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Fluorinated 1,2,4-Triazolo[1,5-*a*]pyrimidine-6-carboxylic Acid Derivatives as Antimycobacterial Agents

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A series of fluorinated 1,2,4-triazolo[1,5-*a*]pyrimidine-6-carboxylic acid derivatives was designed and synthesized as fluoroquinolone analogues. The synthesized compounds were screened against *Mycobacterium tuberculosis* $H_{37}R_v$ strain at 6.25 μg/mL concentration. Compound **4**, the 7-oxo-2-(trifluoromethyl)-4,7-dihydro-1,2,4-triazolo[5,1-*a*]pyrimidine-6-carboxylic acid was found to be a very potent inhibitor, being able to inhibit 92% growth of *M. tuberculosis* $H_{37}R_v$ at 6.25 μg/mL concentration. At the same time, it proofed to be nontoxic to mammalian cells (IC_{50} > 62.5 μg/mL in VERO cells).

Keywords: Antimycobacterial activity / Fluoroquinolone analogues / *Mycobacterium tuberculosis* / 1,2,4-Triazolo[1,5-*a*]pyrimidines

Received: June 9, 208; accepted: September 8, 2008

DOI 10.1002/ardp.200800113

Introduction

Tuberculosis is an old and coming-back disease that spreads at an alarming rate particularly in developing nations such as Sub-Saharan Africa and Southeast Asia. According to the WHO figures, there are two billions infected people worldwide with about 8.8 millions new active disease cases and some 1.6 millions perished from it annually [1].

Fluoroquinolones are broad-spectrum antibacterial agents being subject of intense research for tuberculosis treatment because of their wide spectrum, intense bactericidal activity, and excellent bioavailability [2 – 8]. They were shown to be specific inhibitors of the bacterial DNA gyrase to exert their significant antibacterial activity with a minimum pharmacophore consisting of the 4-pyridone ring with a 3-carboxylic acid group (Fig. 1) [2].

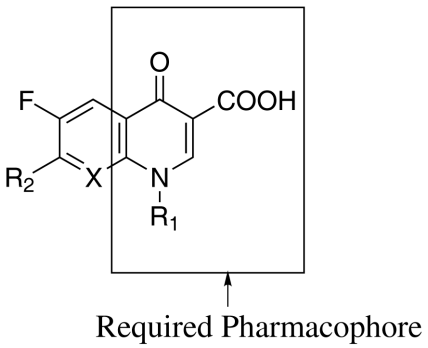

Figure 1. The required pharmacophore of fluoroquinolones.

The antibacterial activity of fluoroquinolines depends not only on the heteroaromatic pharmacophore but also on the nature of the peripheral substituents and their spatial relationship. These substituents exert their influence on antibacterial activity by providing additional affinity for the bacterial enzymes, enhancing the cell penetration, or altering the pharmacokinetics [9, 10]. Several quinolone antibiotics analogues with the pharmacophoric monocyclic 4-pyridone ring alone [11] or fused with five-membered rings such as pyrroles [12, 13], or triazoles [14] have been reported to have good antibacterial activity. Furthermore, the monocyclic 4-pyridone [11] as well as the 1,2,4-triazolo[1,5-*a*]pyrimidine analogues [15] were shown to inhibit of the bacterial DNA gyrase to exert their antibacterial activity in a similar manner to fluoroquinolones.

