second variation, the replacement of the ethoxy unit in the lead drug 8 by a methylene group to give thiocyanate 29 was considered. This drug was easily prepared from 4-phenoxybenzaldehyde (compound 26). On reaction with sodium borohydride, 26 was converted into the alcohol 27, which after treatment with triphenylphosphine in carbon tetrachloride–acetonitrile yielded the chloride 28. Compound 28 reacted with potassium thiocyanate to yield the benzyl thiocyanate 29. To correlate the biological activity of drug 29 with the tetrahydro-2H-pyranyl ether analogue, compound 30 was easily prepared by treating alcohol 27 with 3,4-dihydro-2H-pyran in the presence of pyridinium p-toluenesulfonate (Scheme 2).

The design of sulfur-containing derivatives and closely related compounds against T. cruzi has included the potential activity shown by the lead structure 8. The incorporation of the thiocyanate moiety at the terminal aliphatic chain of 4-phenoxyphenoxy derivatives and closely related compounds gives rise to potent growth inhibitors of T. cruzi. Therefore a new set of related compounds possessing the arylsulfonamido thiocyanate moieties was designed, prepared, and evaluated against the epimastigote forms of T. cruzi which is the preferred form to test new drugs.

Bearing in mind the unusual biological activity exhibited by sulfur-containing derivatives at C-1, it was likely that minor modifications in the vicinity of this position would have a strong effect on the biological activity. The replacement of the oxygen atom between the phenyl group and the aliphatic side chain (C-2) by other heteroatoms (sulfur or nitrogen) was the first modification considered. The designed drugs 11 and 15 were straightforwardly prepared from 4-bromodiphenyl ether (compound 9). This compound treated with metallic magnesium gave rise to the respective Grignard reagent that, on reaction with sulfur followed by reduction with lithium aluminum hydride, produced the respective thiophenol derivative 10 in theoretical yield. Compound 10 was transformed into the tetrahydro-2H-pyran-2-yl ether in a suspension of potassium hydroxide in dimethyl sulfoxide, via a modified Williamson procedure. Cleavage of the tetrahydro-2H-pyranyl group of 11 with pyridinium p-toluenesulfonate produced alcohol 12, which after treatment with tosyl chloride gave tosylate 13 and the undesired chloride derivative (compound 14) as a side product. Tosylate 13 reacted with potassium thiocyanate in N,N-dimethylformamide at 100 °C to yield the desired thiocyanate derivative 15. The nitrogen-containing derivatives (compounds 18, 24, and 25), replacing the oxygen atom at C-1 by a nitrogen atom, were prepared following a similar synthetic approach employing 4-phenoxyaniline (16) as starting material. Attempts to alkylate the nitrogen atom of 16 with 2-bromoethyl tetrahydro-2H-pyran-2-yl ether were unsuccessful; however, the acetylthio derivative 17, with a lower pKa, was easily N-alkylated by treatment with 2-bromoethyl tetrahydro-2H-pyran-2-yl ether to afford compound 18 in almost quantitative yield. Removal of tetrahydro-2H-pyran group with pyridinium p-toluenesulfonate gave alcohol 19. The normal precursor to obtain thiocyanate derivative was the corresponding tosylate because a good leaving group at C-1 has to be present; nonetheless an alkyl halide could be an alternate leaving group. Although 19 could not be tosylated under the usual conditions, this compound was transformed into the respective chloride (compound 20) by treatment with triphenylphosphine in carbon tetrachloride. Surprisingly, when 20 was treated with potassium thiocyanate in dimethylformamide it afforded acetate 21 instead of the expected thiocyanate derivative 25. The formation of compound 21 can be rationalized by the anionic mechanism of the neighbor acetate group. Hydrolysis of 21 with potassium carbonate led to 22 that reacted with triphenylphosphine in carbon tetrachloride to give 23 in good yield. Nucleophilic displacement with potassium thiocyanate afforded 24. As preliminary biological assays had indicated that 18 exhibited similar inhibitory action as the parent drug 8, it was of interest to prepare the acetylated analogue of 24. Therefore, compound 24 reacted with acetic anhydride in pyridine to yield 25 (Scheme 1).

As a second variation, the replacement of the ethoxy unit in the lead drug 8 by a methylene group to give thiocyanate 29 was considered. This drug was easily prepared from 4-phenoxybenzaldehyde (compound 26). On reaction with sodium borohydride, 26 was converted into the alcohol 27, which after treatment with triphenylphosphine in carbon tetrachloride–acetonitrile yielded the chloride 28. Compound 28 reacted with potassium thiocyanate to yield the benzyl thiocyanate 29. To correlate the biological activity of drug 29 with the tetrahydro-2H-pyranyl ether analogue, compound 30 was easily prepared by treating alcohol 27 with 3,4-dihydro-2H-pyran in the presence of pyridinium p-toluenesulfonate (Scheme 2).

Previous biological assays had indicated that the tetrahydro-2H-pyranyl ether 31, in which the aromatic skeleton was a 2,4-dichlorophenyl group instead of a 4-phenoxyphenyl moiety, was 2-fold more active than its analogue 7. Then, it was interesting to prepare the respective thiocyanate derivative from the readily available compound 31, which after hydrolysis of the tetrahydro-2H-pyran group followed by treatment with tosyl chloride and further nucleophilic attack of potassium thiocyanate led to drug 34. To study the influence of
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**Scheme 1**

![Scheme 1](image)

*Reagents: (a) i. Mg/I₂, THF, 65 °C, 15 min, then rt, ii. S₂, 3 h, rt, iii. LiAlH₄, rt, 30 min (100%); (b) BrCH₂CH₂OTHP, KOH/DMSO, rt, 16 h (43% for 11, 90% for 18); (c) PPTs, MeOH, rt, (78% for 12, 73% for 19); (d) CITS/py, rt, 4 h (85% for 13, 9% for 14); (e) KSCN, DMF, 100 °C, 5 h (100% for 15, 95% for 21, 39% for 24, 13% for 29); (f) AcO/py, rt, 16 h (98% for 17, 82% for 25); (g) Ph₃P/Cl₂C, MeCN, rt, overnight (51% for 20, 70% for 23); (h) K₂CO₃, MeOH–H₂O, rt, 3 h; (i) NaBH₄, EtOH, rt, 2 h (84%); (j) PPTs, DHP, Cl₂CH₂, rt, (100%).

**Scheme 2**

![Scheme 2](image)

*Reagents: (a) BrCH₂CH₂OTHP, KOH/DMSO, rt, overnight (77%); (b) PPTs, MeOH, rt, (43% for 32, 78% for 37); (c) CITS/py, rt, 4 h (53% for 33, 92% for 38); (d) KSCN, DMF, 100 °C, 5 h (70% for 34, 63% for 39). The chlorine atom at C-2′ position on biological activity, the 4-phenyl group was envisioned. Therefore, employing 4-chlorophenol (compound 35) as starting material, this compound was transformed into tetrahydropropyl derivative 36, which following a similar protocol as described for 34 was converted into thiocyanate 39 in good yield (Scheme 2).

