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ORIGINAL RESEARCH

Design, synthesis, and characterization of (1-(4-aryl)-1*H*-1,2,3-triazol-4-yl)methyl, substituted phenyl-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylates against *Mycobacterium tuberculosis*

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Abstract: The novel (1-(4-aryl)-1*H*-1,2,3-triazol-4-yl)methyl, substituted phenyl-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate derivatives were synthesized by the click reaction of the dihydropyrimidinones, bearing a terminal alkynyl group, with various substituted aryl azides at room temperature using a catalytic amount of $Cu(OAc)_2$ and sodium ascorbate in a 1:2 ratio of acetone and water as a solvent. The newly synthesized compounds were characterized by a number of spectroscopic techniques, such as infrared, liquid chromatography-mass spectrometry, ^1H, and ^{13}C nuclear magnetic resonance along with single crystal X-ray diffraction. The current procedure for the synthesis of 1,2,3-triazole hybrids with dihydropyrimidinones is appropriate for the synthesis of a library of analogs **7a-l** and the method accessible here is operationally simple and has excellent yields. The title compounds **7a-l** were evaluated for their in vitro antitubercular activity against $H37R_V$ and multidrug-resistant strains of *Mycobacterium tuberculosis* by resazurin microplate assay plate method and it was found that compound **7d** was promising against $H37R_V$ and multidrug-resistant strains of *M. tuberculosis* at 10 and 15 µg/mL, respectively.

Keywords: 1,2,3-triazole, dihydropyrimidinone, click chemistry, antitubercular drug discovery, synthesis

Introduction

The pyrimidine system is an important pharmacophore with abundant occurrence in nature. Natural and synthetic dihydropyrimidine derivatives have a wide range of pharmacological actions, such as anticancer,[1] antiviral,[2,3] antihypertensive,[4] calcium channel blocking,[5] antitubercular,[6] antimicrobial,[7,8] anti-inflammatory,[9,10] and larvicidal and insecticide actions.[11,12] 1,2,3-Triazoles, as a vital class of *N*-heterocyclic compounds, due to their unique chemical and structural properties, have received a great deal of attention over the past few decades and found broad application in medicinal chemistry[13] and particularly as anticancer,[14] antimicrobial,[15] antitubercular,[16] anti-HIV,[17] and antifungal agents.[18] On the other hand, this special class of scaffolds has also found relevance in objective oriented synthesis,[19] bioconjugation,[20] materials and surface science,[21] combinatorial chemistry,[22] and medicinal chemistry.[23] Moreover, 1,2,3-triazole can mimic natural peptides and heterocycles in geometrical shape and interaction function.[24] 1,2,3-Triazoles could be easily constructed by click chemistry reaction[25] and which yielded small molecules with special properties, such as moderate dipole character, hydrogen bonding capability, rigidity, and stability.[26] Heterocyclic[27–29] fluorine-containing compounds have been shown to exhibit promising

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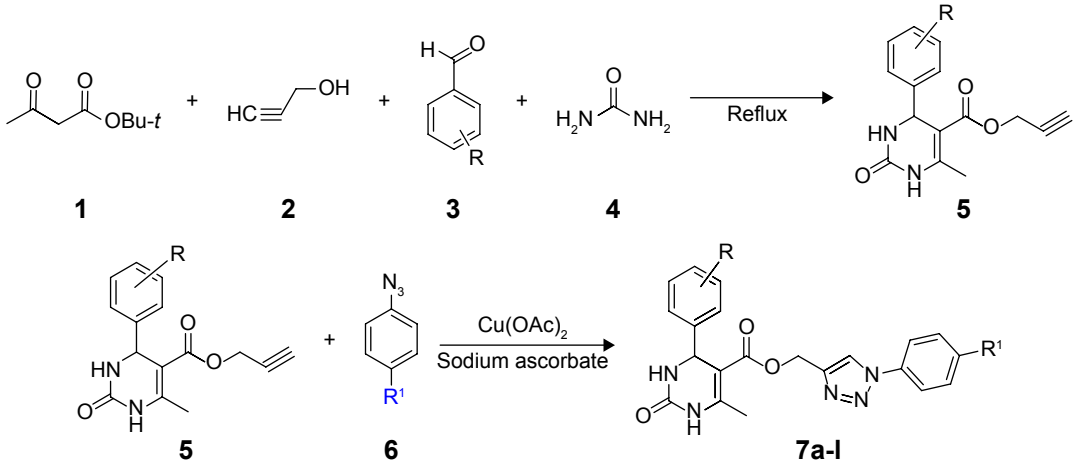


Figure 1 Synthesis of 1,2,3-triazole hybrid with dihydropyrimidinone scaffolds **7a-l**.

anti-tuberculosis (anti-TB) activity,[30] including 1,2,3-triazole analogs, for their promising anti-TB activity.[31] Keeping this in mind and considering the pharmacological significance of dihydropyrimidine and 1,2,3-triazole pharmacophores, in the present investigation it was decided to design and synthesize a series of novel 1,2,3-triazole hybrid with dihydropyrimidinone (DHPM) scaffolds in accordance with Lipinski rule except compound **7d**.[32] The title compounds, (1-(4-aryl)-1-*H*-1,2,3-triazol-4-yl)methyl, substitutedphenyl-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylates **7a-l**, have been tested for safety studies by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay[33] following in vitro antitubercular activity against $H37R_V$ and multidrug-resistant strains of *Mycobacterium tuberculosis* (MDR-MTB) by resazurin microplate assay plate method.