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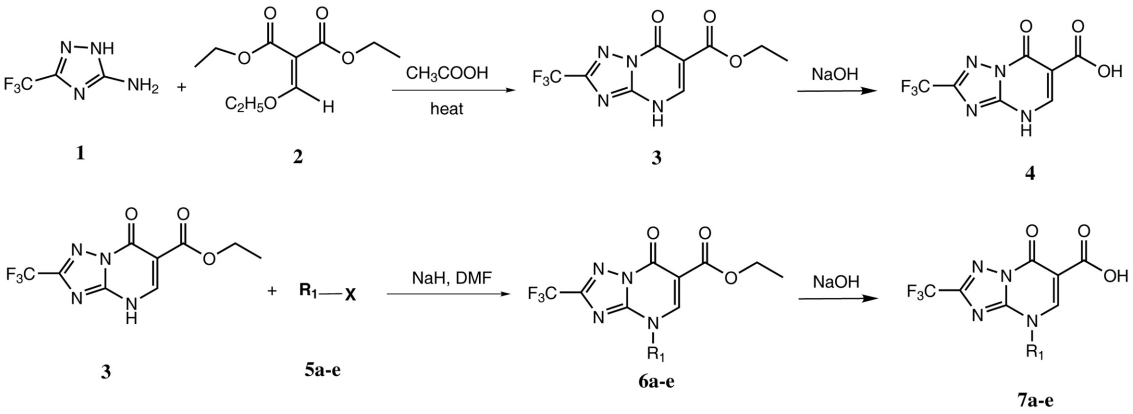
Abbreviation: Tuberculosis Antimicrobial Acquisition & Coordinating Facility (TAACF)



For **5**, **6, 7** R_1 = **a**: CH_3, **b**:C_2H_5, **c**: $-CH_2CH{=}CH_2$, **d**: $-CH_2-C_6H_4$(4 -Br), **e**: $-CH_2-C_6H_4$(4 $-NO_2$)

Scheme 1. Synthesis of the 1,2,4-triazolo[1,5-*a*]pyrimidine-6-carboxylic acid derivatives.

The synthesis and characterizations of 1,2,4-triazolo[1,5-*a*]pyrimidine derivatives received much attention due to their wide biological applications [16–19]. In this paper, we report the synthesis of several new fluorinated-1,2,4-triazolo[1,5-*a*]pyrimidine derivatives as potential fluoroquinolone analogues as antimycobacterial agents. The parameters which were considered for the design of these compounds are the presence of: (*i*) the quinolones pharmacophore (the 4-pyridone ring with 3-carboxylic acid group), (*ii*) the lipophilic CF_3 substituent attached to the 1,2,4-triazolo[1,5-*a*]pyrimidine nucleus, and (*iii*) the simplicity and ease of synthesis of the desired derivatives.

Results and discussion

Chemistry

A simple and straightforward scheme was adopted for the synthesis of fluorinated-1,2,4-triazolo[1,5-*a*]pyrimidine derivatives rather than the tedious and costly synthetic schemes reported for the synthesis of fluoroquinolone derivatives [3, 6, 7].

The synthesis of the target compounds is outlined in Scheme 1. The starting 5-amino-3-trifluoromethyl-1,2,4-triazole **1** was prepared by a reported procedure [20]. Refluxing with diethoxymethylenemalonate (DEEM) **2** in glacial acetic acid afforded 7-oxo-2-(trifluoromethyl)-4,7-dihydro-1,2,4-triazolo[1,5-*a*]pyrimidine-6-carboxylic acid ethyl ester **3**. Alkylation of the resulting ester at the N-4 position with alkyl / aralkyl halides (**5a–e**) in dry DMF and NaH as a base afforded compounds **6a–e** in accordance with previous reports on the alkylation of 1,2,4-triazolo[1,5-*a*]pyrimidines [15, 16]. Base-catalyzed hydrolysis of ethyl esters **3**, and **6a–e** using sodium hydroxide afforded compounds **4** and **7a–e**, respectively. The identities of the compounds obtained were confirmed by elemental analyses, IR, and ^{1}H-NMR spectroscopy.

Antimycobacterial assay

Nine of the synthesized compounds were initially screened for their antimycobacterial activity at 6.25 μg/mL against the $H_{37}R_v$ strain by the Tuberculosis Antimicrobial Acquisition & Coordinating Facility (TAACF) in BACTEC 12B medium using the Microplate Alamar Blue Assay (Table 1) [21].

