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Ethyl 1-Aminotetrazole-5-carboxylate로부터 유도된 헤테로고리 화합물들의 항균 활성 시험

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Antimicrobial Assessment of Some Heterocyclic Compounds Utilizing Ethyl 1-Aminotetrazole-5-carboxylate

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요약. Ethyl 1-aminotetrazole-5-carboxylate (**1**)를 hydrazine hydrate와 반응시켜서 대응하는aminohydrazide **2**를 합성한 후에, 화합물 **2**를carbon disulfide와 반응시켜서 1,3,4-oxadiazole-5-thiol structure **3**을 합성하였다. 얻어진 화합물 **3**을 either chloroacetone 또는ethyl chloroacetate와 반응시켜서S-acyl 1,3,4-oxadiazole 유도체인 **4** 와 **5**를 합성하였으며, 또한 hydrazine hydrate와 반응시켜서 4-amino-1,2,4-triazole-5-thiol 유도체인 **6**을 합성하였으며, 화합물 **6**을 glacial acetic acid와 반응시켜서 6-methyl-1,3,4-triazolo[3,4-*b*]-1,3,4-thiadiazole (**7**)을 합성하였다. 한편, 알려진 방법에 따라서, 화합물 **1**로부터 tetrazolo[5,1-*f*]-1,2,4-triazine **9**을 얻은 다음에, 화합물 **9**를 carbon disulfide와 반응시켜서 8-thione 유도체인 **10**을 합성한 후에, 대응하는 화합물 **11**, **12** 및 **13**을 합성하였다. 얻어진 화합물**13**을 이용하여1,2,4-triazolo[4,3-*d*]tetrazolo[5,1-*f*]-1,2,4-triazines **14**와 **15**를 합성하였다. 새롭게 합성한 화합물들의 화학구조를 확인하였으며, 합성한 화합물들에 대한 항균 활성시험을 수행하였다.

주제어: 1-Aminotetrazole-5-carboxylate, 1,3,4-Oxadiazoles, 1,2,4-Triazole, Oxazole, Fused heterocyclic ring systems, 항균 활성

ABSTRACT. Ethyl 1-aminotetrazole-5-carboxylate (**1**) reacted with hydrazine hydrate to give the corresponding aminohydrazide **2**. Cyclization of **2** by carbon disulfide yielded 1,3,4-oxadiazole-5-thiol structure **3**. Reaction of **3** with either chloroacetone or ethyl chloroacetate furnished S-acyl 1,3,4-oxadiazole derivatives **4** and **5**, respectively. Also compound **3** reacted with hydrazine hydrate afforded 4-amino-1,2,4-triazole-5-thiol derivative **6**. 6-Methyl-1,3,4-triazolo[3,4-*b*]-1,3,4-thiadiazole structure **7** was synthesized by reaction of aminothiol **6** with glacial acetic acid. Diazotization of **1** with sodium nitrite in presence of hydrochloric acid yielding the diazonium salt which on treating with hippuric acid, oxazolone derivative **8** was obtained. Furthermore, tetrazolo[5,1-*f*]-1,2,4-triazine **9** was constructed *via* cyclization of aminoester **1** with formamide. Compound **9** reacted with carbon disulfide to furnish 8-thione derivative **10** which reacting with chloroacetone, ethyl chloroacetate, and hydrazine hydrate, the corresponding chemical structures **11**, **12**, and **13** were synthesized. 1,2,4-Triazolo[4,3-*d*]tetrazolo[5,1-*f*]-1,2,4-triazines **14** and **15** were resulted by treating of compound **13** with triethyl orthoformate, and glacial acetic acid, respectively. The structures of the newly synthesized products were elucidated according to elemental analyses and spectroscopic evidences. Some of the representative members of the prepared compounds were screened for antimicrobial activity.

Keywords: 1-Aminotetrazole-5-carboxylate, 1,3,4-Oxadiazoles, 1,2,4-Triazole, Oxazole, Fused heterocyclic ring systems, Antimicrobial activity

INTRODUCTION

Tetrazoles and their derivatives have received great attention because of their wide range of therapeutic and biological properties.[1,2] They have emerged as antibacterial,[3-6] antiproliferation,[7] anticancer,[7] and anticonvulsant[8] agents. In this article, it is our intention to enlarge the area of the investigation towards tetrazole framework and to evaluate

antimicrobial activities.

