Journal of Pharmaceutical and Applied Chemistry

ISSN: 2357-0210 VOLUME 2, ISSUES1, 2016 www.egyptfuture.org/ojs/

Utility of CyanoaceticAcid Hydrazide in Organic Synthesis: Synthesis and Characterization of Some Novel Heterocycles bearing 1,3,4-Oxadiazole Moeity

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Received: 5 Feb. 2017, Revised: 22 Apr. 2017, Accepted: 25 Apr. 2017.
Published online: 1 May 2017.

Abstract: 2,2'-(1,3,4-oxadiazole-2,5-diyl)diacetonitrile **2** has been prepared expeditiously from 2-cyano-N'-(2-cyanoacetyl)acetohydrazide**1**. Reaction of 2,2'-(1,3,4-oxadiazole-2,5-diyl)diacetonitrile **2** with aromatic aldehydes under mild alkaline conditions afforded the corresponding condensation products **3a-d**, which in turn allowed to react with hydrazine, phenylhydrazine or hydroxylamine to give pyrazole and iosxazole derivatives **(4-6)a-d**. Also compound **3a-d** was reacted with acetylacetone or ethyl acetoacetate to give pyran derivatives **(7,8)a-d**. Condensation of compound **2** with cyclopentanone afforded compound **9** which was reacted with acetylacetone or ethyl acetoacetate to give the corresponding spiropyran derivatives **10** and **11** respectively. The one-pot reaction of compound **2** with carbon disulfide or phenylisocyanate and ethyl chloroacetate under phase transfer catalysis conditions afforded the corresponding thiophene derivatives **12** and **13** respectively. Finally, the reaction of compound **2** with ethylcyanoacetate was studied carefully under non-catalytic and catalytic conditions where compounds **14-16** were obtained respectively. The antimicrobial activity of the obtained compounds was examined. The obtained products were characterised by their elemental and spectral data.

Keywords: Cyanoacetohydrazide, oxadiazole, cyanomethyl, spiro, PTC, antibacterial.

1 Introduction

Oxadiazole derivatives belong to an important group of heterocyclic compounds and have been extensively studied in the last two decades [1]. Among the wide variety of heterocyclic compounds, 1, 3, 4 -oxadiazole derivative played a vital role in medicinal chemistry. Large number of synthetic compounds contain oxadiazolenucleus have been studied for their anti-bacterial[1-6], anti-fungal[6-10], anti-microbial[11-18], anti-viral[19-21], anti-TB[22,23], anti-inflammatory[24,25], and analgesic activities[26].The ability of 1,3,4-oxadiazole heterocyclic compounds to undergo various chemical reactions has made them important for molecule planning because of their privileged structure, which has enormous biological potential.[27,28].

The 1,2,4-oxadiazole unit has been identified in the core of some natural products.[29] Two examples are furnished by the 3-substituted indole alkaloids, phidianidines A and B, reported by Carbone et al.[30] as selective inhibitors of the dopamine transporter DAT and partial agonists of the μ opioid receptor.[31] These molecules were isolated from the aeolid opistho branch Phidiana militaris. The oxadiazole ring system is also present in the molecules of some drugs that are available on the pharmaceutical market as Bredon (anti-inflammatory), Irrigor (anaesthetic, vasodilator) and Libexin (antitussive).[32,33]

2 Experimental

All melting points were determined on a Koffler melting point apparatus and are uncorrected. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Brukeravance 400 MHz spectrometer using TMS as internal reference (chemical shifts in δ, ppm), and IR spectra were obtained on a Nicolet 710 FT-IR spectrometer (KBr, vmax in cm^{-1}).

Synthesis of 2-cyano-N'-(2-cyanoacetyl)acetohydrazide (1):

Method A: an equimolar ratio of cyanoacetic acid hydrazide and ethyl cyanocetate was fused carefully for 15 min. (the temperature should not exceed 100 ºC). The obtained slurry was triturated with light petroleum (40-60ºC) and the formed solid was filtered off and recrystallized from aq. ethanol into colourless needles, yield 70%.

Method B: cyanoacetic acid hydrazide (0.99 g, 0.01 mol)

and 3-(3,5-dimethyl-1H-pyrazol-1-yl)-3-oxopropanenitrile (1.63g, 0.01 mol) in dry dioxan 5 ml was heated under reflux for 30 min. The reaction mixture was lift to cool and the formed solid was collected by filtration, yield 90%. Mp: 156-158 ºC, IR (KBr: υ, cm^{-1}): 3242 (NH), 2190 (CN) and 1670 (CO);^{1}H-NMR (DMSO, δ, ppm): 10.25 (s, 1H, NH), 4.18 (s, 2H, CH_2), ^{13}C-NMR (DMSO, δ, ppm): 168, 153 and 53.

Synthesis of 2,2'-(1,3,4-oxadiazole-2,5-diyl)diacetonitrile (2):

2-Cyano-*N'*-(2-cyanoacetyl)acetohydrazide **1** (1.66 g, 0.01 mol) was dissolved in ethanol (20 mL) and then treated with 0.5 mL concentrated hydrochloric acid. The reaction mixture was then heated under reflux for 2 h and then allowed to cool. The precipitate that formed was filtered off, washed with aqueous ethanol, and recrystallized from ethanol into colorless needles: mp: 158-160 ºC (0.75 g, 84%); IR (KBr: υ, cm^{-1}): 2984 (CH_2), 2192 (CN) and 1634 (C=N); ^{1}H-NMR (DMSO, δ, ppm): 9.38 (s, 1H, NH), 5.36 (s, 1H, =CH), 4.12 (CH_2); ^{13}C-NMR (DMSO, δ, ppm): 166, 152, 116 and 54.

Synthesis of (E)-2-(5-(cyanomethyl)-1,3,4-oxadiazol-2-yl)-3-arylacrylonitrile (3a-h):

A mixture of compound **2** (0.74 g, 0.005 mol), aromatic aldehyde (0.005 mol) and triethylamine (0.5 mL) in absolute ethanol (30 mL) was heated under reflux for 3 h, then allowed to cool. The precipitated solid was filtered off and recrystallized from the proper solvent.

