ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

(ISSN: 0975-8585)

RESEARCH ARTICLE

Antimicrobial Assessment of Some Heterocyclic Compounds Utilizing 5-(1-Aminotetrazol-5-yl)-2-Hydrazino-1,3,4-Oxadiazole.

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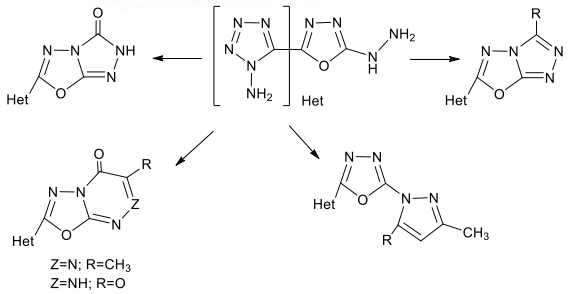
ABSTRACT

The 5-(1-aminotetrazol-5-yl)-2-hydrazino-1, 3, 4-oxadiazole **(2)** was allowed to react with aromatic aldehydes gave the corresponding 2-arylidenehydrazino-1, 3.4-oxadiazole derivatives **3**. Dehydrogenative cyclization of $\mathbf{3_{a-d}}$ *via* bromine in acetic acid or ethanolic ferric chloride yielded the corresponding of 6-(1-aminotetrazol-5-yl)-3-aryl-1,2,4-triazolo[3,4-*b*]-1,3,4-oxadiazoles $\mathbf{4_{a-d}}$. The products $\mathbf{4_{a-d}}$ were accomplished cyclization of hydrazine **2** with appropriate aromatic acid chloride. The cyclic amidrazone **2** reacted with acetic acid and ethyl chloroformate furnished the products of triazolo-oxadiazoles **5** and **6**. The cyclic amidrazone **2** reacted with pyruvic acid or ethyl pyruvate to yield the corresponding hydrazones **7** and **8**, which then cyclized to 1, 3, 4-oxadiazolo [2, 3-*c*]-1, 2, 4-triazine derivative **9**. The 3, 4-dione derivative **10** was synthesized by condensative cyclization of the hydrazine **2** with diethyl oxalate, whereas the reaction of itself hydrazine with acetyl acetone or ethyl acetoacetate resulted the corresponding hydrazone derivatives **11** and **12**. The latter hydrazones were cyclized to yield the pyrazolyl structures **13** and **14**. The new compounds were designed and synthesized their structures were elucidated by spectral analyses in addition to elemental analyses. The synthesized derivatives were tested for their antimicrobial activity against a wide for variety of *Gram*-Positive, *Gram*-negative, and fungal strains.

Keywords: 5-(1-aminotetrazol-5-yl)-2-hydrazino-1, 3, 4-oxadiazole, hydrazones, fused heterocycles, antimicrobial activity.

Graphical Abstract



https://doi.org/10.33887/rjpbcs/2021.12.5.1

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ISSN: 0975-8585

INTRODUCTION

Tetrazoles and their derivatives have great attention because of their wide range of therapeutic and biological properties [1,2]. They have emerged as antibacterial [3-15], antiproliferation [16], anticancer [16], and anticonvulsand [17] agents. In this article, it is our intention to enlarge the area of the investigation towards tetrazole frame work and to evaluate antimicrobial activities.

