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Synthesis and Evaluation of α-ketotriazoles and α,β-diketotriazoles as inhibitors of *Mycobacterium tuberculosis*

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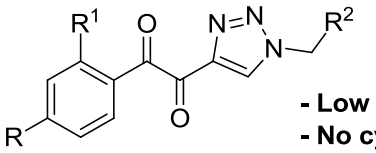

- Low μM MIC values on MT and MDR-MT
- No cytotoxicity on tested human cells

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***Highlights (for review)**

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- A series of α,β-diketotriazoles were synthesized.
- Two compounds displayed inhibitory activity of 7 μM against *M. tuberculosis* H37Rv and multi-drug resistant *M. tuberculosis* strains
- α,β-Diketotriazoles are not cytotoxic on two human cell lines

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Synthesis and Evaluation of α-ketotriazoles and α,β-diketotriazoles as inhibitors of *Mycobacterium tuberculosis*

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Abstract

Two series of α-ketotriazole and α,β-diketotriazole derivatives were synthesized and evaluated for antitubercular and cytotoxic activities. Among them, two α,β-diketotriazole compounds, **6b** and **9b,** exhibited good activities (minimum inhibitory concentration = 7.6 μM and 6.9 μM, respectively) on *Mycobacterium tuberculosis* and multi-drug resistant *M. tuberculosis* strains and presented no cytotoxicity (IC_{50} > 50 μM) on colorectal cancer HCT116 and normal fibroblast GM637H cell lines. These two compounds represent promising leads for further optimization.

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1. Introduction

Tuberculosis (TB) caused by *Mycobacterium tuberculosis* (*M.tb*) remains a major cause of mortality worldwide infecting 8 million and killing two million people annually [1].The mortality and the spread of this disease have been aggravated mainly by its co-infection with Human Immunodeficiency Virus (HIV) [2]. The worsening situation has prompted the World Health Organization (WHO) to declare tuberculosis a global public health threat [1]. Most of the drugs which are classified as a first-line TB treatment were discovered in the 1950's and 1960's. Streptomycin [3] was the first drug used to treat tuberculosis, followed by isoniazid (isonicotinylhydrazine, INH) in the early 1950's [4]. Pyrazinamide (PZA) appeared as a potential drug against TB in 1952 [5]. The two most recent first-line antitubercular drugs are ethambutol (EMB) [6,7] and rifampicin (RIF) [8,9] which were discovered in the 1960's. Today, an improvement of the treatment has been observed by combining these drugs. Strains of *M.tb* resistant to both INH and RIF have been called multidrug resistant (MDR) [10,11]. To treat MDR-TB, the WHO have recommended using second-line drugs such as fluoroquinolones [12], ethionamide [13,14] and cycloserin [15,16,17]. These agents besides being unsuitable for short-course treatment could also be less effective and more toxic. Recently, the emergence of extensively drug-resistant (XDR) strains has been observed [18]. *M.tb* XDR strains are MDR isolates resistant to a fluoriquinolone or a second-line injectable drug [18].

More than ever, there is an urgent need to develop new, potent and fast acting anti-tuberculosis drugs to combat the spread of TB. Different classes of compounds have been reported with antitubercular activities such as cinnamic [19,20,21], phenolic [22], but also five-membered ring heterocycle derivatives. Indeed, over the past ten years, a variety of imidazole [23,24,25], furan [26], 1*H*-1,2,4-triazole [27,28] but also 1*H*-1,2,3-triazole [29-35] derivatives have been reported to have antitubercular activities (Figure 1).

In an ongoing effort to develop novel scaffolds against tuberculosis, we reported a series of α-ketotriazoles and triazoles with good antitubercular activities [36,37]. During our first synthesis of α-ketotriazoles as potential inhibitors of InhA (an essential enzyme involved in the synthesis of the mycobacterial mycolic acids), a side product identified as α,β-diketotriazole was observed. From this observation, a general method for obtaining α,β-diketotriazoles by oxidation of α-ketotriazoles in the presence of catalytic amounts of CuI and 2,9-dimethyl-1,10-phenanthroline as ligand, using air as oxidant, was established [38]. Herein, we report the synthesis of two classes of compounds, namely α-ketotriazoles and α,β-diketotriazoles, their evaluation as inhibitors of *M.tb* strain H37Rv and their cytotoxicity on two human cell lines.