In this communication and in continuation of our work on the development of pharmacologically active heterocyclic compounds[6,34,35] and screening of heterocyclic compounds for properties of polymorphism,[36–38] we have synthesized 1,2,3-triazole hybrid with DHPMs using aryl azide as well as DHPMs having a terminal alkynyl group, which was synthesized by the four component Biginelli-like cyclocondensation reaction (*tert*-butyl β-ketoester, propargyl alcohol, aryl aldehyde, and urea) along with catalytic amount of $Cu(OAc)_2$ and sodium ascorbate in a 1:2 ratio of acetone and water as a solvent at room temperature as shown in Figure 1.

Materials and methods

Chemistry

All the chemicals were purchased from Sigma-Aldrich Corporation (St Louis, MO, USA; analytical grade) and used without further purification. Fourier transform infrared (FTIR) spectra were registered on a Bruker Corporation (Billerica, MA, USA) IFS 55 equinox Fourier transform IR spectrophotometer as KBr discs. ^{1}H- and ^{13}C-nuclear magnetic resonance (NMR) spectra were recorded using a Bruker 400 or 500 MHz spectrometer in the solvents indicated (referenced to the residual ^{1}H signals in the deuterated solvents) using tetramethylsilane (TMS) as an internal standard. Chemical shifts are reported in ppm (δ scale) and coupling constant (*J*) values given in hertz (Hz). The splitting pattern is abbreviated as follows: s, singlet; d, doublet; and m, multiplet. Thin layer chromatography (TLC) analysis of reaction mixtures was performed on Merck (Merck Serono, Darmstadt, Germany) aluminum plates coated with silica gel (60 F254). Compounds were visualized by ultraviolet irradiation at 254 and 366 nm. Merck silica gel (60–120 mesh) was used for column chromatography.

Spectra of the compounds are available as Supplementary Materials.

General procedure for the synthesis of (1-(4-aryl)-1*H*-1,2,3-triazol-4-yl) methyl, substituted phenyl-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylates (**7a-l**)

A 25 mL round bottom flask equipped with a condenser was charged with substituted aryl azides (1.0 mmol) (**6**) as well as DHPMs having terminal alkynyl group (1.0 mmol) (**5**). Compound **5** was synthesized by the four components Biginelli-like cyclocondensation reaction of *tert*-butyl β-ketoester (1.0 mmol) (**1**), propargyl alcohol (1.2 mmol) (**2**), substituted aryl aldehyde (1.0 mmol) (**3**), and urea (1.2 mmol) (**4**) by a reflux method.[39] The entire reaction mixture was allowed to stir for 3 hours at room temperature along with a catalytic amount of $Cu(OAc)_2$ (0.1 mmol) and sodium ascorbate (0.2 mmol) in a 1:2 ratio of acetone and water (2 mL) as a solvent till the reaction was complete. The progress of the reaction was monitored on TLC (4:6 of hexane and ethyl

acetate). After completion of the reaction as indicated on TLC, the contents were concentrated under reduced pressure to remove excess of the acetone and the crude reaction mixture was extracted with ethyl acetate and water. The combined organic extract, after drying over anhydrous sodium sulfate, was again concentrated under reduced pressure to obtain the crude product. For analytically pure products, the final solid mass was purified by column chromatography using the hexane/ethyl acetate (4:6) as the eluent to give the pure products **7a-l** at 77%–92% yield. Physicochemical characteristics of the title compounds are tabulated in Table 1.

(1-(4-(Trifluoromethyl)phenyl)-1*H*-1,2,3-triazol-4-yl)methyl 4-(4-fluorophenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**7a**)

IR (KBr) ν/cm^{-1} 3,333, 2,946, 2,370, 1,670, 1,332, 844, 785, 753. ^{1}H NMR (500 MHz, dimethyl sulfoxide [DMSO]) δ 9.29 (s, 1H), 8.72 (s, 1H), 8.12 (d, *J*=8.5 Hz, 2H), 7.99 (d, *J*=8.6 Hz, 2H), 7.77 (s, 1H), 7.24–7.21 (m, 2H), 7.06 (t, *J*=8.8 Hz, 2H), 5.21–5.17 (m, 3H), 2.27 (s, 3H). ^{13}C NMR (126 MHz, DMSO) δ 165.3, 162.7, 160.7, 152.3, 150.04, 144.2, 141.3, 141.2, 139.6, 128.7, 128.6, 127.6, 123.2, 121.0, 115.6, 115.4, 99.0, 56.6, 53.7, 18.3. Liquid chromatography-mass spectrometry (LCMS): 475.2.