RESULTS AND DISCUSSION

Chemistry

Reaction of 5-(1-aminotetrazol-5-yl)-2-mercapto-1, 3, 4-oxodiazole **(1)**[8] with hydrazine yielded the corresponding 5-(1-aminotetrazol-5-yl)-2-hydrazino-1, 3, 4-oxadiazole **(2)**. Condensation of the former hydrazine with equimolar amount of the appropriate aromatic aldehyde in boiling methanol afforded the corresponding 5-(1-aminotetrazol-5-yl)-2-arylidenehydrazino-1,3,4-oxadiazoles **3$_{a-d}$,** showing the expecting NH in IR absorption as well as ^{1}HNMR signals characteristic of NH, NH_2 (D_2O-exchangeable), methylenic CH=N, and aromatic protons. Their MS revealed the correct molecular ions which were supported by elemental analysis. Subjecting these hydrazone derivatives **3$_{a-d}$** to dehydrogenative cyclization with bromine in acetic acid or ethanolic ferric chloride through un-isolable hydrazonoyl bromides **[A]** and **[B]** intermediates resulted the corresponding of 6-(1-aminotetrazol-5-yl) -3-aryl-1,2,4-triazolo- [3,4-*b*]-1,3,4-oxadiazoles **4$_{a-d}$**. These structures lacked the NH absorption in IR and methylenic proton CH=N signal in ^{1}H NMR. The products **4$_{a-d}$** were also obtained by one-pot cyclization of **2** with aromatic acid chlorides which were formed and concomitantly dehydratively cyclized to structures **4$_{a-d}$.** The aforementioned products **4$_{a-d}$** were proved to be identical in all respects (MP, mixed MP, TLC, and IR) with methods of cyclization mentioned above this article.

Reaction of hydrazine **2** with excess of glacial acetic acid yielded 6-(1-aminotetrazol-5-yl)-3-methyl-1,2,4-triazolo[3,4-*b*]-1,3,4-oxadiazole **5**. None of the possible intermediate of the aforementioned reaction was isolated. On the other hand, heating the hydrazine **2** with excess of ethyl chloroformate in pyridine afforded product, which showed neither ester-carbonyl absorption nor ethyl group signals in the ^{1}H NMR spectrum. The product showed NH and CON absorptions and was, consequently assigned the structure of 6-(1-aminotetrazol-5-yl)-1, 2, 4-triazolo [3, 4-*b*]-1, 3, 4-oxadiazol-3 (2*H*) – one **(6).**

Condensation of **2** with pyruvic acid in boiling methanol caused in the corresponding hydrazone **7** which possessed IR absorption characteristic of OH, NH, and COOH groups. Ethyl pyruvate also reacted with the hydrazine **2** to furnish the corresponding hydrazone **8**. ^{1}H NMR spectrum of the latter contained the triplet and quartet patterns of signals characteristic of ethyl group. Acid-induced heterocyclization of **7** or **8** heating in acetic acid produced one and the same product, which displayed the disappearance of OH and NH absorptions but showed a CON absorption in IR region. The ^{1}H NMR spectrum of this cyclization product revealed no ethyl group pattern. These data together with the correct elemental analysis are compatible with the 7-(1-aminotetrazol-5-yl)-3-methyl-1, 3, 4-oxadiazole [2, 3-*c*)-1, 2, 4-triazin-4(3*H*)-one **(9)**.

Condensative cyclization of 2-hydrazino-1,3,4-oxadiazole structure **2** with equimolar amount of diethyl oxalate gave the corresponding 7-(1-aminotetrazolo-5-yl)-1,3,4-oxadiazolo [2,3-*c*]-1,2,4-triazin-3,4(2*H*)-dione **(10)**. Assignment of this structure and exclusion of possible intermediate hydrazido structure was established by correct elemental analysis as well as the absence of the triplet-quartet patterns of ^{1}H NMR signals characteristic of an ethyl group.

Condensation of the hydrazine **2** with acetyl acetone yielded the corresponding hydrazone derivative **11** which showed IR absorption characteristic of NH and C=O. ^{1}H NMR spectrum of this product revealed the presence of NH (D_2O-exchangeable), methylene, and methyl group signals. Heating **11** with acetic acid in its cyclization to the 5-(1-aminoetetrazol-5-yl)-2-(3, 5-dimethylpyrazol-1-yl)-1, 3, 4-oxadiazole **(13)** which revealed only C=N absorption and lacked NH and C=O absorptions characteristic of the parent hydrazone, and a pyrazolyl CH proton signal in its spectrum.