2. Results and discussion

2.1. Chemistry

Two different methods were employed in the synthesis of α,β-diketotriazoles. The first (**A**, Table 1) consisted of a two-step synthesis as previously described and provides access to the first and second classes of compounds, namely α-ketotriazoles and α,β-diketotriazoles, respectively [36,38]. α-Ketotriazoles were readily available in one-step procedure with TMS deprotection followed by Huisgen 1,3-dipolar cycloaddition (Scheme 1). α,β-Diketotriazoles can then be synthesized from α-ketotriazole in the presence of CuI or $CuCl_2$ and 2,9-dimethyl-1,10-phenanthroline in refluxing acetonitrile.

In an alternative method of preparation (**B**, Table 2), α,β-diketones were synthesized in a one-pot three-step reaction: TMS-deprotection of the trimethylsilyl ynones / Huisgen 1,3-dipolar

cycloaddition between the azides and the deprotected ynones / oxidation of the benzylic moiety (Scheme 1). The yields ranged from 28 to 43% as summarized in Table 1. The two-step procedure afforded higher yields, except for entry 8. The main difference between the two protocols is the absence of water in the oxidation step, when starting directly from α-ketotriazoles (method A). The structure of **9b** was confirmed by X-ray crystallography (Figure 2) [39].

2.2. Biology

2.2.1. Antimycobacterial activity

All the α-ketotriazoles synthesized and their corresponding α,β-diketotriazoles (herein and ref. 38) were evaluated by determining the minimum inhibitory concentration (MIC) on *M. tuberculosis* H37Rv strain (Table 2). Triclosan and INH were used for comparison.

Most of the compounds of the first class, corresponding to α-ketotriazoles, exhibited weak *in vitro* activities against *M. tuberculosis* with MIC values ranging from 20.9 to 360 μM. Interestingly, compounds **5a** and **14a** possessing an octyl chain attached to the triazole core presented the best activities with MIC values of 83.5 and 20.9 μM, respectively. The latter one displayed an activity equivalent to triclosan.

As shown in Table 2, the second class of compounds, corresponding to α,β-diketotriazole derivatives, generally displayed higher activities than α-ketotriazoles, with MIC ranging from 6.9 to 171 μM. This was the case for all compounds possessing a remote aromatic group at the N-1 position of the triazole frame. The only exceptions concern compounds possessing an octyl chain at the N-1 position (**5a**/**5b** and **14a**/**14b**), for which MIC activities were either comparable (**5a** ≈ **5b**) or reversed (**14a** > **14b**). For all α,β-diketotriazole compounds, the

most potent were found to be **6b** and **9b** with MICs of 2.5 μg/mL (7.6 and 6.9 μM, respectively). These compounds present different substituents (*p*-methoxy or 2,4-dichloro) on the aromatic moiety. Among all 2,4-dichloro derivatives **9b-12b** evaluated, those possessing a remote aromatic ring at the *N*-1 position of the triazole ring exhibited comparable *in vitro* activities against *M. tuberculosis* (MIC values: **9b,** 6.9 μM; **10b**, 9.5 μM; **12b**, 13.4 μM), except for the *p*-NO_2 derivative **11b** (MIC = 79.0 μM) .

Furthermore, the cytotoxicity of different α-ketotriazoles and α,β-diketotriazoles was evaluated on two human cell lines, the human colon cancer cell line HCT116 and the human fibroblast cell line GM637 (Table 2). The data showed that the IC_{50} of the different compounds tested were above 50 μM, indicating that these compounds are not cytotoxic against human cell lines.

2.2.2. Bacterial growth inhibition experiments

On the basis of antituberculous activities, compounds **6b** and **9b** both exhibiting MICs of 2.5 μg/mL were selected and evaluated for testing against multi-drug-resistant *M. tuberculosis* strains. The results are reported in Table 3 and show that **6b** and **9b** were active in all of the resistant strains with the same level of efficiency.

