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Article

Synthesis and Antitrypanosomastid Activity of 1,4-Diaryl-1,2,3-triazole Analogues of Neolignans Veraguensin, Grandisin and Machilin G

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Sixteen 1,4-diaryl-1,2,3-triazole compounds derived from the natural products veraguensin, grandisin and machilin G were synthesized, with yields of 78-92%. Biological activity tests against *Leishmania amazonensis* promastigotes showed that three of these compounds were the most active, with maximum inhibitory concentration (IC_{50}) values of 1.1, 3.71 and 7.23 μM. One compound was highly active against *Leishmania infantum*, with an IC_{50} value of 5.2 μM, and one derivative showed an IC_{50} value of 28.6 μM against *Trypanosoma cruzi* trypomastigotes. Regarding structure-activity relationship (SAR), hybrid 1,2,3-triazolic compounds containing a methylenedioxy group, showed the best antileishmanial and antitrypanosomal activities.

Keywords: 1,4-diaryl-1,2,3-triazole derivative, tetrahydrofuran neolignan, click chemistry, antitrypanosomatid activity

Introduction

Leishmaniases are a group of infectious diseases with diverse clinical manifestations, ranging from cutaneous to visceral forms. Visceral leishmaniasis is the most-often fatal protozoan disease, second only to malaria in the number of cases. The disease affects around two million people in endemic areas, with more than 350 million at risk.[1]

The same chemotherapeutic drugs have been used for decades against leishmaniases.[1] Conventional therapeutic options include pentavalent antimony compounds such as, sodium stibogluconate (Pentostam®), *N*-methyl glucamine antimoniate (Glucantime®), amphotericin B, pentamidine, paromomycin, and miltefosine. All these drugs, however, have important limitations regarding toxicity, pronounced side effects or high cost.[2,3]

Recent studies have focused on combinations of these drugs, such as sodium stibogluconate-paromomycin[4] or miltefosine-paromomycin[5] in order to circumvent the resistance problems of different strains related to this disease. Despite the efforts of the Drugs for Neglected Diseases initiative (DNDi) in the clinical studies, only a few candidate drugs are under the development. Therefore, current and new strategies must be expanded in the search for antileishmanial compounds.[4,5]

Chagas disease is also a neglected, potentially lethal disease caused by a protozoan parasite, *Trypanosoma cruzi*. This disorder is an important health problem in Latin America, where it affects about eight million people.[6]

Treatment for Chagas disease includes two drugs, nifurtimox and benznidazole, which are active only in the acute phase of infection. Benznidazole is currently the first-choice drug in most Latin American countries. Unfortunately, narrow therapeutic windows, side effects, and variable susceptibility among *T. cruzi* strains result in low clinical efficacy for this nitroderivative.[7] Recent studies have shown the potential antitrypanosomal activity of nitroheterocyclic fexinidazole. This candidate drug is now in phase II of a clinical trial against Chagas disease in a DNDi initiative for Latin America.[8,9]

In view of the serious health problems related to these two trypanosomatid diseases, it is imperative that new bioactive compounds be developed.

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Natural products, mainly secondary metabolites, are an important source of bioactive compounds, due to their wide chemical diversity.[10,11] However, natural bioactive compounds may possess unsuitable pharmacological characteristics, such as poor oral absorption, high lipophilicity, or cytotoxicity, which limit their use. These various properties can be modulated or improved with the development of synthetic derivatives.[10,12,13]

Among natural products, our research group has focused on the tetrahydrofuran neolignans, a class that exhibits a great number of biological activities including antibacterial, antifungal, antitumor, and anti-inflammatory.[14-23] The neolignans veraguensin **1**, grandisin **2**, and machilin G **3** (Figure 1) have shown antitrypanosomal and antileishmanial activities.[14-23]

Studies on the antitrypanosomal activity of compounds **1**-**3** found maximum inhibitory concentration (IC_{50}) values of 2.3, 3.7 and 2.2 μM, respectively.[21-23] Veraguensin **1** and machilin G **3** showed potential antileishmanial activity, with an IC_{50} value of 18 μg mL^{-1} (48.8 and 50.54 μM, respectively) against *L. donovani*.[16] The synthesis of derivatives could improve these biological effects. In fact, neolignan derivatives with greater hydrosolubility have been synthetized in order to reduce their lipophilicity, which limits *in vivo* studies.[21]

Bioactive compounds obtained via click chemistry strategy have provided chemical libraries of compounds with antitrypanosomal, anticancer, and antituberculosis activities.[24] This methodology could also be used to obtain derivatives of neolignans **1**-**3**, containing the 1,2,3-triazole core, with potential biological activity against neglected diseases such as Chagas and Leishmaniases.

In this context, this study addressed the synthesis of sixteen 1,4-diaryl-1,2,3-triazole derivatives with substitution patterns based on neolignans **1**-**3**, intending to clarify whether substitution of tetrahydrofuran by 1,2,3-triazole core (bioisosterism strategy), could providing compounds with improved antitrypanosomatid activity. Furthermore, click chemistry is a good strategy for synthesis of hybrids analogues of neolignans **1**-**3**, what would permit to obtain preliminary information about structure-activity relationship (SAR) of the compounds **4**-**19** (Figure 2).

Although many studies have described the anticancer activity of triazole compounds with methoxy substitution patterns,[25] to date all possible position isomers of 1,4-diaryl-1,2,3-triazole derivatives of neolignans **1**-**3** have not yet synthesized and tested against *Leishmania sp.* and *T. Cruzi*. For instance, heterocyclic 1,2,3-triazole positional isomers derived from Combrestatin A4 showed different anticancer activities.[26] This led us to consider the need to test the biological activity of possible positional isomers.

Results and Discussion

Chemistry

For the synthesis of 1,4-diaryl-1,2,3-triazoles **4**-**19** it was necessary to synthesize two building blocks: terminal acetylenes and aromatic azides, with appropriate substitution patterns (Scheme 1).[27]

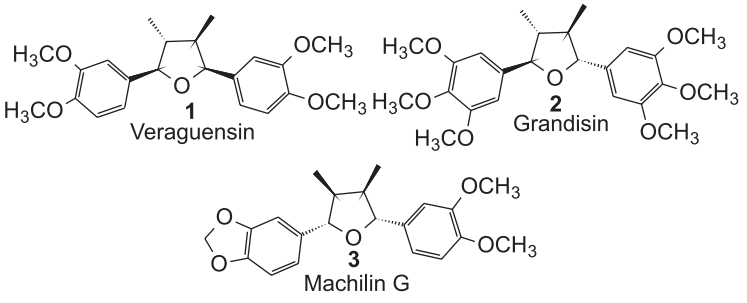


Figure 1. Structures of veraguensin **1**, grandisin **2** and machilin G **3**.