Similarly, condensation of ethyl acetoacetate with hydrazine **2** resulted formation of the hydrazone derivative **12**, which underwent base catalysed cyclization upon heating with sodium ethoxide to provide the 1,2,4-triazolo[3,4-*b*]-1,3,4-oxadiazole **5** or pyrazolyl **14** through the elimination of an ethyl acetate or

an ethyl alcohol molecule. Thus, the evidences of the cyclization of the hydrazone **12** is (i) the melting point and thin layer chromatography of the obtained cyclization product is not similar to the structure **5**, and (ii) spectroscopic data of this product showed OH and lacked any amide absorption bands in IR region; ^{1}H NMR exhibited OH (D_2O-exchangeable) and pyrazolyl CH proton signals. According **11**, the product was decisively assigned as the 5-(1-aminotetrazol-5-yl)-2-(3-hydroxy-5-methylpyrazol-1-yl)-1, 3, 4-oxadiazole **(14)**.

Antimicrobial activity

The antimicrobial activity of the newly synthesized compounds **2,3**$_{a\text{-}d}$, **4**$_{a\text{-}d}$, and **5-14** were evaluated against:

Gram- positive: *Staphylococcus* aureus (*S. aureus*), *Bacillus subtilis* (*B. Sulotilis*), and

Gram-negative: *Escherichia Coli* (*E. coli*) bacterial strains and *Aspergillums niger* (*A. niger*), and *Candida albicans* (*C. abicans*) fungal strains. The minimal inhibitory concentration (*MIC*/*mg*/mL) is displayed in **Table** showing that.

Compounds**: 4**$_a$**, 4**$_b$**, 11,** and **12** exhibit an antimicrobial activity against *S. aureus* (25%); **5**, and **6** against *B. subilis* (25%); **7,8,12** and **14** against *E. Coli* (50%) comparable to that ampicillin. Furthermore, **3b**, **5**,**8**, and **11** possessed an antimycotic activity against *A. niger* (50%) and **3d**, **5**, **11**, and **12** against *C. albican* (50%) comparable to that of clotrimazole.

In conclusion, the foregoing results demonstrated the utility of 5-(1-aminotetrazol-5-yl)-2-hydrazino-1, 3, 4-oxadiazole as synthons for the construction of some condensed heterocyclic nitrogen structures by different cyclization reagents. The antibacterial and antifungal activities of the synthesized compounds were even comparable to ampicillin and clotrimazole.

Table: Antimicrobial activity of synthesized compounds

compound	Minimum inhibitory concentration *(MIC)* in *Mg*/mL				
	Bacterial Strains			*Fungal Strains*	
	S. aureus (+)	*B. subtilis(+)*	*E.Coli (-)*	*A. niger*	*C. albicans*
2	100	100	100	100	100
3a	100	100	>200	100	100
3b	>200	100	100	25	100
3c	100	100	100	100	100
3d	100	100	>200	100	25
4a	50	>200	100	100	100
4b	50	100	100	100	100
4c	>200	100	100	100	100
4d	100	100	100	100	100
5	100	50	>200	25	25
6	>200	50	100	100	100
7	100	100	50	100	100
8	100	100	50	25	100
9	100	>200	>200	100	100
10	100	>200	100	100	100
11	50	100	>200	25	25
12	50	100	50	100	25
13	100	100	100	100	100
14	100	100	20	100	100
Ampicillin	12.5	12.5	25	-	-
Clotrimazole	-	-	-	12.5	12.5

ISSN: 0975-8585

EXPERIMENTAL

General

Melting points were measured with a Gallenkamp apparatus and are uncorrected. The reactions were followed up and the purification of products were carried out on pre- (layer thickness 0.25mm; coated TLC plates Silica Gel-Merck), visualize the spots in Iodine. **IR** spectra were recorded (KBr) on a Shimadzu FT-IR 8101 PC infrared spectrophotometer. The **^{1}H NMR** spectra were determined in *DMSO-d_6* at 300 MHz on a Varian Mercury VX 300 NMR spectrometer and their chemical shifts (δ/ppm) are reported using *TMS* as internal standard. Mass spectra were recorded on a HP model **MS** 5988 spectrometer at electron ionizing energy of 70 eV.

