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Syntheses of *N*-acylhydrazones of 2-hydroxy-3,5-dinitrobenzohydrazide, and their Conversion into 3-Acetyl-2,3-dihydro-1,3,4-oxadiazole

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Abstract:

N-Acylhydrazones **11-17** were prepared *via* reaction of 2-hydroxy-3,5-dinitrobenzohydrazide **3** with aromatic aldehydes **4-6** or with aromatic ketone **7** or aliphatic acyclic ketone **8** and cyclic aliphatic ketones **9-10** under reflux in ethanol. Conversion of hydrazide hydrazone **17** into *N*-acetyl-1,3,4-oxadiazole derivative **19** was achieved *via* reaction with acetic anhydride **18**. The products were characterized by MS, ^{1}H-NMR and ^{13}C-NMR. The structure of **19** was confirmed by X-ray analysis. Compound **19** was evaluated for *in vitro* antibacterial activity against two microorganisms (*S. aureus* and *E. coli*). The obtained results showed that this compound has good inhibition against the tested bacterial pathogens by micro-dilution method with MIC 15.1 µg.

Keywords:

Hydrazide-Hydrazone, 3,5-dinitrosalicylaic acid, 1,3,4-oxadiazole, antibacterial activity

1.Introduction:

Hydrazide-hydrazones have the general formula $R_1R_2C=N-NHCOR$ are organic compounds that received much interest in the field of medicinal chemistry and exploring of new drugs **(Popiołek, 2017, Angelova, et. al., 2022)**. They are playing an important role as pharmacophore in many drugs and they have significant *anti*-bacterial and *anti*-fungal **(Burki et. al., 2020; Krátký et. al., 2021; Morjan et. al., 2014; Pham et. al., 2019; Shabeeb et. al., 2018)** , *anti*-convulsant **(SiN-Ha et. al., 2018; Thorat et. al., 2021)**, *anti*-inflammatory **(M. I. Han et. al., 2019; Zeeshan et. al., 2019)**, *anti*-malarial **(Verma et. al., 2014)** *anti*-tubercular **(Kazakova et. al., 2021; Mokhnache et. al., 2020)** and *anti*-cancer activates **(Atalay et. al., 2020; M. İ. Han et. al., 2021; Samir et. al., 2021)**. The hydrazide-hydrazones are normally prepared through the condensation of acid hydrazide $RC=O-N-HN-H_2$ with different aldehydes and ketones **(Popiołek, 2017; Ramazani et. al., 2011)**. In addition to their own importance; hydrazide-hydrazones are precursors to pharmacologically active 1,3,4-oxadiazoles. Moreover it was reported that compounds containing the 1,3,4-oxadiazole moiety have received much interest due to their potential activities as *anti*-microbial agents **(Desai et. al., 2021; Rao et. al., 2020; Siwach & Verma, 2020)**, *anti*-tumor **(Agarwal et. al., 2016; Liu et. al., 2021; Mohamed Jawed et. al., 2020)**, *anti*-inflammatory agents **(Ahsan et. al., 2011; Koksal et. al., 2021; Mishra et. al., 2005)**. A number of drugs containing a 1,3,4-oxadiazole ring are commercially available. For example; Zibotentan contains a 1,3,4-oxadiazole moity has been approved by the FDA as *anti*-cancer agent **(Siwach & Verma, 2020; Sudeesh & Gururaja, 2017)**. Furamizole **(Singh & Chauhan, 2013; Siwach & Verma, 2020)**, which has strong *anti*bacterial activity, and Raltegravir **(Hicks & Gulick, 2009)**, an *anti*-viral HIV integrate inhibitor drug, are further examples of commercially important 1,3,4-dioxazole-containing drugs (**Figure 1**).

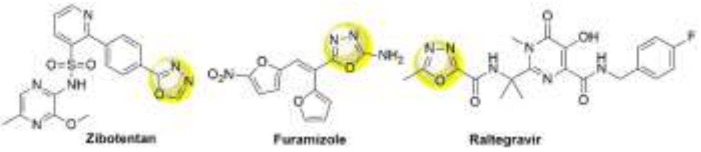
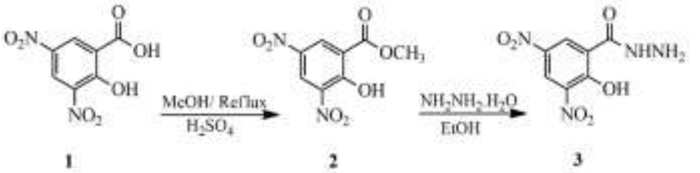


Fig.1: Commercially available drugs contains 1,3,4-oxadiazole ring

This work describes the preparation of *N*-acylhydrazone of 2-hydroxy-3,5-dinitrobenzohydrazide and attempts of their conversion into *N*-acetyl-1,3,4-oxadiazole derivatives.