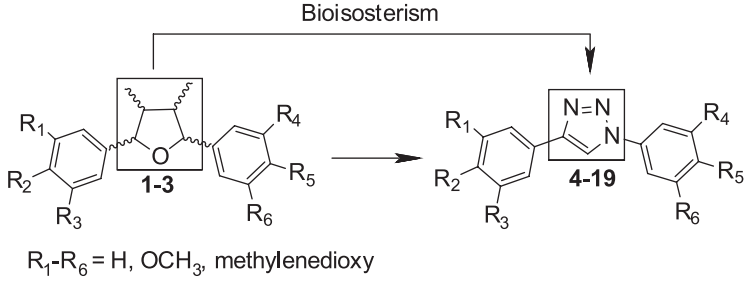


Figure 2. Design of 1,4-diaryl-1,2,3-triazole analogues of neolignans **1**-**3**.

The synthesis of starting materials began by preparing aryl bromides **21b-c** via a bromination reaction of **20b-c** in the presence of *N*-bromosuccinimide (NBS), *p*-TsOH, CH_2Cl_2 and SiO_2.[28] Subsequently, a cross-coupling Sonogashira reaction between bromobenzenes **21a-c** and 2-methyl-3-butyn-2-ol in the presence of $PdCl_2(PPh_3)_2$/CuI, Et_3N provided the acetylene alcohols **22a-c** with 75-85% yields, after 24 h reaction time.[29,30] Retro-Favorski reaction of **22a-c** with KOH under reflux in toluene generated the terminal acetylenes **25a-c** with 79 to 85% yield.[29,30] Ethynyl-1,2,3-trimethoxybenzene **25d** was synthesized by the Corey-Fuchs method (Scheme 1).[25,31]

Next, the aromatic azides **27a-d** were prepared by the reaction of aromatic amines **26a-d** with *t*-BuONO/$TMSN_3$ using the protocol reported by Moses and co-workers[33] (Scheme 2).

The 1,3-dipolar cycloaddition occurred when terminal acetylenes **25a-d** reacted with aryl azides **27a-d** using $CuSO_4.H_2O$, sodium ascorbate and CH_2Cl_2/H_2O 1:1 as solvents, providing the compounds **4-19** in 78 to 92% yield (Table 1).[33]

Synthetic compounds were characterized by nuclear magnetic resonance (NMR) ^1H and ^{13}C, and the unknown compounds **7**, **13**, **17** and **19** were also analyzed by infrared (IR) and high resolution mass spectrometry.

Biological activity

The antileishmanial activity of **4-19** was evaluated against promastigote forms of *L. amazonensis* and *L. infantum*, and the antitrypanosomal activity against trypomastigotes forms of *T. cruzi* (Table 2).

Triazole compounds **4-7** showed low activity against *T. cruzi* (132 to > 200 μM). The triazole **5** derivative from veraguensin exhibited low activity against all trypanosomatids tested (98.5, 62.1 and 132 μM, respectively), and the triazole **6** derivative from grandisin was the least active against all of them (> 200 μM). Compound **7**, containing two methylenedioxy groups, also showed low activity against all species tested.

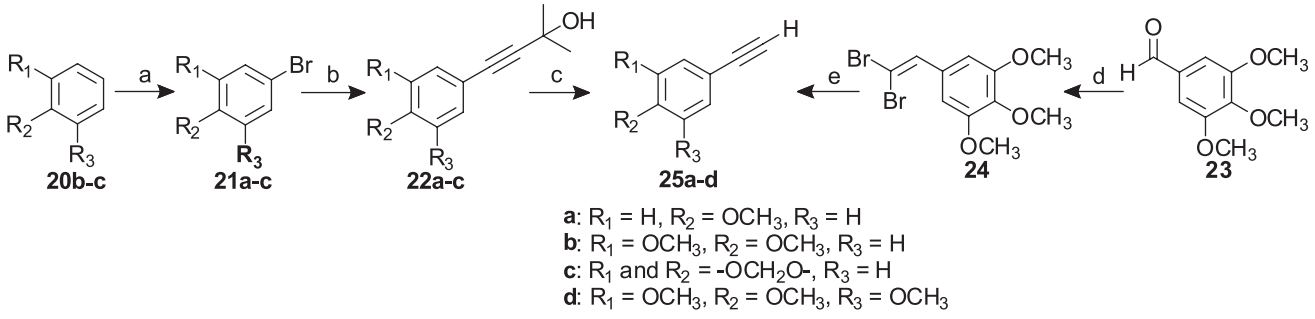
Hybrid derivatives **8-19** showed different activities against *L. amazonensis*, *L. infantum* and *T. cruzi* (Table 2). Of these, the positional isomers also showed different biological activities.

For instance, isomers **8** and **9** exhibited IC_{50} values of 31.7 and 150 μM, respectively, against *L. amazonensis*, and IC_{50} values of > 200 and 53.9 μM against *T. cruzi*. None of these isomers was active against *L. infantum* (> 200 μM).

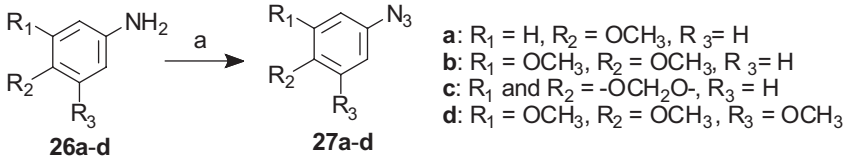
Isomers **10** and **11** showed low or no activity against all parasite species (Table 2). Isomer **12** was less active than **13** against all species, with IC_{50} values of 108.2 and 40.9 μM, respectively against *L. amazonensis*, > 200 and 94.5 μM against *L. infantum*, and > 200 and 28.6 μM against *T. cruzi*. Isomers **14** and **15** showed low activity (Table 2).

Positional isomer **16** was less active than **17**, with IC_{50} values of 96.7 and 1.1 μM, respectively for *L. amazonensis*, 64.6 and 19.5 μM for *L. infantum*, and > 200 and 109.6 μM for *T. cruzi*.

Positional isomers **18** and **19** excelled among all the synthetic compounds, showing high activity against *L. amazonensis* (IC_{50} values of 3.71 and 7.23 μM) and *L. infantum* (15.4 and 5.2 μM). Both also exhibited

