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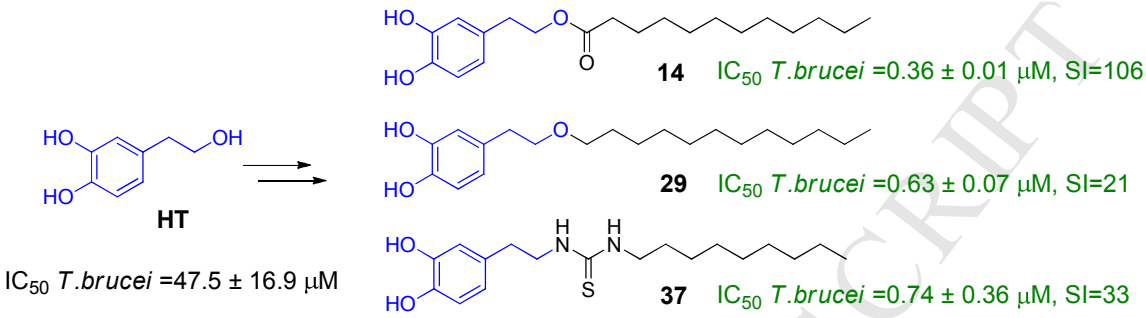
Graphical Abstract



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Tyrosol and hydroxytyrosol derivatives as antitrypanosomal and antileishmanial agents

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Abstract

Trypanosomiasis and leishmaniasis keep being a real challenge for health and development of African countries. Existing treatments have considerable side effects and increase resistance of the parasites. We have measured antitrypanosomal and antileishmanial activity of natural phenols, tyrosol (TYR) and hydroxytyrosol (HT) and several of their esters and metabolites. We found significant IC_{50} values against *Trypanosoma brucei* for HT decanoate ester and HT dodecanoate ester (0.6 and 0.36 μM, respectively). This represents a large increase in activity with respect to HT (79 and 132 fold, respectively). Moreover, both compounds displayed a high selectivity index against MRC-5, a non-tumoral human cell line (118 and 106, respectively). Then, we synthesized a focused library of compounds to explore structure-activity. We found the ether and thiourea analogues of HT decanoate ester and HT dodecanoate ester also showed IC_{50} values against *T.brucei* in the low micromolar range. In conclusion, the di-ortho phenolic ring and medium size alkyl chain are essential for activity whereas the chemical bond among them seems less important.

Keywords

Antitrypanosomal, antileishmanial, tyrosol, hydroxytyrosol

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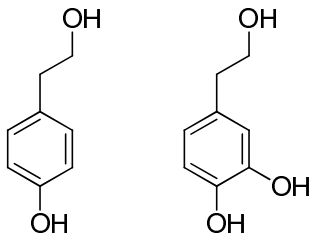
1. Introduction

Infectious diseases caused by protozoan parasites affect millions of people around the world, especially in tropical and subtropical areas. Among them, leishmaniasis (caused by different species of *Leishmania*) is a major global health problem with around 12 million people infected and causing 1-2 million new cases every year. *Trypanosoma brucei*, transmitted by the tsetse fly, is responsible for sleeping sickness in humans and preclude the development of productive livestock and agricultural activity based on domesticated animals where it causes Nagana.[1-2] For these reasons, this parasite is considered to be one of the major root causes of hunger and poverty in sub-Sahara Africa. Current treatments for both diseases present important drawbacks such as high toxicity, increased resistance and variability on their efficacy depending on the different strain of the parasite. Thus, the development of new antiparasitic therapies continues to be necessary.

Natural products have been tested for decades to find new antitrypanosomal and antileishmanial agents. Among them, several phenolics and polyphenols have shown relevant activity. For example, flavonoids such as 7,8-dihydroxyflavone, 3-hydroxyflavone and rhamnetin showed low micromolar IC_{50} values for *Trypanosoma brucei rhodesiense* and others such as luteolin and quercetin also displayed low micromolar IC_{50}s for *Leishmania donovani*[3]. In fact, quercetin glycosides present in the aqueous extract of *Kalanchoe pinnata* were active by oral administration in experimental cutaneous and visceral leishmaniasis infections produced *in vivo*[4-5].

Tyrosol (TYR, **1**) and hydroxytyrosol (HT, **2**) (Figure 1) are natural phenolic antioxidants present in olives and olive oil. They have shown a diverse biological activity such as antibacterial[6-7], antiviral[8], anti-inflammatory[9-12], neuroprotective[13] and anticancer activity,[14] inhibition of human LDL oxidation[15] and prevention of platelet aggregation[16]. Recently, Moradi-Afrapoli *et al*. reported medium activity of TYR against *Trypanosoma brucei rhodesiense* (IC_{50} >15μM)[17]. To the best of our knowledge, HT has not been examined for activity against *T.brucei*. Though, moderate antileishmanial activity was reported for HT against both, promastigotes of *L. infantum*, *L. donovani*, and *L. major*, and against *L. donovani* amastigotes that parasitize J774A.1 macrophages[18].

Figure 1. Chemical structures of tyrosol (TYR, **1**) and hydroxytyrosol (HT, **2**).



The preparation of chemically-modified natural products to improve their antiparasitic activity is still a very active source of potential new drugs. This is the case of chalcone[19] or caracasine acid derivatives[20], or the preparation of hybrids such as *Cinchona* alkaloids with bile acids[21] or caffeine-based chalcones[22]. Tyrosol and hydroxytyrosol derivatives have also been synthesized in order to improve the antioxidant and biological properties of the parent compounds. Hydroxytyrosol fatty acid esters increased the protection of proteins and lipids against oxidation caused by peroxyl radicals in a brain homogenate as an ex vivo model[23]. Similarly, hydroxytyrosol acetate was able to reduce the metabolic imbalance induced by a high-cholesterol diet in rats to a higher extent than HT[24]. Finally, HT alkyl ether derivatives, more stable under biological conditions than HT, have been reported to exert antiproliferative[25], neuroprotective[26-27], antiplatelet and anti-inflammatory effects[28] that are greater than those of HT.

Due to the improved biological activity found for several HT derivatives with respect to HT itself, we decided to investigate the antitrypanosomal and antileishmanial activity of a series of TYR and HT derivatives. We prepared a series of tyrosol and hydroxytyrosol fatty acid esters together with three metabolites of HT and TYR, tyrosol sulfate, tyrosol glucuronate and hydroxytyrosol glucuronate for a first *in vitro* screening. Later, we synthesized a focused library of compounds to explore structure-activity relevance varying the number of phenolic OH groups, the type of chemical bond between the phenolic ring and the alkyl chain, and the length of the alkyl chain.