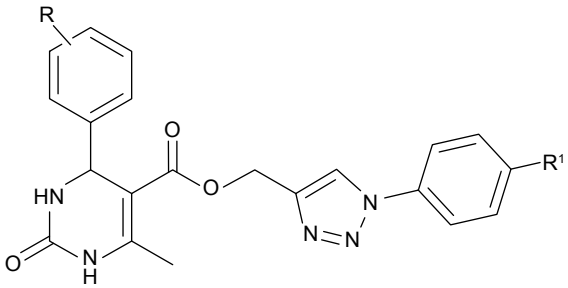
(1-(4-(Trifluoromethyl)phenyl)-1*H*-1,2,3-triazol-4-yl)methyl 4-(3-fluorophenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**7b**)

IR (KBr) ν/cm^{-1} 3,367, 2,979, 2,345, 1,700, 1,636, 1,329, 873, 845, 757. ^{1}H NMR (400 MHz, DMSO) δ 9.31 (s, 1H), 8.80 (s, 1H), 8.10 (d, *J*=7.9 Hz, 2H), 7.96 (d, *J*=8.0 Hz, 2H), 7.79 (s, 1H), 7.27 (d, *J*=6.6 Hz, 1H), 7.06–6.87 (m, 3H), 5.27–5.10 (m, 3H), 2.24 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ 165.2, 163.6, 161.2, 152.3, 150.4, 147.9, 144.2, 139.7, 130.9, 130.8, 127.7, 127.6, 123.2, 122.5, 121.0, 114.6, 114.4, 113.5, 113.3, 98.5, 56.7, 53.8, 18.4. LCMS: 475.4.

(1-(4-(Trifluoromethyl)phenyl)-1*H*-1,2,3-triazol-4-yl)methyl 4-(3-chlorophenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**7c**)

IR (KBr) ν/cm^{-1} 3,363, 2,969, 2,349, 1,695, 1,330, 843, 788, 757. ^{1}H NMR (400 MHz, DMSO) δ 9.33 (s, 1H), 8.80 (s, 1H), 8.11 (d, *J*=8.4 Hz, 2H), 7.96 (d, *J*=8.5 Hz, 2H), 7.79 (s, 1H), 7.34–7.13 (m, 4H), 5.20–5.13 (m, 3H), 2.25 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ 165.2, 158.0, 152.2, 150.5, 147.5, 146.9, 144.2, 133.3, 130.8, 127.7, 127.6, 126.6, 125.2, 123.2, 121.0, 98.4, 56.7, 53.9, 18.4. LCMS: 491.2 (M^{+}), 493.2 (M +2).

Table 1 Physicochemical constants of (1-(4-aryl)-1*H*-1,2,3-triazol-4-yl)methyl, substituted phenyl-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylates **7a-l**



Compound code	Molecular formula (molecular mass)	R	R^{1}	Yield (%)[a,b]	mp (°C)	cLog*P*[c]
7a	$C_{22}H_{17}F_4N_5O_3$ (475)	4-F	CF_3	85	194–196	4.6456
7b	$C_{22}H_{17}F_4N_5O_3$ (475)	3-F	CF_3	87	178–180	4.6456
7c	$C_{22}H_{17}ClF_3N_5O_3$ (491)	3-Cl	CF_3	82	204–206	5.2156
7d	$C_{23}H_{17}F_6N_5O_3$ (525)	4-CF_3	CF_3	89	188–190	5.3856
7e	$C_{22}H_{17}ClF_3N_5O_3$ (491)	4-Cl	CF_3	90	194–196	5.2156
7f	$C_{23}H_{20}F_3N_5O_4$ (487)	4-OCH_3	CF_3	92	196–198	4.4216
7g	$C_{23}H_{20}F_3N_5O_4$ (487)	3-OCH_3	CF_3	87	198–200	4.4216
7h	$C_{23}H_{20}F_3N_5O_3$ (471)	4-CH_3	CF_3	89	202–204	5.0016
7i	$C_{24}H_{22}F_3N_5O_4$ (501)	4-OC_2H_5	CF_3	88	214–216	4.9506
7j	$C_{21}H_{17}ClFN_5O_3$ (441)	4-Cl	F	77	144–146	4.3467
7k	$C_{22}H_{17}F_4N_0O_3$ (475)	CF_3	F	89	184–186	4.5167
7l	$C_{21}H_{17}F_2N_5O_3$ (425)	3-F	F	86	164–166	3.7767

Notes: [a]All of the products were characterized by spectral and physical data. [b]Yields after purification by column chromatography method. [c]cLog*P* was calculated using ChemBioDraw Ultra 13.0 v.

(1-(4-(Trifluoromethyl)phenyl)-1*H*-1,2,3-triazol-4-yl)methyl 6-methyl-2-oxo-4-(4-(trifluoromethyl)phenyl)-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**7d**)

IR (KBr) ν/cm^{-1} 3,412, 2,968, 2,345, 1,700, 1,641, 1,326, 844, 795, 718. ^{1}H NMR (400 MHz, DMSO) δ 9.35 (s, 1H), 8.79 (s, 1H), 8.10 (d, *J*=8.1 Hz, 2H), 7.95 (d, *J*=8.2 Hz, 2H), 7.83 (s, 1H), 7.57 (d, *J*=7.7 Hz, 2H), 7.38 (d, *J*=7.6 Hz, 2H), 5.25–5.12 (m, 3H), 2.25 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ 165.1, 152.2, 150.5, 144.2, 139.6, 127.6, 127.6, 127.6, 127.5, 125.8, 125.7, 123.2, 120.9, 98.3, 59.3, 56.7, 54.1, 18.4. LCMS: 525.1.

