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SYNTHESIS OF 2-TRIFLUOROMETHYL-4,7-DIHYDRO-7-OXO-(1,2,4)TRIAZOLO[1,5-a]PYRIMIDINE-6-CARBOXYLIC ACID DERIVATIVES AS POTENTIAL ANTIMYCO-BACTERIAL AND ANTIMICROBIAL AGENTS

Nawal A. El-Koussi

Department of Medicinal Chemistry, Faculty of Pharmacy, Assiut University, Assiut 71526, Egypt

تم تشييد المركبات المستهدفة بتفاعل ٣ امينو ٥ ثلاثى الفلوروميثيل ١و٢و٤ تريازول مع ثنائى الايثيل ايثوكسى ميثلين مالونات ليعطى المركب ايثيل٢ ثلاثى الفلوروميثيل ٤و٧ ثنائى الهيدرو ٧ اوكسو(١و٢و٤ تريازول)[1,5-a] بيريميدين ٦ كربوكسيلات ٢. وبتفاعل المركب 2 مع هيدروكسيل امين هيدروكلوريد يعطى حمض هيدروكساميك 3 بينما يتفاعل المركب 2 مع الهيدرازين هيدرات يعطى الهيدرازيد 4. وتم تحضير قواعد شيف **5a-g** وكذلك ثيوسيميكربازيد **6a-f** للمركب 4 وقد اختبرت هذه المركبات ضد ميكروب الدرن واظهرالمركبان **5e** و**5 b** فاعلية وتم تعيين IC90 و IC50 لهذه المركبات. واختبرت الفاعلية كمضادات للبكتيريا والفطريات ووجد ان المركبات **3, 5a, 6b** اعلى فى الفاعلية من الامبيسلين ضد البكتيريا الموجبة والسالبة ولها فاعلية تتراوح من نصف الى سدس فاعلية حمض الناليديكسيك والمركبات **3, 5a, 5b, 5c, 5f, 6b** لها فاعلية ضد بسيدوموناس ايروجينوسا بينما حمض الناليدكسيك ليس له اى فاعلية والمركبات **3, 5a, 5b, 6b** لها فاعلية ضد الفطريات.

*Syntheses of the target compounds were achieved by reaction of 3-amino-5-trifluoromethyl-1,2,4-triazole **1** and diethyl-ethoxymethylenemalonate (DEEM) in glacial acetic acid to afford ethyl 2-(trifluoromethyl)-4,7-dihydro-7-oxo[1,2,4]-triazolo[1,5-a]pyrimidine-6-carboxylate **2**. Reaction of compound **2** with hydroxylamine hydrochloride gave hydroxamic acid **3**, while reaction with hydrazine hydrate in methanol gave the corresponding carbohydrazide **4**. Schiff bases of compound **4** with appropriate aldehyde yielded series **5a-g**. Refluxing of hydrazide **4** with appropriate isothiocyanate gave thiosemicarbazides **6a-f.***

*The antimycobacterial evaluation was determined against Mycobacterium tuberculosis $H_{37}Rv$(ATCC 27294). Compound **5e** and **5b** showed activity with IC90(6.672, 7.362 µg/ml respectively)*

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and IC50 (4.627, 6.382 µg/ml respectively). In vitro antibacterial screening for the prepared compounds were determined against certain strains of gram positive and gram negative bacteria. The results showed that compounds ***3, 5a, 6b*** *possessed higher activity than ampicillin against all strains, also the activity range from half to sixth activity of nalidixic acid against E. coli. Compounds* ***3, 5a, 5b, 5c, 5f, 6b*** *exhibited activity against P. aeruginosa, while nalidixic acid possessed no activity. Compounds* ***3, 5a, 5b*** *and* ***6b*** *possessed antifungal activity.*

INTRODUCTION

Fluorine-containing heterocyclic compounds have received considerable interest owing to their potent pharmacological activity[1&2].

Fluoroquinolones have attracted much attention because of their broad spectrum of activity against various bacteria, mycobacteria, and parasites[3].

Among the quinolone class of antibacterial agents, fluoroquinolones have shown promise in curing tuberculosis in combination therapy with anti-tuberculosis drugs. These fluoroquinolones are found to be highly concentrated in the host cells, which further enhance their antimycobacterial action. Fluoroquinolones act by interfering with the action of the bacterial DNA gyrase, which results in the degradation of the chromosomal DNA and leads to termination of chromosomal replication and interference with cell division and gene expression[4]. Antibacterial quinolones have attracted increasing attention as clinically useful drugs[5].