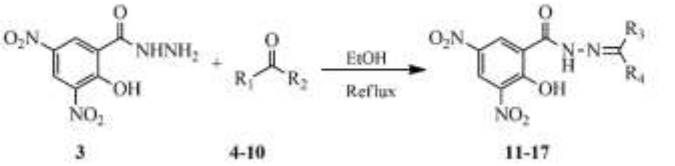
2.Results and Discussion:

The starting material **1** was purchased from Sigma-Aldrich and used without any further purification. Compounds **2** and **3** were synthesized through esterification of **1** with methanol followed by reacting the resulted ester **2** with hydrazine hydrate (**Scheme 1**). The obtained physical and spectroscopic data were in good accordance with the reported data **(Shen et al., 2008)**.



Scheme 1: Syntheses of starting materials

The new series of *N*-acylhydrazones **11-17** was obtained by condensation of 2-hydroxy-3,5-dinitrobenzohydrazide **3** with aldehydes **4-6** or with ketone **7,** or aliphatic acyclic ketone **8** and cyclic aliphatic ketones **9-10**, under reflux in ethanol to yields the target compounds in 75-85% yield (**Scheme 2**).



4) $R_1 = H, R_2 = Ph$
5) $R_1 = H, R_2 = 4\text{-ClPh}$
6) $R_1 = H, R_2 = 4\text{-OMePh}$
7) $R_1 = CH_3, R_2 = Ph$
8) $R_1 = R_2 = CH_3$
9) $R_1 + R_2 = C_5H_{10}$
10) $R_1 + R_2 = C_7H_{14}$

11) $R_1 = H, R_2 = Ph$
12) $R_1 = H, R_2 = 4\text{-ClPh}$
13) $R_1 = H, R_2 = 4\text{-OMePh}$
14) $R_1 = CH_3, R_2 = Ph$
15) $R_1 = R_2 = CH_3$
16) $R_1 + R_2 = C_5H_{10}$
17) $R_1 + R_2 = C_7H_{14}$

Scheme 2: Syntheses of *N*-acylhydrazones

The structure of compounds **11-17** was confirmed by the spectroscopic analyses data. In the ^{1}H-NMR the hydrazine-amide N-H appeared as a broad singlet at ~ 10.5-11.5 ppm in D.M.S.O-d_6. Signals for the two protons corresponding to the core substituted benzene ring were observed as two doublets at ~ δ 8.8 and 8.6 ppm. A singlet peak resonating in the region 8.3-8.7 ppm indicated the formation of the N=CH bond in aldehyde derived compounds **11-13**. Analyses of both ^{1}H-NMR and ^{13}C-NMR spectra for compounds **11-13** showed two signals in ratio of 2:1. These peaks arises from the possibility of formation of possible *E/Z* diastereomers. Two sets of peaks were clearly seen in ^{1}H-NMR or ^{13}C-NMR spectra or both indicating the existence of the stereoisomer's mixture. The spectroscopic data obtained for compound **13** is described here as an illustrative example. The ^{1}H-NMR spectrum for **13** (**Figure 2**) showing two peaks for the hydrazine-amide N-H at 13.9 and 12.6 ppm. The H signals of the aromatic ring holding the two nitro groups also resonated as two sets of doublets for each proton and were seen at 8.8, 8.7, 8.6 and 8.6 ppm. The N=CH proton appeared as two singlet peaks at 8.6 and 8.3 ppm. The protons of the aromatic ring that is holding the OCH_3 group appeared as two sets of doublet peaks with integration value corresponding to 2H at 7.8, 7.7, 7.1 and 7.0 ppm. The two singlet peaks with integration value corresponding to three protons each at 3.83 and 3.81 were assigned for the protons of the OCH_3 group.

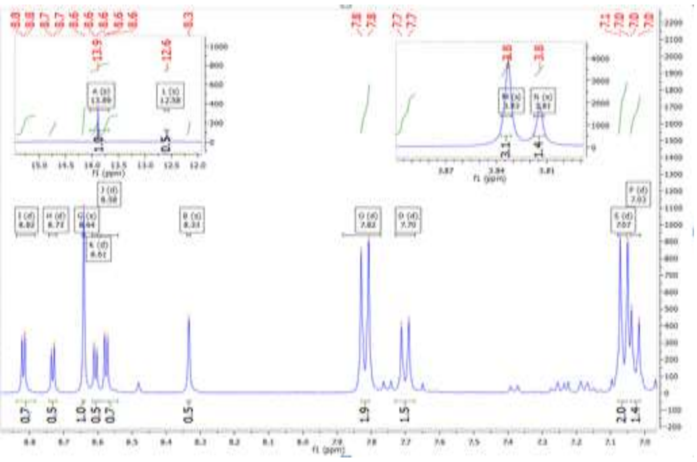


Fig.2: ^{1}H-NMR spectra for compound **13**

The structure of compound **13** and the formation of diastereomers was also confirmed by the ^{13}C-NMR analysis (**Figure 3**). The duplication of some major peaks was clearly shown in the ^{13}C-NMR spectrum. Two peaks at 168.9 and 168.4 ppm are seen for the C=O group and another two peaks for the quaternary C atom holding the OH group are resonated at 164.4 and 162.1 ppm.