For the N-4 unsubstituted 1,2,4-triazolo[1,5-*a*]pyrimidine derivatives **3**, **4**, the ethyl ester **3** was devoid of any antitubercular activity whereas its carboxylic acid analogue **4** showed the best growth-inhibitory activity in this series with 92% growth inhibition. On the other hand, the N-4 substituted derivatives **6a–7e** showed moderate growth inhibitory activity of *M. Tuberculosis* ranging from 26–38%. In a similar manner, the free carboxylic acid derivatives **7d**, **7e** are more potent than their corresponding ethyl esters **6d**, **6e**, respectively. In general, the structure-activity relationship (SAR) of this short series of compounds showed that the biological activity does not depend exclusively on the presence of N-4 substitution but it is probably due to the presence of the 7-oxo-6-carboxylic acid groups on the 1,2,4-triazolo[1,5-*a*]pyrimidine nucleus.

The lipophilicity of the fluoroquinolones is well known to play an important role in the penetration of these compounds especially into bacterial cells [3, 4]. Our results demonstrat that the lipophilic character of the synthesized compounds, as seen from their CLog P-values (calculated using ChemDraw Ultra 9.0 software) is rela-

Table 1. *In-vitro* antimycobacterial activity of the test compounds.

Compound	TAACF ID	Assay	% Inhibition[a)]	MIC (μg/mL)	IC_{50} (μg/mL)[b)]	ClogP[c)]
3	302168	Alamar	0	>6.25	n. d.	−0.65
4	302169	Alamar	92	>6.25	>62.5	−1.34
6a	302170	Alamar	32	>6.25	n. d.	−0.58
6b	302171	Alamar	25	>6.25	n. d.	−0.05
6c	302172	Alamar	28	>6.25	n. d.	0.19
6d	302173	Alamar	29	>6.25	n. d.	2.05
6e	302174	Alamar	26	>6.25	n. d.	0.93
7a	408912	Alamar	n.d	>6.25	n. d.	−1.04
7d	302175	Alamar	31	>6.25	n. d.	1.40
7e	302176	Alamar	38	>6.25	n. d.	0.28
Ciprofloxacin[d)]	–	Alamar	98	2.00	>10	−0.63

TAACF: Tuberculosis Antimicrobial Acquisition and Coordinating Facility.

a) Growth inhibition of *Mycobacterium tuberculosis* $H_{37}R_v$ at a single concentration of 6.25 μg/mL.

b) 50% inhibition concentration in VERO cells.

c) CLog P calculated using ChemDraw Ultra 9.0; n. d. not determined.

d) Data were obtained from reference 8.

tively low, particularly compound **3** (considered a prodrug of the acid **4**) with CLog P: -0.65 in a similar manner as some drugs with antimycobacterial activity, *e. g*. ciprofloxacin (CLog P: −0.63), isoniazide (CLogP: −0.67), and pyrazinamide (CLogP: −0.68).

According to the TAACF program, compound **4** effecting 90% inhibition in this primary screen was further evaluated to determine its actual MIC which was in our case >6.25 μg/mL for compound **4**. Simultaneously, compound **4** was tested for its cytotoxicity, *i.e*. the determination of its 50% inhibitory concentrations (IC_{50}) in VERO cells which in turn found to >62.5 μg/mL. While other compounds effecting <90% inhibition in this primary screening were not generally evaluated further. However, inactive compounds may still have significant inhibitory activity and this data should not be ignored; analogues, derivatives, and alterations in physical properties may confer drastic changes in biological effects. Therefore, synthesis and evaluation of other 1,2,4-triazolo[1,5-*a*]pyrimidine-6-carboxylic acid derivatives are necessary to broaden the structure-activity data.

Antibacterial activity

A preliminary antibacterial activity of compounds **3**, **4**, **6a**, **6d**, **6e**, **7a**, **7d**, and **7e** was evaluated using *in-vitro* growth-inhibitory assay by a modified procedure for disc diffusion method [22] against *Bacillus cereus* and *Escherichia coli* as Gram-positive and Gram-negative bacteria, respectively. Table 2 shows the measured zones of inhibition (in mm) of the tested compounds and the reference drug (nalidexic acid). Compounds **4**, **7a**, **7d** showed moderate to good antibacterial activities against the tested microorganisms with compound **4** showing the same antibacterial activity as nalidixic acid against *B. cereus*.

Table 2. The antibacterial zones of inhibition (in mm) of tested compounds.