5-(1-Aminotetrazol-5-yl)-2-hydrazino-1, 3, 4-oxadiazole (***2***, $C_3H_5N_9O$)

A mixture of mmol **1**[8] and hydrazine hydrate (95%), 10 cm^3) in 15 cm^3 ethanol was refluxed for 2h 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. Yield: 0.82g (81.2%); MP 240-242^{o}C; **IR**: γ=3460, 3380 (NH_2), 3200(NH), 1625 (C=N) cm^{-1}; **^{1}H NMR** (*DMSO-d_6*): δ=11.25 (s, 1H, D_2O-exchangeable NH), 5.72 (s, 2H, D_2O-exchangeable NH_2); **MS**: *m/z* (%) = 183 (M^+, 20).

Synthesis of 5-(1-aminotetrazol-5-yl)-2-arylidenehydrazino-1, 3, 4-oxadiazoles $\mathbf{3_{a-d}}$ *(General Procedure)*

A solution 6 mmol of **2** in 15 cm^3 methanol was added to 6 mmol of appropriate aromatic aldehyde and the mixture was heated at 100^{o}C for 10 min. The reaction mixture was kept at ambient temperature for overnight and the product which separated was filtered off, washed with ether, dried, and crystallized from methanol. The physico-chemical and spectra data of **3a-d** the following:

5-(1-Aminotetrazol-5-yl)-2-benzylidenehydrazino-1, 3, 4-oxadiazole (***3$_a$***, $C_{10}H_9N_9O$)

Yield: 1.20g (81.1 %); pale yellow; MP 168-170^{o}C; **IR**: γ=3325 (NH), 1625 (C=N) cm^{-1}; **^{1}H NMR** (*DMSO-d_6*): δ=11.60 (s, 1H, D_2O-exchangeable NH), 8.20-7.90 (m, 5H, *Ar* H), 7.61 (s, 1H, methylenic H), 5.72 (s, 2H, D_2O- exchangeable NH_2). ppm; **MS**: *m/z* (%) = 271 (M^+, 12), 272 (M^++1, 18).

5-(1-Aminotetrazol-5-yl)-2-p-tolylmethylidenehydrazino-1, 3, 4-oxadiazole (**3$_b$**, $C_{10}H_{11}N_9O$)

Yield: 1.41g (88.1%); yellow; MP 150-152^{o}C; **IR**: γ=3340 (NH), 1610 (C=N) cm^{-1}; **^{1}H NMR** (*DMSO-d_6*): δ=10.87 (s, 1H, D_2O-exchangeable NH), 8.17-7.82 (m, 4H, *Ar* H), 7.71 (s, 1H, methylenic H), 5.61(s, 2H, D_2O- exchangeable NH_2). 2.30 (s, 3H, CH_3) ppm; **MS**: *m/z* (%) = 285 (M^+, 20).

5-(1-Aminotetrazol-5-yl)-2-p-chlorobenzylienehydrazino-1, 3, 4-oxadiazole (**3$_c$**, $C_{10}H_8N_9O$)

Yield: 1.51g (88.8%); yellow; MP 170-172^{o}C; **IR**: γ=3350 (NH), 1610 (C=N) cm^{-1} **MS**: *m/z* (%) = 306 (M^+, 12).

