Triclosan-caffeic acid hybrids: Synthesis, leishmanicidal, trypanocidal and cytotoxic activities

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Abstract

The synthesis, cytotoxicity, anti-leishmanial and anti-trypanosomal activities of twelve triclosan-caffeic acid hybrids are described herein. The structure of the synthesized products was elucidated by a combination of spectrometric analyses. The synthesized compounds were evaluated against amastigotes forms of *L. (V) panamensis*, which is the most prevalent *Leishmania* species in Colombia, and against *Trypanosoma cruzi,* which is the pathogenic species to humans. Cytotoxicity was evaluated against human U-937 macrophages. Eight compounds were active against *L. (V) panamensis* (**18-23, 26** and **30**) and eight of them against *T. cruzi* (**19-22**, **24** and **28-30**) with EC_{50} values lower than 40 µM. Compounds **19-22**, **24** and **28-30** showed higher activities than benznidazole (BNZ). Esters **19** and **21** were the most active compounds for both *L. (V) panamensis* and *T. cruzi* with 3.82 and 11.65 µM and 8.25 and 8.69 µM, respectively. Compounds **19-22**, **24** and **28-30** showed higher activities than benznidazole (BNZ). Most of the compounds showed antiprotozoal activity and with exception of **18**, **26** and **28**, the remaining compounds were toxic for mammalian cells, yet they have potential to be considered as candidates for anti-trypanosomal and anti-leishmanial drug development. The activity is dependent on the length of the alkyl linker with compound **19**, bearing a four-carbon alkyl chain, the most performing hybrid. In general, hydroxyl groups increase both activity and cytotoxicity and the presence of the double bond in the side chain is not decisive for cytotoxicity and anti-protozoal activity.

Keywords: leishmaniasis, chagas disease, *Trypanosoma cruzi*, antiprotozoal activity, cytotoxicity, triclosan, caffeic acid, hybrids.

1. Introduction

Leishmaniasis and Chagas disease are a cause of mortality in various developing countries of tropical and subtropical regions. These diseases are endemic health problems in developing countries. This situation is aggravated by increasing treatment failures with available drugs [1]. Leishmaniasis involves a wide spectrum of clinical manifestations ranging from small cutaneous nodules, plaques or ulcers (cutaneous leishmaniasis), to severe mucosal tissue destruction (mucosal leishmaniasis) or disfunction of vital organs and tissues such as liver, spleen and bone marrow (visceral leishmaniasis). This disease affects to more than 12 million people worldwide and is caused by various species of the *Leishmania* genus that include *L. panamensis*, *L. braziliensis* and *L. guayanensis* (members of the *Viannia subgenus*), as well as *L. mexicana* and *L. amazonensis* (members of the *Leishmania subgenus*). *L. (V) panamensis* is one of the most prevalent leishmania species involved in human cases of cutaneous leishmaniasis in Colombia [2]. On the other hand, Chagas disease, also named American trypanosomiasis, affects about 10 million people mainly in Latin America. This disease is produced by the protozoan parasite *Trypanosoma cruzi* that is transmitted to the mammalian host through the phases of triatomine bugs belonging to *Triatoma, Rhodnius* and *Panstrongylus* genus [3].

Current chemotherapies to treat cutaneous leishmaniasis are based on old drugs, such as pentavalent antimonials, meglumine antimoniate (MA) and for the treatment of Chagas disease sodium stibogluconate (SSG) and nitroaromatic compounds, such as benznidazole (BNZ) and nifurtimox (NF), are usually prescribed. Unfortunately, all of these drugs have severe toxic effects on patients which are associated with high doses and lengths of therapeutic schemes. Moreover, they are no longer as effective as before due to the emergence of drug resistance in the parasite, which is complicating the treatment of these parasitic diseases. [4-6].

Caffeic acid and some of its esters and amides derivatives exhibit a broad spectrum of biological activities including anti-inflammatory [7], antimicrobial [8,9], antioxidant [10], anti-Alzheimer [11], analgesic [12] and anticancer effects [13-15]. In addition, some studies have shown that caffeic acid esters have high anti-leishmanial activity [16-19]. Three caffeic acid esters (**1-3**, see figure 1), which have been isolated from leaves of *Piper sanguineispicum*, showed anti-leishmanial activity with an IC_{50} of 2.0, 10 and 1.8 µM, respectively. In addition, these compounds exhibited moderate cytotoxicity on murine macrophages [16]. The alkyl caffeic acid ester (**4**) was active against axenic amastigotes of *L. panamensis* with an EC_{50} of 0.67 µM although this compound was toxic for mammalian cells [17]. The caffeic acid derivative (**5**), isolated from *V. wallichii*, showed an IC_{50} value of 48.8 µM against *L. major* promastigotes and high cytotoxicity against a J774.1 cell line [18]. Rosmarinic acid (**6**) exhibited

anti-leishmanial activity against both *L. major* and *L. donovani* (IC_{50} of 59.2 and 74.4 µM, respectively) [19]. Isopentyl caffeate (**7**) showed anti-trypanosomal activity against *T. brucei* bloodstream forms with a minimum inhibitory concentration (MIC) of 0.31 µg/ml [20]. Finally, dicaffeoyl acid **8** showed a significant activity against *T. cruzi* with an IC_{50} value of 286.2 µM [21] (Figure 1).

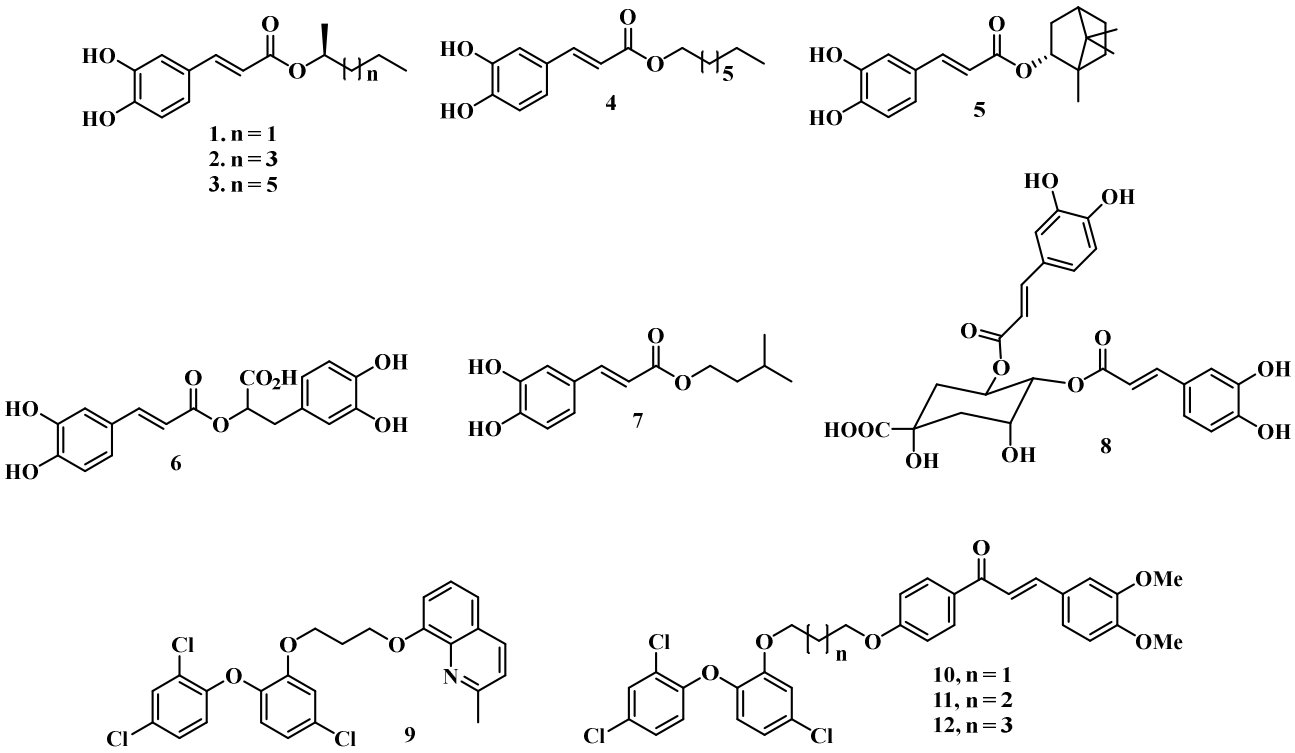


Figure 1. Caffeic acid esters and triclosan derivatives with anti-protozoal activity.

Triclosan is a non-competitive inhibitor of purified enoyl-acyl carrier protein reductase (ENR) which has demonstrated inhibitory *in vitro* activity against *Plasmodium falciparum* [22–25]. A previous study showed that triclosan and triclosan-quinoline hybrids bearing a propyl linker (see compound **9** in figure 1) have *in vitro* activity against axenic and intracellular amastigotes of *L. panamensis* with an effective concentration (EC_{50}) below 24 µg/mL [26]. Triclosan-chalcone hybrids with spacers of three (**10**), four (**11**) and five (**12**) methylene units were active against leishmania parasites (EC_{50} 9.4, 10.2 and 13.5 µg/mL, respectively) and showed no toxicity towards mammalian cells (>200 µg/mL) [27] (see Figure 1).

An emerging strategy in medicinal chemistry and drug discovery relies in the use of hybrid molecules which results from the covalent linking of two molecules with individual intrinsic pharmacological

activity [28,29]. Hybrid molecules bear two distinct pharmacophores with different biological functions and may display dual activity although both entities of the hybrid molecule are not necessarily acting on the same biological target. [30-32]. In the search for new therapeutic alternatives to treat cutaneous leishmaniasis and Chagas disease we have designed and synthesized a series of new triclosan-caffeic acid hybrids (see structures in Figure 2) and we have evaluated their *in vitro* cytotoxicity, anti-leishmanial and anti-trypanosomal activities.