(1-(4-(Trifluoromethyl)phenyl)-1*H*-1,2,3-triazol-4-yl)methyl 4-(4-chlorophenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**7e**)

IR (KBr) ν/cm^{-1} 3,365, 2,966, 2,309, 1,709, 1,638, 1,331, 843, 786, 746. ^{1}H NMR (400 MHz, DMSO) δ 9.30 (s, 1H), 8.75 (s, 1H), 8.11 (d, *J*=8.5 Hz, 2H), 7.96 (d, *J*=8.6 Hz, 2H), 7.76 (s, 1H), 7.27 (d, *J*=8.4 Hz, 2H), 7.17 (d, *J*=8.4 Hz, 2H), 5.31–5.03 (m, 3H), 2.24 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ 165.2, 152.2, 150.2, 144.3, 144.0, 139.7, 132.2, 129.4, 128.7, 128.6, 127.7, 127.6, 123.2, 121.0, 98.6, 56.7, 53.8, 18.4. LCMS: 491.2 (M^{+}), 493.2 (M +2).

(1-(4-(Trifluoromethyl)phenyl)-1*H*-1,2,3-triazol-4-yl)methyl 4-(4-methoxyphenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**7f**)

IR (KBr) ν/cm^{-1} 3,390, 2,964, 2,345, 1,695, 1,638, 1,336, 845, 790, 757. ^{1}H NMR (400 MHz, DMSO) δ 9.21 (s, 1H), 8.68 (s, 1H), 8.10 (d, *J*=8.4 Hz, 2H), 7.96 (d, *J*=8.6 Hz, 2H), 7.66 (s, 1H), 7.08 (d, *J*=8.6 Hz, 2H), 6.76 (d, *J*=8.6 Hz, 2H), 5.18–5.08 (m, 3H), 3.59 (s, 3H), 2.23 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ 165.3, 158.8, 152.4, 149.6, 144.4, 139.6, 137.3, 127.8, 127.6, 127.6, 123.1, 121.0, 114.0, 99.3, 56.6, 55.3, 53.7, 31.1, 18.3. LCMS: 487.4.

(1-(4-(Trifluoromethyl)phenyl)-1*H*-1,2,3-triazol-4-yl)methyl 4-(3-methoxyphenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**7g**)

IR (KBr) ν/cm^{-1} 3,414, 2,964, 2,349, 1,718, 1,642, 1,333, 843, 788, 726. ^{1}H NMR (400 MHz, DMSO) δ 9.25 (s, 1H), 8.74 (s, 1H), 8.10 (d, *J*=8.5 Hz, 2H), 7.97 (d, *J*=8.6 Hz, 2H), 7.72 (s, 1H), 7.15 (t, *J*=7.9 Hz, 1H), 6.82–6.65 (m, 3H), 5.19–5.11 (m, 3H), 3.59 (s, 3H), 2.24 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ 165.3, 159.6, 152.5, 150.0, 146.6, 144.3, 130.0, 127.7, 127.6, 123.2, 121.0, 118.6, 112.8, 112.6, 98.9, 56.7, 55.3, 54.1, 18.3. LCMS: 487.3.

(1-(4-(Trifluoromethyl)phenyl)-1*H*-1,2,3-triazol-4-yl)methyl 6-methyl-2-oxo-4-(p-tolyl)-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**7h**)

IR (KBr) ν/cm^{-1} 3,367, 2,967, 2,349, 1,710, 1,646, 1,324, 841, 792, 703. ^{1}H NMR (400 MHz, DMSO) δ 9.21 (s, 1H), 8.68 (s, 1H), 8.10 (d, *J*=8.5 Hz, 2H), 7.97 (d, *J*=8.6 Hz, 2H), 7.68 (s, 1H), 7.05–6.99 (m, 4H), 5.21–5.09 (m, 3H), 2.23 (s, 3H), 2.12 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ 165.3, 152.4, 149.7, 144.4, 142.2, 139.7, 136.8, 129.2, 127.7, 127.6, 126.6, 125.6, 123.1, 121.0, 99.2, 56.6, 54.0, 20.9, 18.3. LCMS: 471.2.

(1-(4-(Trifluoromethyl)phenyl)-1*H*-1,2,3-triazol-4-yl)methyl 4-(4-ethoxyphenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**7i**)

IR (KBr) ν/cm^{-1} 3,323, 2,982, 2,350, 1,696, 1,644, 1,331, 839, 769, 696. ^{1}H NMR (400 MHz, DMSO) δ 9.20 (s, 1H), 8.65 (s, 1H), 8.09 (d, *J*=8.5 Hz, 2H), 7.96 (d, *J*=8.6 Hz, 2H), 7.65 (s, 1H), 7.05 (d, *J*=8.6 Hz, 2H), 6.72 (d, *J*=8.6 Hz, 2H), 5.26–5.05 (m, 3H), 3.88–3.72 (m, 2H), 2.23 (s, 3H), 1.19 (t, *J*=7.0 Hz, 3H). ^{13}C NMR (101 MHz, DMSO) δ 165.3, 158.1, 152.4, 149.5, 144.4, 139.6, 137.2, 127.8, 127.6, 127.6, 123.0, 121.0, 114.5, 99.3, 63.3, 56.5, 53.7, 18.3, 14.9. LCMS: 501.6.

(1-(4-Fluorophenyl)-1*H*-1,2,3-triazol-4-yl)methyl 4-(4-chlorophenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**7j**)

IR (KBr) ν/cm^{-1} 3,414, 2,960, 2,349, 1,712, 1,640, 1,323, 838, 792, 710. ^{1}H NMR (400 MHz, DMSO) δ 9.36 (s, 1H), 8.63 (s, 1H), 7.93–7.79 (m, 3H), 7.59 (d, *J*=7.9 Hz, 2H), 7.45–7.38 (m, 4H), 5.27–5.09 (m, 3H), 2.25 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ 165.2, 152.2, 150.5, 149.5, 143.8, 127.5, 125.8, 125.8, 123.2, 122.8, 122.8, 117.3, 117.0, 98.4, 56.8, 54.1, 31.1, 23.1, 18.4. LCMS: 441.2 (M^{+}), 443.2 (M +2).

