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# Spectrophotometric investigation of 5-nitroso-6-aminouracil and its methyl derivative in methanol by selective complexation with bivalent metal ions

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#### Abstract:

6-Amino-5-nitrosouracils are synthesized by the condensation reaction of urea or *N*, *N*'-dimethyl urea, cyanoacetic acid and acetic anhydride followed by nitrosation reaction with sodium nitrite. The synthesized compounds are characterized by various spectroscopic techniques like FTIR, NMR, single crystal XRD, and UV-vis absorption spectroscopy. From a single-crystal X-ray crystallography study of DANU, it is found that the compound is crystalline with one water molecule. The binding properties of both compounds with various metal ions are studied using UV-vis spectroscopy, where ANU shows a colour change from colourless to yellow colour-forming complex with cobalt metal ion. While DANU shows a colour change from colourless to dark yellow forming complex with copper and nickel cation, respectively. These compounds showed the job's plots with Cu<sup>2+</sup>, Ni<sup>2+</sup>, and Co<sup>2+</sup> in various stoichiometric ratios to form the respective metal complex. The association/binding constant (Ka) values are calculated by plotting Benesi–Hilderbrand plots of ANU with Co<sup>2+</sup> ion and is found to be 9.524 \* 10<sup>2</sup>. Whereas, DANU with Cu<sup>2+</sup>, Ni<sup>2+</sup> are found to be 3.956 \* 10<sup>3</sup>, 2.041 \* 10<sup>3</sup>, respectively. These cations may be used in metal ions complexation for the respective ligand. The LOD values for ANU-Co<sup>2+</sup>, DANU-Cu<sup>2+</sup> & DANU-Ni<sup>2+</sup> are obtained as 33.9428 μM, 93.8082 μM and 48.396 μM, respectively, whereas the LOQ values are found as 102.857 μM, 284.2675 μM and 146.653 μM, respectively.

# **Keywords:**

Nitrosouracils; Metal ion complexation; Binding constant; Benesi-Hilderbrand plot; Job's plot.

# 1. Introduction:

In recent years, selective complexation of a metal complex with nitrogenous containing organic compounds is one of the major challenges. The successful development of this complex is significant to their applications in molecular recognition, molecular magnetism, crystal engineering, catalysts and pharmaceutical drugs. Uracil derivatives, a nitrogenous containing heterocyclic compounds are widely used for biological and pharmaceutical applications [1-9]. Moreover, they also have a large number of

binding or chelating locations for transition/heavy-metal ions, including the amide as well as heterocyclic nitrogen atoms [10, 11]. 6-Amino-5-nitrosouracils, an important uracil derivative having a good ligating property, may occur in several tautomeric forms: (A) diketonic, (B) nitroso-phenolic as well as (C) keto-oximic form. According to infrared spectroscopic data of 6-amino-5-nitrosouracil (ANU) and 1,3-dimethyl-6-amino-5-nitrosouracil (DANU) in the solid phase, the diketonic forms are most stable [12]. When the ligands are coordinated to metals ions in the anionic form, nitrosophenolic (B) and keto-oxime forms (C) are favored because both have frequently dissociable H atoms, thereby the production of chelate appear most readily (**Scheme 1**). The various tautomeric forms of ANU & DANU may bind with metal atoms in the bidentate mode, through the nitrogen atom of the C5 situated nitroso group and the oxygen atom joined on the C4 position or the nitrogen atom from the 6-amino group. This coordination mode formed a five-membered meta cycle chelate ring to a few metal complexes with 6-amino-5-nitrosouracil derivatives [13-17].



Scheme 1: Structure, naming and various tautomeric forms of the nitrosouracils {(Where R'=R=H, 6amino-5-nitrosouracil (ANU); R'= R=CH<sub>3</sub>, 1,3-dimethyl-6-amino-5-nitrosouracil (DANU)} (A) diketonic (B) nitroso-phenole and (C) keto-oximes.

However, few reports on the metal complexes of 6-amino-5-nitrosouracil are available in the literature, where the most normal coordination modes are observed as bidentate N, N (mode A) and bidentate O, O (mode B) (Scheme 2). Uncommon bis(bidentate bridging (mode C) and tridentate bridging (mode D) patterns are also observed in some polynuclear complex [18,19] and a chain structure, respectively [20]. The rhenium (I) complexes of uracil with a few 5-nitrosouracil appears to be monomeric and performed in a neutral form in a bidentate mode through N(5) and O(4) atoms [21]. Further, J. R. DeMember et al. reported the bidentate coordination including N(1) and O(2) within the case of Ag(I)-uracil complex [22]. While silver (I) complexes of 5-nitrosouracil and its derivatives are prepared and the ligand is O-O facilitated within the keto-oximic form and coordinated by N(5) and O(4) atoms within the other complexes [23,24]. Moreover, Zn(II) & Cd(II) complexes of nitrosouracil are also found where ligand is coordinated to metal atom through C5 mpsition of nitroso group and C-6 position of the amino group [12]. Recently, we have reported that different functional groups in violuric acid (acidification product of nitrosouracil) derivatives, are also providing a large variety of bonding behavior. While the presence of several hydrogen bond donor and acceptor sites together with a uracil ring may

favor diverse non-covalent interactions, thus ensuring a rich metallosupramolecular/coordination chemistry of such compounds [25-28]. However, we are unable to synthesize the metal complexes of nitrosouracils and we believe that a solution is found from our present studies.



Scheme 2: Different coordination modes of 5-nitrosopyrimidines such as (A) bidentate-N,N; (B) bidentate-O,O; (C) bis(bidentate)bridging; (D) tridentate bridging.

#### 2. Experimental Section:

#### 2.1: Reagents

All chemicals are purchased from S. D. fine, Merck, Sigma-Aldrich, and Himedia and used without further purification. Synthesis and spectroscopic analysis are studied using analytical grade solvents.

#### 2. 2: General method

Shimadzu model Iraffinity-1s ce FT-IR spectrophotometer is used for the IR spectra of the compound in the wavelength region of 4000–400 cm<sup>-1</sup>. <sup>1</sup>H NMR and <sup>13</sup>C NMR spectra are taped on a Bruker (AC) 300 MHz and a Bruker DRX-500 MHz FT-NMR spectrometer in the DMSO solvent and TMS used as the internal standard. The electronic spectra are recorded using the Thermo fisher scientific model EV0300 PC UV-vis spectrophotometer in which quartz cuvettes are used for absorption measurements of solution.

