Targeting tuberculosis through a small focused library of 1,2,3-triazoles. Mol Divers

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SHORT COMMUNICATION

Targeting tuberculosis through a small focused library of 1,2,3-triazoles

Guillermo R. Labadie · Agustina de la Iglesia · Héctor R. Morbidoni

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Abstract Looking for new active molecules against *Mycobacterium tuberculosis*, a small focused library of 1,2,3-triazoles was efficiently prepared by click chemistry. Compounds were subsequently tested against different pathogenic and opportunistic mycobacteria including *M. avium* and *M. tuberculosis*. Two of them showed MIC at lower µg/mL concentration for *M. avium* and even below that for *M. tuberculosis,* being more potent that control drugs.

Keywords Tuberculosis · Triazoles · Click chemistry

Introduction

Since the time of the identification of the causing agent of tuberculosis (*Mycobacterium tuberculosis*—Mtb) by Robert Koch more that 120 years ago all the attempts to eradicate this disease have been unsuccessful [1]. Instead of being near to a solution, the situation is so critical that in 1993 WHO declared tuberculosis "a global health emergency" [2]. That situation is caused by the combined effects of the AIDS pandemic, the advent of multidrug (MDR) [3] and extremely drug (XDR) resistant *M. tuberculosis* strains and the decline of public health controls in some parts of the world (notably South East Asia, eastern Europe and the former Soviet Union) [4,5]. There are about 8.9–9 million new cases each year, of which 15% are children, and 1.7–2 million deaths, of which 450,000 are children. In this context, the number of cases is currently rising to 2% per year, and without a coordinated control effort, tuberculosis will infect an estimated 1 billion more people by 2020, killing 70 million [6]. Patients with a faulty immune response due to AIDS or other causes of cellular immunedepression are specially vulnerable to the tubercle bacilli replication leading to up to 31% death rate in the African region [7]. These individuals are also susceptible to infection by non-tuberculous mycobacteria (NTMs) from environmental sources. NTMs are usually cause of infection in AIDS patients as well as in individuals suffering of other chronic pulmonary pathologies, such as cystic fibrosis. Members of this mycobacterial group—specially *M. abscessus* and *M. chelonae*—have recently been linked to skin and soft tissue infections and *M. avium* is frequently a cause of disseminated infections in AIDS patients. For all the mentioned reasons—mainly because of the rise in the detection of MDR and XDR strains—there is an urgent need to develop new antimycobacterial agents with novel mechanism(s) of action that would be active on those strains [8].

Lipoic acid (Fig. 1) is an essential organosulphur compound that activates a number of protein complexes involved in key metabolic processes. Enzymes participating in lipoylation have gained attention because of their implication in bacterial pathogenicity [9]. LipB is part of the lipoic acid biosynthesis catalyzing the octanoyl-[acyl carrier protein]-protein acyltransferase and also plays and essential role in the grow of *M. tuberculosis* [10]. Recently, this enzyme has been crystallized unexpectedly presenting a decanoic acid covalently bound to the sulphur thiol of the Cys176 [11]. Another example of the critical role of fatty acids on *M. tuberculosis*

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G. R. Labadie (✉)
Instituto de Química Rosario (IQUIR-CONICET), Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Suipacha 531, S2002LRK Rosario, Argentina
e-mail: labadie@iquir-conicet.gov.ar

A. de la Iglesia · H. R. Morbidoni
Catedra de Microbiología, Virología y Parasitología, Facultad de Ciencias Médicas, Universidad Nacional de Rosario, Santa Fe 3100, S2002KTR Rosario, Argentina