3. Conclusion

Two series of α-ketotriazole and α,β-diketotriazole derivatives were synthesized. The three-step one-pot synthesis of α,β-diketotriazoles was successfully accomplished with yields ranging from 28 to 43%. The latter approach will be further improved in order to prepare, through automated parallel synthesis, a variety of α,β-diketotriazoles. Among the compounds synthesized and evaluated, two compounds **6b** and **9b** exhibit interesting antituberculosis

activities with MIC values of 2.5 μg/mL and no apparent cytotoxicities toward HCT116 or GM637 human cells. Furthermore, compounds **6b** and **9b** present the same *in vitro* efficiency against MDR-TB strains. In conclusion, it was shown that the potency and low cytotoxicity of these compounds make them good leads for synthesizing new derivatives with enhanced activities. Further studies will focus on the identification of the protein targets of these molecules.

4. Experimental Section

4.1. Material

All chemicals were obtained from Aldrich or Acros Organics and used without further purification. Nuclear magnetic resonance spectra were recorded on a Bruker AC 300 spectrometer (^{1}H and ^{13}C NMR). ^{1}H NMR spectra were recorded at 300 MHz by using $CDCl_3$ (7.26 ppm) as an internal standard. ^{13}C NMR spectra were recorded at 75.0 MHz and are referenced against the central line of the $CDCl_3$ triplet at δ = 77.0 ppm. Mass spectrometry (MS) data were obtained on a ThermoQuest TSQ 7000 spectrometer, high-resolution mass spectra (HRMS) were recorded on a ThermoFinnigan MAT 95 XL spectrometer using electrospray ionization (ESI) methods. Melting points were measured on a Mettler Toledo MP50 melting point system. IR spectra were recorded on a Perkin Elmer 1725. Crystallographic data for compound **9b** [39] were collected on a Bruker-AXS Quazar APEX II diffractometer using a 30 W air-cooled microfocus source (ImS) with focusing multilayer optics at a temperature of 193(2)K, with MoKα radiation (wavelength = 0.71073 Å) using phi- and omega-scans. The data were integrated with SAINT [40], and an empirical absorption correction with SADABS [41] was applied. The structures were solved by direct methods, using SHELXS-97 and refined using the least–squares method on F^2. [42] All non-

H atoms were treated anisotropically. The H atoms were fixed geometrically and treated as a riding model.

4.2. Chemistry

4.2.1. Synthesis of α-ketotriazoles and α,β-diketotriazoles in two steps.

Compounds **1a-6a, 1b-6b, 9a-11a, 9b-11b, 13a**, **13b**, **15a-19a, 15b-19b** were synthesized according to the general procedure and exhibited identical spectral data as indicated in a previous report [38].

4.2.1.1. 2-(4-Methoxyphenyl)-1-(1-phenethyl-1H-1,2,3-triazol-4-yl)ethanone (7a). White powder. M.p. 119.2°C. Yield 60%. IR (ν/cm^{-1}): 3141; 1688; 1616; 1516; 1456; ^{1}H NMR ($CDCl_3$) δ 7.82 (s, 1H); 7.27 (m, 5H); 7.07 (m, 2H); 6.85 (d, *J* = 8.7 Hz, 2H); 4.60 (t, *J* = 7.2 Hz, 2H); 4.34 (s, 2H); 3.77 (s, 3H); 3.21 (t, *J* = 7.3 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 192.4; 158.5; 147.0; 136.3; 130.8; 128.8; 128.5; 127.2; 126.2; 125.9; 113.9; 55.1; 51.7; 45.0; 36.3; HRMS: (DCI/CH_4, m/z) calc. for $C_{19}H_{20}N_3O_2$: 322.1556. Found: 322.1550.

4.2.1.2. 1-(4-Methoxyphenyl)-2-(1-phenethyl-1H-1,2,3-triazol-4-yl)ethane-1,2-dione (7b). Yellow gummy solid. Yield 49%. IR (ν/cm^{-1}): 3116; 1672; 1653; 1600; 1517; ^{1}H NMR ($CDCl_3$) δ 8.00 (s, 1H); 7.99 (d, *J* = 8.9 Hz, 2H); 7.27 (m, 3H); 7.10 (m, 2H); 6.96 (d, *J* = 8.9 Hz, 2H); 4.67 (t, *J* = 7.2 Hz, 2H); 3.88 (s, 3H); 3.24 (t, *J* = 7.2 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 190.4; 185.7; 165.0; 143.9; 136.2; 132.7; 128.9; 128.7; 128.6; 127.4; 125.3; 114.3; 55.6; 52.0; 36.4; HRMS: (DCI/CH_4, m/z) calc. for $C_{19}H_{18}N_3O_3$: 336.1348. Found: 336.1355.