(1-(4-Fluorophenyl)-1*H*-1,2,3-triazol-4-yl)methyl 6-methyl-2-oxo-4-(4-(trifluoromethyl)phenyl)-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**7k**)

IR (KBr) ν/cm^{-1} 3,367, 2,960, 2,349, 1,711, 1,638, 1,313, 835, 787, 691. ^{1}H NMR (400 MHz, DMSO) δ 9.30 (s, 1H),

8.58 (s, 1H), 7.90–7.87 (m, 2H), 7.76 (s, 1H), 7.43 (t, *J*=8.4 Hz, 2H), 7.28 (d, *J*=8.0 Hz, 2H), 7.18 (d, *J*=8.0 Hz, 2H), 5.20–5.09 (m, 3H), 2.24 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ 165.2, 152.2, 150.2, 144.0, 143.9, 133.5, 132.2, 128.7, 128.6, 123.1, 123.0, 122.9, 98.6, 56.8, 53.8, 31.1, 18.4. LCMS: 475.3.

(1-(4-Fluorophenyl)-1*H*-1,2,3-triazol-4-yl) methyl 4-(3-fluorophenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**7l**)

IR (KBr) ν/cm^{-1} 3,324, 3,079, 2,349, 1,663, 1,638, 1,239, 845, 761, 703. ^{1}H NMR (400 MHz, DMSO) δ 9.31 (s, 1H), 8.62 (s, 1H), 7.90–7.87 (m, 2H), 7.79 (s, 1H), 7.43 (t, *J*=8.7 Hz, 2H), 7.28 (m, 1H), 7.06–6.86 (m, 3H), 5.23–5.10 (m, 3H), 2.25 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ 165.2, 163.7, 161.2, 160.9, 152.3, 150.4, 147.9, 147.9, 143.8, 133.5, 130.9, 130.8, 123.2, 123.0, 122.9, 122.5, 117.3, 117.0, 114.6, 114.4, 113.5, 113.3, 98.5, 56.8, 53.8, 18.4. LCMS: 425.4.

Crystal growth and single crystal X-ray study

Title compound **7g** was used to grow single crystals at room temperature (25°C) using benzene as solvent for crystallographic studies. Single crystal data were collected on the Bruker D8 VENTURE diffractometer equipped with CMOS type PHOTON 100 detector using monochromated Mo Kα radiation (λ=0.71073 Å). Unit cell measurement, data collection, integration, scaling, and absorption corrections for the crystal were done using Bruker Apex II software.[40] Data reduction was done by Bruker SAINT suite.[41] The crystal structure was solved by direct methods using SIR 2014[42] and refined by the full matrix least squares method using SHELXL 2014[43] present in the program suite WinGX (version 2014.1, Louis J. Farrugia, Glasgow, Scotland).[44] Absorption correction was applied using SADABS.[45] All non-hydrogen atoms were refined anisotropically and all hydrogen atoms (except H-atoms bonded to N4 and N5) were positioned geometrically and refined using a riding model with Uiso(H) =1.2Ueq. The H-atoms bonded to N4 and N5 were taken directly from difference Fourier maxima. ORTEP (Oak Ridge Thermal Ellipsoid Plot) was generated using Mercury 3.5.1 Cambridge Crystallographic Data Center (CCDC) program.[46] Geometrical calculations were done using PARST[47] and PLATON.[48] Crystallographic and refinement data of the title compound **7g** are tabulated in Table 2.

Table 2 Single crystal data collection and refinement for compound **7g**

Data	Compound code 7g
Formula	$C_{23}H_{20}F_3N_5O_4$
Formula weight	487.3
Temperature (K)	110 (2)
Wavelength (Å)	0.71073
Solvent system, temperature	Benzene, 25°C
CCDC number	1465175
Crystal system	Monoclinic
Space group	$P2_1/c$
a (Å)	17.193 (6)
b (Å)	7.745 (3)
c (Å)	17.544 (7)
α (°)	90
β (°)	116.800 (12)
γ (°)	90
V (Å^3)	2,085.2 (14)
Z′, Z	1, 4
Density (g cm^{-3})	1.553
μ (mm^{-1})	0.126
F (000)	1,008
θ (min, max)	2.327, 29.571
$h_{min, max}$, $k_{min, max}$, $l_{min, max}$	–23 23, –10 10, –24 23
No of reflections	32,355
No of unique reflections/obs reflections	5,826/4,341
No of parameters	326
R_{all}, R_{obs}	0.0693, 0.0444
$wR2_{all}$, $wR2_{obs}$	0.1100, 0.0995
$\Delta\rho_{min, max}$ (eÅ^{-3})	–0.356, 0.378
GOF	1.028

Abbreviations: CCDC, Cambridge Crystallographic Data Center; GOF, goodness of fit.