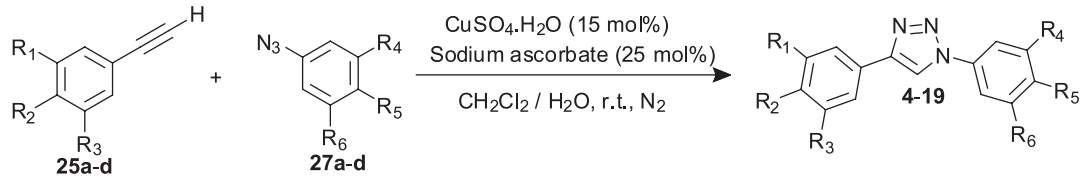


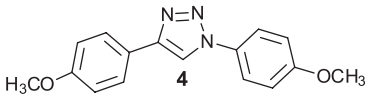
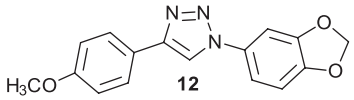
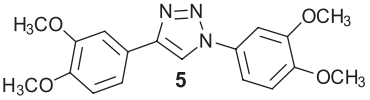
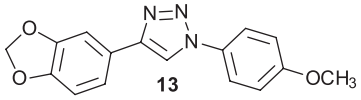
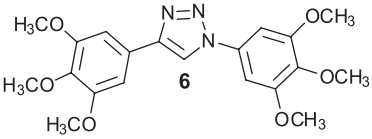
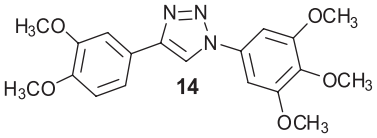
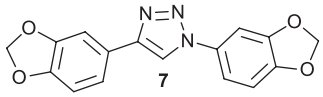
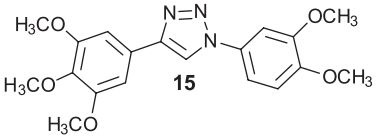
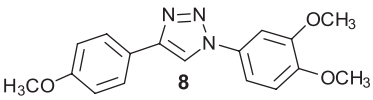
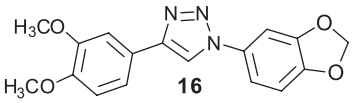
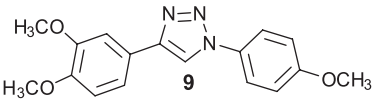
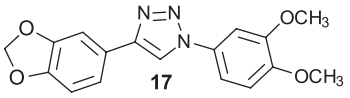
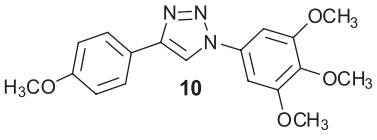
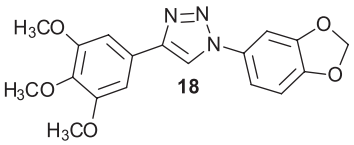
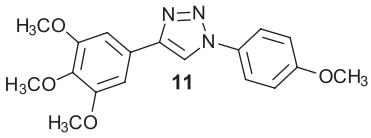
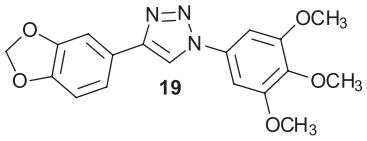
Scheme 1. Synthesis of aryl acetylenes **25a-d**. Reagents and reaction conditions: (a) NBS, TsOH, SiO_2, CH_2Cl_2, **21b** = 87%, **21c** = 79%; (b) 2-methyl-3-butyn-2-ol, $PdCl_2(Ph_3)_2$, CuI, Et_3N, reflux, 24 h, **22a** = 85%, **22b** = 75%, **22c** = 76%; (c) KOH, toluene, reflux, 24 h, **25a** = 85%, **25b** = 83%, **25c** = 79%, (d) $CCBr_4$, CH_2Cl_2, 0 ºC, 5 h, **24** = 74%; (e) tetrahydrofuran (THF), *n*-BuLi, −25 ºC to room temperature, 1 h, **25d** = 82%.



Scheme 2. Synthesis of aryl azides **27a-d**. Reagents and reaction conditions: (a) *t*-BuONO, CH_3CN, 15 min, 0 ºC, then $TMSN_3$, room temperature, 12 h; **27a** = 73%, **27b** = 78%, **27c** = 75%, **27d** = 87%.

Table 1. Synthesis of 1,4-diaryl-1,2,3-triazoles **4**-**19**



Compound[a]	Yield / %	Compound[a]	Yield / %
 **4**	79	 **12**	85
 **5**	92	 **13**	81
 **6**	89	 **14**	82
 **7**	87	 **15**	88
 **8**	80	 **16**	81
 **9**	85	 **17**	90
 **10**	83	 **18**	78
 **11**	86	 **19**	80

[a]Reaction conditions: **25a-d** (1.0 mmol), **27a-d** (1.0 mmol), $CuSO_4.5H_2O$ (0.15 mmol), sodium ascorbate (0.25 mmol), CH_2Cl_2 (3 mL), H_2O (3 mL), room temperature, 24 h.

moderate activity against *T. cruzi* trypomastigotes (IC_{50} values of 108.1 and 56.1 μM, respectively).

Compounds **17**, **18** and **19** were more active against *L. amazonenis* than was pentamidine (IC_{50} 8.9 μM). Compound **17**, an analogue of machilin G **3**, was 8 times more active than pentamidine, while **18** and **19** were 2.5 and 1.2 times more active than pentamidine, respectively (Table 2).

An important criterion in the search for compounds with antiprotozoal activity is their toxicity to mammalian host cells. Compounds **17**, **18** and **19** showed low cytotoxicity, with high selectivity indexes (SI),[34] tens to hundreds of times higher than those of the recommended drugs for leishmaniasis, such as pentamidine and amphotericin B (SI 8.8 and 8.2, respectively) and then benznidazole (SI 13.2) for *T. cruzi,* indicating that these

Table 2. *In vitro* antitrypanosomatid activity of 1,4-diaryl-1,2,3-triazoles **4-19**

Compound	NIH/3T3 IC_{50}[a] / μM	*L. amazonensis* IC_{50}[b] / μM	SI[c]	*L. infantum* IC_{50}[b] / μM	SI[c]	*T. cruzi* IC_{50}[b] / μM	SI[c]
4	> 888.7	123.3	> 7.21	> 200	NC[d]	> 200	NC[d]
5	> 732.4	98.5	> 7.44	62.1	> 11.8	132.0	> 5.6
6	> 622.8	> 200	NC[d]	> 200	NC[d]	> 200	NC[d]
7	> 808.3	92.7	> 8.7	> 200	NC[d]	> 200	NC[d]
8	> 802.9	31.7	> 25.3	> 200	NC[d]	> 200	NC[d]
9	> 802.9	150	> 5.3	> 200	NC[d]	53.9	> 14.9
10	> 732.4	> 200	NC[d]	> 200	NC[d]	> 200	NC[d]
11	> 732.4	125.3	> 5.8	> 200	NC[d]	> 200	NC[d]
12	> 846.6	108.2	> 7.8	> 200	NC[d]	> 200	NC[d]
13	> 846.6	40.9	> 20.7	94.5	> 8.9	28.6	> 29.60
14	103.1	193.6	0.53	92.6	1.1	89.1	1.2
15	> 673.1	122.4	> 5.50	> 200	NC[d]	> 200	NC[d]
16	> 768.5	96.7	> 7.9	64.6	> 11.9	> 200	NC[d]
17	> 768.5	1.1	> 698.6	19.5	> 39.4	109.6	> 7.0
18	> 703.5	3.7	> 189.6	15.4	> 45.7	108.1	> 6.5
19	> 703.5	7.2	> 97.7	5.2	> 135.3	56.1	> 12.5
Pentamidine	78.7	8.9	8.8	–	–	–	–
Amphotericin B	5.7	–	–	0.7	8.2	–	–
Benznidazole	> 96.1	–	–	–	–	7.3	> 13.2
Doxorubicin	2.6	–	–	–	–	–	–

[a]IC_{50}: half maximal inhibitory concentration on fibroblast cells; [b]IC_{50}: half maximal inhibitory concentration on *L. amazonensis*, *L. infantum* and *T. cruzi*; [c]SI: selectivity index: IC_{50} on mammal cells *per* IC_{50} on trypanosomatids; [d]NC: not calculated; [e]positive controls: pentamidine for *L. amazonensis*, amphotericin B for *L. infantum*, benznidazole for *T. cruzi*, and doxorubicin for fibroblast cells.

compounds are potential candidates for further *in vivo* studies (Table 2).