We have recently demonstrated that the substitution of an ortho hydrogen atom of 4-phenoxyphenol by different groups exhibited a marked influence on biological activity. It has been found that the inhibitory potency increases as the substituent size increases. Then, it was reasonable to consider that the replacement in our lead drug 8 of the hydrogen atom at the C-2′ position by different halogen atoms would lead to more active drugs than 8. For the above reasons, 4-phenoxyphenoxynethyl thiocyanates possessing chlorine, bromine, and iodine at C-2′ (drugs 43, 47, and 52, respectively) were designed and synthesized. Consequently, using the halogen-containing derivatives 2-chloro-4-phenoxyphenoxethyl and 2-bromo-4-phenoxyphenoxethyl tetrahydropropyl ethers (compounds 40 and 44, respectively) as starting materials, these compounds were converted into the desired thiocyanate derivatives 43 and 47 following a similar synthetic approach as depicted for compound 15. The iodine derivative 52 was prepared from iodophenol 48, and this compound was converted into the tetrahydropropyl derivative 49, which was transformed into the desired drug employing a similar method for the preparation of 15 (Scheme 3).

Since halogen derivatives were very promising drugs it was decided to introduce a halogen atom at the meta position (C-3). In this case, the designed drug was a simplified model of aryloxyethyl derivatives due to synthetic difficulties to carry out a substitution in 4-phenoxyphenoy derivatives at this site. m-Bromophenol (53) was used as starting material. Following the usual synthetic methodology, 53 reacted with 2-bromoethyl tetrahydro-2H-pyran-2-yl ether to give 54, which after cleavage of the protective group, tosylation and Sn₂ reaction with the thiocyanate ion produced drug 57 (Scheme 4).

To study the influence of the relative spatial alignment of the phenyl groups on the biological activity, it was considered that the replacement of the hydrogen atom at the ortho position in the B ring (C-2′) would drive the phenyl groups to adopt a special conformation by van der Waals interactions. The synthesis of these kinds of 4-phenoxyphenoxethyl derivatives was not direct because the C-2′ is not activated for an electrophilic aromatic substitution. Therefore, the required phenyl intermediates (compounds 60, 68, and 76, respectively) to prepare the designed drugs were synthesized employing a nuleophilic aromatic substitution, an Ullmann
coupling reaction in this case, as the key step. Thus, 3-bromophenol (compound 48) was condensed with 4-nitrochlorobenzene in the presence of cuprous chloride to afford the nitro derivative 58 in good yield. This compound was reduced by catalytic hydrogenation employing palladium on charcoal as catalyst to yield the aniline 59 in very good yield. On reaction with sodium nitrite in sulfuric acid in the presence of urea, 59 was transformed into the phenol 60. This modified Sandmeyer protocol occurred in moderate yield. Following the usual synthetic procedures the intermediate 60 led to compound 61 by treatment with 2-bromoethyl tetrahydro-2H-pyran-2-yl ether, which after tetrahydropyranyl cleavage, followed by tosylation and further nucleophilic displacement with potassium thiocyanate produced the desired thiocyanate 64 in good yield. The use of a group bigger than a bromine atom to replace the H-2′, namely a methyl unit, would give rise to stronger van der Waals forces than those present in compound 64 and its precursors. For that reason, it is quite reasonable to assume that the increment of the substituent size will lead to an increase in the torsion angles of C7′-C1′-O1″-C4″ that is, a replacement out of the plane of one phenyl group with respect to the other one. Therefore, drugs 69 and 72 were synthesized starting from o-cresol via the phenol intermediate 70 employing the same synthetic strategy as depicted for the bromine derivatives 61 and 64. Finally, the introduction of an extra methyl group at C-6′ was also considered in order to bring about strong van der Waals forces. Then, the preparation of compound 77 was successfully carried out employing 2,6-dimethylphenol as starting material; however, the respective Sandmeyer-type reaction to produce the corresponding phenol intermediate was not satisfactory in terms of the yield (Scheme 5).

Another interesting structural variation was the preparation of conformationally constrained 4-phenoxypyran derivatives in order to investigate the influence of a specific restricted spatial disposition of both phenyl groups on biological activity. The skeleton of xanthone (compound 78) would be a suitable template to prepare these rigid drugs. The C-2 and C-4 positions of the xanthone ring should be the more active sites for an electrophilic aromatic substitution reaction because they are para and ortho with respect to phenox group. However, reaction at the C-2 position would be more favorable than at C-4 due to absence of steric hindrances of the incoming electrophile. The nitration reaction at C-2 to form compound 79 was considered as the key step for the preparation of the tetrahydropyranyl and thiocyanate derivatives built on a rigid template because the nitro group can be readily converted into the corresponding phenol intermediate.

The substitution of H-2 by a nitro group was satisfactory and carried out in detriment of the yield by adding 0.5 equiv of the nitrating agent (fuming nitric acid in concentrated sulfuric acid) over a cold diluted solution of 1 equiv of xanthone in concentrated sulfuric acid. Xanthone was always in excess irrespective of the nitrating agent and the forming 2-nitroxanthone was less likely to react with the NO$_2^-$ cation than the starting material 78. Compound 79 was reduced by catalytic hydrogenation to form the rigid aniline 80 that on reaction with sodium nitrite in sulfuric acid at low temperature, and in the presence of urea, led to the hydroxy derivative 81. Treatment of this compound with 2-bromoethyl tetrahydro-2H-pyran-2-yl ether afforded the conformationally constrained drug 82. After the routine three synthetic steps, compound 82 was converted into the rigid thiocyanate 85 in good yield (Scheme 6).
Replacement of the Terminal Phenoxy Group by a Chlorine Atom. The replacement of the terminal phenoxy group and the H-1' by chlorine atoms in our lead drug 8 produced a very active compound. As predicted by the biological activity previously observed for compound 31 (IC₅₀ 93 μM), being almost 50% more active than compound 7, drug 34 proved to be an extremely potent trypanostatic agent being 2-fold more active than 8 with an IC₅₀ of 1.0 μM. In addition at concentrations as low as 40 μM complete growth arrest took place. The presence of the chlorine atom at the ortho position with respect to the ether group seemed to be very important for biological activity. Removal of chlorinated atom at C-1' from compound 34 led to drug 39 that was 15 times less active than the dichloride derivative. Moreover, the precursor tetrahydropyranyl derivative 36 was almost devoid of inhibitory action.

Shortening the Aliphatic Side Chain and Elimination of the Oxygen Atom at C-1'. Although shortening of the aliphatic side chain and deleting the oxygen atom at C-1' gave place to the very potent drug 29, this compound was around 10 times less active than lead structure 8. Unexpectedly, the introduction of the tetrahydropyranyl functionality (compound 30) produced a slight improvement on biological activity (IC₅₀ 102 μM) compared with drug 7.

Introduction of a Halogen Atom at the C-2' Position. Taking into account the increment on biological activity when the thiocyanate moiety replaces the tetrahydropyranyl group when bonded to the same nonpolar skeleton, and considering that the larger the substituent, the more active the drug, it was reasonably expected that an improvement on biological activity would occur by introducing different groups at C-2'. With the exception of chlorine derivative 43, which exhibited an IC₅₀ of 25 μM, the introduction of a halogen atom at the ortho position in the 4-phenoxyphenoxypyridine skeleton produced very active trypanostatic agents. The normal precursor for the preparation of 43 (drug 40) had previously exhibited an IC₅₀ of 93 μM, being one of the most active tetrahydropyranyl ether derivatives ever prepared. Therefore, the introduction of either bromine or iodine into the lead structure brought about two very potent anti-T. cruzi agents exhibiting quite similar biological activities as compound 8. However, the therapeutic index for drugs 47 and 52 was not as good as previously observed for compound 8. These halogen-containing drugs presented IC₅₀ values of 18 and 23 μM, respectively, data that are still far from those reported for 8 (IC₅₀ of 3.5 μM).