Compound	*Bacillus cereus*	*E. Coli*
3	10	0[a)]
4	15	5
6a	0	5
6d	0	5
6e	0	5
7a	10	10
7d	10	10
7e	5	10
Nalidixic acid	15	22

a) 0 = no-inhibition zone at the tested concentration.

Conclusion

A series of 1,2,4-triazolo[1,5-*a*]pyrimidine-6-carboxylic acid derivatives were synthesized and evaluated for antimycobacterial activity. Preliminary results showed most compounds exhibited moderate to good anti-TB activity. Compound **4** was the most promising agent within this series with 92% growth inhibition of *M. tuberculosis* $H_{37}R_v$ at 6.25 μg/mL concentration and IC_{50} > 62.5 μg/mL in VERO cells. These results deserve full attention, especially, because **4** proved to combine the potent anti-TB activity and the non-cytotoxicity to mammalian cells with the ease of synthesis making it a promising lead compound for tuberculosis chemotherapy. Furthermore, compound **4** exhibits the same antibacterial activity against *B. cereus* as nalidixic acid.

Antimycobacterial data were provided by the Tuberculosis Antimicrobial Acquisition and Coordinating Facility (TAACF)

through a research and development contract with the U.S. National Institute of Allergy and Infectious Diseases.

The authors have declared no conflict of interest.

Experimental

Chemistry

Melting points were determined on an electrothermal melting point apparatus and are uncorrected. ^{1}H-NMR spectra were determined on EM-360 (60 MHz; Varian Inc., Palo Alto, CA, USA) in DMSO-d_6 using tetramethylsilane (TMS) as the internal standard and chemical shifts values are given in δ ppm. IR spectra were recorded on 470-Shimadzu infrared spectrophotometer (Shimadzu, Tokyo, Japan) as KBr discs. Elemental microanalysis was performed by the Microanalysis Unit of the Faculty of Science, Assiut University. Product purity was checked by TLC (Kieselgel 60 F_{254}). Yields given are those of the crude products.

5-Amino-3-trifluoromethyl-1,2,4-triazole ***1***

5-Amino-3-trifluoromethyl-1,2,4-triazole was prepared in 92% yield following the reported procedures [20]: Trifluoroacetic acid (15.05 mL, 0.202 mol) was added to aminoguanidine bicarbonate (25 g, 0.184 mol) followed by mixing for 30 minutes at room temperature. Toluene (200 mL) was added thereto, and the mixture was stirred under reflux for 15 h while distilling water off formed by the reaction using a Dean – Stark trap. The reaction mixture was left to stand overnight. Precipitated white crystals were collected by filtration and used directly in the next step without further purification.

7-Oxo-2-(trifluoromethyl)-4,7-dihydro-1,2,4-triazolo[5,1-a]pyrimidine-6-carboxylic acid ethyl ester ***3***

To a stirred solution of 5-amino-3-trifluoromethyl-1,2,4-triazole **1**(0.373 g, 0.0025 mol) in 5 mL glacial acetic acid, diethyl ethoxymethylene malonate (DEEM) **2** (0.86 g, 0.004 mol) was added dropwise. The reaction mixture was refluxed for 3 h and refrigerated overnight. The precipitated solid was filtered off, washed with ethyl acetate, and dried. The crude product was crystallized from acetic acid. Yield: 0.48 g, 70%; m.p.: 280 – 281°C; IR (KBr) ν [cm^{-1}]: 3485, 3135, 1734, 1618, 1578, 1180, 781; ^{1}H-NMR (DMSO-d_6) δ [ppm]: 9.3 (brs, 1H, NH, exchangeable with D_2O), 8.4 (s, 1H, C-H5), 4.1 (q, 2H, CH_2CH_3), 1.1 (t, 3H, CH_2CH_3). Anal. Calcd. for $C_9H_7F_3N_4O_3$: C, 39.1; H, 2.5; N, 20.3. Found: C, 39.4; H, 2.4; N, 20.0.

