Synthesis and Biological Activity of Nitro Heterocycles Analogous to Megazol, a Trypanocidal Lead[#]

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As part of our efforts to develop new compounds aimed at the therapy of parasitic infections, we synthesized and assayed analogues of a lead compound megazol, 5-(1-methyl-5-nitro-1*H*-2-imidazolyl)-1,3,4-thiadiazol-2-amine, CAS no. 19622-55-0), in vitro. We first developed a new route for the synthesis of megazol. Subsequently several structural changes were introduced, including substitutions on the two rings of the basic nucleus, replacement of the thiadiazole by an oxadiazole, replacement of the nitroimidazole part by a nitrofurane or a nitrothiophene, and substitutions on the exocyclic nitrogen atom for evaluation of an improved import by the glucose or the purine transporters. Assays of the series of compounds on the protozoan parasites *Trypanosoma brucei*, *Trypanosoma cruzi*, and *Leishmania donovani*, as either extracellular cells or infected macrophages, indicated that megazol was more active than the derivatives. Megazol was then evaluated on primates infected with *Trypanosoma brucei gambiense*, including late-stage central nervous system infections in combination with suramin. Full recovery was observed in five monkeys in the study with no relapse of parasitemia within a 2 year follow-up. Because there is a lack of efficacious treatments for sleeping sickness in Africa and Chagas disease in South America, megazol is proposed as a potential alternative. The mutagenicity of this compound is at present being reevaluated, and metabolism is also under investigation prior to possible further developments.

Introduction

Although nitro heterocycles have been used for a long time in the treatment of Chagas disease, for example, nifurtimox and benznidazole,[1] or as radiosensitizer in the case of misonidazole,[2] no further important developments were expected from this class of compounds because of the mutagenicity risk that they present. This concern also holds for megazol, a 5-nitroimidazole bearing a thiadiazole ring compound (Figure 1) that exhibits a high curative efficacy in mice infected with different strains of *Trypanosoma cruzi*[3] and also in *Trypanosoma brucei* infected rodents.[4]

However, this limitation should be reconsidered on the following grounds. First, although mutagenic activation of megazol is obtained via a nitro reductase present in the bacteria *Salmonella typhimurium* used in the Ames test,[5] this does not seem to be the case with mammalian nitro reductases.[6] Second, although the number of infected people continues to grow by at least 25 000 new cases every year in Africa,[7] the therapeutic arsenal remains largely inadequate, relying on drugs, either presenting an appreciable toxicity risk such, as melarsoprol,[8] or where the required amount for an effective treatment with difluoromethylornithine (DFMO) is so high (400 g per head) that its use remains restricted to hospital conditions.[9] In this context, we considered that further investigations on megazol were justified.

Several goals were pursued in the present work: (i) to develop a new synthetic route for megazol to obtain large amounts of compound required for pharmacological and toxicological investigations and also for studies on animals with possible veterinary applications; (ii) to obtain substituted analogues for studies on the mode of action in relation to two identified targets, oxygen metabolism[10] and an enzyme of the Krebs cycle, fumarate reductase;[11] (iii) to study the drug uptake into the trypanosome through glucose[12] or purine[13] transporters for which glucoconjugates and other modified structures were synthesized. This paper describes the corresponding syntheses and in vitro biological assays. Preliminary results of in vivo studies are also given.

Results and Discussions

(1) New Synthesis of Megazol. This synthesis was undertaken to increase the poor yield obtained by the previously developed methods[14] (described later in this work for the synthesis of the 4-methyl analogue of megazol), which requires a sealed tube reaction. The purpose was also to find conditions suitable for a large-scale synthesis.

[#] Dedicated to Dr. Benjamin Gilbert, Head of Research at Oswaldo Cruz Foundation in Rio de Janeiro, Brazil, who interested us in the study of megazol.

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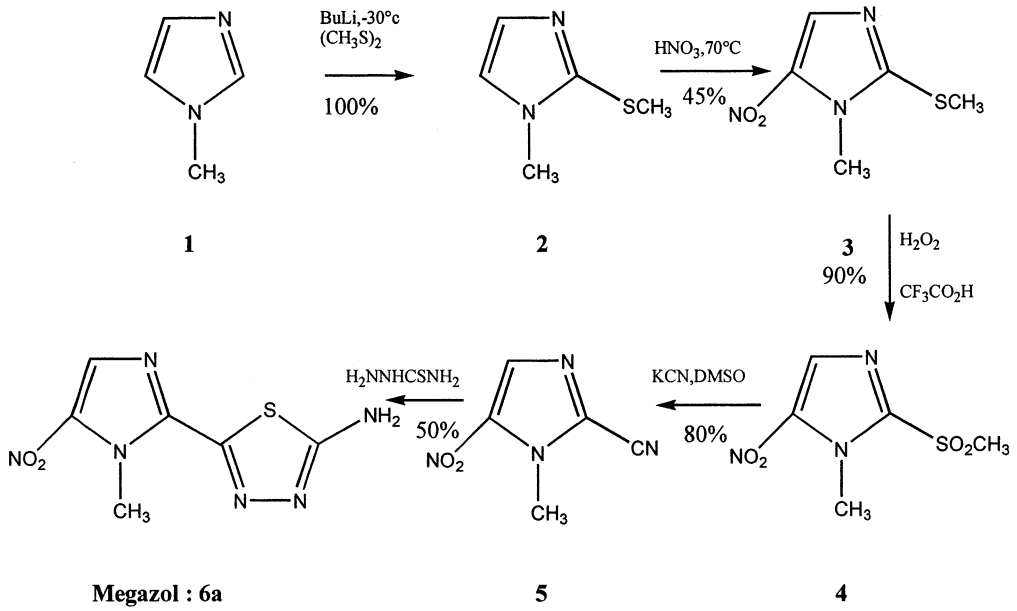


Figure 1. New procedure for megazol synthesis.

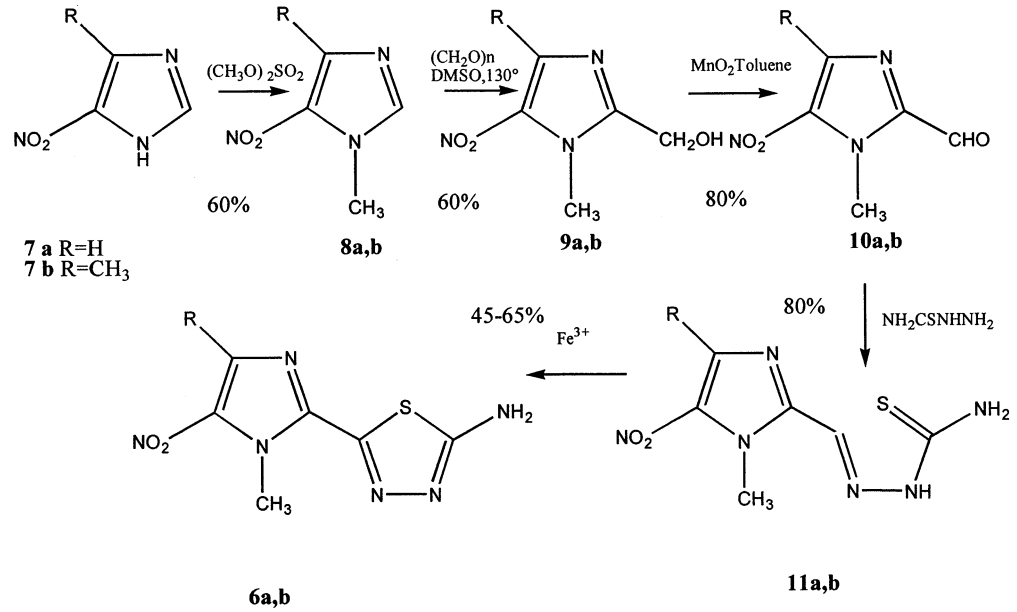


Figure 2. Synthesis of 4-methyl-megazol by the cyanamid procedure.

In the first step, the carbanion at position 2 of 1-methylimidazole **1** was quantitatively thiomethylated with dimethyl disulfide, giving **2**. **2** was then nitrated at 70 °C to the corresponding 5-nitroimidazole **3** (Figure 1). Oxidation by hydrogen peroxide leads nearly quantitatively to the sulfone **4** where a nucleophilic substitution by cyanide anion produces the corresponding carbonitrile **5** in good yield. Finally, a condensation with thiosemicarbazide in trifluoroacetic acid, further cyclization followed by loss of ammonia, and isomerization lead to megazol **6a**.

(2) Synthesis of Megazol Analogues Substituted at Position 4. These compounds were synthesized to determine the specificity of the nitro reductase reducing megazol into the corresponding radical anion and eventually the importance of the substitution at position 4 to the half-life of the latter.

(2.a) Synthesis of the 4 Me-Megazol. This compound was synthesized following the strategy given below (Figure 2), as in the first approach proposed for megazol **6a**:[14] the 4-(5)-Me, 5-(4)-nitroimidazole **7b** was first reacted with dimethyl sulfate, leading to **8b**; the hydroxymethylation at position 2 was then obtained with formaldehyde in DMSO using a sealed tube at 140 °C over 48 h. Oxidation of the corresponding alcohols **9a**,**b** into aldehydes **10a**,**b** was obtained by manganese dioxide in refluxing toluene; the thiosemicarbazone **11a**,**b** led then to the 4-substituted compound through an oxidative cyclization reaction in the presence of ferric ions.

(2.b) Synthesis of Other 4-Substituted Megazol Derivatives. A more general strategy (Figure 3) was developed starting from the dibromo derivative **12**.[15] After almost quantitative and selective methylation to **13** by action of diazomethane, the more reactive bromine

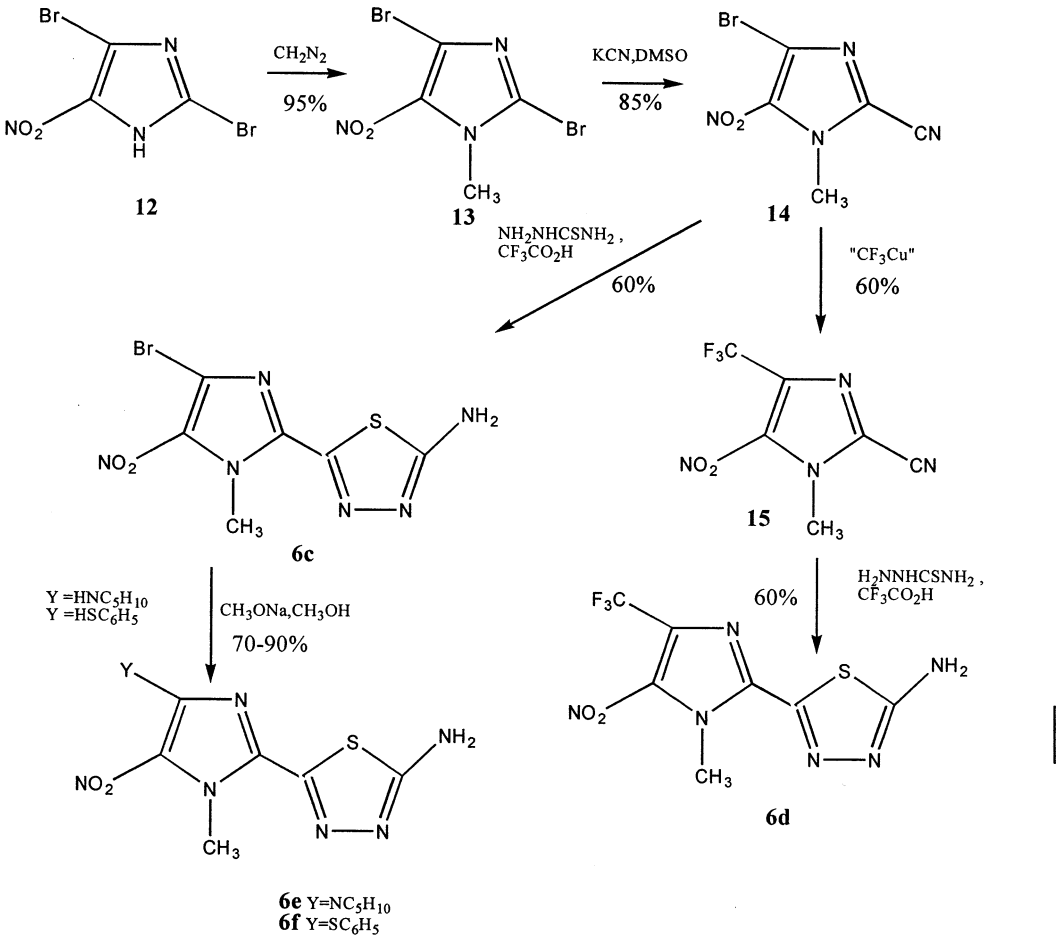


Figure 3. Synthesis of 4-substituted analogues of megazol.

at the 2 position could be selectively substituted by a cyano group.[16] The key compound **14** then led to different substituted derivatives. The reaction of thiosemicarbazide on **14** in trifluoroacetic acid led to the formation of the bromo derivative **6c**. Nucleophilic substitution of the bromine atom in **6c** allowed the introduction of two different electron-donating groups $C_5H_{10}N$ and C_6H_5S. These correspond to soft nucleophiles reacting on a soft center. To complete the study, an electron-withdrawing group CF_3 could be introduced by reacting a soft carbanion synthon of cuprous organometallic on bromoderivative **14**. Further reaction of thiosemicarbazide led to **6d**.