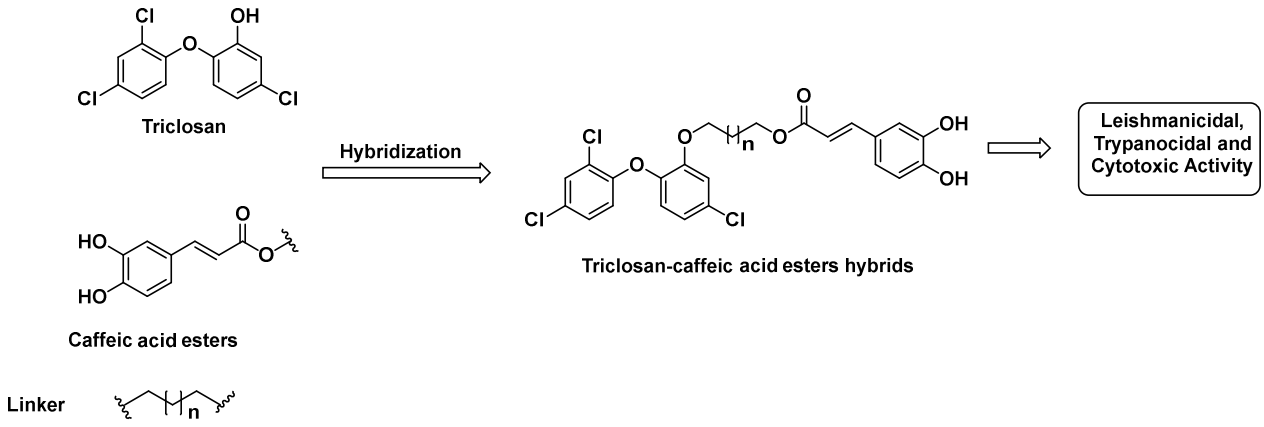


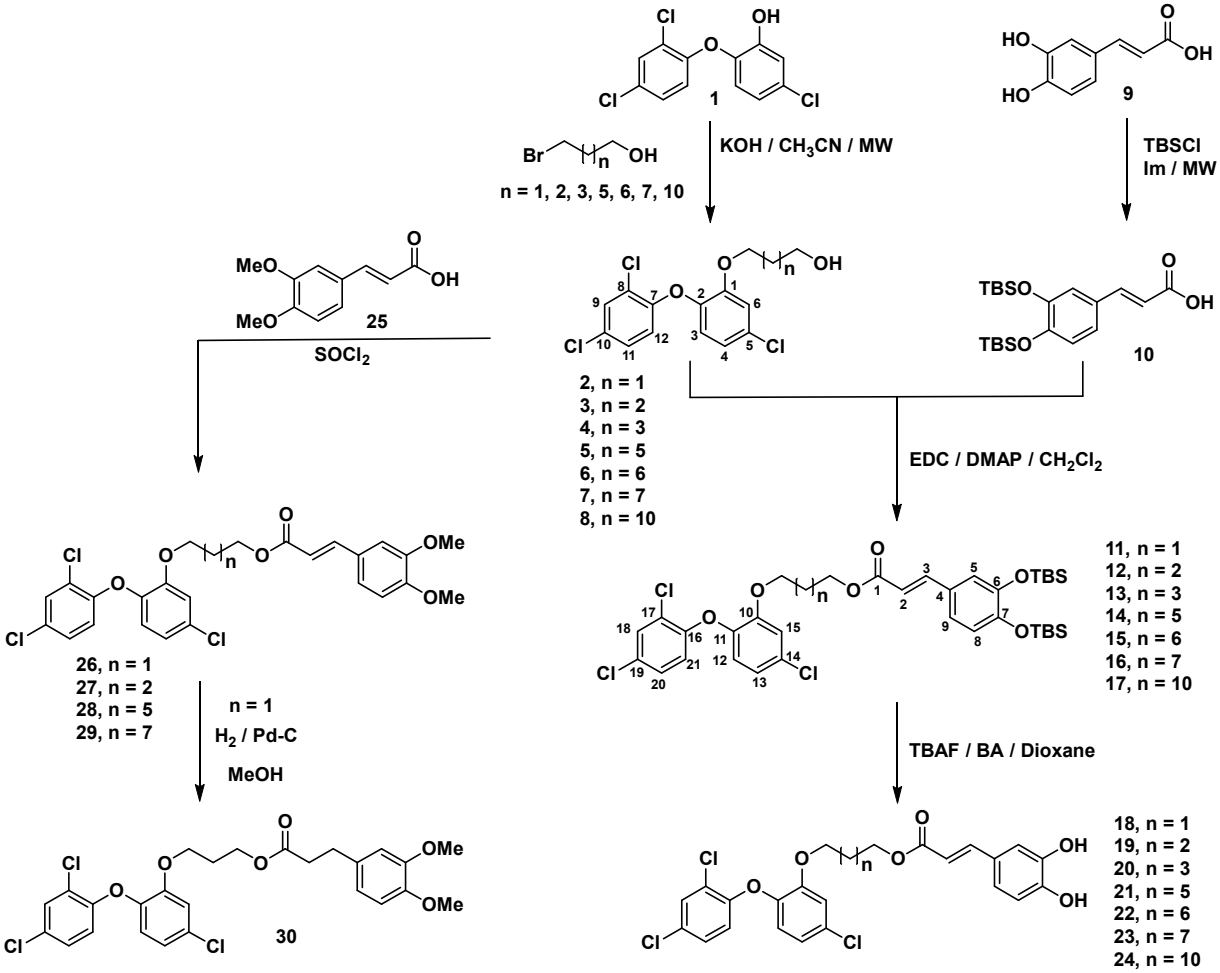
Figure 2. Design of triclosan-caffeic acid hybrids as antiprotozoal agents.

2. Results and discussion

2.1. Chemistry

The synthetic strategy for the preparation of caffeic acid esters is shown in Scheme 1. Thus, reaction of triclosan **1** with ω-bromoalkylalcohols upon microwave assisted Williamson etherification [27] yielded alcohols **2-8** in 61-88%. Caffeic acid silylation with TBSCl afforded silyl-protected caffeic acid **10** upon microwave irradiation [38]. This compound was esterified with ω-bromoalkylalcohols in the presence of EDC and DMAP to provide protected esters **11-17** in 41-60% yields [33]. Deprotection with TBAF and benzoic acid gave rise to esters **18-24** in 50-92% yields [34]. Attempts to directly obtain these compounds by coupling caffeic acid **9** with alcohols **2-8** using different coupling agents, such as EDC/HOBt [35], DCC/HOBt [36] and BOP/Et_3N [37], were fruitless. On the other hand, reaction of methylated caffeic acid **25** with thionyl chloride generated the corresponding acid chloride which in turn was reacted with alcohols **2**, **3**, **5** and **7** to afford esters **26-29** in 40-60% yields. Compound **30** was obtained in 94% yield by catalytic hydrogenation of compound **26** [39].

The structures of all compounds have been established by a combined study of IR, ESI-MS, ^{1}H-NMR, ^{13}C-NMR and COSY spectra. IR spectra exhibit characteristic absorption peaks corresponding to C=O, C=C, C=C_{Ar}, C-O-C, C-H_{Ar} and C-Cl groups. ESI-MS spectra show characteristic $[M+Na]^{+}$ peaks corresponding to their molecular weights. The assignments of all the signals to individual H or C-atoms have been performed on the basis of typical δ-values and *J*-constants. The ^{1}H-NMR spectra of these compounds dissolved in $CDCl_3$ or CD_3OD show signals of –CH_2O- (~4.06 ppm), H-C=C-H_{trans} (~6.21 and 7.50 ppm), -C = C-$H_{triclosan\ ring}$ (~6.92 and 7.46 ppm). ^{13}C-NMR spectra show signals around 62, 115, 124 and 168 ppm, corresponding to –CH_2O-, H-C=C-H_{trans}, C-Cl and C=O, respectively.



Scheme 1. Synthetic pathway to triclosan-caffeic acid hybrids.

2.2. Biological activities

The effect of triclosan-caffeic acid hybrids on cell growth and viability was assessed in human macrophages (U-937 cells) which are the host cells for *L. (V) panamensis* and *T. cruzi* parasites. In addition, the antiparasite activity of these compounds was tested on intracellular amastigotes of *L. (V.)*

panamensis and *T. cruzi* according to the ability of these compounds to reduce the amount of parasite inside infected macrophages. Results are summarized in Table 1.

Table 1. *In vitro* cytotoxicity and antiprotozoal activity of triclosan-caffeic acid hybrids

Compound	Cytotoxicity on U-937 cells	Anti-leishmanial activity on intracellular amastigotes		Anti-trypanosomal activity on intracellular amastigotes	
	LC_{50} (Mean ± SEM) [µM][a]	EC_{50} (Mean ± SEM) [µM][b]	SI[c]	EC_{50} (Mean ± SEM) [µM]	SI
18	>392.34	24.34 ± 0.31	>16.12	52.42 ± 5.04	>7.48
19	12.03 ± 0.77	3.82 ± 0.19	3.16	8.25 ± 1.21	1.46
20	8.18 ± 0.02	15.30 ± 0.17	0.53	29.66 ± 1.81	0.28
21	19.97 ± 1.9	11.65 ± 1.58	1.72	8.69 ± 0.62	2.29
22	40.18 ± 3.81	12.92 ± 3.41	3.11	18.35 ± 0.54	2.19
23	3.62 ± 0.35	30.22 ± 1.74	0.12	41.32 ± 3.87	0.09
24	173.74 ± 30.12	42.55 ± 0.80	4.08	34.21 ± 2.74	5.08
26	>371.87	38.06 ± 1.77	>9.77	>37.19	<10
27	73.39 + 8.55	53.19 ± 4.18	1.38	42.77 ± 7.50	1.72
28	336.75	99.05 ± 5.93	>3.40	27.44 ± 5.93	>12.26
29	106.92 ± 10.48	48.46 ± 2.16	2.21	33.02 ± 3.94	3.24
30	249.52 ± 9.66	40.25 ± 9.03	6.20	33.29 ± 0.52	7.49
Triclosan	193.41 ± 32.86	38.61 ± 2.38	5.01	48.97 ± 4.21	3.95
Caffeic acid	1110.12	1103.46 ± 177.34	>1.0	156.92 ± 18.43	>7.07
Amphotericin B	45.6 ± 2.2	0.054 ± 0.011	842	NA[d]	-
Benznidazole	687.8 ± 16.1	NA[d]	-	40.3 ± 6.92	17.0

Data represent, mean value +/- standard deviation; [a] LC_{50}: Lethal Concentration 50 in µM; [b] EC_{50}: Effective Concentration 50 in µM; [c] SI: Selectivity Index = LC_{50} / EC_{50}; [d]NA: Not applicable.

All compounds, with exception of **18**, **26**, **28** and caffeic acid, were cytotoxic to U-937 cells showing LC_{50} < 200.0 µM. Compound **30** showed moderate cytotoxicity (LC_{50} > 200.0 µM) while compounds **18**, **26**, **28**, caffeic acid and benznidazole had no cytotoxicity (LC_{50} > 300 µM). In turn, amphotericin B showed high cytotoxicity (LC_{50} = 45.6 µM) (Table 1).

The anti-leishmanial and anti-trypanosomal activities were measured by determining the effective concentration 50 (EC_{50}) that corresponds to the concentration of drug that gives the half-maximal reduction of the parasite growth (Table 1). Dose-response relationship shows that compounds **18**-**23**, **26**, **30** and triclosan were active against intracellular amastigotes of *L. (V) panamensis* with EC_{50} < 40 µM. The most actives esters are **18**-**22**, with an EC_{50} of 24.34 µM, 3.82 µM, 15.30 µM, 11.65 µM and 12.92 µM, respectively, followed by **23**, **26** and **30** with an EC_{50} of 30.22 µM, 38.06 µM and 40.25 µM, respectively. As expected, the anti-leishmanial drug amphotericin B showed activity with low EC_{50} values. In turn, compounds **19**, **21**, and **22** were highly active against intracellular amastigotes of *T. cruzi* (Tulahuen strain expressing β-galactosidase) with EC_{50} of 8.25 µM, 8.69 µM and 18.35 µM, respectively, followed by compounds **20**, **24** and **28**-**30** with an EC_{50} of 29.66 µM, 34.21 µM, 27.44 µM, 33.02 µM and 33.29, respectively. In this case, benznidazole showed activity with an EC_{50} of 40.3 µM. Esters **19**-**22**, **24** and **28**-**30** displayed higher anti-trypanosomal activity than benznidazole. However, the SI of these compounds is affected by their cytotoxicity. These results suggest a selective biological activity of the hybrids reported here (with the exception of **20** and **23**) as being more active against *T. cruzi* parasites (Tulahuen strain expressing β-galactosidase) than against U-937 cells.

It can be deduced form table 1 that the activity is related to the length of the alkyl chains and to the even or odd number of carbon atoms of these chains. Thus, for an even number of carbons a decrease in activity was observed with increasing linker length (**19** vs **22** and **24**). In contrast, for an odd number of carbons an increase in activity was achieved with increasing linker length **(18** vs **20** and **21**). The four-carbon alkyl chain was the most determinant for the activity, as compound **19** was the most performing one in both *L. (V) panamensis* and *T. cruzi* parasites. The presence of hydroxyl groups increases cytotoxicity and anti-leishmanial and anti-trypanosomal activities (**18**, **19**, **21**, **23** vs. **26**-**29**). This result is in agreement with the reports for several chalcones and coumarins [40,41]. However, the presence of hydroxyl groups decreased the activity of some of these compounds (**26**, **29** vs **18**, **23**) when they were evaluated against *T. cruzi* (Tulahuen strain expressing β-galactosidase). The effect of the hydroxyl groups may be due to a better molecular recognition ability towards target bioreceptors upon hydrogen bond formation [42]. It is interesting to note the synergistic effect of the parent subunits in the hybrids in comparison with the unlinked cases. For example, triclosan is less active against *L. (V). panamensis* and *T. cruzi* than its hybrids **19**-**22**. This phenomenon can also be observed for all hybrids, as they show increased activity compared to caffeic acid.