7-Oxo-2-(trifluoromethyl)-4,7-dihydro-1,2,4-triazolo[5,1-a]pyrimidine-6- carboxylic acid ***4***

The ethyl ester **3** (0.01 mol) was dissolved in a solution of sodium hydroxide (0.8g, 0.02 mol) in water (15 mL). The reaction mixture was heated under reflux for 3 h. After cooling, the reaction mixture was filtered and acidified by dropwise addition of 4 M HCl. The carboxylic acid precipitated was collected by filtration, washed with water, air dried and recrystallized from the methanol / water. Yield: 82%; m.p.: 238 – 240°C; IR (KBr) ν [cm^{-1}]: 3490, 3140, 1729, 1667, 1622, 1572, 1279, 783; ^{1}H-NMR (DMSO-d_6) δ [ppm]: 9.0 (s, 1H, C-H5), 6.9 (brs, 1H, NH, D_2O exchangeable). Anal. Calcd for $C_7H_3F_3N_4O_3$: C, 33.9; H, 1.2; N, 22.6. Found: C, 33.7; H, 1.4; N, 22.2.

4-Substituted-7-oxo-2-(trifluoromethyl)-4,7-dihydro-1,2,4-triazolo[5,1-a]pyrimidine-6- carboxylic acid ethyl ester ***6a – e***

A solution of compound **3** (2.76g, 0.01mol) in dry DMF (25 mL) was treated with sodium hydride (0.4 g, 0.0167 mol) and stirred at room temperature for 10 minutes. Alkyl or aralkyl halides **5a – e** (0.03 mol) were added and stirring was continued overnight. The reaction mixture was poured over ice-water (100 mL), the resulting precipitate was filtered, washed with water and air dried. The product was recrystallized from methanol / water.

4-Methyl-7-oxo-2-(trifluoromethyl)-4,7-dihydro-1,2,4-triazolo[5,1-a]pyrimidine-6-carboxylic acid ethyl ester ***6a***

Yield: 75%; m.p.: 225°C; IR (KBr) ν [cm^{-1}] 3235, 1749, 1571, 1184, 1144; ^{1}H-NMR (DMSO-d_6) δ [ppm]: 8.3 (s, 1H, C-H5), 4.3 (q, 2H, CH_2CH_3), 4.0 (s, 3H, N-CH_3), 1.3 (t, 3H, CH_2CH_3). Anal. Calcd for $C_{10}H_9F_3N_4O_3$: C, 41.4; H, 3.1; N, 19.3. Found: C, 41.55; H, 3.3; N, 18.9.

4-Ethyl-7-oxo-2-(trifluoromethyl)-4,7-dihydro-1,2,4-triazolo[5,1-a]pyrimidine-6-carboxylic acid ethyl ester ***6b***

Yield: 70%; m.p.: 168-170°C; IR (KBr) ν [cm^{-1}]: 1758, 1679, 1559, 1181; ^{1}H-NMR (DMSO-d_6) δ [ppm]: 9.0 (s, 1H, C-H5), 4.3 (m, 4H, 2 CH_2CH_3), 1.4 (m, 6H, 2 CH_2CH_3). Anal. Calcd. for $C_{11}H_{11}F_3N_4O_3$: C, 43.4; H, 3.6; N, 18.4. Found: C, 43.3; H, 4.0; N, 18.2.

4-Allyl-7-oxo-2-(trifluoromethyl)-4,7-dihydro-1,2,4-triazolo[5,1-a]pyrimidine-6-carboxylic acid ethyl ester ***6c***

Yield: 54%; m.p.: 143 – 145°C; IR (KBr) ν [cm^{-1}]: 1757, 1567, 1222; ^{1}H-NMR (DMSO-d_6) δ [ppm]: 9.1 (s, 1H, C-H5), 6.3 (m, 1H, CH=CH_2), 5.5 (m, 2H, CH=CH_2), 4.9 (m, 2H, N-CH_2), 4.3 (q, 2H, CH_2CH_3), 1.4 (t, 3H, CH_2CH_3). Anal. Calcd. for $C_{12}H_{11}F_3N_4O_3$: C, 45.6; H, 3.5; N, 17.7. Found: C, 45.9; H, 3.4; N, 17.3.