Elimination of the Terminal Phenoxy Moiety and Introduction of a Bromine Atom at the C-3' Position. Replacement of a bromine atom at the meta position (C-3') with respect to the phenol group together with elimination of the terminal phenoxy moiety was at C-1' by a nitrogen (compound 24) produced a dramatic reduction in biological activity compared to our lead structure 8 with IC₅₀ of 141 μM. The decrease in the inhibitory action was not so pronounced with N-acetylated derivatives; certainly, drug 25 exhibited an IC₅₀ close to 15 μM, 10 times more active than 24, while its normal tetrahydropyranyl precursor (compound 18) was a moderate inhibitor of T. cruzi growth (IC₅₀ > 200 μM).

Results and Discussion

The biological data on the epimastigote form of T. cruzi cells were very encouraging. Ketoconazole was the other hand, when the substitution was made with cyanate afforded chiral thiocyante further nucleophilic displacement with potassium thiolate (compound 87), which after reaction with tosyl chloride and nitrogen-containing derivatives did not lead to an enhancement on biological activity. Thus, substitution of the oxygen hydrogen atoms at C-1 by this function would lead to chiral compounds. Thus, regioselective opening of S-propylene oxide by treatment with 4-phenoxyphenol (compound 86) in basic medium lead to optically pure alcohol 87. which after reaction with tosyl chloride and further nucleophilic displacement with potassium thio-cyanate afforded chiral thiocyanate 89 in moderate yield. The enantiomer of 89 was prepared starting from R-propylene oxide using the same synthetic sequence (Scheme 7).

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⁴ IC₅₀ = 32 μM. ⁵ IC₅₀ = 30 μM. ⁶ IC₅₀ = 27 μM. ⁷ IC₅₀ = 18 μM. ⁸ IC₅₀ = 23 μM. ⁹ IC₅₀ = 3.5 μM. ¹ IC₅₀ = 3.5 μM.
very promising for biological activity. In fact, thiocyanate 57 was a very potent trypanostatic agent with an IC₅₀ close to 27 µM, activity that correlates quite properly with the activity exhibited with its precursor, the tehydropyranyl ether 54.

Structural Variations in the B Ring. Structural modifications at the C-2′ or C-3′ positions in the B ring to give thiocyanates 64 and 72 seemed not to have a significant effect on biological activity. In fact, both of these drugs exhibited almost the same inhibitory action as lead drug 8 presenting IC₅₀ values of 2.9 and 2.7 µM, respectively. In addition, their precursors 61 and 69, which showed IC₅₀ values of 138 and 143 µM, respectively, were as active as lead compound 7 (IC₅₀ 138 µM). The above biological data are very meaningful for drug design because the introduction of different groups at C-2′ or C-3′ is not only somewhat difficult but also has no significant consequence on biological activity. Moreover, as we had previously demonstrated that substitutions at C-4″ produced an impairment on the growth inhibitory effect, it can be concluded that any modification in the B ring will not lead to an enhancement on biological activity.

Conformationally Rigid Aryloxylethyl Thiocyanates. Aryloxylethyl thiocyanates built on a rigid template did not present potent action in inhibiting the proliferation of T. cruzi. Accordingly, although conformationally constrained thiocyanate 85 was a rather active compound (IC₅₀ 54 µM), its inhibitory action was far from that observed for other thiocyanates derivatives, namely the lead structure 8. In addition, its precursor (compound 82) showed very modest activity, which at a concentration of 60 µM merely a 25% inhibition was observed.

Branched Derivatives of Lead Drug 8. The introduction of a branched side chain at the proximity of the thiocyanate moiety did not improve the biological activity. On the contrary, neither enantiomer was more active than 8, the R isomer (compound 89) was 10 times less active than 8, while its antipode 92 was almost 30 times less active than our lead drug. A typical dose–response graph for the more active drugs such as compounds 8, 47, 52, and 72 is illustrated in Figure 1.

The more promising drugs (compounds 15, 34, 47, 52, and 72, respectively) were assayed against the intracellular form of the parasite employing compound 8 as positive control. Compound 15 proved to be toxic for myoblasts, while drugs 34 and 52 were devoid of activity against amastigotes. On the other hand, compounds 47 and 72 were highly active trypanostatic agents but to a lesser extent than 8 (IC₅₀ 16.0 µM) exhibiting IC₅₀ values of 64.5 and 41.7 µM, respectively, under the same assays conditions based on differential uracil incorporation (see Table 2). In contrast to compound 15, no toxicity to the host cells, as assessed by phase contrast microscopy observation of detachment, vacuolation, and rounding of the cells, was detected using the concentrations of compounds described.

In conclusion, aryloxylethyl thiocyanate derivatives represent an interesting new family of drugs to control replication of T. cruzi cells that were rationally designed and synthesized. Slight modifications in the closeness to the polar end group produced a marked effect on inhibitory action. Work aimed at exploiting the potential usefulness of these thiocyanate derivatives as well as in vivo studies of the more promising drugs is currently in progress in our laboratory.

**Experimental Section**

The glassware used in air- and/or moisture-sensitive reactions was flame-dried and the experiments were carried out under a dry nitrogen atmosphere. Unless otherwise noted, chemicals were commercially available and used without further purification.

Nuclear magnetic resonance spectra were recorded on a Nicolet Magna 550 spectrometer. Low-resolution mass spectra were obtained on a VG TRIO 2 instrument at 70 eV (direct inlet). High-resolution mass spectrometry (HRMS) were conducted on a VG ZAB BEqQ spectrometer.

Column chromatography was performed employing E. Merck silica gel (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) and was visualized by 254 nm UV light. Melting points were determined using a Fisher-Johns apparatus and are uncorrected. IR spectra were recorded using a Nicolet Magna 550 spectrometer. Low-resolution mass spectra were obtained on a VG TRIO 2 instrument at 70 eV (direct inlet). High-resolution mass spectrometry (HRMS) were conducted on a VG ZAB BEqQ spectrometer.

The results were within ±0.4% of the theoretical values except when otherwise stated.

**Table 2. Growth Inhibition against Intracellular T. cruzi**

<table>
<thead>
<tr>
<th>compd</th>
<th>IC₅₀ (µM)</th>
<th>compd</th>
<th>IC₅₀ (µM)</th>
</tr>
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<tr>
<td>8</td>
<td>16.0</td>
<td>34</td>
<td>&gt;100</td>
</tr>
<tr>
<td>47</td>
<td>64.5</td>
<td>52</td>
<td>&gt;100</td>
</tr>
<tr>
<td>72</td>
<td>41.7</td>
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</tbody>
</table>

**Figure 1.** Dose–response graph for compounds 8, 47, 52, and 72.
partitioned between methylene chloride (70 mL) and an aqueous saturated solution of sodium potassium tartrate (100 mL). The aqueous phase was extracted with methylene chloride (2 × 50 mL), and the combined organic layers were washed with water (2 × 100 mL), dried (MgSO₄), and the solvent was evaporated. The residue was purified by column chromatography eluting with hexanes–EtOAc (50:1) to afford 1.61 g (100% yield) of pure compound 10 as a syrup: IR (film, cm⁻¹) 3447, 3063, 3058, 2978, 2875, 1584, 1482, 1328, 1167, 1045, 756, 692; MS (m/z, relative intensity) 400 (M⁺, 100), 374 (77), 294 (75), 258 (53), 227 (32), 201 (18), 95 (100) (found 400.1717; C₂₁H₂₅O₄N₂ requires 400.1717). Anal. (C₂₁H₂₅O₄N₂) C, H.

