Electronic Supporting Information (ESI)

Exploring Azo-Uracil Based Nickel(II) Complex for Anticancer and Phosphatase like Activities

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1. Materials and methods

1, 3-Dimethyl-6-aminouracil, *p*-amino benzoic acid has been purchased from SigmaAldrich (India). The solvents used are of spectroscopic grade. Other chemicals are of analytical reagent grade and used without further purification. Milli-Q Millipore 18.2 MΩ cm^{-1} water is used whenever required. A Shimadzu Multi Spec 2450 spectrophotometer is used for recording UV−Vis. spectra. FTIR spectra are recorded on a Shimadzu FTIR (model IR Prestige 21 CE) spectrophotometer. Single crystal X-ray diffraction data has been collected on a Bruker X8 APEX-II CCD diffractometer at 100(2) K using graphite-monochromatic Mo-K_α radiation (0.71073 Å) at 150K. The data are processed and corrected for Lorentz and polarization absorption effects. The structure is solved and refined using Bruker SHELXTL Software Package. All non-hydrogen atoms are refined with anisotropic thermal displacements. Hydrogen atoms are included in the structure factor calculation in geometrically idealized positions, with thermal parameters depending on the parent atom, using a riding model. Image was generated by Mercury software

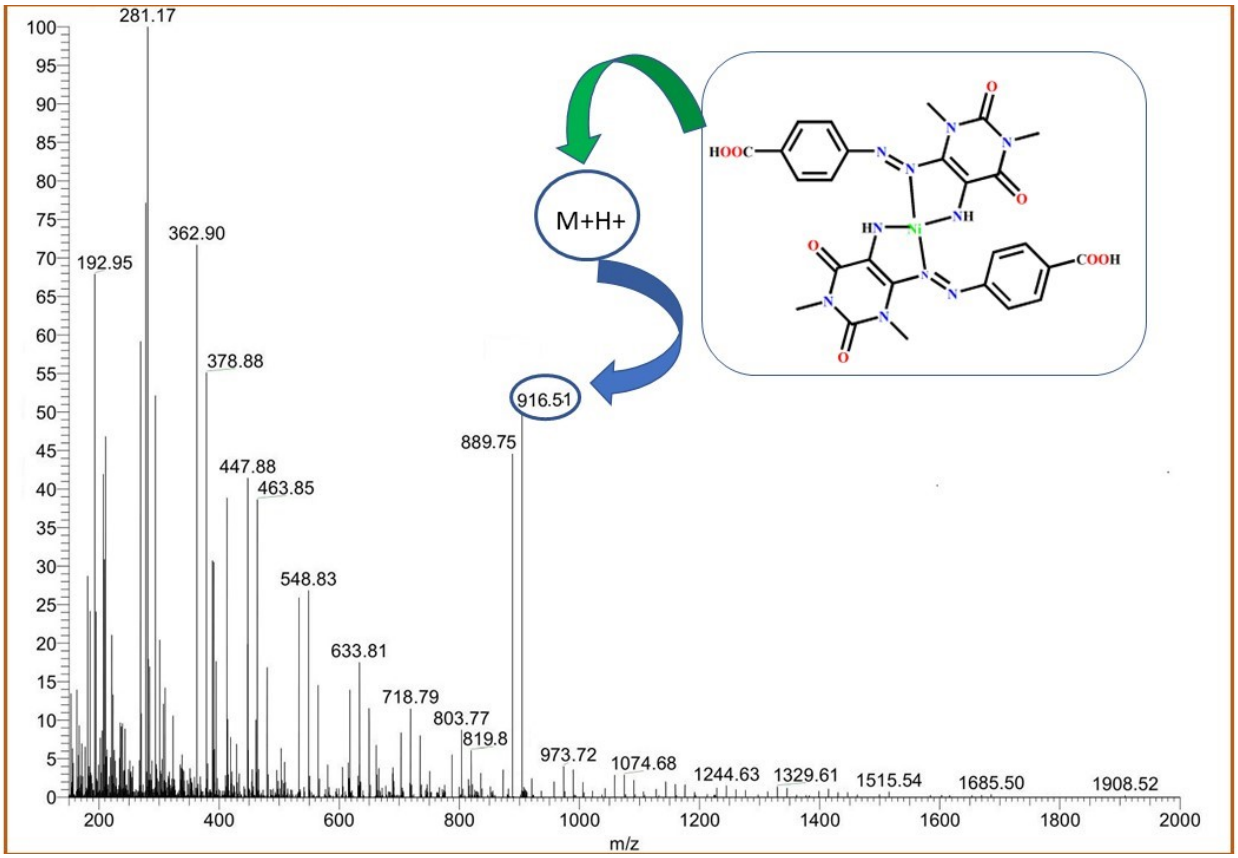

Fig. S1 ESI-MS spectrum of **N1**

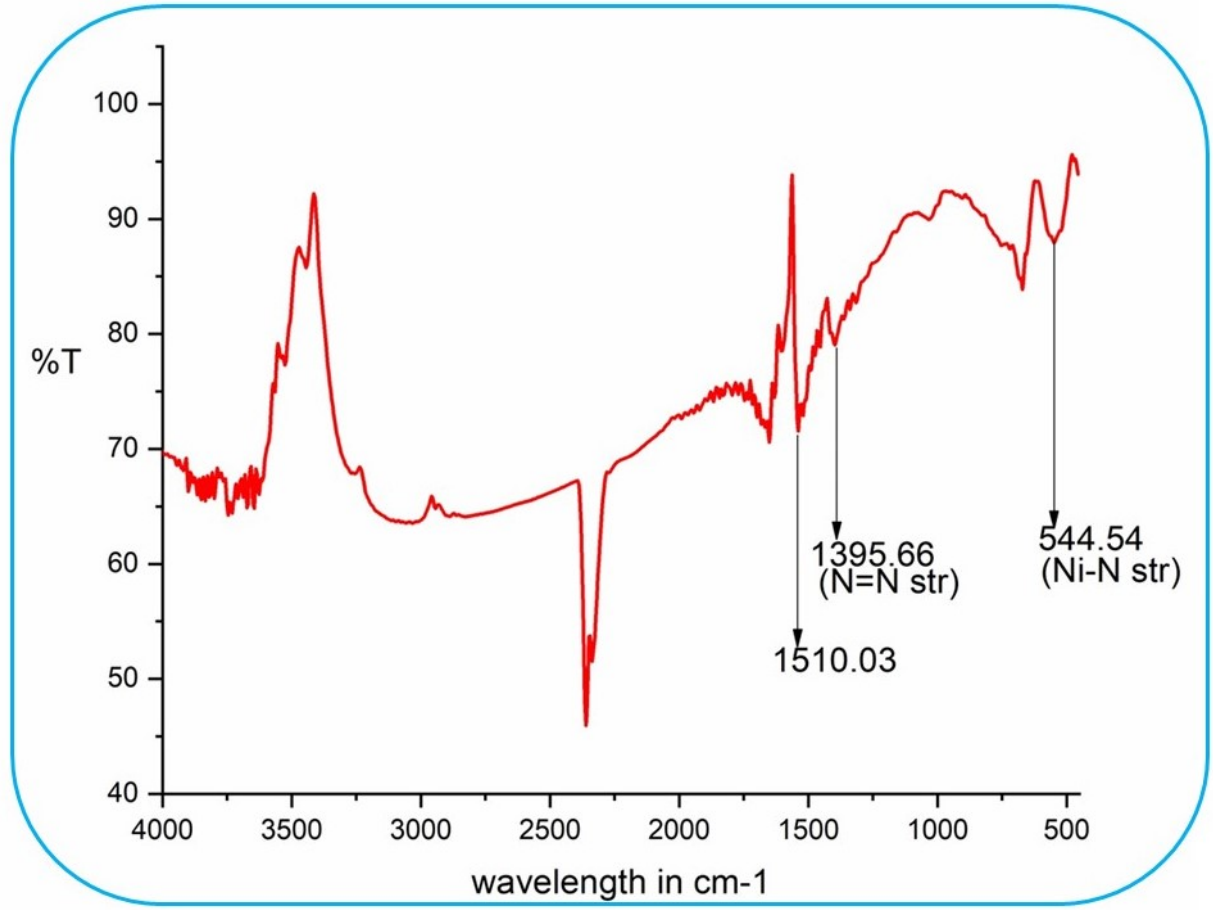

Fig. S2 FTIR spectrum of **N1**

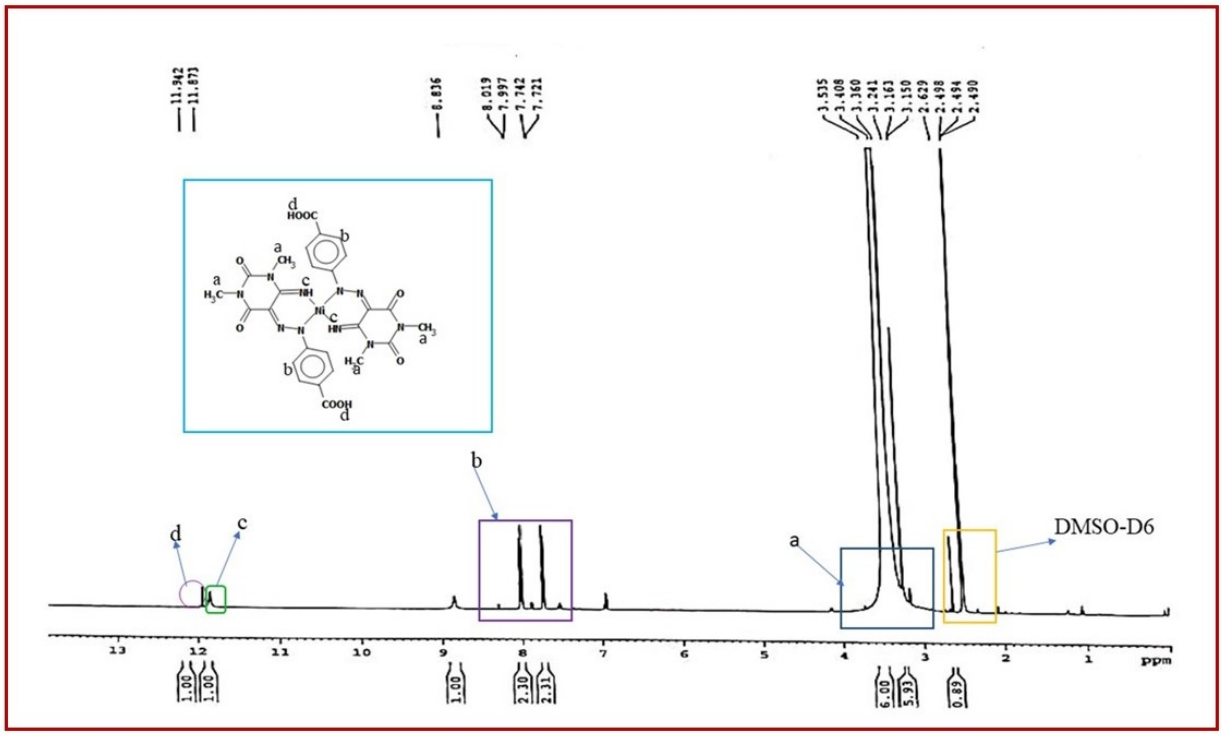

Fig. S3 ^{1}HNMR spectrum of **N1**

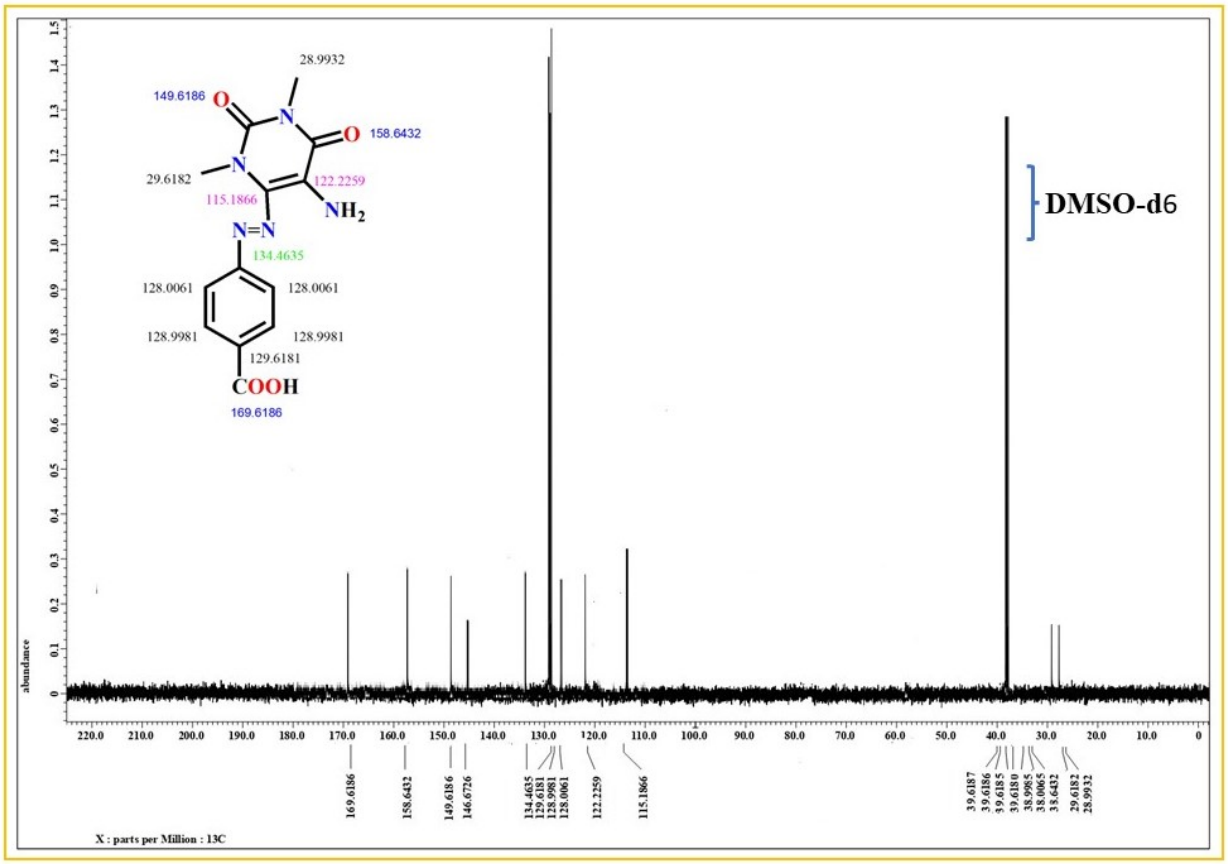

Fig. S4 ^{13}CNMR spectrum of L1 in DMSO-D6