(3) Changes on the Two Heterocyclic Parts of the Reference Megazol Structure. To obtain insight into the importance of the different parts of the megazol frame, different syntheses were prepared.

(3.a) 4-Nitro Megazol. This was completed according to Figure 4 starting from *N*-methylimidazole **1** transformed into 2-cyano-*N*-methylimidazole **16** by action of cyanogen bromide. Further reaction of the thiosemicarbazide led to compound **17**, the nitration of which produced the corresponding 4-nitroimidazole **18**.

(3.b) Replacement of the Thiadiazole Part by an Oxadiazole Ring. This was performed by reaction of the cyanide ion on sulfone **4** (Figure 5). A subsequent reaction of semicarbazide on compound **5** as above led to oxadiazole **19**. This route also allowed the straightforward synthesis of compound **20**, the interest of which is discussed further in the paper.

(3.c) Replacement of the Nitroimidazole Part by a Nitrofurane or a Nitrothiophene. The corresponding syntheses are given on Figure 6. The starting materials were aldehydes **21a** and **21b**, which could be transformed into the corresponding heterocycles either by reaction of semicarbazide or thiosemicarbazide with oxidative cyclization of intermediate **22** or through the cyano intermediate **24** obtained from the corresponding oximes **23**.

(3.d) Replacement of the Extracyclic Amino Group. Through a Sandmeyer reaction, this amino group could be replaced by a chlorine atom (compound **26** in Figure 7). Nucleophilic substitution on the latter in the presence of sodium methylate produced compound **27**.

(4) Changes in Structure in Relationship to Possible Improvement of the Drug Uptake. Different strategies were considered to decrease the effective dose of the reference structure by improving the uptake into the cell.

(4.a) Strategy 1. This was done first by changing the amphiphilic balance of the reference compound through the amides **28a**−**d** (Figure 8) where the nature and the length of the chain were varied. In each case, the recovery of the drug from the prodrug implied the action of a peptidase. It has been shown[17] that these enzymes are abundant in the trypanosome cytosol and microsomes. In this respect also, the sensitivity of the chain length in the prodrug to peptidases was considered.

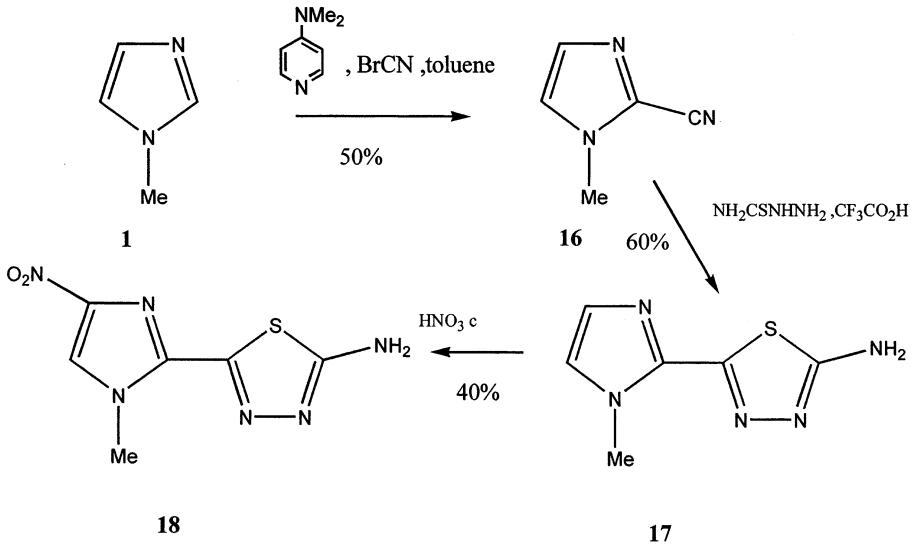


Figure 4. Synthesis of 4-nitro isomer of megazol.

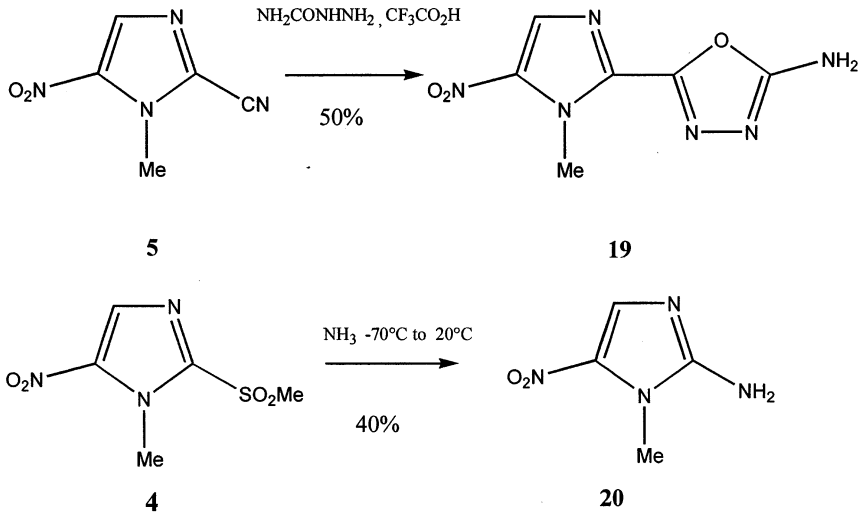


Figure 5. Replacement of thiadiazole ring in megazol by oxadiazole and synthesis of the 2-amino-*N*-methyl-5-nitroimidazole.

(4.b) Strategy 2. Synthesis of a Glucosamino Conjugate. It is known[18] that the African trypanosome relies exclusively on glucose for its energy supply and that the glucose uptake from the blood is ensured by a transporter; this transporter has been cloned and over-expressed.[19] A similar transporter also exists in the South American trypanosome *Trypanosoma cruzi*.[20] To investigate the possible improvement of megazol uptake into the cell via these transporters, the glucosamino conjugate **36** shown in Figure 9 was synthesized. This reaction involves the following steps: peracetylation of glucosamine by classical methods, introduction of a spacer by reaction of succinic anhydride on the latter, affording the intermediate **33**, activation of the acid function by isobutyl chloroformate, reaction on megazol, leading to compound **35**, and deprotection under catalytic conditions of the acetyl groups on the glucose moiety by sodium methylate. The overall yield of this synthesis starting from **29** is 15%.

(5) In Vitro Biological Assays. Three sets of assays were performed.

In the first part (Table 1) compounds were assayed in vitro against the bloodstream trypomastigote form of *Trypanosoma brucei*, the parasite that causes African trypanosomiasis. The purpose was to compare the activity of megazol to that of the reference compound suramin and also to determine a possible effect of the substitution at position 4.

In the second part (Table 2), the representative compounds were assayed against intracellular parasites on mouse peritoneal macrophages infected with either the amastigote form of *Trypanosoma cruzi* or the amastigote form of *Leishmania infantum*. In addition, the assay was also performed on the bloodstream trypomastigote form of another strain of *Trypanosoma brucei* (strain S427). This gave additional information on the ability of the corresponding compounds to cross different membranes.

In the third part (Table 3), all the compounds were assayed on other strains of the intracellular forms of *Trypanosoma cruzi* and *Leishmania donovani* and the activities of the synthesized compounds were compared to the reference drugs sodium stibogluconate for *L. donovani* and nifurtimox for *Trypanosoma cruzi*.

A number of features are shown in Table 1. First, a similar efficacy of megazol compared to the reference suramin is observed (twice in order of MEC values). Second, it is noticeable that substitutions at position 4

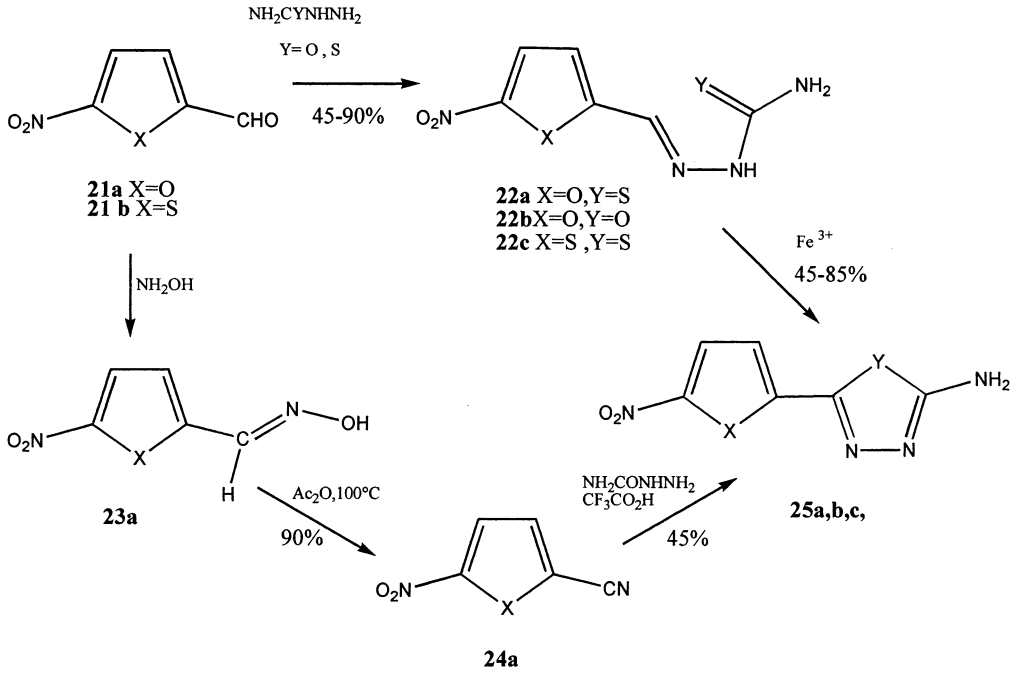


Figure 6. Route to analogues of megazol with nitrofurane and nitrothiophene in place of nitroimidazole.