2-(4-Phenoxypyphenyl)ethyl Thiocyanate (15). To a solution of tosylate 13 (530 mg, 1.3 mmol) in anhydrous N,N-dimethylformamide (5 mL) was added potassium thiocyanate (250 mg, 5.4 mmol). The reaction mixture was heated at 100 °C for 5 h. The mixture was allowed to cool to room temperature and water (50 mL) was added. The aqueous phase was extracted with methylene chloride (2 × 50 mL) and the combined organic layers were washed with an aqueous saturated solution of sodium potassium tartrate (100 mL) and water (2 × 50 mL). The solvent was dried (MgSO₄) and evaporated. The residue was purified by column chromatography (silica gel) using a mixture of hexanes–EtOAc (24:1) as eluent to afford 380 mg (100% yield) of pure compound 15 as a yellow pale oil: Rf 0.58 (hexanes–EtOAc, 1:1); IR (film, cm⁻¹) 3061, 2924, 2853, 2815, 2514, 1582, 1485, 1240, 1167, 868, 756, 692; 1H NMR (CDCl₃) δ 3.08 (m, 2 H, H-1), 3.20 (m, 2 H, H-2), 6.96 (d, J = 8.8 Hz, 2 H, H-3), 6.97–7.42 (m, 5 H, aromatic protons), 7.40 (d, J = 8.8 Hz, 2 H, H-2); 13C NMR (CDCl₃) δ 33.26 (C-1), 35.44 (C-2), 119.23 (C-2′), 119.42 (C-3′), 123.93 (C-4′), 123.87 (C-7′), 132.98 (C-1′), 134.05 (C-2′), 156.34 (C-4′), 157.80 (C-8′); MS (m/z, relative intensity) 287 (M⁺, 83), 274 (3), 215 (20), 201 (96), 77 (100); HRMS calcd for C₁₉H₁₇ON₂S 287.0439, found 287.0441. Anal. (C₁₉H₁₇ON₂S) C, H.

N-(4-Phenoxypyphenyl)acetamide (17). A solution of 4-phenoxylaniline (16, 2.00 g, 10.8 mmol) in anhydrous pyridine (3 mL) was treated with acetic anhydride (2 mL). The reaction mixture was stirred at room temperature overnight. Then 5% hydrochloric acid was added, and the mixture was stirred for 1 h. The aqueous phase was extracted with methylene chloride (2 × 50 mL). The combined organic layers were washed with 5% HCl (3 × 50 mL), and water (2 × 50 mL). The solvent was dried (MgSO₄) and evaporated to afford 2,408 g (98% yield) of pure amide 17 as a brown solid: mp 128–129 °C; IR (KBBr, cm⁻¹) 3290, 3258, 3194, 3136, 3067, 1661, 1607, 1549, 1549, 1506, 1489, 1406, 1373, 1315, 1244, 1085, 831, 754, 691; MS (m/z, relative intensity) 227 (M⁺, 66), 181 (100), 156 (11), 131 (30). Anal. (C₁₅H₁₃ONS₂) C, H.

N-Acetyl-N-(4-phenoxypyphenyl)aminothiolethanol (18). A solution of acetate 17 (3,405 g, 15 mmol) in dimethyl sulfoxide (5 mL) was treated with potassium hydroxide (1.7 g, 30 mmol). The mixture was stirred at room temperature for 5 min. Then, bromothiacyl hydropryranyl ether (3.923 g, 15.75 mmol) was added, and the solution was stirred at room temperature overnight. The reaction was worked up as described for 11. The product was purified by column chromatography (silica gel) eluting with hexanes–EtOAc (7:3) to afford 327 mg (20% yield) of pure compound 18 as a yellow oil: IR (film, cm⁻¹) 3292, 3065, 2941, 2870, 1663, 1506, 1489, 1406, 1207, 1036, 872, 754, 692; 1H NMR (CDCl₃) δ 1.52–1.86 (m, 6 H, H-3′, H-4′, H-5′), 3.09 (t, J = 6.8 Hz, 2 H, H-2), 2.17 (s, 3 H, COCH₃), 3.54 (1 H, H-6′), 3.64 (1 H, H-6′), 3.73–4.03 (m, 2 H, H-1), 4.01 (distorted t, J = 3.2 Hz, 1 H, H-2′), 6.92 (d, J = 8.8 Hz, 2 H, H-3′), 6.91–7.39 (m, 5 H, aromatic protons); 13C NMR (CDCl₃) δ 19.37 (C-2′), 25.19 (C-5′), 29.31 (C-1′), 34.76 (C-5′), 66.43 (C-1′), 98.87 (C-2′), 118.19 (C-2′), 119.26 (C-3′), 123.42 (C-4′), 129.47 (C-2′, C-3′), 132.28 (C-1′), 156.32 (C-4′), 156.94 (C-1′); MS (m/z, relative intensity) 330 (M⁺, 1), 250 (19), 248 (20), 141 (20), 85 (100); HRMS calcd for C₁₉H₁₇ON₂S 330.1290, found 330.1289. Anal. (C₁₉H₁₇ON₂S) C, H.
stirred at room temperature overnight. The solvent was evaporated and the residue was purified by column chromatography (silica gel) eluting with hexanes–EtOAc (7:3) to afford 355 mg (51% yield) of pure chloride 20 as a pale yellow oil. Rf 0.52 (hexanes–EtOAc, 1:1); MS (m/z, relative intensity) 291 (M+, 2), 289 (M+, 6), 271 (20), 247 (5), 211 (10), 198 (100).

(4-Phenoxyphenyl)aminomethyl Acetate (21). A mixture of chloride 20 (154 mg, 0.53 mmol) and potassium thiocyanate (500 mg, 5.1 mmol) in N,N-dimethylformamide (3 mL) was stirred at 100 °C for 6 h. The mixture was treated with potassium thiocyanate (500 mg, 5.1 mmol) and was treated with potassium thiocyanate to give 130 mg (51% yield) of pure acetate 21 as a colorless oil. Rf 0.56 (hexanes–EtOAc, 7:3); MS (m/z, relative intensity) 271 (M+, 24), 211 (13), 198 (100).

2-[(4-Phenoxyphenyl)aminomethyl] ethanol (22). A solution of acetate 21 (250 mg, 0.93 mmol) in methanol–water (10:3) (13 mL) was treated with potassium carbonate. The reaction mixture was suspended in hexane (50 mL) and filtered off and the residue was purified by column chromatography (silica gel) eluting with hexanes–EtOAc (9:1) to give 130 mg (51% yield) of pure compound 22 as a white solid. Rf 0.24 (hexanes–EtOAc, 7:3); MS (m/z, relative intensity) 229 (M+, 29), 198 (100).

(2-Chloroethyl)(4-phenoxyphenyl)amine (23). To a solution of triphenylphosphine (400 mg, 1.5 mmol) in carbon tetrachloride (20 mL) and acetonitrile (5 mL) was added amino alcohol 22 (200 mg, 0.87 mmol). The mixture was stirred at room temperature for 16 h. Then, the solvent was evaporated and the residue was purified by column chromatography (silica gel) eluting with hexanes–EtOAc (9:1) to give 150 mg (70% yield) of pure compound 23 as a colorless oil: IR (film, cm⁻¹) 2957, 2835, 1589, 1508, 1233, 868, 835, 754, 692; MS (m/z, relative intensity) 295 (M+, 24), 274 (M+, 31), 198 (100).