3a: (72%), Mp: 232-234ºC (MeOH), IR (KBr: υ, cm^{-1}): 3321 (NH), 2220, 2192 (2CN); ^{1}H-NMR (DMSO, δ, ppm): 9.38 (s, 1H, NH), 7.65-7.40 (m, 5H, CH-arom.), 5.66 (s, 1H, =CH), 5.45 (s, 1H, =CH).

3b: (87 %) Mp: 246-248 ºC (EtOH), IR (KBr: υ, cm^{-1}): 2985 (CH_2), 2222, 2191 (2CN); ^{1}H-NMR (DMSO, δ, ppm): 7.68-7.46 (m, 4H, CH-arom.), 5.38 (s, 1H, =CH), 4.22 (s, 2H, CH_2).

3c: (82 %) Mp: 263-265 ºC (EtOH), IR (KBr: υ, cm^{-1}): 2977 (CH_2), 2221, 2190 (2CN); ^{1}H-NMR (DMSO, δ, ppm): 7.60-7.40 (m, 4H, CH-arom.), 5.35 (s, 1H, =CH), 4.20 (s, 2H, CH_2), 2.32 (s, 3H, CH_3).

3d: (85 %) Mp: >300 ºC (EtOH), IR (KBr: υ, cm^{-1}): 2985 (CH_2), 2222, 2191 (2CN); ^{1}H-NMR (DMSO, δ, ppm): 7.68-7.46 (m, 4H, CH-arom.), 5.38 (s, 1H, =CH), 4.18 (s, 2H, CH_2), 3.20 (s, 6H, $2CH_3$).

Synthesis of 2-(5-(5-amino-3-aryl-1H-pyrazol-4-yl)-1,3,4-oxadiazol-2-yl)acetonitrile (4a-d), 2-(5-(5-amino-1-phenyl-3-aryl-1H-pyrazol-4-yl)-1,3,4-oxadiazol-2-yl)acetonitrile (5a-d) and 2-(5-(5-amino-3-arylisoxazol-4-yl)-1,3,4-oxadiazol-2-yl)acetonitrile (6a-d):

To a solution of compound **3a-d** (0.001 mol) in ethanol (20 mL), hydrazine, phenylhydrazine or hydroxylamine (0.0015 mol) was added and refluxed for 2h, then left to cool. The formed solid was filtered off and recrystallized from the proper solvent into the desired products **4a-d**, **5a-d** and **6a-d** respectively.

4a: (78 %) Mp: 220-222 ºC (EtOH); IR (KBr: υ, cm^{-1}): 3382, 3309, 3242 (NH_2, NH), 2988 (CH_2), 2198 (CN); ^{1}H-NMR (DMSO, δ, ppm): 9.23 (br, 1H, NH), 7.66-7.46 (m, 5H, CH-arom.), 5.65 (br, 2H, NH_2), 4.20 (s, 2H, CH_2).

4b: (88 %) Mp: 206-208 ºC (EtOH); IR (KBr: υ, cm^{-1}): 3396, 3311, 3240 (NH), 2980 (CH_2), 2198 (CN); ^{1}H-NMR (DMSO, δ, ppm): 9.45 (br, 1H, NH), 7.73-7.43 (m, 4H, CH-arom.), 5.66 (br, 2H, NH_2), 4.23 (s, 2H, CH_2).

4c: (85 %) Mp: 230-232 ºC (EtOH); IR (KBr: υ, cm^{-1}): 3242 (NH), 2980 (CH_2), 2198 (CN); ^{1}H-NMR (DMSO, δ, ppm): 9.25 (br, 1H, NH), 7.60-7.41 (m, 4H, CH-arom.), 5.62 (br, 2H, NH_2), 4.20 (s, 2H, CH_2), 2.30 (s, 3H, CH_3).

4d: (85 %) Mp: 272-274 ºC (EtOH); IR (KBr: υ, cm^{-1}): 3242 (NH), 2980 (CH_2), 2198 (CN); ^{1}H-NMR (DMSO, δ, ppm): 9.25 (br, 1H, NH), 7.65-7.45 (m, 4H, CH-arom.), 5.65 (br, 2H, NH_2), 4.22 (s, 2H, CH_2), 3.18 (s, 6H, $2CH_3$).

5a: (70 %) Mp: 250-252 ºC (EtOH); IR (KBr: υ, cm^{-1}): 3382, 3309 (NH_2), 2198 (CN); ^{1}H-NMR (DMSO, δ, ppm): 7.86-7.22 (m, 10H, CH-arom.), 5.65 (br, 2H, NH_2), 4.21 (s, 2H, CH_2).

5b: (78 %) Mp: 258-260 ºC (EtOH); IR (KBr: υ, cm^{-1}): 3387, 3312 (NH_2), 2197 (CN); ^{1}H-NMR (DMSO, δ, ppm): 7.81-7.33 (m, 9H, CH-arom.), 5.62 (br, 2H, NH_2), 4.20 (s, 2H, CH_2).

5c: (75 %) Mp: >300 ºC (EtOH); IR (KBr: υ, cm^{-1}): 3393, 3311 (NH_2), 2192 (CN); ^{1}H-NMR (DMSO, δ, ppm): 7.80-7.32 (m, 9H, CH-arom.), 5.55 (br, 2H, NH_2), 4.22 (s, 2H, CH_2), 2.30 (s, 3H, CH_3).

5d: (78 %) Mp: >300 ºC (EtOH); IR (KBr: υ, cm^{-1}): 3392, 3310 (NH_2), 2190 (CN); ^{1}H-NMR (DMSO, δ, ppm): 7.80-7.20 (m, 10H, CH-arom.), 5.65 (br, 2H, NH_2), 4.20 (s, 2H, CH_2), 3.21 (s, 6H, $2CH_3$).

6a: (72 %) Mp: 224-226 ºC (EtOH); IR (KBr: υ, cm^{-1}): 3381, 3311 (NH_2), 2193 (CN); ^{1}H-NMR (DMSO, δ, ppm): 7.55-7.36 (m, 5H, CH-arom.), 5.64 (br, 2H, NH_2), 4.20 (s, 2H, CH_2).