4.2.1.3. 1-(1-(4-Methoxyphenethyl)-1H-1,2,3-triazol-4-yl)-2-(4-methoxyphenyl)ethanone (8a). White powder. M.p. 124.7°C. Yield 54%. IR (ν/cm^{-1}): 3121; 1693; 1613; 1515; 1460; ^{1}H NMR ($CDCl_3$) δ 7.80 (s, 1H); 7.26 (d, J = 8.7 Hz, 2H); 6.96 (d, *J* = 8.7 Hz, 2H); 6.84 (d, *J* = 8.7 Hz, 2H); 6.78 (d, J = 8.7 Hz, 2H); 4.56 (t, *J* = 7.1 Hz, 2H); 4.33 (s, 2H); 3.763 (s, 3H); 3.757 (s, 3H); 3.14 (t, *J* = 7.1 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 192.4; 158.7; 158.5; 147.0; 130.8;

129.5; 128.2; 126.3; 125.9; 114.2; 113.9; 55.1; 52.0; 45.0; 36.0; HRMS: (DCI/CH_4, m/z) calc. for $C_{20}H_{22}N_3O_3$: 352.1661. Found: 352.1656.

4.2.1.4. 1-(1-(4-Methoxyphenethyl)-1H-1,2,3-triazol-4-yl)-2-(4-methoxyphenyl)ethane-1,2-dione (8b). Yellow gummy solid. Yield 37%. IR (ν/cm^{-1}): 3132; 1683; 1597; 1573; 1514;1463; ^{1}H NMR ($CDCl_3$) δ 7.99 (m, 3H); 6.98 (t, *J* = 8.9 Hz, 2H); 6.96 (d, *J* = 9.1 Hz, 2H); 6.80 (d, *J* = 8.7 Hz, 2H); 4.62 (t, *J* = 7.1 Hz, 2H); 3.88 (s, 3H); 3.76 (s, 3H); 3.17 (t, *J* = 7.1 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 190.4; 185.8;165.0; 158.8; 143.9; 132.7; 129.6; 128.8; 128.1; 125.3; 114.31; 114.25; 55.6; 55.2; 52.2; 35.5; HRMS: (DCI/CH_4, m/z) calc. for $C_{20}H_{20}N_3O_4$: 366.1454. Found: 366.1458.

4.2.1.5. 2-(2,4-Dichlorophenyl)-1-(1-phenethyl-1H-1,2,3-triazol-4-yl)ethanone (12a). White powder. M.p. 137.2°C. Yield 68%. IR (ν/cm^{-1}): 3135; 1685; 1532; 1474; ^{1}H NMR ($CDCl_3$) δ 7.91 (s, 1H); 7.43 (s, 1H); 7.30 (m, 3H); 7.25 (br s, 2H); 7.12 (m, 2H); 4.68 (t, *J* = 7.2 Hz, 2H); 4.60 (s, 2H); 3.27 (t, *J* = 7.2 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 190.2; 146.9; 136.2; 135.3; 133.5; 132.7; 131.2; 129.2; 128.9; 128.5; 127.3; 127.0; 126.1; 51.9; 43.4; 36.3; HRMS: (DCI/CH_4, m/z) calc. for $C_{18}H_{16}N_3OCl_2$: 360.0670. Found: 360.0681.

4.2.1.6. 1-(2,4-Dichlorophenyl)-2-(1-phenethyl-1H-1,2,3-triazol-4-yl)ethane-1,2-dione (12b). White powder. M.p. 116.5°C. Yield 51%. IR (ν/cm^{-1}): 3133; 1670; 1580; 1533; ^{1}H NMR ($CDCl_3$) δ 8.00 (s, 1H); 7.82 (d, *J* = 8.3 Hz, 1H); 7.45 (d, *J* = 1.8 Hz, 1H); 7.42 (dd, *J* = 8.3 Hz, 1.9 Hz, 1H); 7.27 (m, 3H); 7.08 (m, 2H); 4.71 (t, *J* = 7.1 Hz, 2H); 3.27 (t, *J* = 7.1 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 191.0; 183.3; 143.0; 140.5; 136.1; 134.8; 132.9; 131.6; 130.3; 128.9; 128.8; 128.6; 127.8; 127.3; 52.1; 36.4; HRMS: (DCI/CH_4, m/z) calc. for $C_{18}H_{14}N_3O_2Cl_2$: 374.0463. Found: 374.0468.