Several analogs in which the fused pyridine nucleus of nalidixic acid was replaced by a five membered ring system as thieno[6], isothiazolo[7], furo[8], pyrolo[9-12] and triazolo[13&14] have been reported to have a good antibacterial activity.

A new series of potential antibacterial agents which have a pyridone ring built on a 1,2,4-triazole nucleus was synthesized and evaluated *in vitro* for antibacterial activity[2]. In this research we used 3-amino-5-trifluoromethyl-1,2,4-triazole for the synthesis of new 2-trifluoromethyl-4,7-dihydro-7-oxo-(1,2,4)triazolo[1,5-a]pyrimidine-6-carboxylic acid derivatives, and evaluated their antimycobacterial and antimicrobial activity.

EXPERIMENTAL

Materials and Methods

Melting points were determined on electrothermal melting point apparatus and are uncorrected. Elemental microanalyses were performed on Perkin-Elmer, 240 Elemental Analyzer, at the central laboratory, Assiut University. TLC was carried out using silica gel 60 F_{254} precoated sheets (E. Merck,

Germany) and was visualized using UV lamp at 254 nm. IR spectra were recorded as KBr disks on a 470 Shimadzu IR Spectrophotometer at the Faculty of Pharmacy, Assiut University. ^{1}H -NMR spectra were recorded on a JEOL JNM-AL 300 FT NMR system. Chemical shifts were reported in parts per million (δ) relative to tetramethylsilane (TMS) as internal standard. DMSO-d_6 was used as solvent.

Chemistry

3-Amino-5-trifluoromethyl-1,2,4-triazole (1)

was prepared in 92% yield by reaction of trifluoroacetic acid with aminoguanidine bicarbonate as reported[15].

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

To a stirred solution of 3-amino-5-trifluoromethyl-1,2,4-triazole **1** (0.373 g, 0.0025 mol) in 5 ml glacial acetic acid, diethyl ethoxymethylene malonate (DEEM) (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 (0.48g, 70%). mp 280-281°C. IR (KBr) ν 3485, 3135, 1734, 1618, 1578, 1520, 1165, 788, 625, 587. ^{1}H-NMR (DMSO-d_6) δ: 9.80-8.80 (brs, 1H,NH*); 8.55-8.35 (s, 1H, C-H5); 4.28-3.85 (q, 2H, $\underline{CH_2}CH_3$); 1.30-0.90 ((t, 3H, CH_2-$\underline{CH_3}$). Anal. Calcd. for $C_9H_7F_3N_4O_3$: C, 39.1; H, 2.55; N, 20.3; Found C, 39.45; H, 2.4; N, 20.0 *exchangeable with D_2O

2-(Trifluoromethyl)-4,7-dihydro-7-oxo-1,2,4-triazolo[1,5-a]pyrimidine-6-hydroxamic acid (3)

To a freshly prepared solution of hydroxylamine [prepared from a solution of hydroxylamine hydrochloride (7 g, 0.1 mol) in methanol (30 ml) by addition while cooling to a solution of potassium hydroxide (7 g, 0.125 mol) in methanol (30 ml)], a solution of compound 2 (0.005 mol) in methanol (20 ml) was added portion-wise with stirring at room temperature. After complete addition the mixture was left at room temperature over night. The formed precipitate was filtered off and the potassium salt of hydroxamic acid was dissolved in mixture of water (15 ml), dilute acetic acid (5 ml) and the mixture was allowed to stand for three hours at room temperature. The solid separated was filtered, dried and crystallized from methanol. (Yield 84%, m.p. 226-7°C). IR(KBr) ν3430, 3315, 1647, 1578, 1555.

^{1}H-NMR (DMSO-d_6, δ ppm); 10.72 (s, 1H, CO$\underline{NH}$*); 8.85-8.60 (bs, 2H, $\underline{NH}$* of pyrimidine, $\underline{OH}$*); 7.13 (s, 1H, C-H5). *Exchangeable with D_2O

2-(Trifluoromethyl)-4,7-dihydro-7-oxo-1,2,4-triazolo[1,5-a]pyrimidine-6-carbohydrazide (4)

To a stirred ester 2 (0.83 g, 0.003 mol) in methanol (10 ml) hydrazine hydrate (85%) 0.43 ml (0.365g, 0.007 mol) was added. Stirring was continued at room temperature for 30 minutes and left overnight. The solvent was removed under vacuum, and the residue was crystallized from ethanol. (Yield 75%, m.p. 200-201°C). IR(KBr) ν 3430, 3315, 1668, 1578, 1520, 1165, 788, 625, 587.