4-Phenoxyphenylaminomethyl Thiocyanate (24). A solution of compound 23 (150 mg, 0.66 mmol) in anhydrous dimethylformamide (3 mL) was treated with potassium thiocyanate (200 mg, 2.1 mmol). The mixture was stirred at 100 °C for 6 h. The reaction was worked up as described for 15. The residue was purified by column chromatography (silica gel) eluting with hexanes–EtOAc (19:1) as eluent to afford 63 mg (39% yield) of pure compound 24 as a white solid and 72 mg of unreacted chloride 23. Rf 0.09 (EtOAc); IR (KBr, cm⁻¹) 2981, 2851, 2156, 1491, 1244, 1094, 1032, 827, 667; 1H NMR (CDCl₃) δ 7.26 (t, J = 6.7 Hz, 2 H, H-1), 6.65 (d, J = 6.7 Hz, 2 H, H-2), 7.01 (d, J = 8.8 Hz, 2 H, H-2), 7.02–7.32 (m, 5 H, aromatic protons), 7.34 (d, J = 8.6 Hz, 2 H, H-3); 13C NMR (CDCl₃) δ 26.83 (C-1′), 54.39 (C-2′), 118.75 (C-2′′), 119.32 (C-3′), 123.27 (C-4′), 125.19 (C-2′′′), 129.66 (C-3′′′), 135.72 (C-1′′′), 154.45 (C-4′′), 157.05 (C-1′); MS (m/z, relative intensity) 270 (M+, 100), 243 (4), 215 (9), 210 (10), 198 (16), 84 (74); HRMS calc'd for C₂₀H₁₅NO₂S (M+H⁺) 302.0826, found 302.0827. Anal. (C₂₀H₁₅NO₂S·H₂O) C, H.

N-Acetyl-(4-phenoxyphenyl)-2-thiocyanatoethylamine (25). A solution of compound 24 (30 mg, 0.11 mmol) in pyridine (1 mL) was treated with acetic anhydride (2 mL). The reaction mixture was treated as described for 17. The product was purified by column chromatography (silica gel) eluting with hexanes–EtOAc (4:1) to afford 28 mg (82% yield) of pure compound 25 as a colorless oil: Rf 0.55 (EtOAc); IR (film, cm⁻¹) 3056, 2995, 2933, 2154, 1595, 1516, 1493, 1460, 1430, 1239, 1100, 1076; 1H NMR (CDCl₃) δ 1.56–1.88 (m, 6 H, H-3′′′–H-6′′′), 3.51–3.59 (m, 1 H, H-6′″); MS (m/z, relative intensity) 241 (M+, 9), 208 (M+, 5), 183 (22), 165 (100); HRMS calc'd for C₂₃H₂₂N₂O₂S (M+H⁺) 384.0781, found 384.0780. Anal. (C₂₃H₂₂N₂O₂S) C, H.

4-Phenoxyphenylthiocarbonyl Ethene Ether (30). To a solution of alcohol 27 (158 mg, 0.79 mmol) in anhydrous methylene chloride (50 mL) were added pyridinium 4-toluenesulfonate (20 mg) and 3,4-dihydro-2H-pyran-2-yl Ether (31). The reaction mixture was stirred at room temperature overnight. The organic phase was washed with water (3 × 50 mL), dried (MgSO₄), and the solvent was evaporated. The residue was purified by column chromatography (silica gel) eluting with hexanes–EtOAc to give 22 mg of pure compound 30 as a white solid: IR (film, cm⁻¹) 3238, 2932, 2869, 1508, 1238, 1134, 1038, 871, 692; 1H NMR (CDCl₃) δ 1.56–1.88 (m, 6 H, H-3′′′–H-6′′′), 3.51–3.59 (m, 1 H, H-6″″); MS (m/z, relative intensity) 241 (M+, 4), 197 (100), 155 (10), 91 (22), 77 (40); HRMS calc'd for C₁₅H₁₂O₂N₂S (M+H⁺) 284 (M+, 37), 183 (100), 85 (72). Anal. (C₁₅H₁₂O₂N₂S) C, H.

2,4-Dichlorophenoxycarboxylic Acid (32). A solution of compound 31 (746 mg, 2.6 mmol) in methanol (30 mL) was treated with pyridinium 4-toluenesulfonate (20 mg). The mixture was stirred at room temperature overnight. The reaction was worked up as described for 12. The residue was purified by column chromatography eluting with hexanes–EtOAc (1:1) to afford 230 mg (43% yield) of pure compound 32 as a colorless oil: Rf 0.14 (hexanes–EtOAc, 4:1); IR (film, cm⁻¹) 3238, 2932, 2867, 1558, 1508, 1489, 1328, 1134, 1038, 871, 692; 1H NMR (CDCl₃) δ 1.56–1.88 (m, 6 H, H-3′′′–H-6′′′), 3.51–3.59 (m, 1 H, H-6″″); MS (m/z, relative intensity) 210 (M+, 5), 208 (M+, 27), 206 (M+, 42), 166 (18), 164 (107), 162 (100).

2,4-Dichlorophenylethyl 4-Toluene sulfonate (33). To a solution of alcohol 27 (230 mg, 1.1 mmol) in pyridine (3 mL) was added portionwise tosyl chloride (254 mg, 1.3 mmol) at 0 °C. The reaction mixture was stirred at room temperature overnight. The solvent was evaporated and the residue was purified by column chromatography (silica gel) eluting with hexanes–EtOAc (19:1) to yield 190 mg (53% yield) of pure tosylate 33 as a colorless oil: IR (film, cm⁻¹) 1599, 1558, 1489, 1430, 1377, 1238, 1134, 1038, 871, 692; 1H NMR (CDCl₃) δ 1.56–1.88 (m, 6 H, H-3′′′–H-6′′′), 3.51–3.59 (m, 1 H, H-6″″); MS (m/z, relative intensity) 210 (M+, 5), 208 (M+, 27), 206 (M+, 42), 166 (18), 164 (107), 162 (100).
Aryloxyethyl Thiocyanates as Inhibitors of T. cruzi

2-Chloro-4-phenoxypyphenoxethanol (41). A solution of compound 40 (635 mg, 1.8 mmol) in methanol (20 mL) was treated with pyridinium p-toluenesulfonate (30 mg). The mixture was treated as described for the preparation of compound 12. The residue was purified by column chromatography (silica gel) eluting with hexanes–EtOAc (9:1) to afford 200 mg (42% yield) of pure compound 41 as a colorless oil: IR (film, cm⁻¹) 3385, 3076, 2941, 1596, 1491, 1266, 1216, 1057, 913, 758, 695; MS (m/z, relative intensity) 426 (M⁺, 1), 260 (M⁺, 6), 264 (M⁺, 60), 222 (26), 220 (96), 84 (49), 77 (60), 49 (100).

2-Chloro-4-phenoxypyphenoxethyl 4-Toluenesulfonate (42). A solution of alcohol 41 (180 mg, 0.68 mmol) in pyridine (3 mL) was treated with tosyl chloride (250 mg, 1.3 mmol). After the usual treatment the residue was purified by column chromatography (silica gel eluting with hexanes–EtOAc (19:1) to give 113 mg (40% yield) of pure 42 as a colorless oil: MS (m/z, relative intensity) 420 (M⁺, 6), 418 (M⁺, 15), 221 (4), 219 (10), 199 (100), 155 (31), 91 (86), 77 (47).

2-Chloro-4-phenoxypyphenoxethyl Thiocyanate (43). To a solution of tosylate 42 (120 mg, 0.29 mmol) in dimethylformamide (3 mL) was added potassium thiocyanate (300 mg, 5.0 mmol). The reaction was heated at 100 °C with vigorous stirring for 6 h. The reaction was quenched as described for 15. The residue was purified by column chromatography (silica gel) eluting with hexanes–EtOAc (9:1) to afford 113 mg (42%) of pure tosylate 42 as a colorless oil: IR (film, cm⁻¹) 2918, 2850, 2156, 1481, 1217, 1055, 1024, 924, 876, 824, 961, 1157 (C=O), 1354 (C=S); MS (m/z, relative intensity) 424 (M⁺, 1), 221 (4), 155 (31), 91 (86), 77 (47).