4.2.1.7. 2-(2,4-Dichlorophenyl)-1-(1-nonyl-1H-1,2,3-triazol-4-yl)ethanone (14a). Yellow gummy solid. Yield 63%. IR (ν/cm^{-1}): 2924; 2854; 1684; 1582; 1553; 1437 ; ^{1}H NMR ($CDCl_3$) δ 8.12 (s, 1H); 7.38 (d, *J* = 1.6 Hz, 1H); 7.20 (m, 2H); 4.56 (s, 2H); 4.38 (t, *J* = 7.2 Hz, 2H); 1.90 (m, 2H); 1.23 (m, 12H); 0.85 (t, *J* = 6.7 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 190.2;

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147.1; 135.4; 133.5; 132.7; 131.2; 129.1; 127.0; 125.7; 50.6; 43.3; 31.6; 30.0; 29.2; 29.0; 28.8; 26.3; 22.5; 14.0; HRMS: (DCI/CH_4, m/z) calc. for $C_{19}H_{26}N_3OCl_2$: 382.1453. Found: 382.1447.

4.2.1.8. 1-(2,4-Dichlorophenyl)-2-(1-nonyl-1H-1,2,3-triazol-4-yl)ethane-1,2-dione (14b). White powder. M.p. 60.4°C. Yield 51%. IR (ν/cm^{-1}): 2921; 2852; 1689; 1588; 1471; ^{1}H NMR ($CDCl_3$) δ 8.31 (s, 1H); 7.84 (d, *J* = 8.3 Hz, 1H); 7.46 (d, *J* = 1.9 Hz, 1H); 7.42 (dd, *J* = 8.3 Hz, 2.0 Hz, 1H); 4.46 (t, *J* = 7.3 Hz, 2H); 1.97 (m, 2H); 1.26 (m, 12H); 0.88 (t, *J* = 6.7 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 191.1; 183.5; 143.5; 140.5; 135.0; 133.0; 131.6; 130.5; 128.3; 127.9; 50.8; 31.7; 30.1; 29.2; 29.1; 28.9; 26.3; 22.6; 14.0; HRMS: (DCI/CH_4, m/z) calc. for $C_{19}H_{24}N_3O_2Cl_2$: 396.1259. Found: 396.1247.

4.2.2. Representative procedure for the one-pot synthesis of α,β-diketotriazoles from TMS-ynones and azides.

A typical experimental procedure for the preparation of these compounds from the corresponding trimethylsilylethynyl ketones is described below. $CuCl_2$ (0.1 mol equiv) and sodium ascorbate (0.2 mol equiv) were added at room temperature to a solution of 1-trimethylsilyl-1-alkynyl ketone (1 mol equiv) with azide (1.2 mol equiv) in CH_3CN/H_2O (4/1). Then the reaction mixture was warmed to reflux for 60 min and 2,9-dimethyl-1,10-phenanthroline was added and the reaction was stirred for 20 h. After cooling the reaction mixture to room temperature, H_2O was added and extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The desired product was obtained by purification by flash chromatography (petroleum ether/EtOAc).

4.3. Biology

4.3.1. Growth conditions and minimum inhibitory concentration (MIC) determination. M. tuberculosis H37Rv was used as the reference strain. The strains were grown at 37 °C in

Middlebrook 7H9 broth (Difco), supplemented with 0.05% Tween 80, or on solid Middlebrook 7H11 medium (Difco) supplemented with oleic acid-albumin-dextrose-catalase (OADC). MICs for the new compounds were determined by means of the micro-broth dilution method. Dilutions of *M. tuberculosis* wild-type and clinical isolate cultures (about 10^5–10^6 cfu/ml) were streaked onto 7H11 solid medium containing a range of drug concentrations (0.25 μg/mL to 40 μg/mL). Plates were incubated at 37 °C for about 21 days and the growth was visually evaluated. The lowest drug dilution at which visible growth failed to occur was taken as the MIC value. Results were expressed as the average of at least three independent determinations.