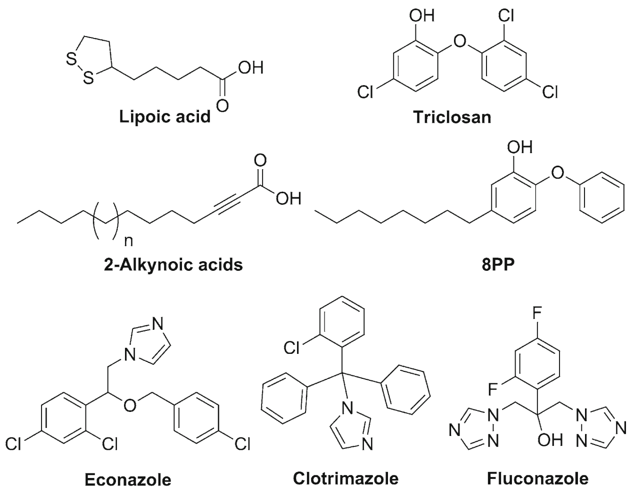


Fig. 1 Antimycobacterial compounds and metabolites

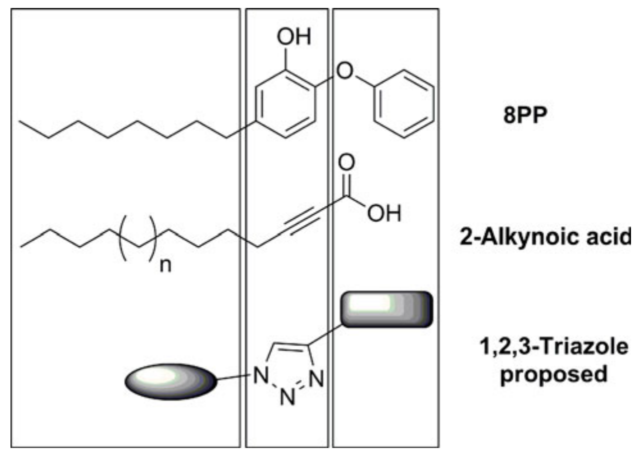


Fig. 2 Strategy used for library design

metabolism was the activity of 2-alkynoic fatty acids (Fig. 1) that inhibited the bacterial growth. These compounds, at toxic concentrations, inhibit fatty acid degradation and synthesis, resulting in cell death. The mechanism of those antibacterial compounds included several pathways to achieve its effect [12]. Another interesting target for the development of new drugs against tuberculosis is the InhA enzyme, the enoyl reductase in the type II fatty acid biosynthesis pathway. This enzyme has been studied as a specific target to develop new chemotherapeutic agents against tuberculosis. In fact, InhA has been validated as a drug target by a temperature-sensitive mutation that causes a phenotypic response similar to isoniazid administration [13]. Triclosan (Fig. 1) is a broad spectrum antimicrobial compound that was long thought to have no specific cellular target until it was recently revealed as a potent inhibitor of the enoyl reductase of many organisms [14]. Different triclosan analogs have been prepared to selectively target *M. tuberculosis* InhA. The alkyl diphenyl ethers have been among the most successful inhibitors derived from triclosan having antitubercular MIC around 2–3 μg/mL, **8PP** (Fig. 1) being the most active of the series [15].

Azoles currently used as antifungal drugs (miconazole, econazole, fluconazole, clotrimazole, among others) have been tested against mycobacteria displaying a good killing activity creating a fresh interest on azole and triazole drugs as potential anti-mycobacterial scaffolds [16]. Econazole (Fig. 1) and clotrimazole (Fig. 1) have shown to be efficient antimycobacterial agents [17,18], even in murine models [19] and in the latent form of the disease [20]. These results have moved different groups to prepare new collections of compounds having triazoles as a "new" antimycobacterial pharmacophore [21–23]. In general, new synthetic 1,2,4-triazoles have been reported as novel antimycobacterial products [24] but recently 1,2,3-isomers have been also introduced, prepared by conventional 1,3-dipolar cycloaddition [25–27]. The introduction of "click chemistry" has significantly changed many fields of science, including drug discovery [28,29]. The simplicity and efficiency of the copper(I)-catalyzed 1,3-dipolar Huisgen cycloaddition introduced by Sharpless [30], allowed to easily synthesize 1,2,3 triazoles. Over the last 15 years many click chemistry approaches have been successfully used to prepare diverse collections of triazoles directed to unspecific targets [31–33] even on solid support [34,35], or focused libraries looking to selectively inhibit enzymes [36,37], or mimicking active natural products [38,39].

Based on the critical role displayed by fatty acid biosynthesis and metabolism and the known and renewed interest on triazoles as antimicobacterial drugs, we decided to combine them as an alternative approach for antitubercular drug design. Our strategy required the preparation of a focused library to find a simple scaffold to develop new drugs based on the known antitubercular compounds **8PP** and **2-alkynoic acid**. To do that, a series of 1,2,3-triazoles were prepared mimicking those two compounds (Fig. 2).

Resultsand discussion

A pool of azides and alkynes were selected and used in our synthesis to guarantee a minimum range of diversity over the products, allowing structural–activity relationship studies. Alkynes **A1–7** and azides were selected as the required building block for our synthesis (Fig. 3). Azide **Z2** were prepared by direct substitution of the bromide with sodium azide in DMF, azides **Z1** and **Z3** were also synthesized in the same way, but the corresponding bromides were prepared starting from the alcohol by substitution with phosphorous tribromide in Et_2O.