5-(1-Aminotetrazol-5-yl)-2-p-nitrobenzylidenehydrazino-1, 3, 4-oxadiazole (**3$_d$**,$C_{10}H_8N_{10}O_3$)

Yield: 1.52g (81.01%); orange; MP 190-192^{o}C; **IR**: γ=3340 (NH), 1630 (C=N) cm^{-1}; **^{1}H NMR** (*DMSO-d_6*): δ=10.68 (s, 1H, D_2O-exchangeable NH), 8.31-7.74 (m, 4H, *Ar* H), 7.22 (s, 1H, methylenic H), 5.68(s, 2H, D_2O- exchangeable NH_2), ppm; **MS**: *m/z* (%) = 316 (M^+, 21).

6-(1-Aminotetrazol-5-yl)-3-aryl-1, 2, 4-triazolo [3, 4-b]-1, 3, 4-oxadiazoles $\mathbf{4_{a-d}}$ *(General procedure)*

Method A. To a solution 4 mmol of the respective hydrazone **3a-d** in 15 cm^3 glacial acetic acid containing 4 mmol bromine in 10 cm^3 glacial acetic acid were added gradually with stirring. The reaction mixture was then warmed on a boiling water-bath for 5 min, left to cool and then poured onto water. The precipitated solid was filtered off, washed thoroughly with water, and crystallized from methanol.

Method B. A solution of 4 mmol of the respective hydrazone $\mathbf{3_{a-d}}$ in 20 cm^3 ethanolic ferric chloride solution (10%) was boiled for 10 min, and then left at room temperature overnight. The separated

product was filtered off, washed with water, dried and crystallized from methanol.

Method C. A mixture of **2** (6 mmol) and appropriate aromatic acid chloride in ethanol (30 cm^3) was refluxed for 3h, after cooling the mass product was filtered off and recrystallized from abs. ethanol.

The aforementioned methods **A**, **B**, and **C** are compatible with the assigned products **4_{a-d}**, the physico-chemical and spectra data the following:

6-(1-Aminotetrazol-5-yl)-3-phenyl-1, 2, 4-triazolo [3, 4-b]-1, 3, 4-oxadiazole (**4_a**, $C_{10}H_7N_9O$)

Yield: method A 0.61g (61.6%); method B 0.52g (52.5%); method C 0.71g (71.7%); MP 170-172°C; **IR**: γ=3345 (NH), 1640 (C=N) cm^{-1}; **^{1}H NMR** (*DMSO-d_6*): *δ*=8.21-7.95 (m, 5H, *Ar* H), 5.71 (s, 2H, D_2O-exchangeable NH_2) ppm; **MS**: *m/z* (%) = 269 (M^+, 20).

6-(1-Aminotetrazol-5-yl)-3-p-tolyl-1, 2, 4-triazolo [3, 4-b]-1, 3, 4-oxadiazole (**4_b**, $C_{11}H_9N_9O$)

Yield: method A 0.61g (61.6%); method B 0.81g (81.8%); method C 0.71g (71.7%); MP 160-162°C; **IR**: γ=3340 (NH), 1630 (C=N) cm^{-1}; **^{1}H NMR** (*DMSO-d_6*): *δ*=8.15-8.10 (m, 4H, *Ar* H), 5.61 (s, 2H, D_2O-exchangeable NH_2), 2.30 ppm (s, 3H, CH_3); **MS**: *m/z* (%) = 284 (M^+ +1, 14).

6-(1-Aminotetrazol-5-yl)-3-p-chlorophenyl-1, 2, 4-triazolo [3, 4-b]-1, 3, 4-oxadiazole (**4_c**, $C_{10}H_8ClN_9O$)

Yield: method A 0.55g (55.5%); method B 0.52g (52.5%); method C 0.54g (54.5%); MP 185-186°C; **IR**: γ=3335 (NH), 1640 (C=N) cm^{-1}; **^{1}H NMR** (*DMSO-d_6*): *δ*=8.12-8.10 (m, 5H, *Ar* H), 5.65 ppm (s, 2H, D_2O-exchangeable NH_2); **MS**: *m/z* (%) = 304 (M^+, 20).