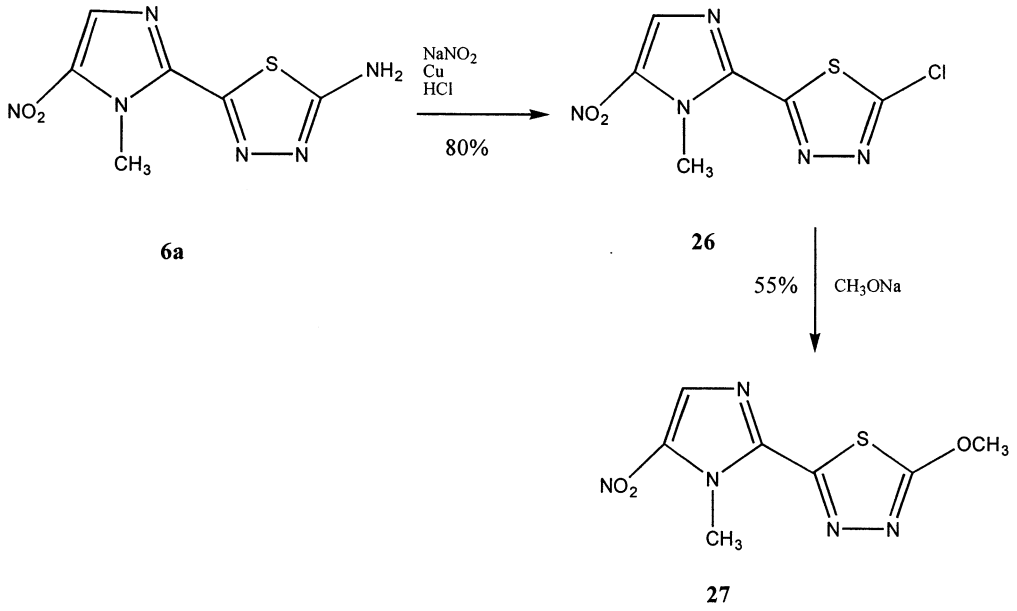


Figure 7. Substitutions of the amino group in the thiadiazole ring of megazol.

on the imidazole moiety either, by electron-donating or withdrawing substituents, reduced or abolished activity. Third, although compound **28a**, the N-acetylated form of megazol, behaves as a prodrug of this compound, this was not observed with the trifluoromethyl analogue, which had no activity.

Finally, the efficacy of the semicarbazone of nitrofurane **22b**, which had the same order of activity as suramin, and surprisingly the inactivity of the corresponding bicyclic analogue **25b** were determined.

Results presented in Tables 2 and 3 show that megazol appears to be the most active compound against *T. brucei*, *T. cruzi*, *Leishmania infantum*, and *L. donovani* with no cytotoxicity to macrophages at active concentrations. Similar results were obtained with the amide **28a**. The enzymatic hydrolysis of this prodrug seems to occur to a lower extent in *Leishmania* compared to the two other parasites, since the difference between **28a** and megazol is more pronounced with the former parasite. Other extra-ring NH_2-protected compounds (**28b**−**d**) show no activity likely because the corresponding protective groups were not hydrolyzed under the action of intracellular peptidases. Protection by an acetyl group as in **28a** corresponds therefore to the only possible precursor. However, this protection does not improve the import of the drug, since the free compound megazol can cross the two membranes of the infected macrophages where it was as active (100% inhibition at 1.5 μM) as against extracellular trypanosomes (1 μM).

Structural changes on the two heterocycles showed the following. (a) Replacement of the sulfur atom of megazol by an oxygen (compound **19**) suppresses activity, and also removing the nitro group (compound **17**)

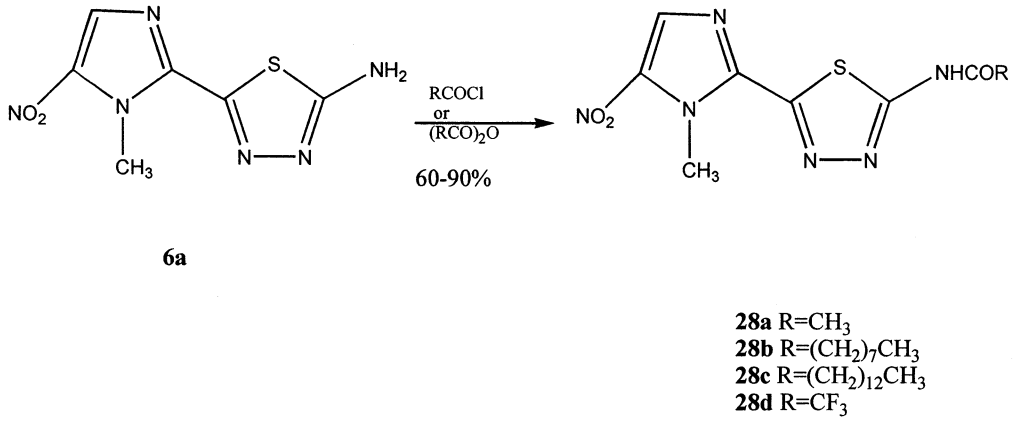


Figure 8. Synthesis of the alkylamides of megazol.

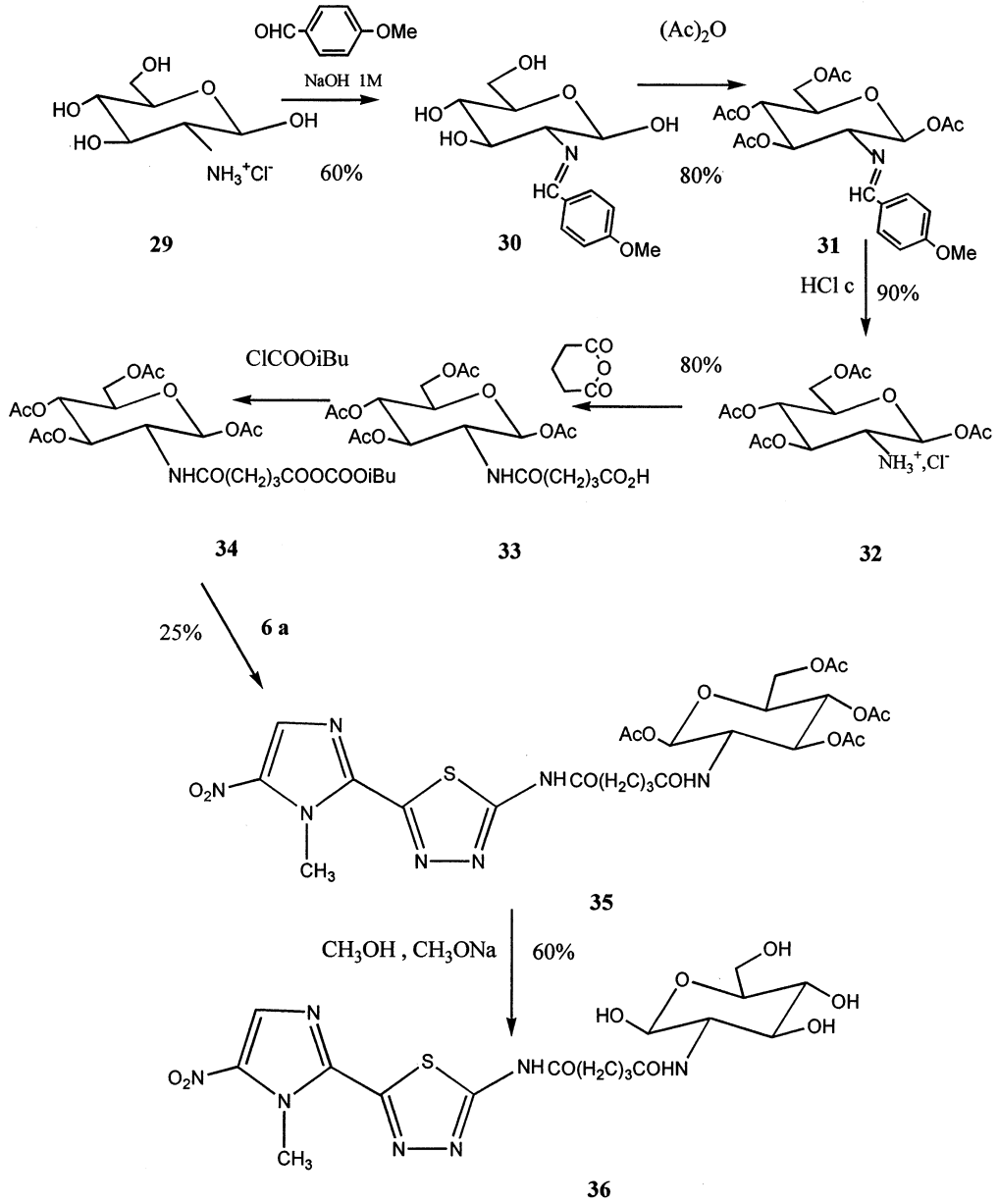


Figure 9. Synthesis of a glucoconjugate of megazol.

or locating the nitro group in position 4 (compound **18**) makes compounds totally inactive. (b) Some activity against *T. brucei* is conserved with sulfone **4**, but this is associated with significant cytotoxicity; other sulfones have been shown to be active, and their activity has been related to the inhibition of a cysteine proteinase.[21] (c) Surprisingly, the open structure **22b**, which combines a nitrofurane such as in nifurtimox and a carbazide

Table 1. In Vitro Activity on *Trypanosoma brucei* of Megazol Analogues

product	EC_{50}, μM	MEC, μM
suramine	0.02	0.8
megazol	0.04	0.4
6c (megazol−4-Br)	0.50	3.30
6b (megazol−4-Me)	>41	>41
6d (megazol−4-CF_3)	25	>34
4 (5-NO_2−2-SO_2Me	8	>49
22b	nd	5
25b	nd	>51
28a (megazol(NHCOCH_3))	0.20	1.60
28d (megazol(NHCOCF_3))	nd	31

Table 2. In Vitro Assays on Different *Trypanosomatidae* of the Main Analogues of Megazol

		% inhibition				
product	model[a]	25 μM	12.5 μM	6.2 μM	3.1 μM	1.5 μM
4	Tb				27	0
	Tc	T	0	0	0	
	Li	T	0	0	0	
	Ct	85	29		28	21
6a (megazol)	Tb			100	100	100
	Tc	99	99	99	99	
	Li	99	99	95	60	
	Ct	20	0	0		
6f (4-SC_6H_5)	Tb	0	0	0	0	
	Tc	80	60	0	0	
	Li	0	0	0	0	
	Ct	40	3	0		
22b	Tb			100	100	100
	Tc	99		40		
	Li	0		0		
	Ct	0	0	0	0	
22c	Tb		0	0	0	
	Tc	90		40		
	Li	40		0		
	Ct	0	0	0	0	
25a	Tb			100	89	0
	Tc			95		
	Li	40		0		
	Ct	4	0	0	0	
28a (NHCOCH_3)	Tb			100	100	70
	Tc	99	99	99	99	
	Li	40	40	0	0	
	Ct	0	0	0		
36	Tb			0	0	
	Tc	99	99	60	20	
	Li	0	0	0	0	
	Ct	0	0	0	0	

[a] Tb = *Trypanosoma brucei*; Tc = *Trypanosoma cruzi*; Li = *Leishmania infantum*; Ct = cytotoxicity; T = toxic for macrophage.

Table 3. In Vitro Activity on Intracellular Forms of *Trypanosoma cruzi* and *Leishmania donovani* of Megazol Analogues

		% inhibition					
product	model[a]	90 μM	30 μM	10 μM	3 μM	ED_{50}	ED_{50} ref
6a	Tc	100	99	99	98	20	10[b]
	Ld	99	75	6			
6e	Tc	41	3	0			
	Ld	0					
11a	Tc	99	93	71	54	3.30	3.50[c]
	Ld	20	0	0	0		

[a] Tb = *Trypanosoma brucei*; Tc = *Trypanosoma cruzi*; Ld = *Leishmania donovani*. [b] Reference is sodium stibogluconate. [c] Reference is Nifurtimox.