2-Chloro-4-phenoxypyphenoxethyl Thiocyanate (44). Compound 45 was prepared from 44 (510 mg, 1.3 mmol) following the method of preparation described for 12. Purification by column chromatography eluting with hexanes–EtOAc (17:1) gave 256 mg (64% yield) of pure alcohol 45 as a colorless oil: IR (film, cm⁻¹) 3291, 2930, 2858, 1589, 1490, 1457, 1217, 1047, 870, 750; MS (m/z, relative intensity) 310 (M⁺, 93), 308 (M⁺, 100), 266 (68), 264 (69), 157 (25), 77 (75).

2-Chloro-2-phenoxypyphenylethanol (45). Alcohol 45 (220 mg, 0.71 mmol) was treated with tosyl chloride according to the general procedure. After the usual workup, 310 mg (94% yield) of pure product was isolated that was purified by column chromatography (silica gel) eluting with hexanes–EtOAc (1:1) to give 290 mg (84% yield) of pure compound 46 as a colorless oil: IR (film, cm⁻¹) 2930, 2858, 1589, 1490, 1360, 1250, 1216, 1207, 1043, 1010, 814, 754, 692; MS (m/z, relative intensity) 464 (M⁺, 6), 462 (M⁺, 6), 310 (15), 308 (13), 266 (33), 264 (33), 199 (86), 155 (32), 91 (73), 77 (100).

2-Chloro-4-phenoxypyphenoxethyl Thiocyanate (47). Compound 44 (273 mg, 0.66 mmol) was treated with potassium thiocyanate as depicted for compound 13. Purification by column chromatography (silica gel) eluting with hexanes–EtOAc (8:1) afforded 162% yield and the residue was purified by column chromatography (silica gel eluting with hexanes–EtOAc (9:1) to afford 105 mg (63% yield) of pure compound 47 as a colorless oil: IR (film, cm⁻¹) 2934, 2876, 1597, 1493, 1287, 1246, 1092, 1053, 916, 824, 667; MS (m/z, relative intensity) 587 (M⁺, 20) 172 (M⁺, 63), 130 (46), 128 (100).

2-Chloro-4-phenoxypyphenoxethyl 4-Toluenesulfonate (46). Alcohol 45 (220 mg, 0.71 mmol) was treated with tosyl chloride according to the general procedure. After the usual workup, 310 mg (94% yield) of pure product was isolated that was purified by column chromatography (silica gel) eluting with hexanes–EtOAc (1:1) to give 290 mg (84% yield) of pure compound 46 as a colorless oil: IR (film, cm⁻¹) 3291, 2930, 2858, 1589, 1490, 1457, 1217, 1047, 870, 750; MS (m/z, relative intensity) 310 (M⁺, 93), 308 (M⁺, 100), 266 (68), 264 (69), 157 (25), 77 (75).

2-Chloro-4-phenoxypyphenoxethyl Thiocyanate (47). Compound 46 (273 mg, 0.66 mmol) was treated with potassium thiocyanate as depicted for compound 13. Purification by column chromatography (silica gel) eluting with hexanes–EtOAc (8:1) afforded 162% yield and the residue was purified by column chromatography (silica gel eluting with hexanes–EtOAc (9:1) to afford 105 mg (63% yield) of pure compound 47 as a colorless oil: IR (film, cm⁻¹) 3291, 2930, 2858, 1589, 1490, 1360, 1250, 1207, 1043, 1010, 814, 754, 692; MS (m/z, relative intensity) 464 (M⁺, 6), 462 (M⁺, 6), 310 (15), 308 (13), 266 (33), 264 (33), 199 (86), 155 (32), 91 (73), 77 (100).

2-Chloro-4-phenoxypyphenoxethyl Thiocyanate (48). Compound 47 (273 mg, 0.66 mmol) was treated with potassium thiocyanate as depicted for compound 13. Purification by column chromatography (silica gel) eluting with hexanes–EtOAc (8:1) afforded 162% yield and the residue was purified by column chromatography (silica gel eluting with hexanes–EtOAc (9:1) to afford 105 mg (63% yield) of pure compound 48 as a colorless oil: IR (film, cm⁻¹) 3291, 2930, 2858, 1589, 1490, 1360, 1250, 1207, 1043, 1010, 814, 754, 692; MS (m/z, relative intensity) 464 (M⁺, 6), 462 (M⁺, 6), 310 (15), 308 (13), 266 (33), 264 (33), 199 (86), 155 (32), 91 (73), 77 (100).
compound 49 as a colorless oil: Rf 0.45 (hexanes–EtOAc, 4:1); IR (film, cm⁻¹) 3063, 2941, 1872, 1578, 1457, 1456, 1265, 1219, 1140, 1034, 881, 756, 692; ¹H NMR (CDCl₃) δ 1.50–1.80 (m, 6 H, H-3′, H-4′, H-5′), 3.56 (m, 1 H, H-6′), 3.83–4.07 (m, 3 H, H-1′, H-6″), 4.17 (distorted t, J = 4.7 Hz, 2 H, H-2″), 4.80 (distorted t, J = 3.1 Hz, 1 H, H-2″), 6.82 (d, J = 8.9 Hz, 1 H, H-6), 6.92–7.09 (m, 4 H, aromatic protons), 7.25–7.34 (m, 2 H, arylmethyl), 7.41 (d, J = 2.8 Hz, 1 H, H-2′′), 7.53 (m, 1 H, aromatic), 10.28 (s, 1 H, =O). MS m/z, relative intensity 440 (M⁺, 10), 352 (32), 213 (129), 120 (100); HRMS calc'd for C₁₅H₁₂₂₉O₃ 440.0485, found 440.0483.

2-ido-4-phenoxypyridoxylates (50). Compound 49 (190 mg, 0.43 mmol) was treated with pyridinium 4-toluene-
sulfonate as described for the preparation of 12 to give 91 mg (59% yield) of pure alcohol 50 as a colorless oil: Rf 0.16 (hexanes–EtOAc, 4:1); IR (film, cm⁻¹) 3366, 3065, 3034, 2936, 2874, 1475, 1456, 1265, 1217, 1043, 889, 816, 750, 692; MS m/z, relative intensity 356 (M⁺, 59), 312 (97), 264 (12), 230 (16), 220 (16), 80 (36), 77 (100).

2-ido-4-phenoxypyridoxyl 4-Toluene-
sulfonate (51). Alcohol 50 (80 mg, 0.22 mmol) was treated with tosyl chloride as described for the preparation of 13 affording 119 mg (100% yield) of pure tosylate 51 as a colorless oil: Rf 0.35 (hexanes–
EtOAc, 4:1); IR (film, cm⁻¹) 3063, 3040, 2926, 2876, 2154, 1585, 1481, 1387, 1267, 1215, 1070, 1038, 878, 713, 692; ¹H NMR (CDCl₃) δ 3.39 (t, J = 5.9 Hz, 2 H, H-1″), 4.31 (t, J = 5.9 Hz, 2 H, H-2″), 6.82 (d, J = 8.9 Hz, 1 H, H-6″), 6.93–7.13 (m, 4 H, aromatic protons), 7.29–7.37 (m, 2 H, aromatic protons), 7.46 (d, J = 2.8 Hz, 1 H, H-2′′), 6.80–7.20 (m, 5 H, aromatic protons); ¹³C NMR (CDCl₃) δ 33.31 (C-1), 67.90 (C-2), 87.04 (C-2′), 113.80 (C-6′′), 112.80 (C-6′′′), 120.11 (C-5′′), 123.33 (C-5′′′), 128.90 (C-3′′), 130.31 (C-3′′′), 152.17 (C-5′′′), 152.94 (C-1′′), 157.39 (C-1′′′); MS m/z, relative intensity 397 (M⁺, 69), 311 (45), 128 (74), 77 (100). Anal. (C₁₅H₁₂₂₉O₃S) C, H.