RESULTS AND DISCUSSION

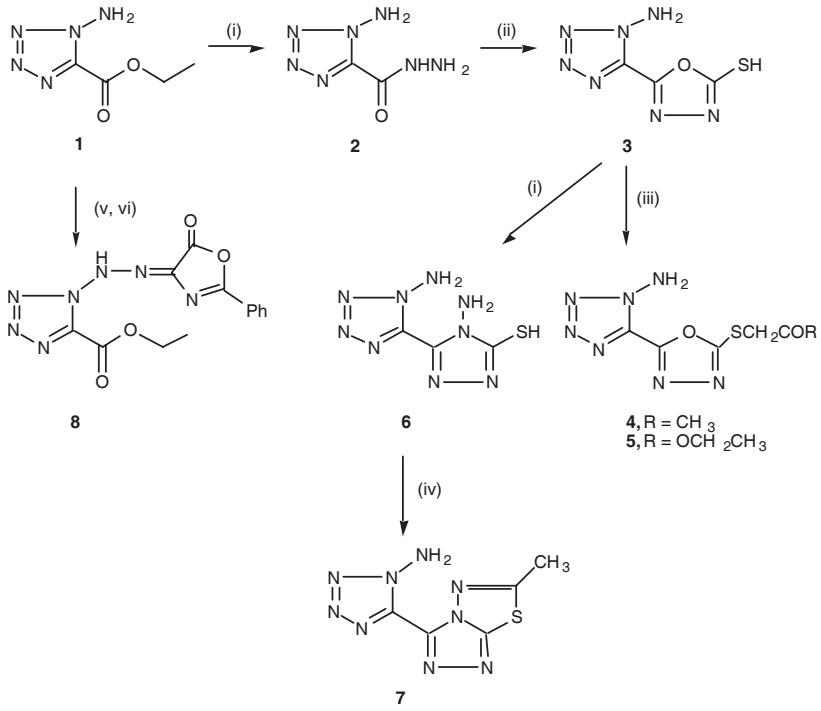
The reaction of ethyl 1-aminotetrazole-5-carboxylate (**1**) with hydrazine hydrate afforded the corresponding 1-aminotetrazole-5-carbohydrazide (**2**), which upon treating with carbon disulfide in pyridine catalyzed, gave 1-amino-5-(5-mercapto-1,3,4-oxadiazol-2-yl)tetrazole (**3**). The formation [5,9,10] of **3** from **2** was supported by elemental analyses and by the absence of absorption bands at 3200 and 1670 cm^{-1} for the respective stretching vibration of NH and CON in its IR spectrum. The acylation of 5-mercapto-1,3,4-oxadiazolyl structure **3** with chloroacetone or ethyl chloroacetate in ethanol containing anhydrous sodium acetate led to the direct formation of S-acylated 1,3,4-oxadiazolyl derivatives **4** and **5**, respectively (*Scheme* 1).

The 1,3,4-oxadiazolyl **3** was also used[5,11] to synthesize 1-amino-5-(4-amino-5-mercapto-1,2,4-triazol-3-yl)tetrazole **(6)** by its reaction with hydrazine hydrate. The mass spectrum of **6** showed molecular ion peak at 198.86 which was in agreement with molecular formula $C_3H_5N_9S$ (*m/z* = 199). The 4-amino-5-mercapto-1,2,4-triazolyl compound **6** was subjected to build a new 1,2,4-triazolo[3,4-b]-1,3,4-thiadiazole derivative **7** *via* the condensation with glacial acetic acid. The ^{1}H NMR spectrum of **7** revealed the presence of signal characteristic of methyl group at 1.94 ppm. This assignment is in harmony with the reported results.[5,12]

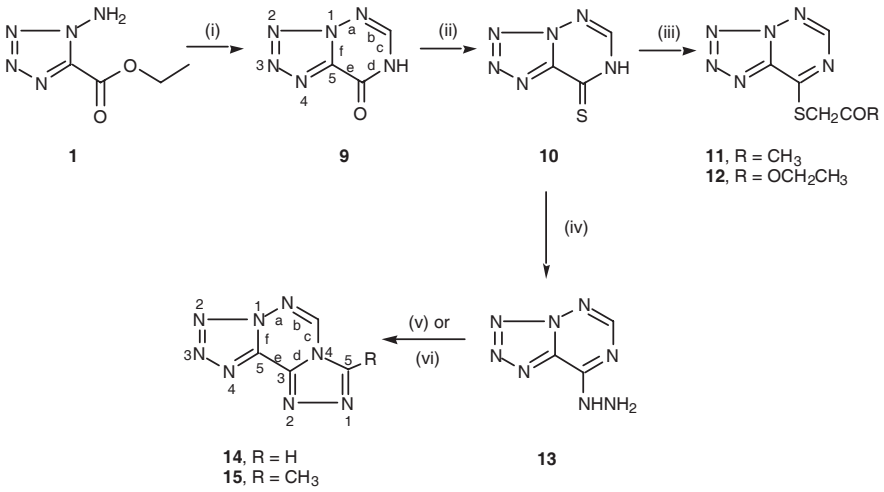
5(4H)-Oxazolones represented[13-16] an important class of heterocycles and interested in their chemistry and continues unabated because of their usefulness as synthons. Thus, diazotization of **1** with sodium nitrite in the presence of hydrochloric acid yielding the diazonium salt which on treating with hippuric acid and acetic anhydride mixture in the presence of sodium acetate trihydrate affording the ethyl 1-[N-(5-oxo-2-phenyloxazol-4-ylidene)-hydrazono] tetrazole-5-carboxylate (**8**).