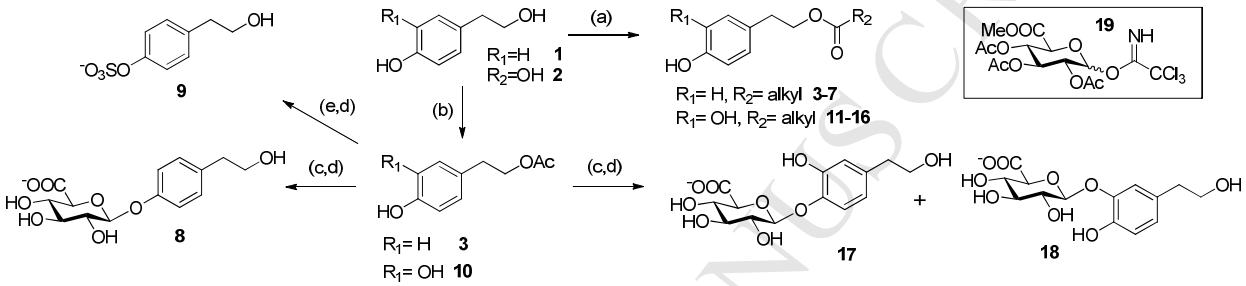
2. Results and Discussion

2.1. Chemistry

Tyrosol and hydroxytyrosol fatty acid esters (**3**-**7** and **11**-**16**, respectively) were synthesized by enzymatic acylation using Novozym 435 and the corresponding vinyl acyl donor in t-butyl methyl ether as reported previously[29-31] (Scheme 1). Tyrosol and hydroxytyrosol glucuronates **8** and **17, 18** were prepared by glycosylation of the acetyl protected TYR or HT derivatives (**3** and **10,** respectively). We used the acetyl protected trichloroacetimidate glucuronosyl derivative

19 as glycosyl donor and boron trifluoride etherate as the catalyst in the glycosylation step. Final acetyl deprotection yielded glucuronate derivatives **8** and **17,18** as described previously[32]. TYR sulfate **9** was prepared following a similar strategy to the one used for the glucuronate derivative. Sulfation of tyrosol acetate **3** was carried out with SO_3•NMe_3 as sulfating reagent, NEt_3 as base, and acetonitrile as solvent at 100 °C under microwave radiation. The reaction afforded the sulfated TYR derivative in good yield. Final acyl deprotection and reverse phase purification gave TYR sulfate **9**.

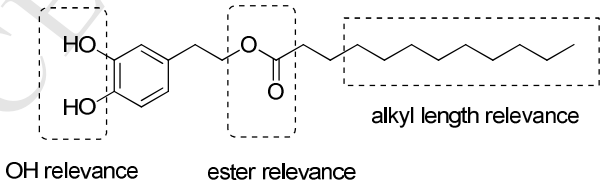
Scheme 1



Reagents and conditions: (a) Vinyl alkyl ester, Novozym435, tBuOMe, 60ºC; (b) EtOAc, Novozym435, 60ºC; (c) **19**, BF_3·OEt_2, CH_2Cl_2; (d) Na_2CO_3, MeOH, H_2O; (e) SO_3·NMe_3, TEA, CH_3CN, 100ºC, 20 min, MW

We identified hydroxytyrosol decanoate ester **13** and hydroxytyrosol dodecanoate ester **14** as relevant hit compounds against *T.brucei* (see below, Table 2) after carrying out the first *in vitro* screening on *T. brucei* and *L. donovani*. We decided then to perform structure-activity studies on **13** and **14** by changing the phenolic hydroxyl groups, the type of chemical bond between the phenolic ring and the alkyl chain, and the length of the alkyl chain (Figure 2) in order to improve its biological activity.

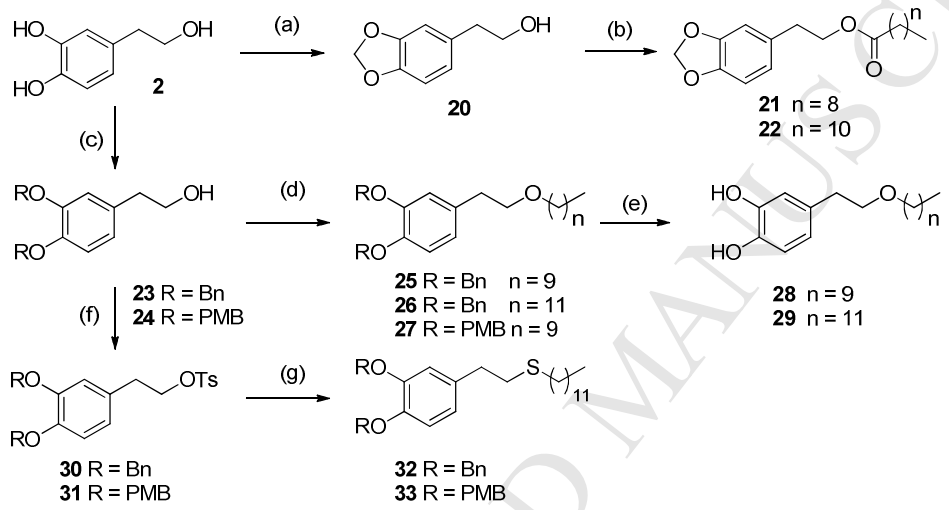
Figure 2. Parts of compound **14** to focus structure-activity studies.



We synthesized a hydroxyl protected version of compound **14** by formation of the acetal derivative **22** and also a shorter version with a decanoate alkyl chain, compound **21** (Scheme 2). The cyclic acetal intermediate **20** was obtained by reaction of HT with CH_2Cl_2 in basic conditions and then acylated using the enzymatic conditions used previously for HT yielding the desired compounds. The ether analogue of **14**, compound **29**, was prepared as reported previously[33] by benzyl protection of the phenolic groups, alkylation with 1-iodododecane under basic conditions and final hydrogenation. The HT ether decanoate derivative **28** was prepared following the same

procedure. We also prepared the p-methoxybenzyl (PMB) protected HT derivative **24** trying to improve the yields of the route. However, deprotection with trifluoroacetic acid of the ether intermediate **27** was unsuccessful. The synthesis of the thioether analogue of **14** was attempted next. Tosylation of derivatives **23** and **24** was followed by nucleophilic displacement with 1-dodecanethiol producing the protected thioether derivatives **32** and **33** with moderate yields. Neither hydrogenation nor reaction under strong acidic conditions yielded the final thioether deprotected products.

Scheme 2



Reagents and conditions: (a) CH_2Cl_2, K_2CO_3, DMF, Reflux; (b) Vinyl alkyl ester, Novozym435, tBuOMe; (c) BrBn or PMBCl, K_2CO_3, Acetone, Reflux; (d) $I(CH_2)_nCH_3$, KOH, DMSO, 50 ºC; (e) H_2, Pd, 4 atm, THF; (f) TsCl, TEA, DCM, 0 ºC; (g) $HS(CH_2)_{11}CH_3$, K_2CO_3, THF.

Dopamine hydrochloride was the starting material for the synthesis of the amide and thiourea analogues of **13** and **14** (compounds **37-40**) (Scheme 3). Dopamine was also synthesized to compare its activity with HT. The thiourea derivatives were synthesized by reaction of the isothiocyanate intermediate **36** with the corresponding primary alkylamine to obtain the products in moderate yields. The amide analogues of **13** and **14** were prepared by reaction of dopamine hydrochloride with the corresponding fatty acids using HATU as the coupling reagent.

