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Synthesis of potential anti-*Trypanosoma cruzi* azole-naftifine analogues by azide–alkyne click reaction

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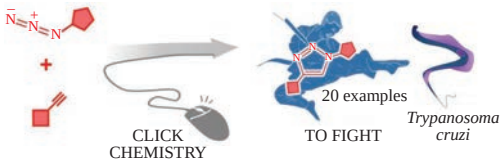
Twenty novel azole-naftifine analogues were obtained using azide–alkyne click reaction. Five of them were more potent than the positive control naftifine revealing an unprecedented antiproliferative effects against *Trypanosoma cruzi*.



Chagas disease (CD), a widespread protozoan infection in Latin America, is caused by the hemoflagellate parasite, *Trypanosoma cruzi*, with incredible ability to infect host cells of the vertebrate host.[1–3] Nowadays, CD is not considered a zoonotic disease that mostly affected poor rural areas in America Latina, but it has become a worldwide major threat to global health.[4] Current treatments for CD are based on two very nonspecific nitroheterocyclic drugs, Nifurtimox and Benznidazole (Figure 1), which require long-term treatments and may lead to severe side effects.[5–7]

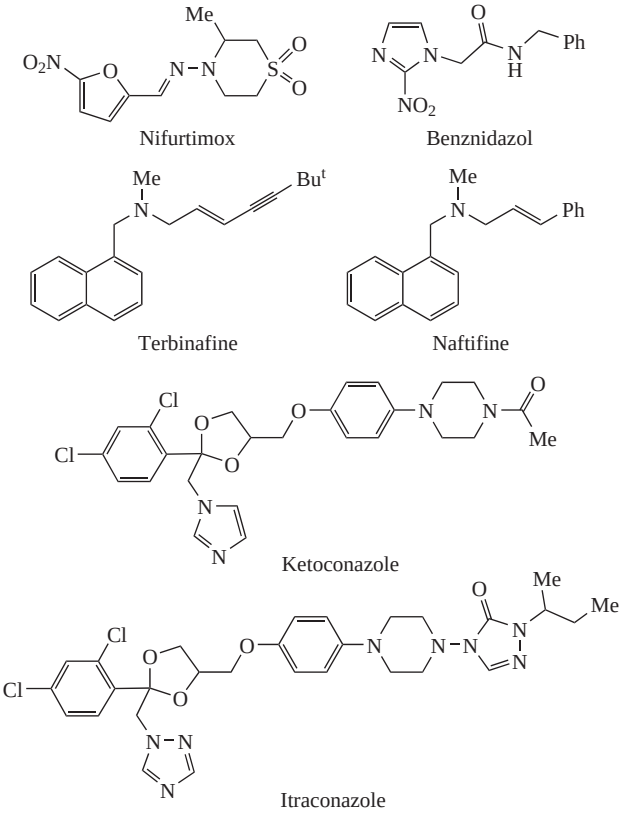
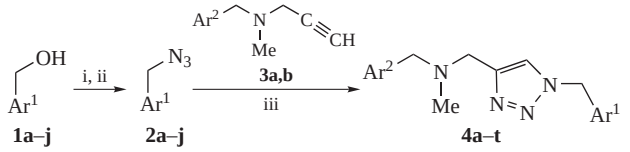


Figure 1 Modern anti-*T. cruzi* agents.

T. cruzi cell viability and proliferation in all stages of its life cycle depends on the availability of ergosterol and related sterols, which are unique to this type of organisms and cannot be fulfilled by cholesterol produced by the host cells.[5] Based on the *T. cruzi*-dependence to ergosterol, the enzymes involved into the ergosterol biosynthesis have been successfully exploited for the development of rational anti-*T. cruzi* agents.[8–12] Allylamines, such as Terbinafine and Naftifine (see Figure 1) are well known antifungal agents which appear to act by preventing *in vitro* and *in vivo* fungal ergosterol biosynthesis *via* a specific and selective inhibition of fungal squalene epoxidase.[13–15] On the other hand, azole antifungal agents, such as Ketoconazole and Itraconazole (see Figure 1) are the best known class of substances capable of inhibiting fungal growth by suppressing ergosterol biosynthesis at the level of the cytochrome P-450-dependent C-14 demethylation of lanosterol.[16–18] Indeed, Terbinafine, Naftifine, Ketoconazole and Itraconazole have been shown to be potent antiproliferative agents against *T. cruzi*, both *in vitro* and *in vivo*.[19–22]

Based on the precedent of the *T. cruzi* antiproliferative effects of allylamines and azoles, herein we report a straightforward approach to obtain new azole-naftifine analogues using the azide–alkyne click reaction. This click-chemistry approach was chosen since it is particularly attractive and has received huge consideration due to its effectiveness in making substances with diverse applications, from drugs to new materials.[23] A total of twenty new analogues were synthesized and their antiproliferative activities against *T. cruzi* were evaluated.