6b: (82 %) Mp: 244-246 ºC (EtOH); IR (KBr: υ, cm^{-1}): 3380, 3316 (NH_2), 2198 (CN); ^{1}H-NMR (DMSO, δ, ppm): 7.51-7.35 (m, 4H, CH-arom.), 5.65 (br, 2H, NH_2), 4.22 (s, 2H, CH_2).

6c: (75 %) Mp: 264-266 ºC (EtOH); IR (KBr: υ, cm^{-1}): 3388, 3310 (NH_2), 2191 (CN); ^{1}H-NMR (DMSO, δ, ppm): 7.56-7.38 (m, 4H, CH-arom.), 5.65 (br, 2H, NH_2), 4.20 (s, 2H, CH_2), 2.30 (s, 3H, CH_3).

6d: (85 %) Mp: >300 ºC (EtOH); IR (KBr: υ, cm^{-1}): 3382, 3316 (NH_2), 2195 (CN); ^{1}H-NMR (DMSO, δ, ppm): 7.55-7.35 (m, 4H, CH-arom.), 5.65 (br, 2H, NH_2), 4.20 (s, 2H, CH_2), 3.20 (s, 6H, $2CH_3$).

Synthesis of 2-(5-(5-acetyl-2-amino-6-methyl-4-phenyl-4H-pyran-3-yl)-1,3,4-oxadiazol-2-yl)acetonitrile (7a-d) and 2-(5-(2-amino-4-phenyl-4,5,6,7-tetrahydrocyclopenta[b]pyran-3-yl)-1,3,4-oxadiazol-2-yl)acetonitrile (8a-d):

An equimolar mixture of compound **3a-d** (0.001 mol) and acetylacetone or cyclopentanone (0.001 mol) in ethanol (20 mL) was treated with triethylamine (0.5 mL) and then refluxed for 3 h. Ethanol was evaporated under reduced pressur and the residual mass was triturated with light petroleum (60-80 ºC). The obtained solid was collected and recrystallized from aq. ethanol into compounds **7a-d** and **8a-d**.

7a: (65 %) Mp: 202-204 ºC (MeOH); IR (KBr: υ, cm^{-1}): 3382, 3316 (NH_2), 2195 (CN), 1668 (CO); ^{1}H-NMR (DMSO, δ, ppm): 7.58-7.35 (m, 5H, CH-arom.), 5.65 (br, 2H, NH_2), 5.18 (s, 1H, CH_{gpyran}), 4.20 (s, 2H, CH_2),2.12 (s, 3H, $COCH_3$), 1.22 (s, 3H, CH_3).

7b: (65 %) Mp: 260-262 ºC (EtOH); IR (KBr: υ, cm^{-1}): 3388, 3310 (NH_2), 2198 (CN), 1666 (CO); ^{1}H-NMR (DMSO, δ, ppm): 7.58-7.35 (m, 4H, CH-arom.), 5.62 (br, 2H, NH_2), 5.18 (s, 1H, CH_{gpyran}), 4.22 (s, 2H, CH_2),2.10 (s, 3H, $COCH_3$), 1.25 (s, 3H, CH_3).

7c: (65 %) Mp: 248-250 ºC (EtOH); IR (KBr: υ, cm^{-1}): 3385, 3303 (NH_2), 2190 (CN), 1665 (CO); ^{1}H-NMR (DMSO, δ, ppm): 7.51-7.38 (m, 4H, CH-arom.), 5.60 (br, 2H, NH_2), 5.16 (s, 1H, CH_{gpyran}), 4.20 (s, 2H, CH_2),2.32 (s, 3H, CH_3), 2.10 (s, 3H, $COCH_3$), 1.22 (s, 3H, CH_3).

7d: (65 %) Mp: 255-257 ºC (EtOH); IR (KBr: υ, cm^{-1}): 3382, 3315 (NH_2), 2195 (CN), 1669 (CO); ^{1}H-NMR (DMSO, δ, ppm): 7.55-7.31 (m, 4H, CH-arom.), 5.60 (br, 2H, NH_2), 5.22 (s, 1H, CH_{gpyran}), 4.20 (s, 2H, CH_2), 3.18 (s, 6H, $2CH_3$),2.10 (s, 3H, $COCH_3$), 1.25 (s, 3H, CH_3).

8a: (65 %) Mp: 198-200 ºC (MeOH); IR (KBr: υ, cm^{-1}): 3356, 3310 (NH_2), 2198 (CN), 1665 (CO); ^{1}H-NMR (DMSO, δ, ppm): 7.53-7.35 (m, 5H, CH-arom.), 5.60 (br, 2H, NH_2), 5.15 (s, 1H, CH_{gpyran}), 4.22 (s, 2H, CH_2),1.62-1.22 (m, 6H, cyclic CH_2).

8b: (85 %) Mp: 255-258 ºC (EtOH); IR (KBr: υ, cm^{-1}): 3359, 3303 (NH_2), 2193 (CN), 1674 (CO); ^{1}H-NMR (DMSO, δ, ppm): 7.58-7.42 (m, 4H, CH-arom.), 5.61 (br, 2H, NH_2), 5.18 (s, 1H, CH_{gpyran}), 4.20 (s, 2H, CH_2),1.60-1.25 (m, 6H, cyclic CH_2).

8c: (78 %) Mp: 248-250 ºC (EtOH); IR (KBr: υ, cm^{-1}): 3367, 3311 (NH_2), 2190 (CN), 1668 (CO); ^{1}H-NMR (DMSO, δ, ppm): 7.55-7.35 (m, 4H, CH-arom.), 5.62 (br, 2H, NH_2), 5.18 (s, 1H, CH_{gpyran}), 4.18 (s, 2H, CH_2),2.20 (s, 3H, CH_3),1.62-1.20 (m, 6H, cyclic CH_2).