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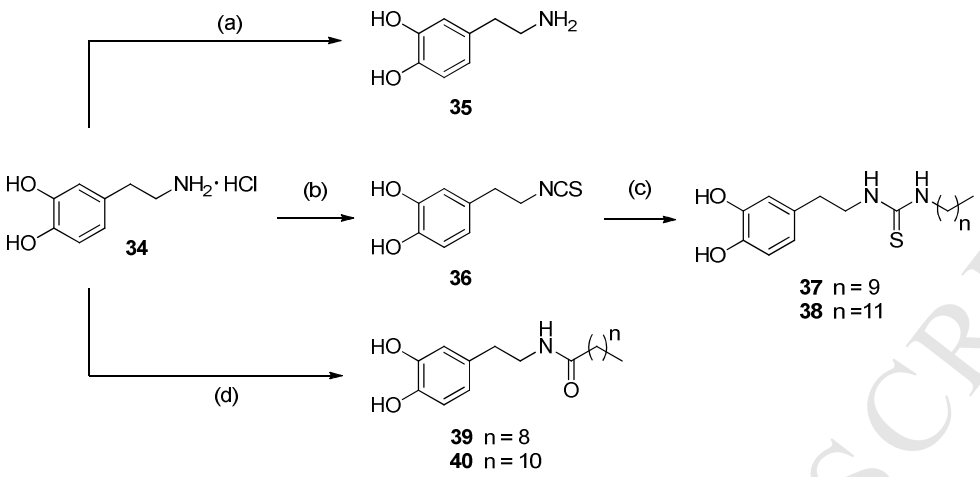
Scheme 3



Reagents and conditions: (a) KOH, MeOH; (b) CS_2, TEA, MEOH, THF; (c) $H_2N(CH_2)_nCH_3$, TEA, Pyridine; (d) $CH_3(CH_2)_nCOOH$, HATU, TEA, THF.

2.2. Biological evaluation

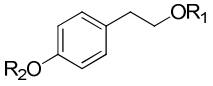
The *in vitro* antiparasitic activities of TYR, HT and their derivatives **3**-**18** were evaluated against *T. brucei brucei* and against axenic amastigotes of *L. donovani*. Those compounds exhibiting over 40% inhibition of axenic amastigotes at 20 μM were also evaluated on intracellular amastigotes of *L. donovani*. The cytotoxicity of these compounds was also evaluated against a human non-tumoral lung cell line (MRC-5). Suramin and amphotericin B were used as positive drug controls for *T. brucei* and *L. donovani*, respectively. Selectivity indices (SI) were calculated according to the formula: IC_{50} (MRC-5) / IC_{50} parasite. The activity gain (AG) of each compound with respect to the reference compound (TYR or HT) was calculated according to the formulas: IC_{50} (TYR) / IC_{50} compound and IC_{50} (HT) / IC_{50} compound.

Table 1 presents the antiparasitic and cytotoxic data for TYR and its derivatives **3**-**9**. IC_{50} values against *T. brucei* ranged from 10 to 62.7 μM and selectivity indices from 1.5 to 5. None of the TYR derivatives improved the activity of TYR itself as can be observed from the AG values. In the case of *L. donovani*, compounds **4** and **5** showed inhibition over 40% of axenic amastigotes at 20 μM concentration. These two derivatives were evaluated on intracellular amastigotes and showed IC_{50} values >10 μM. Aissa *et al.*[34] measured antileishmanial activity against *L. major* and *L. infantum* parasite species for tyrosol fatty acid esters from C2 to C18 chain length. They found the best IC_{50} values for medium chain tyrosyl derivatives **5**-**7** (e.g. tyrosol decanoate **6**: 65 and 132 μM, against *L. major* and *L. infantum*, respectively). The authors proposed this effect could be due to the surfactant activity reported for these medium chain tyrosyl fatty acid esters.[35]

When HT and its derivatives **10**-**18** were evaluated against *T. brucei* (Table 2), compounds **13**-**16** showed IC_{50}s in the low micromolar range (0.36-2.43 μM). Remarkably, hydroxytyrosol

decanoate **13** and hydroxytyrosol dodecanoate **14** displayed a high selectivity index (118 and 101, respectively) and a large increase in activity with respect to HT (AG values of 79 and 132, respectively). A length of the alkyl chain around 10 to 12 carbons seems to be the optimum on this series. When these compounds were examined against axenic amastigotes of *L. donovani*, three of them (compounds **11-13**) exhibited over 40% inhibition at 20 μM concentration. When they were evaluated on intracellular amastigotes, the best IC_{50} value was displayed by hydroxytyrosol decanoate **13** (8.44 μM). Kyriazis et al.[18] had previously reported IC_{50} data for hydroxytyrosol of 393 μM against *L. donovani* and in the same range for *L. infantum* and *L. major*. We have observed that the addition of a fatty acid ester group to HT improves its activity with respect to HT against *L. donovani* although only to IC_{50}s in the low micromolar range.

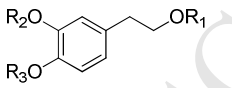
Table 1. Cytotoxic, trypanocidal and leishmanicidal activities of tyrosol derivatives.



Compounds	R_1	R_2	Cytotoxicity MRC-5 IC_{50} (μM)	*T.brucei* S-16 IC_{50} (μM)	SI	AG	*L.donovani* Axenic amastigotes % inhib. (20μM)	Intracellular amastigotes IC_{50} (μM)	SI	AG
TYR (1)	H	H	>500	>10	> 5	--	50	>10	5	--
3	$COCH_3$	H	113.6 ± 11.3	79.4 ± 23.1	1.4	0.13	28	n.d.	--	--
4	$CO(CH_2)_4CH_3$	H	166.4 ± 0.6	59.1 ± 2.3	2.8	0.16	44	>10	16.6	1
5	$CO(CH_2)_6CH_3$	H	133.0 ± 9.8	56.8 ± 8.3	2.3	0.22	46	>10	13.3	1
6	$CO(CH_2)_8CH_3$	H	171.5 ± 15.7	81.4 ± 19.3	2.1	0.16	34	n.d.	--	--
7	$CO(CH_2)_{10}CH_3$	H	67.2 ± 0.2	16.2 ± 3.8	4.1	0.52	32	n.d.	--	--
8	H	GlcAc	110.2 ± 14.4	61.5 ± 23.8	1.8	0.17	36	n.d.	--	--
9	H	$SO_3^-K^+$	106.4 ± 17.2	41.6 ± 12.6	2.6	0.16	36	n.d.	--	--
Suramin			--	0.038 ± 0.003				--		
Eflornitin			--	0.026 ± 0.002				--		
Amphotericin B			--	--				0.10 ± 0.01		

Experiments on *L. donovani* were carried out in axenic amastigotes and intracellular amastigotes.
SI= Selectivity Index (IC_{50} MRC-5 / IC_{50} parasite); AG= Activity gain (IC_{50} tyrosol / IC_{50} compound)

Table 2. Cytotoxic, trypanocidal and leishmanicidal activities of hydroxytyrosol derivatives.