Our initial efforts commenced by converting available benzylic alcohols **1a–j** into benzylic azides **2a–j** (Scheme 1). Transformation of benzylic alcohols **1** to the intermediate mesylates was achieved by adding mesyl chloride (MsCl) to a solution of the alcohol in CH_2Cl_2. In our first attempt, MsCl was added in a single shot, but the reaction furnished only the dibenzylic ethers instead of the desirable mesylates. To our delight, the mesylates were formed on slow addition of MsCl into the reaction medium. The main absorption bands in IR spectra for the mesylates (for crude mesylates) were recorded in the region of 1330–1338 and 1160–1174 cm^{-1}, corresponding to the symmetrical and asymmetrical stretching, respectively, of SO_2 bonds, common



1, **2**: **a** Ar^1 = Ph
b Ar^1 = 4-FC_6H_4
c Ar^1 = 4-ClC_6H_4
d Ar^1 = 4-BrC_6H_4
e Ar^1 = 4-IC_6H_4
f Ar^1 = 4-$F_3CC_6H_4$
g Ar^1 = 4-$F_3COC_6H_4$
h Ar^1 = 2,4-$F_2C_6H_3$
i Ar^1 = 3,4-$F_2C_6H_3$
j Ar^1 = 2,4,6-$Cl_3C_6H_2$

3a, **4a–j** Ar^2 = 1-naphthyl
3b, **4k–t** Ar^2 = anthracen-9-yl

4a,**k** Ar^1 = Ph
4b,**l** Ar^1 = 4-FC_6H_4
4c,**m** Ar^1 = 4-ClC_6H_4
4d,**n** Ar^1 = 4-BrC_6H_4
4e,**o** Ar^1 = 4-IC_6H_4
4f,**p** Ar^1 = 4-$F_3CC_6H_4$
4g,**q** Ar^1 = 4-$F_3COC_6H_4$
4h,**r** Ar^1 = 2,4-$F_2C_6H_3$
4i,**s** Ar^1 = 3,4-$F_2C_6H_3$
4j,**t** Ar^1 = 2,4,6-$Cl_3C_6H_2$

Scheme 1 *Reagents and conditions*: i, MsCl, Et_3N, CH_2Cl_2, –50 °C; ii, NaN_3, DMSO, room temperature; iii, $CuSO_4·5H_2O$ (aq.), Na-ascorbate, CH_2Cl_2, room temperature.

to all mesylates.[24] Since the obtained mesylates have shown some tendency to decompose, the crude product was purified only by washing with water and solvent removal. The prepared mesylates readily underwent azidation with sodium azide in DMSO at room temperature within 15 h[24] (see Scheme 1). The desirable azides **2a**–**j** were obtained in 70 to 99% yields in relation to starting alcohols **1a**–**j**. The IR spectra of the isolated products showed the characteristic azido group absorption band at 2080–2100 cm^{-1} which is in agreement with reported data.[24] With azides **2a**–**j** in hand, we next coupled them, without purification, with propargylamines **3a**,**b** (diluted in CH_2Cl_2) in the presence of $CuSO_4·5H_2O$, sodium ascorbate (diluted in water) at room temperature for 1–5 h, providing the desired azole-naftifine analogues **4** in 55–98% yields (see Scheme 1). In fact, azide–alkyne click reaction worked well for a variety of substituents on the aromatic ring of azides **2a**–**j**, affording good to excellent yields of the desired azole-naftifine analogues, except for reaction of azide **2i** with propargylamine **3b** (55%). The structures of the azole-naftifine analogues **4** were confirmed by spectroscopic and spectrometric analyses. Along with the expected signals for the proposed structures for the azole-naftifine analogues, the common signal for non-hydrogenated

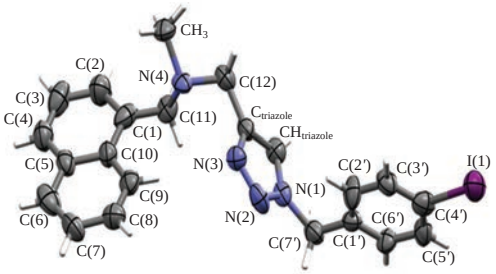


Figure 2 The ORTEP-3 drawing of the azole-naftifine analogue **4e**. Ellipsoids are at 30% probability level and an arbitrary labeling of atoms is displayed.