Safety studies

The safety of the test compounds **7a-l** was evaluated by an MTT assay. The MTT cytotoxicity assay was used to evaluate the cytotoxic effect of the most promising compounds against peripheral blood mononuclear cells according to the protocol described.[49] Cells were pipetted (90 µL of cell culture, 1×10^5 cells/mL) into each well of 96-well microtiter plates, and the outer wells were filled with phosphate-buffered saline in order to prevent the medium from evaporation during incubation. Thereafter, plates were incubated at 37°C for 24 hours. Each well of the plate was then treated with 10 µL of the compounds (1,000–5 µg/mL). In the control wells, the negative control DMSO and media were added. Thereafter, the plates were incubated for 2 days at 37°C in a humidified incubator that contained a 5% CO_2 atmosphere. After the incubation time, 20 µL of MTT reagent (5 mg/mL) was further added to individual well. The plate was then incubated for a further 4 hours at 37°C (5% CO_2 incubator). The media were then removed after incubation, and an aliquot of 100 µL DMSO was added to each well in order to dissolve the formazan crystals that were formed in metabolically active cells. Thereafter, the plates were incubated for an extra hour. The absorbance of the formazan was evaluated at 590 nm using an ELISA plate reader.

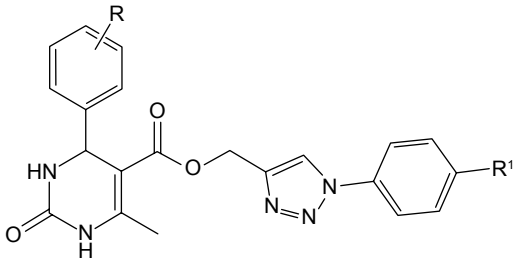
Antitubercular activity

Resazurin microplate assay plate method

The susceptibility of clinical isolates comprising of both fully sensitive and MDR TB isolates were evaluated against test compounds **7a-l** by the colorimetric resazurin microplate assay plate method.[50] An amount of 100 µL of Middlebrook 7H9 (Becton, Dickinson and Company, New Jersey, USA) broth was aseptically prepared and dispensed in each of the wells of a 96-well flat-bottomed microtiter plate with lids (Lasec, Ndabeni, South Africa). Each of the test compounds **7a-l** was weighed out accordingly, dissolved in the appropriate solvent, and filter sterilized using a 0.2 micron polycarbonate filter. Stock solutions of the test samples were aliquoted into cryovials and stored at –20°C. An amount of 100 µL of the test samples was added to each of the well containing Middlebrook 7H9 broth supplemented with 0.1% casitone, 0.5% glycerol, and 10% oleic acid, albumin, dextrose, and catalase. The test samples were then further serially diluted two-fold directly in the broth of the microtiter plate to a desired concentration ranging from 40 to 0.625 µg/mL.

Inoculums from clinical isolates were prepared fresh from Middlebrook 7H11 agar plates by scraping and resuspending loopful of colonies into Middlebrook 7H9 broth containing glass beads. The inoculum turbidity was adjusted to a McFarland number 1 standard and further diluted 1:10 in M7H9 broth prior to addition (100 µL) to each of the test samples and drug-free wells. A growth control and a sterile control were also included for each isolate. Sterile M7H9 broth was added to all perimeter wells to avoid evaporation during the incubation. The plate was covered, sealed in a plastic bag, and incubated at 37°C. After 8 days of incubation, 30 µL of 0.02% working solution of resazurin salt was inoculated into each microtiter well. The plates were then incubated overnight and read the following day. A positive reaction resulted in a color change from blue to pink owing to the reduction of resazurin to rezarufin, which confirmed MTB cell viability/growth and hence drug resistance. The minimum inhibitory concentrations were defined as the minimum drug concentration to inhibit the growth of the organism with no color changes present in the well. The anti-TB results of title compounds **7a-l** are tabulated in Table 3.

Table 3 In vitro antitubercular activity of title compounds **7a-l** against *Mycobacterium tuberculosis*



Compound code	R	R^1	MIC (µg/mL)	
			$H37R_v$	MDR-MTB*
7a	4-F	CF_3	10	20
7b	3-F	CF_3	20	20
7c	3-Cl	CF_3	20	NA
7d	4-CF_3	CF_3	10	15
7e	4-Cl	CF_3	NA	NA
7f	4-OCH_3	CF_3	10	NA
7g	3-OCH_3	CF_3	20	20
7h	4-CH_3	CF_3	10	NA
7i	4-C_2H_5	CF_3	NA	NA
7j	4-Cl	F	NA	NA
7k	CF_3	F	15	NA
7l	3-F	F	20	20

Note: *These isolates were found to be resistant to the first line antibiotics, rifampicin (1 µg/mL), and isoniazid (0.2 µg/mL).
Abbreviations: MDR-MTB, multidrug-resistant strains of *Mycobacterium tuberculosis*; MIC, minimum inhibitory concentration; NA, not active.

Results and discussion

Chemistry

As a continuing aspect of our earlier work[6,37,51] and after much efforts over the years to develop efficient synthetic procedures for multicomponent reactions under greener conditions, there was a requirement to synthesize a huge library of diversified 1,2,3-triazole hybrids with DHPMs analogs with reduced time, outstanding yields, and excellent biological activities. The DHPMs with terminal alkynyl group were synthesized following a previously reported procedure.[52] Aromatic azides were synthesized in high yields from arenediazonium tosylates and sodium azide in water at room temperature. An in situ diazotization followed by azidation in the presence of *p*-toluenesulfonic acid allows the direct transformation of aromatic amines.[53]

For our initial studies, 4-trifluoromethyl aryl azide and prop-2-yn-1-yl 4-(4-fluorophenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate were chosen as model substrates (entry 1; compound **7a**, Table 1) with a catalytic amount of $Cu(OAc)_2$ and sodium ascorbate in a 1:2 ratio of acetone and water (2 mL) as a solvent. A whole reaction mixture of 4-trifluoromethyl aryl azide and terminal alkynyl DHPMs was stirred at room temperature (25°C). The starting material was consumed within 3 hours as indicated by TLC analysis. It was observed that when aryl azide, terminal alkynyl DHPMs, $Cu(OAc)_2$, and sodium ascorbate were used in the ratio of 1:1:0.1:0.2 in 2 mL of a mixture of 1:2 ratio of acetone and water as a solvent, they gave the best result. After workup and purification by silica gel column

chromatography, the final desired product 1,2,3-triazole hybrid with DHPMs was isolated in 85% yield.