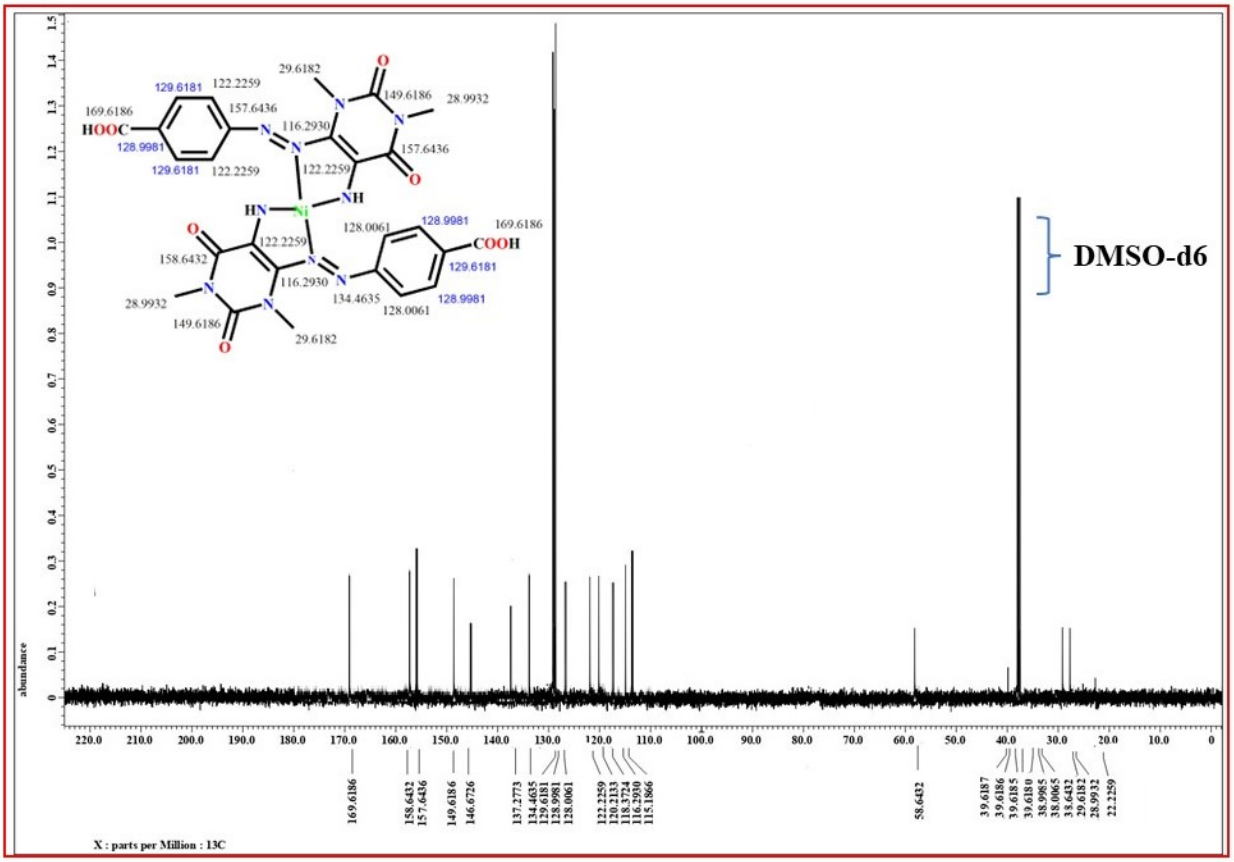

Fig. S5 ^{13}CNMR spectrum of N1 in DMSO-D6

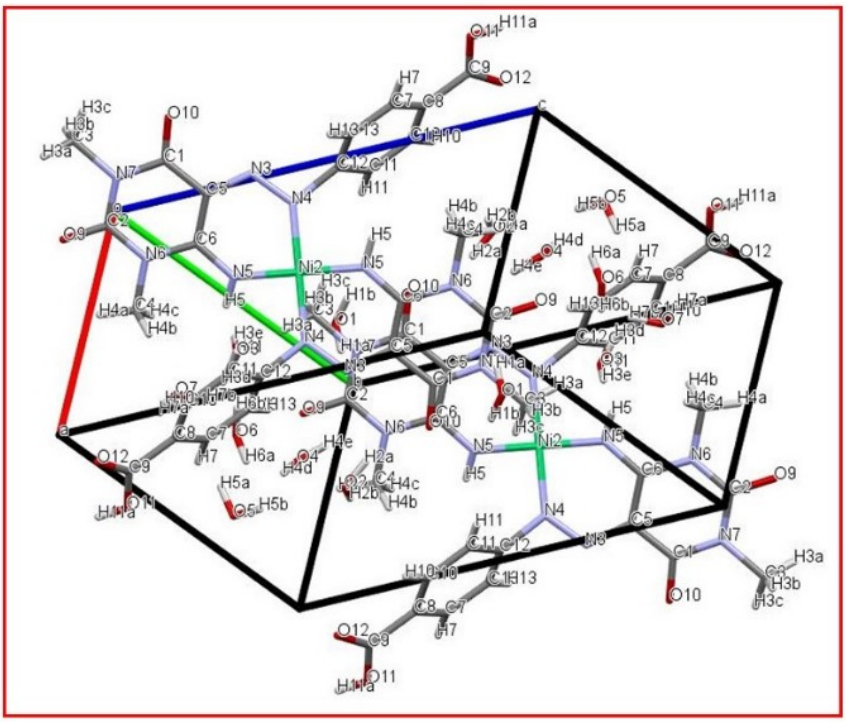

Fig. S6 Packing pattern of **N1**

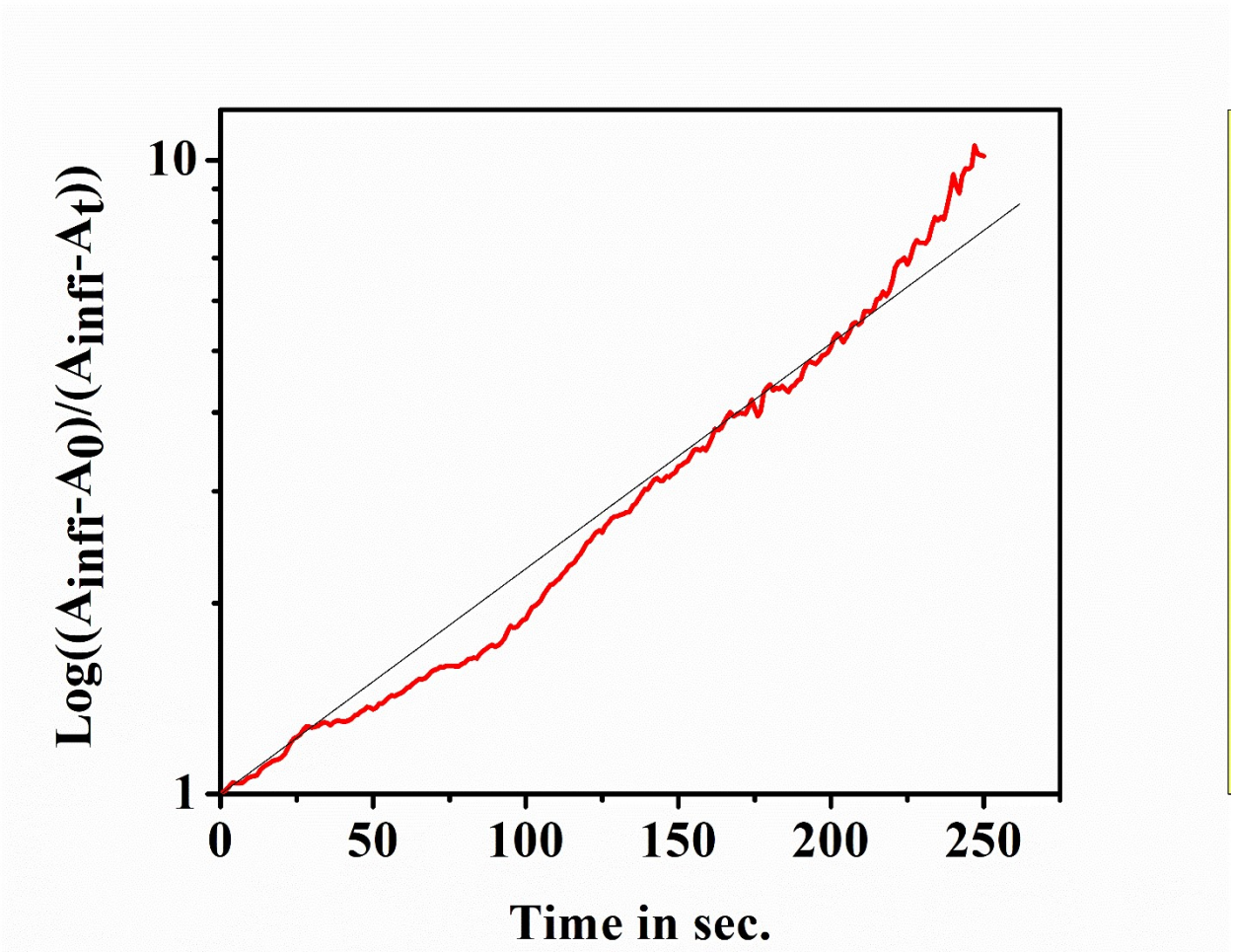

Fig. S7 Determination of rate constant for the **N1** (0.001 mmol) catalysed hydrolysis of ***p*-NPP** under pseudo first order condition (media: MeOH–H_2O, 1:4, v/v, PBS buffer, pH 7.4, room temperature).