The aminoester **1** was condensed with formamide to produce tetrazolo[5,1-*f*]-1,2,4-triazin-8(7H)one (**9**) which in turn was subjected to react with carbon disulfide in pyridine to give 8 (7H) thinone derivative **10**. The ^{1}H NMR spectrum revealed an exchangeable imino proton at 11.65 ppm and absence of SH proton signal, thus indicating its existence as the thione tautomer. The latter was reacted with chloroacetone or ethyl chloroacetate in ethanol containing anhydrous sodium acetate to provide the corresponding compounds **11** and **12**. The thione **10** was also used to synthesize 8-hydrazino derivative **13** by its reaction with hydrazine hydrate.

Finally, the hydrazine **13** was converted into 1,2,4-tria-



***Scheme* 1.** (i) $NH_2NH_2{\cdot}H_2O$; (ii) CS_2/ppyridine; (iii) $ClCH_2COR/CH_3COONa$; (iv) CH_3COOH; (v) $NaNO_2$/HCl; (vi) $PhCONHCH_2COOH/(CH_3CO)_2O/CH_3COONa$



***Scheme* 2.** (i) $HCONH_2$; (ii) CS_2/ppyridine; (iii) $ClCH_2COR/CH_3COONa$; (iv) $NH_2NH_2{\cdot}H_2O$; (v) $HC(OCH_2CH_3)_3$; (vi) CH_3COOH

zolo[4,3-*d*]tetrazolo [5,1-*f*]-1,2,4-triazine derivatives **14** and **15** by refluxing with triethyl orthoformate or glacial acetic acid (*Scheme* 2). Elemental and spectra data of the latter compounds are consistent with the structure assigned to their compounds (*cf.* Experimental).

ANTIMICROBIAL ACTIVITY

Ten compounds were selected and evaluated *in vitro* for their antimicrobial properties against *Gram*- positive (*Staphylococcus aureus*) and *Gram* negative bacteria (*Escherichia coli*) in addition to a fungus (*Candida albicans*) microorganisms. The activities of these compounds were used for the determination [17,18] of antibacterials and antifungal activity is summarized in *Table* 1 showing that compounds **4**, **7**, **8**, **10** and **15** exhibited activity comparable 25% of the ampicillin against *S. aureus* while the effect of **6**,**10** and **15** were 50% of the ampicillin against *E. coli*, the activity of **5** and **12** and both **4, 13** and **15** were 50% against *C. albicans* comparable to clotrimazole, respectively. The compound **3** possessed lower activity than the reference standards (ampicillin and clotrimazole) against the test organisms.

***Table* 1.** Antimicrobial Activity of Synthesized Compounds (*MIC* in μg/mL)

Compound No.	*S. aureus*	*E. coli*	*C. albicans*
3	100	> 200	100
4	50	> 200	50
5	100	100	25
6	> 200	50	> 200
7	50	100	100
8	50	> 200	100
10	50	50	> 200
12	> 200	> 200	25
13	100	100	50
15	50	50	50
Ampicillin	12.5	25	—
Clotrimazole	—	—	12.5

EXPERIMENTAL

All melting points were determined in capillary tubes in a stuart scientific SMP1 apparatus. IR spectra were recorded on a satellite 1000 spectrophotometer using KBr wafer technique. ^{1}H NMR spectra were obtained on a Varian Mercury VXR 3000 and Jeol ECA 500 spectrometers and chemical shifts are expressed in δ/ppm using *TMS* as an internal strandard. MS were recorded on a HP model MS-5988 spectrometer at electron ionizing energy of 70 eV. Microanalyses were performed by the Microanalytical Unit, Cairo University, Egypt. Compound **1** was synthesized[19] from benzaldehyde hydrazone with ethyl triethoxyacetate in presence of sodium azide followed by hydrolysis with concentrated hydrochloric acid.