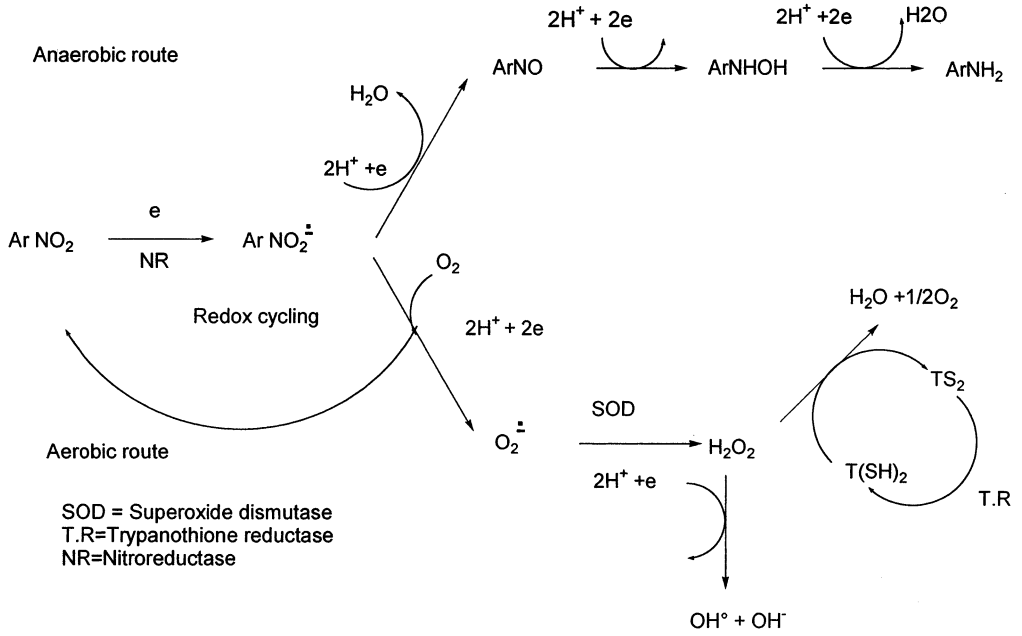
chain, appears as active as megazol on *T. brucei*, but the activity of this compound is lower against other parasites and the cyclic analogue with oxadiazole ring **25b** loses activity (see also Table 1). **25c** (megazol with a nitrothiophene in place of a nitroimidazole) is also inactive. (d) Compound **20** obtained from sulfone **4** has been prepared to evaluate the activity of the nitroimidazole part of megazol associated with the recognition motif NH_2−C=N− by the purine transporter.[13] This compound was totally inactive, which implies that the two rings in megazol are required for activity. (e) Suppressing the extra-cyclic amino group as in compound **27** strongly reduces activity (only active at 25 μM on *T cruzi* culture, with 90% inhibition). (f) The inactivity of the glucosaminoconjugate of megazol **36** suggests that this molecule is not internalized by the glucose transporter in *T. brucei*. Until now, only glucose and fructose derivatives with minor changes have been investigated in their affinities for this transporter[22] and little is known for complex molecules.

The glucose transporter in *T. cruzi* appears to be less specific, since an activity is noticed down to 3 μM concentration for the glucosaminoconjugate of megazol.

(6) In Vivo Assays with Megazol. (6.a) Assays on Mice. In vivo, a single injection of megazol at 20 mg kg^{-1} cured mice of acute *T. brucei brucei* AnTat 1.9 infection. A single dose of 80 mg kg^{-1}, given either intraperitoneally (ip) or per os (po), was effective 48 h after infection, when parasitemia levels were very high and infected mice were difficult to cure. This result confirms that of Winkelmann et al.[23] who cured mouse *T. brucei* infection with megazol at 25 mg kg^{-1} administered PO.

For subacute *T. brucei brucei* AnTat 1.1E infections in mice, megazol or suramin administered alone did not show any effect on the progression of the disease. In contrast, a combination treatment of megazol and suramin proved to be extremely effective. These results confirm the interest in testing drugs in combinations. If treatment is delayed until day 21 postinfection, suramin is totally ineffective, since the central nervous system (CNS) has become involved, and this drug is poorly transported across the blood−brain barrier.[24] In such animals, the effect of suramin is to eliminate the peripheral parasitemia followed by subsequent relapse, indicating that parasites persist in brain tissue during the aparasitemic period. Megazol alone, even at the highest doses (80 mg kg^{-1} d^{-1} for 4 consecutive days, given ip), like suramin, failed to act against the CNS infection. On day 100 postinjection, mice displayed several neurological signs: lack of coordination, equilibrium problems and disorientation with circling movements, altered motor function with difficulty in movement, and sometimes paralysis, somnolence, and rare convulsive fits. With a combination of suramin (20 mgkg^{-1} j^{-1} for 1 day, given ip) and megazol (80 mg kg^{-1} j^{-1} for 4 consecutive days, given ip or po), parasitemia quickly disappeared and all animals treated showed improvement of their general state from the first week after treatment. One case of relapse was noted following each mode of megazol administration. The surviving animals had no relapses 6 months after treatment. The results of histological examination of brain tissues removed at various times were as follows. In untreated mice, a severe meningitis and a perivasculitis with mild to strong infiltration of mononuclear cells in the parenchymal perivascular spaces were observed. From the second month after combined chemotherapy, the CNS

Scheme 1. Bioreduction Routes for Nitro Compounds



inflammatory lesions were completely resolved and the brain appeared histologically normal. These two animals with relapses presented a degree of inflammatory lesions similar to that of untreated animals. A regression of astrocytosis was also observed that seemed to be correlated with the presence of inflammatory lesions in the CNS depending on the time after treatment.[25]

(6.b) Assays on Monkeys. The efficacy of megazol was tested at the Centre International de Recherche Médicale (Franceville, Gabon) on vervet monkeys *Cercopithecus aethiops pygerethrus* infected intravenously with *T. b. gambiense* MBA (first isolated from a Zairian patient and kindly donated by D. Le Ray, Institute of Tropical Medicine Prince Leopold, Antwerpen, Belgique; unpublished results). The study consisted of two groups of animals: one group treated in the first stage of the disease with bloodstream infection only (5 vervets) with megazol alone (100 mg kg^{-1} j^{-1} for 1 day, given po from 18 to 53 days postinfection); one group treated during the second stage when CNS is involved with trypanosomes in the CSF (1 vervet, 69 days postinfection) with megazol (100 mg kg^{-1} j^{-1} for 1 day, given po) followed by suramin (20 mg kg^{-1} j^{-1} for 1 day, given ip). Biological and clinical parameters returned to normal within 2 weeks following treatment in both groups, and the posttherapeutic followup of these animals showed no relapse over 2 years.[26]

Discussion

The most salient features of this work are as follows.

First, for the synthesis of megazol, the proposed route represents a real improvement compared to the previous synthesis.[14] The introduction of a cyano substituent at position 2 via the sulfone **4** followed by reaction of thiosemicarbazine on the former and cyclization under acidic conditions makes this route more convenient than the previous reaction in a sealed tube. Also, the suppression of contaminant metal avoids environmental problems.

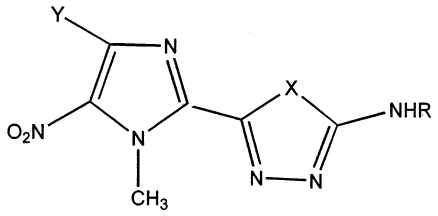
Second, of the different compounds investigated, unsubstituted megazol was the most active compound as long as the nitro group is at position 5, since the isomer **18** with the nitro group at position 4 is totally inactive. This point had previously been noticed for other nitroimidazoles when substituted on the imidazole ring at position 4.[8] The decreased activity after substitution on the reference structure also holds when changes are made on the thiadiazole ring. Substitution of sulfur by an oxygen abolishes activity; any substitution on the extra-cyclic amino group has the same consequence, except when this substitution is acetyl.

Two targets of these nitro compounds were examined: the detoxification system, since megazol induces an oxidative stress as shown from ESR experiments,[27] and fumarate reductase, an enzyme of the Krebs cycle.[11] Because this latter metabolic pathway is absent in *T. brucei*,[28] the efficacy of megazol via oxidative stress can be inferred.

The oxidative stress induced by megazol suggests a first reduction by a nitro reductase (Scheme 1). Several reductases were assayed in in vitro tests and k_{cat}/K_M values in the range of 2×10^2 to 5×10^3 M^{-1} s^{-1} were found.[27] Because the bimolecular rate constant for the reaction of the nitro radical anion with oxygen, as determined by pulse radiolysis,[29] is about 5×10^6 M^{-1} s^{-1}, it indicates that the entire process is controlled by the nitro reductase step. This enzyme has not been identified yet, but the results suggest that it should be sensitive to any structural change, since substitution at position 4 either abolishes or suppresses activity. Alternatively, the difference in biological efficacy of 4-substituted derivatives may rely on the change in structure of the compound that no longer would be planar owing to steric interactions between the substituent at position 4 and the nitro group. As we showed previously,[30] the most efficient compound in the series, megazol, has a planar structure, a feature that might be a requirement for delocalization and consequently for the optimized half-life of the nitro radical anion.

The loss of activity corresponding to the replacement of the sulfur atom by an oxygen (megazol and compound **19**) cannot be ascribed to a dramatic change in the

Table 4. Calculated log *P* Values of Megazol Analogues



	Y	X	R	log *P*
megazol		S	H	0.58
oxadiazol		O	H	−1.17
		S	$COCH_3$	0.56
		S	$COCF_3$	0.50
		S	$CO(CH_2)_{12}CH_3$	5.34
	CF_3	S	H	0.38
	C_6H_5S	S	H	1.72
	$(CH_2)_5N$	S	H	0.83

properties of the corresponding radical anion, since ESR studies indicated a behavior similar to that of megazol.[27] But the significant change in calculated log *P* values (see Table 4) indicates that the suppression of the biological activity of this compound may be due to the lack of import into the cell. It is indeed generally considered that passive diffusion of compounds into cells is suppressed for compounds with negative log *P* values.[31]

The substitution on the amino group indicated that only the acetylated derivative gave activity, although lower than that of the reference compound. This implies that the active form has to bear a free amino group and that the acetyl group may first have to be hydrolyzed by cytosolic peptidases. Increasing the lipophilic character by linking an aliphatic chain (see Table 4) to this amino group is irrelevant. The inactivity of compounds **28b** and **28c** indicates that the corresponding alkyl chains are not enzymatically hydrolyzed. It was alternatively considered that this amino group should be free for recognition of the compound by the purine transporter. Previous work showed that adenine/adenosine moiety and compounds such as melarsoprol are recognized by their $C{=}N{-}NH_2$ frame.[13]

We showed in another work using tritiated megazol that this moiety is not necessary, since although uptake can be via this purine transporter, a large part of the compound is internalized via passive diffusion.[32]

As far as the glucose transporter is concerned, although the glucosaminoconjugate of megazol **36** exhibits a significant affinity for the glucose transporter (K_i = 0.80 mM against K_M = 0.94mM for glucose[22]), the glucosamino conjugate showed no activity, possibly because of lack of uptake. This situation bears some similarity to results obtained with cancer cells where glucoconjugates of different anticancer agents exhibit a strong affinity for the glucose transporter but no activity on the cell.[33]

The last comment concerning in vitro assays is related to the differences observed in the *T. brucei* and *T. cruzi* cultures with compounds such as **6f**, **36** (Table 2), and **17** (Table 3). Although no efficacy is observed in the former, an activity was found on *T. cruzi* cultures. This result is particularly significant for compound **17** deprived of the nitro group. This result could relate to the observation made by Turrens et al.[11] in the identification of fumarate reductase as target in *T. cruzi* for different heterocycles including those without nitro substituents. The in vivo assays on mice and subsequently on monkeys showed that combined therapy of megazol with suramin was highly effective in the treatment of experimental *T. brucei* and *T. gambiense* infections with CNS involvement.

The efficacy of treatment of experimental HAT in mice by a combination of melarsoprol with suramin has been reported.[34] Combination therapy in this case enabled the treatment of trypanosomiasis with CNS involvement, whereas it has been shown that treatment with either of the drugs alone is not effective at this stage of the disease.[35] So far, no treatment has been suggested for treating advanced stages of *T. brucei* infections when a high perivascular infiltration of mononuclear cells invades the CNS associated with neurological disorders such as those observed in patients with sleeping sickness.[36]

Therefore, the combined therapy of sleeping sickness at the CNS infection stage by megazol and suramin as a nontoxic alternative to previous treatments should be considered.

In this regard, toxicity and mutagenicity studies are presently being performed before clinical trials are considered.