^{1}H-NMR (DMSO-d_6, δ ppm); 10.3 (bs, 1H, CONH*), 8.92-8.72 (bs, 2H, NH* of pyrimidine, C-H5), 4.02 -3.58 (bs, 2H, $CONHNH_2$*) *Exchangeable with D_2O.

General method for the synthesis of Schiff bases 5a-g

A solution of **4** (0.188 g, 0.72 mmol) in EtOH (40 ml) and the appropriate aldehyde (0.76 mmol) was heated under reflux for 4 h. The precipitate obtained from the hot ethanolic solution or after cooling was purified by crystallization from EtOH. Physical data of the synthesized compounds are shown in Table 1.

N-Benzylidine-2-(trifluoromethyl)-4,7-dihydro-7-oxo-1,2,4-triazolo [1,5-a]pyrimidine-6-carbohydrazide (5a)

IR(KBr) ν 3440, 1713, 1627, 1535, 1508, 1163, 782, 614.

^{1}H-NMR (DMSO-d_6, δ ppm); 12.32 (s, 1H, CONH*), 8.82 (s, 1H, NH*of pyrimidine), 8.23(s, 1H, C-H5), 7.95 (s, 1H, N=CH), 7.48-7.37 (m, 5H, ArH). *Exchangeable with D_2O

N-(*p*-Methoxybenzylidine)-2-(trifluoromethyl)-4,7-dihydro-7-oxo-1,2,4-triazolo[1,5-a]pyrimidine-6-carbohydrazide (5b)

IR(KBr) ν 3440, 1690, 1630, 1607, 1529, 1504, 1180, 788, 614.

^{1}H-NMR (DMSO-d_6, δ ppm); 11.49 (s, 1H, CONH*), 8.82 (s, 1H, NH*of pyrimidine), 8.34 (s, 1H, C-H5), 7.93 (s, 1H-N=CH), 7.63-7.57 (d, 2H, ArH), 6.99-6.96 (d, 2H, ArH), 3.78 (s, 3H, OCH_3). *Exchangeable with D_2O

N-(3,5-Dimethoxybenzylidine)-2-(trifluoromethyl)-4,7-dihydro-7-oxo-1,2,4-triazolo [1,5-a]pyrimidine-6-carbohydrazide (5c)

IR(KBr) ν 3440, 1680, 1636, 1605, 1535, 1504, 1180, 775, 635.

^{1}H-NMR (DMSO-d_6, δ ppm); 12.90 (s, 1H, CONH*), 9.54 (s, 1H, NH* of pyrimidine), 7.90 (s, 2H, N=CH, C-H5), 7.75-7.54 (m, 2H, ArH), 7.43-7.40 (m, 1H, ArH), 3.80 (s, 6H, 2(OCH_3)). *Exchangeable with D_2O

N-(4-Hydroxy-3-methoxybenzylidine)-2-(trifluoromethyl)-4,7-dihydro-7-oxo-1,2,4-triazolo[1,5-a] pyrimidine-6-carbohydrazide (5d)

IR(KBr) ν 3530, 3390, 1629, 1535, 1504, 1165, 788, 614.

^{1}H -NMR (DMSO-d_6, δ ppm); 10.72 (s, 1H, CONH*), 9.74 (bs, 1H, OH*), 8.88 (s, 1H, NH* of pyrimidine), 8.80 (s, 1H, C-H5), 7.83

(s, 1H, N=CH), 7.36-7.34 (m, 1H, ArH), 7.24-7.20 (m, 1H, ArH), 6.98-6.90 (m, 1H, ArH), 3. 90-3. 80 (s, 3H, OCH_3). *Exchangeable with D_2O.

N-(*p*-Dimethylaminobenzylidine)-2-(trifluoromethyl)-4,7-dihydro-7-oxo-1,2,4-triazolo [1,5-a]pyrimidine-6-carbohydrazide (5e)

IR(KBr) ν 3525, 3390, 1626, 1515, 1171, 788, 612.