Alkynes **A1–7** were purchased from different commercial providers. With the necessary building blocks at hand, the triazoles were prepared following conditions originally reported [30] (Scheme 1).

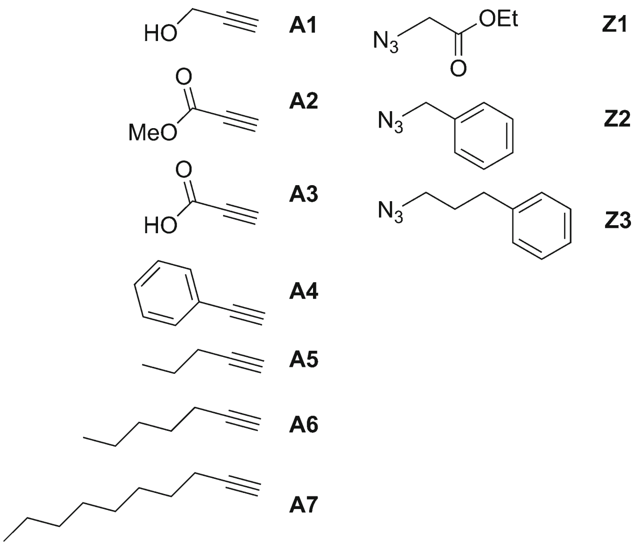
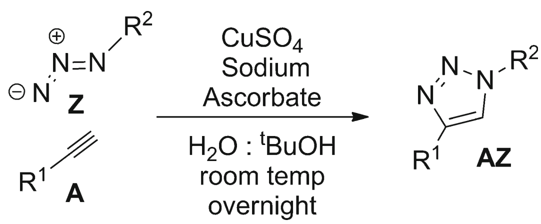


Fig. 3 Alkynes (**A1–7**) and azides (**Z1–3**) used in triazole synthesis



Scheme 1 Triazoles synthesis by copper catalyzed Hüisgen cycloaddtion conditions

Reactions were conducted in a parallel solution synthesis fashion under copper(II) sulphate catalytic conditions in water:*t*-BuOH (1:1) using sodium ascorbate as reductant. A collection of 16 compounds **AZ** were obtained with an 84% average yield after purification (Table 1).

Products were completely characterized by 1D-, 2D-NMR experiments and HRMS. The products prepared have a 1,4-substitution pattern as was expected based on the original description of this methodology and by several other authors after that [31–39].

Minimum inhibitory concentration (MIC) of the compounds was determined against *M. tuberculosis* H37Rv and four non-tuberculous mycobacterial species. Of those, three (*M. fortutitum*, *M. avium* and *M. abscessus*) are increasingly becoming important opportunistic pathogens. The fourth species, *M. smegmatis* is a non-pathogenic rapid growing mycobacteria, that is usually used for rapid testing of compounds. The MIC value for Rifampicin used as control drug against Mtb, was 0.5 µg/mL while MIC values for econazole and fluconazole were of 2.5 µg/mL and ≥100 µg/mL, respectively.

Compounds were tested for their activity against *M. tuberculosis* H37Rv, of those eight (compounds **A1Z1**, **A1Z2**, **A1Z3**, **A2Z2**, **A3Z1**, **A3Z2**, **A4Z1** and **A5Z1**) were not active at 100 µg/mL, three displayed activity at 50 µg/mL (compounds **A2Z3**, **A6Z2** and **A7Z2**) and four (compounds **A4Z2**, **A4Z3**, **A5Z2** and **A6Z1**) showed activity at 25 µg/mL (Table 1).

The remaining compound (**A7Z1**) has an MIC value of 0.50 µg/mL (Table 1) fivefold more active than econazole. Compounds showing the highest activity against *M. avium* were **A4Z3** and **A7Z1** with MIC values of 1.6 µg/mL, and 3.1 µg/mL, respectively, while compounds **A4Z2**, **A7Z2**, showed activity at 25 µg/mL on non-tuberculous mycobacteria. These results are promising, but will need a complete study over different strains because previous reports [40] have established that *M. avium* presents differences in cell envelope composition between strains and biotypes that will produce different responses to the same compound. None of the tested molecules displayed activity against the other mycobacterial species tested (Table 1), probably due to NTMs species having very low membrane permeability. None of these compounds showed cytotoxic effects on Vero cells, at 4.76 µg/mL, the higher concentration tested. That provides a minimum selectivity index (MIC antimycobacterial/MIC cytotoxicity) of 9.5 for compound **A7Z1** and 3.0 for compound **A4Z3**. It is important to remark that activity of the series of the aliphatic triazoles with the carboethoxy group (**A5Z1**, **A6Z1** and **A7Z1**) increase their activity as long as the number of carbon on the chain is growing. That can be explained based on the change of the lipophilicity of the compounds. To demonstrate that, log *D* of the library were calculated using adequate software (Table 1). As we predicted, the calculated values showed the expected trend: 1.77 for **A5Z1**, 2.83 for **A6Z1** and 4.42 for **A6Z1** showing a good correlation between MIC and log *D* for this small series.