4-p-Bromobenzyl-7-oxo-2-(trifluoromethyl)-4,7-dihydro-7-oxo-1,2,4-triazolo[5,1-a] pyrimidine-6-carboxylic acid ethyl ester ***6d***

Yield: 83%; m.p.: 161-162°C; IR (KBr) ν [cm^{-1}]: 1755, 1692, 1585, 983, 559, 522; ^{1}H-NMR (DMSO-d_6) δ [ppm]: 9.4 (s, 1H, C-H5), 7.8 (dd, 4H, Ar-H), 5.6 (d, 2H, N-CH_2-Ar), 4.2 (q, 2H, CH_2CH_3), 1.2 (t, 3H, CH_2CH_3). Anal. Calcd. for $C_{16}H_{12}BrF_3N_4O_3$: C, 43.2; H, 2.7; N, 12.3. Found: C, 43.4; H, 2.5; N, 12.3.

4-p-Nitrobenzyl-7-oxo-2-(trifluoromethyl)-4,7-dihydro-1,2,4-triazolo[5,1-a]pyrimidine-6-carboxylic acid ethyl ester ***6e***

Yield: 90%; m.p.: 180 – 182°C; IR (KBr) ν [cm^{-1}]: 1747, 1556, 1513, 1337, 1180, 845; ^{1}H-NMR (DMSO-d_6) δ [ppm]: 9.5 (s, 1H, C-H5), 8.0 (dd, 4H, Ar-H), 5.8 (d, 2H, N-CH_2-Ar), 4.3 (q, 2H, CH_2CH_3), 1.3 (t, 3H, CH_2CH_3). Anal. Calcd for $C_{16}H_{12}F_3N_5O_3$: C, 46.7; H, 2.9; N, 17.0. Found: C, 46.4; H, 2.7; N, 17.1.

4-Substituted-7-oxo-2-(trifluoromethyl)-4,7-dihydro-1,2,4-triazolo[5,1-a]pyrimidine-6-carboxylic acid ***7a – e***

The corresponding ethyl esters **6a – e** (0.01 mol) were dissolved in a solution of sodium hydroxide (0.8 g, 0.02 mol) in water (15 mL). The reaction mixture was heated under reflux for 3 h. After cooling, the reaction mixture was filtered and acidified by

dropwise addition of 4 M HCl. The carboxylic acid precipitated was collected by filtration, washed with water, air dried, and recrystallized from the methanol/water.

*4-Methyl-7-oxo-2-(trifluoromethyl)-4,7-dihydro-1,2,4-triazolo[5,1-a]pyrimidine-6-carboxylic acid **7a***

Yield: 70%; m.p.: 175 – 177°C; IR (KBr) ν [cm^{-1}]: 3550, 3410, 1683, 1571, 1184, 620; ^{1}H-NMR (DMSO-d_6) δ [ppm]: 9.18 (s, 1H, C-H5), 3.9 (d, 3H, N-CH_3). Anal. Calcd. for $C_8H_5F_3N_4O_3$: C, 36.6; H, 1.9; N, 21.3. Found: C, 36.4; H, 1.7; N, 20.9.

*4-Ethyl-7-oxo-2-(trifluoromethyl)-4,7-dihydro-1,2,4-triazolo[5,1-a]pyrimidine-6-carboxylic acid **7b***

Yield: 65%; m.p.: >280°C; IR (KBr) ν [cm^{-1}]: 3410, 3350, 1677, 1566, 1181, 620; ^{1}H-NMR (DMSO-d_6) δ [ppm]: 8.8 (s, 1H, C-H5), 4.3 (q, 2H, CH_2CH$_3$), 1.3 (t, 3H, CH$_2$CH_3). Anal. Calcd. for $C_9H_7F_3N_4O_3$: C, 39.1; H, 2.5; N, 20.3. Found: C, 39.3; H, 2.7; N, 20.5.