Regarding SAR, hybrid 1,2,3-triazole compounds **13** and **17** and positional isomers **18** and **19**, containing the methylenedioxy group present in machilin G **3**, were the most active against the trypanosomatids, indicating that this group is responsible for the high antileishmanial activity and moderate antitrypanosomal activity of these compounds.

The replacement of tetrahydrofuran by the 1,2,3-triazole core increases the antileishmania activity, since an IC_{50} of 18 μg mL^{-1} (48.8 μM) of machilin G **3** against promastigotes of *L. donovani* was reported.[16] Last, the different biological activities of 1,2,3-triazole positional isomers synthesized in this study are notable.

Conclusions

In summary, this article describes the synthesis and the antitrypanosomatid activities of 16 1,4-diaryl-1,2,3-triazole compounds **4**-**19**. Compound **13** was the most active agaisnt *T. cruzi*. The 1,2,3-triazole compound **17**, an analogue of machilin G, and the hybrid compounds **18** and **19**, analogues of grandisin **2** and machilin G **3**, showed very good activity against *L. amazonensis* and *L. infantum*.

Experimental

General remarks

All solvents were distilled before use according to the standard procedure. All reactions were performed under an atmosphere of dry nitrogen and monitored by thin-layer chromatography (TLC) using prepared plates (Silica Gel 60 F254 on aluminum). The chromatograms were examined under both 254 and 360 nm UV light or with the developing agent ethanolic vanillin and heat. Flash column chromatography was performed on silica gel 60 (particle size 200-400 mesh ASTM, purchased from Aldrich) and eluted with hexane or hexane/ethyl acetate in different ratios. Melting points were determined using Fisatom 430D equipment. Infrared (IR) spectra were recorded on Nicolet iS5 spectrometer from Thermo Scientific. The ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ solutions

using a Brucker 75 or 300 MHz spectrometer, as noted. Chemical shifts (δ) are expressed as parts *per* million (ppm) downfield from tetramethylsilane as the internal standard. High-resolution electrospray ionization mass spectrometry (HR-ESI-MS) measurements were carried out on a quadrupole time-of-flight instrument (UltrOTOF-Q, BrukerDaltonics, Billerica, MA). Compound **21a** was purchased from Sigma-Aldrich.

General procedure for the preparation of aryl bromides (**21b-c**)

To a solution of compounds **20b** and **20c** (75 mmol) in dichloromethane (210 mL) containing TsOH (10 mmol), silica gel G 60 230-400 mesh (37 g) in nitrogen atmosphere at 0 °C, was added NBS (75 mmol) slowly. The reaction was stirred at room temperature by 3 hours. The work up was performed with 300 mL of saturated $NaHCO_3$ solution and the product was extracted with ethyl acetate (3 × 150 mL). The organic phase was dried over $MgSO_4$ and the solvent removed under reduced pressure. The products were purified by distillation at low pressure (3 mmHg).

4-Bromo-1,2-dimethoxybenzene (**21b**)[35]

Yield: 87%; colorless oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.84 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 6.73 (d, 1H, *J* 8.3 Hz, Ph-H), 6.98 (d, 1H, *J* 1.6 Hz, Ph-H), 7.03 (dd, 1H, *J* 8.3, 1.6 Hz, Ph-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 55.97, 112.56, 112.66, 114.80, 123.34, 148.36, 149.78.

5-Bromo-1,3-benzodioxole (**21c**)[36,37]

Yield: 79%; colorless oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.92 (s, 2H, CH_2), 6.64 (d, 1H, *J* 8.2 Hz, Ph-H), 6.91 (dd, 1H, *J* 8.2, 1.9 Hz, Ph-H), 6.92 (d, 1H, *J* 1.9 Hz, Ph-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 101.59, 109.54, 112.29, 113.06, 124.30, 147.01, 148.61.

General procedure for the preparation of acetylene alcohols (**22a-c**)

To a solution of the bromines **21a-c** (3.0 mmol) in triethylamine (15 mL), $PdCl_2(PPh_3)_2$ (0.075 mmol), CuI (0.15 mmol) in nitrogen atmosphere was added 2-methyl-3-butyn-2-ol (11.0 mmol). The mixture was stirred under refluxed for 20 hours. Then, the excess triethylamine was removed by distillation, and the reaction was extracted with ethyl acetate, dried over $MgSO_4$, and the solvent removed under reduced pressure. The products were purified by column chromatography on silica gel using hexane/ethyl acetate as eluent.

4-(4-Methoxyphenyl)-2-methyl-3-butyn-2-ol (**22a**)[38]

Yield: 85%; yellow crystal; mp 53 ºC; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.59 (s, 6H, $2CH_3$), 2.07 (s, 1H, OH), 3.78 (s, 3H, OCH_3), 6.81 (d, 2H, *J* 8.8 Hz, Ar-H), 7.33 (d, 2H, *J* 8.8 Hz, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 31.56, 55.25, 65.65, 81.99, 92.38, 113.86, 114.81, 133.07, 159.53.

4-(3,4-Dimethoxyphenyl)-2-methyl-3-butyn-2-ol (**22b**)[39]

Yield: 75%; yellow crystal; mp 48-50 ºC; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.59 (s, 6H, $2CH_3$), 2.11 (sl, 1H, OH), 3.84 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 6.75 (d, 1H, *J* 8.3 Hz, Ar-H), 6.89 (d, 1H, *J* 1.8 Hz, Ar-H), 6,99 (dd, 1H, *J* 8.2, 1.8 Hz, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 31.03, 31.55, 55.86, 55.87, 65.65, 82.13, 92.27, 110.92, 114.39, 114.89, 124.87, 148.54, 149.41.

4-(1,3-Benzodioxol-5-yl)-2-methyl-3-butyn-2-ol (**22c**)[40]

Yield: 76%; yellow crystal; mp 44 ºC; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.57 (s, 6H, $2CH_3$), 2.21 (sl, 1H, OH), 5.93 (s, 2H, CH_2), 6.70 (d, 1H, *J* 8.0 Hz, Ar-H), 6.83 (d, 1H, *J* 1.6 Hz, Ar-H), 6.90 (dd, 1H, *J* 8.1, 1.6 Hz, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 31.50, 61.57, 81.94, 92.14, 101.24, 108.34, 111.64, 115.97, 126.21, 147.32, 147.79.

General procedure for the preparation of terminal acetylenes (**25a-c**)

To a solution of compounds **22a**, **22b** and **22c** (47 mmol, 1.0 equiv) in toluene (353 mL), were added KOH (141 mmol, 3.0 equiv). The reaction was stirred under reflux in nitrogen atmosphere by 18 hours. Toluene was evaporated under reduced pressure, the residue diluted with ethyl acetate (150 mL) and then, it was added a saturated solution of NH_4Cl (100 mL). The products were extracted with ethyl acetate (3 × 100 mL) and washed with water (3 × 100 mL). After organic phase was dried over anhydrous $MgSO_4$, the solvent was removed under reduced pressure and the residue purified by column chromatography on silica gel using hexane/ethyl acetate as eluent.