Therefore, inhibitory activity of this series of compounds correlates with an increased value of the lipophilicity index in good agreement with previous observations for major antitubercular drugs and some recent reports of newly introduced active compounds [41,42].

In conclusion, we have found through a small focused collection of carefully selected 1,2,3-triazoles a very simple active scaffold against *M. tuberculosis*. The most promising candidate as antitubercular agent was compound **A7Z1** (Fig. 4) being five times more active that econazole.

Also, we have found that **A4Z3** (Fig. 4) was active against *M. avium* at 1.6 µg/mL. A complete study of the most active compounds with MRD strains is currently being conducted together with experiments to validate their mechanism of action.

Table 1 Antymycobacterial, cytotoxity activities and yields for 1,2,3-triazoles **AZ**

Compound	*M. avium* MIC[a]	*M. tuberculosis* MIC[a]	Cytotoxicity[b] IC_{50}	log *D*[c] pH 7.4	Yield[d] (%)
A1Z1	>100	>100	NC[e]	−0.94 ± 0.57	85
A1Z2	>100	>100	NC	0.21 ± 0.54	78
A1Z3	>100	>100	NC	1.05 ± 0.54	90
A2Z2[f]	>100	>100	NC	2.23 ± 0.57	79
A2Z3	>100	50	NC	2.21 ± 0.77	70
A3Z1	>100	>100	NC	−3.19	78
A3Z2	>100	>100	NC	−2.03	72
A4Z1	>100	>100	NC	2.00 ± 0.58	94
A4Z2[g]	25	25	NC	3.15 ± 0.56	86
A4Z3[g]	1.6	25	NC	3.99 ± 0.55	93
A5Z1	>100	100	NC	1.77 ± 0.56	72
A5Z2	>100	25	NC	2.91 ± 0.53	78
A6Z1	>100	25	NC	2.83 ± 0.56	79
A6Z2	100	50	NC	3.97 ± 0.53	96
A7Z1	3.1	0.5	NC	4.42 ± 0.52	95
A7Z2	25	50	NC	5.57 ± 0.53	96
Rifampicin		0.5			
Econazole		2.5			
Fluconazole		≥100			

[a] Minimum inhibitory concentration expressed in μg/mL
[b] Vero cells, concentration expressed in μg/mL
[c] Calculated using ACD labs 6.0
[d] After purification
[e] NC: not cytotoxic (up to the maximum dose tested 4.76 μg/mL)
[f] Ethyl ester reported by Appukkuttan et al. [43]
[g] Reported by Lee et al. [31] without a complete characterization