*4-Allyl-7-oxo-2-(trifluoromethyl)-4,7-dihydro-1,2,4-triazolo[5,1-a]pyrimidine-6-carboxylic acid **7c***

Yield: 60%; m.p.: 162 – 163°C; IR (KBr) ν [cm^{-1}]: 3540, 3410, 2990, 1707, 1567, 1173, 785, 619; ^{1}H-NMR (DMSO-d_6) δ [ppm]: 9.1 (s, 1H, C-H5), 6.3 (m, 1H, CH=CH$_2$), 5.5 (m, 2H, CH=CH_2), 4.9 (m, 2H, N-CH_2). Anal. Calcd. for $C_{10}H_7F_3N_4O_3$: C, 41.6; H, 2.4; N, 19.4. Found: C, 41.5; H, 2.3; N, 19.8.

*4-p-Bromobenzyl-7-oxo-2-(trifluoromethyl)-4,7-dihydro-1,2,4-triazolo[5,1-a]pyrimidine-6-carboxylic acid **7d***

Yield: 76%; m.p.: 196 – 199°C; IR (KBr) ν [cm^{-1}]: 3545, 3410, 1665, 1576, 1187; ^{1}H-NMR (DMSO-d_6) δ [ppm]: 9.3 (s, 1H, C-H5), 7.8 (dd, 4H, Ar-H), 5.6 (d, 2H, N-CH_2-Ar). Anal. Calcd. for $C_{14}H_8BrF_3N_4O_3$: C, 40.3; H, 1.9; N, 13.4. Found: C, 40.2; H, 1.8; N, 13.6.

*4-p-Nitrobenzyl-7-oxo-2-(trifluoromethyl)-4,7-dihydro-1,2,4-triazolo[5,1-a]pyrimidine- 6-carboxylic acid **7e***

Yield: 83%; m.p.: 150 – 152°C; IR (KBr) ν [cm^{-1}]: 3455, 1660, 1511, 1336, 1180; ^{1}H-NMR (DMSO-d_6) δ [ppm]: 9.5 (s, 1H, C-H5), 8.0 (dd, 4H, Ar-H), 5.8 (d, 2H, N-CH_2-Ar). Anal. Calcd. for $C_{14}H_8F_3N_5O_3$: C, 43.8; H, 2.1; N, 18.2. Found: C, 43.5; H, 2.3; N, 18.4.

Antimycobacterial assay

The primary antimycobacterial evaluation was performed at the National Hansen's Disease Programs (NHDP) TAACF facilities, Baton Rouge, LA, USA. The screening was conducted at a single concentration of 6.25 μg/mL against *Mycobacterium tuberculosis* $H_{37}R_v$ (*ATCC 27294*) in BACTEC 12B medium using the Microplate Alamar Blue Assay (MABA) [19]. Compounds exhibiting fluorescence are tested in the BACTEC 460-radiometric system. The MIC is defined as the lowest concentration effecting a reduction in fluorescence of 90% relative to controls.

Determination of mammalian cell cytotoxicity (IC_{50})

Concurrent with the determination of MICs, compounds are tested for cytotoxicity (IC_{50}) in VERO cells at concentrations of 62.5 μg/mL or ten times the MIC for *M. tuberculosis* $H_{37}R_v$. After 72 h exposure, viability is assessed on the basis of cellular conversion of MTT to a formazan product with the Promega Cell Titer 96 Nonradioactive Cell Proliferation Assay.

Antibacterial activity

Authentic pure cultures of the microorganisms were obtained from the bacteriological lab. Botany Dept., Assiut University. Nutrient agar (15 mL) was poured into each of the sterilized plates (9 cm in diameter), then inoculated with 1 mL of the bacterial cell suspension, and the plates were shaken gently to homogenize and to dry. The antibacterial activity of the tested compounds was determined by modification of the paper disc diffusion method [22] as follows:

Sterile (6 mm) filter-paper disks (Whatman) were impregnated with solutions of the tested compounds and nalidixic acid (100 μg/mL in DMSO). In addition, other discs were impregnated with the solvent (DMSO) and served as control. The impregnated discs were then dried for 1 h and placed in the center of each plate. The seeded plates were incubated at 37°C. The radii of the inhibition zones (in mm) were measured after 48 h of the incubation period. Results are given in Table 2.

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