^{1}H-NMR (DMSO-d_6, δ ppm); 11.90 (1H, CONH*), 8.88 (s, 1H, NH* of pyrimidine), 8.67 (s, 1H, C-H5), 7.59 (s, 1H, N=CH), 7.66-7.53 (d, 2H, ArH), 6.72-6.63 (d, 2H, ArH), 2.94-2.87 (s, 6H, $N(CH_3)_2$). *Exchangeable with D_2O.

N-(*p*-Chlorobenzylidine)-2-(trifluoromethyl)-4,7-dihydro-7-oxo-1,2,4-triazolo[1,5-a]pyrimidine-6-carbohydrazide (5f)

IR(KBr) ν 3440, 3390, 1627, 1568, 1536, 1508, 1171, 788, 617.

^{1}H-NMR (DMSO-d_6, δ ppm); 11.69 (s, 1H, CONH*), 8.88 (s, 1H, NH* of pyrimidine), 8.80 (s, 1H, C-H5), 7.97 (s, 1H, N=CH), 7.71-7.65 (d, 2H, ArH), 7.58-7.47 (d, 2H, ArH). *Exchangeable with D_2O.

N-((Pyridin-3-yl)methylene-2-(trifluoromethyl)-4,7-dihydro-7-oxo-1,2,4-triazolo [1,5-a]pyrimidine-6-carbohydrazide (5g)

IR(KBr) ν 3440, 3390, 1629, 1565, 1536, 1506, 1171, 785, 612.

^{1}H-NMR (DMSO-d_6, δ ppm); 12.33 (s, 1H, CONH*), 8.92 (s, 1H, NH* of pyrimidine), 8.85 (s, 1H, C-H5), 8.64-8.61 (m, 2H, pyr), 8.48 (s, 1H, pyr), 8.24-8.20 (d, 1H, pyr), 7.58 (s, 1H, N=CH). *Exchangeable with D_2O.

N^4-Substituted-2-(trifluoromethyl)-4,7-dihydro-7-oxo-[1,2,4]triazolo [1,5-a]pyrimidine-6-carboxylic acid thiosemicarbazides 6a-f

To a stirred hydrazide **4** (2.62 g, 0.01 mol) in ethanol (40 ml); a solution of the appropriate isothiocyanate (0.01 mol) in ethanol (10 ml) was added. The mixture was refluxed for two hours. The reaction mixture was cooled and the separated solid was filtered, washed with ethanol and crystallized from appropriate solvent. Physical data are listed in Table 1.

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

IR(KBr) ν 3435, 3390, 1630, 1607, 1533, 1502, 1171, 785, 607.

^{1}H-NMR (DMSO-d_6, δ ppm); 10.85 (br. s, 1H, CONH*); 9.45 -9.30 (br. s, 1H, NH*CS); 8.72 (s, NH* of pyrimidine), 8.30 (m, 1H, NH*CH_3); 8.18 (s, 1H, C-H5), 3.30 (s, 3H, CH_3). *Exchangeable with D_2O.

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

IR; 3438, 3390,1627, 1607, 1535, 1502, 1175, 785, 612.

^{1}H–NMR (DMSO-d_6, δ ppm); 10.90 (br. s, 1H, CONH*); 9.60 (br. s, 1H, NH*CS), 8.72-8.65 (s, 1H, NH* of pyrimidine), 8.22 (t, 1H,

NH*CH_2), 7.85 (s, 1H, C-H5), 3.80-3.70 (m, 2H, CH_2 CH_3); 1.24 -1.15 (t, 3H, CH_2CH_3) *Exchangeable with D_2O.

Table 1: Physical data of the target compounds.