8d: (80 %) Mp: >300 ºC (EtOH); IR (KBr: υ, cm^{-1}): 3365, 3310 (NH_2), 2196 (CN), 1665 (CO); ^{1}H-NMR (DMSO, δ, ppm): 7.58-7.32 (m, 4H, CH-arom.), 5.60 (br, 2H, NH_2), 5.18 (s, 1H, CH_{gpyran}), 4.20 (s, 2H, CH_2),3.20 (s, 6H, $2CH_3$),1.62-1.20 (m, 6H, cyclic CH_2).

Synthesis of 2-(5-(cyanomethyl)-1,3,4-oxadiazol-2-yl)-2-cyclopentylideneacetonitrile (9):

An equimolar mixture of compound **2** (1.48 g, 0.01 mol) and cyclopentanone (0.89 mL, 0.01 mol) in ethanol (25 mL) was treated with triethylamine (0.5 mL) and then was refluxed for 2 h. The reaction mixture was left to cool and the formed solid was filtered off and recrystallized from acetonitrile into pale yellow needles.

9: (65 %) Mp: 224-226 ºC (MeCN); IR (KBr: υ, cm^{-1}): 2221, 2198 (2CN); ^{1}H-NMR (DMSO, δ, ppm): 4.18 (s, 2H, CH_2), 1.68-1.22 (m, 8H, cyclic CH_2);^{13}C-NMR (DMSO, δ, ppm): 153, 151, 122, 116, 112, 101, 53, 22, 16.

Synthesis of 2-(5-(10-acetyl-7-amino-9-methyl-8-oxaspiro[4.5]deca-6,9-dien-6-yl)-1,3,4-oxadiazol-2-yl)acetonitrile (10) and ethyl 9-amino-10-(5-(cyanomethyl)-1,3,4-oxadiazol-2-yl)-7-methyl-8-oxaspiro[4.5]deca-6,9-diene-6-carboxylate (11):

An equimolar mixture of compound **9** (1.07 g, 0.005 mol) and acetylacetone or ethyl acetoacetate (0.005 mol) in ethanol (25 mL) was treated with triethylamine (0.5 mL). The reaction mixture was refluxed for 4 h, solvent was removed under reduced pressure and the residual mass was then poured into 50 mL cold water. The formed solid was collected by filtration and recrystallized from aq. ethanol.

10: (72 %) Mp: 244-246 ºC (EtOH); IR (KBr: υ, cm^{-1}): 3396, 3301 (NH_2), 2196 (CN), 1667 (CO); ^{1}H-NMR (DMSO, δ, ppm): 5.66 (br, 2H, NH_2), 4.18 (s, 2H, CH_2),2.10 (s, 3H, CH_3), 1.68-1.18 (m, 11H, cyclic CH_2+CH_3)**.**

11: (70 %) Mp: 238-240 ºC (EtOH); IR (KBr: υ, cm^{-1}): 3390, 3305 (NH_2), 2195 (CN), 1666 (CO); ^{1}H-NMR (DMSO, δ, ppm): 5.65 (br, 2H, NH_2), 4.18 (s, 2H, CH_2),3.98 (q, 2H, CH_{2ester}), 2.10 (s, 3H, CH_3), 1.66-1.12 (m, 14H, cyclic CH_2+$2CH_3$)**.**

Synthesis of 3-amino-4-(5-(cyanomethyl)-1,3,4-oxadiazol-2-yl)-5-thioxo-4,5-dihydrothiophene-2-carbonitrile (12) and 3-amino-4-(5-(cyanomethyl)-1,3,4-oxadiazol-2-yl)-5-(phenylimino)-4,5-dihydrothiophene-2-carbonitrile (13):

(1.48 g, 0.01 mol) of Compound **2** was dissolved in dry dioxan and treated with anhydrous K_2CO_3 (~ 7 g). The reaction mixture was stirred at room temperature for 15 min. and was then treated with carbon disulfide (0.75 mL, 0.0125 mol) or phenylisocyanate (1.05 mL, 0.01 mol) and followed by a catalytic amount of tetrabutylammonium bromide (TBAB). The reaction mixture was then stirred at 60 ºC for 1 h, followed by addition of ethyl chloroacetate (1.06 mL, 0.01 mol) and then further stirred for another 3h. Carbonate was removed by filtration and the dioxan layer was evaporated under reduced pressure. The residual mass was then triturated with light petroleum (40-60 ºC) and the formed solid was collected by filtration.

12: (65 %) Mp: 244-246 ºC (EtOH/DMF 3:1); IR (KBr: υ, cm^{-1}): 3380, 3296 (NH_2), 2195 (CN), 1723 (CO); ^{1}H-NMR

(DMSO, δ, ppm): 5.55 (br, 2H, NH_2), 5.12 (s, 1H, CH), 4.16 (s, 2H, CH_2), 3.98 (q, 2H, CH_2), 1.22 (t, 3H, CH_3).

13:(72 %) Mp: 276-278 ºC (EtOH/DMF 3:1); IR (KBr: υ, cm^{-1}): 3388, 3303 (NH_2), 2192 (CN), 1726 (CO); ^{1}H-NMR (DMSO, δ, ppm): 7.64-7.41 (m, 5H, CH-arom.), 5.59 (br, 2H, NH_2), 5.11 (s, 1H, CH), 4.18 (s, 2H, CH_2), 3.99 (q, 2H, CH_2), 1.20 (t, 3H, CH_3).

Synthesis of (2E,5E)-2,5-(3-(2-cyanoacetyl)-1,3,4-oxadiazolidine-2,5-diylidene)diacetonitrile 14:

(2E,5E)-2,5-(3-(2-cyanoacetyl)-1,3,4-oxadiazolidine-2,5-diylidene)diacetonitrile

An equimolar mixture of compound **2** (1.48 g, 0.01 mol) and ethyl cyanoacetate (1.07 mL, 0.01 mol) in ethanol (~25 mL) was heated under reflux for 3 h. The reaction was monitored by TLC and after reaction completion, solvent was removed by evaporation and the residual solid was collected and recrystallized from DMF into pale yellow needles and was identified as compound **14**.

14: (65 %) Mp: 218-220 ºC (DMF); IR (KBr: υ, cm^{-1}): 3228 (NH), 2202, 2190 (CN), 1696 (CO); ^{1}H-NMR (DMSO, δ, ppm): 9.23 (br, 1H, NH), 5.65 (s, 1H, =CH), , 5.35 (s, 1H, =CH), 4.02 (s, 2H, CH_2).