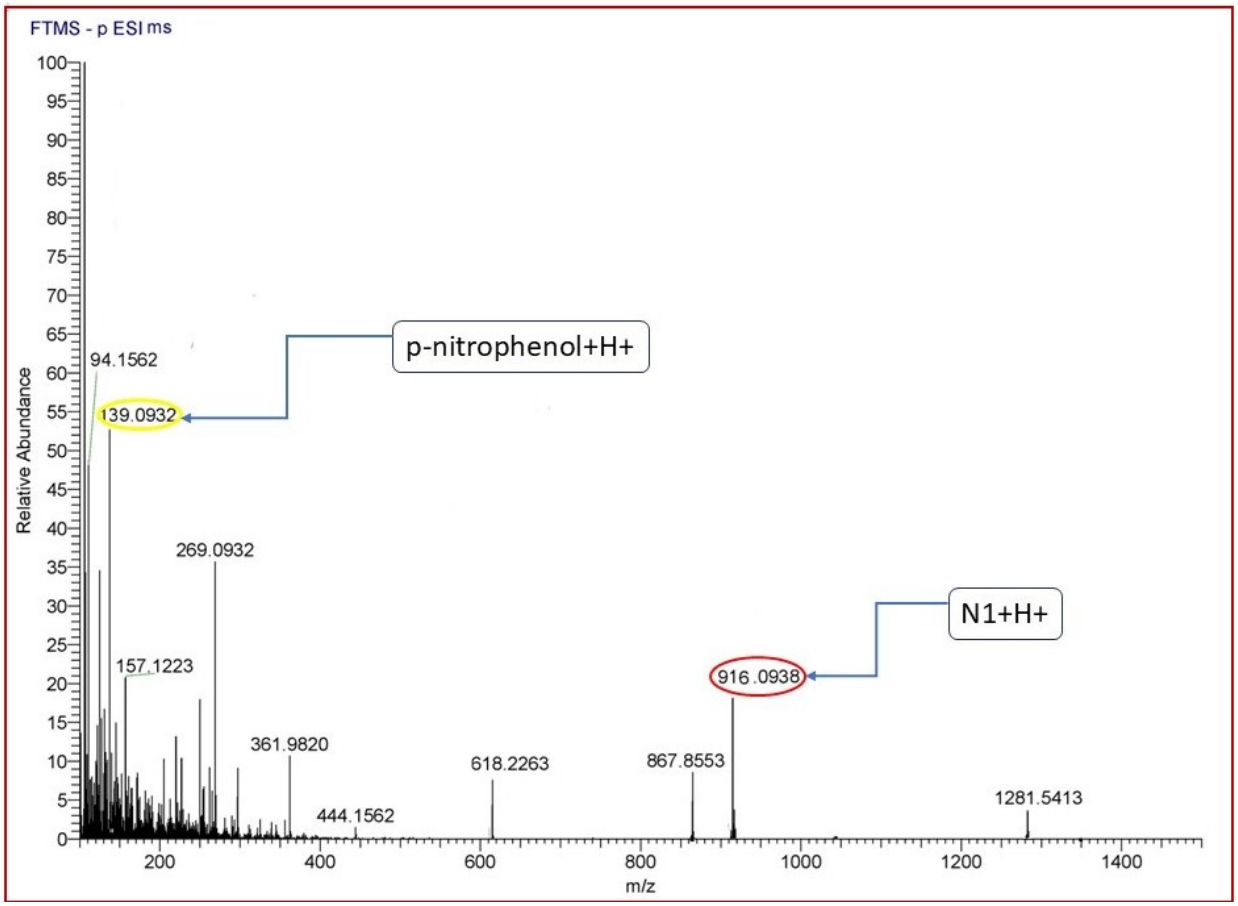

Fig. S8 ESI-MS spectrum of the [**N1+*p*-NPP]** system

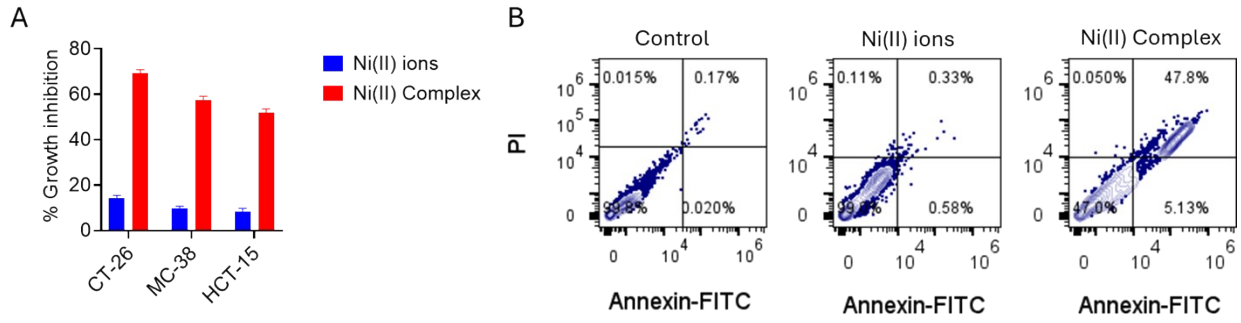

Fig. S9 Comparison of cytotoxic and apoptotic effects of N1 and free Ni^{2+}: (A) Percentage cytotoxicity in CT26 cells treated with N1 and free Ni^{2+} ion as $[Ni(NO_3)_2 \cdot 6H_2O]$ at 0.04 µg mL^{-1}, demonstrating significantly higher cytotoxicity of the N1 relative to the Ni^{2+} salt alone. Data are expressed as mean ± SD (n = 3); (B) Representative flow cytometric dot plots of Annexin V–FITC/PI staining showing apoptotic populations in untreated control cells, free Ni^{2+} treated cells, and N1 treated cells. Quadrants represent viable (Annexin V^-/PI^-), early apoptotic (Annexin V^+/PI^-), late apoptotic (Annexin V^+/PI^+), and necrotic (Annexin V^-/PI^+) cell populations. The N1 treated group shows a pronounced increase in apoptotic cells compared to free Ni^{2+}, confirming that the observed biological activity predominantly arises from the N1. All experiments have been performed under identical conditions and analysed by flow cytometry.

Table S1 Crystal data and structure refinement parameters for **N1**

Parameters	**Data**
CCDC	2003428
Empirical formula	C26 H24 N10 Ni O8,7(H4 O2)
Formula weight	915.47
Crystal system	Triclinic
Space group	P -1
Hall group	-P 1
Temparature	296 K
Wavelength	0.71073
a/Å	7.89(2)
b/Å	8.49(2)
c/Å	15.86(4)
α/°	80.77(4)
β/°	79.09(5)
γ/°	89.38(4)

Volume/Å^3	1030(4)
Z	1
ρ_{calc} g/cm^3	1.436
μ/mm^{-1}	0.565
F(000)	482
F(000′)	482.62
Θmax	24.26
Index ranges (h, k, lmax)	9,9,18
Reflections collected (R)	0.1031(1234)
wR_2 (Reflection)	0.2471(1945)
S	1.151
Npar	292
Data completeness	1.000