On the other hand, the presence of a double bond in the side chain is not decisive for cytotoxicity and anti-protozoal activity (**26** vs. **30**). A similar result was found for cinnamic acid bornyl ester and its hydrogenated derivative (39.6 and 50.2 µM, respectively) [43]. However, we have previously reported on cinnamic acid alkyl ester derivatives and we have shown a decrease in activity upon saturation of the double bond of these molecules [19]. Based on this fact, we proposed as their mechanism of action a nucleophilic addition of amino acid residues on the Michael acceptor system of these compounds [19, 44, 45]. However, according to the results achieved in this work we can suggest that the activity of triclosan-caffeic acid hybrids could be due to the presence of the triclosan unit. Two mechanisms of action for similar compounds have been reported. The first one could be related with the inhibition of the enzymatic systems of the protozoan by blocking the utilization of iron by the parasite, a mode of action which was observed for the African species [46]. The second one could be related with the loss of mitochondrial transmembrane potential [47].

3. Conclusions

The synthesis, cytotoxicity and activity against *L. (V) panamensis* and *T. cruzi* amastigotes of twelve triclosan-caffeic acid hybrids are reported. Eight of them were active against *L. (V) panamensis* (**18-23, 26** and **30**) and eigth of them against *T. cruzi* (**19-22**, **24** and **28-30**) with EC_{50} values lower than 40 µM, with **19** and **21** being the most active compounds against both *L. (V) panamensis* and *T. cruzi* with 3.82 and 11.65 µM and 8.25 and 8.69 µM, respectively. Compounds **19-22**, **24** and **28-30** showed higher activities than benznidazole (BNZ). Studies on an animal model of leishmaniasis are needed to confirm the results observed *in vitro*. Except for **18**, **26** and **28**, most of the compounds showed antiprotozoal activity but were toxic for mammalian cells. However, they have potential to be considered as candidates for anti-trypanosomal and anti-leishmanial drug development although more studies on toxicity using other cell lines are needed to discriminate whether the toxicity shown by these compounds is against tumor or non-tumor cells. The activity is dependent on the length of the alkyl linker being compound **19**, with a four-carbon alkyl chain, the most performing one. In general, hydroxyl groups increase both activity and cytotoxicity and the presence of a double bond in the side chain is not decisive for cytotoxicity and anti-protozoal activity. The mechanism of action of these compounds needs to be addressed and will be the objective of further studies.

4. Experimental section

4.1. Chemical synthesis

4.1.1. General remarks

Microwave reactions were carried out in a CEM Discover microwave reactor in sealed vessels (monowave, maximum power 300 W, temperature control by IR sensor, fixed temperature). ^{1}H and ^{13}C NMR spectra were recorded on a Varian instrument operating at 500 and 125 MHz, respectively. The signals of the deuterated solvent ($CDCl_3$ or CD_3OD) were used as reference ($CDCl_3$: δ = 7.27 ppm for ^{1}H NMR and δ = 77.00 ppm for ^{13}C NMR; CD_3OD: δ = 3.31 and 4.87 ppm for ^{1}H NMR and δ = 49.2 ppm for ^{13}C NMR). Carbon atom types (C, CH, CH_2, CH_3) were determined by using the DEPT or APT pulse sequence. Signal were assigned using two-dimensional heteronuclear correlations (COSY and HSQC). High resolution mass spectra were recorded using electrospray ionization mass spectrometry (ESI-MS). A QTOF Premier instrument with an orthogonal Z-spray-electrospray interface (Waters, Manchester, UK) was used operating in the W-mode. The drying and cone gas was nitrogen set to flow rates of 300 and 30 L/h, respectively. Methanol sample solutions (ca. 1×10^{-5} M) were directly introduced into the ESI spectrometer at a flow rate of 10 µL/min. A capillary voltage of 3.5 kV was used in the positive scan mode, and the cone voltage set to *U*c = 10 V. For accurate mass measurements, a 2 mg/L standard solution of leucine enkephalin was introduced via the lock spray needle at a cone voltage set to 85 V and a flow rate of 30 µL/min. IR spectra were recorded on a Spectrum RX I FT-IR system (Perkin-Elmer, Waltham, MA, USA) in KBr disks. Silica gel 60 (0.063–0.200 mesh, Merck, Whitehouse Station, NJ, USA) was used for column chromatography, and precoated silica gel plates (Merck 60 F254 0.2 mm) were used for thin layer chromatography (TLC).

*4.1.2. General procedure for the synthesis of triclosanalkyl-alcohols **2**-**8**:*

Triclosan, (3.1 mmol, 900 mg), potassium hydroxide (4.0 mmol, 224 mg) and acetonitrile (10 mL), were placed into in a 50 mL flat-bottomed flask equipped with a magnetic stirring bar. The mixture was stirred and heated to reflux under microwave irradiation for a period of 5 min. Then, ω-bromoalkylalcohols (3.2 mmol) were added and the reaction mixture was refluxed for 30 minutes under microwave irradiation (200 W). The crude reaction mixture was evaporated under reduced pressure and the residue was purified by column chromatography over silica gel eluting with Hexanes and a mixture

of Hexanes-Ethyl acetate (9:1 ratio) to obtain the alkyltriclosanalcohols in yields ranging between 61%–88%. Monitoring of the reaction progress and product purification was carried out by TLC.

*4.1.2.1. 3-(5-chloro-2-(2,4-dichlorophenoxy)phenoxy)propan-1-ol (**2**).* Yield 73% (2.19 mmol, 759.7 mg); clear oil; ^{1}H-NMR ($CDCl_3$, 500 MHz): δ 1.88–1.94 (CH_2, m), 3.65 (-CH_2OH, t, *J* = 5.9 Hz), 4.10 (-CH_2O-, t, *J* = 5.9 Hz), 6.67 (H_3, d, *J* = 8.8 Hz), 6.92-6.94 (H_4, H_{12}, m), 7.01 (H_6, $s_{apparent}$), 7.11 (H_{11}, dd, *J* = 8.8, 2.5 Hz), 7.44 (H_9, d, *J* = 2.5 Hz); ^{13}C-NMR ($CDCl_3$, 125 MHz): δ 31.64 (CH_2), 59.78 (-CH_2OH), 66.87 (-OCH_2-), 114.83 (C_6), 118.02 (C_3), 121.20 (C_{12}), 121.68 (C_4), 124.52 (C_5), 127.59 (C_{11}), 128.04 (C_8), 130.21 (C_9), 130.50 (C_{10}), 143.08 (C_2), 150.64 (C_1), 152.21 (C_7).

*4.1.2.2. 4-(5-chloro-2-(2,4-dichlorophenoxy)phenoxy)butan-1-ol (**3**).* Yield 61% (1.83 mmol, 660.8 mg); clear oil; ^{1}H-NMR ($CDCl_3$, 500 MHz): δ 1.48–1.56 (CH_2, m), 1.72–1.79 (CH_2, m), 3.60 (-CH_2OH, t, *J* = 6.4 Hz), 3.98 (-CH_2O-, t, *J* = 6.2 Hz), 6.66 (H_3, d, *J* = 8.8 Hz), 6.92-6.95 (H_4, H_{12}, m), 6.97 (H_6, d, *J* = 2.1), 7.10 (H_{11}, dd, *J* = 8.8, 2.5 Hz), 7.44 (H_9, d, *J* = 2.5 Hz); ^{13}C-NMR ($CDCl_3$, 125 MHz): δ 25.44 (CH_2), 29.03 (CH_2), 62.39 (-CH_2OH), 68.93 (-OCH_2-), 114.79 (C_6), 117.95 (C_3), 121.94 (C_{12}), 121.16 (C_4), 124.46 (C_5), 127.57 (C_{11}), 127.86 (C_8), 130.13 (C_9), 130.65 (C_{10}), 143.07 (C_2), 150.78 (C_1), 152.47 (C_7).

*4.1.2.3. 5-(5-chloro-2-(2,4-dichlorophenoxy)phenoxy)pentan-1-ol (**4**).* Yield 62% (1.87 mmol, 702.3 mg); clear oil; ^{1}H-NMR ($CDCl_3$, 500 MHz): δ 1.24–1.32 (CH_2, m), 1.47–1.55 (CH_2, m), 1.62–1.70 (CH_2, m), 3.58 (-CH_2OH, t, *J* = 6.4 Hz), 3.92 (-CH_2O-, t, *J* = 6.2 Hz), 6.63 (H_3, d, *J* = 8.3 Hz), 6.92 (H_4, dd, *J* = 8.3, 2.2), 6.96 (H_6, d, *J* = 2.2), 6.98 (H_{12}, d, *J* = 8.8), 7.08 (H_{11}, dd, *J* = 8.8, 2.5 Hz), 7.43 (H_9, d, *J* = 2.5 Hz); ^{13}C-NMR ($CDCl_3$, 125 MHz): δ 22.02 (CH_2), 28.66 (CH_2), 32.20 (CH_2), 62.66 (-CH_2OH), 68.90 (-OCH_2-), 114.69 (C_6), 117.65 (C_3), 120.90 (C_{12}), 122.23 (C_4), 124.24 (C_5), 127.48 (C_{11}), 127.62 (C_8), 130.04 (C_9), 130.65 (C_{10}), 142.88 (C_2), 150.95 (C_1), 152.60 (C_7).

*4.1.2.4. 7-(5-chloro-2-(2,4-dichlorophenoxy)phenoxy)heptan-1-ol (**5**).* Yield 64% (1.93 mmol, 780.2 mg); clear oil; ^{1}H-NMR ($CDCl_3$, 500 MHz): δ 1.17–1.25 (CH_2, m), 1.25–1.34 (CH_2, m), 1.49–1.58 (CH_2, m), 1.59–1.67 (CH_2, m), 3.64 (-CH_2OH, t, *J* = 6.5 Hz), 3.91 (-CH_2O-, t, *J* = 6.2 Hz), 6.63 (H_3, d, *J* = 8.5 Hz), 6.92 (H_4, dd, *J* = 8.5, 2.4), 6.96 (H_6, d, *J* = 2.4), 6.98 (H_{12}, d, *J* = 8.8), 7.08 (H_{11}, dd, *J* = 8.8, 2.6 Hz), 7.43 (H_9, d, *J* = 2.6 Hz); ^{13}C-NMR ($CDCl_3$, 125 MHz): δ 25.58 (CH_2), 25.67 (CH_2), 28.84 (CH_2), 28.95 (CH_2), 32.65 (CH_2), 62.97 (-CH_2OH), 69.97 (-OCH_2-), 114.67 (C_6), 117.57 (C_3), 120.82 (C_{12}), 122.30 (C_4), 124.22 (C_5), 127.45 (C_{11}), 127.56 (C_8), 130.05 (C_9), 130.67 (C_{10}), 142.85 (C_2), 151.04 (C_1), 152.68 (C_7).

*4.1.2.5. 8-(5-chloro-2-(2,4-dichlorophenoxy)phenoxy)octan-1-ol (**6**).* Yield 65% (2.02 mmol, 841 mg); pale yellow oil; ^{1}H-NMR ($CDCl_3$, 500 MHz): δ 1.09–1.42 ($4CH_2$, m), 1.50–1.75 ($2CH_2$, m), 3.67 (-CH_2OH, t, *J* = 6.5 Hz), 3.93 (-CH_2O-, t, *J* = 6.2 Hz), 6.65 (H_3, d, *J* = 8.8 Hz), 6.90-6.99 (H_4, H_6, m), 7.02 (H_{12}, d, *J* = 8.8), 7.11 (H_{11}, dd, *J* = 8.8, 2.4 Hz), 7.45 (H_9, d, *J* = 2.4 Hz); ^{13}C-NMR ($CDCl_3$, 125 MHz): δ 25.68 ($2CH_2$), 28.92 (CH_2), 29.21 (CH_2), 29.32 (CH_2), 32.77 (CH_2), 63.00 (-CH_2OH), 68.98 (-OCH_2-), 114.61 (C_6), 117.61 (C_3), 120.82 (C_{12}), 122.37 (C_4), 124.21 (C_5), 127.52 (C_{11}), 127.60 (C_8), 130.08 (C_9), 130.72 (C_{10}), 142.82 (C_2), 151.11 (C_1), 152.72 (C_7).