Compd. No.	R	M.P [°C]	Yield [%]	Molecular Formula (M.Wt)	Microanalysis Calcd./Found C	H	N
3	-NHOH	226-7 Ethanol	70	$C_7H_4F_3N_5O_3$ 263.13	31.95 31.72	1.53 1.43	6.62 6.41
4	-$NHNH_2$	200-01 Ethanol	75	$C_7H_5F_3N_6O_2$ 262.15	32.07 32.43	1.92 2.22	2.06 2.48
5a	-NHN=CH-C_6H_5	236-7 Ethanol	73	$C_{14}H_9F_3N_6O_2$ 350.26	48.01 48.35	2.59 2.73	3.99 4.18
5b	-NHN=CH-*p*-$OCH_3C_6H_4$	254-6 Ethanol/ DMF	83	$C_{15}H_{11}F_3N_6O_3$ 380.28	47.38 47.53	2.92 3.21	22.10 22.41
5c	-NHN=CH-3,5-$(OCH_3)_2C_6H_3$	257-8 Ethanol/ DMF	98	$C_{16}H_{13}F_3N_6O_4$ 410.31	46.84 46.75	3.19 3.27	20.48 20.56
5d	-NHN=CH-3-OCH_3-4-OH-C_6H_3	239-40 Ethanol/ DMF	94	$C_{15}H_{11}F_3N_6O_4$ 396.28	45.46 45.57	2.80 2.99	21.21 21.54
5e	-NHN=CH-*p*-$N(CH_3)_2$ C_6H_4	265-6 Ethanol/ DMF	73	$C_{16}H_{14}F_3N_7O_2$ (393.32	48.86 48.73	3.59 3.42	24.93 24.76
5f	-NHN=CH-*p*-ClC_6H_4	246-7 Ethanol/ DMF	90	$C_{14}H_8ClF_3N_6O_2$ 384.70	43.71 43.52	2.10 2.34	21.85 21.74
5g	-NHN=CH-C_5H_4N	288-9 Ethanol/ DMF	65	$C_{13}H_8F_3N_7O_2$ 351.24	44.45 44.82	2.30 2.54	27.91 27.80
6a	-$NHNHCSNHCH_3$	233-5 Ethanol	72	$C_9H_8F_3N_7O_2S$ 335.27	32.24 32.51	2.41 2.31	29.24 29.55
6b	-$NHNHCSNHC_2H_5$	221-3 Ethanol	60	$C_{10}H_{10}F_3N_7O_2S$ 349.29	34.39 34.65	2.89 2.64	28.07 28.31
6c	-$NHNHCSNHC_3H_5$	197-8 Ethanol	40	$C_{11}H_{10}F_3N_7O_2S$ 361.30	36.57 36.74	2.79 2.63	7.14 7.02
6d	-$NHNHCSNHC_6H_5$	203-4 Ethanol	60	$C_{14}H_{10}F_3N_7O_2S$ 397.34	42.32 42.68	2.54 2.98	24.68 25.02
6e	-NHNHCSNH-*p*-CH_3 C_6H_4	194-6 Ethanol	60	$C_{15}H_{12}F_3N_7O_2S$ 411.36	43.80 43.56	2.94 2.53	23.83 24.11
6f	-NHNHCSNH-c.hexyl	210-2 Ethanol	50	$C_{14}H_{16}F_3N_7O_2S$ 403.38	41.68 42.03	4.00 4.31	24.31 24.66

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

IR(KBr) ν 3438, 3395, 1654, 1631, 1541, 1503, 1181, 785, 613.

^{1}H-NMR (DMSO-d_6, δ ppm); 10.51 (s, 1H, CONH*), 9.31 (s, 1H, NH*CS), 8.72 (s, NH* of pyrimidine), 8.18 (s, 1H, C-H5), 5.79-5.75 (m, 1H, CH=CH_2), 5.10-5.01 (m, 2H, CH= CH_2), 4.10-4.00 (m, 2H, NH-CH_2). *Exchangeable with D_2O.

N^4- Phenyl- 2-(trifluoromethyl)-4,7-dihydro-7-oxo[1,2,4]triazolo[1,5-a] pyrimidine-6- carboxylic acid thiosemicarbazide 6d

IR(KBr) ν 3535, 3390, 1652, 1629, 1540, 1505, 1185, 784, 612.

^{1}H -NMR (DMSO-d_6, δ ppm); 10.51 (s, 1H, CONH*), 9.78 (s, 1H, NH*CS), 8.75 (s, 1H, NH* of pyrimidine), 8.74-8.70 (m, 2H, NH*-ph, C-H5), 7.27-7.09 (m, 5H, ArH). *Exchangeable with D_2O.

N^4- *p*-Tolyl -2-(trifluoromethyl)-4,7-dihydro-7-oxo[1,2,4] triazolo [1,5-a] pyrimidine-6-carboxylic acid thio-semicarbazide 6e

IR(KBr) ν 3435, 3390, 1630, 1607, 1533, 1502, 1181, 783, 607.