4.3.2. M. tuberculosis clinical isolates and drug susceptibility testing. Three *M. tuberculosis* MDR isolates were collected at the Sondalo Division of the Valtellina and Valchiavenna, Italy, hospital authority in the 2012. Their resistance profile is shown in Table 3. All clinical isolates were grown in BACTEC™ MGIT™ 960 and Lowenstein–Jensen slants. Drug susceptibility testing for all first-line antitubercular drugs was performed with the BACTEC™ MGIT™ 960 System (Becton-Dickinson Diagnostic Systems, Sparks, Maryland) for isoniazid (0.1 μg/ml; 0.4 μg/ml), rifampicin (1 μg/ml), streptomycin (1 μg/ml; 4 μg/ml), ethambutol (5 μg/ml) and pyrazinamide (100 μg/ml), in accordance with the manufacturer's instructions.. MIC determination to second-line drugs (cycloserine, 50 μg/ml; amikacin, 1-5 μg/ml; ciprofloxacin, 2 μg/ml; ethionamide, 5 or 10 μg/ml; para-aminosalicylic acid, 4 or 8 μg/ml; ofloxacin, 10 μg/ml) was also performed by the MGIT™ 960 System.

4.3.3.Cytotoxicity

Human colon cancer cell line HCT116 (ATCC) and human fibroblasts (GM637 cell line) were cultured in DMEM supplemented with 10% fetal calf serum. For cytotoxicity evaluation, 3000 cells were seeded per well in 96-wells plates and, 24 h later, were treated

with concentrations ranging from 100 nM to 50 µM (8 replicates for each). After 4 days of treatment, the cytotoxicity of each compound was measured by using the WST-1 kit (Roche).

Acknowledgments

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[39] Crystal data for **9b**: $C_{17}H_{11}Cl_2N_3O_2$, M=360.19, Orthorhombic, space group P*ca2*$_1$, a=13.2479(11)Å, b=7.8752(7)Å, c=30.784(3)Å, V=3211.7(5) Å^3, Z=8, crystal size 0.20 x 0.12 x 0.12 mm^3, 45337 reflections collected (5423 independent, Rint=0.0669), 434 parameters, R1 [I>2σ(I)]= 0.0466, wR2 [all data]= 0.1217, largest diff. peak and hole: 0.401 and -0.328 e.Å-3. CCDCC 923093 contains the supplementary crystallographic data. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

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Table(s)

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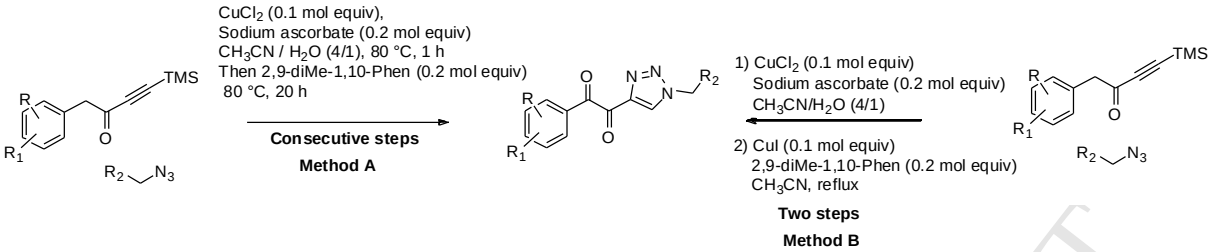
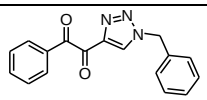
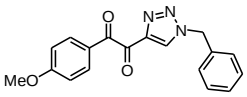
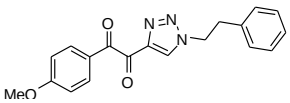
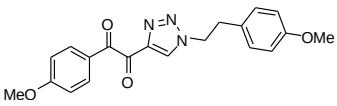
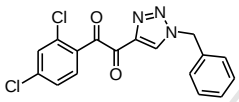
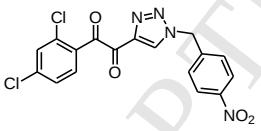
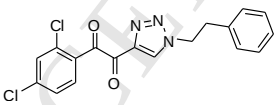
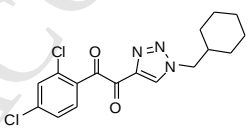
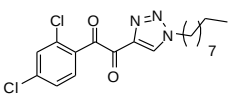
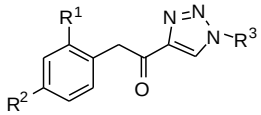
Table 1. One-pot reaction to obtain α,β-diketotriazoles from TMS-ynones and azides.