Synthesis of (E)-7-amino-2-(cyanomethylene)-5-oxo-3,5-dihydro-2H-[1,3,4]oxadiazolo[3,2-a]pyridine-6-carbonitrile 15:

Compound **14** (0.5g) was dissolved in absolute ethanol (25 mL) and few drops of TEA. The reaction mixture was heated under reflux for 2hrs, olvent was removed by evaporation and the residual solid was collected and recrystallized from DCM into pale yellow crystals and was identified as compound **15**.

15: (55 %) Mp: 202-204 ºC (DCM); IR (KBr: υ, cm^{-1}): 3390, 3305, 3224 (NH_2, NH), 2210, 2190 (2CN), 1698 (CO); ^{1}H-NMR (DMSO, δ, ppm): 9.22 (br, 1H, NH), 6.65 (s, 1H, =CH), 5.60 (br, 2H, NH_2), 5.35 (s, 1H, =CH).

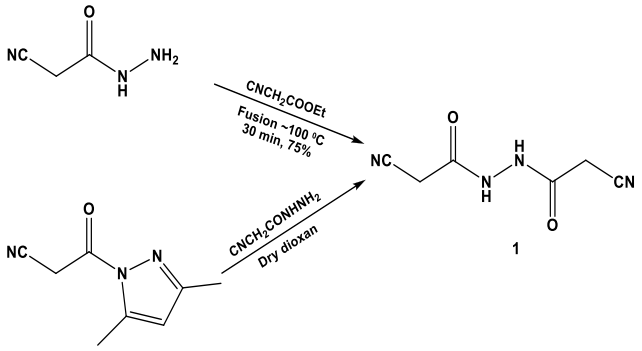
Synthesis of(E)-5-amino-2-(cyanomethylene)-7-oxo-3,7-dihydro-2H-[1,3,4]oxadiazolo[3,2-a]pyridine-6,8-dicarbonitrile15:

An equimolar mixture of compound **2** (1.48 g, 0.01 mol) and ethyl cyanoacetate (1.07 mL, 0.01 mol) in ethanol was treated with few drops of TEA catalyst. The reaction mixture was then heated under reflux for 3 h, solvent was evaporated under reduced pressure and the residual mass was triturated with light petroleum (40-60 ºC) and the formed solid was collected and recrystallized from ethanol into colorless cubes and was identified as compound **15**.

15: (65 %) Mp: 260-263 ºC (DMF); IR (KBr: υ, cm^{-1}): 3386, 3308, 3235 (NH_2, NH), 2202, 2192 (CN), 1696 (CO); ^{1}H-NMR (DMSO, δ, ppm): 9.23 (br, 1H, NH), 5.60 (br, 2H, NH_2), 5.35 (s, 1H, =CH).

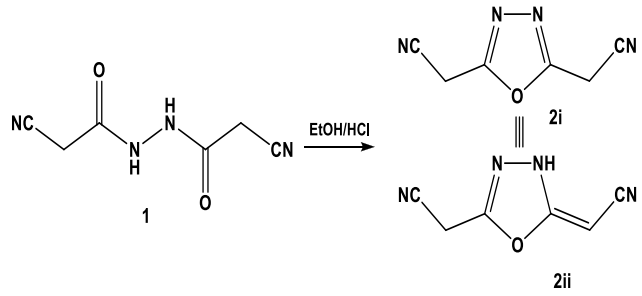
3 Results and Discussion

Various methods of synthesis of 1,3,4-oxadiazoles[34] are available in the literature. However, these methods suffer from disadvantages such as long reaction time, severe reaction conditions[35].The key intermediate 2-cyano-*N'*-(2-cyanoacetyl)acetohydrazide**1** was readily available from the solvent-free reaction of cyanoacetic acid hydrazide with ethyl cyanoacetate or from the reaction of cyanoacetichydrazide with 3-(3,5-dimethyl-*1H*-pyrazol-1-yl)-3-oxopropanenitrile in dry dioxan, Scheme 1.



Scheme 1.

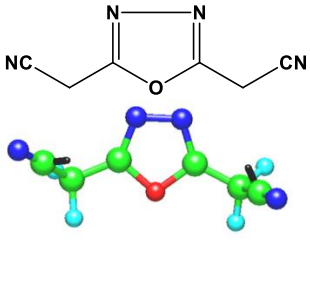
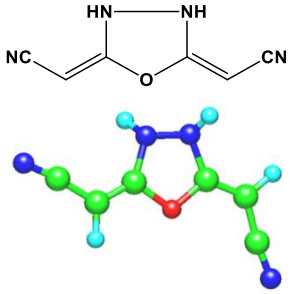
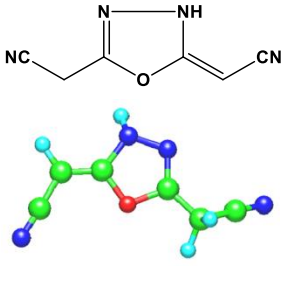
The structure of compound **1** was confirmed on the basis of its elemental and spectral data. The IR spectrum revealed absorption bands at 3242, 2190 and 1670 cm^{-1} assignable to NH, CN and amidic carbonyl groups respectively. The ^{1}H-NMR spectrum displayed two singlet signals at δ 10.25 and δ 4.18 ppm characteristic of NH and CH_2 protons respectively, where the ^{13}C-NMR spectrum showed three signals at δ 168, 152 and 53 ppm characteristic for CO, CN and CH_2 carbon respectively. When compound **1** was boiled in ethanol containing few drops of conc. hydrochloric acid2,2'-(1,3,4-oxadiazole-2,5-diyl)diacetonitrile **2** was obtained as a sole product, Scheme 2.



Scheme 2.