The practicality of optimized reaction conditions was further extended to the synthesis of more functionalized 1,2,3-triazole hybrid DHPM derivatives **7b-l** and experiments were performed by making use of a wide range of aryl azides. It was found that in all the cases the reaction occurred smoothly. We have also reacted a variety of DHPMs analogs having both electron-releasing and -withdrawing substituent to synthesize the diversified DHPMs derivatives with 1,2,3-triazole linkage and all the results are appended in Table 1. The partition coefficient of the title compounds was calculated by ChemBioDraw Ultra 13.0v (PerkinElmer Inc., Waltham, MA, USA) and the results were in the range of 3.7767–5.3856. The purity of the compounds was confirmed by high performance liquid chromatography and it was over 99%.

Crystallographic studies

Test compound **7g** emerged as one of the promising compounds for anti-TB activity from the series subjected to single crystal X-ray studies. The compound crystallizes in the centrosymmetric monoclinic space group $P2_1/c$ with one molecule in the asymmetric unit (Z=4). *ORTEP* is shown in Figure 2. Structural investigation shows that the DHPM ring of the molecule exists in a boat-like conformation due to minimization of the steric repulsion between ester moiety containing a triazole ring with *meta* methoxy phenyl ring. The *para* trifluoro substituted benzene ring and triazole unit remain almost in the same molecular plane whereas the methoxy phenyl substituted DHPM unit is almost perpendicular to the plane. Crystal packing is mainly controlled by strong intermolecular N-H…O dimeric motif along the crystallographic axis *a* and weak C-H…O hydrogen bonding dimers along the axis *c* (shaded regions in Figure 3A). In addition, molecules form centrosymmetric dimers via N-H…N, C-H…N, C-H…π (Cg1) hydrogen bonds and weak π…π stacking interactions along the crystallographic *b*-axis wherein these dimers are linked with weak C-H…F (involving H20, F2) hydrogen bond and F…F (involving F1, F3) interactions (Figure 3B). Hence, it is noteworthy to mention that interactions involving fluorine atom are one of the important contributors to the overall packing.[54–56] The list of all the intermolecular interactions is given in Table 4.

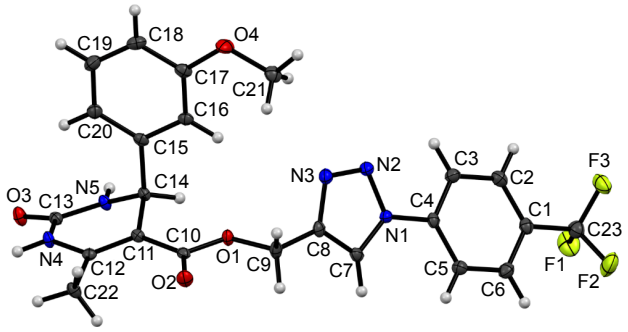


Figure 2 ORTEP (Oak Ridge Thermal Ellipsoid Plot) of the compound **7g** drawn with 50% ellipsoidal probability.

Safely studies

Test compounds **7a-l** were evaluated for safely studies by an MTT assay and it was found that up to 500 µg/mL no toxicity on PBM cell lines was observed.

Antitubercular activity

Anti-TB activity of the test compounds **7a-l** was evaluated against $H37R_v$ and MDR-MTB by resazurin microplate assay plate method and the results are tabulated in Table 3. Compound **7d** with trifluoro methyl group at fourth position of two-phenyl ring appeared as a promising agent against $H37R_v$ and MDR-MTB at 10 and 15 µg/mL, respectively. However, compound **7f** with methoxy group on phenyl ring of pyrimidine nucleus and trifluoromethyl group on phenyl ring of triazole ring exhibited activity at 10 µg/mL against $H37R_v$ and no activity against MDR-MTB. Test compounds **7b**, **7g**, and **7l** exhibited similar activity against $H37R_v$ and MDR-MTB in spite of varying functional groups on phenyl rings that are on pyrimidine and triazole nucleus.

Conclusion

We have established an operationally simple and straightforward one-pot synthesis for the synthesis of 1,2,3-triazole hybrid with DHPMs analogs via click chemistry. The purity of the compound was over 99% and yield of the compounds was excellent. Compound **7d** emerged as a promising compound from the series for anti-TB activity. Crystallographic studies for the compound **7g** revealed that the interplay of strong (such as N-H…O, N-H…N, C-H…O) and weak interactions (eg, C-H…F, C-H…π, F…F) stabilizes the overall crystal packing in the solid state.