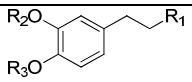


Compounds	R_1	R_2, R_3	Cytotoxicity MRC-5 IC_{50} (μM)	*T.brucei* S-16 IC_{50} (μM)	SI	AG	*L.donovani* Axenic amastigotes % inhib. (20μM)	Intracellular amastigotes IC_{50} (μM)	SI	AG
HT (2)	H	H, H	>50	47.5 ± 16.9	> 1	--	0	n.d.	--	--
10	$COCH_3$	H, H	>50	43.4 ± 14.0	> 1	1	48	>10	--	--
11	$CO(CH_2)_2CH_3$	H, H	71.5 ± 15.5	9.4 ± 1.4	8	5	48	>10	7.1	--
12	$CO(CH_2)_6CH_3$	H, H	103.5 ± 15.2	9.6 ± 0.8	11	5	45	>10	10.3	--
13	$CO(CH_2)_8CH_3$	H, H	71.1 ± 6.9	0.6 ± 0.24	118	79	54	8.44 ± 1.6	8.4	--
14	$CO(CH_2)_{10}CH_3$	H, H	38.1 ± 2.4	0.36 ± 0.01	106	132	56	>10	--	--
15	$CO(CH_2)_{12}CH_3$	H, H	> 50	2.29 ± 0.48	> 22	21	53	>10	--	--
16	$CO(CH_2)_{14}CH_3$	H, H	> 50	2.43 ± 0.33	> 21	20	52	>10	--	--
17-18	H	GlcAc, H	118.8 ± 21.5	62.3 ± 16.8	2	0.8	31	n.d.	--	--

Experiments on *L. donovani* were carried out in axenic amastigotes and intracellular amastigotes.
SI= Selectivity Index (IC_{50} MRC-5 / IC_{50} parasite); AG= Activity gain (IC_{50} hydroxytyrosol / IC_{50} compound)

After performing this first *in vitro* screening on *T. brucei* and *L. donovani* for TYR, HT and their derivatives **3**-**18**, we decided to focus our efforts only on new compounds against *T. brucei*. We carried out a structural-activity study of analogues of hydroxytyrosol fatty acid esters **13** and **14** (Table 3) in order to probe the relevance of the phenolic hydroxyl groups, the type of chemical bond between the phenolic ring and the length of the alkyl chain.

Table 3. Cytotoxic and trypanocidal activities of hydroxytyrosol derivatives **19**-**40**.



			Cytotoxicity MRC-5	*T.brucei* S-16		
Compounds	R_1	R_2, R_3	IC_{50} (μM)	IC_{50} (μM)	SI	AG
21	$OCO(CH_2)_8CH_3$	$-CH_2-$	>50	>10 (n=2)	> 5	> 5
22	$OCO(CH_2)_{10}CH_3$	$-CH_2-$	>50	>10 (n=3)	> 5	> 5
25	$O(CH_2)_9CH_3$	Bn, Bn	>50	>10 (n=3)	> 5	> 5
27	$O(CH_2)_9CH_3$	PMB, PMB	>50	>10 (n=2)	> 5	> 5
28	$O(CH_2)_9CH_3$	H, H	>50	1.29 ± 0.21	> 38	37
29	$O(CH_2)_{11}CH_3$	H, H	13.19 ± 2.90	0.63 ± 0.07	21	75
32	$S(CH_2)_{11}CH_3$	Bn, Bn	>50	>10 (n=2)	> 5	5
33	$S(CH_2)_{11}CH_3$	PMB, PMB	>50	>10 (n=2)	> 5	5
35	NH_2	H, H	68.9 ± 15.11	>10 (n=2)	7	5
36	NCS	H, H	21.13 ± 8.78	9.87 ± 1.02	2	5
37	$NHSNH(CH_2)_8CH_3$	H, H	24.8	0.74 ± 0.36	33	64
38	$NHSNH(CH_2)_{10}CH_3$	H, H	2.96 ± 1.20	0.77 ± 0.28	4	62
39	$NHCO(CH_2)_8CH_3$	H, H	>50	>10 (n=4)	> 5	5
40	$NHCO(CH_2)_{10}CH_3$	H, H	>50	8.74 ± 1,15	> 5	5

SI= Selectivity Index (IC_{50} MRC-5 / IC_{50} *T.brucei*); AG= Activity gain (IC_{50} hydroxytyrosol / IC_{50} compound)

We found that the phenolic hydroxyl groups seem to be essential for activity against *T. brucei* since changing them to a methylene acetal (compounds **21** and **22**) or protecting them with benzyl or p-methoxybenzyl groups (compounds **25**, **27**, **32** and **33**) increased their IC50 values (> 10 μM) with respect to those of **13** and **14**. The type of bond linking the alkyl chain to the HT scaffold on these compounds (ester, ether or thioether) appears to be much less important for their activity. When both phenolic hydroxyl groups and the medium size alkyl chain still remain as part of the structure of the compounds, the functional group connecting them seems to be less relevant. Actually, the hydroxytyrosol ether analogues **28** and **29**, and the thiourea analogues **37** and **38** showed similar IC_{50} values (0.63 to 1.29 μM) to those of the ester derivatives **13** and **14**. In contrast, the amide derivatives **39** and **40** presented slightly worse antitrypanosomal activity (>10 and 8.74 μM, respectively) than the ester derivatives. However, none of the new compounds displayed better selectivity index than compounds **13** and **14** and their activity gain with respect to HT was lower too. Finally, the length of the alkyl chain seems optimum at 10-12 carbon atoms independently of the bond connecting it to the HT scaffold. Only a two-fold difference is observed for the ester and ether derivatives with decyl and dodecyl chains.

Hydroxytyrosol decanoate **13** and hydroxytyrosol dodecanoate **14** possess relevant surfactant properties, even better than medium chain tyrosyl fatty acid esters.[35] So, it could be argued that **13** and **14** are acting as nonspecific detergents disrupting the parasite's membrane. Another possibility is they could be inhibiting fatty acid biosynthesis, through inhibition of FabG, FabI or

FabZ enzymes. The structural similarity of **13** and **14** with *trans*-2-hexadecenoyl-(*N*-acetylcysteamine)-thioester, a FabI inhibitor,[36-37] or with 2-, 5-, 6-, and 9-hexadecynoic acids (HDAs), inhibitors of FabG, FabI and FabZ of *P.falciparum*,[38] could be pointing to these potential therapeutic targets. In both cases, the inhibitors possess long alkyl chains (13 to 16 atoms in length) together with one or two groups at the end of the compound capable of accepting or donating hydrogen bonds resembling the scaffolds of compounds **13** and **14**.

3. Conclusions

We have examined the trypanocidal and leishmanicidal activities of several tyrosol and hydroxytyrosol fatty acid esters together with three of TYR and HT metabolites. We found notable IC_{50} values against *T. brucei* for HT decanoate ester **13** and HT dodecanoate ester **14** (0.6 and 0.36 μM, respectively) and against *L. donovani* for **14** (8.44 μM). We synthesized several analogues of **13** and **14** to investigate structure-activity relationship in *T. brucei*. We observed that the di-ortho phenolic ring and the medium size alkyl chain are essential for activity but the nature of the chemical bond among them is not so relevant.

4. Experimental Section

4.1. General

All chemicals were used without further purification, unless otherwise noted. All reactions were monitored by TLC on precoated Silica-Gel 60 plates F254, and detected by heating with 5% sulfuric acid in ethanol or Mostain (500 ml of 10% H_2SO_4, 25g of $(NH_4)_6Mo_7O_{24}•4H_2O$, 1g $Ce(SO_4)_2•4H_2O$). Products were purified by flash chromatography with Silica gel 60 (200-400 mesh). Low resolution mass spectra were obtained on an ESI/ion trap mass spectrometer. High resolution mass spectra were obtained on an ESI/quadrupole mass spectrometer. NMR spectra were recorded on a 300, 400 or 500 MHz [300 or 400 MHz (1H), 75 or 100 (^{13}C)] NMR spectrometers, at room temperature for solutions in $CDCl_3$, D_2O or CD_3OD. Chemical shifts are referred to the solvent signal. 2D experiments (COSY, TOCSY, ROESY, and HMQC) were done when necessary to assign the new compounds. Chemical shifts are in ppm. Data were processed using manufacturer software, raw data were multiplied by shifted exponential window function prior to Fourier transform, and the baseline was corrected using polynomial fitting.