The structure of compound **2** was found to be with accordance with its spectral data. The IR spectrum of compound **2** showed three characteristic maxima, 3321 cm^{-1} for NH group, 2212 cm^{-1} for CN group and 2195 cm^{-1} for the other CN group. The ^{1}H-NMR spectrum showed three singlet bands at δ 9.38, 5.36 and δ 4.18 ppm for NH, =CH and CH_2 protons respectively. The structure of compound **2**

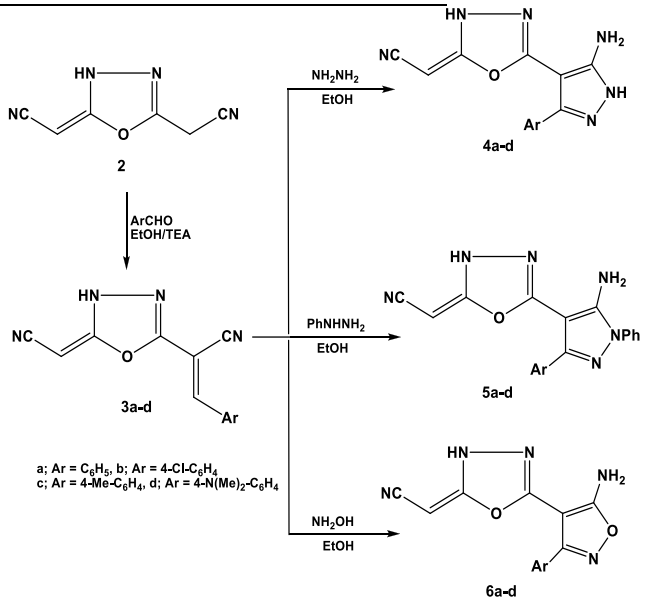
Table 1 indicates the heat of formation for each structure.

Structure	**I**	**II**	**III**
			
H. O. F.	88.3565 Kcal/mol	106.5980 Kcal/mol	98.5292 Kcal/mol

was examined theoretically using WinMOPACsemiempirical calculations using PM3 method[36] where three different possible structure were suggested for compound **2** and according to the results of calculations structure **I** has the lowest heat of formation indicating the it is the more stable structure or in other word the more contributing structure, Table 1.

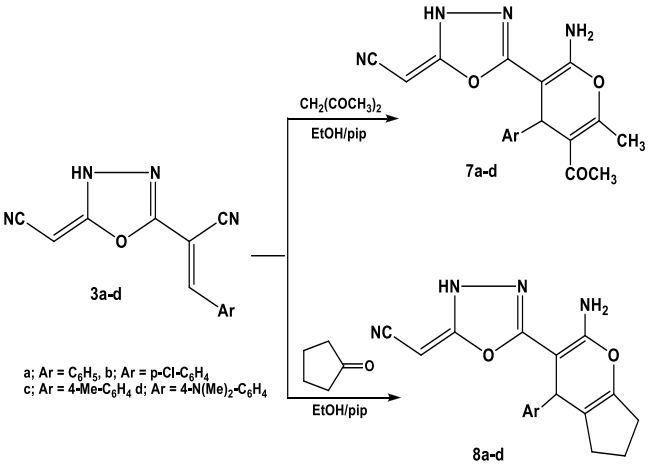
The reactivity of compound **2** towards aromatic aldehydes was investigated. Thus treatment of compound **2** with aromatic aldehydes in ethanol in the presence of TEA catalyst afforded the corresponding condensation products (*E*)-2-(5-(cyanomethyl)-1,3,4-oxadiazol-2-yl)-3-arylacrylonitrile**3a-d**. The spectroscopic data of the isolated compounds was in complete accordance with the proposed structures of compounds **3a-d**, Scheme 3. The reactivity of *(E)*-2-(5-(cyanomethyl)-1,3,4-oxadiazol-2-yl)-3-arylacrylonitrile**3a-d** towards Michael-type addition was investigated, where the reaction of compound **3a-d** with hydrazine, phenylhydrazine or hydroxylamine in ethanol afforded 2-(5-(5-amino-3-phenyl-*1H*-pyrazol-4-yl)-1,3,4-oxadiazol-2(*3H*)-ylidene)acetonitrile**4a**-d, 2-(5-(5-amino-1,3-diphenyl-*1H*-pyrazol-4-yl)-1,3,4-oxadiazol-2(*3H*)-ylidene)acetonitrile**5a-d** or 2-(5-(5-amino-3-phenylisoxazol-4-yl)-1,3,4-oxadiazol-2(*3H*)-ylidene)acetonitrile**6a-d** respectively, Scheme 3.

Spiro compounds represent an important class of naturally occurring heterocycles characterized by highly pronounced biological properties.[37,38] In this context, we explore the synthetic versatility of compound **2** for the synthesis of spiro compounds. Thus, treatment of 2,2'-(1,3,4-oxadiazole-2,5-diyl)diacetonitrile**2** with cyclopentanone in refluxing ethanol in the presence of TEA catalyst afforded the corresponding condensation product namely:2-(5-(cyanomethyl)-1,3,4-oxadiazol-2-yl)-2-cyclopentylideneacetonitrile**9**. Treatment of compound **2** active methylene compounds e.g. acetylacetone or ethyl acetoacetate afforded the corresponding spiroheterocycles **10** and **11** respectively, Scheme 5.

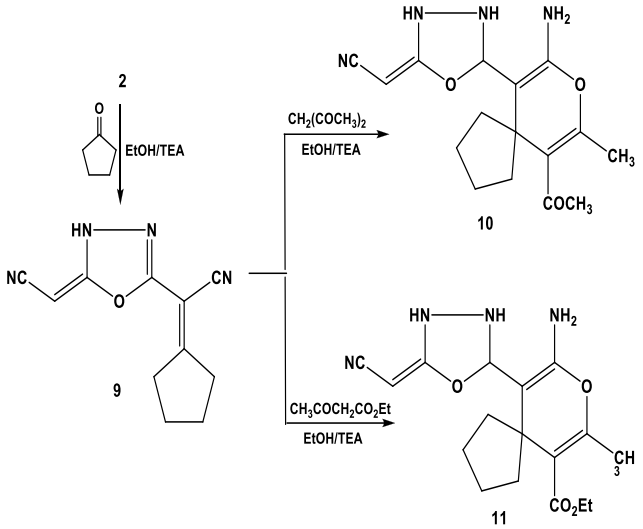


Scheme 3

On the other hand, when compounds **3a-d** were allowed to react with acetylacetone or cyclopentanone in absolute ethanol in the presence of piperidine catalyst afforded the corresponding pyran derivatives **7a-d** and **8a-d** respectively, Scheme 4.