^{1}H -NMR (DMSO-d_6, δ ppm); 10.50 (s, 1H, CONH*), 9.72 (s, 1H, NH*CS), 8.75 (s, 1H, NH* of pyrimidine), 8.59 (s, 1H, C-H5), 7.90 (s, 1H, NH*-ph), 7.40-7.37 (d, 2H, ArH), 7.15-7.12 (d, 2H, Ar H), 2.26-2.25 (s, 3H, CH_3) *Exchangeable with D_2O.

N^4-Cyclohexyl-2-(trifluoromethyl)-4,7-dihydro-7-oxo[1,2,4]triazolo [1,5-a]pyrimidine-6-carboxylic acid thiosemicarbazide 6f

IR(KBr) ν 3525, 3390, 1628, 1647, 1549, 1525, 1165, 610.

^{1}H -NMR (DMSO-d_6, δ ppm); 10.50 (br. s, 1H, CONH*); 9.60 (br. s, 1H, NH*CS); 8.70 (s, 1H, NH* of pyrimidine), 8.59 (s, 1H, C-H5), 4.20 (br. s, 1H, NH*C_6H_{11}); 2.00-1.00 (m, 11H, C_6H_{11}) *Exchangeable with D_2O.

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}Rv(ATCC 27294) in BACTEC 12B medium using the Microplate Alamar Blue Assay (MABA). Compounds exhibiting fluorescence are tested in BACTEC 460-radiometric system[16]. Compounds effecting <90% inhibition in the primary screen are not evaluated further.

Antimicrobial assay

The synthesized compounds were screened *in vitro* for their anti-bacterial activity against the following strains *Escherichia coli* (*E. coli*, ATCC 25922), *Serratia marcescens*, *DMS* 1608, *Pseudomonas aeruginosa* as gram negative bacteria. *Bacillus cereus*, and *Staphylococcus aureus* ATCC

25923 as Gram positive bacteria. Antifungal activity against *Aspergillus niger*, *Aspergillus flavus*, *Candida albicans*, *Scopulariopsis brevicaulis*, *Fusarium oxysporum* and *Geotrichum candidum*.

Method

Solutions of the tested compounds, ampicillin trihydrate, nalidixic acid and clotrimazole were dissolved in DMSO and were tested at a concentration 2000 µg /ml. The twofold dilution of the compounds and references were prepared. Cultures were grown on nutrient agar medium containing peptone 5 g/l, beef extract 3 g/l and NaCl 3 g/l. The mixture was incubated at 121°C and 1.5 atm for 20 min, distributed in sterile plates (20 ml per plate) and allowed to solidify. The tested bacterial or fungal species were firstly grown for 48 hr, then 1ml of each suspension was poured on the solidified agar medium and thoroughly distributed on agar surface. Cups were made in the solidified agar (6/plate) with the aid of sterile cork borer. Aliquots of 0.1 ml of solutions of the tested compounds as well as the reference drugs were pipette into the appropriate cup. The last cup was used as control for the solvent. The plates were left for one hr at room temperature to attain prediffusion. After 24 hr of incubation at 37° for bacteria and 48 hr of incubation at 30° for fungi, the diameters of inhibition zones(mm) were measured and the minimal inhibitory concentrations (MICs) were determined. MICs were recorded as the minimum concentration of a compound that visually inhibits the growth of tested microorganisms. The results are summarized in Table **3**.