#### 2. 3: Synthesis of ANU and DANU

ANU and DANU are prepared by using the modification of the reported method [29]. The one-pot synthesis process is carried out using cyanoacetic acid (85g), urea (60.06 g) and 125 ml of acidic anhydride in a flask at 60°C for 3 hours. After that, 5M sodium hydroxide solution (10 ml) is included gradually to the chilled mixture and stirred continuously until the 6-aminouracil is precipitated out. A solution of 83 g of sodium nitrite in 500 ml of water is included in the chilled agitated mixture and then it is acidified by the drop-by-drop addition of acidic acid over an hour of time duration. The reaction mixture is chilled; the red-violet precipitate is filtered and washed with an aqueous ethanol solution (**Scheme-3**). Yield 141 g. (82%), m.p. 320°C. In the case of DANU, we have used N, N-dimethyl urea

(88.11g) instead of urea. The rest procedure is the same as in the case of ANU synthesis. Yield 162 g. (80%) m.p. 233 °C.



Scheme 3: Synthesis of 6-amino-5-nitrosouracils (Where R'= R= H, for ANU & R'= R= CH<sub>3</sub>, DANU)

### FTIR, NMR and elemental analysis of ANU:

IR (KBr, cm<sup>-1</sup>),  $v_{max}$ : 3506, 3441, 3281, 3178, 2901, 2804, 2363, 2338, 1691, 1627, 1541, 1516, 1447, 1418, 1381, 1315, 1256, 1229, 1136, 1086, 986, 931, 816, 764, 706, 694, 658, 552, 513, 447, 440. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, 400 MHz):  $\delta_{\rm H}$  11.5 (br s, 1H, NH of uracil ring), 11.0 (br s, 1H, NH of uracil ring), 3.75 (br s, 2H, NH<sub>2</sub>). <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>, 100 MHz):  $\delta_{\rm C}$  162 (C6), 151 (C2), 147 (C4), 140 (C5). CHNS analysis: C<sub>4</sub>H<sub>4</sub>N<sub>4</sub>O<sub>3</sub> (156.10) calcd. C = 30.47, H = 2.58, N = 35.89; found C = 30.44, H = 2.57, N = 35.93.

#### FTIR, NMR and elemental analysis of DANU:

IR DANU (KBr, cm<sup>-1</sup>),  $v_{max}$ : 3545, 3315, 3011, 2361, 1713, 1653, 1585, 1512, 1462, 1439, 1381, 1329, 1288, 1236, 1140, 1076, 1049, 951, 920, 824, 766, 716, 679, 629, 496, 426. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, 400 MHz):  $\delta_{\rm H}$  3.5 (s, 3H, NCH<sub>3</sub> of uracil ring), 3.57 (s, 3H, NCH<sub>3</sub> of uracil ring), 9.1 (br s, 2H, NH<sub>2</sub>). <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>, 100 MHz):  $\delta_{\rm C}$  161 (C6), 150 (C2), 146 (C4), 140 (C5), 29 (NCH<sub>3</sub>) 27 (NCH<sub>3</sub>). CHNS analysis: C<sub>6</sub>H<sub>10</sub>N<sub>4</sub>O<sub>4</sub> (202.17) calcd. C = 35.65, H = 4.99, N =27.71; found C = 35.63, H = 4.97, N = 27.73.

#### 2. 4. X-Ray crystallography:

The single-crystal X-ray diffraction data are collected at 293 K with Mo K $\alpha$  radiation ( $\lambda = 0.71073$  Å) using a Bruker Smart Apex II CCD di  $\Box$  ractometer equipped with an Oxford Cryostream low-temperature device and a fine focus sealed-tube X-ray source (graphite monochromated) operating at 50 kV and 30 mA. APEX3 software [30] is used for the preliminary determination of the unit cell. Using Olex [31-32] the structure is solved with the Superflip [33] structure solution program using the Charge Flipping solution method and refined with the ShelXL [34] refinement package using Least Squares minimization. All the non-hydrogen atoms are refined anisotropically and the hydrogen atoms are placed based on the Fourier difference maps.

#### 2. 5. Procedures of the metal ions complexation.

# 2. 5. 1. UV-vis analysis of ANU and DANU for metal ion detection

The electronic absorption spectra of ANU and DANU are considered in natural solvents of different hydrogen holding parameters (dH), viz. non-polar:  $C_6H_{14}$  (0.0) and CHCl<sub>3</sub> (5.7); polar-aprotic: CH<sub>2</sub>Cl<sub>2</sub> (7.1), DMSO (10.2) and DMF (11.3); polar protic: EtOH (19.4) and MeOH (22.3) over the wavelength range of 260–500 nm. The cation identification behavior of complexation ANU and DANU with transition and heavy metal ions (Fe<sup>2+</sup>, Ni<sup>2+</sup>, Cu<sup>2+</sup>, Zn<sup>2+</sup>, Cd<sup>2+</sup>, Mo<sup>2+</sup>, Mn<sup>2+</sup>, Co<sup>2+</sup> Ag<sup>2+</sup>, Hg<sup>2+</sup>) is studied using UV-vis absorption spectroscopy.

# 2. 5. 2. Sample preparation, binding constant and LOD/LOQ value determination

The absorption spectrum of ANU and DANU with different metal ions was taken, in which the inclusion of five equivalents of different cations ( $50\mu$ L, 1 x  $10^{-2}$  M, in water) to a 5 x  $10^{-5}$  M solution of ANU and DANU in CH<sub>3</sub>OH. The Co<sup>2+</sup> ion is able to perturb the absorption spectra of ANU and the Cu<sup>2+</sup>, Ni<sup>2+</sup> ions are capable to perturb the absorption spectrum of DANU productively. The association constant is determined using the Benesi-Hilderbrand plots (or double-reciprocal plots) as shown in Eq. (1) [35]

$$\frac{1}{A - Ao} = \frac{1}{Ka * (Amax - Ao) * [M2+]} + \frac{1}{Amax - Ao}$$
(1)  
Ka value is calculated by Eq (2)

$$Ka = \frac{1}{\text{Slope}}$$
(2)

The limit of detection (LOD) and limit of qualification (LOQ) value are calculated by Eq. (3) and (4).

$$LOD = \frac{3.3 * Standard deviation}{Slope}$$
(3)  
$$LOQ = \frac{10 * Standard deviation}{Slope}$$
(4)

For the confirmation of metal complexation and its stoichiometry, we studied the job's plots for ANU & DANU with  $\text{Co}^{2+}$ ,  $\text{Cu}^{2+}$ , and  $\text{Ni}^{2+}$  ions at  $\lambda_{\text{max}}$  455 nm, 405 nm, and 450 nm, respectively.