*4.1.2.6. 9-(5-chloro-2-(2,4-dichlorophenoxy)phenoxy)nonan-1-ol (**7**).* Yield 77% (2.31 mmol, 998.4 mg); clear oil; ^{1}H-NMR ($CDCl_3$, 500 MHz): δ 1.14–1.26 ($4CH_2$, m), 1.26–1.32 (CH_2, m), 1.32–1.40 (CH_2, m), 1.53–1.66 (CH_2, m), 3.65 (-CH_2OH, t, *J* = 6.8 Hz), 3.91 (-CH_2O-, t, *J* = 6.4 Hz), 6.63 (H_3, d, *J* = 8.8 Hz), 6.92 (H_4, dd, *J* = 8.8, 2.3), 6.96 (H_6, d, *J* = 2.3), 6.98 (H_{12}, d, *J* = 8.8), 7.08 (H_{11}, dd, *J* = 8.8, 2.5 Hz), 7.43 (H_9, d, *J* = 2.5 Hz); ^{13}C-NMR ($CDCl_3$, 125 MHz): δ 25.67 (CH_2), 25.69 (CH_2), 28.89 (CH_2), 29.11 (CH_2), 29.28 (CH_2), 29.41 (CH_2), 32.77 (CH_2), 63.04 (-CH_2OH), 69.04 (-OCH_2-), 114.68 (C_6), 117.60 (C_3), 120.80 (C_{12}), 122.27 (C_4), 124.21 (C_5), 127.44 (C_{11}), 127.60 (C_8), 130.05 (C_9), 130.61 (C_{10}), 142.87 (C_2), 151.09 (C_1), 152.67 (C_7).

*4.1.2.7. 12-(5-chloro-2-(2,4-dichlorophenoxy)phenoxy)dodecan-1-ol (**8**).* Yield 88% (2.65 mmol, 1255 mg); clear oil; ^{1}H-NMR ($CDCl_3$, 500 MHz): δ 1.15–1.26 ($5CH_2$, m), 1.26–1.41 ($3CH_2$, m), 1.54–1.65 ($2CH_2$, m), 3.65 (-CH_2OH, t, *J* = 6.5 Hz), 3.90 (-CH_2O-, t, *J* = 6.3 Hz), 6.63 (H_3, d, *J* = 8.6 Hz), 6.92 (H_4, dd, *J* = 8.6, 2.0), 6.96 (H_6, d, *J* = 2.0), 6.98 (d, *J* = 8.8, H_{12}), 7.08 (H_{11}, dd, *J* = 8.8, 2.5 Hz), 7.42 (H_9, d, *J* = 2.5 Hz); ^{13}C-NMR ($CDCl_3$, 125 MHz): δ 25.69 (CH_2), 25.72 (CH_2), 28.90 (CH_2), 29.19 (CH_2), 29.41 (CH_2), 29.46 (CH_2), 29.50 (CH_2), 29.53 (CH_2), 29.56 (CH_2), 32.81 (CH_2), 63.07 (-CH_2OH), 69.07 (-OCH_2-), 114.68 (C_6), 117.62 (C_3), 120.77 (C_{12}), 122.25 (C_4), 124.26 (C_5), 127.44 (C_{11}), 127.61 (C_8), 130.05 (C_9), 130.63 (C_{10}), 142.95 (C_2), 151.10 (C_1), 152.66 (C_7).

*4.1.3. General procedure for the synthesis of protected esters **11-17**:*

Caffeic acid (10 g, 55.51 mmol), tert-butyldimethylsilyl chloride (66.61 mmol, 10 g) and imidazole (111.02 mmol, 7.6 g) were placed into a 50 mL sealed tube. The mixture was heated under microwave irradiation (50 W) for a period of 10 min Then, the reaction mixture was poured into water and extracted with ethyl acetate. The combined organic phases were dried over anhydrous $MgSO_4$ and the solvent was evaporated under reduced pressure. The crude products were purified by column chromatography over silica gel eluting with Hexanes and a mixture of Hexanes-Ethyl acetate (8:2 ratio) affording silyl protected caffeic acid **10** in 52% yield (28.87 mmol, 11.8g). Alkyltriclosan

alcohols **2-8** (0.7 mmol) were dissolved in dry CH_2Cl_2 (20 mL) and treated sequentially with silyl protected caffeic acid **10** (0.73 mmol, 300mg), DMAP (1.2 mmol) and EDC (1.2 mmol). The reaction mixture was stirred for 24 h and filtered. The resulting solution was concentrated under reduced pressure and the residue was purified by column chromatography over silica gel eluting with Hexanes and a mixture of Hexanes-Ethyl acetate (9:1 ratio) affording compounds **11-17** in yields ranging between 41%–60%.

*4.1.3.1. 3-(5-chloro-2-(2,4-dichlorophenoxy)phenoxy)propyl (E)-3-(3,4-bis((tert-butyldimethylsilyl)oxy)phenyl)prop-2-enoate (**11**).* Yield 41% (0.284 mmol, 212 mg); pale yellow oil; ^{1}H-NMR ($CDCl_3$, 500 MHz): δ 0.25 (CH_3-Si), 0.26 (CH_3-Si), 1.03 (C<u>H</u>$_3$-C-Si), 1.04 (C<u>H</u>$_3$-C-Si), 2.00-2.15 (CH_2, m), 4.11 (-CH_2O-, t, *J* = 6.1 Hz), 4.20 (-CH_2O-, t, *J* = 6.2 Hz), 6.23 (H_2, d, *J* = 16.0 Hz), 6.71 (H_{12}, d, *J* = 8.8 Hz), 6.86 (H_8, d, *J* = 8.8 Hz), 6.96-70.1 (H_{13}, H_{15}, H_{21}, m), 7.02-7.08 (H_5, H_9, m), 7.13 (H_{20}, dd, *J* = 8.8, 2.5 Hz), 7.47 (H_{18}, d, *J* = 2.5 Hz), 7.59 (H_3, d, *J* = 16.0 Hz); ^{13}C-NMR ($CDCl_3$, 125 MHz): δ -4.02 (CH_3-Si), -4.06 (CH_3-Si), 18.47 (-C-Si), 18.53 (-C-Si), 25.62 (CH_2), 25.83 (CH_2), 25.90 (<u>C</u>H_3-C-Si), 25.93 (<u>C</u>H_3-C-Si), 28.58 (CH_2), 60.63 (-OCH_2-), 65.64 (-OCH_2-), 114.95 (C_8), 115.37 (C_5), 118.01 (C_{15}), 120.49 (C_2), 121.17 (C_{12}), 121.31 (C_{21}), 122.06 (C_8), 122.33 (C_9), 124.48 (C_{14}), 127.67 (C_{17}), 127.94 (C_{20}), 128.05 (C_4), 130.24 (C_{18}), 130.56 (C_{19}), 143.22 (C_{11}), 144.97 (C_3), 147.20 (C_6), 149.54 (C_{10}), 151.71 (C_{16}), 152.39 (C_7), 167.12 (C = O).

*4.1.3.2. 4-(5-chloro-2-(2,4-dichlorophenoxy)phenoxy)butyl (E)-3-(3,4-bis((tert-butyldimethylsilyl)oxy)phenyl)prop-2-enoate (**12**).* Yield 43% (0.304 mmol, 231 mg); pale yellow oil; ^{1}H-NMR ($CDCl_3$, 500 MHz): δ 0.93 (CH_3-Si), 0.95 (C<u>H</u>$_3$-C-Si), 1.56-1.70 (CH_2, m), 1.71-1.87 (CH_2, m), 4.00 (-CH_2O-, t, *J* = 6.1 Hz), 4.14 (-CH_2O-, t, *J* = 6.0 Hz), 6.23 (H_2, d, *J* = 16.0 Hz), 6.67 (H_{12}, d, *J* = 8.6 Hz), 6.79-6.89 (H_8, H_{13}, H_{21}, m), 6.92-7.06 (H_5, H_9, H_{15}, m), 7.08-7.16 (H_{20}, m), 7.46 (H_{18}, d, *J* = 2.4 Hz), 7.59 (H_3, d, *J* = 16.0 Hz); ^{13}C-NMR ($CDCl_3$, 125 MHz): δ 3.55 (CH_3-Si), 4.03 (CH_3-Si), 18.02 (-C-Si), 18.47 (-C-Si), 25.07 (CH_2), 25.63 (CH_2), 25.68 (<u>C</u>H_3-C-Si), 25.92 (<u>C</u>H_3-C-Si), 45.48 (2CH_2), 63.54 (-OCH_2-), 68.39 (-OCH_2-), 114.17 (C_8), 114.36 (C_5), 114.73 (C_{15}), 115.75 (C_2), 117.75 (C_{12}), 121.07 (C_{21}), 121.80 (C_8), 122.28 (C_9), 124.33 (C_{14}), 126.22 (C_{17}), 127.59 (C_{20}), 127.81 (C_4), 130.16 (C_{18}), 130.69 (C_{19}), 142.97 (C_{11}), 145.60 (C_3), 146.03 (C_6), 148.95 (C_{10}), 150.86 (C_{16}), 152.61 (C_7), 167.69 (C = O).

*4.1.3.3. 5-(5-chloro-2-(2,4-dichlorophenoxy)phenoxy)pentyl (E)-3-(3,4-bis((tert-butyldimethylsilyl)oxy)phenyl)prop-2-enoate (**13**).* Yield 59% (0.41 mmol, 317 mg); pale yellow oil; ^{1}H-NMR ($CDCl_3$, 500 MHz): δ 0.23 (CH_3-Si), 1.00 (C<u>H</u>$_3$-C-Si), 1.01 (C<u>H</u>$_3$-C-Si), 1.52–1.61 (CH_2, m),

1.62–1.74 (2CH_2, m), 3.95 (-CH_2O-, t, *J* = 6.3 Hz), 4.14 (-CH_2O-, t, *J* = 6.8 Hz), 6.23 (H_2, d, *J* = 16.0 Hz), 6.64 (H_{12}, d, *J* = 8.8 Hz), 6.83 (H_8, d, *J* = 8.8 Hz), 6.93 (H_{13}, dd, *J* = 8.8, 2.2), 6.96 (H_{15}, d, *J* = 2.2), 6.98 (H_{21}, d, *J* = 8.7), 7.03 (H_5, $s_{apparent}$), 7.06 (H_9, dd, *J* = 8.8, 2.5 Hz), 7.09 (H_{20}, dd, *J* = 8.7, 2.5 Hz), 7.41 (H_{18}, d, *J* = 2.5 Hz), 7.57 (H_3, d, *J* = 16.0 Hz); ^{13}C-NMR ($CDCl_3$, 125 MHz): δ -4.06 (CH_3-Si), -4.10 (CH_3-Si), 18.43 (-C-Si), 18.48 (-C-Si), 25.87 (CH_3-C-Si), 25.89 (CH_3-C-Si), 22.18 (CH_2), 28.40 (2CH_2), 64.10 (-OCH_2-), 68.76 (-OCH_2-), 114.67 (C_8), 114.79 (C_5), 117.60 (C_{15}), 117.74 (C_2), 120.92 (C_{12}), 121.01 (C_{21}), 122.17 (C_8), 122.28 (C_9), 124.23 (C_{14}), 127.48 (C_{17}), 127.57 (C_{20}), 127.76 (C_4), 130.01 (C_{18}), 130.74 (C_{19}), 142.98 (C_{11}), 144.60 (C_3), 146.26 (C_6), 150.94 (C_{10}), 152.60 (C_{16}), 152.68 (C_7), 167.25 (C = O).