RESULTS AND DISCUSSION

Chemistry

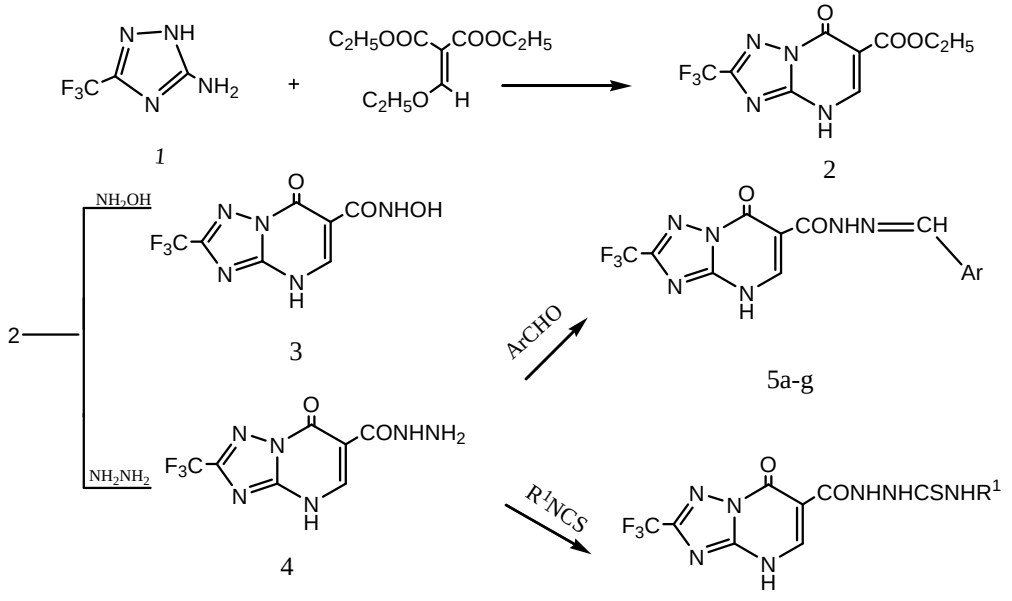
The synthesis of the target compounds was achieved by the route depicted in Scheme 1. Reaction of 3-amino-5-trifluoromethyl-1,2,4-triazole **1** with diethyl ethoxymethyl-enemalonate (DEEM) in glacial acetic acid afforded ethyl 2-(trifluoromethyl)-4,7-dihydro-7-oxo [1,2,4]-triazolo[1,5-a]pyrimidine-6-carboxylate **2** as a single product, as evident by TLC and IR spectra by the appearance of two CO group. Compound **2** gave hydroxamic acid **3** when reacted with hydroxylamine hydrochloride**.** Reaction of compound **2** with hydrazine hydrate in methanol afforded the corresponding hydrazide **4**. Condensation of hydrazide **4** with aromatic aldehydes led to corresponding Schiff bases **5 a-g**. The structures were confirmed on the basic of elemental analyses, spectral data. ^{1}H-NMR showed downfield signals at δ 10.72-12.9 (CONH), 7.95-7.58 (N=CH) and disappearance of signals of NH_2 in addition of aromatic protons which appeared at the expected chemical shifts. Refluxing of hydrazide **4** with appropriate isothiocyanate gave thiosemicarbazides **6a-f**. The structures were confirmed on the

basic of elemental analyses, spectral data. ^{1}H-NMR showed signals at δ 10.9-10.5 (CONH), 9.3-9.78 (NHCS), and 4.00-8.74 (N^4-H). All other aromatic and aliphatic protons were observed at expected regions. Physical data of the synthesized compounds are shown in Table 1.

Antimycobacterial activity

The synthesized compounds **3,4,5(a-g)** and **6(a-f)** were tested for their primary antimycobacterial activity against *Mycobacteria tuberculosis* H_{37}Rv, IC90 and IC50 were determined as shown in Table 2.

Compound **5e** and **5b** showed activity with IC90 (6.672, 7.362 µg/mL respectively) and IC50 (4.627, 6.382 µg/mL respectively) while compounds **5c** and **5d** showed weak activity with IC90 >100 and IC50 (82.237, 29.67). The remaining compounds showed no activity. From the above results we can conclude that Schiff bases as a unit appears to be a useful scaffold for the *antimycobacterial* agents.



Scheme 1: Synthesis of the target compounds.

Table 2: Antitubercular *in vitro* activity of the test compounds expressed as IC90, IC50 of *mycobacterium tuberculosis* $H_{37}Rv$.

Compd. No.	Sample ID	Assay	IC90	IC50	Activity
3	408898	MABA	>100	>100	Inactive
4	408897	MABA	>100	>100	Inactive
5a	408905	MABA	>100	>100	Inactive
5b	408907	MABA	7.362	6.382	Active
5c	408908	MABA	>100	82.237	Weakly active
5d	408909	MABA	>100	29.670	Weakly active
5e	408906	MABA	6.672	4.627	Active
5f	408910	MABA	>100	>100	Inactive
5g	408911	MABA	>100	>100	Inactive
6a	408899	MABA	>100	>100	Inactive
6b	408900	MABA	>100	>100	Inactive
6c	408901	MABA	>100	>100	Inactive
6d	408902	MABA	>100	>100	Inactive
6e	408903	MABA	>100	>100	Inactive
6f	408904	MABA	>100	>100	Inactive

Conc µg/mL

IC50, IC90: is the concentration where a drug inhibits the TB strain by 50% or 90%. Compounds are considered active if IC90 ≤ 10 µg/mL.