1-Ethynyl-4-methoxybenzene (**25a**)[40]

Yield: 85%; colorless oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.98 (s, 1H, CH), 3.79 (s, 3H, OCH_3), 6.83 (d, 2H, *J* 8.9 Hz, Ar-H), 7.41 (d, 2H, *J* 8.9 Hz, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 55.26, 75.74, 83.63, 113.90, 114.12, 133.56, 159.90.

4-Ethynyl-1,2-dimethoxybenzene (**25b**)[41]

Yield: 83%; white solid; mp 73 ºC; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.95 (s, 1H, CH), 3.82 (s, 3H, OCH_3), 3.83 (s, 3H, OCH_3), 6.74 (d, 1H, *J* 8.3 Hz, Ar-H), 6.93 (d, 1H, *J* 1.8 Hz,

Ar-H), 7.05 (dd, 1H, *J* 8.2, 1.8 Hz, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 55.83, 75.63, 83.72, 110.84, 114.12, 114.61, 125.43, 148.50, 149.79.

5-Ethynyl-1,3-benzodioxole (**25c**)[42]

Yield: 79%; yellow oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.95 (s, 1H, CH), 5.96 (s, 2H, CH_2), 6.73 (d, 1H, *J* 8.0 Hz, Ar-H), 6.91 (d, 1H, *J* 1.4 Hz, Ar-H), 7.00 (dd, 1H, *J* 7.9, 1.4 Hz, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 75.50, 83.52, 101.33, 108.40, 112.02, 115.28, 126.87, 147.36, 148.27.

Synthesis of 5-(2,2-dibromovinyl)-1,2,3-trimethoxybenzene (**24**)[43]

To a solution of CBr_4 (100 mmol, 1.0 equiv) in dry CH_2Cl_2 (100 mL) under nitrogen atmosphere, at 0 °C, was added dropwise a solution of PPh_3 (200 mmol) in dry CH_2Cl_2 (100 mL). After 1 h was added 3,4,5-trimethoxybenzaldehyde **23** (50 mmol) in dry CH_2Cl_2 (50 mL). The reaction was stirred by 3 h. Then, the reaction was extracted with ethyl acetate (4 × 100 mL), organic phase was dried over anhydrous $MgSO_4$ and the solvent was removed under reduced pressure. The residue was purified by column chromatography on silica gel using hexane/ethyl acetate 9:1 as eluent to give **24** as a yellow crystal in 74% yield; mp 59 ºC; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.83 (s, 9H, $3OCH_3$), 6.77 (s, 2H, Ar-H), 7.38 (s, 1H, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 56.16, 60.84, 88.77, 105.79, 130.54, 136.59, 138.36, 152.99.

General procedure for the preparation of 5-ethynyl-1,2,3-trimethoxybenzene (**25d**)[43]

To a solution of 5-(2,2-dibromovinyl)-1,2,3-trimethoxybenzene **24** (14.08 g, 40 mmol, 1.0 equiv) in tetrahydrofuran (THF) (60 mL) at –25 °C, under nitrogen atmosphere, and vigorous stirred, was added dropwise *n*-BuLi (78.63 mL, 92 mmol, 2.3 equiv). After complete addition of *n*-BuLi, the reaction solution was kept at room temperature for one hour. Then, it was added 100 mL of saturated solution of NH_4Cl and the product was extracted with ethyl acetate (3 × 100 mL). The organic phase was dried over anhydrous $MgSO_4$ and the solvent was removed under reduced pressure. The residue purified by column chromatography on silica gel using hexane/ethyl acetate as eluent to give **25d** as white solid; 83% yield; mp 68-69 ºC; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.01 (s, 1H, CH), 3.83 (s, 3H, OCH_3), 3.87 (s, 6H, $2OCH_3$), 6.71 (s, 2H, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 55.99, 60.11, 79.70, 83.71, 109.11, 116.80, 138.64, 152.87.

General procedure for the preparation of azides (**27a-d**)[32]

To a solution of anilines **26a-d** (20 mmol, 1.0 equiv) in acetonitrile (76 mL) under nitrogen atmosphere, at 0 °C, was added *t*-BuONO (43 mmol, 2.15 equiv) and $TMSN_3$ (32.6 mmol, 1.63 equiv) dropwise. After 15 minutes, the reaction was kept at ambient temperature for 12 hours. Then, 40 mL of water was added and reaction solution was extracted with ethyl acetate (3 × 100 mL). The organic phase was dried over anhydrous $MgSO_4$ and the solvent was removed under reduced pressure. The residue purified by column chromatography on silica gel using hexane/ethyl acetate 8:2 as eluent.

1-Azide-4-methoxybenzene (**27a**)[26]

Yield: 73%; yellow solid; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.78 (s, 3H, OCH_3), 6.87 (d, 2H, *J* 9.0 Hz, Ar-H), 6.94 (d, 2H, *J* 8.9 Hz, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 55.49, 115.05, 119.93, 132.26, 156.93.

4-Azide-1,2-dimethoxybenzene (**27b**)[25]

Yield: 78%; orange solid; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.84 (s, 6H, $2OCH_3$), 6.49 (d, 1H, *J* 2.5 Hz, Ar-H), 6.58 (dd, 1H, *J* 8.4, 2.4 Hz, Ar-H), 6.81 (d, 1H, *J* 8.6 Hz, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 55.92, 56.17, 103.15, 110.40, 112.01, 132.72, 146.48, 149.95.

1,3-Benzodioxol-5-yl-azide (**27c**)[25]

Yield: 75%; brown oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.95 (s, 2H, CH_2), 6.47 (dd, 1H, *J* 8.2, 2.3 Hz, Ar-H); 6.51 (d, 1H, *J* 2.1 Hz, Ar-H); 6.75 (d, 1H, *J* 8.3 Hz, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 100.66; 101.59; 108.75; 111.58; 133.74; 145.01; 148.64.

5-Azide-1,2,3-trimethoxybenzene (**27d**)[26]

Yield: 87%; yellow solid; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.78 (s, 3H, OCH_3), 3.82 (s, 6H, $2OCH_3$), 6.21 (s, 2H, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 56.17, 61.00, 96.40, 135.35, 135.64, 154.07.

General procedure for the preparation of triazole derivatives (**4-19**)

To a solution of terminal acetylenes **25a-d** (2 mmol, 1.0 equiv) and azides **27a-d** (2 mmol, 1.0 equiv) in dichloromethane (2 mL) and water (2 mL), were added $CuSO_4.5H_2O$ pentahydrate (0.128 mmol, 0.064 equiv) and sodium ascorbate (0.352 mmol, 0.176 equiv). The reaction mixture was stirred for 24 h. Then was added a saturated solution of NH_4Cl (30 mL) and the product was extracted with dichloromethane (3 × 20 mL). The organic

phase was dried over anhydrous $MgSO_4$, and the solvent was evaporated under reduced pressure. The product was purified by recrystallization from ethyl acetate.