4.2. Synthesis

*Preparation of 2-(benzo[d][1,3]dioxol-5-yl)ethyl decanoate (**21**)*

To a solution of **20**[39] (45 mg, 0.271 mmol) in *tert*-butyl methyl ether (3 ml), vinyl decanoate (0.061 ml, 0.271 mmol) and *Candida antarctica lipase* (Novozym 435, 200 mg) were added. The reaction was stirred by an orbital shaker for 24h at 55 ºC and then the enzyme was

separated and washed. The solvent was concentrated and purified by silica-gel flash column chromatography using a mixture of hexane/ethyl acetate, 9:1 (v/v). Evaporation of the solvents yielded 18.6 mg of the pure product as a white solid (Yield = 22%; Rf = 0.67 (Hexane/ethyl acetate, 4:1)). ^{1}H NMR (300 MHz, $CDCl_3$) δ = 6.77 – 6.47 (m, 3H, CH_{arom}), 5.86 (s, 2H, OCH_2O), 4.16 (t, *J* = 7.0 Hz, 2H, CH_2O), 2.77 (t, *J* = 6.9 Hz, 2H, CH_2CH_2O), 2.21 (t, *J* = 7.5 Hz, 2H, CH_2COO), 1.51 (d, *J* = 6.2 Hz, 2H, CH_2CH_2COO), 1.19 (s, 12H, CH_2), 0.81 (t, *J* = 5.4 Hz, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 174.0 (COO), 147.9 ($C^3_{ipso}O$), 146.4 ($C^4_{ipso}O$), 131.9 (C^1_{ipso}), 122.0 (C^6_{arom}), 109.6 (C^2_{arom}), 108.5 (C^5_{arom}), 101.1 (OCH_2O), 65.1 (C_1), 35.1 (C_2), 34.6 (CH_2COO), 32.1 (CH_2), 29.7 (CH_2), 29.5 (CH_2), 29.4 (CH_2), 25.2 (CH_2), 22.9 (CH_2), 14.4 (CH_3); ESI-HRMS [M + Na] calcd for $C_{19}H_{28}O_4$ 343.1885, found 343.1875.

*Preparation of 2-(benzo[d][1,3]dioxol-5-yl)ethyl dodecanoate (**22**)*

Following the same procedure used to prepare compound **21**, with vinyl dodecanoate (0.075 ml, 0.289 mmol) as the acylating agent, afforded 71.5 mg as a white solid (Yield = 71%; R_f = 0.65 (Hexane/ethyl acetate, 4:1)). ^{1}H NMR (300 MHz, $CDCl_3$) δ = 6.72 – 6.53 (m, 3H, CH_{arom}), 5.86 (s, 2H, OCH_2O), 4.16 (t, J = 7.0 Hz, 2H, CH_2O), 2.77 (t, J = 7.0 Hz, 2H, C**H$_2$**CH_2O), 2.21 (t, J = 7.5 Hz, 2H, CH_2COO), 1.58 – 1.47 (m, 2H, C**H$_2$**CH_2COO), 1.19 (s, 16H, CH_2), 0.81 (t, J = 6.6 Hz,3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 174.0 (COO), 147.9 ($C^3_{ipso}O$), 146.4 ($C^4_{ipso}O$), 131.9 (C^1_{ipso}), 122.0 (C^6_{arom}), 109.6 (C^2_{arom}), 108.5 (C^5_{arom}), 101.1 (OCH_2O), 65.1 (C_1), 35.1 (C_2), 34.6 (CH_2COO), 32.2 (CH_2), 29.9 (CH_2), 29.7 (CH_2), 29.6 (CH_2), 29.5 (CH_2), 29.4 (CH_2), 25.2 (CH_2), 22.9 (CH_2), 14.4 (CH_3); ESI-HRMS [M + Na] calcd for $C_{21}H_{32}O_4$ 371.2198, found 371.2204.

*Preparation of 2-(3,4-bis(benzyloxy)phenyl)ethan-1-ol (**23**)*

Benzyl bromide (1.62 ml, 13.62 mmol) was added to a mechanically stirred degassed suspension of hydroxytyrosol (1 g, 6.49 mmol) and potassium carbonate (3.59 g, 25.9 mmol) in dry acetone (20 ml) and the resulting mixture was refluxed for 12 h. The reaction was then filtered through a celite © pad and purified by silica-gel flash chromatography column (hexane/ethyl acetate, 3:1 (v/v) as solvents) obtaining 1.68 g of the desired product as a white solid (Yield = 77%; R_f = 0.16 (hexane: ethyl acetate, 3:1)). The spectroscopic data coincide with the previous report[33].

*Preparation of 2-(3,4-bis(4-methoxybenzyloxy)phenyl)ethan-1-ol (**24**)*

Following the same procedure used to prepare compound **23**, with hydroxytyrosol (175 mg, 1.135 mmol), potassium carbonate (377 mg, 2.72 mmol) and 4-methoxybenzyl chloride (0.339 ml, 2.497 mmol) in DMF (2 ml), afforded 249.5 mg of the pure product as a white solid (yield = 56%; R_f = 0.35 (Hexane/ethyl acetate, 2:1)). Solvent purification mixture: Hexane/ethyl acetate, 4:1 (v/v). ^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.23 (dd, *J* = 8.7, 2.0 Hz, 4H, H_{PMB}), 6.76 (dd, *J* = 8.5, 1.8 Hz 5H, H_{PMB} and H'2), 6.70 (d, *J* = 1.9 Hz, 1H, H'5), 6.61 (dd, *J* = 8.1, 2.0 Hz, 2H, H'6), 4.94 (s, 2H, $CH_2^{PMB}O$), 4.93 (s, 2H $CH_2^{PMB}O$), 3.69 (s, 3H, CH_3O), 3.69 (s, 3H, CH_3O), 3.66 (t, *J* = 6.4 Hz, 2H, CH_2OH), 2.64 (t, *J* = 6.5 Hz, 2H, CH_2CH_2OH); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 159.3 (C_{ipso}), 149.1 (C_{ipso}), 147.9 (C_{ipso}), 131.8 (C_{ipso}), 129.5 (C_{ipso}), 129.4 (C_{ipso}), 129.1

(C^{PMB}_{arom}), 129.0 (C^{PMB}_{arom}), 121.9 (C^{6}_{arom}), 116.5 (C^{2}_{arom}), 115.8 (C^{5}_{arom}), 113.8 (C^{PMB}_{arom}), 71.4 ($C^{PMB}H_2O$), 71.2 ($C^{PMB}H_2O$), 63.7 (CH_2OH), 55.3 (CH_3O), 38.7 (**C**H_2CH_2OH); ESI-HRMS [M + Na] calcd for $C_{24}H_{26}O_5$ 417.1678, found 417.1658.