#### 3. Results and discussion:

### 3. 1. FTIR and NMR spectral studies:

For ANU the v(N–H) stretching of primary amines at 6-position of the uracil ring generally appear at 3506 cm<sup>-1</sup>. Whereas, the two v(N-H) stretching vibrations are observed at 3281cm<sup>-1</sup>, 3178 cm<sup>-1</sup> due to the two secondary amino groups at N1 and N3 positions of uracil ring, respectively. The sharp intense peaks appeared at 1691 cm<sup>-1</sup> is due to the v(C=O) stretching vibration of two oxo groups at positions 2 and 4 of the uracil ring, respectively. The v(C=C) stretching frequency of conjugated double bond between C5 and

C6 of ANU ring appears at 1627 cm<sup>-1</sup>. The v(N=O) stretching vibration of the C5 position of uracil ring has appeared at 1541 cm<sup>-1</sup>. Whereas, for DANU the v(N–H) stretching frequency of primary amines of the uracil ring at the 6-position appears at 3545 cm<sup>-1</sup>. However, two v(N-H) stretching vibrations are not observed as two protons at N1 and N3 positions are replace with a methyl group in DANU. The sharp intense peaks appeared at 1713 cm<sup>-1</sup> is due to the v(C=O) stretching vibration of oxo groups at positions 2 and 4 of the uracil ring, respectively. The v(C=C) stretching frequency of conjugated double bond between C5 and C6 of ring appears at 1653 cm<sup>-1</sup>. The v(N=O) stretching frequency of C5 position of uracil ring is observed at 1512 cm<sup>-1</sup>. The IR spectral data of ANU & DANU are shown in **Fig. S1** of the supporting information.

ANU shows a <sup>1</sup>H NMR chemical shift values at 11 ppm and 11.5 ppm for two protons N1(H) and N3(H), respectively. A broad peak appeared in ANU at 3.75 ppm due to the water molecule present in the DMSO- $d_6$  solvent merged with amino group protons attached at C6. For DANU, <sup>1</sup>H NMR chemical shift values are observed at 3.5 ppm and 3.57 ppm due to the two NCH<sub>3</sub> protons of uracil ring, respectively. The amine protons attached at C6 position showed the broad chemical shift value at 9.1 ppm. The proton NMR spectra of both ANU and DANU are shown in **Fig. S2** of supporting information. In the case of <sup>13</sup>C NMR of ANU, the C6 is deshielded and peak appears at 162 ppm due to the presence of an electron-donating NH<sub>2</sub> group. Other peaks at 151 ppm and 147 ppm are observed which may be due to the presence of two oxo groups at positions 2 and 4 of uracil ring, respectively. Due to the effect of substituent electron-withdrawing NO group, the C5 is a little bit shielded and chemical shift value is observed at 140 ppm. Similarly, in the case of <sup>13</sup>C NMR of DANU, peaks appear at 161 ppm, 150 ppm, 146 ppm and 140 ppm is due to the C6, two oxo groups (2 and 4 position) and C5 of uracil ring, respectively. In addition, peaks appear at 27 ppm and 29 ppm is due to the two NCH<sub>3</sub> protons of uracil ring (**Fig. S3** of supporting information).

#### 3. 2. Crystal structure of DANU:

DANU is crystallized with one water molecule as shown in the ORTEP diagram (**Fig. 1**). The details of the crystallographic data are listed in **Table 1**. The molecule is orthorhombic in nature with a space group pnma. DANU having C5-C6 bond length is greater than that of uracil molecule; this may be the mesomeric effect of the presence of -NH2 group at C6 position and -NO group at the C5 position. The -NO group have a negative inductive effect due to which it attracts bonding electron towards itself and affects the C5-C6 bond length. Selected bond lengths and angles are shown in **Table S1** of supporting information. Moreover, DANU shows intramolecular hydrogen bonding with a nitrogen atom of the nitroso group and the oxygen atom of the carbonyl group of the same uracil ring as well as with the water molecule. Intermolecular hydrogen bonding with the nitrogen atom of the amino group with a water

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molecule and oxygen atom of the carbonyl group of DANU and nitrogen atom of the amino group of another DANU molecule are shown in **Fig. 2**.



Fig. 1: ORTEP representation of compound with the ellipsoid contour 50% probability.

Empirical Formula	$C_6H_8N_4O_3$ . $H_2O_3$	
CCDC number	1979301	
Temperature/K	298 K	
Crystal system	Orthorhombic	
Space group	P n m a	
a [ Å]	13.9694(14)	
<i>b</i> [ Å]	6.3336(6)	
<i>c</i> [ Å]	9.6251(9)	
α [ <sup>0</sup> ]	90	
β [ <sup>0</sup> ]	90	
γ [ <sup>0</sup> ]	90	
Volume/Å <sup>3</sup>	851.60(14)	
Z	8	
λ[Å]	0.71073	
$\rho_{calcd} [gcm^{-3}]$	1.577	
μ [mm <sup>-1</sup> ]	0.133 mm <sup>-1</sup>	
F[000]	424	

Table 1: Crystallographic data and structure refinement of DANU.

$2^{\Theta}$ range for data	2.57 to 28.34°	
collection/°		
Index ranges	$-18 \le h \le 18, -8 \le k \le 8,$	
	- 12≤l≤ 12	
Parameters	93	
Final R indexes R1	0.0454	
wR2	0.1229	
Parameters	93	



Fig. 2: Intermolecular and intramolecular hydrogen bonding as shown in DANU.

# 3. 3. UV-vis absorption and solvatochromic studies:

The UV-vis spectra showed a broad peak with a shoulder at a longer wavelength (**Fig. 3**). There are welldefined maximum absorption bands in the wavelength range  $319\pm5$  nm as observed in the spectrum. ANU and DANU showed an equilibrium mixture of tautomeric forms (**Scheme 2**) or deprotonated and protonated forms of the tautomeric form in these solvents. Solvatochromic study of ANU and DANU are carried out in various solvents (hexane, MeOH, EtOH, EtOAc, DCM, DMSO, DMF, and CHCl<sub>3</sub>). ANU shows a solvatochromic study with DMSO, DMF, MeOH, and EtOH. Other solvents like hexane, EtOAc, DCM, CHCl<sub>3</sub> did not responded in solvatochromic properties with ANU ligand due to poor solubility parameter. Whereas, in the case of DANU maximum absorption with DMSO, DMF, EtOH and EtOAc and less absorption (hypochromic shift) with DCM, MeOH, CHCl<sub>3</sub>, and hexane are observed. The effect of solvents to the electronic transition of both ANU & DANU i.e. their bathochromic & hypsochromic shift in terms of  $\lambda_{max}$  are shown in **Table 2**.