Experimental Section

Biological Assays. Results in Table 1 were determined at the Institute of Neurologic Epidemiology and Tropical Neurology of Limoges Medical Faculty, for *Trypanosoma brucei brucei* AnTat 1−9 maintained in cell-free conditions as described previously[4] and in a density of 10^5 trypanosomes/mL. The compounds were tested in 10-fold dilutions covering final concentrations from 50 to 0.01 μM. Each dilution and control was tested in 16 replicates. The plates were incubated for 48 h (37 °C, CO_2 = 5% in air). Results are expressed as either 50% effective concentration EC_{50} or 100% minimal effective concentration (MEC_{100}), the drug concentration that kills 100% of the population within 48 h.

The results in Table 2 were obtained by Dr. L. Maes at the Janssen Research Foundation in the Department of Parasitology.

For trypomastigotes of *Trypanosoma brucei* strain S427, they were derived from axenic culture in HMI-18 medium, incubated at different drug levels in HMI medium in a 96-microwell tissue culture plate. Parasite multiplication was assessed after 3 days of incubation at 37 °C in 5% CO_2. Drug activity was semiquantatively scored as the percent reduction of parasite multiplication compared to untreated control cultures. Scoring was performed either microscopically or colorimetrically.

For trypomastigotes of *Trypanosoma cruzi* strain Tulahen CL2, primary mouse peritoneal macrophages were seeded in 96-well microplates at 30 000 cells/well. After 24 h, about 100 000 trypomastigotes of *T. cruzi* were added per well, together with 2-fold dilutions of the drug. The cultures were incubated at 37 °C in 5% CO_2 for 4 days. To cope with the problem of a specific toxicity, noninfected cultures were treated likewise to enable the calculation of a selectivity index. Drug activity was semiquantitavely scored as the percent reduction of the total parasite load (free trypomastigotes and intracellular amastigotes) compared to untreated control cultures. Scoring was determined microscopically.

For amastigotes of *Leishmania infantum* (MOHM/MA BE/67), primary mouse peritoneal macrophages were seeded in 16-well Labtek culture slides at 30 000 cells per well. After 24 h, amastigotes of *L. infantum* (derived from the spleen of an infected donor animal) were added at an infection ratio of 10/1 together with 2-fold dilutions of the drug. The cultures were incubated at 37 °C in 5% CO_2 for 5 days. Treatment of

uninfected control cultures was also included to determine a selectivity index. Drug activity was semiquantitavely scored as the percentage reduction of the total parasite load or the number of infected macrophages in Wright stained preparations. Scoring was performed microscopically.

Cytotoxicity. MRC-5 cells were seeded in 96-well microtiterplates. Drugs were added in 2-fold dilutions. The cultures were incubated at 37 °C in 5% CO_2 for 7 days. Cytotoxicity was photometrically determined as the percent reduction of extinction after addition of MTS in treated cultures compared to untreated control cultures.

The results in Table 3 were carried out in the Department of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine.

For *Leishmania donovani* (MHOM/ET/67/L82), amastigotes, derived from hamster spleen, were used to infect mouse intraperitoneal macrophages in Labteck chamber slides. Infected macrophages were maintained in the presence of drugs in the range 90−3 μM in quadruplicate cultures for 7 days at 37 °C. Drug activity was measured from a percentage of macrophages cleared of amastigotes in treated cultures. Pentostam (sodium stibogluconate) was used as a positive control.

For trypomastigotes of *Trypanosoma cruzi* (MHOM/BR/OO/Y) derived from rat, L6 myoblast cultures were used to infect mouse peritoneal macrophages in medium containing drugs from 90 to 3 μM for 72 h at 37 °C. Drug activity was determined from the percent of infected macrophages in treated cultures. Nifurtimox was used as a positive control.

The standard deviation for all results given in Tables 1−3 is ±3%.

Synthesis. Melting points were determined on an Electrothermal capillary melting point apparatus and were uncorrected.

^{1}H and ^{13}C NMR spectra were recorded respectively on Brucker AC80 (80 MHz) and AC200 (50 MHz) spectrometers. All chemical shifts are reported in ppm relative to TMS as internal standard. Mass spectra were run on a Nermag R1010 spectrometer at Paul Sabatier University, Toulouse, France. All chemicals were purchased from Aldrich or Fluka and were used without additional purification.

DMF and DMSO were dried by stirring over 4 Å molecular sieves. THF was distilled over sodium in the presence of the benzophenone ketyl as indicator prior to use.

Reactions and column chromatographic separations were followed by thin-layer chromatography using silica gel (with 254 nm fluorescent indicator) on alumina plates.

1-Methyl-2-(methylsulfanyl)-1*H*-imidazole (2). Butyllithium 1.6 M (55 mL) was slowly added (10 min) by syringe through a septum cap to a 1-methylimidazole (6.56 g, 80 mmol) solution in THF (60 mL) at −30 °C. After 10 min at this temperature, dimethyl disulfide was added by syringe (9 mL, 90 mmol). The yellow, creamy precipitate formed was magnetically agitated for an hour at room temperature. After vacuum evaporation of THF, water (30 mL) was added to the residue, which was extracted by dichloromethane (3 × 50 mL). After the mixture was dried with magnesium sulfate and evaporation, **2** was obtained as a crude oil (10.5 g, 100% yield) and nitrated directly without further purification. ^{1}H NMR-($CDCl_3$) δ 2.53 (s,3H,SCH_3), 3.53 (s,3H,NCH_3), 6.84 (d,1H,J = 1.5 Hz), 6.98 (d,1H,J = 1.5 Hz); ^{13}C NMR ($CDCl_3$) δ 16.1 (SCH_3), 32.8 (NCH_3), 122.0 (C5), 128.7 (C4), 142.8 (C2); mass spectrum (DCI/NH_3) m/z 129 (M^+ + 1, 100), 146 (M^+ + 18, 30).

1-Methyl-2-(methylsulfanyl)-5-nitro-1*H*-imidazole (3). **2** (10.5 g, 80 mmol) was added slowly by dropping funnel to 69% nitric acid (30 mL) at 70 °C, and the mixture was maintained for 1 h. The mixture was then poured in cold water and neutralized with saturated $NaHCO_3$ aqueous solution. The yellow precipitate formed was extracted with dichloromethane (3 × 30 mL). The organic phase dried with magnesium sulfate gave, after evaporation, a yellow solid purified by flash chromatograghy on silica gel (CH_2Cl_2), giving pure **3** (6.2 g, 45% yield). Mp 285 °C; ^{1}H NMR ($CDCl_3$) δ 2.71 (s, 3H, SCH_3), 3.85 (s, 3H, NCH_3), 7.97 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 14.4 (SCH_3), 33.64 (NCH_3), 133.6 (C4), 151.8 (C2) (C5, no apparent); mass spectrum (DCI/NH_3) m/z 174 (M^+ + 1, 100).

1-Methyl-2-(methylsulfonyl)-5-nitro-1*H*-imidazole (4). To **3** (6.2 g,36 mmol) in trifluoroacetic acid (5 mL) was added hydrogen peroxide (30%,5 mL). The mixture was stirred for 1 h at 40 °C, then cooled and neutralized with saturated $NaHCO_3$ aqueous solution. After extraction with CH_2Cl_2 (2 × 20 mL) and the usual workup, **4** (6.6 g, 90% yield) was obtained. ^{1}H NMR ($CDCl_3$) δ 3.45 (s, 3H, SO_2CH_3), 4.28 (s, 3H, NCH_3), 7.93 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 35 (SO_2CH_3), 42.25 (NCH_3), 130.4 (C4), 146.4 (C2) (C5, not apparent); mass spectrum (DCI/NH_3) m/z 206 (M^+ + 1, 100), 223 (M^+ + 18, 25).

1-Methyl-5-nitro-1*H*-2-imidazolecarbonitrile (5). Finely ground potassium cyanide (2.1 g,30 mmol) in DMSO (10 mL) mixed with **4** (5.4 g, 27 mmol) was stirred at 80 °C for a half-hour.The mixture was poured into ice−water (50 mL) and extracted with dichloromethane (3 × 20 mL). The crude solid obtained after evaporation was purified by flash chromatography (petroleum ether/CH_2Cl_2, 1/1) giving pure **5** (3.28 g, 80% yield). ^{1}H NMR ($CDCl_3$) δ 4.19 (s, 3H, NCH_3), 8.03 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 35.7 (NCH_3), 109.2 (CN), 125 (C2), 132 (C4) (C5, not apparent); IR 2246 cm^{-1} (CN); mass spectrum (DCI/NH_3) m/z 153 (M^+ + 1, 100), 170 (M^+ + 18, 40).

5-(1-Methyl-5-nitro-1*H*-2-imidazolyl)-1,3,4-thiadiazol-2-amine (6a). Megazol (Original Synthesis). Thiosemicarbazide (2 g,22 mmol) mixed with **5** (3.28 g,22 mmol) was heated in trifluoroacetic acid (10 mL) at 60 °C for 15 h. The reaction medium was poured into ice−water (20 mL) and neutralized with saturated $NaHCO_3$ aqueous solution. The yellow precipitate formed was filtered on a sintered glass filter, washed with water (2 × 5 mL), and dried under vacuum to give **6a** after recrystallization in acetone (2.5 g, 50% yield). Mp 270 °C; ^{1}H NMR (DMSO-d_6) δ 4.32 (s, 3H, NCH_3), 7.8 (broad (b), 2H, NH_2), 8.2 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 35.00 (s, NCH_3), 133.1 (C4), 140.1 (C5), 141.4 (C2), 48.2 (C2*), 169.9 (C5*); mass spectrum (EI) m/z 227 (M^+ + 1, 80). Anal. ($C_6H_6N_6O_2S$) C, H, N.

5-(1,4-Dimethyl-5-nitro-1*H*-2-imidazolyl)-1,3,4-thiadiazol-2-amine (6b). 11b (2.1 g, 8.8 mmol) and ammonium iron-(III) sulfate (17.35 g, 36 mmol) were stirred in refluxing water (200 mL) for 5 h. The solid formed was filtered and washed with water. **6b** was obtained (1.3 g, 60% yield) after recrystallization (acetone/DMF, 1:1). Mp 285 °C; ^{1}H NMR (DMSO-d_6) δ 2.09 (s, 3H, CH_3), 4.29 (s, 3H, NCH_3), 7.82 (s, 2H, NH_2); ^{13}CNMR (DMSO-d_6) δ 15.75 (CH_3), 35.37 (NCH_3), 136.77 (C4), 139.20 (C2), 143.93 (C2*), 148.09 (C5), 169.9 (C5*); mass spectrum (EI) m/z 241 (M^+ + 1). Anal. ($C_7H_8N_6O_2S$) C, H, N.

4-Methyl-5-nitro-1*H*-imidazole (7b). To a mixture of anhydrous nitric acid (8 mL) and concentrated sulfuric acid (8 mL) was added at 0 °C 4-methylimidazole (3.2 g, 38 mmol). After 30 min at room temperature, the mixture was poured onto ice. After neutralization with sodium hydroxide, the yellow precipitate formed was filtered,washed with cold water (10 mL), recrystallized in water, and dried under vacuum. An amount of 4.4 g of **7b** was obtained (90% yield). Mp 260 °C; ^{1}HNMR (acetone-d_6) δ 2.69 (s, 3H, CH_3), 7.59 (s, 1H), 10.13 (s, 1H); ^{13}C NMR (acetone-d_6) δ 11.6 (CH_3), 133.7 (C2), 139.9 (C4) (C5, not apparent); mass spectrum (DCI/NH3) m/z 128 (M^+ + 1, 100)

1-Methyl-5-nitro-1*H*-imidazole (8a). 4-Nitroimidazole (5.4 g, 48 mmol) and dimethyl sulfate (5.3 mL) were heated in refluxing dioxane (20 mL) for 2 h. The solvent was then evaporated under vacuum, and the oily acidic residue was neutralized by a $NaHCO_3$ saturated aqueous solution. The solid formed and the aqueous solution were extracted with dichloromethane (4 × 20 mL). After the mixture was dried with magnesium sulfate and after evaporation of the organic phase, crude **8a** was obtained (3.8 g, 62% yield) and used without further purification. Mp 60 °C; ^{1}H NMR ($CDCl_3$) δ 3.93 (s, 3H, NCH_3), 7.5 (s, 1H), 7.87 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 35.2 (NCH_3), 133.07 (C4), 141.7 (C2) (C5, not apparent). Anal. ($C_4H_5N_3O_2$) C, H, N.

