Electronic Supplementary Information (ESI)

Catalytic Application of Green-Synthesized ZnO Nanoparticles in the Synthesis of 1*H*-pyrazolo[1,2-a]pyridazine-5,8-diones and Evaluation of Their Anti-Cancer Properties†

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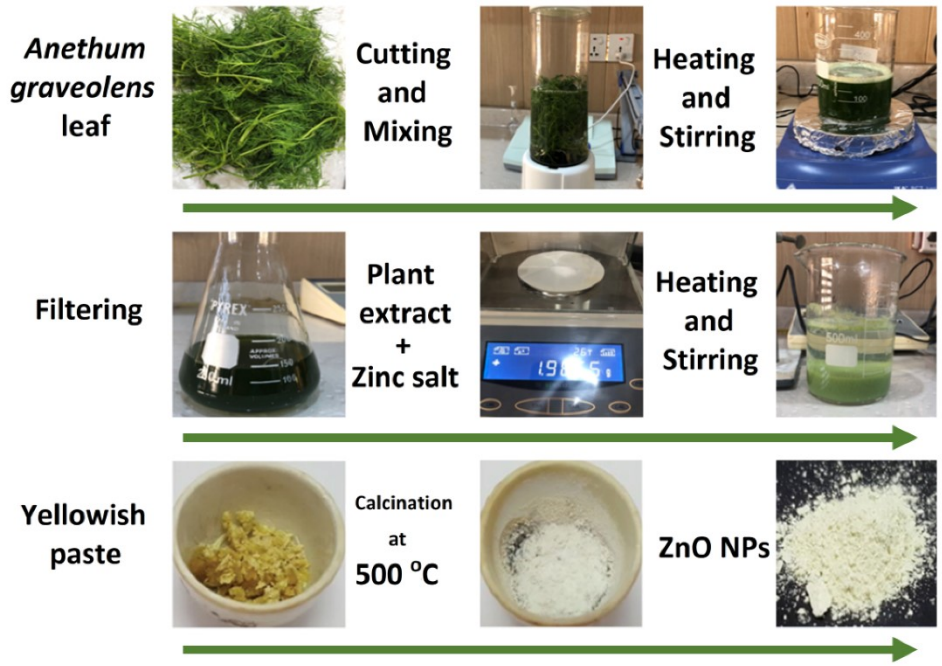


Fig.S1: Graphical demonstration of ZnO NPs formation process utilizing *Anethum graveolens* leaf extract and zinc acetate dehydrate

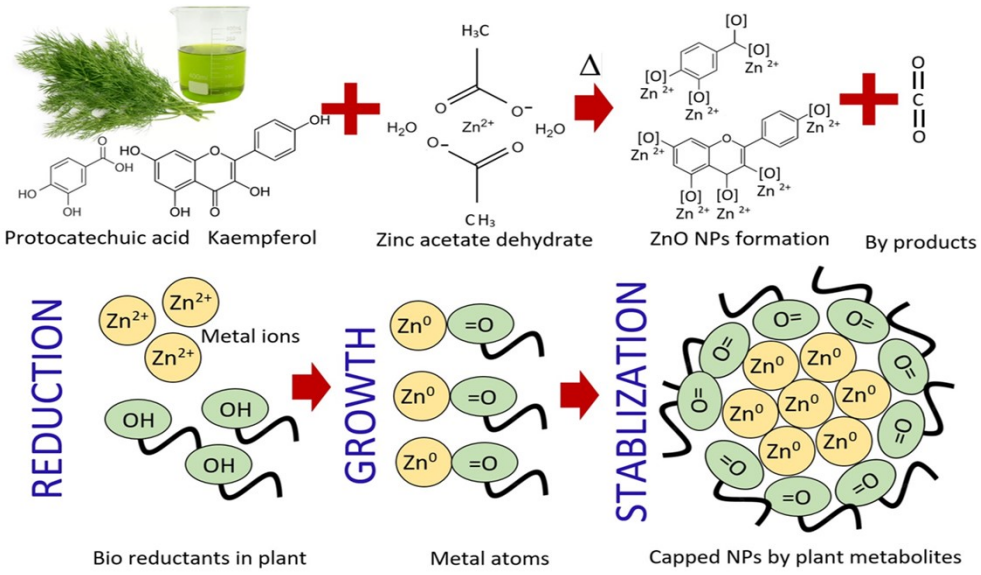


Fig. S2: The plausible mechanism of synthesizing ZnO NPs using *Anethum graveolens* leaf extract.

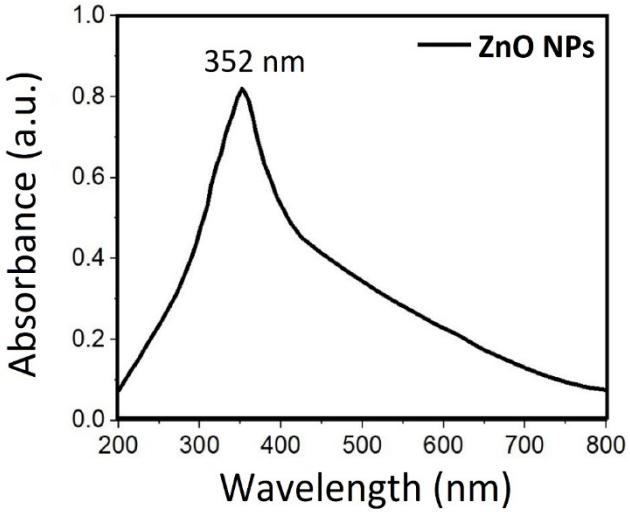


Fig. S3: UV-Vis spectra of ZnO NPs prepared using *Anethum graveolens* leaf extract

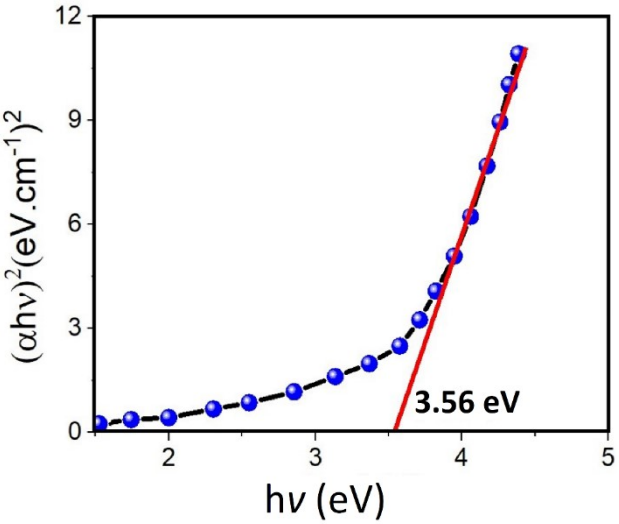


Fig. S4: Tauc plot of ZnO NPs prepared using *Anethum graveolens* leaf extract

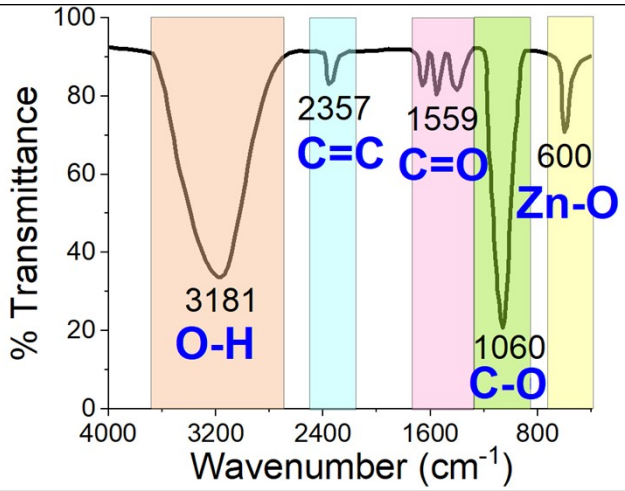


Fig. S5: FTIR spectrum of ZnO NPs synthesized using Anethum graveolens leaf extract