**Fig. 3.** UV-vis absorption spectra of (a) (ANU), (b) (DANU) with different solvents. Solvents showing \* mark does not appear in the graph.

Table 2: Effect of solvent on the electronic absorption properties of ANU and DANU.

	Solvents	A (ANU)	B (DANU)
	Hexane	$(\lambda_{\max} (nm)) $	$(\lambda_{\max} (nm))$ 298
	DCM	*	324
	Chloroform	*	323
	Ethyl acetate	*	314
	Methanol	318	318
	Ethanol	320	322
	DMF	320	324
	DMSO	324	322
*Not observed any prominent peak due to solubility problem.			

#### 3. 4. Metal ions complexation study:

The absorption spectrum of nitrosouracils with different metal ions in the methanol solvent shows two absorption bands at 227 nm and 318 nm due to  $\pi$ - $\pi$ \* and n- $\pi$ \* electronic transition, respectively. To observe the effects of various metal ions, five equivalents of different cations (50µL, 1 x 10<sup>-2</sup> M, in water) is taken to a (5 ml, 5 x 10<sup>-5</sup> M, in CH<sub>3</sub>OH) solution of nitrosouracils. Co<sup>2+</sup> ion is able to perturb the absorption spectra of ANU and the Cu<sup>2+</sup>, Ni<sup>2+</sup> ions are capable to perturb the absorption spectrum of DANU productively as shown in **Fig. 4**. As shown in **Fig. 4** (a), all metals follow the same wavelength pattern except Co<sup>2+</sup> ions in the case of ANU ligand. The colour change is observed after the addition of

 $Co^{2+}$  ions in the case of ANU ligand. Similarly, in **Fig. 4** (b), the colour of DANU changes after the addition of 5 equivalent copper and nickel metal ions but other metal ions didn't show the change in colour. All metals follow the same wavelength pattern except  $Cu^{2+}$  and  $Ni^{2+}$  metal ions in the case of DANU. If we add aqueous  $Co^{2+}$  ions to the ANU solution, it will lead to the loss in the absorption band from 334 nm and display of new broadband between 334-420 nm due to the interaction of the paramagnetic  $Co^{2+}$  with ANU {**Fig. 5(a)**}. In case of the DANU-metal complex, copper ions showed hypsochromic shift upon complexation with DANU ligand, whereas nickel showed a bathochromic shift upon complexation with DANU ligand. On adding aqueous  $Cu^{2+}$  ions to the DANU solution, the absorption band at 318 nm disappeared and a new peak in the longer wavelength in the range at 350-500 nm due to the interaction of the paramagnetic  $Cu^{2+}$  with DANU {**Fig. 5(b)**}. On adding aqueous  $Ni^{2+}$  ions to the DANU solution, it led to a bathochromic shift in the absorption band from 318 nm to 350 nm {**Fig. 5(c)**}. The colour change of ANU with  $Co^{2+}$  cations and DANU with  $Cu^{2+}$ ,  $Ni^{2+}$  cations is due to the appearance of new charge transfer.

Both ANU and DANU may act as a bidentate ligand because there are two binding sites i) nitrogen atom of  $5^{th}$  positioned nitroso group of uracil and ii) nitrogen atom of  $6^{th}$  positioned amino group of uracil. In this metal complexation HSAB principle works. i.e. soft acids bind with soft bases and hard acids bind with hard bases. Both ANU and DANU acts as a soft acid analogous because there is delocalization of electron takes place from C6 attached lone pair present on nitrogen atom of amino group to C5 attached nitroso group. In case of DANU, two methyl group positioned at N1 and N3 atoms increases the delocalistion of electrons to some extend due to electron donating nature of methyl group. Therefore, it has more tendency to bind or form complexes with soft metal atoms. Transition metal like cobalt, copper and nickel atoms are soft bases in nature. Based on this HSAB principle, ANU form complexation with Co<sup>2+</sup> cations and DANU forms complexation with Cu<sup>2+</sup> and Ni<sup>2+</sup> cations.





Fig. 4: Absorption spectra of the compounds (a) ANU and (b) DANU with different metals ions (cations).

To determine the binding ability and the limit of detection the UV-vis absorption titration is later implemented upon consecutive addition of 1-10 equivalents of  $Co^{2+}$  ions with ANU and  $Cu^{2+}$ ,  $Ni^{2+}$  ions to the DANU solution. On consecutive addition of  $Co^{2+}$  with ANU ligand, the maximum absorbance wavelength at 319 nm reduced continuously and shoulders extended up to 475 nm as shown in **Fig. 5(a)**. The formation of the complex between ANU and  $Co^{2+}$  ions in solution is confirmed by the formation of the isosbestic point at 331 nm in UV-vis absorption titration. In another case, DANU with the successive addition of  $Cu^{2+}$ , the absorbance wavelength at 318 nm decreased continuously and  $\lambda_{max}$  shifted to shorter wavelength with extension shoulders up to 500 nm as shown in **Fig. 5(b**). The formation of the complex between DANU and  $Cu^{2+}$  ions in solution is confirmed by the formation of the absorbance at wavelength 318 nm decreased continuously with the successive addition of Ni<sup>2+</sup>, the absorbance at wavelength 318 nm decreased continuously with the extended shoulders up to 500 nm as shown in **Fig. 5(c)**. The formation of a complex between DANU and nickel ions in solution is confirmed by the formation of the isosbestic point at 334 nm in UV-vis absorption titration.

Finally, LOD and LOQ values calculated with relative standard deviation is shown in **Fig. S4 to Fig. S6** of supporting information. The absorbance calibration values are normalized between the least intensity and the extreme intensity and after that, a straight regression curve is fitted to these normalized data to get the slope. The calculated LOD and LOQ values are found to be 33.9428  $\mu$ M & 102.857 $\mu$ M, respectively for the ANU-Co<sup>2+</sup> complex. Similarly, the LOD and LOQ values are found to be 93.8082  $\mu$ M & 284.2675  $\mu$ M for DANU-Cu<sup>2+</sup> complex, respectively and 48.396  $\mu$ M & 146.653  $\mu$ M for DANU-Ni<sup>2+</sup> complex, respectively.