1,4-Bis(4-ethoxyphenyl)-1*H*-1,2,3-triazole (**4**)[25]

Yield: 79%; yellow solid; mp 205-206 ºC; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.84 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 6.97 (d, 2H, *J* 8.6 Hz, Ar-H), 7.02 (d, 2H, *J* 9.0 Hz, Ar-H), 7.65 (d, 2H, *J* 9.0 Hz, Ar-H), 7.81 (d, 2H, *J* 8.6 Hz, Ar-H), 8.01 (s, 1H, CH, *Tr-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 55.33, 55.63, 114.31, 114.77, 117.02, 122.14, 123.07, 127.13, 130.62, 148.09, 159.74, 159.78; HRMS (ESI+) *m/z* calcd. for $C_{16}H_{15}N_3O_2$ $[M + H]^+$: 282.1242; found: 282.1242; *Tr: triazole hydrogen.

1,4-Bis(3,4-dimethoxyphenyl)-1*H*-1,2,3-triazole (**5**)[44]

Yield: 92%; yellow solid; mp 156-157 ºC; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.90 (s, 3H, OCH_3), 3.92 (s, 3H, OCH_3), 3.95 (s, 3H, OCH_3), 3.96 (s, 3H, OCH_3), 6.91 (d, 1H, *J* 8.2 Hz, Ar-H), 6.94 (d, 1H, *J* 8.4 Hz, Ar-H), 7.19 (dd, 1H, *J* 8.6, 2.5 Hz, Ar-H), 7.34 (dd, 1H, *J* 8.3, 1.9 Hz, Ar-H), 7.37 (d, 1H, *J* 2.4 Hz, Ar-H); 7.51 (d, 1H, *J* 1.7 Hz, Ar-H), 8.05 (s, 1H, *Tr-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 55.94, 56.01, 56.18, 56.22, 105.02, 109.08, 111.20, 111.40, 112.42; 117.27; 118.27; 123.30, 130.70, 148.14, 149.26, 149.36, 149.39, 149.78; HRMS (ESI+) *m/z* calcd. for $C_{18}H_{19}N_3O_4$ $[M + H]^+$: 342.1454; found: 342.1458; *Tr: triazole hydrogen.

1,4-Bis(3,4,5-trimethoxyfenyl)-1*H*-1,2,3-triazole (**6**)[25]

Yield: 89%; yellow solid; mp 172-174 ºC; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.88 (s, 6H, $2OCH_3$), 3.94 (s, 12H, $4OCH_3$), 6.97 (s, 2H, Ar-H), 7.12 (s, 2H, Ar-H); 8.07 (s, 1H, *Tr-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 56.31, 56.50, 60.99, 61.09, 98.60, 103.10, 117.71, 125.76, 132.88, 138.42, 148.27, 153.73, 153.96; HRMS (ESI+) *m/z* calcd. for $C_{20}H_{23}N_3O_6$ $[M + H]^+$: 402.1665; found: 402.1677; *Tr: triazole hydrogen.

1,4-Bis(1,3-benzodioxol-5-yl)-1*H*-1,2,3-triazole (**7**)

Yield: 87%; light-yellow solid; mp 214-215 ºC; IR (KBr): ν / cm^{-1} 3104, 2989, 2888, 2796, 1500, 1477, 1359, 1257, 1216, 1182, 1108, 1037, 935, 817, 808, 607; ^{1}H NMR (300 MHz, DMSO-d_6) δ 6.07 (s, 2H, CH_2), 6.16 (s, 2H, CH_2), 7.02 (d, *J* 8.6 Hz, 1H, Ar-H), 7.12 (d, *J* 8.5 Hz, 1H, Ar-H), 7.38 (dd, 1H, *J* 8.4, 2.2 Hz, Ar-H), 7.43 (m, 2H, Ar-H), 7.49 (d, 1H, *J* 2.2 Hz, Ar-H), 9.05 (s, 1H, *Tr-H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 101.67, 102.27, 102.61, 106.11, 109.12, 109.26, 114.11,119.44, 119.57, 124.80, 131.55, 147.42, 147.66, 147.87, 148.28, 148.66; HRMS (ESI+) *m/z* calcd. for $C_{16}H_{11}N_3O_4$ $[M + H]^+$: 310.0828; found: 310.0833; *Tr: triazole hydrogen.

1-(3,4-Dimethoxyphenyl)-4-(4-methoxyphenyl)-1*H*-1,2,3-triazole (**8**)[25]

Yield: 80%; white solid; mp 163-165 ºC; ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.84 (s, 3H, OCH_3), 3.93 (s, 3H, OCH_3), 3.96 (s, 3H, OCH_3), 6.96 (m, 3H, Ar-H), 7.18 (dd, 1H, *J* 8.4, 2.3 Hz, Ar-H), 7.38 (d, 1H, *J* 8.6 Hz, Ar-H), 7.12 (d, 1H, *J* 2.3 Hz, Ar-H), 7.81 (d, 2H, *J* 8.7 Hz, Ar-H), 8.03 (s, 1H, *Tr-H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 55.35, 56.20, 56.25; 87.58, 104.96, 111.13, 112.36, 114.30, 117.09, 122.98, 127.15, 130.73, 148.13, 149.32; 149.32, 149.75, 159.76; HRMS (ESI+) *m/z* calcd. for $C_{17}H_{17}N_3O_3$ $[M + H]^+$: 312.1348, found: 312.1350; *Tr: triazole hydrogen.

4-(3,4-Dimethoxyphenyl)-1-(4-methoxyphenyl)-1*H*-1,2,3-triazole (**9**)[25,45]

Yield: 85%; white solid; mp 141-143 ºC; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.86 (s, 3H, OCH_3), 3.91 (s, 3H, OCH_3), 3.97(s, 3H, OCH_3), 6.92 (d, 1H, *J* 8.3 Hz, Ar-H), 7.02 (m, 2H, Ar-H), 7.34 (dd, 1H, *J* 8.3, 1.5 Hz, Ar-H), 7.53 (d, 1H, *J* 1.5 Hz, Ar-H), 7.66 (m, 2H, Ar-H), 8.03 (s, 1H, *Tr-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 55.64, 55.98, 56.04, 109.09, 111.42, 114.79, 117.23, 118.26, 122.18, 123.40; 130.60; 149.25; 149.39; 159.83; HRMS (ESI+) *m/z* calcd. for $C_{17}H_{17}N_3O_3$ $[M + H]^+$: 312.1348; found: 312.1347; *Tr: triazole hydrogen.

4-(4-Methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)-1*H*-1,2,3-triazole (**10**)[25]

Yield: 83%; yellow solid; mp 180-181 ºC; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.83 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 3.92 (s, 6H, $2OCH_3$), 6.97 (m, 4H, Ar-H), 7.81 (d, 2H, *J* 8.8 Hz, Ar-H), 8.04 (s, 1H, *Tr-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 55.35, 56.45, 61.06, 98.48, 114.32, 117.08, 122.87, 127.16, 133.00, 138.28, 153.92, 159.83; HRMS (ESI+) *m/z* calcd. for $C_{18}H_{19}N_3O_4$ $[M + H]^+$: 342.1454; found: 342.1443; *Tr: triazole hydrogen.