3-Bromophenoxypyridoxyl Tetrahydro-2H-pyran-2-yl Ether (52). A solution of 3-bromophenoxypyridoxyl (1.73 g, 10 mmol) in dimethyl sulfoxide (10 mL) was treated with potassium hydride (600 mg, 11 mmol). The mixture was stirred at room temperature for 10 min. Then, 4-chloronitrobenzene (1.57 g, 10 mmol) and cupric chloride (50 mg) were added and the mixture was stirred at room temperature overnight. The reaction was worked up as described for 11. The product was purified by column chromatography (silica gel, eluting with EtOAc (50:1) to afford 2.099 g (71% yield) of pure compound 52 as a colorless oil: Rf 0.20 (hexanes–EtOAc, 19:1); IR (film, cm⁻¹) 1580, 1516, 1489, 1341, 1240, 1111, 1061, 895, 847, 775, 750, 671; MS m/z, relative intensity 295 (M⁺, 100), 293 (M⁺, 92), 265 (14), 263 (13), 168 (54), 157 (16), 155 (19), 139 (41), 128 (33).

3-Bromophenoxypyridoxylaniline (53). A solution of compound 52 (1.5 g, 5.1 mmol) in ethyl acetate (50 mL) in the presence of paladium on charcoal (100 mg) was treated with hydrogen at 3 atm, and the mixture was stirred at room temperature overnight. The mixture was filtered, and the solvent was evaporated to give 1.220 g (91% yield) of pure amine 53 as a pale yellow oil that was used as such in the next step: Rf 0.61 (hexanes–EtOAc, 4:1); IR (film, cm⁻¹) 2824, 2594, 2575, 1570, 1504, 1485, 1246, 1198, 831, 779, 692; MS m/z, relative intensity 265 (M⁺, 5), 263 (M⁺, 5), 185 (100), 156 (17), 108 (99), 80 (69).

3-Bromophenoxypyridoxylphenol (60). To a solution of amine 59 (1.00 g, 3.8 mmol) in glacial acetic acid (12 mL) was added sulfuric acid (3.6 mL). The mixture was cooled to 0 °C, and a solution of sodium nitrite (6.3 g) in water (30 mL) was added dropwise. The reaction mixture was stirred at 0 °C for 20 min. Then, urea (120 mg) and cold water (12 mL) was added. This mixture was kept at 0 °C and was carefully added to a boiling mixture of water (25 mL) and concentrated sulfuric acid (6 mL). After the addition was complete the reaction was stirred for an additional 10 min at the boiling temperature. The mixture was allowed to cool and was partitioned between water (100 mL) and methylene chloride (75 mL). The aqueous layer containing phenol (350 mg) was water (12 mL) was added. The aqueous layer was extracted with dichloromethane (2 × 70 mL), and the combined organic layers were washed with brine (2 × 50 mL), dried (MgSO₄), and the solvent was evaporated. The residue was purified by column chromatography eluting with hexanes–EtOAc (9:1) to yield 374 mg (37% yield) of pure phenol 60 as a colorless oil: Rf 0.32 (hexanes–EtOAc, 4:1); IR (film, cm⁻¹) 3377, 1592, 1508, 1489, 1362, 1217, 1096, 1070, 847, 815, 692; MS m/z, relative intensity 266 (M⁺, 11), 264 (M⁺, 12), 186 (100), 157 (21), 129 (16), 106 (9), 78 (52), 65 (1), 44 (16), 33 (20), 25 (2)

3-Bromophenoxypyridoxyl Tetrahydro-2H-pyran-2-yl Ether (61). Phenol 60 (359 mg, 1.36 mmol) was condensed with bromomethyl tetrahydroxypropyl ether (358 mg, 1.7 mmol) as described for 11. The product was purified by column chromatography (silica gel) eluting with hexanes–EtOAc (9:1) as eluent to afford 320 mg (60% yield) of pure compound 61 as a colorless oil: Rf 0.24 (hexanes–EtOAc, 10:1); ¹H NMR
A colorless oil: Rf purified by column chromatography (silica gel) eluting with hexanes—EtOAc (9:1) to yield 3.356 g (63% yield) of pure compound.

4-(2-Methylphenoxy)phenoxyethanol (70). Ether 69 (405 mg, 1.2 mmol) was treated as described for the preparation of 12. After the usual workup and evaporation of the solvent afforded 333 mg (100% yield) of pure alcohol 70 as a colorless oil: Rf 0.19 (hexanes—EtOAc, 3:1); IR (film, cm⁻¹) 3362, 2934, 2868, 1585, 1506, 1460, 1299, 1229, 1018, 1052, 850, 834, 762; MS (m/z, relative intensity) 328 (M⁺, 100), 257 (125), 176 (100), 85 (100); HRMS calc'd for (C₁₉H₂₂O₅) 328.1675, found 328.1680.

4-(2-Methylphenoxyl)phenoxyethyl 4-Toluenesulfonate (71). Alcohol 70 (207 mg, 0.85 mmol) was treated with tosyl chloride as depicted for 13. The product was purified by column chromatography (silica gel) using hexanes—EtOAc (9:1) as eluent to afford 227 (67% yield) of pure tosylate 71 as a colorless oil and 30 mg of unreacted alcohol 70: Rf 0.32 (hexanes—EtOAc, 3:1); IR (film, cm⁻¹) 2928, 1510, 1492, 1456, 1365, 1233, 1172, 1027930, 822, 774, 665; MS (m/z, relative intensity) 398 (M⁺, 28), 199 (100), 155 (40), 91 (95).

4-(2-Methylphenoxyl)phenoxyethyl Thiocyanate (72). Tosylate 71 (196 mg, 0.68 mmol) was treated with potassium thiocyanate as described for 15. The residue was purified by column chromatography (silica gel) eluting with hexanes—EtOAc (93:7) to afford 97 mg (50% yield) of pure thiocyanate 72 as a colorless oil: Rf 0.12 (hexanes—EtOAc, 9:1); IR (film, cm⁻¹) 3067, 2927, 2869, 2161, 1588, 1512, 1471, 1301, 1235, 1207, 1120, 1044, 880, 833, 763; 'H NMR (CDCl₃) 0.26 (s, 3 H, PhMe), 3.29 (t, 2 H, -CH₂Br), 4.26 (t, 2 H, -CH₂-). After the usual workup and evaporation of the solvent afforded 333 mg (100% yield) of pure compound.
anes–EtOAc, 4:1); \(^1\)H NMR (CDCl\(_3\)) \(\delta 1.53–1.85\) (m, 6 H, H-3\(^{'-}\), H-4\(^{-}\), H-5\(^{-}\)), 2.12 (s, 6 H, PhMe), 3.51 (m, 1 H, H-6\(^{-}\)), 3.75–4.00 (m, 3 H, H-3, H-6\(^{-}\)), 4.02–4.12 (m, 2 H, H-2, H-4), 6.69 (distorted t, J = 2.9 Hz, 1 H, H-2\(^{-}\)), 6.65 (d, J = 9.2 Hz, 2 H, H-2), 6.62 (d, J = 9.1 Hz, 2 H, H-3), 7.05 (m, 3 H, aromatic protons); \(^1\)C NMR (CDCl\(_3\)) \(\delta 16.29\) (PhMe), 19.33 (C-4\(^{-}\)), 25.39 (C-3\(^{-}\)), 30.48 (C-5\(^{-}\)), 62.12 (C-2\(^{-}\)), 65.90 (C-1), 68.05 (C-2), 98.93 (C-2\(^{-}\)), 121.52 (C-4\(^{-}\)), 124.81 (C-5\(^{-}\)), 131.20 (C-3\(^{-}\)), 131.50 (C-2), 151.53 (C-4), 152.11 (C-1), 153.39 (C-1\(^{-}\)); MS (m/z, relative intensity) 342 (M\(^+\), 26), 214 (32), 129 (100); HRMS calculated for C\(_{19}\)H\(_{20}\)O\(_2\) 342.1831, found 342.1834.