**Fig. 5:** Change in absorption spectra of nitrosouracil ligands upon gradational addition of (a) ANU with  $Co^{2+}$  ions (1-10 equiv.) ( $\Delta A$  at  $\lambda_{max} = 455$  nm) as a function of  $Co^{2+}$  ions concentration. (b). DANU with  $Cu^{2+}$  ions (1-10 equiv.) ( $\Delta A$  at  $\lambda_{max} = 405$  nm) as a function of  $Cu^{2+}$  ions concentration. (c). DANU with  $Ni^{2+}$  ions (1-10 equiv.) ( $\Delta A$  at  $\lambda_{max} = 450$  nm) as a function of  $Ni^{2+}$  ions concentration.

# 3. 4. 1. Calculation of the binding association constant using the Benesi-Hilderbrand plots

Benesi-Hilderbrand plot (or double-reciprocal plot) is shown in **Fig. 6** [35], where; A is the experimentally measured absorption intensity,  $A_0$  is the absorption intensity of free nitrosouracil and  $A_{max}$  is the saturated absorption intensity of the ANU-Co<sup>2+</sup>, DANU-Cu<sup>2+</sup>, and DANU-Ni<sup>2+</sup> complexes, respectively. The association constant (Ka) is graphically estimated by plotting  $1/[A-A_0]$  versus  $1/[Co^{2+}]$ ,  $1/[Cu^{2+}]$  and  $1/[Ni^{2+}]$  as shown in **Fig. 6**. The graph is plotted between (a)  $1/\Delta A$  against  $1/[Co^{2+}]$  in case of ANU ligand (b)  $1/\Delta A$  against  $1/[Cu^{2+}]$  in case of DANU ligand and (c)  $1/\Delta A$  against  $1/[Ni^{2+}]$  in case of DANU ligand to obtain association constant (ka) value as shown in **Fig. 6**. The resultant graph is a straight line (matched as Benesi-Hilderbrand condition) and the Ka value is achieved from the slope and intercept of the line. The Ka value is found to be  $9.524 \times 10^2$  (R<sup>2</sup> = 0.985) for ANU-Co<sup>2+</sup>,  $3.956 \times 10^3$  (R<sup>2</sup> = 0.9629) for DANU-Cu<sup>2+</sup>complex and  $2.041 \times 10^3$  (R<sup>2</sup> = 0.9899) for DANU-Ni<sup>2+</sup> complex, respectively. The values revealed that the ANU has high attachment towards Cu<sup>2+</sup>, Ni<sup>2+</sup> ions. From the Ka values of DANU-Cu<sup>2+</sup> and DANU-Ni<sup>2+</sup>, it is clear that Cu<sup>2+</sup> metal ions have high affection towards DANU as compare to Ni<sup>2+</sup> metal ions.



Fig. 6: Benesi–Hilderbrand plots of nitrosouracil ligands with cations for estimation of the association constant or binding constant for (a)  $\text{Co}^{2+}$  ions with ANU ligand (where  $\Delta A$  is at  $\lambda_{max}$ = 455 nm). (b) For  $\text{Cu}^{2+}$  ions with DANU (where  $\Delta A$  is at  $\lambda_{max}$ = 405 nm). (c) For Ni<sup>2+</sup> ions with DANU (where  $\Delta A$  is at  $\lambda_{max}$ = 450 nm).

# 3. 4. 2. Determination of stoichiometry using job's plots method

Job's plots method helps to determine the stoichiometry of the metal complex [36]. Using the Job's plots method the coordination between metal ions and ANU, DANU are studied for the determination of molar ratio as shown in **Fig. 7.** In this method, each experiment is observed with divergent concentrations of ANU-Co<sup>2+</sup> ions, DANU-Cu<sup>2+</sup> ions, and DANU-Ni<sup>2+</sup> ions, respectively. The plots achieved by calculating the absorbance at 455 nm, 405 nm and 450 nm for nine specimens of the sample with molar fraction of respective nitrosouracil (ANU-co<sup>2+</sup>, DANU-Cu<sup>2+</sup>, and DANU-Ni<sup>2+</sup>) from 0 to 1. The maximum absorbance intensity at 455 nm, 405 nm, and 450 nm is observed when the molar fraction of respective nitrosouracil (ANU-Co<sup>2+</sup>, DANU-Cu<sup>2+</sup>, and DANU-Ni<sup>2+</sup>) is 0.2, 0.5, 0.7, which showed that the 1:2, 1:1, 2:1 stoichiometry is the considerable binding mode nitrosouracil-metal ions. (ANU-co<sup>2+</sup>, DANU-Cu<sup>2+</sup>, and DANU-Ni<sup>2+</sup>)



**Fig. 7**: Job's plots for the determination of (a) 1:2 stoichiometry upon ANU-Co<sup>2+</sup> ions complexation (where,  $\Delta A$  is at  $\lambda_{max} = 455$  nm). (b) 1:1 stoichiometry upon DANU-Cu<sup>2+</sup> ions complexation (where,  $\Delta A$  is at  $\lambda_{max} = 405$  nm) (c) 2:1 stoichiometry upon complexation of DANU-Ni<sup>2+</sup> ions complexation (where,  $\Delta A$  is at  $\lambda_{max} = 450$  nm).

3. 5. Comparative study of ANU and DANU after the complexation of Co<sup>2+</sup>, Cu<sup>2+</sup>, and Ni<sup>2+</sup> cations. The ANU and DANU can binds to the Co<sup>2+,</sup> Cu<sup>2+</sup>, and Ni<sup>2+</sup> ions via binding positions enclosing of amino nitrogen at the C-6 position and a nitroso group nitrogen at the C-5 position, respectively. All reported crystal structures show the above-mentioned binding positions for metal complex formation [16-17, 37-40]. Thus, the lone pair of electrons of the amino and nitroso groups of the ANU and DANU is delocalized to the vacant orbital of Co<sup>2+</sup>, Cu<sup>2+</sup>, and Ni<sup>2+</sup>, respectively. This electron donation or charge transfer results in increment to a colour change from colourless to clear yellow solution. The cobalt, copper, and nickel are in +2-oxidation state and hence there should be two negative charges as a counter anion for charge noninterference in case of  $Co^{2+}$ ,  $Cu^{2+}$ , and  $Ni^{2+}$  ions. Hence, we suggest the deprotonation of -NH<sub>2</sub> group and the formation of two SO<sub>4</sub><sup>2-</sup> counter ion in the case of ANU and DANU at the complex formula. ANU binds with  $Co^{2+}$  ions in the 1:2 stoichiometry. However, DANU binds with Cu<sup>2+</sup> and Ni<sup>2+</sup> metal ions in the 1:1 and 2:1 stoichiometry ratio. Additionally, for confirmation of the binding of the Co<sup>2+</sup>, Cu<sup>2+</sup>, and Ni<sup>2+</sup>, we performed FTIR spectroscopic studies on ANU and DANU by adding  $Co^{2+}$ ,  $Cu^{2+}$ , and Ni<sup>2+</sup> solutions, respectively. We have taken 1:2 molar ratio of ANU:  $Co^{2+}$  cations, 1:1 molar ratio of DANU: Cu<sup>2+</sup> cations and 2:1 molar ratio of DANU: Ni<sup>2+</sup> cations for the FTIR spectroscopic conformation.