1-(4-Methoxyphenyl)-4-(3,4,5-trimethoxyphenyl)-1*H*-1,2,3-triazole (**11**)[25]

Yield: 86%; white solid; mp 166-167 ºC; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.87 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 3.94 (s, 6H, $2OCH_3$), 7.02 (d, 2H, *J* 9.0 Hz, Ar-H), 7.12 (s, 2H, Ar-H), 7.67 (d, 2H, *J* 8.9 Hz), 8.09 (s, 1H, *Tr-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 55.64, 56.27, 60.96, 103.02, 114.79, 117.68, 122.19, 125.99, 130.50, 138.29, 153.70, 159.89; HRMS (ESI+) *m/z* calcd. for $C_{18}H_{19}N_3O_4$ $[M + H]^+$: 342.1454; found: 342.1451; *Tr: triazole hydrogen.

1-(1,3-Benzodioxol-5-yl)-4-(4-methoxyphenyl)-1*H*-1,2,3-triazole (**12**)[25]

Yield: 85%; white solid; mp 186-187 ºC; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.84 (s, 3H, OCH_3), 6.06 (s, 2H, CH_2), 6.89 (d, 1H, *J* 8.3 Hz, Ar-H), 6.97 (d, 1H, *J* 8.6 Hz, Ar-H), 7.16 (dd, 1H, *J* 8.3, 2.0 Hz, Ar-H), 7.28 (d, 1H, *J* 2.0 Hz, Ar-H), 7.80 (d, 2H, *J* 8.6 Hz, Ar-H), 7.98 (s, 1H, *Tr-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 55.33, 102.09, 102.74, 108.49, 113.15, 114.12, 114.30, 117.11, 122.90, 127.12, 131.63, 147.94, 148.61, 159.79; HRMS (ESI+) *m/z* calcd. for $C_{16}H_{13}N_3O_3$ $[M + H]^+$: 296.1035; found: 296.1028; *Tr: triazole hydrogen.

4-(1,3-Benzodioxol-5-yl)-1-(4-methoxyphenyl)-1*H*-1,2,3-triazole (**13**)

Yield: 81%; white solid; mp 175-176 ºC; IR (KBr) ν / cm^{-1} 3106, 3012-2794, 1608, 1519, 1481, 1255, 1230, 1214, 1108, 1037, 935, 838, 811; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.86 (s, 3H, OCH_3), 6.00 (s, 2H, CH_2), 6.87 (d, 1H, *J* 7.6 Hz, Ar-H), 7.02 (d, 2H, *J* 8.5 Hz, Ar-H), 7.37 (m, 2H, Ar-H), 7.65 (d, 2H, *J* 8.5 Hz, Ar-H), 7.99 (s, 1H, *Tr-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 55.64, 101.22, 106.48, 108.72, 114.80, 117.23, 119.56, 122.15, 124.57, 130.57, 147.74, 148.18, 159.84; HRMS (ESI+) *m/z* calcd. for $C_{16}H_{13}N_3O_3$ $[M + H]^+$: 296.1035; found: 296.1036; *Tr: triazole hydrogen.

4-(3,4-Dimethoxiphenyl)-1-(3,4,5-trimethoxyphenyl)-1*H*-1,2,3-triazole (**14**)[25]

Yield: 82%; white solid; mp 141-142 ºC; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.88 (s, 3H, OCH_3), 3.91 (s, 3H, OCH_3), 3.93 (s, 6H, 2 OCH_3), 3.97 (s, 3H, OCH_3), 6.92 (d, 1H, *J* 8.3 Hz, Ar-H), 6.97 (s, 2H, Ar-H), 7.35 (dd, 1H, *J* 8.3, 1.6 Hz, Ar-H), 7.52 (d, 1H, *J* 1.6 Hz, Ar-H), 8.06 (s, 1H, *Tr-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 55.98, 56.05, 56.48, 61.06, 98.60, 109.11, 111.43, 117.24, 118.33, 123.20, 132.99, 138.40, 149.37, 149.42, 153.95; HRMS (ESI+) *m/z* calcd. for $C_{19}H_{21}N_3O_5$ $[M + H]^+$: 372.1559; found: 372.1561; *Tr: triazole hydrogen.

1-(3,4-Dimetyhoxyphenyl)-4-(3,4,5-trimethoxyphenyl)-1*H*-1,2,3-triazole (**15**)[25]

Yield: 88%; yellow solid; mp 136-137 ºC; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.88 (s, 3H, OCH_3), 3.94 (s, 9H, 3OCH_3), 3.97 (s, 3H, OCH_3), 6.95 (d, 1H, *J* 8.6 Hz, Ar-H), 7.18 (m, 3H, Ar-H), 7.38 (d, 1H, *J* 1.9 Hz, Ar-H), 8.11 (s, 1H, *Tr-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 56.21, 56.27, 56.28, 60.96, 103.03, 105.06, 111.23, 112.55, 126.02, 130.73, 138.34, 149.49, 149.84, 153.73; HRMS (ESI+) *m/z* calcd. $C_{19}H_{21}N_3O_5$ $[M + H]^+$: 372.1559; found: 372,1571; *Tr: triazole hydrogen.

1-(1,3-Benzodioxol-5-yl)- 4-(3,4-dimethoxyphenyl)-1*H*-1,2,3-triazole (**16**)[25]

Yield: 81%; brown solid; mp 152-153 ºC; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.90 (s, 3H, OCH_3), 3.96 (s, 3H, OCH_3), 6.06 (s, 2H, CH_2), 6.89 (d, 1H, *J* 8.3 Hz, Ar-H), 6.91 (d, 1H, *J* 8.3 Hz, Ar-H), 7.16 (dd, 1H, *J* 8.3, 2.2 Hz, Ar-H), 7.27 (d, 1H, *J* 2.1 Hz, Ar-H), 7.32 (dd, 1H, *J* 8.3, 1.8 Hz, Ar-H), 7.51 (d, 1H, *J* 1.8 Hz, Ar-H), 8.00 (s, 1H, *Tr-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 55.95, 56.01, 102.10, 102.70, 108.48; 109.02, 111.37, 114.14, 117.31, 118.25; 123.22, 131.60, 147.99, 148.13, 148.33, 149.27, 149.36; HRMS (ESI+) *m/z* calcd. $C_{17}H_{15}N_3O_4$ $[M + H]^+$: 326.1141; found: 326.1136; *Tr: triazole hydrogen.

4-(1,3-Benzodioxol-5-yl)-1-(3,4-dimethoxyphenyl)-1*H*-1,2,3-triazole (**17**)

Yield: 90%; light-brown solid; mp 151-152 ºC; IR (KBr) ν / cm^{-1} 3133-3099, 3014-2834, 1602, 1515, 1481, 1440, 1378, 1346, 1267, 1234, 1214, 1137, 1035, 931, 885, 813, 769; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.93 (s, 3H, OCH_3), 3.96 (s, 3H, OCH_3), 6.00 (s, 2H, CH_2), 6.87 (d, 1H, *J* 7.9 Hz, Ar-H), 6.95 (d, 1H, *J* 8.6 Hz, Ar-H), 7.18 (dd, 1H, *J* 8.6, 2.4 Hz, Ar-H), 7.37 (m, 3H, Ar-H), 8.00 (s, 1H, *Tr-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 56.20, 56.24, 101.23, 104.97, 106.46, 108.72, 111.16, 112.36, 117.26, 119.56, 124.47, 130.67, 147.75, 148.17, 149.38, 149.78; HRMS (ESI+) *m/z* calcd. $C_{17}H_{15}N_3O_4$ $[M + H]^+$: 326.1141; found: 326.1134; *Tr: triazole hydrogen.