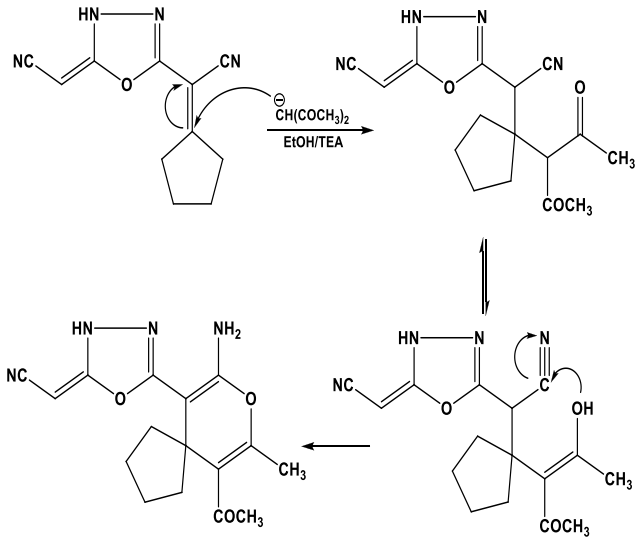


Scheme 4.



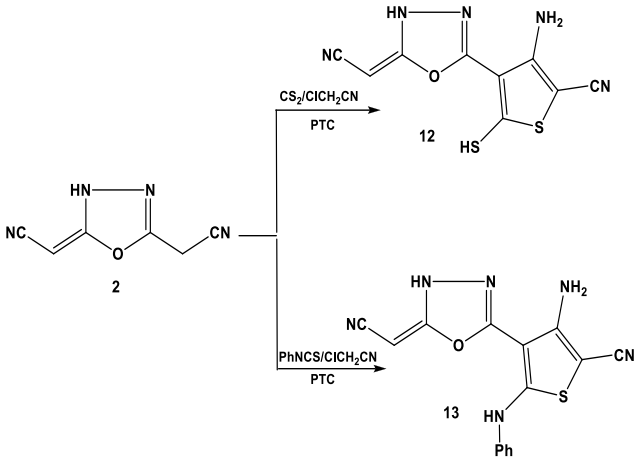
Scheme 5.

The reaction mechanism was assumed to proceed *via* a preliminary nucleophilicattact of the carbanion formed from acetylacetone or ethyl acetoacetate onto the olefinic bond of compound **9** followed by another nucleophilic attack of the enolic OH to the CN group and subsequent cyclisation, Scheme 6.



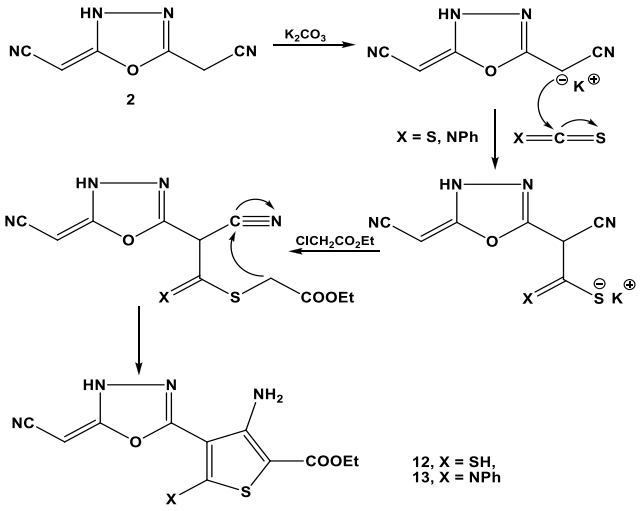
Scheme 6.

Furthermore, the one-pot reaction of compound **2**, carbon disulfide and chloroacetonitrile under phase transfer catalysis conditions [benzene/K_2CO_3/TBAB] resulted the formation of thiophene derivative **12**, where the one-pot reaction of compound **2** with phenylisothiocyanate and chloroacetonitrile under the same conditions gave the thiophene derivative **13**, Scheme 7.



Scheme 7.

The formation of compounds **12** and **13** was assumed to proceed *via* a preliminary nucleophilic attack the carbanion formed from compound **2** onto the polarized C=S bond in CS_2 or PhNCS followed by S-alkylation of the addition product by ethyl chloroacetate and subsequent cyclization *via* another nucleophic attack onto the CN group, Scheme 8.



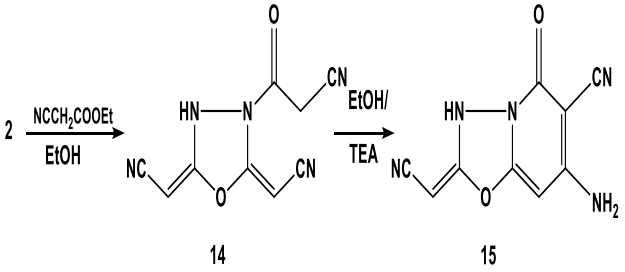
Scheme 8

Finally, the reaction of compound **2** with ethyl cyanoacetate in different reaction conditions was extensively studied, where compound **2** was allowed to react with ethyl cyanoacetate in ethanol without using any catalyst afforded(*2E,2'E*)-2,2'-(3-(2-cyanoacetyl)-1,3,4-oxadiazolidine-2,5-diylidene)diacetonitrile **14** which gave7-amino-2-(cyanomethyl)-5-oxo-5*H*-[1,3,4]oxadiazolo[3,2-a]pyridine-6-carbonitrile **15** after heating in ethanol in the presence of a catalytic amount of TEA, Scheme 9.

Table 2. Antimicrobial Activities of the newly Synthesized Compounds.