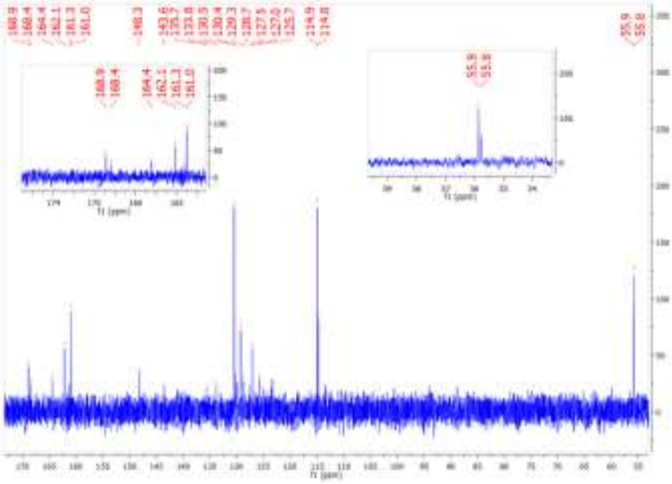
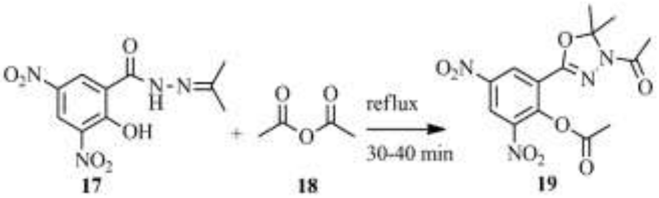


Fig.3: ^{13}C-NMR spectra for compound **13**

The quaternary carbon of the phenyl group that is bonded to the OCH_3 group appeared as two peaks at 161.3 and 161.0. The duplication of carbon atom of C=N group were also seen in the spectrum at 148 ppm. The spectrum shown two peaks for the OCH_3 group at 55.9 and 55.8 ppm providing an unambiguous evidence about the formation of a mixture of diastereomers.

We also tried to convert the synthesized compounds into 1,3,4 oxadiazole. Thus, refluxing compound **17** in acetic anhydride **18** for 30–40 min provided the corresponding 1,3,4-oxadiazole **19** (**Scheme 3**), which was fully characterized by the different spectroscopic analyses.



Scheme 3: Conversion of **17** *into* 1,3,4-oxadiazol

Analysis of compound **19** showed conversion of *N*-acylhydrazone **17** into the *N*-acetyl 1,3,4-oxadiazole **19** with concurrent acetylation of the phenolic OH. Fortunately, suitable crystals of compound **19** enabled X-ray crystal structure determination and further proved the structure of **19** (**Figure 4**). (**CCDC 2003006**).

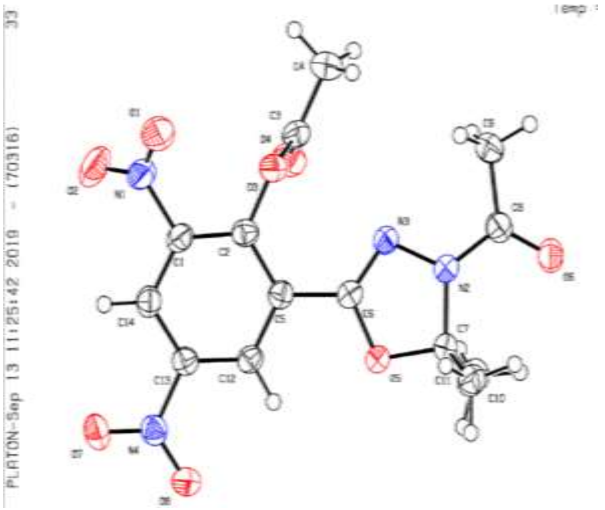


Fig. 4: X-ray structure for compound **19**

***Anti*bacterial Activity Evaluation of 19**

The agar well diffusion method was utilized to carry out the initial screening of compound **19** to evaluate its *anti*-bacterial activity. A lawn of standardized bacterial inoculum was spread into the surface of Muller-Hinton agar plates. A well is dug into the agar using sterile glass pasteur pipette. 100.0 µl of the stock solution was added to the well. The plate was left for about 30 minutes at the refrigerator to enable the diffusion of **19**. The plate was then incubated for 24 hours at 37 ^{o}C. The zone of inhibition is measured and reported in mm. All tests were implemented in triplicates and the Minimum Inhibitory Concentration (MIC) test was performed using the micro-broth dilution method. A sterile 96 microtiter plates were utilized. A stock solution prepared by dissolving **19** in D.M.S.O and was diluted and transferred into the first well and serial dilutions were performed. The standardized bacterial inoculum (0.5 Mac. Farland standard) was added to all wells and the plates were incubated for 24h at 37 ^{o}C. The *E. Coli*. ATCC 25922 (G. Negative) and *S. aureus* ATCC 29213 (G. positive) were used as bacterial strains. *Anti*-microbial activity was determined by adding 20 µl of 0.5% aqueous 2,3,5-Triphenyl-tetrazolium chloride (TTC). MIC was defined as the lowest concentration of the test compound that inhibited visible growth, as indicated by color change. Compound **19** showed similar *anti*bacterial activity against both *E. Coli*. and *S. aureus* in the two assays used. An average zone of inhibition of 27 mm for *E. Coli*. and 26 mm for *S. aureus* were recorded. However, in MIC assay a 15.2 µg value was obtained for **19** against the two test organisms.