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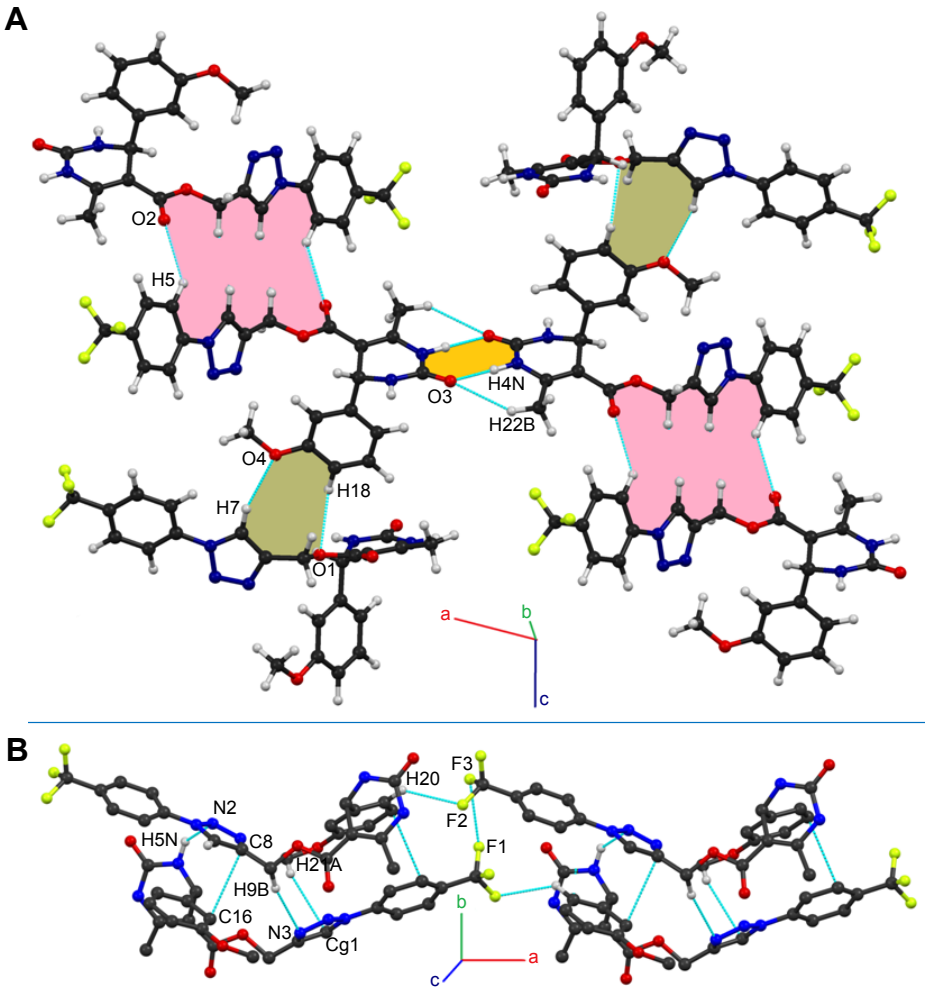

Figure 3 Packing network of compound **7g** (**A**) down the *ac* plane associated with N-H…O and CH…O dimers (noninteracting H-atoms have been removed for clarity); (**B**) molecules forming centrosymmetric dimers via N-H…N, C-H…N, C-H…π (Cg1) hydrogen bonds and π…π stacking connected down the *ab* plane through weak C-H…F hydrogen bond and F…F interactions.

Table 4 Intermolecular interactions in **7g**

Motifs	D–H…A	Symmetry	Geometry		
			D…A/Å	H…A/Å	∠D–H…A/°
I	N4-H4N…O3	–x+1, –y+1, –z+2	2.829 (2)	1.80	177
II	C9-H9B…N3	–x, y–1/2, –z+3/2	3.857 (2)	2.79	168
III	C18-H18…O1	x, –y+1/2, z–1/2	3.478 (2)	2.54	145
IV	C21-H21A…Cg1	–x, y–1/2, –z+3/2	3.415 (2)	2.60	132
V	C8 (π)…(π) C16	–x, y–1/2,–z+3/2	3.292 (2)	–	–
VI	C6-H6…(π) C13	–x, –y+1, –z+2	3.741 (3)	2.81	145
VII	C22-H22C…O3	x, y–1, z	3.460 (2)	2.50	147
VIII	C22-H22B…O3	–x+1, –y+1, –z+2	3.606 (2)	2.70	141
IX	C20-H20…F2	x+1, y, z	3.510 (2)	2.68	134
X	C5-H5…O2	–x, –y, –z+2	3.425 (2)	2.57	136
XI	N5-H5N…O2	x, y+1, z	3.667 (2)	2.86	136
XII	N5-H5N…N2	–x, y+1/2, –z+3/2	3.069 (2)	2.25	134
XIII	C19-H19…F3	x+1, y, z	3.572 (2)	2.78	130
XIV	C22-H22A…F3	x+1, –y+1/2, z+1/2	3.445 (2)	2.66	129
XV	C7-H7…O4	x, –y+1/2, z+1/2	3.353 (2)	2.66	121
XVI	C21-H21B…O4	–x, –y, –z+1	3.370 (2)	2.66	122
XVII	C21-H21A…N1	–x, y–1/2, –z+3/2	3.601 (2)	2.92	121
XVIII	F1…F3	–x–1, y+1/2, –z+3/2	3.026 (2)	–	–

Note: Cg1 refers to the center of gravity of ring formed by C7-C8-N3-N2-N1.

Disclosure

The authors report no conflicts of interest in this work.

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