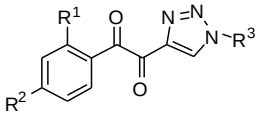
Entry	Product		Yield[a] (%) Method A	Overall yield[a] (%) Method B (step 1/step 2)
1		**1b**	35	49
2		**6b**	28	33
3		**7b**	ND[b]	30 (60 / 49)
4		**8b**	ND[b]	20 (54 / 37)
5		**9b**	30	52
6		**11b**	43	61
7		**12b**	ND[b]	35 (68 / 51)
8		**13b**	41	33
9		**14b**	ND[b]	32 (63 / 51)

a. Yields for isolated compounds.
b. Not determined.

Table 2. Compounds tested as inhibitors of *M. tuberculosis* growth.



Compounds Xa



Compounds Xb

Compound	R^1	R^2	R^3	MIC (μg/ml)/ (μM)	Cytotoxicity (μM)
Triclosan	---	---	---	10/34.5	ND
INH	---	---	---	0.05/0.4	ND
1a	H	H	Bn	100/360.6	>50
1b	H	H	Bn	50/171.6	>50
2a	H	H	4-NO_2-Bn	100/310.3	>50
2b	H	H	4-NO_2-Bn	4/11.89	>50
3a	H	H	3,5-diMeO-Bn	>100/>296.4	ND
3b	H	H	3,5-diMeO-Bn	4/11.38	>50
4a	H	H	3,5-diMe-Bn	>100/>327.4	>50
4b	H	H	3,5-diMe-Bn	16/50	>50
5a	H	H	octyl	25/83.5	>50
5b	H	H	octyl	16/51	ND
6a	H	OMe	Bn	ND	ND
6b	H	OMe	Bn	**2.5/7.6**	>50
7a	H	OMe	$Ph(CH_2)_2$	>32/>112.0	ND
7b	H	OMe	$Ph(CH_2)_2$	>32/>95.4	>50
8a	H	OMe	4-MeO$Ph(CH_2)_2$	>32/>91.1	ND
8b	H	OMe	4-MeO$Ph(CH_2)_2$	>32/>87.6	ND
9a	Cl	Cl	Bn	100/288.9	>50
9b	Cl	Cl	Bn	**2.5/6.9**	>50
10a	Cl	Cl	3,5-di-MeO-Bn	100/246.1	ND
10b	Cl	Cl	3,5-di-MeO-Bn	4/9.51	>50

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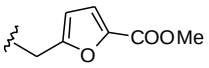
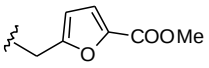
11a	Cl	Cl	4-NO_2-Bn	100/255.6	ND
11b	Cl	Cl	4-NO_2-Bn	32/79.0	>50
12a	Cl	Cl	$Ph(CH_2)_2$	>32/>88.8	ND
12b	Cl	Cl	$Ph(CH_2)_2$	5/13.4	>50
13a	Cl	Cl	cyclohexyl	ND	ND
13b	Cl	Cl	cyclohexyl	16/43.7	ND
14a	Cl	Cl	Octyl	8/20.9	ND
14b	Cl	Cl	Octyl	32/80.7	>50
15a	Cl	Cl		ND	ND
15b	Cl	Cl		16/39.16	ND
16a	H	4-NO_2	Bn	ND	ND
16b	H	4-NO_2	Bn	32/95.1	>50
17a	H	4-NO_2	3,5-di-MeO-Bn	ND	ND
17b	H	4-NO_2	3,5-di-MeO-Bn	64/161.4	>50
18a	MeO	H	Bn	ND	ND
18b	MeO	H	Bn	4/12.44	>50
19a	MeO	H	3,5-di-MeO-Bn	ND	ND
19b	MeO	H	3,5-di-MeO-Bn	8/21.0	>50

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Table 3. Activities against multi-drug-resistant *M. tuberculosis* strains.