Experimental

General method for the synthesis of *N*-acylhydrazones (11-17)

A mixture of 2-hydroxy-3,5-dinitrobenzohydrazide **3** (0.01 mol) and aldehydes or ketones **4-10** (0.01 mol) in ethanol (30 mL) was stirred under reflux until a solid precipitate had formed (30-90 min). The reaction mixture was allowed to cool to room temperature, and the solid precipitate was filtered and recrystallized from ethanol to give the compounds **11-17** in 75-85% yield.

Synthesis of *N*-acetyl 1,3,4-oxadiazole 19

N-Acylhydrazone **17** (0.01 mol) in acetic anhydride **18** (10ml) was stirred under reflux. After 30-40 min TLC indicated that most of the starting material had been converted into product. The reaction mixture was allowed to cool to room temperature and then quenched by pouring in water (10 mL). After stirring for 15 min to allow hydrolysis of acetic anhydride, water and acetic acid were removed *invacuo*. The organic layer residue was extracted with CH_2Cl_2 (2 x 20 mL) and dried over $MgSO_4$. Solvent was removed *invacuo* and the crude product was purified by flash chromatography (Hexane → Hexane: Et_2O, 1:4) to give pure products in 82% yield.

N'-Benzylidene-2-hydroxy-3,5-dinitrobenzohydrazide 11

Mp. (202-204 °C); Yields (80%); ^{1}H-NMR (400 MHz, D.M.S.O) δ 13.9 (s, 1H, N-H), 12.6 (s, 1H, N-H), 8.8 (d, *J* = 3.3 Hz, 1H, Ar-**H**), 8.7 (s, 1H, N=C**H**), 8.6 (d, *J* = 3.3 Hz, 1H, Ar-**H**), 8.4 (s, 1H, N=C**H**), 7.9 (dd, *J* = 7.2, 2.1 Hz, 3H), 7.8 – 7.7 (m, 2H, Ph), 7.5 (dd, *J* = 8.0, 2.4 Hz, 3H), 7.5 – 7.4 (m, *J* = 7.3, 4.8 Hz, 2H) mixture of diastereomers. ^{13}C-NMR (100 MHz, D.M.S.O) δ 168.8, 168.6 (**C**=O), 162.0, 161.8 (**C**-OH), 148.4 (N=**C**H), 141.27, 141.11 (Ar**C**NO_2), 134.9, 134.3 (Ar**C**NO_2), 131.9, 131.7, 130.5, 130.3, 129.4, 129.2, 128.8, 128.5, 127 (aromatic carbons) mixture of diastereomers. L.R.M.S (ES$^+$) 329 [M - H]$^+$; H.R.M.S (TOF-ES$^-$) *m/z* calcd. for $C_{14}H_9O_6N_4$ [M - H]$^+$ 329.0528, found 329.0529.

N'-(4-Chlorobenzylidene)-2-hydroxy-3,5-dinitrobenzohydrazide 12

Mp. (204-206 °C); Yields (83%); ^{1}H-NMR (400 MHz, D.M.S.O) δ 14.0 (s, 1H, N-H), 13.5 (s, 1H, N-H), 12.6 (s, 1H, N-H), 8.8 (d, *J* = 3.3 Hz, 1H, Ar-**H**), 8.8 (d, *J* = 3.4 Hz, 1H, Ar-H), 8.7 (d, *J* = 3.4 Hz, 1H, Ar-**H**), 8.7 (s, 1H, N=C**H**), 8.6 (d, *J* = 3.3 Hz, 1H, Ar-**H**), 8.6 (d, *J* = 3.3 Hz, 1H, Ar-**H**), 8.5 (d, *J* = 3.4 Hz, 1H, Ar-**H**), 8.42 (s, 1H, N=C**H**), 8.3 (s, 1H, N=C**H**), 7.9 (d, *J* = 8.5 Hz, 2H, ClPh-**H**), 7.8 (d, *J* = 8.5 Hz, 2H, ClPh-**H**), 7.7 (d, *J* = 8.5 Hz, 2H, 2H, ClPh-**H**), 7.6 (d, *J* = 8.5 Hz, 2H, 2H, ClPh-**H**), 7.5 (d, *J* = 8.5 Hz, 2H, 2H, ClPh-**H**), 7.4 (d, *J* = 8.5 Hz, 2H, 2H, ClPh-**H**). ^{13}C NMR (100 MHz, D.M.S.O) δ 168.9, 168.4 (**C**=O), 161.9, 161.2 (Ar**C**-OH), 147.1 (N=**C**H), 140.9, 136.5, 134.8, 134.2, 133.9, 133.1, 132.9, 132.7, 130.5, 130.4, 130., 129.6, 129.4, 129.3, 125.7, 125.2 (mixture of diastereomers). L.R.M.S (ES$^+$) 363 [M - H]$^+$; H.R.M.S (TOF-ES$^-$) *m/z* calcd. for $C_{14}H_8O_6N_4Cl$ [M - H]$^+$ 363.0138, found 363.0140.