1-Aminotetrazole-5-carbohydrazide (2)

A mixture of 1.51 g **1** (0.01 mol) and 2 mL hydrazine hydrate was refluxed in 15 mL methanol for 3 h. The solid separated from the hot mixture was filtered off and recrystallized from methanol to give 1.23 g (89%) of **2**; mp. 275 - 277 °C; IR (KBr): $\bar{\nu}$ = 3400, 3350 (NH_2), 3290 (NH), 1670 (CO) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ = 10.80 (s, 1H, NH), 5.74, 4.30 (2s, 2H each, $2NH_2$) ppm. Found: C,16.45; H, 3.30; N, 68.40%. $C_2H_5N_7O$ (143) required: C, 16.78; H, 3.50; N, 68.53%.

1-Amino-5-(5-mercapto-1,3,4-oxadiazol-2-yl) tetrazole (3)

A mixture of 1.12 g **2** (0.008 mol) and 5 mL carbon disulfide in 15 mL pyridine was heated on a water bath for 3 h, and then cooled. The separated product was filtered off, washed several times with ethanol and recrystallizred from methanol to give 1.15 g (79%) of **3**; mp. 230 - 232 °C; IR (KBr): $\bar{\nu}$ = 3390, 3350 (NH_2), 2550 (SH), cm^{-1}; ^1H NMR (DMSO-d_6): δ = 5.81 (s, 2H, NH_2), 3.70 (s, 1H, SH) ppm. Found: C,19.22; H, 1.31; N, 53.22 %. $C_3H_3N_7OS$ (185) required: C, 19.46; H, 1.62; N, 52.97 %.

1-Amino-5(5-acetonylthio-1,3,4-oxadiazol-2-yl) tetrazole (4)

A mixture of 0.72 g **3** (0.004 mol) and 0.36 g chloroacetone (0.004 mol) in 15 mL ethanol containing 2 g anhydrous sodium acetate was refluxed for 1 h. After cooling, the collected product was filtered off, washed with water and recrystallized from methanol to yield 0.53 g (56%) of **4**, mp. 220 - 222 °C; IR (KBr): $\bar{\nu}$ = 3400, 3350 (NH_2), 1750 (CO) cm^{-1}; ^1H NMR (DMSO-d_6): δ= 5.70 (s, 2H, NH_2), 4.25 (s, 2H, CH_2), 2.30 (s, 3H, CH_3) ppm; MS: *m/z* (%) = 242 (M^+ + 1, 28). Found : C, 30.22; H, 3.23; N, 40.43%. $C_6H_7N_7O_2S$ (241) required: C, 29.88; H, 2.91; N, 40.66%.

1-Amino-5-(5-ethoxycarbonylmethylthio-1,3,4-oxadiazol-2-yl) tetrazole (5)

Compound **5** was prepared form **3** 0.54 g (0.003 mol) and 0.40 g ethyl chloroacetate (0.003 mol) as previously described for the preparation of **4**. It was crystallized from methanol, yield 0.47 g (60%) mp. 214 - 216 °C; IR (KBr): $\bar{\nu}$ = 3480, 3380 (NH_2), 1770 (CO) cm^{-1}; ^1H NMR (DMSO-d_6): δ = 5.72 (s, 2H, NH_2), 4.40 (q, 2H, CH_2), 4.25 (s, 2H, CH_2), 1.25 (t, 3H, CH_3) ppm. Found: C, 31.44; H, 3.62; N, 35.84%, $C_7H_9N_7O_3S$ (271) required: C, 31.00; H, 3.32; N, 36.16.

1-Amino-5-(4-amino-5-mercapto-1,2,4-triazol-3-yl) tetrazole (6)

A solution of 0.54 g **3** (0.003 mol), in ethanol 15 mL, was treated with hydrazine hydrate (95%, 5 mL) was refluxed for 2 h, diluted with cold water, and acidified by hydrochloric acid. The precipitated product was filtered, washed with water, and recrystallized from methanol to afford 0.37 g (64%) of **6**; mp. 245 - 247 °C; IR (KBr): $\bar{\nu}$ = 3390, 3350 (NH_2), 2600 (SH) cm^{-1}; ^1H NMR (DMSO-d_6): δ = 5.81, 5.74 (2s, 2H each, $2NH_2$), 3.92 (s, 1H, SH) ppm; MS: *m/z* (%) = 198.86 (M^+, 17), Found: C,17.81; H, 2.94; N, 63.45%. $C_3H_5N_9S$ (199) required: C, 18.09; H, 2.51, N, 63.32%.