6-(1-Aminotetrazol-5-yl)-3-p-nitrophenyl-1, 2, 4-triazolo [3, 4-b]-1, 3, 4-oxadiazole (**4_d**, $C_{10}H_6N_{10}O_3$)

Yield: method A 0.46g (46.5%); method B 0.51g (51.5%); method C 0.54g (54.5%); MP 205-206°C; **^{1}H NMR** (*DMSO-d_6*): *δ*=8.12-8.10 (m, 5H, *Ar* H), 5.71ppm (s, 2H, D_2O-exchangeable NH_2); **MS**: *m/z* (%) = 314 (M^+, 25).

6-(1-Aminotetrazol-5-yl)-3-methyl-1, 2, 4-triazolo [3, 4-b]-1, 3, 4-oxadiazole (**5**, $C_5H_5N_9O$)

A mixture of 6 mmol of **2** 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 0.92g (81.4%); MP 218-220°C; **IR**: γ=3340 (NH), 1630 (C=N) cm^{-1}; **^{1}H NMR** (*DMSO-d_6*): *δ*=5.62 (s, 2H, D_2O-exchangeable NH_2), 2.42 (s, 3H, CH_3) ppm; **MS**: *m/z* (%) = 208 (M^+, 18).

6-(1-Aminotetrazol-5-yl)-1, 2, 4-triazolo [3, 4-b]-1, 3, 4-oxadiazol-3(2H)-one (**6**, $C_4H_3N_9O_2$)

A suspension of **2** (6 mmol) in 2 cm^3 pyridine was treated with excess of ethyl chloroformate and the mixture was treated under reflux for 3h. The reaction mixture was poured onto ice-water and the product which separated was filtered off, washed with water, and crystallized from methanol. Yield: 0.85g (74.6%); MP 210°C; **IR**: γ=3300 (NH), 1690 (CON), 1625 (C=N) cm^{-1}; **^{1}H NMR** (*DMSO-d_6*): *δ*=11.85 (s, 1H, D_2O-exchangeable), 5.75 (s, 2H, D_2O-exchangeable NH_2) ppm; MS: *m/z* (%) = 209 (M^+, 26).

*Synthesis of **7** and **8** (General Procedure)*

To a solution of **2** (6 mmol) in 10 cm^3 methanol, 6 mmol pyruvic acid or ethyl pyruvate was added and the mixture was kept at ambient temperature for 24h or heated at reflux for 1h. The product which separated was filtered off, washed with ether, and crystallized from methanol to provide **7** and **8**.

5-(1-Aminotetrazol-5-yl)-2-pyruvic acid hydrazino-1, 3, 4-oxadiazole (**7**, $C_6H_7N_9O_3$)

Yield: 0.92g (66.7%); MP 175°C; **IR**: γ=3450 (OH), 3225 (NH), 1715 (C=O), 1625 (C=N) cm^{-1}; **^{1}H NMR** (*DMSO-d_6*): *δ*=12.52 (s, 1H, D_2O-exchangeable OH), 11.84(s, 1H, D_2O-exchangeable NH), 5.72 (s, 2H, D_2O-exchangeable NH_2), 2.50 (s, 3H, CH_3) ppm; **MS**: *m/z* (%) = 253 (M^+, 27).

5-(1-Aminotetrazol-5-yl)-2-ethyl pyruvate hydrazino-1, 3, 4-oxadiazole (**8**, $C_8H_{11}N_9O_3$)

Yield: 1.21g (78.6%); MP 185°C; **IR**: γ=3210 (NH), 1730 (C=O), 1600 (C=N) cm^{-1}; **^{1}H NMR** (*DMSO-d_6*): *δ*=11.61 (s, 1H, D_2O-exchangeable NH), 5.85 (s, 2H, D_2O-exchangeable NH_2), 3.85 (q, 2H, CH_2CH_3), 2.55 (s, 3H, CH_3), 1.35 (t, 3H, CH_2CH_3) ppm; **MS**: *m/z* (%) = 281 (M^+, 30).