Antimicrobial activity

The synthesized compounds were tested for their *in-vitro* antimicrobial activity against *Escherichia coli* (E. coli, ATCC 25922), *Serratia marcescens*, DMS 1608, *Pseudomonas aeruginosa* as gram negative bacteria. *Bacillus cereus*, and *Staphylococcus aureus* ATCC 25923 as Gram positive bacteria. Antifungal activity against *Aspergillus niger*, *Aspergillus flavus*, *Candida albicans*, (WT-S) *Scopulariopsis brevicaulis*, *Fusarium oxysporum* and *Geotrichum candidum* by the method described by Kwon-Chung and Bennett[17]. The tesed compounds were allowed to diffuse readily from wells into the medium inoculated with micro-organism. Ampicillin trihydrate, Nalidixic acid and Clotrimazole were used as standard antibacterial and antifungal agents, respectively. As shown in Table 3. Some of the compounds tested were illustrated significant antibacterial and anti-fungal activity when compared with reference drugs.

Table 3: Antimicrobial activity of the synthesized compounds.

In vitro activity-zone of inhibition in mm(MIC in µg /ml)											
No.	*E. Coli*	*S. marcescens*	*B. cereus*	*P. aeruginosa*	*Staf. Aureus*	*Asp niger*	*Asp. flavus*	*Can. albicans*	*Scop. brevicaulis*	*Fus. Oxysporum*	*Geo. candidum*
3	12(75)	12(125)	16(250)	30(1000)	10(250)	16(250)	10(25)	12(500)	10(250)	-	16(125)
5a	16(25)	16(25)	16(50)	8(125)	12(500)	14(250)	10(250)	8(500)	18(250)	22(500)	14(75)
5b	8(250)	8(500)	8(250)	8(250)	-	-	-	-	-	-	-
5c	8(50)	10(500)	10(250)	10(250)	-	-	-	-	-	-	-
5d	-	20(125)	-	-	12(125)	-	-	12(50)	14(250)	-	10(50)
5e	-	-	-	-	-						
5f	12(500)	16(500)	17(500)	19(500)	14(500)	-	-	-	10(500)	-	-
5g	11(50)	10 (125)	-	-	-						
6b	10(75)	12(125)	12(75)	8(250)	-	12(500)	14(500)	12(75)	-	8(500)	8(250)
6c	-	-	-	-	-						
6d	-	-	-	-	-	-	-	-	-	-	-
6e						-	-	-	-	-	-
Nali	14(12.5)	20(12.5)	20(25)	-	26(25)						
Ampi	12(500)	-	8(250)	-	12(500)						
Clotri						22(25)	21(25)	20(25)	26(25)	22(25)	21(25)

The MIC values were generally within the range of (25-500 μg /mL) against all evaluated strains. In comparing their MIC values, compounds **3, 5a, 6b** showed higher activity than ampicillin against almost strains, and activity range from half to sixth activity of nalidixic acid against E. coli. Compounds **5b**, **5c, 5d, 5f, 5g** showed higher activity or similar activity as ampicillin against certain strains as shown in Table 2. Compounds **3, 5a, 5b, 5c, 5f, 6b** showed activity against *P. aeruginosa*, while nalidixic acid possessed no activity. Compounds **3, 5a, 5d, 6b** possess antifungal activity with MIC values range from (75-500 μg /mL) which is comparable to or less than clotrimazole against *A. flavus*, *G. candidum* and *C. albicans*.

Conclusion

It was found that the most active derivative of the tested compounds against certain strains of bacteria and fungi was 2-(trifluoromethyl)-4,7-dihydro-7-oxo-1,2,4-triazolo[1,5-a] pyrimidine-6-hydroxamic acid **3**. The Schiff bases bearing electron donating groups in *p*-position of the phenyl ring possessed good antimycobacterial activity (**5e** and **5b**). The unsubstituted Schiff base, N-Benzylidine-2-(trifluoromethyl)-4,7-dihydro-7-oxo-1,2,4-triazolo[1,5-a] pyrimidine-6-carbohydrazide **5a** was found to be the most active member of series **5,** substitution on the phenyl ring decrease activity. In thio-semicarbazide series **6,** it was found that compound **6b** bearing ethyl group at N^4 of thiosemicarbazide was the only active compound against bacteria and fungi.

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