2-Nitroanisole (79). To a solution of xanthone (78; 50.0 g, 255 mmol) in concentrated sulfuric acid (14 mL) cooled at 0 °C was added dropwise fuming nitric acid (0.9 mL, 12.8 mmol in concentrated sulfuric acid (3 mL). The reaction mixture was stirred at 0 °C for 40 min. The mixture was poured onto crushed ice. The solid was filtered off and was washed with cold water until pH 7. The residue was used as such in the next step without further purification. An analytical sample was purified by column chromatography (silica gel) eluting with hexanes–EtOAc (4:1) as eluent to afford pure compound 79 as a white solid: mp 195–198 °C; R\(_f\) 0.50 (toluene); IR (KBr, cm\(^{-1}\)) 1668, 1614, 1533, 1462, 1348, 833, 770, 746, 669; \(^1\)H NMR (CDCl\(_3\)) \(\delta 7.34–7.58\) (m, 3 H, H-5, H-7), 7.64 (d, J = 9.1 Hz, 1 H, H-4), 7.81 (m, 1 H, H-6), 8.35 (dd, J = 8.0, 1.6 Hz, 1 H, H-8), 8.55 (dd, J = 9.2, 2.8 Hz, 1 H, H-3), 9.21 (d, J = 2.8 Hz, 1 H, H-1); \(^1\)C NMR (CDCl\(_3\)) \(\delta 118.21 (C-5), 121.56 (C-4), 125.27 (C-4\(^{-}\)), 126.75 (q), 129.73 (C-3), 129.01 (C-8), 135.89 (C-6), 155.88 (C-5\(^{-}\)), 159.20 (C-4\(^{-}\)); MS (m/z, relative intensity) 241 (M\(^+\), 36), 196 (100), 168 (49), 139 (88). Aminoxanate (80). A suspension of compound 79 in ethyl acetate (100 mL) in the presence of palladium on charcoal as catalyst (200 mg) was treated with hydrogen at 3 atm in a Parr apparatus. The mixture was shaken at room temperature for 6 h. The mixture was filtered and the solvent was evaporated. The residue was purified by column chromatography (silica gel) eluting hexanes–EtOAc as eluent to afford 2.465 g (46% yield from xanthone, 91% yield taken the equivalents of nitric acid used) of pure compound 80 as a yellow solid: mp 190–192 °C; IR (KBr, cm\(^{-1}\)) 3412, 3321, 1641, 1618, 1593, 1491, 1466, 1327, 1209, 1147, 881, 756, 625; R\(_f\) 0.72 (hexanes–EtOAc, 1:1); \(^1\)H NMR (CDCl\(_3\)) \(\delta 7.18\) (dd, J = 8.8, 2.8 Hz, 1 H, H-3), 7.32–7.55 (m, 3 H, H-5, H-7, H-5\(^{-}\)), 7.37 (d, J = 8.9 Hz, 1 H, H-4), 7.72 (m, 1 H, H-6), 8.39 (dd, J = 8.0, 1.5 Hz, 1 H, H-8), 8.48 (s, 1 H, H-1); \(^1\)C NMR (CDCl\(_3\))–CD\(_2\)OD, 296.47, 207.56, 121.43, 124.56, 125.27 (C-4\(^{-}\)), 126.57 (C-3), 129.01 (C-8), 135.89 (C-6), 155.88 (C-5\(^{-}\)), 159.20 (C-4\(^{-}\)); MS (m/z, relative intensity) 241 (M\(^+\), 36), 196 (100), 139 (88). 2-Hydroxyxanthonne (81). A solution of compound 80 (770 mg, 3.65 mmol) in glacial acetic acid (10 mL) was treated as described for the preparation of 60 to afford 280 mg (36% yield) of pure compound 81 as a red solid: R\(_f\) 0.10 (hexanes–EtOAc, 3:2); mp 176–177 °C; IR (KBr, cm\(^{-1}\)) 2342, 1769, 1628, 1487, 1460, 1348, 1237, 1153, 1089, 876, 821, 788, 757, 626; \(^1\)H NMR (CDCl\(_3\)) \(\delta 7.31–7.63\) (m, 7 H, aromatic protons), 7.48 (m, 2 H, H-5, H-8), 8.28 (dd, J = 8.0, 1.5 Hz, 1 H, H-8), 10.47 (s, 1 H, OH); \(^1\)C NMR (CDCl\(_3\)) \(\delta 108.69 (C-1), 117.72 (C-5), 118.97 (C-4), 120.58 (C-8), 121.70 (C-9a), 123.38 (C-7), 124.47 (C-2), 126.00 (C-8), 134.56 (C-6), 150.09 (C-4), 153.37 (C-2), 156.02 (C-5\(^{-}\)); MS (m/z, relative intensity) 212 (M\(^+\), 54), 184 (99), 46 (96), 55 (85), 43 (100).

2-(3,5-Dimethyl-4-hydroxyphenyl)acetophenone (82). A solution of phenol 81 (270 mg, 1.3 mmol) in methyl sulfoxide (3 mL) was treated as depicted for 11. The product was purified by column chromatography eluting with hexanes–EtOAc (19:1) to afford 200 mg (45% yield) of pure compound 82 as a white solid: mp 292–294 °C; IR (KBr, cm\(^{-1}\)) 3046, 2924, 2894, 2274, 1597, 1504, 1489, 1364, 1225, 1190, 1045, 928, 903, 768, 665, 555; MS (m/z, relative intensity) 298 (M\(^+\), 8), 274 (24), 211 (100), 184 (25), 155 (47); HRMS calculated for C\(_{17}\)H\(_{16}\)O\(_2\)S 279.0490, found 279.0459.

2-(4-Phenylthio)acetophenone (83). A solution of 4-phenylphenol (86; 1.0 g, 5.4 mmol) in methyl sulfoxide (3 mL) was treated with S-propylene oxide (320 mg, 4.0 mmol, 5.5 mmol) and the mixture was stirred at room temperature for 6 h. The reaction mixture was quenched as depicted for 11, and the residue was purified by column chromatography employing a mixture of hexanes–EtOAc (1:1) as eluent to afford 266 mg (20% yield) of alcohol 87 as a white solid: R\(_f\) 0.51 (toluene–EtOAc, 4:1); mp 67–69 °C; [\(\alpha\)]\(_D\) +18.2 (c 1.0, CHCl\(_3\)).
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Supporting Information Available: NMR spectral data for compounds 12, 14, 17, 19, 23, 27, 28, 32, 33, 35, 37, 38, 41, 42, 45, 46, 50, 51, 55, 56, 58–60, 62, 63, 66, 68, 70, 71, 74, 76, 87, and 88 and tables of data needed to calculate percent inhibition. This material is available free of charge via the Internet at http://pubs.acs.org.

References