1,4-Dimethyl-5-nitro-1*H*-imidazole (8b). Starting from **7b** (4.4 g, 31 mmol), the same procedure as for **8a** was used

and **8b** was obtained (2.65 g, 60% yield). Mp 210 °C; ^{1}H NMR ($CDCl_3$) δ 2.58 (s, 3H, CH_3), 3.94 (s, 3H, NCH_3), 7.44 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 16.24 (CH_3), 36.2 (NCH_3), 139.5 (C2), 143.9 (C5), 145.15 (C4); mass spectrum (DCI/NH_3) *m*/*z* 142 ($M^+ + 1$, 100).

(1-Methyl-5-nitro-1*H*-2-imidazolyl)methanol (9a). In a sealed glass tube, paraformaldehyde (3.6 g, 120 mmol) and **8a** (3.8 g, 28 mmol) in DMSO solution (20 mL) were introduced. The tube was heated at 140 °C for 48 h. Subsequently the solution was poured into water (200 mL) and extracted with ethyl acetate (3 × 50 mL). After the mixture was dried ($MgSO_4$) and after evaporation of the solvent under vacuum, the solid was recrystallized (THF/heptane, 1:1) and **9a** was isolated (2.63 g, 60% yield). Mp 110 °C; ^{1}H NMR (DMSO-d_6) δ 3.9 (s, 3H, NCH_3), 4.5 (d, 2H, CH_2, J = 6 Hz), 5.6 (t, 1H, OH, J = 6 Hz), 7.9 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 33.2 (NCH_3), 56 (CH_2), 131.4 (C4), 139.1 (C5), 152.3 (C2). Anal. ($C_5H_7N_3O_3$) C, H, N.

(1,4-Dimethyl-5-nitro-1*H*-2-imidazolyl)methanol (9b). Starting with **8b** (2.65 g, 18.6 mmol), the same procedure as for **9a** was applied with paraformaldehyde (2.4 g,80 mmol), and **9b** was obtained (2.20 g, 70% yield). Mp 110 °C; ^{1}H NMR (DMSO-d_6) δ 2.4 (s,3H,CH_3), 3.87 (s, 3H, NCH_3), 4.53 (d, 2H, CH_2, J = 6 Hz), 5.6 (t, 1H, OH, J = 6 Hz); ^{13}C NMR (DMSO-d_6) δ 15.53 (CH_3), 33.63 (NCH_3), 55.57 (CH_2OH), 140.6 (C2), 142.6 (C5), 149.93 (C4). Anal. ($C_6H_9N_3O_3$) C, H, N.

1-Methyl-5-nitro-1*H*-2-imidazolcarbaldehyde (10a). 9a (2.6 g,17 mmol) in toluene (50 mL) and activated manganese dioxide (7.2 g, 85 mmol) were refluxed for 4 h. After filtration, the mixture was evaporated. The residue was purified by flash chromatography (CH_2Cl_2) and gave **10a** (2.1 g, 80% yield). Mp 86 °C; ^{1}H NMR ($CDCl_3$) δ 4.4 (s, 3H, NCH_3), 8.1 (s,1H), 9.9 (s, 1H, CHO); ^{13}C NMR ($CDCl_3$) δ 34.3 (NCH_3), 132.7 (C4), 143.1 (C2), 163.7 (CHO) (C5, not apparent). Anal. ($C_5H_5N_3O_3$) C, H, N.

1,4-Dimethyl-5-nitro-1*H*-2-imidazolcarbaldehyde (10b). 9b (2.20 g, 13 mmol) in toluene (50 mL) was refluxed with manganese dioxide (5.6 g, 65 mmol) for 3 h. After filtration of the manganese salt, toluene evaporation, and recrystallization (hexane), **10b** was obtained (1.43 g, 80% yield). Mp 115 °C; ^{1}H NMR ($CDCl_3$) δ 2.62 (s, 3H, CH_3), 4.3 (s, NCH_3), 9.89 (s, 1H, CHO); ^{13}C NMR (acetone-d_6) δ 15.93 (CH_3), 34.63 (NCH_3), 130.04 (C4), 132.2 (C2), 183.65 (CHO) (C5, not apparent). Anal. ($C_6H_7N_3O_3$) C, H, N.

2-[(*E*)-1-(1-Methyl-5-nitro-1*H*-2-imidazolyl)methylidene]-1-hydrazinecarbothioamide (11a). 10a (2g, 13 mmol) and thiosemicarbazide (1.42 g, 15 mmol) were stirred in DMSO (10 mL) at room temperature for 16 h. The mixture was poured into water (50 mL) and a yellow precipitate was formed, filtered, washed with dichloromethane, and recrystallized (ethyl acetate/acetone, 1:1) to afford **11a** (2.6 g, 11 mmol). Mp 240 °C; ^{1}H NMR (DMSO-d_6) δ 4.15 (s, 3H, NCH_3), 8.11 (s, 1H, CH=N), 7.75−8.54 (b, 2H, NH_2), 8.2 (s, 1H, H4), 11.8 (s, 1H, NH). In (DMSO-d_6/D_2O), signals 7.15−8.54 (b, 2H, NH_2) and 11.8 (s, 1H, NH) disappear. ^{13}C NMR (DMSO-d_6) δ 35.07 (NCH_3),133.3 (CH=N), 133.4 (C4), 140.2 (C5), 144.6 (C2), 178.4 (C=S); mass spectrum (EI) *m*/*z* 229 ($M^+ + 1$, 80). Anal. ($C_6H_8N_6O_2S$) C, H, N.

5-(1-Methyl-5-nitro-1*H*-2-imidazolyl)-1,3,4-thiadiazol-2-amine (6a). Megazol (14). 11a (2.6 g, 11 mmol) and ammonium iron(III) sulfate (21g, 44 mmol) in water (200 mL) were stirred and refluxed for 5 h. The solid formed was filtered, washed with water, and gave **6a** (1.1 g, 40% yield). Analytical data are given above.

2-[(*E*)-1-(1,4-Dimethyl-5-nitro-1*H*-2-imidazolyl)methylidene]-1-hydrazinecarbothioamide (11b). The procedure was similar to that for **11a**, using **10b** (1.43 g,11 mmol) and thiosemicarbazide (1.14 g, 12 mmol) in DMSO (10 mL). After 16 h of reaction, hydrolysis, filtration, and recrystallization (ethanol), **11b** was obtained (2.1 g, 80% yield). Mp 255 °C; ^{1}H NMR (DMSO-d_6) δ 2.5 (s, 3H, CH_3), 3.35 (b, 2H, NH_2), 4.11 (s, 3H, NCH_3), 8.08 (s, 1H, CH=N), 11.8 (s, 1H, NH). The 3.35 and 11.8 signals disappear after adding D_2O. ^{13}C NMR (DMSO-d_6) δ 15.62 (CH_3), 35.45 (NCH_3), 133.17 (C4), 142.56 (C2), 143.9 (C5), 178.3 (C=S); mass spectrum (EI) *m*/*z* 243 ($M^+ + 1$, 100). Anal. ($C_7H_{10}N_6O_2S$) C, H, N.

2,4-Dibromo-5-nitro-1*H*-imidazole (12). 12 was prepared as described in ref 37 by adding bromine to 5-nitroimidazole. ^{13}C NMR (DMSO-d_6) δ 126.1 (C4), 120.0 (C2) (C5 not apparent).

2,4-Dibromo-1-methyl-5-nitro-1*H*-imidazole (13). 12 (2.7 g,10 mmol) in ether (20 mL) was added to diazomethane (12 mmol) in ether solution at 20 °C and left for 1 h at room temperature. After the mixture was dried (magnesium sulfate) and after evaporation of ether, **13** was obtained (2.7 g, 95% yield) as a yellow solid that was used without further purification. Mp 160 °C; ^{1}H NMR ($CDCl_3$) δ 4.0 (s,CH_3); ^{13}C NMR ($CDCl_3$) δ 37.6 (NCH_3), 119.6 (C4), 126.0 (C2), 137.1 (C5); mass spectrum (DCI/NH_3) *m*/*z* 284 ($M^+ + 1$, 52), 286 ($M^+ + 3$, 100), 288 ($M^+ + 5$, 50).

4-Bromo-1-methyl-5-nitro-1*H*-2-imidazolecarbonitrile (14). 13 (2.85 g, 10 mmol) was mixed with finely ground potassium cyanide (0.7 g, 11 mmol) in DMSO (10 mL) and heated for 2 h at 80 °C. The solution was poured into water (100 mL) and extracted with dichloromethane (3 × 20 mL). The solid obtained after drying ($MgSO_4$) the organic phase and after solvent evaporation was purified by flash chromatography (ethyl acetate/hexane, 3:1) to give pure **14** (2 g, 85% yield). Mp 101 °C; IR 2247 cm^{-1} (CN); ^{1}H NMR ($CDCl_3$) δ 4.18 (s, NCH_3); ^{13}CNMR ($CDCl_3$) δ 37.3 (NCH_3), 108.2 (CN), 120.1 (C4), 124.0 (C2), 137.1 (C5); mass spectrum (EI) *m*/*z* 230 (M^+, 20), 232 ($M^+ + 2$, 20). Anal. ($C_5H_3BrN_4O_2$) C, H, N.

1-Methyl-5-nitro-4-(trifluoromethyl)-1*H*-2-imidazolecarbonitrile (15). 15 was synthesized from **14** with "CF_3Cu" generated by the Burton process as previously described.[15]

5-(4-Bromo-1-methyl-5-nitro-1*H*-2-imidazolyl)-1,3,4-thiadiazol-2-amine (6c). 6c was prepared from **14** with thiosemicarbazide in trifluoroacetic acid, similar to the preparation of **6a** from **5**, described above.

5-[-1-Methyl-5-nitro-4-(trifluoromethyl)-1*H*-2-imidazolyl]-1,3,4-thiadiazol-2-amine (6d). 6d was prepared from **15**, similar to preparation described for **6c** previously.[15]

5-(-1-Methyl-5-nitro-4-piperidino-1*H*-2-imidazolyl)-1,3,4-thiadiazol-2-amine (6e). 6e was synthesized from **6c** with piperidine in the presence of sodium methylate, as previously described.[15]

5-[-1-Methyl-5-nitro-4-(phenylsulfanyl)-1*H*-2-imidazolyl]-1,3,4-thiadiazol-2-amine (6f). 6f was synthesized from **6c** with thiophenol, as previously described.[15]

1-Methyl-1*H*-2-imidazolecarbonitrile (16). 4-*N*,*N*-Dimethylaminopyridine (6.1 g, 50 mmol) and cyanogen bromide (5.3 g, 50 mmol) in DMF (20 mL) under argon at room temperature generated a yellow precipitate of the cyanoaminopyridinium salt to which was added by syringe 1-methylimidazole (1.7 mL, 20 mmol). After 15 h of being stirred, the mixture was poured into a $NaHCO_3$ saturated solution (100 mL) and extracted with dichloromethane (4 × 25 mL). After evaporation of the organic phase and flash chromatography (CH_2Cl_2), **16** as a brown oil (1.1 g, 50% yield) was obtained. ^{1}H NMR ($CDCl_3$) δ 3.85 (s, 3H, NCH_3), 7.06 (d, 1H, H5, J = 1.5 Hz), 7.16 (d, 1H, H4, J = 1.5 Hz); ^{13}C NMR ($CDCl_3$) δ 34.1 (CH_3N), 110.9 (CN), 124.4 (C5), 131.6 (C4) (C2 not apparent); IR 2235 cm^{-1} (CN); mass spectrum (EI) *m*/*z* 108 ($M^+ + 1$, 20).