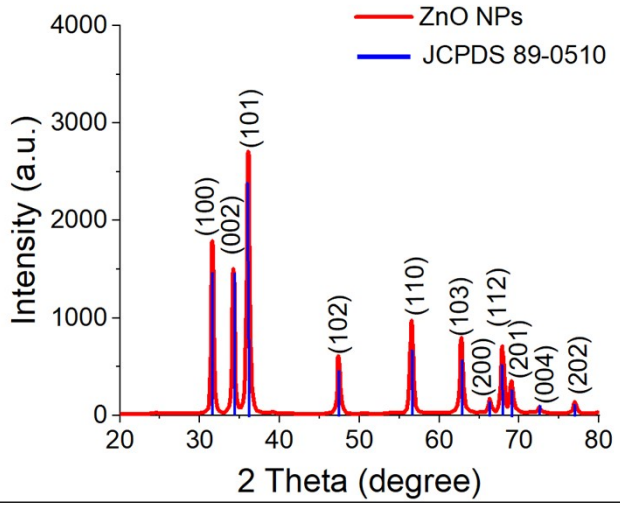


Fig. S6: XRD patterns of ZnO NPs synthesized using *Anethum graveolens* leaf extract

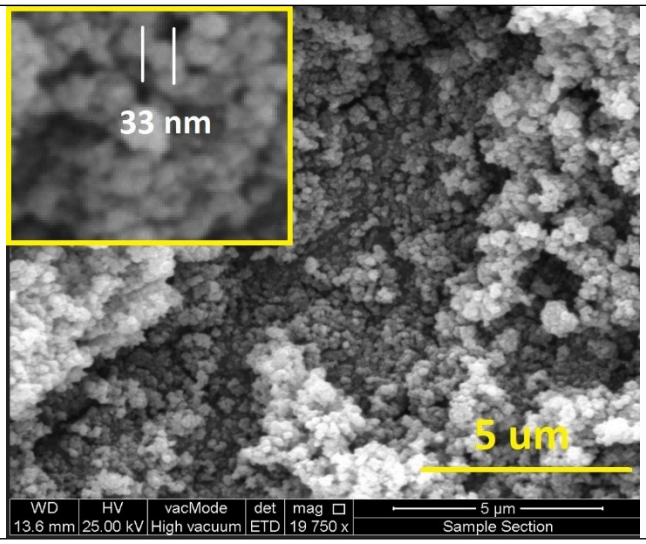


Fig. S7: SEM analysis of ZnO NPs using Anethum graveolens leaf extract

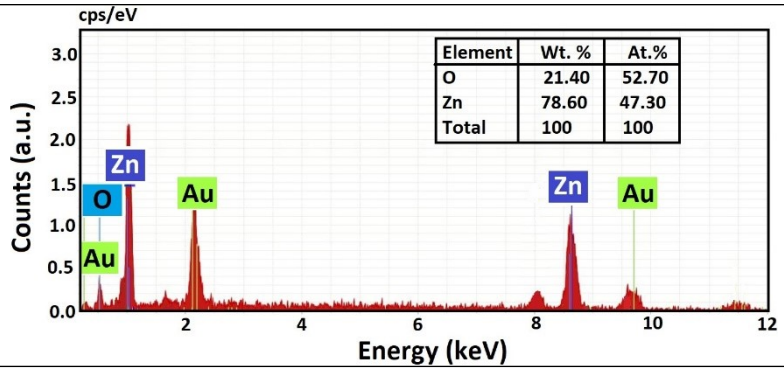


Fig. S8: EDX Analysis of ZnO NPs using *Anethum graveolens* leaf extract

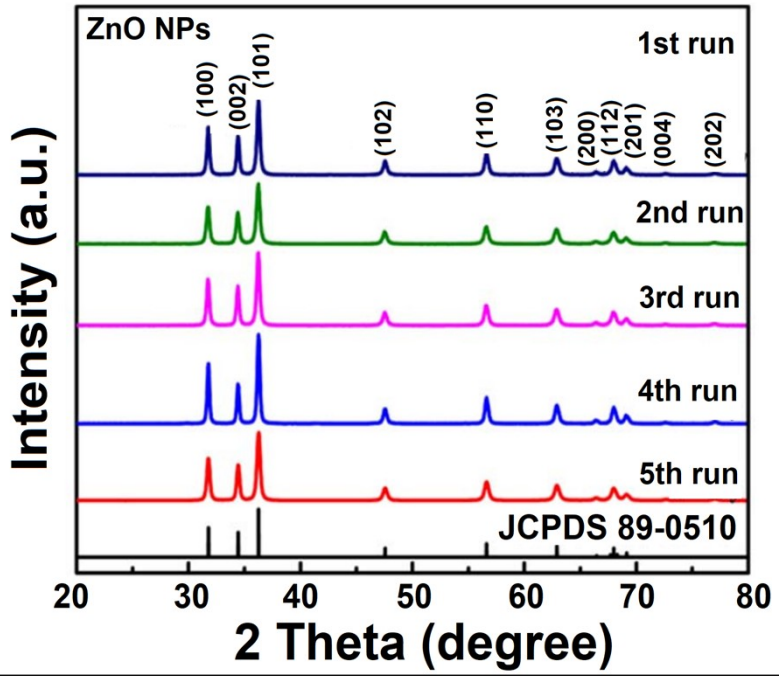


Fig. S9: XRD diffraction patterns of the hexagonal wurtzite ZnO NPs (JCPDS No. 89-0510) and reusing after 5 runs

2. General Information about Experimentation (Chemicals, Reagents, and Characterization)

All commercially available chemicals were obtained from Merck Company and were used without further purification. The described catalyst was prepared according to the mentioned procedure.

A Stuart model SMP3 apparatus was used for measuring melting points, melting points are uncorrected. Nuclear magnetic resonance (^{1}HNMR and ^{13}CNMR) spectra were recorded on Bruker Avance 400 MHz FT NMR spectrometers (run at the University of Tehran and the University of Isfahan, Iran).

Infrared (IR) spectroscopy was conducted on a Perkin Elmer GX FT-IR spectrometer. Mass spectra were recorded on a Shimadzu QP 1100 BX Mass Spectrometer (University of Tehran, Iran). Elemental analyses were recorded on an Eager 300 for EA1112 instrument (University of Tehran, Iran).

Zinc acetate dehydrates $C_4H_6O_4Zn.2H_2O$, molecular weight 219.50 g/mol, as well as NaOH Molecular weight 40 g/ mol have been used in this study.

The heating supply for synthesizing ZnO NPs was a Hot Plate Stirrer. PAN analytical X' Pert PRO (Cu Kα =1.5406 Å) was used to perform X-ray diffraction (XRD) analyses. The scanning percentage was 1 /min in the 2θ assortment from 20° to 80°.

A double-beam UV spectrophotometer (Super Aquarius spectrophotometer) was utilized to record UV–Vis spectral analysis to confirm the ZnO NPs production. Scanning electron microscopy (SEM) (Quanta 4500) was utilized to evaluate morphology and particle dispersion.