2-Hydroxy-*N'*-(4-methoxybenzylidene)-3,5-dinitrobenzohydrazide 13

Mp. (212-214 °C); Yields (82%); ^{1}H-NMR (400 MHz, D.M.S.O) δ 13.9 (s, 1H, N-H), 12.6 (s, 1H, N-H), 8.8 (d, *J* = 3.3 Hz, 1H, Ar**H**), 8.7 (d, *J* = 3.3 Hz, 1H, Ar**H**), 8.6 (s, 1H, C**H**), 8.6 (d, *J* = 3.3 Hz, 1H, Ar**H**), 8.6 (d, *J* = 3.3 Hz, 1H, Ar**H**), 8.3 (s, 1H, C**H**), 7.8 (d, *J* = 8.7 Hz, 2H, Ar**H**), 7.7 (d, *J* = 8.7 Hz, 2H, Ar**H**), 3.83 (s, 3H, OCH_3), 3.81 (s, 3H, OCH_3) mixture of diastereomers. ^{13}C-NMR (100 MHz, D.M.S.O) δ 168.9, 168.4 (**C**=O), 164.4, 162.1 (Ar**C**-OH), 161.3, 161.0 (Ar**C**-OCH_3), 148.3 (N=**C**H), 143.5 (Ar**C**-NO_2), 135.6 (Ar**C**-NO_2), 130.5, 130.4 (**C**H-Ar), 129.3 (**C**H-Ar), 127.0 (**C**-Ar), 125.7 (**C**HAr), 114.9, 114.8 (**C**H-Ar), 55.86, 55.76 (O**C**H_3) mixture of diastereomers. L.R.M.S (ES$^+$) 359 [M - H]$^+$; H.R.M.S (TOF-ES$^-$) *m/z* calcd. for $C_{15}H_{11}O_7N_4$ [M - H]$^+$ 359.0633, found 359.0635.

2-Hydroxy-3,5-dinitro-*N'*-(1-phenylethylidene)benzohydrazide 14

Mp. (216-218 °C); Yields (80 %) ^{1}H-NMR (400 MHz, D.M.S.O) δ 13.9 (s, 1H, N-H), 8.9 (d, *J* = 3.3 Hz, 1H, Ar-**H**), 8.6 (d, *J* = 3.3 Hz, 1H, , Ar-**H**), 7.86 (dd, *J* = 7.9, 1.7 Hz, 2H, , Ar-**H**), 7.49 – 7.39 (m, 3H, Ar-**H**), 2.36 (s, 3H, C**H**$_3$). ^{13}C-NMR (100 MHz, D.M.S.O) δ 169.1 (**C**=O), 161.7 (**C**-OH), 151.3 (N=**C**), 141.2, 138.6, 130.4, 129.6, 128.8, 126.9, 124.9, 123.6 (Aromatic carbons), 14.7 (**C**H$_3$). L.R.M.S (ES$^+$) 343 [M **-** H]$^+$; H.R.M.S (TOF-ES$^-$) *m/z* calcd. for $C_{15}H_{11}O_6N_4$ [M - H]$^+$ 343.0684, found 343.0685.

2-Hydroxy-3,5-dinitro-*N'*-(propan-2-ylidene)benzohydrazide 15

Mp. (228-229 °C); Yields (85%); ^{1}H-NMR (400 MHz, D.M.S.O) δ14.0 (s.1H,OH), δ10.4 (s,1H,N-H), δ 8.8 (d, *J* = 3.3 Hz, 1H, Ar-**H**), 8.7 (d, *J* = 3.3 Hz, 1H, Ar-**H**), 8.6 (d, *J* = 3.3 Hz, 1H, Ar-**H**), 8.58 (d, *J* = 3.3 Hz, 1H, Ar-**H**), 2.10 (s, 3H, C**H**$_3$), 2.0 (s, 3H, C**H**$_3$). ^{13}C-NMR (100 MHz, D.M.S.O) δ 168.4 (**C**=O), 164.5 (**C**-OH), 161.5 (**C**=N), 140.9 (**C**-NO_2), 130.3 (**C**H-Ar), 129.3 (**C**-NO_2) 124.9 (**C**H-Ar), 123.1 (**C**C=O) 24.8 (**C**H$_3$), 18.6 (**C**H$_3$). L.R.M.S (ES$^+$) 281 [M **-** H]$^+$; H.R.M.S (TOF-ES$^-$) *m/z* calcd. for $C_{10}H_9O_6N_4$ [M - H]$^+$ 281.0767, found 281.0856.

N'-Cyclohexylidene-2-hydroxybenzohydrazide 16

Mp. (226-227 °C); Yields (78%); ^{1}H-NMR (400 MHz, D.M.S.O-H_6) δ 12.8 (s, 1H, O**H**), 12.02 (s, 1H, N-H), 8.9 (d, *J* = 7.2 Hz, 1H, Ar-**H**), 8.3 (d, *J* = 6.5 Hz, 1H, Ar-**H**), 7.9 (s, 2H, Ar-**H**), 3.3 (m, 4H, cyclohexane), 2.6 (m, 6H, cyclohexane).). ^{13}C-NMR (100 MHz, D.M.S.O-H_6) δ 165.0 (**C**=O), 163.9 (Ar**C**-OH), 159.2 (N=**C**), 135.9, 133.4, 122.5, 120.8, 119.6 (aromatic carbons), 29.7, 28.4, 27.8, 26.7 (aliphatic carbons). L.R.M.S (ES$^+$) 231 [M - H]$^+$; H.R.M.S (TOF-ES$^-$) *m/z* calcd. for $C_{13}H_{15}O_2N_2$ [M - H]$^+$ 231.1139, found 231.1134.