† *Crystal data for* **4e**. Crystals of $C_{22}H_{21}IN_4$ (M = 468.33) are triclinic, space group $P\bar{1}$, at 293(2) K: a = 5.5291(4), b = 12.9638(9) and c = 15.4547(11) Å, α = 70.88(3)°, β = 84.50(4)°, γ = 77.72(5)°, V = 1022.3(3) $Å^3$, Z = 2 (Z' = 1), d_{calc} = 1.521 g cm^{-3}, μ = 1.580 mm^{-1}, 3.23° ⩽ θ ⩽ 26.62°, 7101 reflections collected, 2639 unique [$I > 2\sigma(I)$], F(000) = 468, number of refined parameters 255. The refinement converged to GOF on F^2 1.258, final R factors were R_1 = 0.0711 and wR_2 = 0.2368 [for $I > 2\sigma(I)$], R_1 = = 0.1003 and wR_2 = 0.2491 (for all reflections). Largest diff. peak/hole: 1.641/–1.377 e $Å^{-3}$.

CCDC 1039072 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre *via* http://www.ccdc.cam.ac.uk.

Table 1 Susceptibility of *T. cruzi* epimastigotes (Y strain) to azole-naftifine analogues and Naftifine.[a]

Compound	EC_{50} / μM[b]	Compound	EC_{50} / μM[b]
4a	25.36 ± 4.89	**4o**	25.56 ± 11.07
4d	22.11 ± 3.46	**4h**	19.08 ± 5.28
4n	29.96 ± 8.67	Naftifine	59.80 ± 8.21

[a] Values are expressed as the mean ± standard deviation. The EC_{50} values for the presented azole-naftifine analogues were statistically significant by *t*-test ($p < 0.05$ were considered significant) in relation to the EC_{50} values obtained for Naftifine.

carbon atom of the triazole ring at δ 146.0 ppm and the signal related to the C–H bond of triazole moiety at δ 122.0 ppm were observed in all ^{13}C NMR spectra. In addition, the high resolution mass spectra of products **4** revealed the signal of m/z for the expected ion $[M + H]^+$ of these compounds.

The structure of the azole-naftifine analogue **4e** was confirmed by X-ray analysis.† There is only one molecule of **4e** in the asymmetric unit (Figure 2), its molecular backbone is completely twisted due to different degrees of rotation around its several open-chain single bonds. The torsions on the bond bridging rings and open-chain deviate markedly from the values expected for a planar conformation (0° or 180°). For instance, the C(10)–C(1)–C(11)–N(4) and C(6′)–C(1′)–C(7′)–N(1) torsions on the bonds connecting peripheral naphthalene and benzene rings to the open-chain are –69(2)° and 23(2)°, respectively. The central triazole ring is also bent relative to the open-chain, which can be described by the N(3)–$C_{triazole}$–C(12)–N(4) and C(1′)–C(7′)–N(1)–$CH_{triazole}$ torsions of –48(2)° and 90(2)°, respectively. As a result of these rotations, naphthalene and benzene rings are not coplanar to the triazole mean plane, forming angles of 49.5(5)° and 79.4(5)° with it, respectively. Double bonds in the triazole ring were clearly identified between $CH_{triazole}$ and $C_{triazole}$ carbons [1.37(2) Å] and between N(2) and N(3) nitrogen atoms [1.31(2) Å].

Next we turn our attention to the study of the anti-*T. cruzi* properties of the twenty new azole-naftifine analogues synthesized. To determine the effects on parasite survival, 10^6 ml^{-1} *log*-phase epimastigotes (Y strain) were incubated in culture medium at 28 °C in the presence of different concentrations of the azole-naftifine analogues **4** (0–100 μM). After 4 days, cell viability was determined using a colorimetric assay (MTT).[25] Four independent experiments were performed in triplicate and naftifine was used as a positive control. Among twenty, five test compounds had effect on *T. cruzi* proliferation under the concentration of 100 μM (Table 1). Azole-naftifine analogue **4h**, which bears the same di-flurophenyl ring of fluconazole was the most potent compound, being 3-fold more potent than naftifine (Table 1). Azole-naftifine analogues **4a**,**d**,**n**,**o** were roughly 2-fold more potent than naftifine. The unprecedented antiproliferative effects against *T. cruzi* of these azole-naftifine analogues seems promising for further exploration of their molecular scaffold for developing drugs that are more effective to fight *T. cruzi*.

In summary, twenty novel azole-naftifine analogues were synthesized in 52–95% overall yield in two steps from readily accessible benzylic alcohols and propargylamines. The unique results of this work are the first application of azide–alkyne click reaction to obtain novel azole-naftifine analogues and the antiproliferative effects of such a class of compounds against *T. cruzi*. Five of new compounds were disclosed as promising lead ones to develop new anti-*T. cruzi* agents.

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2018.03.029.

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