7-(1-Aminotetrazol-5-yl)-3-methyl-1, 3, 4-oxadiazolo [2, 3-c]-1, 2, 4-triazin-4(3H)-one (**9**, $C_6H_5N_9O_2$)

A mixture of **7** or **8** (4 mmol) and 10 cm^3 acetic acid was heated under reflux for 2h and then evaporated to dryness. The obtained residue was crystallized from methanol. Yield: 0.61g (65.6%); MP 220°C; **IR**: γ=1690 (CON), 1620 (C=N) cm^{-1}; **^{1}H NMR** (*DMSO-d_6*): *δ*=5.90 (s, 2H, D_2O-exchangeable NH_2), 2.60 (s, 3H, CH_3) ppm; **MS**: *m/z* (%) = 235 (M^+, 13).

7-(1-Aminotetrazol-5-yl)-1-3-4-oxadiazolo [2, 3-c]-1, 2, 4-triazin-3, 4(2H)-dione (**10**, $C_5H_3N_9O_3$)

A mixture of **2** (6 mmol) and diethyl oxalate 6 mmol was heated under reflux for 1h after attaining room temperature, the mixture was triturated with methanol and the product which separated was filtered off and crystallized from methanol. Yield: 0.93g (71.8%); MP 225°C; **IR**: γ=3325 (NH), 1690, 1660 (CON), 1590 (C=N) cm^{-1}; **^{1}H NMR** (*DMSO-d_6*): *δ*=12.10 (s, 1H, D_2O-exchangeable NH), 5.75 (s, 2H, D_2O-exchangeable NH_2) ppm; **MS**: *m/z* (%) = 237 (M^+, 12).

*Synthesis of **11** and **12** (General Procedure)*

To a solution of **2** (6 mmol) in 15 cm^3 methanol was added to 6 mmol acetyl acetone or ethyl acetoacetate and the mixture was heated under reflux for 2h. The separated product was filtered off, washed with ether and crystallized from methanol to performed **11** and **12**.

5-(1-Aminotetrazol-5-yl)-2-acetylacetonehydrazino-1, 3, 4-oxadiazole (**11**, $C_7H_{11}N_9O_2$)

Yield: 1.13g (81.9%); MP 175°C; **IR**: γ=3240 (NH), 1700 (C=O), 1625 (C=N) cm^{-1}; **^{1}H NMR** (*DMSO-d_6*): *δ*=11.85 (s, 1H, D_2O-exchangeable NH), 5.80 (s, 2H, D_2O-exchangeable NH_2), 4.12 (s, 2H, CH_2), 2.35, 2.20 (2s, 3H each, $2CH_3$) ppm; **MS**: *m/z* (%) = 253 (M^+, 18).

5-(1-Aminotetrazol-5-yl)-2-(3, 5-dimethylpyrazol-1-yl) 1, 3, 4-oxadiazole (**13**, $C_7H_9N_9O$)

A solution of **11** (4 mmol) in 10 cm^3 glacial acetic acid was heated under reflux for 2h and then evaporated dryness under reduced pressure. The obtained residue was crystallized from methanol. Yield: 0.53g (57.0%); MP 195-197°C; **IR**: γ=1625 (C=N) cm^{-1}; **^{1}H NMR** (*DMSO-d_6*): *δ*=5.75 (s, 2H, D_2O-exchangeable NH_2), 5.25 (2, 1H, pyrazolyl CH), 2.30, 2.25 (2s, 3H each, $2CH_3$) ppm; **MS**: *m/z* (%) = 235 (M^+, 7).