N'-Cyclooctylidene-2-hydroxybenzohydrazide 17

Mp. (226-227 °C); Yields (75%); ^{1}H-NMR (400 MHz, D.M.S.O-H_6) δ 12.8 (s, 1H, ArCO**H**), 12.2 (s, 1H, N-H), 9.1 (d, *J* = 7.3 Hz, 1H, C**H**), 8.5 (t, *J* = 7.1 Hz, 1H, C**H**), 8.1 (dd, *J* = 15.1, 7.7 Hz, 2H, 2x C**H**), 3.5 (dd, *J* = 12.5, 5.7 Hz, 4H, 2 x **CH**$_2$), 2.85 (s, 4H, 2 x **CH**$_2$), 2.67 – 2.40 (m, 6H, 3 x **CH**$_2$). ^{13}C NMR (100 MHz, D.M.S.O-H_6) δ 165.9 (**C**=O), 164.1 (Ar**C**-OH), 159.1 (N=**C**), 135.9, 133.4, 122.5, 120.8, 119.6 (aromatic carbons), 38.9, 30.8, 29.8, 28.5, 27.8, 26.7 (aliphatic carbons). L.R.M.S (ES$^+$) 259 [M - H]$^+$; H.R.M.S (TOF-ES$^-$) *m/z* calcd. for $C_{15}H_{19}O_2N_2$ [M - H]$^+$ 259.1452, found 259.1452.

1-(5-(2-Hydroxy-3,5-dinitrophenyl)-2,2-dimethyl-1,3,4-oxadiazol-3(2H)-yl)ethanone 19

Mp. (78-80 °C); Yields (82%); ^{1}H-NMR (400 MHz, $CDCl_3$) δ 9.0 (d, *J* = 2.7 Hz, 1H), 8.9 (d, *J* = 2.7 Hz, 1H), 2.5 (s, 3H, CH_3), 2.3 (s, 3H, CH_3), 1.9 (s, 6H, 2 x CH_3). ^{13}C-NMR (100 MHz, $CDCl_3$) δ 167.1 (C=O), 167.0 1 (C=O), 147.9, 146.8, 144.9, 127.9 (Ar-C), 123.85 (CH-Ar), 122.5 (CH-Ar), 101.5 (OC=N), 24.8 (2 x CH_3), 22.5 (CH_3C=O), 20.8 (CH_3C=O) L.R.M.S (ES+) 257 [M + Na]+; H.R.M.S (TOF-ES-) m/z calcd. for $C_9H_{15}O_7$ [M + H]+ 235.0812, found 235.0805.

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References

Agarwal, M., Singh, V., Sharma, S. K., Sharma, P., Ansari, M. Y., Jadav, S. S., Yasmin, S., Sreenivasulu, R., Hassan, M. Z., Saini, V., & Ahsan, M. J. (2016). Design and synthesis of new 2,5-disubstituted-1,3,4-oxadiazole analogues as *anti*cancer agents. *Medicinal Chemistry Research, 25*(10), 2289-2303. doi:https://doi.org/10.1007/s00044-016-1672-1

Ahsan, M. J., Samy, J. G., Khalilullah, H., Nomani, M. S., Saraswat, P., Gaur, R., & Singh, A. (2011). Molecular properties prediction and synthesis of novel 1,3,4-oxadiazole analogues as potent *anti*microbial and *anti*tubercular agents. *Bioorganic & Medicinal Chemistry Letters, 21*(24), 7246-7250. doi:https://doi.org/10.1016/j.bmcl.2011.10.057

Angelova, V. T., Pencheva, T., Vassilev, N., K-Yovkova, E., Mihaylova, R., Petrov, B., & Valcheva, V. (2022). Development of New Antimycobacterial Sulfonyl Hydrazones and 4-Methyl-1,2,3-thiadiazole-Based Hydrazone Derivatives. *11*(5), 562. doi: https://doi.org/10.3390/antibiotics11050562

Atalay, P., İMamoĞLu, N., Küçükgüzel, Ş. G., & Han, I. (2020). Synthesis, characterization and *anti*cancer activity of novel hydrazide-hydrazones derived from ethyl paraben. *Journal of Research in Pharmacy, 24*, 341-349. doi:http://10.35333/jrp.2020.156

Burki, S., Burki, Z. G., Haider, S., Mehjabeen, & Ahmed, I. (2020). Synthesis and characterization of Schiff base of nicotinic hydrazide as *anti*bacterial agent along with in vivo wound healing activities and atomic force microscopic study of bacterial cell wall affected by synthesized compound. *Pakistan Journal of Pharmaceutical Sciences, 33*(2), 675-683. doi:http://dx.doi.org/10.36721/PJPS.2020.33.2.REG.675-683.1

CCDC: Cambridge crystallographic data centre for small molecule CCDC for compound **19** (**2003006**).