Compound	MIC (μg/ml)			
	M. tuberculosis	***M. tuberculosis* clinical isolates**		
	$H_{37}R_v$	**IC1[a]**	**IC2[a]**	**IC3[a]**
	μg/mL / μM	**μg/mL / μM**	**μg/mL / μM**	**μg/mL / μM**
6b	2.5 / 6.9	2.5 / 6.9	2.5 / 6.9	2.5 / 6.9
9b	2.5 / 7.6	2.5 / 7.6	2.5 / 7.6	2.5 / 7.6

[a]*Mtb* clinical isolate: IC1 drug resistance profile: resistant to streptomycin, isoniazid, rifampicin, ethambutol; IC2 drug resistance profile: resistant to streptomycin, isoniazid, rifampicin, ethambutol, pyrazinamide, ethionammide, capreomicin; IC3 drug resistance profile: resistant to streptomycin, isoniazid, rifampicin, ethambutol, pyrazinamide, ethionammide.

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Figure(s)

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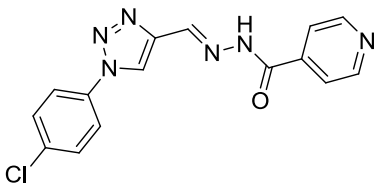
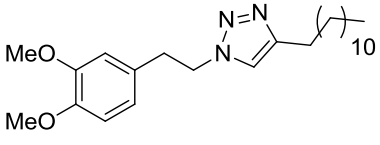
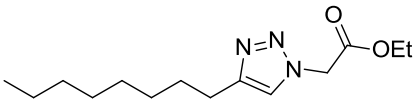


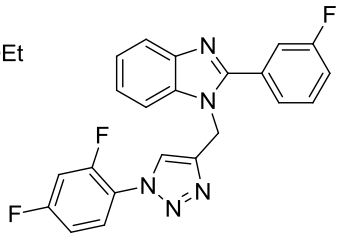
MIC H37Rv (μg/mL) 0.62 [30]



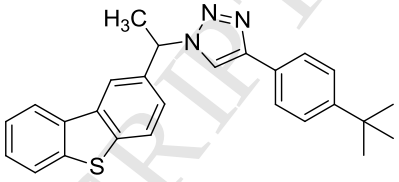
MIC H37Rv (μg/mL) 0.25 [37]



MIC H37Rv (μg/mL) 1.90 [31]



MIC H37Rv (μg/mL) 0.32 [32]



MIC H37Rv (μg/mL) 0.78 [33]

Figure 1. Different triazole-based anti-TB drugs.

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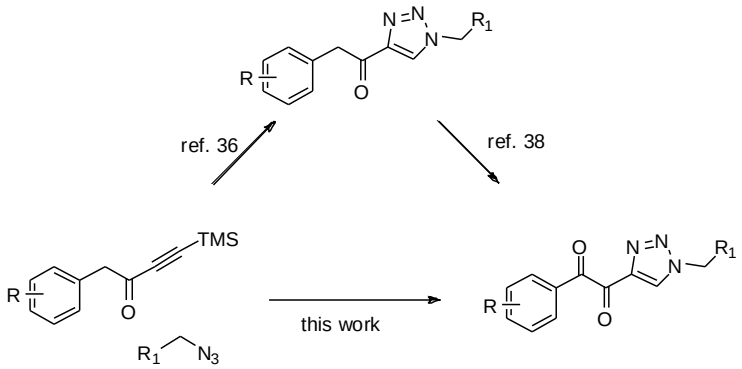

Scheme 1. Two methods to synthesize α,β-diketotriazole derivatives.

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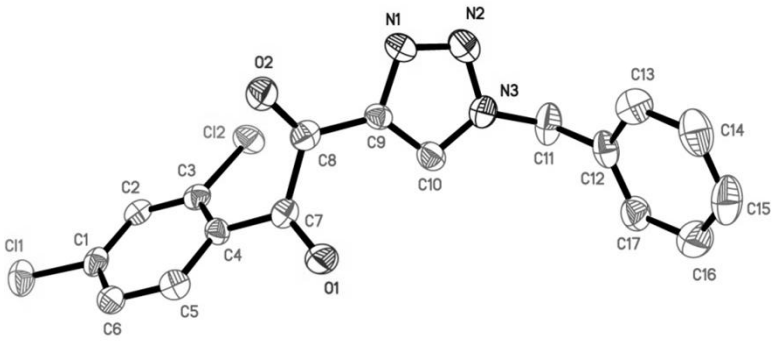

Figure 2. Molecular view of **9b**. Only one of the two independent molecules in the asymmetric unit is shown. Hydrogen atoms are omitted for clarity (crystallographic data for **9b** are provided in the Supporting Information)

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