*4.1.3.4. 7-(5-chloro-2-(2,4-dichlorophenoxy)phenoxy)heptyl (E)-3-(3,4-bis((tert-butyldimethylsilyl)oxy)phenyl)prop-2-enoate (**14**).* Yield 60% (0.420 mmol, 337 mg); pale yellow oil; ^{1}H-NMR ($CDCl_3$, 500 MHz): δ 0.14 (CH_3-Si), 0.91 (CH_3-C-Si), 0.92 (CH_3-C-Si), 1.09–1.30 (3CH_2, m), 1.50–1.63 (2CH_2, m), 3.82 (-CH_2O-, t, *J* = 6.2 Hz), 4.10 (-CH_2O-, t, *J* = 6.8 Hz), 6.16 (H_2, d, *J* = 16.0 Hz), 6.54 (H_{12}, d, *J* = 8.8 Hz), 6.74 (H_8, d, *J* = 8.9 Hz), 6.83 (H_{13}, dd, *J* = 8.8, 2.4), 6.87 (H_{15}, d, *J* = 2.4), 6.90 (H_{21}, d, *J* = 8.6), 6.92-6.95 (H_5, H_9, m), 6.99 (H_{20}, dd, *J* = 8.6, 2.6 Hz), 7.34 (H_{18}, d, *J* = 2.6 Hz), 7.49 (H_3, d, *J* = 16.0 Hz); ^{13}C-NMR ($CDCl_3$, 125 MHz): δ -4.07 (CH_3-Si), -4.11 (CH_3-Si), 18.42 (-C-Si), 18.47 (-C-Si), 25.62 (CH_2), 25.83 (CH_2), 25.86 (CH_3-C-Si), 25.89 (CH_3-C-Si), 28.66 (CH_2), 28.85 (CH_2), 28.86 (CH_2), 64.42 (-OCH_2-), 68.96 (-OCH_2-), 114.69 (C_8), 115.86 (C_5), 117.57 (C_{15}), 120.37 (C_2), 120.83 (C_{12}), 121.11 (C_{21}), 122.19 (C_8), 122.30 (C_9), 124.24 (C_{14}), 127.44 (C_{17}), 127.62 (C_{20}), 128.07 (C_4), 130.06 (C_{18}), 130.67 (C_{19}), 142.88 (C_{11}), 144.50 (C_3), 147.15 (C_6), 149.35 (C_{10}), 151.05 (C_{16}), 152.66 (C_7), 167.34 (C = O).

*4.1.3.5. 8-(5-chloro-2-(2,4-dichlorophenoxy)phenoxy)octyl (E)-3-(3,4-bis((tert-butyldimethylsilyl)oxy)phenyl)prop-2-enoate (**15**).* Yield 54% (0.378 mmol, 306 mg); pale yellow oil; ^{1}H-NMR ($CDCl_3$, 500 MHz): δ 0.25 (CH_3-Si), 0.26 (CH_3-Si), 1.03 (CH_3-C-Si), 1.04 (CH_3-C-Si), 1.14-1.45 (2CH_2, m), 1.56–1.69 (4CH_2, m), 3.94 (-CH_2O-, t, *J* = 6.4 Hz), 4.23 (-CH_2O-, t, *J* = 6.5 Hz), 6.28 (H_2, d, *J* = 16.1 Hz), 6.66 (H_{12}, d, *J* = 8.8 Hz), 6.86 (H_8, d, *J* = 8.7 Hz), 6.96 (H_{13}, dd, *J* = 8.8, 2.0), 6.98-7.02 (H_{21}, H_{15}, m), 7.03-7.08 (H_5, H_9, m), 7.11 (H_{20}, dd, *J* = 8.7, 2.0 Hz), 7.47 (H_{18}, d, *J* = 2.0 Hz), 7.61 (H_3, d, *J* = 16.1 Hz); ^{13}C-NMR ($CDCl_3$, 125 MHz): δ -4.03 (CH_3-Si), -4.07 (CH_3-Si), 18.47 (-C-Si), 18.53 (-C-Si), 25.69 (CH_2), 25.91 (CH_3-C-Si), 25.93 (CH_3-C-Si), 28.62 (CH_2), 28.79 (CH_2), 28.91 (CH_2), 29.14 (CH_2), 29.17 (CH_2), 29.20 (CH_2), 64.50 (-OCH_2-), 68.94 (-OCH_2-), 114.62 (C_8), 117.59 (C_5), 120.44 (C_{15}), 120.83 (C_2), 121.16 (C_{12}), 122.27 (C_{21}), 122.33 (C_8), 122.36 (C_9), 125.81 (C_{14}),

127.51 (C_{17}), 127.66 (C_{20}), 128.08 (C_4), 130.11 (C_{18}), 130.72 (C_{19}), 142.93 (C_{11}), 144.59 (C_3), 148.34 (C_6), 149.44 (C_{10}), 151.12 (C_{16}), 152.62 (C_7), 167.46 (C = O).

*4.1.3.6. 9-(5-chloro-2-(2,4-dichlorophenoxy)phenoxy)nonyl (E)-3-(3,4-bis((tert-butyldimethylsilyl)oxy)phenyl)prop-2-enoate (**16**).* Yield 49% (0.344 mmol, 286 mg); pale yellow oil; ^{1}H-NMR ($CDCl_3$, 500 MHz): δ 0.22 (CH_3-Si), 0.23 (CH_3-Si), 1.00 (C<u>H</u>$_3$-C-Si), 1.01 (C<u>H</u>$_3$-C-Si), 1.15–1.46 (5CH_2, m), 1.56–1.66 (CH_2, m), 1.67–1.76 (CH_2, m), 3.91 (-CH_2O-, t, *J* = 6.2 Hz), 4.20 (-CH_2O-, t, *J* = 6.8 Hz), 6.25 (H_2, d, *J* = 16.0 Hz), 6.63 (H_{12}, d, *J* = 8.8 Hz), 6.83 (H_8, d, *J* = 8.8 Hz), 6.83 (H_{13}, dd, *J* = 8.8, 2.1), 6.96 (H_{15}, d, *J* = 2.1), 6.98 (H_{21}, d, *J* = 8.9), 7.01-7.04 (H_5, H_9, m), 7.08 (H_{20}, dd, *J* = 8.9, 2.4 Hz), 7.43 (H_{18}, d, *J* = 2.4 Hz), 7.57 (H_3, d, *J* = 16.0 Hz); ^{13}C-NMR ($CDCl_3$, 125 MHz): δ -4.06 (CH_3-Si), -4.10 (CH_3-Si), 18.43 (-C-Si), 18.48 (-C-Si), 25.70 (CH_2), 25.87 (<u>C</u>H_3-C-Si), 25.90 (<u>C</u>H_3-C-Si), 25.95 (CH_2), 28.77 (CH_2), 28.91 (CH_2), 29.13 (CH_2), 29.18 (CH_2), 29.37 (CH_2), 29.68 (CH_2), 64.53 (-OCH_2-), 68.04 (-OCH_2-), 114.70 (C_8), 115.90 (C_5), 117.60 (C_{15}), 120.38 (C_2), 120.80 (C_{12}), 121.11 (C_{21}), 122.19 (C_8), 122.30 (C_9), 124.23 (C_{14}), 127.44 (C_{17}), 127.61 (C_{20}), 128.09 (C_4), 130.06 (C_{18}), 130.67 (C_{19}), 142.92 (C_{11}), 144.48 (C_3), 147.16 (C_6), 149.34 (C_{10}), 151.11 (C_{16}), 152.70 (C_7), 167.37 (C = O).

*4.1.3.7. 12-(5-chloro-2-(2,4-dichlorophenoxy)phenoxy)dodecyl (E)-3-(3,4-bis((tert-butyldimethylsilyl)oxy)phenyl)prop-2-enoate (**17**).* Yield 52% (0.366 mmol, 319 mg); pale yellow oil; ^{1}H-NMR ($CDCl_3$, 500 MHz): δ 0.22 (CH_3-Si), 0.23 (CH_3-Si), 1.00 (C<u>H</u>$_3$-C-Si), 1.01 (C<u>H</u>$_3$-C-Si), 1.14–1.47 (8CH_2, m), 1.57–1.66 (CH_2, m), 1.67–1.75 (CH_2, m), 3.91 (-CH_2O-, t, *J* = 6.3 Hz), 4.20 (-CH_2O-, t, *J* = 6.8 Hz), 6.24 (H_2, d, *J* = 16.0 Hz), 6.63 (H_{12}, d, *J* = 8.8 Hz), 6.83 (H_8, d, *J* = 8.8 Hz), 6.92 (H_{13}, dd, *J* = 8.8, 2.2), 6.96 (H_{15}, d, *J* = 2.2), 6.98 (H_{21}, d, *J* = 8.8), 7.01-7.04 (H_5, H_9, m), 7.08 (H_{20}, dd, *J* = 8.8, 2.5 Hz), 7.42 (H_{18}, d, *J* = 2.5 Hz), 7.57 (H_3, d, *J* = 16.0 Hz); ^{13}C-NMR ($CDCl_3$, 125 MHz): δ -4.06 (CH_3-Si), -4.10 (CH_3-Si), 18.43 (-C-Si), 18.48 (-C-Si), 25.70 (CH_2), 25.87 (<u>C</u>H_3-C-Si), 25.90 (<u>C</u>H_3-C-Si), 25.98 (CH_2), 28.79 (CH_2), 28.92 (CH_2), 29.20 (CH_2), 29.30 (CH_2), 29.47 (CH_2), 29.51 (CH_2), 29.55 (CH_2), 29.68 (CH_2), 64.57 (-OCH_2-), 68.08 (-OCH_2-), 114.70 (C_8), 115.90 (C_5), 117.61 (C_{15}), 120.38 (C_2), 120.78 (C_{12}), 121.11 (C_{21}), 122.18 (C_8), 122.27 (C_9), 124.24 (C_{14}), 127.44 (C_{17}), 127.61 (C_{20}), 128.10 (C_4), 130.06 (C_{18}), 130.65 (C_{19}), 142.88 (C_{11}), 144.46 (C_3), 147.19 (C_6), 149.34 (C_{10}), 151.12 (C_{16}), 152.67 (C_7), 167.38 (C = O).

*4.1.4. General procedure for the synthesis of esters **18-24:***

Compounds **11-17** (0.25 mmol) were dissolved in dioxane (8 mL) and treated with benzoic acid (BA) (0.03 mmol) and TBAF (1 mmol). The mixture was then heated at reflux for 12 h, cooled, and

neutralized by addition of solid $NaHCO_3$. After filtration, the solution was evaporated in vacuo, and the residue was subjected to column chromatography on silica gel (EtOAc-MeOH, 1:1) affording compounds **18-24** in yields between 50-98%.

*4.1.4.1. 3-(5-chloro-2-(2,4-dichlorophenoxy)phenoxy)propyl (E)-3-(3,4-dihydroxyphenyl) prop-2-enoate (**18**).* Yield 73% (0.183 mmol, 93 mg); pale yellow oil; IR (KBr, cm-1): ν_{max} 3483 (OH), 2927 (C-H), 1685 (C=O), 1600 (C=C), 1477 (C=C_{Ar}), 1269 (C-O-C), 1184 ((C=O)-O), 800 (C-H_{Ar}), 700 (C-Cl). ^{1}H-NMR (CD_3OD-$CDCl_3$, 300 MHz): δ 1.87–2.06 (CH_2, m), 4.06 (-CH_2O-, t, *J* = 6.5 Hz), 6.21 (H_2, d, *J* = 16.0 Hz), 6.06 (H_{12}, d, *J* = 8.9 Hz), 6.78 (H_8, d, *J* = 8.1 Hz), 6.92 (H_{13}, dd, *J* = 8.8, 1.7 Hz), 6.95-7.05 (H_{15}, H_{21}, m), 7.10-7.18 (H_5, H_9, H_{20}, m), 7.46 (H_{18}, d, *J* = 2.5 Hz), 7.50 (H_3, d, *J* = 16.0 Hz); ^{13}C-NMR (CD_3OD-$CDCl_3$, 75 MHz): δ 18.13 (CH_2), 28.18 (CH_2), 65.34 (-OCH_2-), 61.17 (-OCH_2-), 113.56 (C_{15}), 113.78 (C_5), 114.67 (C_8), 115.08 (C_2), 117.43 (C_{12}), 120.95 (C_{21}), 121.60 (C_{13}), 122.17 (C_9), 123.75 (C_{14}), 126.30 (C_{17}), 127.48 (C_{20}), 127.60 (C_4), 129.68 (C_{19}), 130.56 (C_{18}), 142.85 (C_{11}), 145.38 (C_6), 145.54 (C_3), 148.17 (C_7), 150.89 (C_{10}), 152.63 (C_{16}), 167.68 (C = O). EIMS: m/z 531.0145 $[M + Na]^+$, Calcd. for $C_{24}H_{19}Cl_3O_6$: 531.0129.