5-(1-Aminotetrazol-5-yl)-2-ethyl acetoacetatehydrazino-1, 3, 4-oxadiazole (**12**, $C_8H_{13}N_9O_3$)

Yield: 1.22g (78.7%); MP 190-192°C; **IR**: γ=3295(NH), 1735 (C=O), 1615 (C=N) cm^{-1}; **^{1}H NMR** (*DMSO-d_6*): *δ*=12.15 (s, 1H, D_2O-exchangeable NH), 5.78 (s, 2H, D_2O-exchangeable NH_2), 4.20 (q, 2H, CH_2CH_3) 4.10 (s, 2H, CH_2), 2.40 (s, 3H, CH_3), 1.28 (t, 3H, CH_2CH_3) ppm ; **MS**: *m/z* (%) = 284 (M^++1, 12).

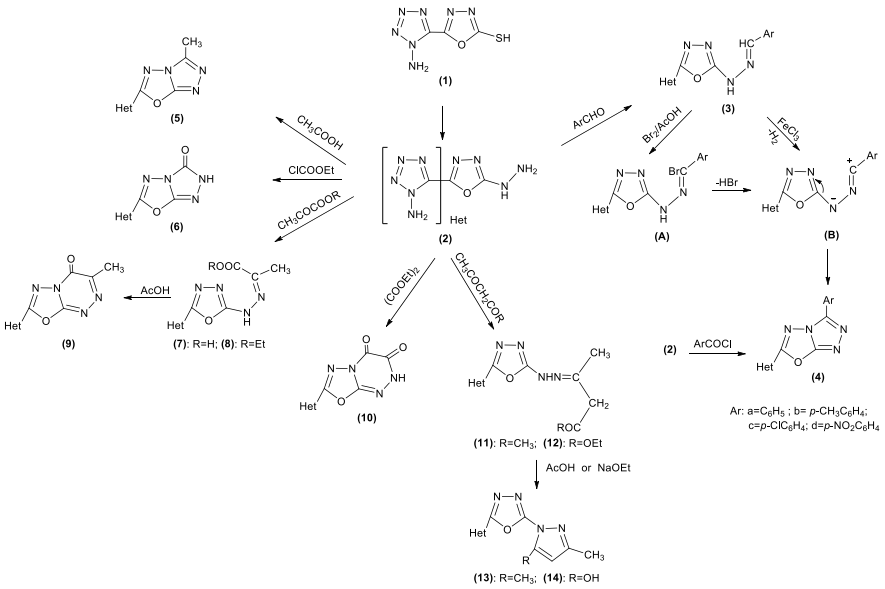
5-(1-Aminotetrazol-5-yl)-2-(3-hydroxy-5-methylpyrazol-1yl)-1, 3, 4-oxadiazole (**14**, $C_6H_7N_9O_2$)

A solution of **12** (3 mmol) in 15 cm^3 freshly prepared 0.1M sodium ethoxide was heated under reflux for 2h. The resulting solution was neutralized with acetic acid and product which separated was filtered off and crystallized from methanol. Yield: 0.54g (64.3%); MP 235-237°C; **IR**: γ=3400 (OH), 1615 (C=N) cm^{-1}; **^{1}H NMR** (*DMSO-d_6*): *δ*=12.35 (s, 1H, D_2O-exchangeable OH), 5.80 (s, 2H, D_2O-exchangeable NH_2), 5.30, (s, 1H, pyrazolyl CH), 2.35 (s, 3H, CH_3) ppm ; **MS**: *m/z* (%) = 237 (M^+, 30).

Biological Screening

The antimicrobial activity of some the synthesized compounds were determined *in vitro* against a

variety of bacteria. The tests were carried out using disc diffusion method[18, 19] against *Gram*-positive bacteria and *Gram*-negative bacteria were dissolved in *DMF*, and activity mentioned on 1000 ppm. Agar plates were surface inoculated uniformly from fresh broth culture of the *Gram* bacteria. The discs were incubated at 25°C for 1h to permit good diffusion then incubated at 28°C for 24h, and the zones of inhibition were measured.



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