Table S2 Selected bond lengths [Å] and angles [°] for **N1**

Atoms	**Bond length (Å)**	**Atoms**	**Bond angle (°)**
Ni2 N5	1.828(12)	N5 Ni2 N4	87.9(5)
Ni2 N5	1.828(12)	N5 Ni2 N4	92.1(5)
Ni2 N4	1.855(14)	N5 Ni2 N4	92.1(5)
Ni2 N4	1.855(14)	N5 Ni2 N4	87.9(5)
N3 N4	1.329(16)	N4 Ni2 N4	180.0
N3-C5	1.314(18	C5 N3 N4	120.0(14)
C12-N4	1.484(17)	N2 N1 C11	109.4(8)
N3 C5	1.294(12)	N2 N1 Ni1	129.3(7)
N5 C5	1.392(14)	C12 N4 Ni2	122.4(9)
N5 C8	1.344(14)	C5 N3 Ni1	130.2(9)
N5 C10	1.452(15)	C5 N5 C8	122.1(11)
O11 C7	1.214(14)	C5 N5 C10	121.1(9)
O10 C8	1.237(14)	C8 N5 C10	116.7(10)
C5 C6	1.408(15)	N3 C5 N5	120.8(11)
O3 C13	1.220(14)	N3 C5 C6	119.9(10)
C11 C1	1.364(14)	N5 C5 C6	119.2(9)
C11 C4	1.397(15)	C1 C11 C4	121.3(10)
N4 C8	1.354(16)	C1 C11 N1	118.4(11)
N4 C7	1.387(16)	C4 C11 N1	120.2(9)
N4 C9	1.505(13)	C8 N4 C7	125.9(9)
C1 C2	1.421(15)	C8 N4 C9	117.4(10)

C12 C2	1.380(16)	C7 N4 C9	116.6(12)
C12 C3	1.379(14)	C11 C1 C2	119.2(12)
C12 C13	1.474(16)	C2 C12 C3	118.3(10)
C6 C7	1.449(14)	C2 C12 C13	118.8(10)
C4 C3	1.389(15)	C3 C12 C13	122.9(12)
O4 C13	1.266(15)	N2 C6 C5	124.4(8)
		N2 C6 C7	115.2(11)
		C5 C6 C7	119.6(10)
		C3 C4 C11	117.9(9)
		C12 C2 C1	120.6(10)
		O10 C8 N4	121.1(10)
		O10 C8 N5	120.7(13)
		N4 C8 N5	118.2(11)
		O11 C7 N4	121.2(10)
		O11 C7 C6	124.1(12)
		N4 C7 C6	114.7(12)
		C4 C3 C12	122.7(12)
		O3 C13 O4	122.4(12)
		O3 C13 C12	121.3(13)
		O4 C13 C12	116.3(11)

Table S3 Results from TD-DFT calculation on **L1**

Compound	**Electronic Transitions**	**Energy[a] (eV)**	**Wavelength (nm)**	**f[b]**	**Transitions involved**
L1	$S_0 \longrightarrow S_1$	2.2434 eV	552.66 nm	0.0002	HOMO $\longrightarrow$ LUMO
	$S_0 \longrightarrow S_2$	3.7392 eV	331.58 nm	0.5782	HOMO-1 $\longrightarrow$ LUMO HOMO $\longrightarrow$ LUMO + 1
	$S_0 \longrightarrow S_3$	3.8327 eV	323.49 nm	0.0477	HOMO -1 $\longrightarrow$ LUMO HOMO $\longrightarrow$ LUMO + 1

Table S4 TD-DFT calculation results of N1

Compound	**Electronic Transitions**	**Energy[a] (eV)**	**Wavelength (nm)**	**f[b]**	**Transitions involved**
N1	$S_0 \longrightarrow S_1$	1.3068 eV	948.77 nm	0.0125	HOMO-1 $\longrightarrow$ LUMO HOMO $\longrightarrow$ LUMO HOMO $\longrightarrow$ LUMO + 1
	$S_0 \longrightarrow S_2$	1.6430 eV	754.60 nm	0.0053	HOMO-3 $\longrightarrow$ LUMO HOMO-2 $\longrightarrow$ LUMO HOMO-1 $\longrightarrow$ LUMO HOMO-1 $\longrightarrow$ LUMO+1 HOMO $\longrightarrow$ LUMO
	$S_0 \longrightarrow S_3$	1.7957 eV	690.46 nm	0.0198	HOMO-3 $\longrightarrow$ LUMO HOMO-2 $\longrightarrow$ LUMO + 2 HOMO $\longrightarrow$ LUMO HOMO $\longrightarrow$ LUMO + 1 HOMO-1 $\longrightarrow$ LUMO + 9

Table S5. Structural parameters of L1 derived from TDDFT studies

Multiplicity	1
Number of electrons	158
Number of alpha electrons	79
Number of beta electrons	79
Number of basis functions	224
Number of independent functions	224
Number of point charges in /Mol/	0
Number of translation vectors	0
Number of Atoms	35

Calculation Type	SP
Calculation Method	RB3LYP TD-FC
Basis Set	3-21G
Charge	0
Spin	Singlet
Solvation	None
E(TD-HF/TD-DFT)	-1071.7464 Hartree
RMS Gradient Norm	0 Hartree/Bohr

Dipole Moment	4.6361928 Debye
Point Group	C1

Table S6. Structural and relevant parameters of N1 derived from TDDFT studies

Multiplicity	1
Number of electrons	342
Number of alpha electrons	171
Number of beta electrons	171
Number of basis functions	473
Number of independent functions	473
Number of point charges in /Mol/	0