*4.1.4.2. 4-(5-chloro-2-(2,4-dichlorophenoxy)phenoxy)butyl (E)-3-(3,4-dihydroxyphenyl)prop-2-enoate (**19**).* Yield 62% (0.155 mmol, 81 mg); pale yellow solid; m.p. 154–156 °C; IR (KBr, cm-1): ν_{max} 3483 (OH), 2924 (C-H), 1685 (C=O), 1600 (C=C), 1473 (C=C_{Ar}), 1274 (C-O-C), 1190 ((C=O)-O), 804 (C-H_{Ar}), 700 (C-Cl). ^{1}H-NMR (DMSO-D_6, 500 MHz): δ 1.40–1.50 (CH_2, m), 1.56–1.64 (CH_2, m), 4.01 (-CH_2O-, t, *J* = 6.1 Hz), 6.23 (H_2, d, *J* = 16.0 Hz), 6.73 (H_{12}, d, *J* = 8.8 Hz), 6.76 (H_8, d, *J* = 8.0 Hz), 6.97 (H_{13}, dd, *J* = 8.1, 2.0), 7.00-7.09 (H_{12}, H_{15}, m), 7.15 (H_{20}, dd, *J* = 8.6, 2.4), 7.24-7.30 (H_5, H_9, m), 7.45 (H_3, d, *J* = 16.0 Hz), 7.64 (H_{18}, d, *J* = 2.4 Hz); ^{13}C-NMR (DMSO-D_6, 125 MHz): δ 24.50 (CH_2), 25.00 (CH_2), 64.07 (-OCH_2-), 68.09 (-OCH_2-), 114.73 (C_{15}), 114.80 (C_5), 115.68 (C_8), 117.90 (C_2), 120.82 (C_{12}), 121.26 (C_{21}), 122.63 (C_{13}), 123.02 (C_9), 125.46 (C_{14}), 126.65 (C_{17}), 128.16 (C_{20}), 129.50 (C_4), 129.57 (C_{19}), 129.85 (C_{18}), 142.16 (C_{11}), 144.92 (C_6), 145.53 (C_3), 148.34 (C_7), 150.61 (C_{10}), 152.22 (C_{16}), 166.38 (C = O). EIMS: m/z 545.0301 $[M + Na]^+$, Calcd. for $C_{25}H_{21}Cl_3O_6$: 545.0287.

*4.1.4.3. 5-(5-chloro-2-(2,4-dichlorophenoxy)phenoxy)pentyl (E)-3-(3,4-dihydroxyphenyl)prop-2-enoate (**20**).* Yield 50% (0.125 mmol, 67 mg); pale yellow solid; m.p. 126-128°C; IR (KBr, cm-1): ν_{max} 3471 (OH), 2933 (C-H), 1689 (C=O), 1602 (C=C), 1477 (C=C_{Ar}), 1273(C-O-C), 1186 ((C=O)-O), 812 (C-H_{Ar}), 700 (C-Cl). ^{1}H-NMR (DMSO-D_6, 500 MHz): δ 1.13–1.22 (CH_2, m), 1.47–1.58 (2CH_2, m), 3.97 (-CH_2O-, t, *J* = 6.0 Hz), 4.01 (-CH_2O-, t, *J* = 6.6 Hz), 6.23 (H_2, d, *J* = 16.0 Hz), 6.73 (H_{12}, d, *J* =

8.7 Hz), 6.76 (H_8, d, *J* = 8.2 Hz), 6.98 (H_{13}, dd, *J* = 8.7, 2.0), 7.02-7.07 (H_9, H_{15}, m), 7.16 (H_{21}, d, *J* = 8.8 Hz), 7.26 (H_5, d, *J* = 2.3 Hz), 7.28 (H_{20}, dd, *J* = 8.8, 2.6), 7.46 (H_3, d, *J* = 16.0 Hz), 7.65 (H_{18}, d, *J* = 2.6 Hz); ^{13}C-NMR (DMSO-D_6, 125 MHz): δ 26.96 (CH_2), 33.04 (CH_2), 33.22 (CH_2), 68.73 (-OCH_2-), 73.64 (-OCH_2-), 119.10 (C_{15}), 119.17 (C_5), 120.00 (C_8), 120.92 (C_2), 123.11 (C_{12}), 126.00 (C_{21}), 126.50 (C_{13}), 127.90 (C_9), 128.23 (C_{14}), 130.67 (C_{17}), 131.83 (C_{20}), 133.43 (C_4), 134.78 (C_{19}), 135.11 (C_{18}), 147.36 (C_{11}), 150.10 (C_6), 150.78 (C_3), 153.62 (C_7), 155.95 (C_{10}), 157.51 (C_{16}), 171.7 (C = O). EIMS: m/z 559.0458 $[M + Na]^+$, Calcd. for $C_{26}H_{23}Cl_3O_6$: 559,0466.

*4.1.4.4. 7-(5-chloro-2-(2,4-dichlorophenoxy)phenoxy)heptyl (E)-3-(3,4-dihydroxyphenyl)prop-2-enoate (**21**).* Yield 92% (0.230 mmol, 130 mg); pale yellow oil; IR (KBr, cm-1): v_{max} 3387 (OH), 2935 (C-H), 1689 (C=O), 1598 (C=C), 1496 (C=C_{Ar}), 1269 (C-O-C), 1190 ((C=O)-O), 800 (C-H_{Ar}), 704 (C-Cl). ^{1}H-NMR (CD_3OD, 300 MHz): δ 1.17–1.38 ($3CH_2$, m), 1.47–1.72 ($2CH_2$, m), 3.89 (-CH_2O-, t, *J* = 6.0 Hz), 4.15 (-CH_2O-, t, *J* = 6.4 Hz), 6.26 (H_2, d, *J* = 16.0 Hz), 6.61 (H_{12}, d, *J* = 8.8 Hz), 6.78 (H_8, d, *J* = 8.2 Hz), 6.90-7.00 (H_9, H_{15}, m), 7.01-7.10 (H_5, H_{13}, H_{21}, m), 7.14 (H_{20}, dd, *J* = 8.8, 2.5), 7.46 (H_{18}, d, *J* = 2.5 Hz), 7.54 (H_3, d, *J* = 16.0 Hz); ^{13}C-NMR (CD_3OD, 75 MHz): δ 25.43 (CH_2), 25.63 (CH_2), 28.35 (CH_2), 28.59 (CH_2), 28.65 (CH_2), 64.17 (-OCH_2-), 68.45 (-OCH_2-), 113.71 (C_{15}), 113.84 (C_5), 114.23 (C_8), 115.10 (C_2), 116.91 (C_{12}), 120.51 (C_{21}), 121.55 (C_{13}), 122.56 (C_9), 123.47 (C_{14}), 126.27 (C_{17}), 127.01 (C_{20}), 127.45 (C_4), 129.56 (C_{19}), 130.78 (C_{18}), 142.35 (C_{11}), 145.40 (C_6), 145.44 (C_3), 148.21 (C_7), 151.25 (C_{10}), 153.00 (C_{16}), 168.62 (C = O). EIMS: m/z 587.0771 $[M + Na]^+$, Calcd. for $C_{28}H_{27}Cl_3O_6$: 587.0772.

*4.1.4.5. 8-(5-chloro-2-(2,4-dichlorophenoxy)phenoxy)octyl (E)-3-(3,4-dihydroxyphenyl)prop-2-enoate (**22**).* Yield 92% (0.230 mmol, 133 mg); pale yellow oil; IR (KBr, cm-1): v_{max} 3421 (OH), 2926 (C-H), 1683 (C=O), 1600 (C=C), 1469 (C=C_{Ar}), 1267 (C-O-C), 1188 ((C=O)-O), 806 (C-H_{Ar}), 702 (C-Cl). ^{1}H-NMR (CD_3OD-$CDCl_3$, 300 MHz): δ 1.11–1.49 ($4CH_2$, m), 1.69–1.81 (CH_2, m), 1.58-1.69 (CH_2, m), 3.94 (-CH_2O-, t, *J* = 6.2 Hz), 4.23 (-CH_2O-, t, *J* = 6.7 Hz), 6.30 (H_2, dd, J = 15.8 Hz), 6.66 (H_{12}, d, *J* = 8.8 Hz), 6.91 (H_{21}, d, *J* = 8.1 Hz), 6.96 (H_{13}, dd, *J* = 8.8, 2.4 Hz), 6.98-7.01 (H_{15}, H_{21}, m), 7.02-7.06 (H_5, H_9, m), 7.11 (H_{20}, dd, *J* = 8.8, 2.5 Hz), 7.46 (H_{18}, d, *J* = 2.5 Hz), 7.62 (H_3, d, *J* = 15.8 Hz); ^{13}C-NMR (CD_3OD-$CDCl_3$, 75 MHz): δ 25.68 (CH_2), 25.91 (CH_2), 28.72 (CH_2), 28.91 (CH_2), 29.13 (CH_2), 29.17 (CH_2), 64.80 (-OCH_2-), 68.99 (-OCH_2-), 114.36 (C_{15}), 114.63 (C_5), 115.46 (C_8), 115.59 (C_2), 117.65 (C_{12}), 120.85 (C_{13}), 122.33 (C_9), 124.24 (C_{14}), 127.46 (C_{17}), 127.52 (C_{20}), 127.66 (C_4), 130.10 (C_{19}), 130.71 (C_{18}), 142.84 (C_{11}), 143.97 (C_6), 144.93 (C_3), 146.50 (C_7), 151.07 (C_{10}), 152.68 (C_{16}), 167.95 (C = O). EIMS: m/z 601.0933 $[M + Na]^+$, Calcd. for $C_{29}H_{29}Cl_3O_6$: 601,0904.

*4.1.4.6. 9-(5-chloro-2-(2,4-dichlorophenoxy)phenoxy)nonyl (E)-3-(3,4-dihydroxyphenyl)prop-2-enoate (**23**).* Yield 81% (0.203 mmol, 126 mg); pale yellow oil; IR (KBr, cm-1): v_{max} 3406 (OH), 2929 (C-H), 1689 (C=O), 1606 (C=C), 1477 (C=C_{Ar}), 1269 (C-O-C), 1190 ((C=O)-O), 810 (C-H_{Ar}), 744 (C-Cl). ^{1}H-NMR (CD_3OD-$CDCl_3$, 300 MHz): δ 1.06–1.45 (5CH_2, m), 1.47–1.61 (CH_2, m), 1.62–1.75 (CH_2, m), 3.88 (-CH_2O-, t, *J* = 6.0 Hz), 4.16 (-CH_2O-, t, *J* = 6.6 Hz), 6.24 (H_2, d, *J* = 16.0 Hz), 6.60 (H_{12}, d, *J* = 8.9 Hz), 6.78 (H_8, d, *J* = 8.9 Hz), 6.88-6.97 (H_{13}, H_{15}, H_{21}, m), 6.99-7.06 (H_5, H_9, m), 7.10 (H_{20}, dd, *J* = 8.9, 2.4 Hz), 7.42 (H_{18}, d, *J* = 2.4 Hz), 7.53 (H_3, d, *J* = 16.0 Hz); ^{13}C-NMR (CD_3OD-$CDCl_3$, 75 MHz): δ 25.56 (CH_2), 25.77 (CH_2), 28.51 (CH_2), 28.76 (CH_2), 28.94 (CH_2), 29.20 (CH_2), 64.40 (-OCH_2-), 68.64 (-OCH_2-), 113.87 (C_{15}), 114.00 (C_5), 114.35 (C_8), 115.20 (C_2), 117.07 (C_{12}), 120.60 (C_{21}), 121.68 (C_{13}), 122.48 (C_9), 123.67 (C_{14}), 126.35 (C_{17}), 127.19 (C_{20}), 127.41 (C_4), 129.69 (C_{19}), 130.80 (C_{18}), 142.45 (C_{11}), 145.31 (C_6), 145.52 (C_3), 148.05 (C_7), 151.18 (C_{10}), 152.88 (C_{16}), 168.24 (C = O). EIMS: m/z 615.1084 $[M + Na]^+$, Calcd. for $C_{30}H_{31}Cl_3O_6$: 615.1080.