3-(1-Aminotetrazol-5-yl)-6-methyl-1,2,4-triazolo [3,4-b]-1,3,4-thiadiazole (7)

A mixture of aminothiol 0.61g **6** (0.003 mol) and glacial acetic acid 10 mL was refluxed for 1 h. The mixture was evaporated under reduced pressure, and the obtained residue was crystallized from methanol to give 0.41 g (60%) of **7**; mp. 260 - 261 °C; IR (KBr): $\bar{\nu}$ = 3380, 3355 (NH_2) cm^{-1}; ^1H NMR (DMSO-d_6): δ = 5.78 (s, 2H, NH_2), 1.94 (s, 3H, CH_3) ppm; MS: *m/z* (%) = 224 (M^+ + 1, 30). Found C, 27.11; H. 2.65; N, 56.44% . $C_5H_5N_9S$(223) required: C, 26.91; H, 2.24; N, 56.50%.

Ethyl 1-[*N*-(5-oxo-2-phenyloxazol-4-ylidene)-hydrazono] tetrazole-5-carboxylate (8)

A solution of aminoester 1.48 g **1** (0.009 mol) in glacial acetic acid 20 mL and concentrated hydrochloric acid 5 mL was cooled in an ice-salt bath and diazotized with sodium nitrile 0.65 g. Sodium acetate trihydrate 1.28 g was added, followed by a freshly prepared solution of hippuric acid 1.69 g (0.009 mol) in acetic anhydride 10 mL, which added rapidly, with heating at 90 °C for 2 h. The mixture was cooled, almost completely precipitated within 2 h, filter the formed solid and recrystallized methanol to yield 2.54 g (82%) orange crystals of **8**; mp. 190 - 191 °C; IR (KBr): $\bar{\nu}$ = 3279 (NH), 1774, 1712 (CO) cm^{-1}; ^1H NMR (DMSO-d_6): δ= 10.28 (s, 1H, exchangeable with D_2O, NH), 8.21-7.42 (m, 5H, ArH), 4.15 (q, 2H, CH_2), 1.35 (t, 3H, CH_3) ppm. MS: *m/z* (%) = 330 (M^+ + 1, 16.3). Found: C, 47.64; H, 3.21; N, 30.22%. $C_{13}H_{11}N_7O_4$ (329) required: C, 47.42; H, 3.34; N, 29.79%.

Tetrazolo[5,1-f]-1,2,4-triazin-8-(7*H*)-one (9)

A solution of 2.23 g **1** (0.01 mol) in 10 mL formamide was refluxed for 2 h. The solid product which separated from the mixture was filtered off and recrystallized from methanol to yield 0.84 g (78%) of **9**, mp. 230 - 231 °C; IR (KBr): $\bar{\nu}$ = 3260 (NH), 1670 (CO) cm^{-1}; ^1H NMR (DMSO-d_6): δ = 11.81 (s, 1H, exchangeable with D_2O, NH), 6.22 (s, 1H, CH) ppm; MS: *m/z* (%) = 138 (M^+, 7.4). Found : C, 25.88; H, 1.92; N, 61.01%. $C_3H_2N_6O$ (138) required: C, 26.09; H, 1.45; N, 60.87%.

Tetrazolo[5,1-f]-1,2,4-triazin-8(7*H*)-thione (10)

A mixture of 0.52 g **9** (0.004 mol) and 5 mL carbon disulfide in 15 mL pyridine was heated under reflux for 3 h, and then cooled at ambient temperature, poured onto ice-water, the crude solid that precipitated was filtered, washed with water and recrystallized from methanol to produce 0.44 g (76%) yellow crystrals of **10**; mp. 220 - 222 °C; IR (KBr):

$\bar{\nu}$ = 3227 (NH), 1176 (CS) cm^{-1}; ^1H NMR (DMSO-d_6): δ = 11.65 (s, 1H, exchangeable with D_2O, NH), 5.92 (s, 1H, CH) ppm. Found : C, 23.21; H, 1.56; N, 54.98%. $C_3H_2N_6S$ (154) required: C, 23.38; H, 1.30; N, 54.55%.