EDX (Energy Dispersive X-ray Analysis) in SEM was utilized to determine the elemental configuration of the produced nanostructures, while FT-IR analysis was done on a Perkin Elmer.

FTIR spectrophotometer with a steadfastness of 4 cm^{-1} was utilized for the examination of the available functional groups in the utilized plant extract and the NPs correspondingly.

Thin layer chromatography (TLC) was performed using 60 mesh silica gel plates and visualized with short wavelength UV light (254 nm).

1H NMR and 13C NMR were all recorded using DMSO-d_6 as a solvent on a Bruker 400 MHz spectrometer at 298 K (400 MHz for 1H and 100 MHz for 13C).

3-amino-5,8-dioxo-1-phenyl-5,8-dihydro-1H-pyrazolo[1,2-a]pyridazine-2-carbonitrile (**1**)

IR, HNMR, and CNMR spectral data are identical to those reported in ref. [1]

3-amino-1-(2-bromophenyl)-5,8-dioxo-5,8-dihydro-1H-pyrazolo[1,2-a]pyridazine-2-carbonitrile (**2**)

IR, HNMR, and CNMR spectral data are identical to those reported in ref. [1-2]

3-amino-1-(2-chlorophenyl)-5,8-dioxo-5,8-dihydro-1H-pyrazolo[1,2-a]pyridazine-2-carbonitrile (**3**)

IR, HNMR, and CNMR spectral data are identical to those reported in ref. [1-2]

3-amino-1-(2,4-dichlorophenyl)-5,8-dioxo-5,8-dihydro-1H-pyrazolo[1,2-a]pyridazine-2-carbonitrile (**4**)

IR, HNMR, and CNMR spectral data identical to those reported in ref. [1-2]

3-amino-1-(3-chlorophenyl)-5,8-dioxo-5,8-dihydro-1H-pyrazolo[1,2-a]pyridazine-2-carbonitrile (**5**)

IR, HNMR, and CNMR spectral data are identical to those reported in ref. [1-2]

3-amino-1-(2-methoxyphenyl)-5,8-dioxo-5,8-dihydro-1H-pyrazolo[1,2-a]pyridazine-2-carbonitrile (**6**)

IR, HNMR, and CNMR spectral data are identical to those reported in ref. [1-2]

3-amino-1-(3-nitrophenyl)-5,8-dioxo-5,8-dihydro-1H-pyrazolo[1,2-a]pyridazine-2-carbonitrile (**7**)

IR, HNMR, and CNMR spectral data are identical to those reported in ref. [1]

3-amino-1-(2,6-dichlorophenyl)-5,8-dioxo-5,8-dihydro-1H-pyrazolo[1,2-a]pyridazine-2-carbonitrile (**8**)

IR, HNMR, and CNMR spectral data are identical to those reported in ref. [1-2]

2.1. *Characterization data for 3-amino-1-(2-fluorophenyl)-5,8-dioxo-5,8-dihydro-1H-pyrazolo[1,2-a]pyridazine-2-carbonitrile (Entry 9)*

m.p.:259-262 °C (dec.); ^{1}H NMR (400 MHz, DMSO-d_6):□ δ = 6.21 (s, 1H), 7.04-7.20 (dd, 2H), 7.42-7.47 (m, 1H), 7.74 (d, 1H), 8.04-8.07 (t, 1H), 8.56 (s, 2H, NH_2) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ = 58.36, 59.95, 113.68, 115.40, 116.6, 125.35, 129.12, 136.56, 150.53, 152.85, 153.80, 156.01, 159.27, 161.31; IR (KBr, cm^{-1}): 3384, 3265, 3185, 3062, 2202, 1698, 1677, 1644, 1586, 1562, 1476, 1436, 1371, 1336, 1288, 1255, 1123, 1048, 1022, 992, 848, 753, 702, 611, 560, 487; MS, m/z (%): 284 (M^+). Anal.Calcd.for. $C_{14}H_9FN_4O_2$: C: 59.16, H: 3.19, N: 19.71 %. Found: C: 59.11; H: 3.22; N: 19.69%.

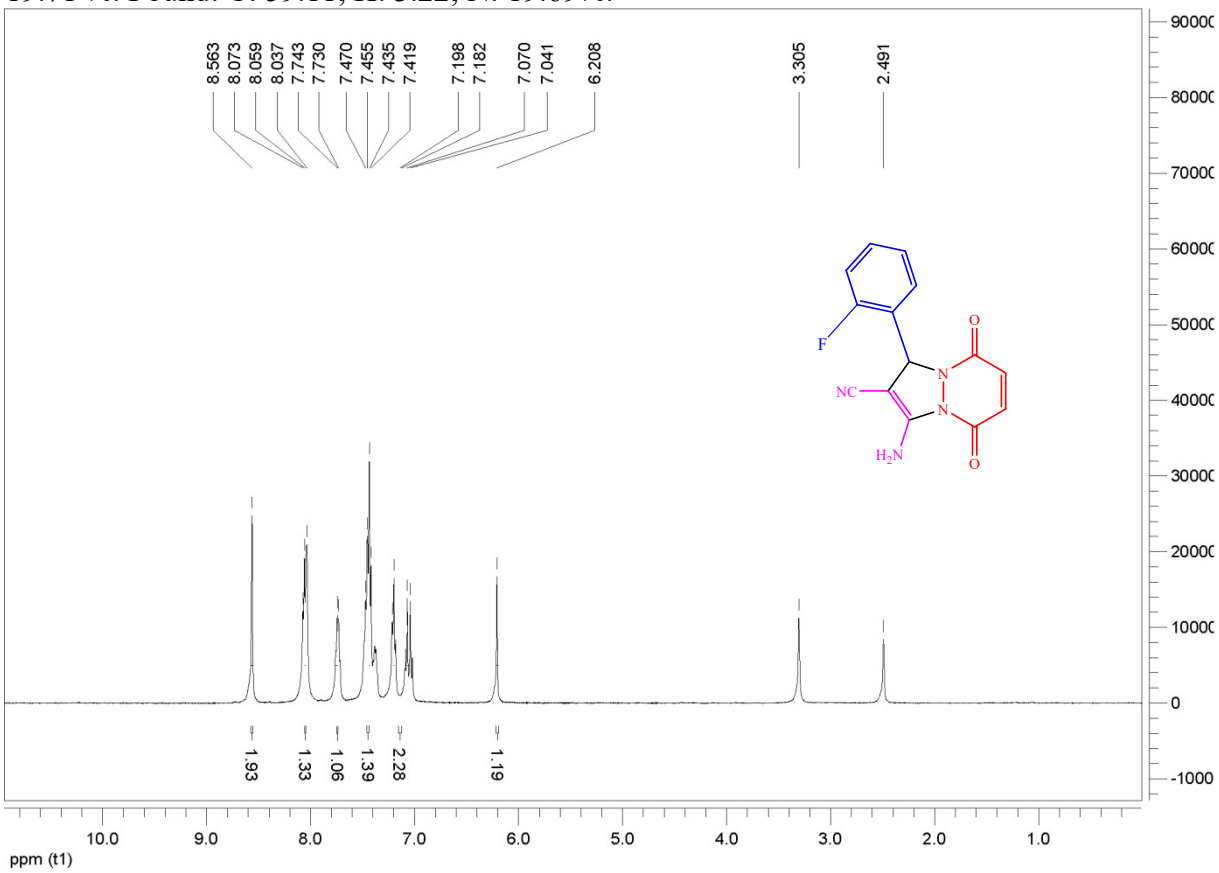


Fig. S10 The ^{1}H NMR (400MHz) spectrum of 3-amino-1-(2-fluorophenyl)-5,8-dioxo-5,8-dihydro-1*H*-pyrazolo[1,2-a]pyridazine-2-carbonitrile (Entry 9**)**