From FTIR spectroscopy of ANU-Co<sup>2+</sup> complexation solution formed in a 1:2 molar ratio between ANU: Co<sup>2+</sup> metal ion in methanol solvent shows that the change in the spectroscopic peak at 3317 cm<sup>-1</sup> is due to v(N-H) stretching vibration of the amino group. However, for ANU we observed a peak at 3506 cm<sup>-1</sup> due to v(N-H) stretching vibration of the same amino group. The sharp change of peak from 3506 cm<sup>-1</sup> to 3317 cm<sup>-1</sup> indicates the successful interaction of the amino group to the corresponding metal ions. The v(N-H) stretching of cyclic secondary amines appear at 3282 cm<sup>-1</sup>, 3178 cm<sup>-1</sup> and shifted to 2945 cm<sup>-1</sup> and 2831 cm<sup>-1</sup>, respectively. The v(C=O) stretching vibration for oxo groups the uracil ring is shifted from 1691 cm<sup>-1</sup> to 1699 cm<sup>-1</sup>. Similarly, the v(C=C) stretching vibration is also shifted from 1627 cm<sup>-1</sup> to 1652 cm<sup>-1</sup>. The v(N=O) stretching of ANU is shifted to 1516 cm<sup>-1</sup> from 1541 cm<sup>-1</sup> in a complexation solution as shown in **Fig 8(a)**.

FTIR spectroscopic data of DANU-Cu<sup>2+</sup> and DANU-Ni<sup>2+</sup> complexation is also found to vary from original spectra as shown in **Fig 8(b)**. DANU shows the v(N-H) stretching frequency of an amino group at 3545 cm<sup>-1</sup>, while that of DANU-Cu<sup>2+</sup> and DANU-Ni<sup>2+</sup> complexation solution shows a broad v(N-H) stretching frequency for the amino group at 3303 cm<sup>-1</sup> and 3317 cm<sup>-1</sup>, respectively. The sharp changes in the stretching frequency of amino group of DANU corresponds to their metal complexation and indicates successful interaction of their corresponding metal complexation. The sharp intense peaks appeared at 1713 cm<sup>-1</sup> and 1653 cm<sup>-1</sup> of 'oxo' groups the uracil ring has remained same without any sharp changes. Less intense peaks are seen at 1647 cm<sup>-1</sup> and 1516 cm<sup>-1</sup> in case of DANU-Cu<sup>2+</sup> complexation

solution and 1651 cm<sup>-1</sup> and 1516 cm<sup>-1</sup> in the case of DANU-Ni<sup>2+</sup> complexation solution is due to C=C stretching and N-H bending vibration, respectively. The v(N=O) stretching frequency of DANU at 1512 cm<sup>-1</sup> is shifted to 1541 cm<sup>-1</sup> after the respective complexation of Cu<sup>2+</sup> and Ni<sup>2+</sup> metal ions.

FTIR data reveals that both ANU and DANU can bind to the Co<sup>2+,</sup> Cu<sup>2+</sup>, and Ni<sup>2+</sup> ions as a bidentate coordination mode via their binding sites of amino nitrogen at the C-6 position and nitroso nitrogen at the C-5 position, respectively. The vibration frequency at 3744-3740 cm<sup>-1</sup> is also observed additionally of respective metal complexation of ANU and DANU with Co<sup>2+,</sup> Cu<sup>2+</sup>, and Ni<sup>2+</sup> ions, respectively. This may be due to the intramolecular O-H stretching frequency of water molecule, which corresponds to participate in complex formation. Therefore, the expected binding sites of ANU and DANU as a bidentate N-N-mode and the possible structures are shown in **Fig. 9**.



data of nitrosouracils after metal complexation in methanol solvent; (a) ANU with  $Co^{2+}$  metal ion in 1:2 mole ratio, (b) DANU with  $Cu^{2+}$  and  $Ni^{2+}$  metal ions in 1:1 and 2:1 mole ratio, respectively.



**Fig. 9**: Expected condition for binding of  $\text{Co}^{2+}$  ion with ANU and  $\text{Cu}^{2+}$ ,  $\text{Ni}^{2+}$  with DANU as displayed by the colour change. (Where M = transition metal i.e.  $\text{Co}^{2+}$ ,  $\text{Cu}^{2+}$ , and  $\text{Ni}^{2+}$ )

# 4. Conclusion:

Herewith, we have reported a successful synthesis of ANU and DANU and their characterization by using various spectroscopic techniques like FTIR, NMR, single-crystal X-ray crystallography, and UV-vis absorption spectroscopy.  $\text{Co}^{2+}$ ,  $\text{Cu}^{2+}$  and  $\text{Ni}^{2+}$  cations are used in metal ions complexation for ANU and DANU, respectively. The association constant (Ka) values as obtained from Benesi–Hilderbrand plots of ANU with  $\text{Co}^{2+}$  and DANU with  $\text{Cu}^{2+}$ ,  $\text{Ni}^{2+}$  cations are 9.524 \* 10<sup>2</sup> M, 3.956 \* 10<sup>3</sup> M, 2.041 \* 10<sup>3</sup> M, respectively. It concludes that copper ions have more tendency towards the formation of a complex with DANU than nickel-metal ion. ANU- $\text{Co}^{2+}$  job's plot showed a 1:2 stoichiometry upon ANU- $\text{Co}^{2+}$  ions complexation, DANU- $\text{Cu}^{2+}$  and DANU- $\text{Ni}^{2+}$  job's plots showed 1:1 and 2:1 stoichiometry upon DANU with  $\text{Cu}^{2+}$ ,  $\text{Ni}^{2+}$  ions complexation, respectively. The same as the stoichiometric molar ratio of respective metal complexes cations are confirmed by FTIR spectroscopy. The calculated LOD values for ANU- $\text{Co}^{2+}$ , DANU- $\text{Cu}^{2+}$  & DANU- $\text{Ni}^{2+}$  are found to be 33.9428 µM, 93.8082 µM and 48.396 µM, respectively. Whereas the LOQ values are obtained as 102.857 µM, 284.2675 µM and 146.653 µM, respectively. As uracil derivatives are bioactive compounds, so we believe that their metal complex may be used as potential drugs in pharmaceutical and biomedical research.