Desai, N. C., Vaghani, H. V., Jethawa, A. M., & Khedkar, V. M. (2021). In silico molecular docking studies of oxadiazole and pyrimidine bearing heterocyclic compounds as potential *anti*microbial agents. *Archiv der Pharmazie, n/a*(n/a), e2100134. doi:https://doi.org/10.1002/ardp.202100134

Han, M. İ., Atalay, P., Tunç, C. Ü., Ünal, G., Dayan, S., Aydın, Ö., & Küçükgüzel, Ş. G. (2021). Design and synthesis of novel (S)-Naproxen hydrazide-hydrazones as potent VEGFR-2 iN-Hibitors and their evaluation in vitro/in vivo breast cancer models. *Bioorganic & Medicinal Chemistry, 37*, 116097. doi:https://doi.org/10.1016/j.bmc.2021.116097

Han, M. I., Bekçi, H., Uba, A. I., Yıldırım, Y., Karasulu, E., Cumaoğlu, A., Karasulu, H. Y., Yelekçi, K., Yılmaz, Ö., & Küçükgüzel Ş, G. A.-O. (2019). Synthesis, molecular modeling, in vivo study, and *anti*cancer activity of 1,2,4-triazole containing hydrazide–hydrazones derived from (S)-naproxen. *Archiv der Pharmazie, 352*(6), 1800365. doi:https://doi.org/10.1002/ardp.201800365

Hicks, C., & Gulick, R. M. (2009). Raltegravir: The First HIV Type 1 Integrase IN-Hibitor. *Clinical Infectious Diseases, 48*(7), 931-939. doi:https://doi.org/10.1086/597290

Kazakova, O. B., Medvedeva, N. I., Smirnova, I. E., Lopatina, T. V., & Veselovsky, A. V. (2021). The Introduction of Hydrazone, Hydrazide, or Azepane Moieties to the Triterpenoid Core EN-Hances an Activity Against M. tuberculosis. *Medicinal Chemistry, 17*(2), 134-145. doi:https://doi.org/10.2174/157340641666620011516170

Koksal, M., Dedeoglu-Erdogan, A., Bader, M., Gurdal, E. E., Sippl, W., Reis, R., Ozgurbuz, M., Sipahi, H., & Celik, T. (2021). Design, synthesis, and molecular docking of novel 3,5-disubstituted-1,3,4-oxadiazole derivatives as iNOS iN-Hibitors. *Archiv der Pharmazie, n/a*(n/a), e2000469. doi:https://doi.org/10.1002/ardp.202000469

Krátký, M., Konečná, K., Brablíková, M., Janoušek, J., Pflégr, V., Maixnerová, J., Trejtnar, F., & Vinšová, J. (2021). Iodinated 1,2-diacylhydrazines, benzohydrazide-hydrazones and their analogues as dual *anti*microbial and cytotoxic agents. *Bioorganic & Medicinal Chemistry, 41*, 116209. doi:https://doi.org/10.1016/j.bmc.2021.116209

Liu, L., Yao, Z., Wang, S., Xie, T., Wu, G., Zhang, H., Zhang, P., Wu, Y., Yuan, H., & Sun, H. (2021). Syntheses, Biological Evaluations, and Mechanistic Studies of Benzo[c][1,2,5]oxadiazole Derivatives as Potent PD-L1 IN-Hibitors with In Vivo *Anti*tumor Activity. *Journal of medicinal chemistry, 64*(12), 8391-8409. doi:https://doi.org/10.1021/acs.jmedchem.1c00392

Mishra, P., Rajak, H., & Mehta, A. (2005). Synthesis of Schiff bases of 2-amino-5-aryl-1,3,4-oxadiazoles and their evaluation for *anti*microbial activities. *The Journal of General and Applied Microbiology, 51*(2), 133-141. doi:https://doi.org/10.2323/jgam.51.133

Mohamed Jawed, A., Lakshya, B., Shally, M., Rajan, S., Mohd. Zaheen, H., Mohammed, H. G., Mohamed Afroz, B., Surender Singh, J., Tuniki, B., Yassine, R., Sandhya, R., Habibullah, K., Vasubabu, G., & Afzal, H. (2020). Synthesis, *Anti*proliferative, and *Anti*oxidant Activities of Substituted N-[(1,3,4-Oxadiazol-2-yl) Methyl] Benzamines. *Letters in Drug Design & Discovery, 17*(2), 145-154. doi:http://dx.doi.org/10.2174/1570180816666181113110033

Mokhnache, K., Karbab, A., Soltani, E.-K., Bououden, W., Ouhida, S., Arrar, L., Esteban, M. A., Charef, N., & Mubarak, M. S. (2020). Synthesis, characterization, toxic substructure prediction, hepatotoxicity evaluation, marine pathogenic bacteria iN-Hibition, and DFT calculations of a new hydrazone derived from isoniazid. *Journal of Molecular Structure, 1221*, 128817. doi:https://doi.org/10.1016/j.molstruc.2020.128817

Morjan, R. Y., Mkadmh, A. M., Beadham, I., Elmanama, A. A., Mattar, M. R., Raftery, J., Pritchard, R. G., Awadallah, A. M., & Gardiner, J. M. (2014). *Anti*bacterial activities of novel nicotinic acid hydrazides and their conversion into N-acetyl-1,3,4-oxadiazoles. *Bioorganic & Medicinal Chemistry Letters, 24*(24), 5796-5800. doi:https://doi.org/10.1016/j.bmcl.2014.10.029