Number of translation vectors	0
Number of Atoms	69

Calculation Type	SP
Calculation Method	RTD-B3LYP-FC
Basis Set	6-31G
Charge	0
Spin	Singlet
Solvation	None
E(TD-HF/TD-DFT)	-3643.382 Hartree
RMS Gradient Norm	0 Hartree/Bohr
Dipole Moment	0.0028231188 Debye
Point Group	C1

Table S7. Structural and other parameters for N1- *p*-NPP interaction derived from TDDFT studies

Multiplicity	1
Number of electrons	454

Number of alpha electrons	227
Number of beta electrons	227
Number of basis functions	615
Number of independent functions	615
Number of point charges in /Mol/	0
Number of translation vectors	0
Number of Atoms	89

Calculation Type	SP
Calculation Method	FOPT
Basis Set	3-21G
Charge	0
Spin	Singlet

Solvation	None
E(TD-HF/TD-DFT)	-4741.384978 Hartree
RMS Gradient Norm	0.000759 Hartree/Bohr
Dipole Moment	12.717439 Debye
Point Group	C1

B. <u>Justification for alerts at level A and B for N1</u>

Alert level A
PLAT417_ALERT_2_A Short Inter D-H..H-D H1B ..H2B . 1.70 Ang. -1+x,y,z = 1_455
PLAT417_ALERT_2_A Short Inter D-H..H-D H3D ..H3D . 1.74 Ang. 1-x,1-y,2-z = 2_667
PLAT417_ALERT_2_A Short Inter D-H..H-D H4D ..H6A . 1.72 Ang. x,y,z = 1_555
PLAT417_ALERT_2_A Short Inter D-H..H-D H5A ..H6A . 1.08 Ang. x,y,z = 1_555
PLAT417_ALERT_2_A Short Inter D-H..H-D H5B ..H6A . 1.70 Ang. x,y,z = 1_555
PLAT417_ALERT_2_A Short Inter D-H..H-D H6B ..H7B . 1.14 Ang. x,y,z = 1_555

Hydrogen atoms were placed in calculated positions and refined more times. The short H···H contacts arise from idealized hydrogen geometry and do not represent significant intermolecular interactions.

Alert level B
ALERT_3_B Ratio Observed / Unique Reflections (too) Low .. 37%
These alerts are generated because the crystal used here is not of very high quality although we have made several attempts to obtain better quality crystal but every time the data we have similar crystallographic issue . Besides the diffraction quality of the crystal is weak. Despite this, the molecular structure and connectivity are clearly established.

ALERT_3_B Poor Data / Parameter Ratio……..6.66
The alarm of poor data/parameter ratio is due to the crystal which diffracted quite weakly at high angle.

ALERT_2_B ADP of Atom O6 is N.P.D. or (nearly) 2D.

The ADP of atom O6 appears slightly non-positive definite due to weak electron density associated with a solvent molecule and possible thermal motion.

ALERT_2_B Atom O11 has ADP max/min Ratio ..... 4.4 prolate

These alerts are generated because the crystal used here is not of very high quality although we have made several attempts to obtain better quality data but every time we have similar crystallographic issue, besides this there is a large amount of disorder in the structure due to thermal motion.

ALERT_3_B Low Bond Precision on C-C Bonds 0.01989

This alert is generated because there is a large amount of disorder in the structure due to thermal motion. Nevertheless, the connectivity and overall molecular geometry are well established.

ALERT_2_B Short Inter D-H..H-D H3D ..H6B . 1.91 Ang. x,y,z = 1_555
.ALERT_2_B Short Inter D-H..H-D H6A ..H7B . 2.07 Ang. x,y,z = 1_55

Hydrogen atoms were placed in calculated positions and refined more times. The short H···H contacts arise from the idealized geometry of the hydrogen atoms and are not chemically significant.

ALERT_2_B D-H Bond Without Acceptor O7 --H7A

Atom O7 corresponds to a solvent molecule and the hydrogen atom H7A which does not participate in a significant hydrogen bond interaction in the crystal packing.

ALERT_3_B Missing FCF Reflection(s) Below Theta(Min)[Deg]= 5.15 Note
1 0 0, -1 1 0, 0 1 0, 1 1 0, 0 2 0, -1 -1 1, 0 -1 1, 1 -1 1, -1 0 1, 0 0 1, 1 0 1, -1 1 1, 0 1 1, 1 1 1, 0 2 1, -1 -1 2, 0 -1 2, 1 -1 2, -1 0 2, 0 0 2, 1 0 2, -1 1 2, 0 1 2, 1 1 2, (5 More Missing: see the .ckf listing file)
ALERT_3_B Missing FCF Refl Between Thmin & STh/L= 0.578 1365 Report
2 0 0, 2 1 0, 3 1 0, 4 1 0, -7 2 0, -2 2 0, 1 2 0, 2 2 0, 8 2 0, -5 3 0, 1 3 0, 2 3 0, 3 3 0, 4 3 0, 6 3 0, 7 3 0, 8 3 0, 0 4 0, 1 4 0, 2 4 0, 3 4 0, 4 4 0, 5 4 0, 6 4 0, 7 4 0, 8 4 0, -1 5 0, 1 5 0, 2 5 0, 3 5 0, (1335 More Missing: see the .ckf listing file)

Some reflections are missing in the FCF file because very weak reflections were omitted during data reduction. The structure refinement was performed using the available reflections and the overall data completeness and refinement statistics remain satisfactory.