*Preparation of 1,2-bis((4, 4'-methoxybenzyl)oxy)-4-(2-(decyloxy)ethyl)benzene (**27**)*

Compound **24** (50 mg, 0.127 mmol) and potassium hydroxide (49.8 mg, 0.887 mmol) were added to DMSO (2 ml) in a round-bottomed flask. 1-Iododecane (0.081 ml, 0.380 mmol) was then added and the reaction was heated to 50 ºC and stirred overnight. The reaction mixture was diluted with hydrochloric acid (5%, 25 mL) and extracted with CH_2Cl_2 (3 x 25 mL). The combined organic layers were then dried with $MgSO_4$, filtered and concentrated. The crude was purified by silica-gel flash column chromatography eluting with hexane/ethyl acetate, 3:1 (v/v) obtaining 40.5 mg of the pure product as a white solid (Yield = 60%; R_f = 0.8 (hexane/ethyl acetate, 2:1)). ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.27 (dd, *J* = 8.5, 4.7 Hz, 4H, H_{PMB}), 6.84 – 6.73 (m, 6H, $H_{PMB,}$ H'2 and H'5), 6.68 – 6.61 (m, 1H, H'6), 4.97 (s, 2H, $CH_2^{PMB}O$), 4.95 (s, 2H, $CH_2^{PMB}O$), 3.73 (s, 3H, CH_3O), 3.72 (s, 3H, CH_3O), 3.48 (t, *J* = 7.3 Hz, 2H, CH_2O), 3.33 (t, *J* = 6.7 Hz, 2H, CH_2O), 2.70 (t, *J* = 7.2 Hz, 2H, CH_2CH_2O), 1.49 (dd, *J* = 13.5, 6.8 Hz, 2H, CH_2C_{ipso}), 1.18 (s, 16H, CH_2), 0.80 (t, *J* = 6.6 Hz, 3H, CH_3CH_2); ^{13}C NMR (126 MHz, $CDCl_3$) δ = 159.3 (C_{ipso}), 159.2 (C_{ipso}), 149.0 (C_{ipso}), 147.6 (C_{ipso}), 132.6 (C_{ipso}), 129.6 (C_{ipso}), 129.5 (C_{ipso}), 129.1 (C^{PMB}_{arom}), 129.0 (C^{PMB}_{arom}), 121.7 (C^{6}_{arom}), 116.4 (C^{2}_{arom}), 115.7 (C^{5}_{arom}), 113.8 (C^{PMB}_{arom}), 113.8 (C^{PMB}_{arom}), 71.9 (CH_2O), 71.4 (CH_2O), 71.2 (CH_2O), 71.1 (CH_2O), 55.3 (CH_3O), 35.9 (CH_2), 31.9 (CH_2), 29.8 (CH_2), 29.6 (CH_2), 29.6 (CH_2), 29.5 (CH_2), 29.3 (CH_2), 26.2 (CH_2), 22.7 (CH_2), 14.1 (**C**H_3CH_2); ESI-HRMS [M + Na] calcd for $C_{34}H_{46}O_5$ 557.3243, found 557.3237.

*Preparation of 3,4-bis(benzyloxy)phenethyl 4-methylbenzenesulfonate (**30**)*

Tosyl chloride (0.855 g, 4.49 mmol) was dissolved in CH_2Cl_2 (1.5 ml) in a 10 mL flask under argon atmosphere. Compound **23** (1 g, 2.99 mmol) and triethylamine (1.250 ml, 8.97 mmol) were dissolved in CH_2Cl_2 (1.5 ml) in a second 10 mL flask under argon atmosphere and then cooled to 0 ºC. The tosyl chloride solution was added at a rate of 0.75 mL/h (time = 2h) whilst stirring and the reaction was then left to react for another 21h at room temperature. The reaction mixture was then washed with water (3 x 20mL) and the organic phases were combined, concentrated and purified by silica gel column eluting with hexane/ethyl acetate, 3:1 (v/v) to give a yellow oil (Yield = 98%). ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.71 (d, *J* = 8.2 Hz, 2H, H^{Ts}), 7.48 (d, *J* = 7.0 Hz, 4H, H^{Bn}), 7.39 (dd, *J* = 16.2, 8.5 Hz, 6H, H^{Bn}), 7.30 (d, *J* = 8.1 Hz, 2H, H^{Ts}), 6.87 (d, *J* = 8.1 Hz, 1H, H^{HT}), 6.75 (s, 1H, H'2), 6.67 (d, *J* = 8.1 Hz, 1H, H^{HT}), 5.14 (d, *J* = 15.3 Hz, 4H, CH_2O^{Bn}), 4.19 (t, *J* = 7.0 Hz, 2H, CH_2O), 2.89 (t, *J* = 7.9 Hz, 2H, CH_2CH_2O), 2.44 (s, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 149.2 (C_{ipso}), 148.2 (C_{ipso}), 144.9 (C_{ipso}), 137.6 (C_{ipso}), 137.4 (C_{ipso}), 133.3 (C_{ipso}), 130.0 (C_{ipso}), 129.8 (C_{arom}), 128.7 (C_{arom}), 128.1 (C_{arom}), 127.6 (C_{arom}), 127.5 (C_{arom}), 122.1 (C_{arom}), 116.1 (C_{arom}), 115.5 (C_{arom}), 71.6 (CH_2O), 71.6 (CH_2O), 71.0 (CH_2O), 35.1 (**C**H_2CH_2O), 21.9 (CH_3); ESI-HRMS [M + Na] calcd for $C_{29}H_{28}O_5S$ 511.1555, found 511.1561.

*Preparation of 3,4-bis((4-methoxybenzyl)oxy)phenethyl 4-methylbenzenesulfonate (**31**)*

Following the same procedure used to prepare compound **30**, with compound **24** (200 mg, 0.507 mmol) as starting material, tosyl chloride (145 mg, 0.761 mmol) and triethylamine (0.212 ml, 1.521 mmol) in 3 ml of DCM, afforded 184 mg of pure product as a yellow oil (Yield = 66%, R_f = 0.46 (hexane/ethyl acetate/Acetone, 4:2:1). ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.52 (d, *J* = 8.2 Hz, 2H, H^{Ts}), 7.20 (d, *J* = 7.3 Hz, 4H, H^{PMB}), 7.10 (d, *J* = 8.0 Hz, 2H, H^{Ts}), 6.74 (dd, *J* = 8.6, 2.9 Hz, 4H, H^{PMB}), 6.68 (d, *J* = 8.2 Hz, 1H, H'5), 6.57 (s, 1H, H'2), 6.48 (d, *J* = 8.1 Hz, 1H, H'6), 4.88 (s, 2H, CH_2O^{PMB}), 4.84 (s, 2H, CH_2O^{PMB}), 4.01 (t, *J* = 6.9 Hz, 2H, CH_2O), 3.64 (s, 3H, OCH_3), 3.63 (s, 3H, OCH_3), 2.69 (t, *J* = 6.9 Hz, 2H, CH_2CH_2O), 2.24 (s, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 159.6 (C_{ipso}), 159.6 (C_{ipso}), 149.4 (C_{ipso}), 148.3 (C_{ipso}), 145.0 (C_{ipso}), 133.2 (C_{ipso}), 130.1 (C_{ipso}), 129.8 (C_{ipso}), 129.7 (C_{ipso}), 129.6 (C_{arom}), 129.4 (C_{arom}), 129.3 (C_{arom}), 128.1 (C_{arom}), 122.1 (C_{arom}), 116.4 (C_{arom}), 115.8 (C_{arom}), 114.1 (C_{arom}), 71.5 (CH_2O), 71.4 (CH_2O), 71.1 (CH_2O), 55.5 (CH_3O), 35.1 (CH_3O), 21.9 (CH_3); ESI-HRMS [M + Na] calcd for $C_{31}H_{32}O_7S$ 571.1766, found 571.1771.