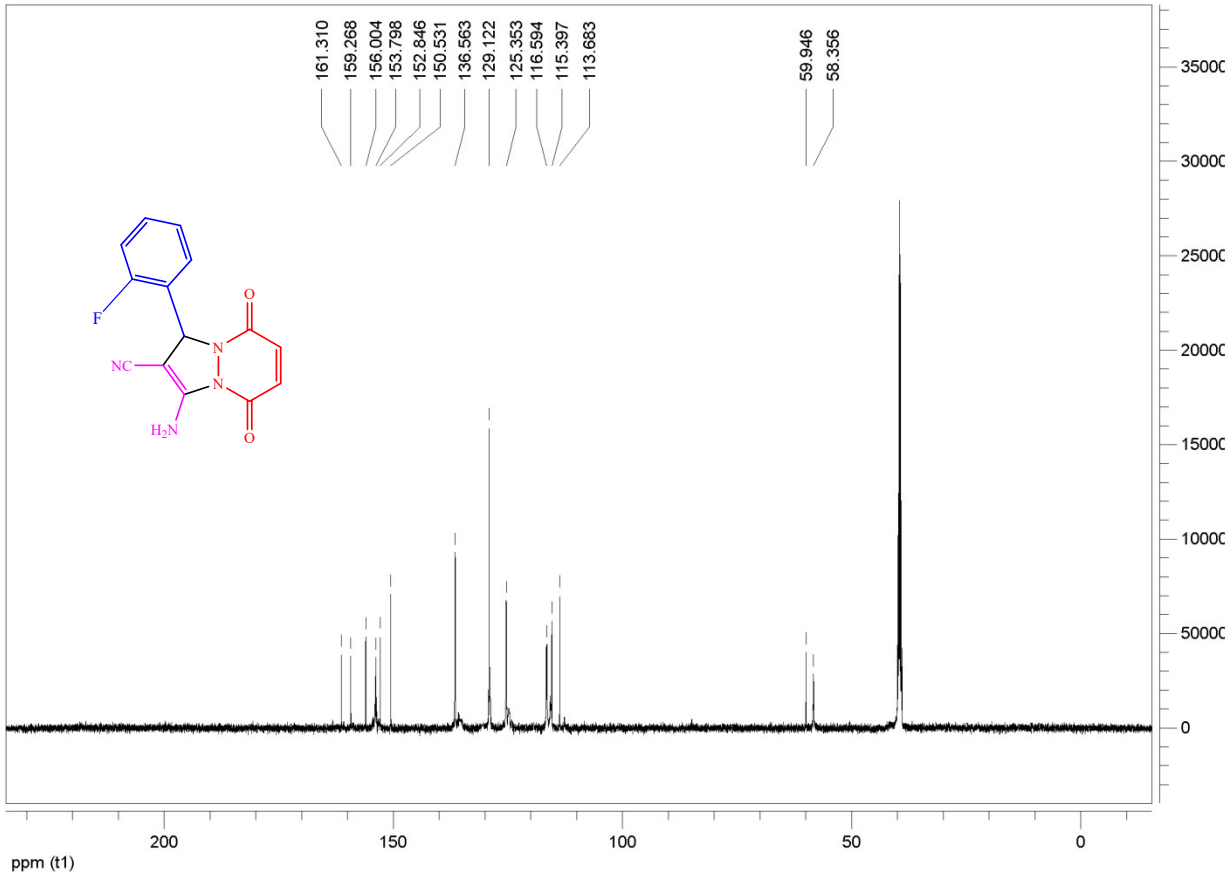


Fig. S11 The ^{13}C NMR (100MHz) spectrum of 3-amino-1-(2-fluorophenyl)-5,8-dioxo-5,8-dihydro-1*H*-pyrazolo[1,2-a]pyridazine-2-carbonitrile (Entry 9)

2.2. Characterization data for 3-amino-1-(2,3-dichlorophenyl)-5,8-dioxo-5,8-dihydro-1H-pyrazolo[1,2-a]pyridazine-2-carbonitrile (Entry 10)

m.p.: 277-280 °C (dec.); ^{1}H NMR (400 MHz, DMSO-d_6): δ = 6.37 (s, 1H), 7.03-7.12 (dd, 2H), 7.37 (t, 1H), 7.53 (d, 1H), 7.63 (d, 1H), 8.08 (s, 2H, NH_2) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ = 59.41, 60.12, 115.24, 128.63, 128.80, 128.98, 130.36, 130.47, 135.36, 135.47, 135.54, 150.93, 152.96, 156.05 ppm; IR (KBr, cm^{-1}): 3383, 3263, 3065, 2195, 1671, 1662, 1648, 1561, 1438, 1374, 1284, 1255, 1151, 1121, 846, 802, 559, 488; MS, m/z (%): 334 (M^+). Anal.Calcd.for. $C_{14}H_8Cl_2N_4O_2$: C: 50.17, H: 2.41, N: 16.72 %. Found: C: 50.15; H: 2.44; N: 16.69%.