*4.1.4.7. 12-(5-chloro-2-(2,4-dichlorophenoxy)phenoxy)dodecyl (E)-3-(3,4-dihydroxyphenyl)prop-2-enoate (**24**).* Yield 80% (0.20 mmol, 127 mg); pale yellow solid; m.p. 78-80°C; IR (KBr, cm-1): v_{max} 3483 (OH), 2924 (C-H), 1689 (C=O), 1598 (C=C), 1463 (C=C_{Ar}), 1271 (C-O-C), 1174 ((C=O)-O), 804 (C-H_{Ar}), 700 (C-Cl). ^{1}H-NMR (CD_3OD, 300 MHz): δ 1.13–1.48 (8CH_2, m), 1.55–1.68 (CH_2, m), 1.69–1.82 (CH_2, m), 3.93 (-CH_2O-, t, *J* = 6.0 Hz), 4.23 (-CH_2O-, t, *J* = 6.4 Hz), 6.26 (H_2, d, *J* = 16.0 Hz), 6.66 (H_{12}, d, *J* = 8.8 Hz), 6.88 (H_8, d, *J* = 8.0 Hz), 7.07-7.17 (H_9, H_{15}, m), 6.92-7.05 (H_5, H_{13}, H_{21}, m), 7.11 (H_{20}, dd, *J* = 8.6, 2.4), 7.45 (H_{18}, d, *J* = 2.2 Hz), 7.58 (H_3, d, *J* = 16.0 Hz); ^{13}C-NMR (CD_3OD, 75 MHz): δ 25.75 (CH_2), 26.01 (CH_2), 28.74 (CH_2), 28.93 (CH_2), 29.26 (CH_2), 29.33 (CH_2), 29.55 (4CH_2), 64.90 (-OCH_2-), 69.10 (-OCH_2-), 114.41 (C_{15}), 114.63 (C_5), 115.32 (C_8), 115.50 (C_2), 117.70 (C_{12}), 120.80 (C_{21}), 122.30 (C_{13}), 124.26 (C_9), 127.29 (C_{14}), 127.52 (C_{17}), 127.66 (C_{20}), 130.10 (C_4), 130.70 (C_{19}), 134.30 (C_{18}), 139.42 (C_{11}), 142.87 (C_6), 144.18 (C_3), 145.10 (C_7), 151.09 (C_{10}), 152.67 (C_{16}), 168.15 (C = O). EIMS: m/z 657.1553 $[M + Na]^+$, Calcd. for $C_{33}H_{37}Cl_3O_6$: 657.1561.

*4.1.5. General procedure for the synthesis of esters (**26-29**)*

3,4-dimethoxy cinnamic acid **25** (0.96 mmol, 200 mg) and thionyl chloride (5 mL) were placed in a 50 ml 3-neck round-bottom flask equipped with a magnetic stirring bar. The mixture was heated to reflux for 4h. The reaction mixture was then concentrated under reduced pressure and the residue was added to a solution of alcohols **2-8** (0.9 mmol) in dichloromethane. The resulting mixture was stirred for 4h, transferred to a separation funnel and then 20 mL of an aqueous solution of potassium carbonate was added. The organic layer was washed with water, separated, dried on anhydrous sodium sulfate, filtered

and concentrated under reduced pressure. The residue was chromatographed over silica gel (Hexanes–Ethyl acetate, different ratios) to obtain the esters **26-29** in yields between 40-60%.

*4.1.5.1. 3-(5-chloro-2-(2,4-dichlorophenoxy)phenoxy)propyl (E)-3-(3,4-dimethoxyphenyl) prop-2-enoate (**26**).* Yield 46% (0.41 mmol, 221 mg); white solid; m.p. 112–114 °C; IR (KBr, cm-1): ν_{max} 2949 (C-H), 1705 (C=O), 1627 (C=C), 1510 (C=C_{Ar}), 1269 (C-O-C), 1172 ((C=O)-O), 798 (C-H_{Ar}), 705 (C-Cl). ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 2.01-2.12 (CH_2, m), 3.93 (2OCH_3), 4.09 (-CH_2O-, t, *J* = 6.1 Hz), 4.18 (-CH_2O-, t, *J* = 6.0 Hz), 6.30 (H_2, d, *J* = 16.0 Hz), 6.67 (H_{12}, d, *J* = 8.7 Hz), 6.88 (H_8, d, *J* = 8.4 Hz), 6.95 (H_{15}, $s_{apparent}$), 7.04-7.15 (H_5, H_9, H_{13}, H_{20}, H_{21}, m), 7.43 (H_{18}, d, *J* = 2.4 Hz), 7.62 (H_3, d, *J* = 16.0 Hz); ^{13}C-NMR ($CDCl_3$, 75 MHz): δ 28.56 (CH_2), 55.88 (OCH_3), 55.96 (OCH_3), 60.64 (-OCH_2-), 68.71 (-OCH_2-), 109.60 (C_8), 111.03 (C_5), 114.91 (C_{15}), 115.46 (C_2), 118.01 (C_{12}), 121.28 (C_{21}), 122.06 (C_8), 122.70 (C_9), 124.44 (C_{14}), 127.30 (C_{17}), 127.67 (C_{20}), 128.01 (C_4), 130.20 (C_{18}), 130.51 (C_{19}), 143.20 (C_{11}), 144.9 (C_3), 149.20 (C_6), 151.70 (C_{10}), 151.17 (C_{16}), 152.37 (C_7), 167.00 (C = O). EIMS: m/z 559.0458 $[M + Na]^+$, Calcd. for $C_{26}H_{23}Cl_3O_6$: 559.0449.

*4.1.5.2. 4-(5-chloro-2-(2,4-dichlorophenoxy)phenoxy)butyl (E)-3-(3,4-dimethoxyphenyl)prop-2-enoate (**27**).* Yield 40% (0.36 mmol, 200 mg); pale yellow oil; IR (KBr, cm-1): ν_{max} 2935 (C-H), 1706 (C=O), 1633 (C=C), 1513 (C=C_{Ar}), 1260 (C-O-C), 1159 ((C=O)-O), 807 (C-H_{Ar}), 701 (C-Cl). ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 1.51–1.59 (CH_2, m), 1.66–1.74 (CH_2, m), 3.84 (2OCH_3), 3.90 (-CH_2O-, t, *J* = 6.1 Hz), 4.07 (-CH_2O-, t, *J* = 6.4 Hz), 6.22 (H_2, d, *J* = 16.0 Hz), 6.57 (H_{12}, d, *J* = 8.8 Hz), 6.80 (H_8, d, *J* = 8.4 Hz), 6.85 (H_{13}, dd, *J* = 8.8, 2.0), 6.86 (H_{15}, d, *J* = 2.0), 6.96-7.06 (H_9, H_5, H_{20}, H_{21}, m), 7.35 (H_{18}, d, *J* = 2.3 Hz), 7.54 (H_3, d, *J* = 16.0 Hz); ^{13}C-NMR ($CDCl_3$, 75 MHz): δ 25.10 (CH_2), 25.64 (CH_2), 55.90 (OCH_3), 55.97 (OCH_3), 63.68 (-OCH_2-), 68.45 (-OCH_2-), 109.68 (C_8), 111.08 (C_5), 114.81 (C_{15}), 115.75 (C_2), 117.75 (C_{12}), 121.10 (C_{21}), 122.22 (C_8), 122.52 (C_9), 124.36 (C_{14}), 127.42 (C_{17}), 127.54 (C_{20}), 127.80 (C_4), 130.19 (C_{18}), 130.62 (C_{19}), 143.03 (C_{11}), 144.67 (C_3), 149.23 (C_6), 151.83 (C_{10}), 151.14 (C_{16}), 152.61 (C_7), 167.10 (C = O). EIMS: m/z 573.0614 $[M + Na]^+$, Calcd. for $C_{27}H_{25}Cl_3O_6$: 573.0604.

*4.1.5.3. 7-(5-chloro-2-(2,4-dichlorophenoxy)phenoxy)heptyl (E)-3-(3,4-dimethoxyphenyl)prop-2-enoate (**28**).* Yield 60% (0.54 mmol, 321 mg); pale yellow solid; m.p. 63-65 °C; IR (KBr, cm-1): ν_{max} 2934 (C-H), 1708 (C=O), 1632 (C=C), 1510 (C=C_{Ar}), 1258 (C-O-C), 1159 ((C=O)-O), 807 (C-H_{Ar}), 753 (C-Cl). ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 1.14–1.27 (CH_2, m), 1.27–1.36 (CH_2, m), 1.36–1.46 (CH_2, m), 1.54–1.65 (CH_2, m), 1.66–1.75 (CH_2, m), 3.89 (2OCH_3), 3.90 (-CH_2O-, t, *J* = 6.4 Hz), 4.20 (-CH_2O-, t, *J* = 6.7 Hz), 6.32 (H_2, d, *J* = 16.0 Hz), 6.61 (H_{12}, d, *J* = 8.8 Hz), 6.86 (H_8, d, *J* = 8.7 Hz), 6.91 (H_{13}, dd,

J = 8.8, 2.3), 6.94 (H_{15}, d, *J* = 2.3), 6.97 (H_{21}, d, *J* = 8.5), 7.06 (H_5, d, *J* = 2.6 Hz), 7.07 (H_9, dd, *J* = 8.7, 2.6 Hz), 7.10 (H_{20}, dd, *J* = 8.5, 2.5 Hz), 7.43 (H_{18}, d, *J* = 2.5 Hz), 7.63 (H_3, d, *J* = 16.0 Hz); ^{13}C-NMR ($CDCl_3$, 75 MHz): δ 25.60 (CH_2), 25.82 (CH_2), 28.64 (CH_2), 28.83 ($2CH_2$), 55.85 (OCH_3), 55.92 (OCH_3), 64.41 (-OCH_2-), 68.93 (-OCH_2-), 109.65 (C_8), 111.04 (C_5), 114.65 (C_{15}), 115.96 (C_2), 117.57 (C_{12}), 120.81 (C_{21}), 122.26 (C_8), 122.52 (C_9), 124.20 (C_{14}), 127.44 (C_{17}), 127.43 (C_{20}), 127.58 (C_4), 130.02 (C_{18}), 130.63 (C_{19}), 142.84 (C_{11}), 144.43 (C_3), 149.20 (C_6), 151.01 (C_{10}), 151.06 (C_{16}), 152.65 (C_7), 167.21 (C = O). EIMS: m/z 615.1084 $[M + Na]^+$, Calcd. for $C_{30}H_{31}Cl_3O_6$: 615.1086.

*4.1.5.4. 9-(5-chloro-2-(2,4-dichlorophenoxy)phenoxy)nonyl (E)-3-(3,4-dimethoxyphenyl)prop-2-enoate (**29**).* Yield 40% (0.36 mmol, 224 mg); pale yellow oil; IR (KBr, cm-1): v_{max} 2932 (C-H), 1705 (C=O), 1633 (C=C), 1513 (C=C_{Ar}), 1259 (C-O-C), 1139 ((C=O)-O), 808 (C-H_{Ar}), 702 (C-Cl). ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 1.08–1.47 ($5CH_2$, m), 1.54–1.65 (CH_2, m), 1.66–1.78 (CH_2, m), 3.89 (-CH_2O-, t, *J* = 6.4 Hz), 3.91 ($2OCH_3$), 4.21 (-CH_2O-, t, *J* = 6.5 Hz), 6.34 (H_2, d, *J* = 16.0 Hz), 6.62 (H_{12}, d, *J* = 8.8 Hz), 6.86 (H_8, d, *J* = 8.3 Hz), 6.91 (H_{13}, dd, *J* = 8.8, 2.2), 6.95 (H_{15}, d, *J* = 2.2), 6.98 (H_{21}, d, *J* = 8.5), 7.04-7.10 (H_5, H_9, m), 7.11 (H_{20}, dd, *J* = 8.5, 2.1 Hz), 7.42 (H_{18}, d, *J* = 2.5 Hz), 7.64 (H_3, d, *J* = 16.0 Hz); ^{13}C-NMR ($CDCl_3$, 75 MHz): δ 25.71 (CH_2), 25.99 (CH_2), 28.77 (CH_2), 28.91 (CH_2), 29.16 (CH_2), 29.21 (CH_2), 29.41 (CH_2), 55.85 (OCH_3), 55.94 (OCH_3), 64.58 (-OCH_2-), 68.93 (-OCH_2-), 109.54 (C_8), 111.00 (C_5), 114.55 (C_{15}), 115.95 (C_2), 117.54 (C_{12}), 120.78 (C_{21}), 122.38 (C_8), 122.61 (C_9), 124.15 (C_{14}), 127.42 (C_{17}), 127.49 (C_{20}), 127.54 (C_4), 130.03 (C_{18}), 130.70 (C_{19}), 142.75 (C_{11}), 144.51 (C_3), 149.17 (C_6), 151.05 (C_{10}), 151.09 (C_{16}), 152.71 (C_7), 167.33 (C = O). EIMS: m/z 643.1397 $[M + Na]^+$, Calcd. for $C_{32}H_{35}Cl_3O_6$: 643.1382.