*Preparation of (3,4-bis(benzyloxy)phenethyl)(dodecyl)sulfane (**32**)*

To a mechanically stirred two socket flask under argon atmosphere was potassium carbonate (141 mg, 1.025 mmol) suspended in acetonitrile (5 ml) and heated to reflux. In a second and third flask under argon atmosphere was dodecane-1-thiol (0.054 ml, 0.225 mmol) dissolved in acetonitrile (1 ml) and compound **30** (100 mg, 0.205 mmol) dissolved in acetonitrile (2 ml), respectively. The second and third flask solutions were simultaneously added to the refluxing two socket flask and left to react for 48 h. The reaction product was then filtered, concentrated and purified by silica-gel flash chromatography column using a mixture of hexane/ethyl acetate, 9:1 (v/v) as solvents to obtain 50 mg of the desired product as a yellow oil (Yield = 47 %, R_f = 0.83 (Hexane: ethyl acetate – 4:1)). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.37 (dd, *J* = 8.0, 2.4 Hz, 4H, H^{Bn}), 7.26 (dtd, *J* = 8.6, 6.7, 1.8 Hz, 6H, H^{Bn}), 6.79 (d, *J* = 8.2 Hz, 1H, H'5), 6.73 (d, *J* = 1.9 Hz, 1H, H'2), 6.64 (dd, *J* = 8.1, 1.9 Hz, 1H, H'6), 5.07 (s, 2H, CH_2O), 5.05 (s, 2H, CH_2O), 2.74 – 2.64 (m, 2H, CH_2S), 2.64 – 2.55 (m, 2H, CH_2S), 2.46 – 2.34 (m, 2H, $C_{ipso}C\mathbf{H_2}CH_2$), 1.52 – 1.45 (m, 2H, $C\mathbf{H_2}CH_2S$), 1.18 (s, 18H, CH_2), 0.80 (t, *J* = 6.6 Hz, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 149.3 (C_{ipso}), 147.8 (C_{ipso}), 137.7 (C_{ipso}), 137.6 (C_{ipso}), 134.5 (C_{ipso}), 128.7 (C^{Bn}), 128.0 (C^{Bn}), 128.0 (C^{Bn}), 127.6 (C^{Bn}), 127.6 (C^{Bn}), 121.6 (C^6_{arom}), 116.0 (C^2_{arom}), 115.5 (C^5_{arom}), 71.7 (OCH_2), 71.7 (OCH_2), 36.2 (SCH_2), 34.0 (SCH_2), 32.6 (CH_2), 32.2 (CH_2), 29.9 (CH_2), 29.9 (CH_2), 29.8 (CH_2), 29.6 (CH_2), 29.5 (CH_2), 29.2 (CH_2), 23.0 (CH_2), 14.4 (CH_3). ESI-HRMS [M + H] calcd for $C_{34}H_{46}O_2S$ 519.3297, found 519.3295.

*Preparation of (3,4-bis((4-methoxybenzyl)oxy)phenethyl)(dodecyl)sulfane (**33**)*

Following the same procedure used to prepare compound **32**, with compound **31** (100 mg, 0.182 mmol), potassium carbonate (126 mg, 0.911 mmol) and dodecane-1-thiol (0.048 ml, 0.2 mmol), afforded 97 mg of pure product as a yellow oil (Yield = 92 %, R_f = 0.3 (Hexane: ethyl acetate, 6:1)). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.26 (dd, *J* = 8.4, 4.6 Hz, 4H, H^{PMB}), 6.86 – 6.75 (m, 5H, H^{PMB} and H'5), 6.72 (d, *J* = 1.5 Hz, 1H, H'2), 6.63 (d, *J* = 8.1 Hz, 1H, H'6), 4.97 (s, 2H, CH_2O), 4.95 (s, 2H, CH_2O), 3.72 (s, 3H, CH_3O), 3.72 (s, 3H, CH_3O), 2.75 – 2.65 (m, 2H, CH_2S),

2.65 – 2.56 (m, 2H, CH_2S), 2.42 (t, *J* = 7.4 Hz, 2H, $C_{ipso}C\mathbf{H_2}CH_2$), 1.57 – 1.42 (m, 2H, $C\mathbf{H_2}CH_2S$), 1.18 (s, 18H, CH_2), 0.80 (t, *J* = 6.5 Hz, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 159.6 (C_{ipso}), 159.5 (C_{ipso}), 149.3 (C_{ipso}), 147.9 (C_{ipso}), 134.5 (C_{ipso}), 129.8 (C_{ipso}), 129.7 (C_{ipso}), 129.4 (C_{arom}), 129.3 (C_{arom}), 121.6 (C_{arom}), 116.3 (C_{arom}), 115.9 (C_{arom}), 114.1 (C_{arom}), 71.6 (CH_2O), 71.6 (CH_2O), 55.5 (CH_3O), 36.2 (SCH_2), 34.1 (SCH_2), 32.6 (CH_2), 32.2 (CH_2), 30.0 (CH_2), 29.9 (CH_2), 29.9 (CH_2), 29.8 (CH_2), 29.6 (CH_2), 29.6 (CH_2), 29.2 (CH_2), 23.0 (CH_2), 14.4 (CH_3); ESI-HRMS [M + H] calcd for $C_{36}H_{50}O_4S$ 579.3508, found 579.3486.

*Preparation of 4-(2-isothiocyanatoethyl)benzene-1,2-diol (**36**)*

Triethylamine (1.617 ml, 11.60 mmol) was added to a stirring suspension of dopamine hydrochloride **34** (2 g, 10.55 mmol) in THF (26 ml). Methanol (20.80 ml) was slowly added to dissolve the dopamine hydrochloride, forming a clear solution. Carbon disulfide (3.17 ml, 52.7 mmol) was added to the mixture which was stirred for 1 h at 28 ºC under argon atmosphere. The yellowish reaction mixture was cooled to 0ºC and hydrogen peroxide (0.942 ml, 30% in water) was added drop wise (30%). The solution was immediately acidified with concentrated hydrochloric acid and then concentrated *in vacuo*. The resulting mixture was filtered and rinsed with water. The filtrate was extracted with ethyl acetate and the combined organic layers were dried over $MgSO_4$, filtered, and concentrated affording 740 mg of crude product as an oil (Yield = 36%). The spectroscopic data coincide with the previous report[40-41].