1-(1,3-Benzodioxol-5-yl)-4-(3,4,5-trimethoxyphenyl)-1*H*-1,2,3-triazole (**18**)[25]

Yield: 78%; a yellow solid; mp 126-127 ºC; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.87 (s, 3H, OCH_3), 3.93 (s, 6H, OCH_3), 6.07 (s, 2H, CH_2), 6.90 (d, 1H, *J* 8.3 Hz, Ar-H), 7.11 (sl, 2H, Ar-H), 7.18 (dd, 1H, *J* 8.4, 2.1 Hz, Ar-H), 7.28 (d, 1H, *J* 2.0 Hz, Ar-H), 8.05 (s, 1H, *Tr-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 56.23, 60.94, 102.12, 102.69, 102.97, 108.47, 114.14, 117.77, 125.82, 131.49, 138.28, 148.04, 148.63, 153.67; HRMS (ESI+) *m/z* calcd. for $C_{18}H_{17}N_3O_5$ $[M + H]^+$: 356.1246; found: 356.1253; *Tr: triazole hydrogen.

4-(1,3-Benzodioxol-5-yl)-1-(3,4,5-trimethoxyphenyl)-1*H*-1,2,3-triazole (**19**)

Yield: 80%; light-brown solid; mp 182-183 ºC; IR (KBr) ν / cm^{-1} 2996-2796, 1604, 1511, 1482, 1452, 1419, 1309, 1238, 1128, 1037, 997, 827, 779; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.87 (s, 3H, OCH_3), 3.92 (s, 6H, 2OCH_3), 5.99 (s, 2H, CH_2), 6.86 (d, 1H, *J* 8.0 Hz, Ar-H), 6.96 (s, 2H, Ar-H), 7.36 (m, 2H, Ar-H), 8.02 (s, 1H, *Tr-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 56.43, 61.03, 98.45, 101.24, 106.43,

108.70, 117.27, 119.57, 124.32, 132.89, 138.32, 147.81, 148.18, 153.91; HRMS (ESI+) *m/z* calcd. for $C_{18}H_{17}N_3O_5$ $[M + H]^+$: 356.1246; found: 356.1244; *Tr: triazole hydrogen.

Antileishmanial assays

Leishmania amazonensis

Synthetic compounds were tested in triplicates in 96-well microplates, with final concentrations from 0.78 to 50 μg mL^{-1}. *L. amazonensis* promastigotes (IFLA/BR/1967/PH8 strain, 1×10^6 parasites mL^{-1}) in exponential phase cultivated in Schneider's Insect Medium were added to the plates and then incubated at 26 °C for 72 h. 20 μL (5 mg mL^{-1}) of thiazolyl blue tetrazolium bromide-MTT (Sigma-Aldrich®) were added in each well and incubated at 37 °C, 5% CO_2 for 2 h as described by Marques *et al*.[46] Pentamidine (Sigma-Aldrich) was used as positive control. Dimethyl sulfoxide (DMSO, Vetec) in Schneider's Insect Medium was used as negative control at the concentration used to solubilize the highest concentration of the test samples and did not interfered on cell viability (1%). A non-linear dose-response regression curve was used to calculate the half maximum inhibitory concentration (IC_{50}).

Leishmania infantum

The compounds were applied in triplicate under promastigotes forms of *Leishmania infantum* (0.5, 2.0, 8.0 and 32.0 μM) previously adjusted to 1×10^6 cells mL^{-1} in a 96 well plate. The plate was incubated in a humidified atmosphere at 22 °C for 72 hours. After this period the viability of the promastigotes was performed by XTT tetrazol salt, which was added in 50 mL of a XTT solution containing PMS (phenazine methosulfate), in the proportion of 1 mg mL^{-1} of XTT to 0.001 mg mL^{-1} of PMS. After, the plate was incubated in humid atmosphere at 37 °C and 5% CO_2 for 4 hours, protected from light. At the end, the reaction results were obtained in spectrophotometer (Biotek) at 450 nm. As positive control only culture medium was used, and parasites with 1.5% DMSO as used as negative control.

The percentage of activity was calculated by the following formula:

$$\% \text{ Activity} = 100 - [(N - Y) / (N - P)] \times 100 \quad (1)$$

where: Y = optical density reading of cells and wells with different concentrations of the compounds; N = optical density reading of parasites in wells with 1.5% DMSO; P = optical density reading of the wells with only culture medium.

Trypanocidal assays

The assays against trypomastigote forms of *T. cruzi* were carried out in 96 well microplates. The trypomastigotes were obtained from supernatant of cellular culture. The concentration of parasites was adjusted to 10^6 forms mL^{-1} in RPMI medium, supplemented by antibiotics and fetal bovine serum. The compounds were added at concentrations of 0.5, 2.0, 8.0 and 32.0 μM. All the compounds were evaluated in triplicate. The materials were incubated for 24 hours at 4 °C. After this period, to each well was added 10 μL solution of FluoReporter lacZ/Galactosidase quantitative Kit (Life Technologies), and the plates were incubated again for 30 minutes. The colorimetric reaction was quantified by fluorescence microplate reader (BIOTEK) at 386 nm excitation and 448 nm emissions.

For both kinds of assays the percentages of parasite lysis were determined from the following formula:

$$\% \text{ lysis} = 100 - \{[(X - CP) / (PC - NC)]\} \times 100 \quad (2)$$

where: X = optical density value of the samples; CP = optical density value of the positive controls; CN = optical density value of the negative controls.

Culture medium was used as positive control (CP) and medium with DMSO 0.6% as negative control.

Cytotoxicity assay

Fibroblasts (NIH/3T3) obtained from Rio de Janeiro Cell Bank (Brazil), were seeded in 96 well plates (1×10^4 cells mL^{-1}) and incubated with synthetic compounds at 37 °C, 5% CO_2 for 48 h at the concentrations of 0.25 to 250 μg mL^{-1} to calculate the IC_{50}. Amphotericin B (Sigma-Aldrich) was used as the reference drug at the concentrations of 0.025 to 25 μg mL^{-1}. Cell growth was estimated by the sulforhodamine B colorimetric method (SRB).[47] DMSO (Vetec) was used as negative control at the concentration used to solubilize the highest concentration of the test samples and did not interfered on cell viability. The percentage of growth was calculated as described by Monks *et al*.[48] IC_{50} was determined by non-linear regression analysis (Microcal Origin Version 6.0 e Microsoft Office Excel 2007). Selectivity index (SI) was calculated according to de Medeiros *et al*.[49]

Supplementary Information

Supplementary data are available free of charge at http://jbcs.sbq.org.br as PDF file.

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