5-(1-Methyl-1*H*-2-imidazolyl)-1,3,4-thiadiazol-2-amine (17). 16 (0.55 g, 5 mmol) mixed with thiosemicarbazide (0.5 g, 5 mmol) and heated at 60 °C in trifluoroacetic acid (5 mL) gave **17** (0.55 g, 60% yield) after similar treatment of the reaction mixture as above for **6a**−**f** . ^{1}H NMR (DMSO-d_6) δ 3.96 (s, 3H, NCH_3), 6.00 (d, 1H, J = 1.5 Hz), 7.3 (d, 1H, J = 1.5 Hz); ^{13}C NMR (DMSO-d_6) δ 34.7 (NCH_3), 124.7 (C5), 128.4 (C4), 137.7 (C2), 149.8 (C2′), 168.2 (C5′); mass spectrum (EI) *m*/*z* 182 ($M^+ + 1$, 20). Anal. ($C_6H_7N_5S$) C, H, N.

5-(1-Methyl-4-nitro-1*H*-2-imidazolyl)-1,3,4-thiadiazol-2-amine (18). 17 (0.36 g, 2 mmol) was added to a sulfonitric solution (2 mL of H_2SO_4 (conc), 2 mL of anhydrous HNO_3) at 0 °C. After half an hour, the mixture was poured into ice (5 mL). The yellow precipitate formed was filtered, washed with water (5 mL), and dried under vacuum, giving **18** (0.2 g, 40%

yield). Mp 265 °C; ^{1}H NMR (DMSO-d_6) δ 4.39 (s, 3H, NCH_3), 7.2 (NH_2), 8.21 (s,1H); ^{13}C NMR (DMSO-d_6) δ 35.0 (NCH_3), 133.3 (C5), 140.2 (C4), 142.4 (C2), 153.5 (C2′), 171.5 (C5′); mass spectrum (EI) *m/z* 227 ($M^+ + 1$, 10). Anal. ($C_6H_6N_6O_2S$) C, H, N.

5-(1-Methyl-5-nitro-1*H*-2-imidazolyl)-1,3,4-oxadiazol-2-amine (19). 1-Methyl-2-cyano-5-nitroimidazole **5** (600 mg, 4 mmol) and semicarbazide hydrochloride (500 mg, 4.5 mmol) were mixed in 10 mL of trifluoroacetic acid and refluxed at 70 °C for 15 h. Then the solution was poured into ice−water (10 mL) and carefully neutralized with $NaHCO_3$ saturated solution. The yellow solid formed was filtered and washed with ether and water. After crystallization (ethanol/water, 2:1), **19** was obtained (420 mg, 50% yield). Mp 285 °C; ^{1}H NMR (DMSO-d_6) δ 4.25 (s,NCH_3), 7.94 (s,NH_2), 8.11 (s,H4); ^{13}C NMR (DMSO-d_6) δ 34.53 (NCH_3), 130.65 (C4), 140.05 (C5), 141.34 (C2), 157.0 (C2′), 159.77 (C5′). Anal. ($C_6H_6N_6O_3$) C, H, N.

1-Methyl-5-nitro-1*H*-2-aminoimidazole (20). The sulfone **4** (205 mg, 1 mmol) was mixed at −70 °C with gaseous ammonia condensed in the reaction flask by a Dewar condenser. After 2 h, the medium was warmed to room temperature. After ammonia evaporation, the yellow solid formed was extracted with ethyl acetate (2 × 10 mL), dried ($MgSO_4$), recovered after solvent evaporation, and purified by flash chromatography (CH_2Cl_2/CH_3OH, 90:10), and **20** was obtained (60 mg, 40% yield). Mp 205 °C; ^{1}H NMR (CH_3OD) δ 3.71 (s, 3H, NCH_3), 7.8 (1H, H4); mass spectrum (DCI/NH_3) *m/z* 143 ($M^+ + 1$, 100).

2-[(*E*)-1-(5-Nitro-2-furyl)methylidene]-1-hydrazinecarbothioamide (22a). Thiosemicarbazide (455 mg, 5 mmol) mixed with 5-nitrofurane-2-carboxaldehyde **21a** (760 mg, 5 mmol) was stirred for 15 h at room temperature in DMSO (10 mL). After the same workup as that described for **11a**, **22a** was obtained (950 mg, 90% yield). ^{1}H NMR (DMSO-d_6) δ 7.24 (b, NH), 7.67−7.68 (m, 2H), 7.9 (b, NH_2), 11.82 (s, 1H, CH═N); ^{13}C NMR (DMSO-d_6) δ 113.7 (C4), 114.8 (C3), 130.2 (CH═N), 151.5 (C5), 151.9 (C2), 176.2 ($CSNH_2$); mass spectrum (EI) *m/z* 214 (M^+). Anal. ($C_6H_6N_4O_3S$) C, H, N.

2-[(*E*)-1-(5-Nitro-2-furyl)methylidene]-1-hydrazinecarboxamide (22b). Semicarbazide hydrochloride (444 mg, 4 mmol) dissolved in DMSO (5 mL) was added to sodium hydride (110 mg,4 mmol) suspended in DMSO (3 mL). After 5 min when all hydrogen bubbling had ceased, 5-nitrofurane-2-carboxaldehyde (570 mg, 4 mmol) diluted in DMSO (2 mL) was added. After 18 h at room temperature, the brown solution was poured into water (50 mL) and a dark precipitate was filtered and purified after dissolution in acetone by flash chromatography (CH_2Cl_2/methanol, 90:10) to give **22c** (400 mg, 55% yield). ^{1}H NMR (DMSO-d_6) δ 6.57 (s, NH), 7.22 (d, H4), 7.77 (d, H3), 10.76 (s, CH═N); ^{13}C NMR (DMSO-d_6) δ 112.0 (C4), 115.1 (C3), 127.3 (CH═N), 151.1 (C2), 153.0 (C5), 155.8 ($CONH_2$); mass spectrum (DCI/NH3) *m/z* 216 ($M^+ + 18$, 100).

2-[(*E*)-1-(5-Nitro-2-thienyl)methylidene]-1-hydrazinecarbothioamide (22c). 21b (0.830 g, 5.3 mmol) and thiosemicarbazide (0.48 g, 5.4 mmol) in DMSO (5 mL) were stirred at room temperature for 18 h. The mixture was poured into water (20 mL), and the formed precipitate was filtered, washed with water, and dried under vacuum, leading to **22b** (1.09 g, 90% yield). ^{1}H NMR (DMSO-d_6) δ 7.5 (d,1H), 8 (d,-1H), 11.75 (s,NH_2); ^{13}C NMR (DMSO-d_6) δ 129 (C═N), 130.2 (C3), 135.2 (C4), 146.6 (C2), 150.6 (C5), 178.1 (C═S). Anal. ($C_6H_6N_4O_2S_2$) C, H, N.

5-Nitro-2-furyl Cyanide (24a). Nitrofuroxime **23a**, from Aldrich (2 g, 12 mmol), was refluxed in acetic anhydride (50 mL) at 140 °C for 3 h. After evaporation of the solvent under vacuum, the oily residue was recrystallized (hexane/ethyl acetate, 3:1), giving **24a** (1.5 g, 90% yield). ^{1}H NMR ($CDCl_3$) δ 7.29 (d, 1H), 7.38 (d, 1H); ^{13}C NMR ($CDCl_3$) δ 109.1 (CN), 111.2 (C3), 123.8 (C4), 127.0 (C2), 144.4 (C5); mass spectrum (EI) *m/z* 138 (M^+, 35).

5-(5-Nitro-2-furyl)-1,3,4-thiadiazol-2-amine (25a). 22a (425 mg, 2 mmol) mixed in water (20 mL) with ferric chloride hexahydrate (2.7 g, 10 mmol) was refluxed for 4 h. The yellow precipitate formed was filtered, washed with water, and dried under vacuum to give **25a** (275 mg, 65% yield). ^{1}H NMR (DMSO-d_6) δ 7.22 (d, 1H, H4, J = 5 Hz), 7.72 (d, 1H, H3, J = 5 Hz), 7.77 (b, NH_2); ^{13}C NMR (DMSO-d_6) δ 111.6 (C4), 114.9 (C3), 144.7 (C5), 147.6 (C2), 151.2 (C′2), 170.0 (C′5); mass spectrum (EI) *m/z* 212 (M^+, 10). Anal. ($C_6H_4N_4O_3S$) C, H, N.

5-(5-Nitro-2-furyl)-1,3,4-oxadiazol-2-amine (25b). 5-Nitro-2-furyl cyanide **24a** (700 mg, 5 mmol) and semicarbazide hydrochloride (560 mg, 5 mmol) were stirred in refluxed trifluoroacetic acid (20 mL) for 15 h. The cold solution (0 °C) was then carefully neutralized with a $NaHCO_3$ aqueous solution. The orange solid obtained was filtered, washed with water, and dried in a vacuum, giving **25b** (450 mg, 45% yield). ^{1}H NMR (DMSO-d_6) δ 6.33 (s, NH_2), 7.30 (d, H4), 7.80 (d, H3); ^{13}C NMR (DMSO-d_6) δ 111.3 (C3), 114.9 (C4), 133.5 (C2), 140.6 (C5), 150.8 (C′2), 157.0 (C′5); mass spectrum (DCI/NH_3) *m/z* 231 ($M^+ + 2NH_3$, 100).

5-(5-Nitro-2-thienyl)-1,3,4-thiadiazol-2-amine (25c). 22c (1.09 g, 4.4 mmol) mixed in water (50 mL) with ferric chloride hexahydrate (5.4 g, 20 mmol) was refluxed for 4 h. The yellow precipitate formed was filtered, washed with water, and dried under vacuum to give **25c** (0.90 g, 85% yield). Mp 245 °C; ^{13}C NMR (DMSO-d_6) δ 126.4 (C3), 130.6 (C4), 140.5 (C5), 148.6 (C2), 149.7 (C5′), 170.5 (C2′); mass spectrum (DCI/NH_3) *m/z* 229 ($M^+ + 1$, 70), 246 ($M^+ + 18$, 100), 263 ($M^+ + 34$, 40).

2-Chloro-5-(1-Methyl-4-nitro-1*H*-2-imidazolyl)-1,3,4-thiadiazole (26). To megazol (**6a**) (230 mg,1 mmol) dissolved in a mixture of concentrated hydrochloric acid and acetic acid (1:5) was added copper powder (10 mg) and sodium nitrite (10 mmol). After 30 min of being stirred at 0 °C, the reaction medium was left at room temperature for 15 h and then poured into water (20 mL) and extracted with dichloromethane (3 × 20 mL). After being dried ($MgSO_4$) and after evaporation of the organic phase, the residue was recrystallized (THF/heptane, 1:2) to give **26** (200 mg, 80% yield). Mp 135 °C; ^{1}H NMR (DMSO-d_6) δ 4.4 (s,NCH_3), 8.30 (s,H4); ^{13}C NMR (DMSO-d_6) δ 35.3 (NCH_3), 133.0 (C4), 139.3 (C5), 140.9 (C2), 155.8 (C′2), 162.5 (C′5). Anal. ($C_6H_4N_5O_2SCl$) C, H, N.

2-Methoxy-5-(1-Methyl-5-nitro-1*H*-2-imidazolyl)-1,3,4-thiadiazole (27). 26 (250 mg, 1 mmol) was stirred with sodium methylate (210 mg, 4 mmol) in methanol (10 mL) for 4 h at room temperature. After evaporation, the residue was extracted with ethyl acetate and purified by column chromatography (CH_2Cl_2/CH_3OH, 95:5). **27** was obtained as an oil (130 mg, 55% yield). ^{1}H NMR ($CDCl_3$) δ 4.25 (s, 3H, NCH_3), 4.48 (s, 3H, OCH_3), 8.00 (1H, H4); ^{13}C NMR (DMSO-d_6) δ 35.56 (NCH_3), 60.32 (OCH_3), 133.0 (C4), 135.78 (C2), 140.9 (C5), 154.6 (C′2), 177.1 (C′5). Anal. ($C_7H_7N_5O_3S$) C, H, N.