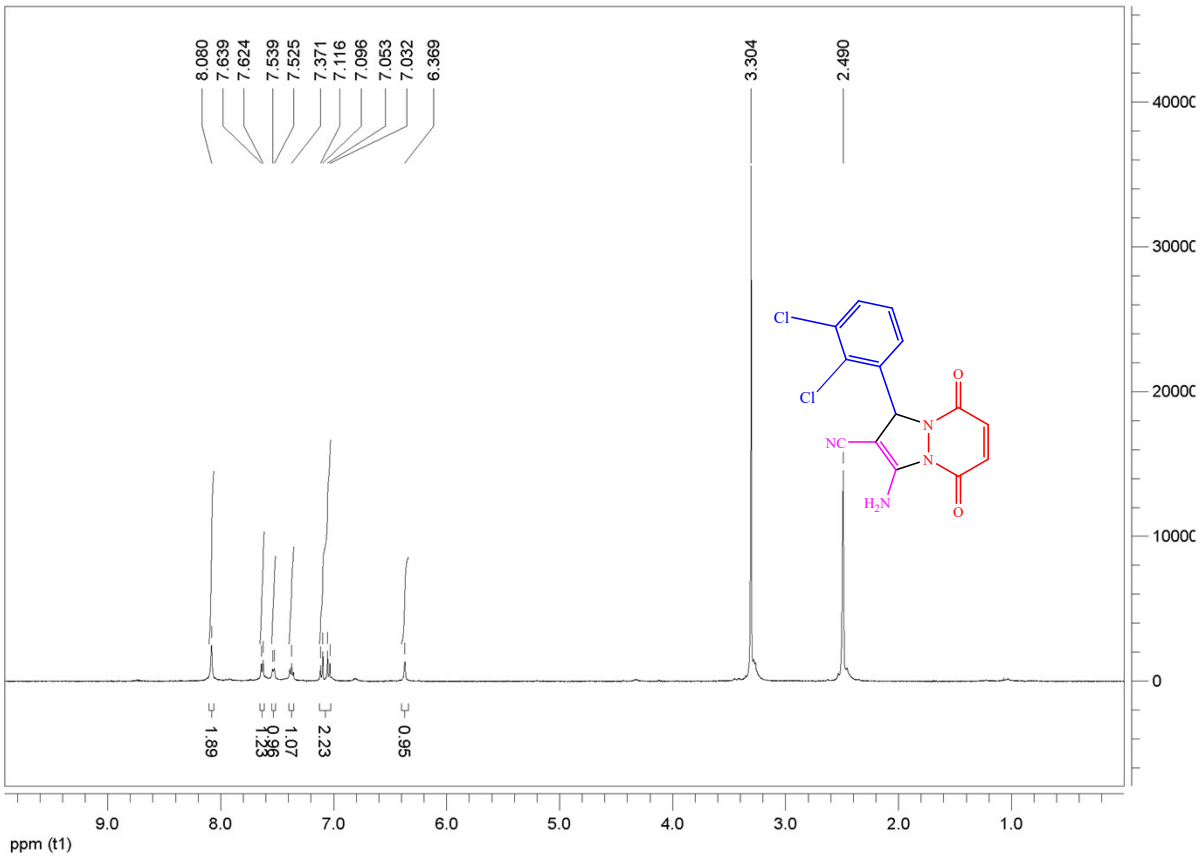


Fig. S12 The ^{1}H NMR (400MHz) spectrum of 3-amino-1-(2,3-dichlorophenyl)-5,8-dioxo-5,8-dihydro-1*H*-pyrazolo[1,2-a]pyridazine-2-carbonitrile (Entry 10)

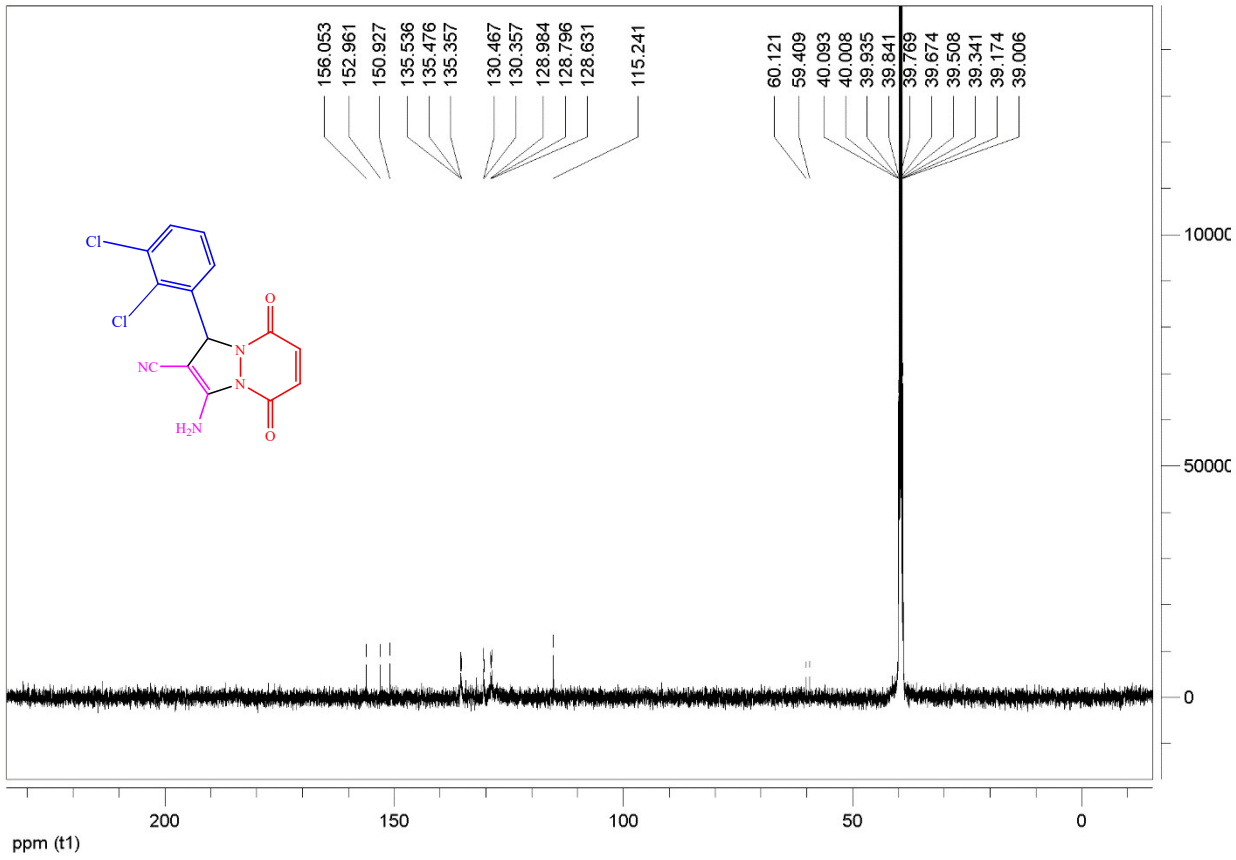


Fig. S13 The ^{13}C NMR (100MHz) spectrum of 3-amino-1-(2,3-dichlorophenyl)-5,8-dioxo-5,8-dihydro-1*H*-pyrazolo[1,2-a]pyridazine-2-carbonitrile (Entry 10)

2.3. Characterization data for 3-amino-1-(2,6-di fluorophenyl)-5,8-dioxo-5,8-dihydro-1H-pyrazolo[1,2-a]pyridazine-2-carbonitrile (Entry 11)

m.p.: 264-268 °C (dec.); ^{1}H NMR (400 MHz, DMSO-d_6): δ = 6.35 (s, 1H), 7.06-7.16 (m, 4H), 7.45-7.48 (t, 1H), 8.12 (s, 2H, NH_2) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ = 53.9, 57.9, 115.2, 131.3, 135.1, 135.3, 135.5, 135.7, 150.9, 152.7, 155.8, 159.3, 161.3 ppm; IR (KBr, cm^{-1}): 3367, 3250, 3175, 3075, 2933, 2200, 1701, 1672, 1624, 1592, 1471, 1434, 1374, 1293, 1145, 1001, 853, 780, 603, 484; MS, m/z (%): 302 (M^+). Anal.Calcd.for. $C_{14}H_8F_2N_4O_2$: C: 55.64, H: 2.67, N: 18.54 %. Found: C: 55.60; H: 2.69; N: 18.52%.