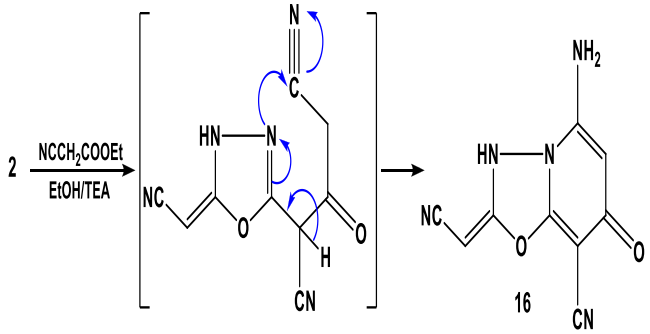
Comp. No.	Inhibition zone (mm)						
	Gram-negative		Gram-positive		Fungi		Yeast
	E. Coli	*p. putida*	*B. subtilis*	*S. lactis*	*A. niger*	*P. sp.*	*C. albicans*
4a	7	9	10	9	8	8	0
4b	14	15	10	14	10	12	0
4c	12	12	8	8	10	10	0
4d	8	10	6	8	10	10	0
5a	12	12	8	12	12	10	0
5b	15	12	13	13	11	8	0
5c	14	12	12	12	8	8	0
5d	10	10	10	10	8	8	0
6a	10	11	10	10	8	8	0
6b	15	13	12	11	10	10	0
6c	13	12	8	8	10	12	0
6d	10	10	10	11	8	8	0
7a	11	10	10	10	8	8	0
7b	12	12	11	10	8	8	0
7c	14	12	12	12	8	8	0
7d	10	10	10	11	8	8	0
8a	10	11	13	11	10	8	0
8b	14	12	14	15	12	12	2
8c	12	12	10	8	7	6	0
8d	10	8	8	8	6	6	0
10	12	10	11	10	8	8	0
11	13	10	12	8	7	7	0
12	15	15	14	12	12	10	2
13	15	15	10	10	12	12	4
15	8	7	6	6	6	7	0
16	7	8	8	10	8	6	0
Ampicillin	24	20	19	22	24	14	14

E. coli = Escherichia coli; P. putida = Pseudomonas putida; B. subtilis = Bacillus subtilis; S. lactis = Streptococcus lactis; A. niger = Aspergillusniger; P. sp. = Penicilliumsp; C. albicans = Candida albicans. The sensitivity of microorganisms to the tested compounds is identified in the following manner *: Highly sensitive = Inhibition zone 15-20 mm; Moderately sensitive = Inhibition zone: 10-15 mm; Slightly sensitive = Inhibition zone: 5-10 mm; Not sensitive = Inhibition zone: 0 mm;* Each result represents the average of triplicate readings. The newly synthesized compounds were screened for their antimicrobial activities and they showed moderate to potent activity against their corresponding pathogens.



Scheme 9

At the same time the reaction of compound **2** with ethyl cyanoacetate was proceeded in ethanol the presence of TEA catalyst where 5-amino-2-(cyanomethylene)-7-oxo-3,7-dihydro-2*H*-[1,3,4]oxadiazolo[3,2-a]pyridine-6,8-dicarbonitrile **16** was obtained, Scheme 10.



Scheme 10

The elemental and spectral data of the obtained compounds were with accordance with their proposed structures.

3.1 Antimicrobial evaluation

The newly synthesized heterocyclic compounds listed in

Table 1 were tested for their antimicrobial activity against the following microorganisms: Escherichia coli, Pseudomonas putida, Bacillus subtilis, Streptococcus lactis, Aspergillus niger, Penicillium sp. and Candida albicans. The preliminary screening of the investigated compounds was performed using the filter paper disc-diffusion method. The most active compounds were **4b**, **5b**, **6b,c**, **7b,c**, **8b,c**, **11**,**12**, and **13**, which were slightly inhibitory to the microorganisms. The rest of compounds showed weak to moderate sensitivity to the tested organisms, and the results are summarized in Table 1.

3.2 Antimicrobial screening

The newly synthesized heterocyclic compounds were tested for their antimicrobial activity against the following microorganisms: (a) Gram-negative: Escherichia coli and Pseudomonas putide; (b) Gram-positive: Bacillus subtilis and Streptococcus lactis; (c) Fungi: Aspergillusniger and Penicillium sp.; (d) Yeast: Candida albicans

3.3 Procedure (Filter paper diffusion method)[39]

Proper concentrations of microbial suspensions were prepared from 1 (forbacteria) to 3 (for yeast and fungi)-day-old liquid stock cultures incubated on a rotary shaker (100 rbm). In the case of fungi, 5 sterile glass beads were added to each culture flask. The mycelia were then subdivided by mechanical stirring at speed No.1 for 30 minutes. Turbidity of microorganisms was adjusted with a spectrophotometer at 350 nm to give an optical density of 1.0. Appropriate agar plates were aseptically surface inoculated uniformly by a standard volume (ca. 1 ml) of the microbial broth culture of the tested microorganism, namely E. coli, P. putide, B. subtilis, S. Lactis. A. Niger, Penicillium sp. and C. albicans.

Whatman No. 3 filter paper discs of 10 mm diameter were sterilized by autoclaving for 15 minutes at 121°C. Test compounds were dissolved in 80% ethyl alcohol to give final concentration of 5 μg/ml. The sterile discs were impregnated with the test compounds (5 μg/disc). After the impregnated discs have been air dried, they were placed on the agar surface previously seeded with the organism to be tested. Discs were gently pressed with forceps to insure thorough contact with the media. Three discs were arranged per dish, suitably spaced apart, i.e. the discs should be separated by a distance that is equal to or slightly greater than the sum of the diameters of inhibition produced by each disc alone. Each test compound was conducted in triplicate. Plates were kept in the refrigerator at 5°C for 1 hour to permit good diffusion before transferring them to an incubator at 37°C for 24 hours for bacteria and at 30°C for 72 hours for yeast and fungi.

4 Conclusion

In this context, we have reported a simple and facile synthesis for a variety of fused and spiroheterocycles bearing 1,3,4-oxadiazole moiety. The biological activity of the synthesized oxadiazole derivatives was evaluated, where they revealed different potencies against bacteria and fungi. Plausible mechanisms to account for the formation of the suggested products were formulated.

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