N1-[5-(1-Methyl-5-nitro-1*H*-2-imidazolyl)-1,3,4-thiadiazol-2-yl]acetamide (28a). Acetyl chloride (0.8 mL, 10 mmol) was added, with syringe through a septum cap, to **6a** (0.8 g, 3.5 mmol) in THF (20 mL) under nitrogen at room temperature. After 3 h, the precipitate formed was filtered and recrystallized (ethanol/acetone, 2:1), giving **28a** (0.72 g, 75% yield). ^{1}H NMR (DMSO-d_6) δ 2.24 (s, 3H, $COCH_3$), 4.39 (s, 3H, NCH_3), 8.25 (s, 1H, H4); ^{13}C NMR (DMSO-d_6) δ 22.33 (CH_3-CO), 35.23 (CH_3N), 133.09 (C4), 140.6 (C2), 141.04 (C5), 153.93 (CONH), 159.74 (C2′), 169.17 (C5′); mass spectrum (EI) *m/z* 269 ($M^+ + 1$, 10). Anal. ($C_8H_8N_6O_3S$) C, H, N.

N1-[5-(1-Methyl-5-nitro-1*H*-2-imidazolyl)-1,3,4-thiadiazol-2-yl]nonamide (28b). From **6a** (0.225 g,1 mmol) with nonanoyl chloride (1 mL, 5 mmol) in THF (20 mL), the same procedure as for **28a** was used during 3 h. **28b** recrystallized (ethanol) in 60% yield was obtained. Mp 257 °C; ^{1}H NMR (DMSO-d_6) δ 0.85 (s, 3H, CH_3), 1.25 (m, 14H, $(CH_2)_7$), 4.39 (s, 3H, NCH_3), 8.26 (s, 1H, H4); ^{13}C NMR (DMSO-d_6) δ 13.6 (CH_3), 21.9−34.8 ($(CH_2)_7$), 35.2 (NCH_3), 133.1 (C4), 140.5 (C2) (C5 absent), 153.8 (CONH), 159.9 (C′2), 172.2 (C′5); mass spectrum (DCI/NH_3) *m/z* 367 ($M^+ + 1$, 100), 384 ($M^+ + 18$, 15). Anal. ($C_{15}H_{22}N_6O_3S$) C, H, N.

N1-[5-(1-Methyl-5-nitro-1*H*-2-imidazolyl)-1,3,4-thiadiazol-2-yl]tetradecamide (28c). The same procedure as for **28a** but with **6a** (1 mmol) and myristoyl chloride (5 mmol) in THF gave **28c** in 60% yield. Mp 247 °C; ^{1}H NMR (DMSO-d_6) δ 0.84 (s, 3H, CH_3), 1.23 (m, 24H, $(CH_2)_{12}$), 4.39 (s, 3H, NCH_3), 8.27

(s, 1H, H4); mass spectrum (DCI/NH_3) *m/z*: 437 ($M^+ + 1$, 100), 454 ($M^+ + 18$,5). Anal. ($C_{20}H_{32}N_6O_3S$) C, H, N.

N1-[5-(1-Methyl-5-nitro-1*H*-2-imidazolyl)-1,3,4-thiadiazol-2-yl]-2,2,2,trifluoroacetamide (28d). To **6a** (226 mg,1 mmol) suspended in THF (5 mL) was added trifluoroacetic anhydride (2 mL), Instantaneously the suspension was dissolved. After 5 min the THF was evaporated under vacuum. The oily residue neutralized with an aqueous solution of $NaHCO_3$ gave a yellow precipitate of **28d**, which was washed with water and dried under vacuum (300 mg, 90% yield). ^{13}C NMR (DMSO-d_6) δ 35.0 (NCH_3), 117.0 (q, CF_3, $^1J_{CF} = 186$ Hz), 132.9 (C4), 140.5 (C2) (C5 absent), 141 (C2′), 167.7 (CO), 170 (C5′); mass spectrum (DCI/NH_3) *m/z* 323 ($M^+ + 1$, 66), 340 ($M^+ + 18$, 52). Anal. ($C_8H_5F_3N_6O_3S$) C, H, N.

6-(Hydroxymethyl)-3-[4-methoxybenzylideneamino]-tetrahydropyran-2,4,5-triol (30). Paramethoxybenzaldehyde (0.136 g, 1 mmol) was added by syringe to a glucose amine hydrochloride **29** (0.215 g, 1 mmol) in NaOH solution (20 mL, 20 mmol) at 0 °C. After the mixture was stirred for 12 h at room temperature, **30** was obtained as a white solid (60% yield). ^1H NMR (D_2O) δ 3.84 (s, 3H, OCH_3), 6.7−6.9 (m, 4H, phenyl), 8.23 (s, 1H, HC=N).

6-[(Acetyloxy)methyl]-3-[4-methoxybenzylideneamino]-tetrahydro-2,4,5-triacetyloxypyrane (31). 30 (0.298 g, 1 mmol) in pyridine (10 mL) in an ice bath was mixed with acetic anhydride (0.3 mL, 5 mmol) and stirred for 48 h at room temperature. After azeotropic solvent evaporation with toluene, **31** obtained in 80% yield was used directly without further purification. ^1H NMR (D_2O) δ 1.84−2.06 (m, 12H, CH_3COO), 3.8 (s, 3H, OCH_3), 4.01−4.31 (m, 3H, H5, H6), 5.09−5.40 (m, 3H, H2, H3, H4), 5.90 (d, 1H, $J = 7$ Hz, H1), 6.81−7.68 (d, 4H, phenyl), 8.13 (s, 1H, CH=N); ^{13}C NMR (D_2O) δ 20.5−20.6 (CH_3COO), 55.43 (CH_3O), 61.86 (C6′), 68.06 (C2′), 72.7−73.2 (C3′,4′,5′), 91.2 (C1′), 114.1, 128.3, 130.3, 162.3 (aromatic carbons), 164.3 (CH=N), 168.8−170.7 (CH_3COO); IR (KBr/cm^{-1}) 2918 (CH_3), 1750−1740 (COO).

2,5-Di(acetyloxy)-6-[(acetyloxy)methyl]-3-ammoniotetrahydro-4-pyranyl Acetate Chloride (32). Concentrated hydrochloric acid was carefully added in an acetone solution of **31** kept at 0 °C in an ice bath to reach pH 5.The precipitate formed was filtered and washed with acetone (yield 90%). ^1H NMR (D_2O) δ 2.06−2.25 (m, 12H, CH_3COO), 5.95 (d, 1H, $J =$ 8 Hz, H1); ^{13}C NMR (D_2O) δ 22.6−22.7(CH_3COO), 54.0 (C2′), 64.0 (C6′), 70.5 (C3′), 73.4 (C4′), 74.7 (C5′), 92.9 (C1′), 175.7−176.1 (CH_3COO).

5-Oxo-5-({2,4,5-tri(acetyloxy)-6-[(acetyloxy)methyl]tetrahydro-2*H*-3-pyranyl}amino)pentanoic acid (33). Glutaric anhydride (0.30 g, 2 mmol) and **32** (0.384 g, 1 mmol) in pyridine (10 mL) with 4-dimethylaminopyridine (0.04 g, 1 mmol) were stirred for 48 h at room temperature. After evaporation of pyridine in the presence of toluene and chromatography (CH_2Cl_2/CH_3OH, 95:5), **33** was obtained (80% yield). ^1H NMR ($CDCl_3$) δ 1.71−2.10 (m, 12H, CH_3COO), 2.16−2.34 (m, 6H, $(CH_2)_3$), 3.71−4.41 (m, 3H, H5, H6), 4.80−5.30 (m, 2H, H3, H4), 5.60−5.70 (m, 1H, H2), 6.65 (d, 1H, $J = 7$ Hz, H1); ^{13}C NMR (CD_3OD) δ 20.5−20.6 (CH_3COO), 32.57−35.O ($(CH_2)_3$), 52.44 (C2′), 61.69 (C6′), 66.3 (C5′), 72.5 (C3′C4′), 92.3 (C1′), 169.6−171.4 (CH_3COO), 173.3 (CONH), 177.4 (COO); IR (KBr/cm^{-1}) 3365 (COOH), 2968 (CH_3), 1750 (COO).

3-Methylpentanoic-4-oxo-4-({2,4,5-(acetyloxy)-6-[(acetyloxy)methyl]tetrahydro-2*H*-3-pyranyl}amino)-pentanoic Anhydride (34). *N*-Methylmorpholine (0.12 mL, 1.1 mmol) and isobutyl chloroformate (0.15 mL, 1.1 mmol) at −40 °C were mixed with **33** (1 mmol) under argon in THF (10 mL) for 10 min, and **34** formed in situ was not isolated.

2,5-Di(acetyloxy)-6-[(acetyloxy)methyl]-3-[(5-{[5-(1-methyl-5-nitro-1*H*-2imidazolyl)-1,3,4-thiadiazol-2-yl]amino}-5-oxopentanoyl)amino]tetrahydro-2*H*-4-pyranyl Acetate (35). Megazol (225 mg, 1 mmol) was added to the above mixture containing **34** and kept at ambient temperature for 2 h. After evaporation under vacuum and flash chromatography (CH_2Cl_2/CH_3OH, 95:5), **35** was isolated (230 mg, yield 25%). ^1H NMR (DMSO-d_6) δ 1.92−2.04 (m, 12H, CH_3COO), 1.6−2.11 (m, 6H, $CH_2CH_2CH_2$), 3.27 (NH), 3.90−4.10 (m, 3H, H5, H6), 4.37 (s, 3H, NCH_3), 4.70−5.30 (m, 2H, H3, H4), 5.67−5.76 (m, 1H, H2), 7.98 (d, 1H, $J = 7$ Hz, H1), 8.26 (s, 1H, C4); ^{13}C NMR (DMSO-d_6) δ 20.21−20.36 (CH_3COO), 20.53 (CH_2), 33.99.34.46 (CH_2CH_2), 35.13 (NCH_3), 51.77 (C2′), 61.39 (C6′), 67.99 (C5′), 71.48 (C5′), 71.48 (C3′), 72.07 (C4′), 91.64 (C1′), 132.95 (C4), 140.46 (C5), 141.09 (C2), 153.62 (C2*), 159.77 (C5*), 166.59−169.75 ($OOCCH_3$), 171.46−171.74 (CONH); mass spectrum (DCI/NH_3) *m/z* 670 ($M^+ + 1$, 20). Anal. ($C_{25}H_{31}N_7O_{13}S$) C, H, N.

N1-[5-(1-Methyl-5-nitro-1*H*-2-imidazolyl)-1,3,4-thiadiazol-2-yl]-N5-[2,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2*H*-3-pyranyl]pentanediamide (36). Sodium methylate (0.22 mmol) was mixed and stirred with **35** (70 mg, 0.1 mmol) dissolved in methanol (10 mL) at room temperature for 2 h. Deprotection was monitered by TLC (AcOEt). The mixture was treated with acid Dowex 50WA resin. After methanol evaporation under vacuum, compound **36** was recovered and recrystallized in ethanol (60% yield). ^1H NMR (DMSO-d_6) δ 1.83−2.17 (m, 6H, $CH_2CH_2CH_2$), 3.09−4.10 (m, 5H, H3, H4, H5, H6), 4.38 (s, 3H, NCH_3), 4.95 (m, 1H, H2), 7.62 (d, 1H, 8 Hz, H1), 8.24 (s, 1H, C4). ^{13}C NMR (DMSO-d_6) 20.57 (CH_2), 34.14 ($COCH_2$), 35.19 (NCH_3), 54.16 (C2′), 61.04 (C6′), 70.40 (C3′), 70.73 (C4′), 71.98 (C5′), 90.51 (C1′), 133.07 (C4), 140.58 (C5), 141.01 (C2), 153.66 (C2*), 159.67 (C5*), 171.62 (CONH), 171.76 (CONH); mass spectrum, FAB (matrix, 3-nitrobenzyl alcohol/DMSO) *m/z* 502 ($M^+ + 1$).

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