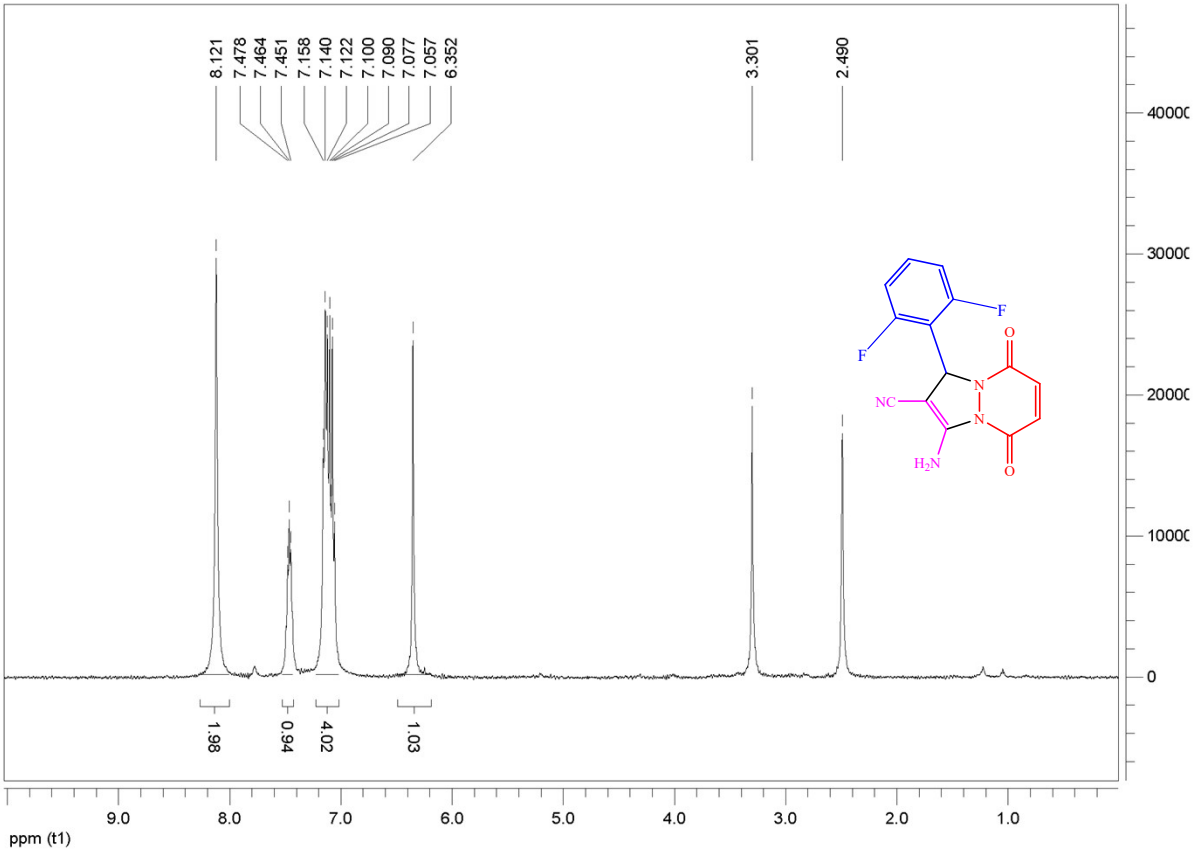


Fig. S14 The ^{1}H NMR (400MHz) spectrum of 3-amino-1-(2,6-di fluorophenyl)-5,8-dioxo-5,8-dihydro-1*H*-pyrazolo[1,2-a]pyridazine-2-carbonitrile (Entry 11)

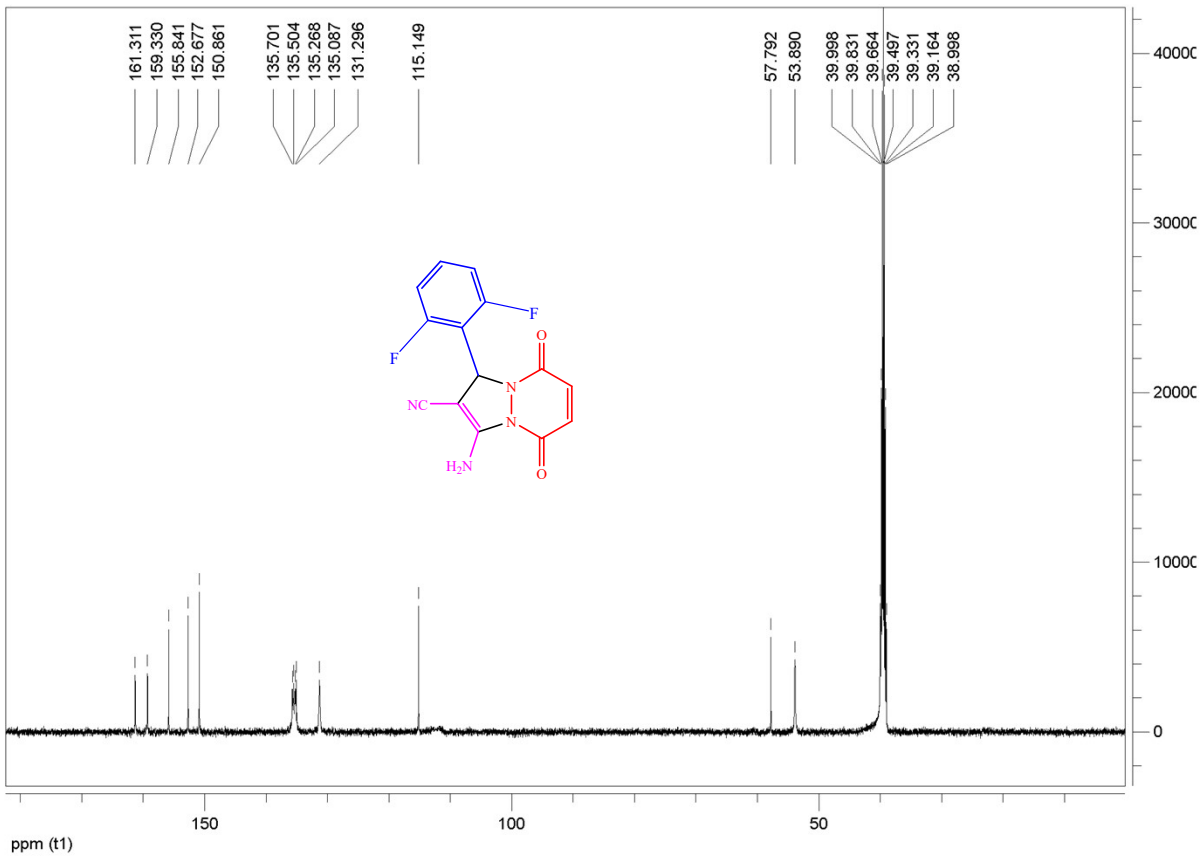


Fig. S15 The ^{13}C NMR (100MHz) spectrum of 3-amino-1-(2,6-di fluorophenyl)-5,8-dioxo-5,8-dihydro-1*H*-pyrazolo[1,2-a]pyridazine-2-carbonitrile (Entry 11**)**

2.4. Characterization data for methyl 4-(3-amino-2-cyano-5,8-dioxo-5,8-dihydro-1H-pyrazolo[1,2-a]pyridazine-1-yl)benzoate (Entry 12)

m.p.: 274-276 °C (dec.); ^{1}H NMR (400 MHz, DMSO-d_6): δ = 3.88 (s, 3H), 6.77 (s, 1H), 7.34-7.45 (dd, 2H), 7.66-8.08 (m, 4H), 8.11 (s, 2H, NH_2) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ = 49.6, 53.8, 58.6, 113.9, 135.5, 139.5, 140.1, 144.0, 144.5, 146.9, 148.5, 154.7, 156.2, 174.9 ppm; IR (KBr, cm^{-1}): 3443, 3348, 3219, 2955, 2195, 1721, 1642, 1437, 1286, 1192, 1113, 862, 706; MS, m/z (%):324 (M^+). Anal.Calcd.for. $C_{16}H_{12}N_4O_4$: C: 59.26, H: 3.73, N: 17.28 %. Found: C: 59.25; H: 3.75; N: 17.26%.