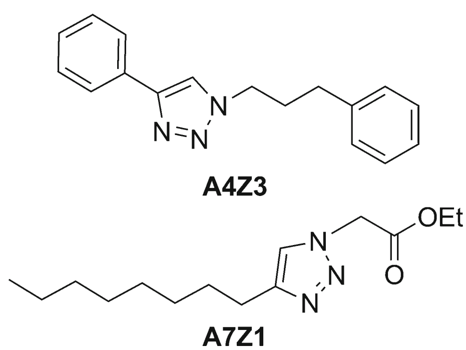


Fig. 4 Most active compounds

Experimental

General methods

Chemical reagents were purchased from commercial suppliers and were used without further purification unless otherwise noted. All the solvents (hexane, ethyl acetate, CH_2Cl_2, Et_2O) were distilled prior to use. Yields were calculated for material judged homogenous by thin layer chromatography and nuclear magnetic resonance (NMR). Reaction progress was monitored by thin layer chromatography (TLC) performed on silica gel 60 F_{254} pre-coated aluminium sheets. Spots were visualized under 254 nm UV lamp and/or by dipping the TLC plate into a solution of *p*-anisaldehyde or phosphomolibdic acid followed by heating with a heat gun. Flash column chromatography was performed using silica gel 60 (230–400 mesh). ^1H and ^{13}C NMR spectra were recorded on a Bruker Avance II 300 MHz (75.13 MHz) using $CDCl_3$ as solvent unless otherwise noted. Chemical shifts for proton nuclear magnetic resonance (^1H NMR) spectra are reported in parts per million relative to the signal of tetramethylsilane at 0 ppm (internal standard) and coupling constants (*J*) are reported in Hertz (Hz). Chemical shifts for carbon nuclear magnetic resonance (^{13}C NMR) spectra are reported in parts per million relative to the centre line of the $CDCl_3$ triplet at 76.9 ppm. Assignments of proton resonances were confirmed by correlated spectroscopy. ESI-HRMS were recorded at the University of Mississippi, Department of Medicinal Chemistry on a Waters LC-QTof micro.

General procedure for azides and alkynes reaction under click chemistry conditions

Alkyne (1 eq) and the azide (1 eq) were suspended in mL/eq of ^{t}BuOH:H_2O (1:1) and then aqueous 1 M $CuSO_4$ solution and finally aqueous 1 M sodium ascorbate solution were added and the mixture stirred overnight at room temperature. Brine was added and the solution was extracted with dichloromethane. The combined organic extracts were dried over sodium sulphate and evaporated. The resulting residue was purified by column chromatography over silica gel using an increasing AcOEt/hexane gradient to afford desired pure products.

Compound **A1Z1** *ethyl 2-(4-(hydroxymethyl)-1H-1,2, 3-triazol-1-yl)acetate*

^{1}H NMR: 7.62 (s, 1H), 5.07 (s, 2H), 4.64 (s, 2H), 4.15 (q, $J=6.9$ Hz, 2H), 1.20 (t, $J=6.9$ Hz, 3H); ^{13}C NMR: 166.6 (C), 148.2 (C), 123.7 (C), 62.3 (CH_2), 55.8 (CH_2), 50.8 (CH_2), 14.0 (CH_3); ESI-HRMS Calcd for (M + H^+) $C_7H_{12}N_3O_3$ 186.0879; found 186.0871.

Compound **A1Z2** *(1-benzyl-1H-1,2,3-triazol-4-yl)methanol*

^{1}H NMR: 7.45 (s, 1H), 7.30 (m, 3H), 7.20 (m, 2H), 5.42 (s, 2H), 4.67 (s, 2H); ^{13}C NMR: 148.3 (C), 134.6 (C), 129.0 (CH), 128.7 (CH), 128.1 (CH), 122.0 (CH), 54.1 (CH_2); ESI-HRMS Calcd for (M + H^+) $C_{10}H_{12}N_3O_2$ 190.0980; found 190.0972.

Compound **A1Z3** *(1-(3-phenylpropyl)-1H-1,2,3-triazol-4-yl)methanol*

^{1}H NMR: 7.36 (s, 1H), 7.10–7.30 (m, 5H), 4.74 (s, 2H), 4.28 (t, $J=7.2$ Hz, 2H), 2.60 (t, $J=7.2$ Hz, 2H), 2.18 (q, $J=7.2$, 2H); ^{13}C NMR: 148.0 (C), 128.6 (CH), 128.4 (CH), 126.3 (CH), 122.0 (C), 56.0 (CH_2), 49.5 (CH_2), 32.4 (CH_2), 31.6 (CH_2). HRMS Calcd for (M+H^+) $C_{12}H_{16}N_3O_2$; 218.1293; found 218.1293.

Compound **A2Z2** *methyl 1-benzyl-1H-1,2,3-triazole-4-carboxylate*

^{1}H NMR: 7.97 (s, 1H), 7.41 (m, 3H), 7.29 (m, 2H), 5.58 (s, 2H), 3.93 (s, 3H); ^{13}C NMR: 161.1 (C), 140.3 (C), 133.7 (C), 129.4 (CH), 129.2 (CH), 128.3 (CH), 127.4 (CH), 54.5 (CH_2), 52.2 (CH_3); ESI-HRMS Calcd for (M + H^+) $C_{11}H_{12}N_3O_2$ 218.0930; found 218.0931.

Compound **A2Z3** *(methyl 1-(3-phenylpropyl)-1H-1,2, 3-triazole-4-carboxylate*

^{1}H NMR: 8.10 (s, 1H), 7.30–7.10 (m, 5H), 4.39 (t, $J=7.2$), 3.92 (s, 3H), 2.63 (t, $J=7.2$, 2H), 2.25 (q, $J=7.2$, 2H); ^{13}C NMR: 161.2 (C), 139.9 (C), 139.8 (C), 128.7 (CH), 128.4 (CH), 127.5 (CH), 126.6 (CH), 52.2 (CH_3), 49.9 (CH_2), 32.3 (CH_2), 31.5 (CH_2); HRMS Calcd for (M+H^+) $C_{13}H_{16}N_3O_2$ 246.1253; found 246.1243.