*Preparation of 1-(3,4-dihydroxyphenethyl)-3-decylthiourea (**37**)*

Compound **36** (355.7 mg, 1.822 mmol) and decan-1-amine (0.364 ml, 1.820 mmol) were dissolved in pyridine (10 ml) to give a yellow solution. The reaction was stirred for 3 h and triethylamine (0.254 ml, 1.822 mmol) was then added. The reaction was stirred for 1 h and then concentrated, diluted with 20 mL of hydrochloric acid (5%) and extracted with ethyl acetate (3 x 20 mL). The combined organic phases were concentrated and purified via silica-gel flash column chromatography while eluting with hexane/ethyl acetate, 2:1 (v/v) to give 338 mg of the pure product as a yellow solid (Yield = 53% R_f = 0.2 (hexane/ethyl acetate - 2:1)). ^{1}H NMR (300 MHz, $CDCl_3$) δ = 6.72 (d, *J* = 8.1 Hz, 2H, C^2_{arom} and C^5_{arom}), 6.56 (d, *J* = 7.9 Hz, 1H, C^6_{arom}), 3.64 (t, *J* = 7.1 Hz, 2H, CH_2N), 3.39 (m, 2H, CH_2N), 2.74 (t, *J* = 7.1 Hz, 2H, $C\mathbf{H_2}C_{ipso}$), 1.55 (t, 2H, $C\mathbf{H_2}CH_2N$), 1.31 (s, 16H, CH_2), 0.91 (t, *J* = 6.7 Hz, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 202.0 (NCN), 145.1 (C^4_{arom}), 143.6 (C^3_{arom}), 130.8 (C^1_{arom}), 120.0 (C^6_{arom}), 115.8 (C^2_{arom} or C^5_{arom}), 115.3 (C^2_{arom} or C^5_{arom}), 34.6 (CH_2), 32.0 (CH_2), 29.6 (CH_2), 29.3 (CH_2), 26.9 (CH_2), 22.6 (CH_2), 13.4 (CH_3); ESI-HRMS [M + H] calcd for $C_{19}H_{32}N_2O_2S$ 353.2252, found 353.2263.

*Preparation of 1-(3,4-dihydroxyphenethyl)-3-dodecylthiourea (**38**)*

Following the same procedure used to prepare compound **37**, from dodecan-1-amine (0.42 mL, 1.822 mmol), afforded 392.7 mg of the pure product as a yellow solid (Yield = 57% R_f = 0.5 (hexane/ethyl acetate - 1:1)). ^{1}H NMR (300 MHz, $CDCl_3$) δ = 6.68 (d, *J* = 8.0 Hz, 2H, C^2_{arom} and C^5_{arom}), 6.53 (dd, *J* = 8.0, 2.0 Hz, 1H, C^6_{arom}), 3.61 (s, 2H, CH_2N), 3.30 (dt, *J* = 3.2, 1.6 Hz, 2H, CH_2N), 2.71 (t, *J* = 7.2 Hz, 2H, $C\mathbf{H_2}C_{ipso}$), 1.56 – 1.46 (m, 2H, $C\mathbf{H_2}CH_2N$), 1.29 (s, 18H, CH_2), 0.89 (t, *J* = 6.2 Hz, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 196.0 (NHCNH), 146.3 (C^4_{arom}),

144.5 (C^3_{arom}), 131.9 (C^1_{arom}), 121.1 (C^6_{arom}), 116.9 (C^2_{arom} or C^5_{arom}), 116.4 (C^2_{arom} or C^5_{arom}), 35.7 (CH_2), 35.7 (CH_2), 33.1 (CH_2), 30.7 (CH_2), 30.4 (CH_2), 28.0 (CH_2), 23.7 (CH_2), 14.4 (CH_3); ESI-HRMS [M + H] calcd for $C_{21}H_{36}N_2O_2S$ 381.2576, found 381.2563.

4.3. Biological assays.

4.3.1. In vitro antitrypanosomal activity

Bloodstream forms (BSF) of *T. brucei brucei* 'single marker' S427 (S16) were grown at 37 ºC, 5% CO2 in HMI-9 medium supplemented with 10% (heat-inactivated fetal bovine serum, hiFBS). Drug susceptibility assay was performed as described in (Carvalho L et al, 2015). Briefly, parasites (1×10^4 BSF per mL) were incubated in 96-well plates with increasing concentration of drugs/compounds for 72 h at 37 ºC, 5% CO_2 in culture medium. Cell proliferation was determined using the alamarBlue® assay[42]. The Alamar Blue assay to determine drug sensitivity of African trypanosomes (*T.b. rhodesiense* and *T.b. gambiense*) in vitro. Fluorescence was recorded with an Infinite® F200 microplate reader (Tecan Austria GmbH, Austria) equipped with 550 and 590 nm filters for excitation and emission wavelengths, respectively.

4.3.2. In vitro antileishmanial activity

The experiments of drug susceptibility on *L. donovani* were carried out in axenic amastigotes and intracellular amastigotes. Axenic *L. donovani* MHOM/ET/67/HU3 amastigote parasites were grown in Schneider medium supplemented with 20% hiFBS, pH 5.4 at 37 ºC and 5% CO_2. 10^6 axenic amastigotes/ml in a 96-well plate were incubated with increasing concentrations of compounds for 72 h at 37 °C, followed by a resazurin-based assay, as described.[43] Additionally, we use intracellular amastigotes of a *L. donovani* (MHOM/ET/67/HU3) line with luciferase gene integrated into the parasite genome.[44] The susceptibility of intracellular *L. donovani* amastigotes to synthesized compounds was determined using the Luciferase Assay System Kit (Promega, Madison, Wis.) as previously described.[43] Briefly, macrophage-differentiated-THP-1 cells were plated at a density of $3x10^4$ macrophages/well in 96-well white polystyrene microplates and were infected at a macrophage/parasite ratio of 1:10 with late-stage

promastigotes. Infected cell cultures were incubated at different compound concentrations in RPMI 1640 medium plus 10% hiFBS at 37 °C with 5% CO_2 for 72 h and then lysed. Luminescence intensity was measured as indicative of the intracellular parasite growth, according to the instructions of the supplier.

4.3.3. Cytotoxicity assay

Human myelomonocytic cell line THP-1 were grown at 37 ºC and 5% CO_2 in RPMI-1640 supplemented with 10% hiFBS, 2 mM glutamate, 100 U/mL penicillin and 100 μg/mL streptomycin. 3 x 10^4 cells/well in 96-well plates were differentiated to macrophages with 20 ng/mL of PMA treatment for 48 h followed by 24 h of culture in fresh medium.[43] MRC-5 cells, a SV-40 transformed human fetal lung fibroblast cell line, were maintained at 37 ºC and 5% CO_2 in DMEM supplemented with 10% hiFBS, 100 U/mL penicillin and 100 μg/mL streptomycin. Cells were harvested by treatment with 0.05% (w/v) trypsin plus 0.48 mM EDTA for 5 min, diluted to 4 x 10^4 cell/ml in 96-well plates and incubated at 37 ºC and 5% CO_2 before toxicity assay.[43] Cellular toxicity of all compounds was determined using the colorimetric MTT-based assay after incubation at 37 °C for 72 h in the presence of increasing concentrations of compounds.[43] The results are expressed as EC_{50} values, as the concentration of compound that reduce cell growth by 50% versus untreated control cells.

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Highlights

- Tyrosol and hydroxytyrosol alkyl derivatives were synthesized in 2-3 steps.
- Their antitrypanosomal and antileishmanial activity is reported.
- Compounds **13** and **14** showed the best IC_{50} against Trypanosoma brucei (0.36-0.6 μM).
- Compounds **13** and **14** displayed a high selectivity index (SI=101-118).
- Two phenol groups and medium size alkyl chain are essential for activity.

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