Pham, V. H., Phan, T. P. D., Phan, D. C., & Vu, B. D. (2019). Synthesis and Bioactivity of Hydrazide-Hydrazones with the 1-Adamantyl-Carbonyl Moiety. *Molecules, 24*(21), 4000. doi: https://doi.org/10.3390/molecules24214000

Popiołek, Ł. (2017). Hydrazide-hydrazones as potential *anti*microbial agents: overview of the literature since 2010. *Medicinal chemistry research : an international journal for rapid communications on design and mechanisms of action of biologically active agents, 26*(2), 287-301. doi:10.1007/s00044-016-1756-y.

Ramazani, A., Ahmadi, Y., & Mahyari, A. (2011). One-Pot Efficient Synthesis of Fully Substituted 1,3,4-Oxadiazole Derivatives from (N-Isocyanimino)triphenylphosphorane, Carboxylic Acids, and Aromatic Bis-Aldehydes. *Synthetic Communications, 41*(15), 2273-2282. doi:https://doi.org/10.1080/00397911.2010.501480

Rao, A. T., Shree, A. J., Reddy, A. V. K., & Zyryanov, G. V. (2020). Synthesis and molecular properties of novel fluoroquinolone citrate conjugates linked to 1,3,4-oxadiazoles as *anti*bacterial and *anti*cancer agents. *AIP Conference Proceedings, 2280*(1), 050047. doi:https://doi.org/10.1063/5.0023473

Samir, M., Ramadan, M., Abdelrahman, M. H., Abdelbaset, M. S., Abourehab, M. A. S., Abdel-Aziz, M., & Abuo-Rahma, G. E.-D. A. (2021). 3,7-bis-benzylidene hydrazide ciprofloxacin derivatives as promising *anti*proliferative dual TOP I & TOP II isomerases iN-Hibitors. *Bioorganic Chemistry, 110*, 104698. doi:https://doi.org/10.1016/j.bioorg.2021.104698

Shabeeb, I., Issa, L., Shtaiwi, M., al-shalabi, E., Younes, E., Okasha, R., & Abu sini, M. (2018). New Hydrazide-Hydrazone Derivatives of Quinoline 3-Carboxylic Acid Hydrazide: Synthesis, Theoretical Modeling and *Anti*bacterial Evaluation. *Letters in Organic Chemistry, 16*. doi:https://doi.org/10.2174/157017861666181227122326

Singh, R., & Chauhan, A. (2013). Biological importance of 1, 3, 4-Oxadiazole derivatives. *Int J Adv Biol Res, 3*, 140-149.

SiN-Ha, R., Singh, U. V. S., Khosa, R. L., & Jain, J. (2018). *Anti*convulsant Activity of Isonicotinic Acid Hydrazone Derivatives Using MES, scPTZ and Rotorod Neurotoxicity Models. *Journal of Applied Pharmaceutical Sciences and Research, 1*. doi:https://doi.org/10.31069/japsr.v1i01.13058

Shen, Y.-D., Zhang, S.-W., Lei, H.-T., Wang, H., Xiao, Z.-L., Jiang, Y.-M., & Sun, Y.-M. (2008). Design and Synthesis of Immunoconjugates and Development of an Indirect ELISA for Rapid Detection of 3, 5-Dinitrosalicyclic Acid Hydrazide. *Molecules, 13* (9) doi:10.3390/molecules13092238

Siwach, A., & Verma, P. K. (2020). Therapeutic potential of oxadiazole or furadiazole containing compounds. *BMC Chemistry, 14*(1), 70. doi:https://doi.org/10.1186/s13065-020-00721-2

Sudeesh, K., & Gururaja, R. (2017). Facile synthesis of some novel derivatives of 1, 3, 4-oxadiazole derivatives associated with quinolone moiety as cytotoxic and *anti*bacterial agents. *Org Chem Curr Res, 6*(2), 2-5. doi:https://doi.org/10.4172/2161-0401.1000183

Thorat, B. R., Mali, S. N., Rani, D., & Yamgar, R. S. (2021). Synthesis, In silico and In vitro Analysis of Hydrazones as Potential *Anti*tuberculosis Agents. *Current Computer-Aided Drug Design* doi: https://doi.org/10.2174/1573409916666200302120942

Verma, G., Marella, A., Shaquiquzzaman, M., Akhtar, M., Ali, M. R., & Alam, M. M. (2014). A review exploring biological activities of hydrazones.

Journal of pharmacy & bioallied sciences, 6(2), 69-80. doi:https://10.4103/0975-7406.129170

Zeeshan, S., Naveed, M., Khan, A., Atiq, A., Arif, M., Ahmed, M. N., Kim, Y. S., & Khan, S. (2019). N-Pyrazoloyl and N-thiopheneacetyl hydrazone of isatin exhibited potent *anti*-inflammatory and *anti*-nociceptive properties through suppression of NF-κB, MAPK and oxidative stress signaling in animal models of inflammation. *Inflammation Research, 68*(7), 613-632. doi:https://10.1007/s00011-019-01245-9