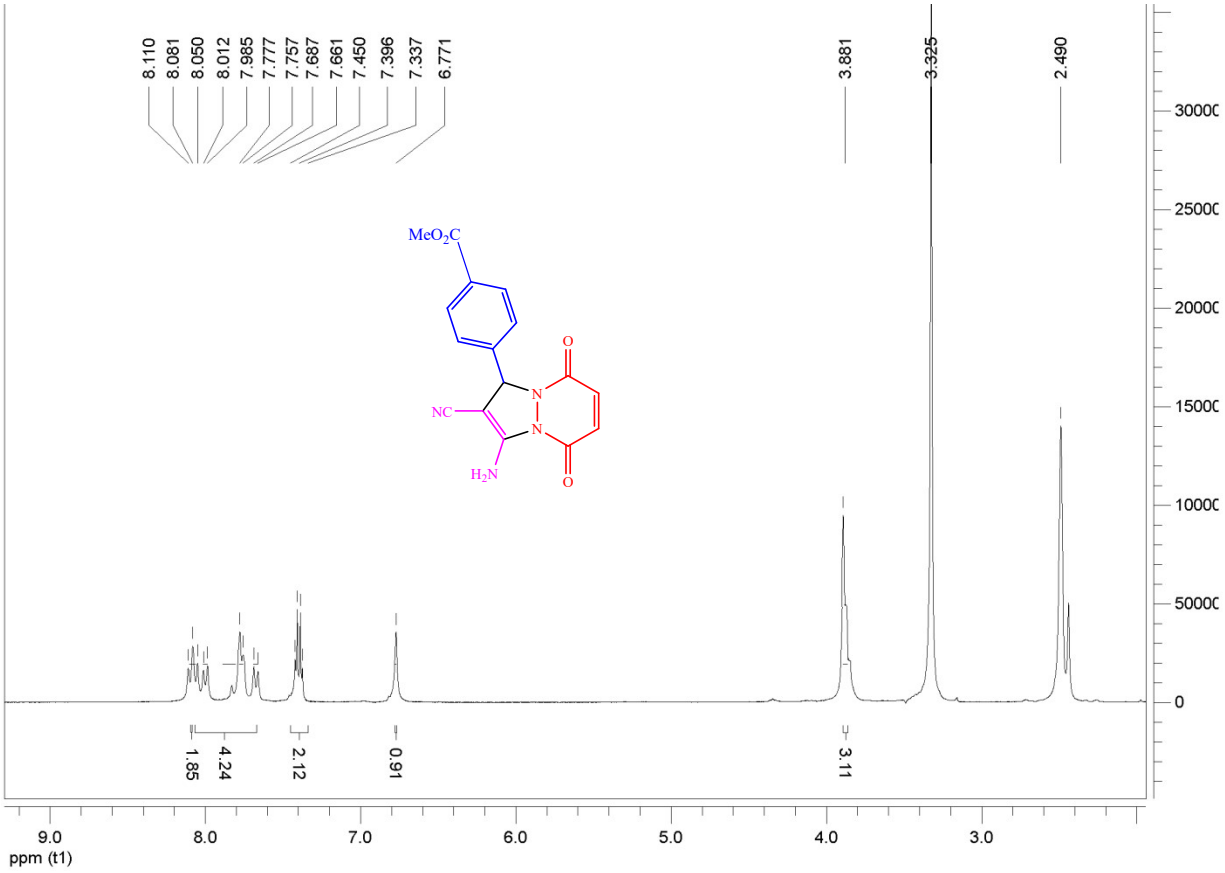


Fig. S16 The ^{1}H NMR (400MHz) spectrum of methyl 4-(3-amino-2-cyano-5,8-dioxo-5,8-dihydro-1*H*-pyrazolo[1,2-a]pyridazine-1-yl)benzoate (Entry 12)

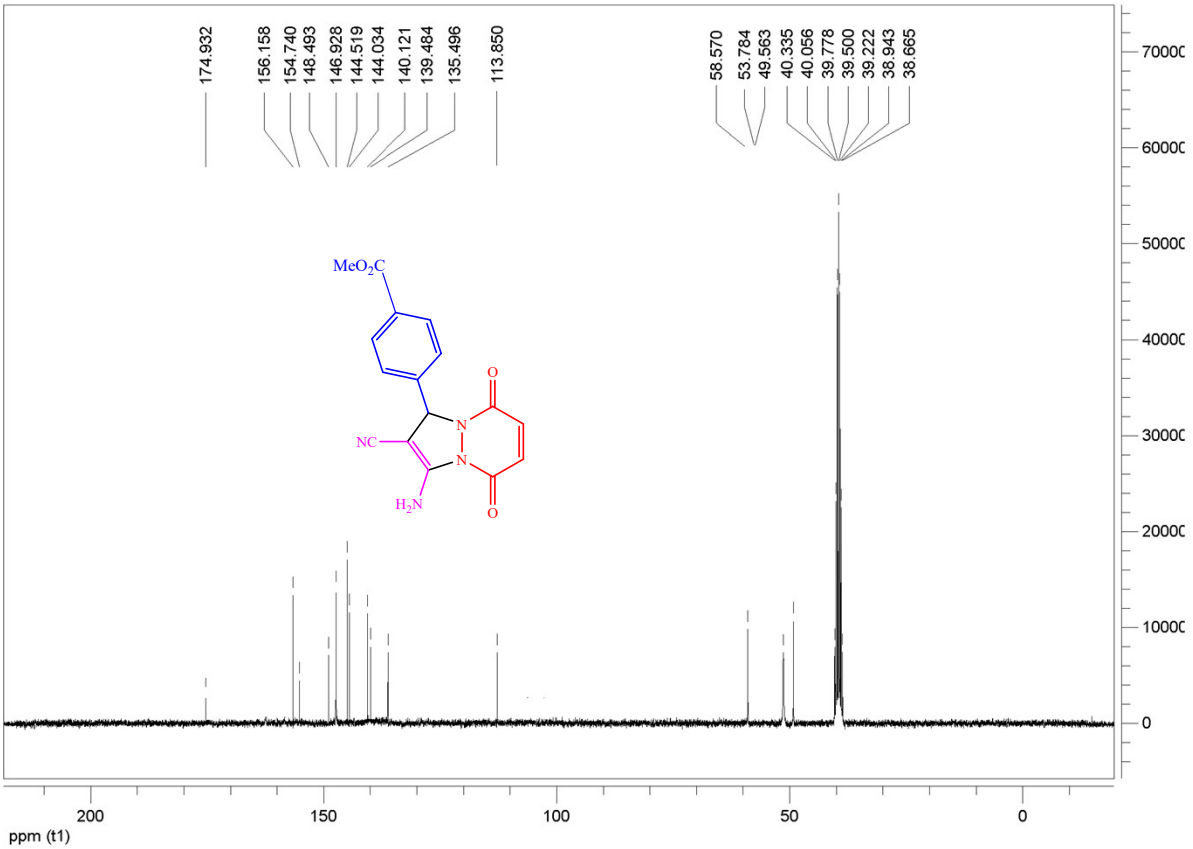


Fig. S17 The ^{13}C NMR (100MHz) spectrum of methyl 4-(3-amino-2-cyano-5,8-dioxo-5,8-dihydro-1*H*-pyrazolo[1,2-a]pyridazine-1-yl)benzoate (Entry 12)