Compound **A3Z1** *1-(2-ethoxy-2-oxoethyl)-1H-1,2, 3-triazole-4-carboxylic acid*

^{1}H NMR (DMSO-d_6): 13.09 (bs, 1H), 8.75 (s, 1H), 7.35 (m, 5H), 5.63 (s, 2H); ^{13}C NMR: 167.7 (C), 163.2 (C), 139.8 (C), 129.4 (CH), 59.6 (CH_2), 52.5 (CH_2), 13.9(CH_3); ESI-HRMS Calcd for (M + H^+) $C_7H_{10}N_3O_4$ 200.0671; found 200.0667.

Compound **A3Z2** *1-benzyl-1H-1,2,3-triazole-4-carboxylic acid*

^{1}H NMR (DMSO-d_6): 13.09 (bs, 1H), 8.75 (s, 1H), 7.35 (m, 5H), 5.63 (s, 2H); ^{13}C NMR (DMSO-d_6): 163.1 (C), 140.4 (C), 136.0 (C), 129.3 (CH), 129.3 (CH), 128.8 (CH), 128.5 (CH), 53.6; ESI-HRMS Calcd for (M + H^+) $C_{10}H_{10}N_3O_2$ 204.0777; found 204.0777.

Compound **A4Z1** *ethyl 2-(4-phenyl-1H-1,2,3-triazol-1-yl)acetate*

^{1}H NMR: 7.90 (s, 1H), 7.84 (dd, $J_1=1.5$, $J_2=0.6$, 2H), 7.50–7.30 (m, 3H), 5.21 (s, 2H), 4.29 (q, $J = 7.2$, 2H), 1.32 (t, $J = 7.2$, 3H); ^{13}C NMR: 166.3 (C), 148.2 (C), 130.4 (C), 128.8 (CH), 128.3 (CH), 125.8 (CH), 121.0 (CH), 51.0 (CH_2), 62.5 (CH_2), 14.1 (CH_3); ESI-HRMS Calcd for (M + H^+) $C_{12}H_{14}N_3O_2$ 232.1097; found 232.1086.

Compound **A4Z2** *(1-benzyl-4-phenyl-1H-1,2,3-triazole*

^{1}H NMR: 7.80 (dd, $J_1 = 8.4$, $J_2 = 1.5$, 2H), 7.66 (s, 1H), 7.42–7.30 (m, 8H), 5.58 (s, 2H); ^{13}C NMR: 148.2 (C), 134.7 (C), 130.6 (CH), 129.2 (CH), 128.8 (CH), 128.2 (CH), 128.1 (CH), 125.5 (CH), 119.5 (CH), 54.2 (CH_2); ESI-HRMS Calcd for (M + H^+) $C_{15}H_{14}N_3$ 236.1188; found 236.1189.

Compound **A4Z3***, 4-phenyl-1-(3-phenylpropyl)-1H-1,2, 3-triazole*

^{1}H NMR: 7.82 (m, 2H); 7.70 (s, 1H); 7.46–7.16 (m, 8H); 4.83 (t, $J = 6.9$, 2H), 2.69 (t, $J = 7.8$, 2H), 2.29 (t, $J = 6.9$, 2H); ^{13}C NMR: 147.6 (C), 140.1 (C), 130.6 (C), 128.7 (CH), 128.5 (CH), 128.4 (CH), 128. 0 (CH), 126.3 (CH), 119.5 (CH), 49.5 (CH_2), 32.4 (CH_2), 31.4 (CH_2). ESI-HRMS Calcd for (M + H^+) $C_{17}H_{18}N_3$ 264.1501; found 264.1501.

Compound **A5Z1** *ethyl 2-(4-propyl-1H-1,2,3-triazol-1-yl)acetate*

^{1}H NMR: 7.41 (s, 1H), 5.11 (s, 2H), 4.25 (q, $J = 7.2$, 2H), 2.71 (t, $J = 7.5$, 2H), 1.71 (m, $J = 7.5$, 2H), 1.29 (t, $J = 7.2$, 3H), 0.97 (t, $J = 7.2$, 3H); ^{13}C NMR: 166.5 (C), 148.4 (C), 122.2 (CH), 62.1 (CH_2), 50.7 (CH_2), 27.5 (CH_2), 22.5

(CH_2), 13.9 (CH_3), 13.6 (CH_3); ESI-HRMS Calcd for ($M + H^+$) $C_9H_{16}N_3O_2$ 198.1243; found 198.1244.