8-Acetonylthiotetrazolo[5,1-f]-1,2,4-triazine (11)

A mixture of 0.32 g **10** (0.002 mol) and 0.19 g chloroacetone (0.002 mol) in 15 mL ethanol containing 2 g anhydrous sodium acetate was refluxed for 1 h. After cooling, the solid product was filtered off, washed with water and recrystallized from methanol to give 0.31g (71%) of **11**; mp. 205 - 207 °C ; IR (KBr): $\bar{\nu}$ = 1740 (CO) cm^{-1}; ^1H NMR (DMSO-d_6): δ = 6.15 (s, 1H, CH), 4.30 (s, 2H, CH_2), 2.25 (s, 3H, CH_3) ppm. Found: C, 34.61; H, 3.02; N, 40.36%. $C_6H_6N_6OS$ (210) required: C, 34.29; H, 2.86; N, 40.00%.

8-Ethoxycarbonylmethylthiotetrazolo[5,1-f]-1,2,4-triazine (12)

Compound **12** was prepared from **10** 0.21g (0.001 mol) and 0.19 g ethyl chloroacetate (0.001 mol) as just described for the preparation of **11**. It was crystallized from methanol, yield 0.19 g (61%) mp. 195 - 196 °C; IR (KBr): $\bar{\nu}$ = 1770 (CO) cm^{-1}; ^1H NMR (DMSO-d_6): δ = 5.92 (s, 1H, CH), 4.45 (q, 2H, CH_2), 4.20 (s, 2H, CH_2), 1.30 (t, 3H, CH_3) ppm; MS: *m/z* (%) = 224 (M^+; 16.2). Found : C, 37.41; H, 3.82; N, 38.01%. $C_7H_8N_6OS$ (224) required: C, 37.50; H, 3.57; N, 37.50%.

8-Hydrazinotetrazolo[5,1-f]-1,2,4-triazine (13)

A mixture of 0.41 g **10** (0.003 mol) and hydrazine hydrate (95%, 5 mL) in 15 mL ethanol was refluxed for 2 h and then left to cool, diluted with water, and acidified with hydrochloric acid. The mass product was filtered, washed with water, and recrystallized from methanol to give 0.31 g (77%) of **13**; mp. 190 - 192 °C; IR (KBr): $\bar{\nu}$ = 3460, 3380 (NH_2), 3200 (NH) cm^{-1}; ^1H NMR (DMSO-d_6): δ = 11.25 (s, 1H, NH), 5.90 (s, 1H, CH), 4.32 (br s, 2H, NH_2) ppm. Found: C, 24.12; H, 2.42; N, 74.02%. $C_3H_4N_8$ (152) required: C, 23.68; H, 2.63; N, 73.68%.

1,2,4-Triazolo[4,3-d]tetrazolo[5,1-f]-1,2,4-triazine (14)

A mixture of 0.22 g **13** (0.001 mol) and 5 mL triethyl orthoformate was heated at reflux for 2 h and then evaporated under reduced pressure. The obtained residue was crystallized from methanol to yield 0.12 g (52%) of **14**; mp. 235 - 237 °C; ^1H NMR (DMSO-d_6): δ = 6.02, 5.80 (2 s, 1H each, 2 CH); MS: *m/z* (%) = 163 (M^+ + 1, 26.7). Found: C, 29.21; H, 1.56; N, 68.82%. $C_4H_2N_8$ (162) required: C, 29.63; H, 1.24; N, 69.14%.

8-Methyl-1,2,4-triazolo[4,3-d]tetrazolo [5,1-f]-1,2,4-triazine (15)

A mixture of of 0.26 g **13** (0.002 mol) and glacial acetic acid 10 mL was refluxed for 1h. The mixture was evaporated under reduced pressure, and the resulted residue was crystallized from methanol to give 0.16 g (53%) of **15**; mp. 219 - 221 °C; ^1H NMR (DMSO-d_6): δ = 6.14 (s, 1H, CH), 2.40 (s, 3H, CH_3) ppm; MS: *m/z* (%) = 176 (M^+; 29). Found: C, 33.89; H, 2.51; N, 64.01%. $C_5H_4N_8$ (176) required: C, 34.09; H, 2.27; N, 63.64%.

BIOLOGICAL SCREENING

The screened compounds, ampicillin trihydrate and clotrimazole were dissolved in DMSO at concentration 1600 μg/mL. The minimal inhibitory concentrations (*MIC*) were measured in μg/mL at the end of the incubation period for 24 to 48 h at 36 °C. Controls for the DMSO microorganisms and media microorganisms were also undertaken.

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