3. UV–Vis spectra of *Anethum graveolens* leaf

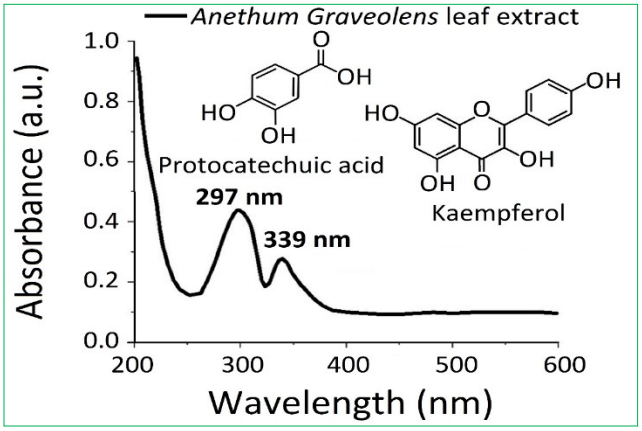
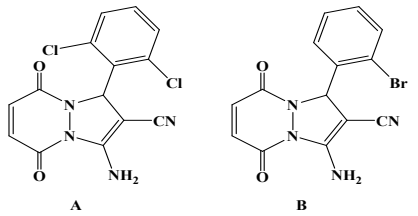
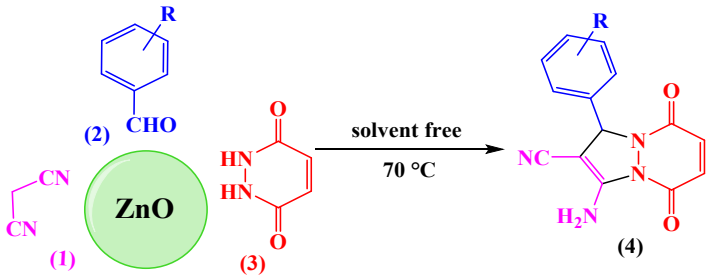


Fig. S18: UV–Vis spectra of *Anethum graveolens* leaf extract and its corresponding major phytochemicals

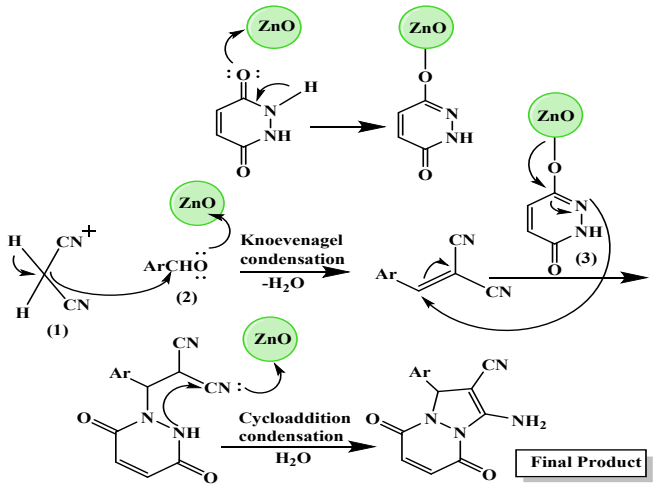


Scheme S1: Structures of two 1H-pyrazolo[1,2-a]pyridazine-5,8-dione derivatives as bactericidal compounds



R= **a:** H; **b:** 2-Br; **c:** 2-Cl; **d:** 2,4-diCl; **e:** 3-Cl; **f:** 2-OCH_3; **g:** 3-NO_2; **h:** 2,6-diCl; **i:** 2-F; **j:** 2,3-diCl; **k:** 2,6-diF; **l:** 4-CO_2Me

Scheme S2: Synthesis of 1H-pyrazolo[1,2-a]pyridazine-5,8-diones using green nano-ZnO as a heterogeneous, nanocatalyst



Scheme S3: A reasonable mechanism for the synthesis of 1*H*-pyrazolo[1,2-a]pyridazine-5,8-diones

Table S1: ZnO crystalline size computed using Debye–Scherrer equation and the corresponding XRD data from Figure 7

Peak No.	Planes	Pos. (°2Th.)	FWHM (o2Th.)	Size (nm)
1	100	31.634	0.276	28.971
2	002	34.287	0.256	32.501
3	101	36.084	0.236	35.373
4	102	47.402	0.354	23.495
5	110	56.46	0.315	28.634
6	103	62.753	0.354	26.271
7	200	66.273	0.315	30.128
8	112	67.872	0.236	40.539
9	201	69.117	0.394	24.508
10	004	72.399	0.945	10.422
11	202	76.859	0.394	25.765
			Average size	**27.873**

Table S2: Synthesis of 1*H*-pyrazolo[1,2-a]pyridazine-5,8-diones **(4a-l)** using nano-ZnO, as a green catalyst[a]

[a] Reaction conditions: maleic hydrazide (1mmol), benzaldehyde derivatives (1mmol), malononitrile (1.3 mmol) using catalytic amount (3 mg) of green synthesized nano-ZnO, at 70 °C and solvent-free conditions. [b] Yields refer to isolated pure products.

Entry	Product	Time (min)	Yield (%)[b]	m.p (°C)/Lit. mp
1	**4a**	40	70	289-292/(291-294[25])
2	**4b**	20	85	285-289/(287-290[25,26])
3	**4c**	25	80	270-272/(271-274[25,26])
4	**4d**	25	75	271-274/(273-276[25,26])
5	**4e**	35	75	278-281/(277-280[25,26])
6	**4f**	25	80	260-263/(258-261[25,26])
7	**4g**	30	80	270-273/(269-271[25])
8	**4h**	20	85	272-275/(273-276[25,26])
9	**4i**	40	70	259-262
10	**4j**	30	95	277-280
11	**4k**	20	95	264-268
12	**4l**	60	70	274-276

Table S3: Synthesis of 3-amino-1-(2-bromophenyl)-5,8-dioxo-5,8-dihydro-1*H*-pyrazolo[1,2-a]pyridazine-2-carbonitrile under different conditions [a]

Entry	Solvent (5ml)	Condition	Time (min)	Yield (%)[b]
1	Ethanol	r.t.	20	20
2	Ethanol	Reflux	20	60
3	Solvent-free	r.t.	20	10
4	Solvent-free	70 °C	20	85

[a] Reaction conditions: maleic hydrazide (1mmol), 2-bromobenzaldehyde (1mmol), malononitrile (1.3mmol) using a catalytic amount of green nano-ZnO, at different conditions. [b] Separated yield.

Table S4: Synthesis of 3-amino-1-(2-bromophenyl)-5,8-dioxo-5,8-dihydro-1*H*-pyrazolo[1,2-a]pyridazine-2-carbonitrile in the presence of different amounts of catalyst.[a]

Entry	Catalyst (mg)	Time (min)	Yield (%)[b]
1	1	20	20
2	1.5	20	35
3	2	20	45
4	2.5	20	60
5	3	20	85

[a] Reaction conditions: maleic hydrazide (1mmol), 2-bromobenzaldehyde (1mmol), malononitrile (1.3mmol) using a catalytic amount of green nano-ZnO, at 70 °C and solvent-free condition.

Table S5: Cytotoxic activities (IC_{50}) of 4a-l against human cancer cell lines [a]

Entry	Product	MCF-7	A375	Fibroblast ($Hu0_2$)
1	**4a**	>100	>100	-
2	**4b**	>100	>100	-
3	**4c**	>100	16	99.34±0.2
4	**4d**	35	40	-
5	**4e**	48	>100	-
6	**4f**	>100	>100	-
7	**4g**	75	>100	-
8	**4h**	9	80	84.32±1.3
9	**4i**	60	30	-
10	**4j**	>100	11.4	77.28 ±0.9
11	**4k**	75	>100	-
12	**4l**	14	60	78.04±0.7
Paclitaxel	-	3.125	5.315	67.89±0.2

[a] In vitro cytotoxic activity (IC50) of synthesized compounds 4a-4l on three cell lines (MCF-7, A375, Fibroblast). All IC_{50} are based on micromolar (μM), 48h.

4. References

1. A. Khoraamabadi-zad, M. Azadmanesh, R. Karamian, M. Asadbegy, and M. Akbari, *RSC Adv.*, **4**, 47721 (2014).
2. R. Ghorbani-Vaghei, J. Mahmoodi, and Y. Maghbooli*, Appl. Organomet. Chem.*, **31**, 3717 (2017).