Compound **A5Z2** *benzyl-4-proyl-1H-1,2,3-triazole*

^{1}H NMR: 7.41–7.32 (m, 3H), 7.26–7.22 (m, 2H), 7.18 (s, 1H), 5.49 (s, 2H), 2.67 (t, $J = 7.8$, 2H), 1.67 (m, 2H), 0.95 (t, $J = 6.9$, 3H); ^{13}C NMR: 148.6 (C), 135.1 (C), 129.0 (CH), 128.5 (CH), 127.9 (CH), 53.9 (CH_2), 27.7 (CH_2), 22.6 (CH_2), 13.7 (CH_3); ESI-HRMS Calcd for ($M + Na^+$) $C_{12}H_{15}N_3O_2Na$ 224.1170; found 224.1164.

Compound **A6Z1** *ethyl 2-(4-pentyl-1H-1,2,3-triazol-1-yl)acetate*

^{1}H NMR: 7.41 (s, 1H), 5.12 (s, 2H), 4.26 (q, $J = 7.3$, 2H), 2.73 (t, $J = 7.3$, 2H), 1.65 (m, 4H), 1.20–1.40 (m, 10H), 1.30 (t, $J = 7.2$, 3H), 0.87 (t, $J = 0.69$, 3H); ^{13}C NMR: 166.5 (C), 148.9 (C), 121.9 (CH), 62.2 (CH_2), 50.7 (CH_2), 31.8 (CH_2), 29.3 (CH_2), 29.2 (CH_2), 25.6 (CH_2), 22.6 (CH_2), 14.0 (CH_3); ESI-HRMS Calcd for ($M + H^+$) $C_{11}H_{20}N_3O_2$ 226.1556; found 226.1554.

Compound **A6Z2** *benzyl-4-pentyl-1H-1,2,3-triazole*

^{1}H NMR: 7.35 (m, 3H), 7.26 (m, 2H), 7.17 (s, 1H), 5.49 (s, 2H), 2.68 (t, $J = 7.7$, 2H), 1.67 (m, 2H), 1.31 (m, 4H), 0.88 (t, $J = 7.0$, 3H); ^{13}C NMR: 149.0 (C), 135.1 (C), 129.0 (CH), 128.6 (CH), 127.9 (CH), 120.5 (CH), 53.9 (CH_2), 31.4 (CH_2), 29.1 (CH_2), 25.7 (CH_2), 22.4 (CH_2), 14.0 (CH_3); ESI-HRMS Calcd for ($M + H^+$) $C_{14}H_{20}N_3$ 230.1657; found 230.1662.

Compound **A7Z1***, ethyl 2-(4-octyl-1H-1,2,3-triazol-1-yl)acetate*

^{1}H NMR: 7.40 (s, 1H), 5.11 (s, 2H), 4.26 (q, $J = 7.2$, 2H), 2.73 (t, $J = 7.5$, 2H), 1.65 (m, 4H), 1.20–1.40 (m, 10H), 1.30 (t, $J = 7.2$, 3H), 0.87 (t, $J = 0.69$, 3H); ^{13}C NMR: 166.5 (C), 148.9 (C), 121.9 (CH), 62.2 (CH_2), 50.7 (CH_2), 31.8 (CH_2), 29.3 (CH_2), 29.2 (CH_2), 25.6 (CH_2), 22.6 (CH_2), 14.0 (CH_3); ESI-HRMS Calcd for ($M + H^+$) $C_{14}H_{26}N_3O_2$ 268.2025; found 268.2022.

Compound **A7Z2** *benzyl-4-octyl-1H-1,2,3-triazole*

^{1}H NMR: 7.36 (m, 3H), 7.25 (m, 2H), 7.17 (s, 1H), 5.49 (s, 2H), 2.68 (t, $J = 7.5$, 2H), 1.63 (q, $J = 7.5$, 2H), 1.20–1.30 (m, 10H), 0.87 (t, $J = 6.3$, 3H); ^{13}C NMR: 148.9 (C), 135.1 (C), 129.01 (CH), 128.5 (CH), 127.9 (CH), 120.5 (CH), 53.9 (CH_2), 31.8 (CH_2), 29.3 (CH_2), 29.2 (CH_2), 25.7 (CH_2), 22.6 (CH_2), 14.1 (CH_3); ESI-HRMS Calcd for ($M + H^+$) $C_{17}H_{26}N_3$ 272.2127; found 272.2127.