*4.1.6. 3-(5-chloro-2-(2,4-dichlorophenoxy)phenoxy)propyl 3-(3,4-dimethoxyphenyl)propanoate (**30**)*

A solution of **26** (100 mg, 0.185 mmol) in metanol was added under hydrogen to a suspention of Pd-C 10% (5mg) in dry metanol (10 mL). The reaction was monitored by NMR until consumption of the starting material. Filtration afforded compound **30** (90.2 mg, 0.167 mmol, 94 %).

Pale yellow oil; IR (KBr, cm-1): v_{max} 2935 (C-H), 1735 (C=O), 1498 (C=C_{Ar}), 1263 (C-O-C), 1157 ((C=O)-O), 804 (C-H_{Ar}), 763 (C-Cl). ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 1.90-1.97 (CH_2, m), 2.60 (H_2, t, *J* = 7.9 Hz), 2.89 (H_3, t, *J* = 7.9 Hz), 3.85 (OCH_3), 3.87 (OCH_3), 3.95 (-CH_2O-, t, *J* = 6.2 Hz), 4.05 (-CH_2O-, t, *J* = 6.2 Hz), 6.65 (H_{12}, d, *J* = 8.8 Hz), 6.71-6.75 (H_8, H_{13}, H_{15}, m), 6.79 (H_{21}, d, *J* = 8.8 Hz), 6.92 – 6.97 (H_9, H_5, m), 7.08 (H_{20}, dd, *J* = 8.8, 2.4 Hz), 7.43 (H_{18}, d, *J* = 2.4 Hz); ^{13}C-NMR ($CDCl_3$, 75 MHz): δ 28.47 (CH_2), 30.55 (C_3), 35.98 (C_2), 55.83 (OCH_3), 55.92 (OCH_3), 60.65 (-OCH_2-), 65.57 (-OCH_2-), 111.35 (C_8), 111.07 (C_5), 114.92 (C_{15}), 118.01 (C_{12}), 120.08 (C_{21}), 121.30 (C_8), 122.00 (C_9),

124.49 (C_{14}), 127.60 (C_{17}), 128.00 (C_{20}), 130.18 (C_4), 130.47 (C_{18}), 133.03 (C_{19}), 143.23 (C_{11}), 147.55 (C_6), 148.92 (C_{10}), 150.62 (C_{16}), 152.34 (C_7), 172.64 (C = O). EIMS: m/z 561.0614 $[M + Na]^+$, Calcd. for $C_{26}H_{25}Cl_3O_6$: 561.0618.

4.2. Biological activity assays

The compounds were subjected to *in vitro* evaluation as regards their cytotoxicity, anti-leishmanial and anti-trypanosomal activity against U-937 human cells and against intracellular amastigotes of *L. (V) panamensis* and *T. cruzi*, respectively.

4.2.1. In vitro Cytotoxicity

The cytotoxic activity of the compounds was assessed based on the viability of the human promonocytic cell line U-937 (ATCC CRL-1593.2™) evaluated by the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay following the methodology described previously [48]. Briefly, cells grown in tissue flasks were harvested and washed with phosphate buffered saline (PBS) by centrifuging. Cells were counted and adjusted at 1 $\times 10^6$ cells/mL of RPMI-1640 supplemented with complete 10% Fetal Bovine Serum (FBS) and 1% antibiotics (100 U/mL penicillin and 0.1 mg/mL streptomycin). One hundred µL were dispensed into each well of a 96-well cell-culture plate and then 100 mL of RPMI-1640 and the corresponding concentrations of the compounds were added, starting at 200 µg/mL in duplicate. Plates were incubated at 37 °C, 5% CO_2 during 72 h in the presence of compound. The effect of compounds was determined by measuring the activity of the mitochondrial dehydrogenase by adding 10 µL/well of MTT solution (0.5 mg/mL) and incubation at 37 °C for 3h. The reaction was stopped by adding 100 µL/well of 50% isopropanol solution with 10% sodium dodecyl sulfate and 30 min incubation. Cell viability was determined based on the quantity of formazan produced according to the intensity of color (absorbance) registered as optical densities (O.D) obtained at 570 nm in a spectrophotometer (Varioskan™ Flash Multimode Reader - Thermo Scientific, USA). Cells cultured in absence of compounds were used as control of viability (negative control), while amphotericin B (AmB) was used as control for cytotoxicity (non-cytotoxic and cytotoxic drugs, respectively). Assays were conducted in two independent runs with three replicates per each concentration tested.

4.2.2. In vitro anti-leishmanial activity

The activity of compounds was evaluated on intracellular amastigotes of *L. (V) panamensis* transfected with the green fluorescent protein gene (MHOM/CO/87/UA140pIR-GFP) [49]. The effect of each compound was determined according to the inhibition of the infection evidenced by both decrease of

the infected cells and decrease of intracellular parasite load. Briefly, U-937 human cells at a concentration of 3×10^5 cells/mL in RPMI 1640 and 0.1 μg/mL of phorbol-12-myristate-13-acetate (PMA) were dispensed into each well of a 24-well cell culture plate and then infected with 5 days-old promastigotes in a 15:1 parasites per cell ratio. Plates were incubated at 34 °C, 5% CO_2 during 3 h and cells were washed two times with PBS to eliminate not internalized parasites. One mL of fresh RPMI 1640 supplemented with 10% FBS and 1% antibiotics was added into each well, cells were incubated again to guarantee multiplication of intracellular parasites. After 24 h of infection, culture medium was replaced by fresh culture medium containing each compound at 20 μg/mL or lower (based on the cytotoxicity showed previously by each compound), plates were incubated at 37 °C, 5% CO_2. After 72 h, inhibition of the infection was determined. For this, cells were removed from the bottom plate with a trypsin/EDTA (250 mg) solution; recovered cells were centrifuged at 1100 rpm during 10 min at 4 °C, the supernatant was discarded and cells were washed with 1 mL of cold PBS and centrifuged at 1100 rpm during 10 min at 4 °C. The supernatant was discarded and cells were suspended in 500 μL of PBS and analyzed by flow cytometry (FC 500MPL, Cytomics, Brea, CA, US. All determinations for each compound and standard drugs were carried out in triplicate, in two independent experiments (Buckner et al., 1996; Pulido et al., 2012). Activity of the tested compounds was carried out in parallel with infection progress in culture medium alone and in culture medium with AmB as anti-leishmanial drugs (positive controls). Compounds that showed percentages of inhibition higher than 50% to 20 or fewer μg/mL were then evaluated at four additional concentrations to determine the effective concentration 50 (EC_{50}). Here, infected cells were exposed against each concentration of compounds during 72 h; then, cells were removed and tested by flow cytometry as described before.

4.2.3. In vitro anti-trypanosomal activity

Compounds were tested on intracellular amastigotes of *T. cruzi*, Tulahuen strain transfected with β-galactosidase gene (donated by Dr. F. S. Buckner, University of Washington) [50]. The activity was determined according to the ability of the compounds to reduce the infection of U-937 cells by *T. cruzi*. Following the procedure described above, anti-parasite activity was initially screened at a single concentration of 20 mg/mL. In this case, 100 μL of U-937 human cells at a concentration of 2.5×10^5 cells/mL in RPMI-1640, 10% SFB and 0.1 μg/mL of PMA were placed in each well of 96-well plates and then infected with phase growth epimastigotes in 5:1 (parasites per cell) ratio and incubated at 34 °C, 5% CO_2. After 24 hours of incubation, 20 μg/mL of each compounds were added to infected cells. After 72 h of incubation, the effect of all compounds on the viability of intracellular amastigotes was determined by measuring the β-galactosidase activity by spectrophotometry adding 100 μM CPRG and

0.1% nonidet P-40 to each well. After 3 h of incubation, plates were read at 570 nm in a spectrophotometer (Varioskan™ Flash Multimode Reader - Thermo Scientific, USA) and intensity of color (absorbance) was registered as O.D. compounds that showed inhibition percentages higher than 50% were evaluated again at four concentrations selected according to the LC_{50} previously obtained for each compound. Infected cells exposed to benznidazol (BNZ) were used as control for anti-trypanosomal activity (positive control) while infected cells incubated in culture medium alone were used as control for infection (negative control). Non-specific absorbance was corrected by subtracting the O.D of the blank. Determinations were done by triplicate in at least two independent experiments [51].

4.2.4. Statistical Analysis

Cytotoxicity was determined according to viability and mortality percentages obtained for each isolated experiment (compounds, amphotericin B, Benznidazole and culture medium alone). The results were expressed as 50 lethal concentrations (LC_{50}) that corresponds to the concentration necessary to eliminate 50% of cells and calculated by Probit analysis [52]. Percentage of viability was calculated by Equation 1, where the O.D of control, corresponds to 100% of viability. In turn, mortality percentage corresponds to 100%–% viability:

$$\% \text{ Viability} = (\text{O.D Exposed cells}) / (\text{O.D Control cells}) \times 100 \quad (1)$$

The degree of toxicity was graded according to the LC_{50} value using the following scale: high cytotoxicity: $LC_{50} < 200$ μM; moderate cytotoxicity: LC_{50} in the 200-300 μM range, and potentially non-cytotoxicity: $LC_{50} > 300$ μM.

Anti-leishmanial activity was determined according to the percentage of infected cells and parasite load obtained for each experimental condition by flow cytometry. The percentage of infected cells was determined as the number of positive events by double fluorescence (green for parasites and red for cells) using dotplot analysis. On the other hand, the parasitic load was determined by analysis of mean fluorescence intensity (MFI) of fluorescent parasites [48]. The parasite inhibition was calculated by equation 2, where the MFI of control, corresponds to 100% of parasites. In turn, inhibition percentage corresponds to 100% – % Parasites. Results of anti-leishmanial activity were expressed as EC_{50} determined by the Probit method [52]:

$$\% \text{ Parasite} = (\text{MFI Exposed parasites}) / (\text{MFI Control parasites}) \times 100 \quad (2)$$

Similarly, anti-trypanosomal activity was determined according to the percentage of infected cells and parasite load obtained for each experimental condition by colorimetry. The parasite inhibition was

calculated by equation 3, where the O.D of control corresponds to 100% of parasites. In turn, the inhibition percentage corresponds to 100% – % Parasites. Results of anti-trypanosomal activity were also expressed as EC_{50} determined by the Probit method [52]:

$$\% \text{ Parasite} = (\text{O.D Exposed parasites}) / (\text{O.D Control parasites}) \times 100 \quad (3)$$

The anti-leishmanial or anti-trypanosomal activities were graded according to the EC_{50} value using the following scale: High activity: EC_{50} < 40 μM, moderate activity: EC_{50} in the 40-80 μM range, potentially non activity: EC_{50} > 80 μM.

The selectivity index (SI), was calculated by dividing the cytotoxic activity and the anti-leishmanial or anti-trypanosomal activity using the following formula: $SI = CL_{50}/CE_{50}$. Cytotoxic compound: LC_{50}<100 μg/mL.

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Conflict of interest

The authors declare no conflict of interest.

Supplementary data

Supplementary data associated with this article can be found at the online version

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