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#### **References**:

- G.M. Ziarani, N.H. Nasab, N. Lashgari, Synthesis of heterocyclic scaffolds through 6aminouracil-involved multicomponent reactions, RSC Adv. 6 (2016) 38827–38848. https://doi.org/10.1039/C6RA02834A.
- A.A. Mustafa, A.A. Alhaider, A.A. Hijazi, Study on the renal and cardiovascular activities of aminouracil derivatives, Pharm. Sci. 6 (1989) 394–398. https://doi.org/10.1023/A:1015979314978.
- 3. C. Bayrak, Vibrational spectroscopic study of 6-aminouracil tetracyanonickelate complexes, Hacettepe J. Biol. & Chem., 40 (4) (2012) 419–426.

- A. Palasz, D. Ciez, In search of uracil derivatives as bioactive agents. Uracils and fused uracils: Synthesis, biological activity and applications. Eur. J. Med. Chem. 97 (2015) 582-611. https://doi.org/10.1016/j.ejmech.2014.10.008.
- G. Lu, X. Li, K. Mohamed O, D. Wang, F. Meng, Design, synthesis and biological evaluation of novel uracil derivatives bearing 1, 2, 3-triazole moiety as thymidylate Synthase (TS) inhibitors and as potential antitumor drugs. Eur. J. Med. Chem. 171 (2019) 282–296. https://doi.org/10.1016/j.ejmech.2019.03.047.
- M. Chen, W. Li, C. Ma, K. Wu, H. He, K. Wang, Fluorometric determination of the activity of uracil-DNA glycosylase by using graphene oxide and exonuclease-I assisted signal amplification, Microchim. Acta 186 (2019) 1–7. doi:10.1007/s00604-019-3247-6.
- S.M. Kim, M. Lee, S.Y. Lee, S.M. Lee, E.J. Kim, J.S. Kim, J. Ann, J. Lee, J. Lee, Synthesis and biological evaluation of 3-(2-aminoethyl) uracil derivatives as gonadotropin-releasing hormone (GnRH) receptor antagonists, Eur. J. Med. Chem. 145 (2018) 413–424. https://doi.org/10.1016/j.ejmech.2017.12.095.
- 8. X. Liu, Q. Zhang, Y. Qiao, L. Duan, G. Gao, Anti-fatigue adhesive and tough hydrogels regulated by adenine and uracil, Polym. Chem. 9 (2018) 4535–4542. https://doi.org/10.1039/c8py00868j.
- C.D. Mol, A.S. Arvai, R.J. Sanderson, G. Slupphaug, B. Kavli, H.E. Krokan, D.W. Mosbaugh, J.A. Tainer, Crystal structure of human uracil-DNA glycosylase in complex with a protein inhibitor: Protein mimicry of DNA, Cell. 82 (1995) 701–708. https://doi.org/10.1016/0092-8674(95)90467-0.
- B. Lippert, Multiplicity of metal ion binding patterns to nucleobases, Coord. Chem. Rev. 202 (2000) 487–516. https://doi.org/10.1016/S0010-8545(00)00260-5.
- 11. S.M. Teleb, Spectroscopic studies on Co(II) and Cu(II) complexes of 6-amino-1-methyl-5nitrosouracil and its 6-methylamine derivative, Spectrochim. Acta, Part A 60 (2004) 3093–3100. https://doi.org/10.1016/j.saa.2004.01.032.
- J.M. Salas-Pergrin, M.N. Moreno-Carretero, E. Colacio-Rodriguez, Zn(II),Cd(II) and Hg(II) complexes of 6-amino-3-methyl-5-nitroso-uracil, Can. J. Chem. 63 (1985) 3573-3576. https://doi.org/10.1016/0584-8539(86)80142-8.

- M. Bakir, Optical, electrochemical and structural studies on the first rhenium compound of di-2pyridylketonephenylhydrazone (dpkphh), fac-Re(CO)<sub>3</sub>(dpkphh)Cl, Inorganica Chim. Acta 332 (2002) 1–6. https://doi.org/10.1016/S0020-1693(02)00708-9.
- M.A. Romero, J. Ruiz, M.P. Sanchez, M.N. Moreno, F. Nieto, A novel type of tetradentate uracil derivative in a dodecahedral eight-coordinate chelate complex: Catena-bis(.mu.-6-amino-3methyl-5-nitrosouracilato-N5,04,N1,O2)cadmium(II), Inorg. Chem. 25 (1986) 1498–1501. https://doi.org/10.1021/ic00229a040.
- M.N. Moreno, J.M. Salas, E. Colacio, M.P. Sanchez, F. Nieto, Structure of bis( 4-amino-1,2-dih ydro-1-methyl-5-nitroso-2-oxo-6-pyrimidinolato-O,N)-diaquazinc(II) dihydrate, Acta Cryst. C42 (1986) 407–410. https://doi.org/10.1107/S0108270186095999.
- J. M. Salas, M. A. Romero, M. P. Sánchez, M. N. Moreno, M. Quirós, J. Molina, R. Faure, Molecular orbital calculation on 6-amino-1,3-dimethyl-5-nitrosouracil: Crystal structure of its copper complex, Polyhedron, 11 (1992) 2217–2222. https://doi.org/10.1016/S0277-5387(00)83699-2.
- G. Ferguson, J.N. low, M. Quirs-olozabal, J.M. Salas-peregrin, F. Hueso-urena, M.N. Morenocarretero, Mixed complexes of 6-aminouracil derivatives synthesis, spectral properties and crystral structure of 6-amino-5-nitro1,3-dimethyl-5-ethanol(o)-copper (II) nitrate, Polyhedron. 15 (19) (1996) 3233-3239. https://doi.org/10.1016/0277-5387(96)00051-4.
- J. Faus, F. Lloret, M. Julve, J.M. Clemente-juan, M.C. Munoz, X. Solans, M. Font-bardia, Synthesis, crystal structure and magnetic properties of an octanuclear nickel(II) complex with a hexahedro-Ni<sub>8</sub> core, Angew. Chem. Int. Ed. Engl. 35, (1996) 1485–1487. https://doi.org /10.1002/anie.199614851.
- E. Colacio, C. Lopez-magana, V. Mckee, A. Romerosa, Novel square-planar cyclic tetranuclear copper complex containing oximate bridges. Synthesis, crystal structure and magnetic properties of tetrakis[diaqua(µ-1,3-dimethylviolurato)copper(II)] tetraperchlorate dehydrate, J. Chem. Soc. Dalton Trans. (1999) 2923–2926. https://doi.org / 10.1039/A904012I.
- C. Ruiz-valero, A. Monge, E. Gutierrez-puebla, E. Gutierrez-rios, Structure of [1,3-dimethyl-2,4,5,6(IH, 3H)-pyrimidinetetrone-5-oximato][1,3-dimethyl-2,4,5,6(IH,3H)-pyrimidinetetrone-5 oxime]silver(I) [Ag(C<sub>6</sub>H<sub>6</sub>N<sub>3</sub>0<sub>4</sub>)(C<sub>6</sub>H<sub>7</sub>N<sub>3</sub>0<sub>4</sub>)], Acta Cryst. C39, (1983) 1214–1216. https://doi.org/10.1107/S0108270183007970.