Bacterial strains

Mycobacterium tuberculosis strain H37Rv as well as clinical isolates of *M. fortuitum*, *M. avium* subsp. *avium* and *M. abscessus* were kindly provided by Dr. L. Barrera (Instituto Nacional de Microbiología "C.G. Malbrán", Argentina). *M. smegmatis* strain mc^{2}155 was the kind gift from Dr. W. R. Jacobs (A. Einstein College of Medicine, NY, USA). All the mycobacterial strains were grown in Middlebrook 7H9 broth (DifcoLaboratories, Detroit, MI, USA) supplemented with 1/10 v/v of ADS (a solution containing 50 g/L BSA fraction V, 20 g/L dextrose and 8.1 g/L NaCl), glycerol (1% w/v) herein designated 7H9-ADS-G for short. Tween 80 was added to prevent clumping (0.05% w/v for cultures of *M. tuberculosis* and *M. avium*, 0.2% w/v for cultures of *M. abscessus*, *M. fortuitum* and *M. smegmatis*). When needed, solid media (Middlebrook 7H11 supplemented with ADS (1/10 v/v) and glycerol (1% v/v) was used. All strains were grown at 37 °C under gentle agitation.

Anti-mycobacterial activity assay

Stock solutions for all the tested compounds were made in DMSO at 10 mg/mL. Working solutions were made by dilution in the above described 7H9-ADS-G medium at a final concentration of 400 μg/mL.

Antimycobacterial activity was determined by a twofold dilution of the compounds in Middlebrook 7H9-ADS-G medium. For this purpose 96-well plates (Falcon, Cat number 3072, BectonDickinson, Lincoln Park, NJ) were used. The 96-well plates received 100 μL of Middlebrook 7H9 broth and a serial twofold dilution of the compounds was made directly on the plate. The initial and final drug concentrations tested were 100 and 0.8 μg/mL, respectively. Four compounds were tested in duplicate in each microtiter plate, Rifampicin (from 2 to 0.16 μg/mL; stock solution prepared as a 10 mg/mL solution in methanol) was used as control drug. For the sake of simplicity of the 96 wells plate design, control azole drugs were tested in parallel. The azole drugs used, Econozale (20 mg/mL in DMSO stock solution, 80 μg/mL working solution) and fluconazole (20 mg/mL in DMSO stock solution, 200 μg/mL working solution) were tested in the range of 20–0.16 μg/mL and 100–0.81 μg/mL, respectively. Two rows were used for growth control (medium and inoculum alone) and sterility control (medium alone). The inoculum was prepared as a 1/25 dilution of a fresh mid-log *M. tuberculosis* H37Rv suspension (O.D equivalent to Mc Farland 1.0 scale value) made in Middlebrook 7H9-ADS-G. A 100-μL aliquot (containing approximately 10^6 Colony Forming Units) was used to inoculate the wells except for the row used for sterility testing. Plates were sealed with Parafilm and incubated at 37 °C for 5 days. After this time, 22 μL of a freshly prepared solution of the tetrazolium dye 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT, Sigma, 2.5 mg/mL in 1:1 water: 20% (w/v) Tween 80) was added to the first growth control well plate in the growth control row and reincubated at 37 °C for 24 h. A change from yellow to purple colour indicated that the plate was ready to be fully developed in which case 22 μL of the MTT solution was added to the rest of the wells. If the colour change was not observed the second well in the growth control row was developed as describe above and incubated for a further 24 h. The operation was repeated if necessary until a colour change was clearly seen in the growth control well, at which time the remaining drug containing wells were developed. In our experience, colour change in the growth control row was seen in the first well.

Minimum inhibitory concentration (MIC) was defined as the lowest drug concentration preventing mycobacterial growth and colour change.

Cytotoxicity assay

The in vitro cytotoxicity was determined against mammalian kidney fibroblasts (VERO). The assay was performed in 96-well tissue culture-treated plates as described earlier [44]. Briefly, cells were seeded to the wells of the plate (25,000 cells/well) and incubated for 24 h. Samples were added and plates were again incubated for 48 h. The number of viable cells was determined by neutral red assay. IC_{50} values were determined from logarithmic graphs of growth inhibition versus concentration. Doxorubicin was used as a positive control, while DMSO was used as vehicle control.

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