- A.S. El-tabl, M.M.A. El-waheed, M.A. Wahba, N.A.El-Halim Abou El-Fadl, Synthesis, characterization, and anticancer activity of new metal complexes derived from 2-hydroxy-3-(hydroxyimino)-4-oxopentan-2-ylidene)benzohydrazide, J. Inorg. Biochem. 99 (8) (2015) 1-16. https://doi.org/10.1155/2015/126023.
- 22. J.R. Demember, F.A. Wallace, Uracil and its interaction with silver ion in aqueous alkaline media, J. Am. Chem. Soc. 97 (1975) 6240-6245. https://doi.org/10.1021/ja00854a050.
- J.M. Salas-Peregrin, M.N. Moreno-Carretero, M.A. Romero-Molina, E. Colacio-Rodriguez, Silver(I) complexes of 6-amine-5 nitroso-uracil and its methylated derivatives, Rev. Chim. Miner. 21 (1984) 233-238.
- J.M. Salas-Peregrin, M.N. Moreno-Carretero, J.de D. Lopez-Gonzalez, C. Valenzuela-Calahorro, Thermal behaviour of silver/I/ complexes of 4-amino-5-nitroso-uracil derivatives, J. Thermal Anal. 26 (1983) 251-256. https://doi.org/10.1007/BF01913209.
- 25. R. Banik, S. Roy, L. Dlhan, J. Titis, R. Boca, A.M. Kirillov, A.D. Martin, A. Bauza, A. Frontera, A. Rodriguez-Dieguez, Self-assembly synthesis, structure, topology, and magnetic properties of a mononuclear Fe(III)-Violurate derivative: A combined experimental and theoretical study, Dalton Trans., 45 (41) (2016) 16166–16172. https://doi.org/10.1039/c6dt03139k.
- 26. R. Banik, S. Roy, A.M. Kirillov, A. Bauza, A. Frontera, A. Rodríguez-Diéguez, J.M. Salas, W. Maniukiewicz, S.K. Das, S. Das, Two mixed-ligand cadmium(II) compounds bearing 5-nitrosopyrimidine and N-donor aromatic blocks: Self-assembly generation, structural and topological features, DFT studies, and Hirshfeld surface analysis, CrystEngComm. 18 (2016) 5647–5657. https://doi.org/10.1039/c6ce00989a.
- 27. R. Banik, S. Roy, A. Bauza, A. Frontera, A. Rodríguez-Diéguez, J.M. Salas, A.M. Kirillov, S. Chowdhury, S. K. Das, S. Das, Supramolecular interactions through lone Pair(Lp)-π and hydrogen bonding in cobalt(III) and manganese(II) derivatives of N,N'-dimethylvioluric acid: A combined experimental and theoretical study. Inorganica Chim. Acta, 435 (2015) 178–186. https://doi.org/10.1016/j.ica.2015.06.018.
- R. Banik, S. Roy, A. Bauza, A. Frontera, S. Das, Cadmium(II) complexes containing N,N'dimethylviolurate as ligand or counteranion: synthesis, characterization, crystal structures and DFT Study, RSC Adv. 5 (14) (2015) 10826–10836. https://doi.org/10.1039/c4ra10251g.
- 29. F.F. Blicke, H.C. Godt, Jr. Reactions of 1,3-dimethyl-5,6-diaminouracil, J. Am. Chem. Soc. 76

(1954) 2798-2800. https://doi.org/10.1021/ja01639a058

- 30. APEX3; Bruker AXS inc. Madison, Wisconsin, USA.
- 31. X. Jiang, Y. Sun, J. Yao, Y. Cao, M. Kai, N. He, X. Zhang, Y. Wang, R. Wang, Core scaffoldinspired concise synthesis of chiral spirooxindole-pyranopyrimidines with broad-Spectrum anticancer potency, Adv. Synth. Catal. 354, (2012) 917–925. https://doi.org/10.1002/adsc.201100792.
- O.V. Dolomanov, L.J. Bourhis, R.J. Gildea, J.A.K. Howard, H. Puschmann, OLEX2 : a complete structure solution, refinement and analysis program, J. Appl. Crystallogr. 42 (2009) 339–341. https://doi.org/10.1107/S0021889808042726.
- 33. L. Palatinus, G. Chapuis, Superflip a computer program for the solution of crystal structures by charge flipping in arbitrary dimensions, J. Appl. Crystallogr. 40 (2007) 786–790. https://doi.org/10.1107/S0021889807029238.
- G. Oszlanyi, A. Suto, The charge flipping algorithm, foundations of crystallography, Acta Cryst. A 64 (2008) 123–134. https://doi.org/10.1107/S0108767307046028.
- 35. X. Bao, Q. Cao, X. Wu, H. Shu, B. Zhou, Y. Geng, J. Zhu, Design and synthesis of a new selective fluorescent chemical sensor for Cu<sup>2+</sup> based on a pyrrole moiety and a fluorescein conjugate, Tetrahedron Lett. 57 (8) (2016) 942–948. https://doi.org/10.1016/j.tetlet.2016.01.056.
- P. Job, Formation and stability of inorganic complexes in solution, Ann. Chim. 9 (1928) 113–203.
- 37. R. Kivekas, A. Pajunen, E. Colacio, J. M. Dominguez-Vera, J. M. Moreno, A. Romerosa, Structural characterization of closely related mononuclear Cu(II) complexes of 5-nitrosooxime pyrimidines. A combined X-ray and EPR study, Acta Chem. Scand. 51 (1997) 1051-1057.
- 38. E. Colacio, J. M. Dominguez-Vera, A. Escuer, R. Kivekas A. Romerosa, Heterodinuclear copper(II)-nickel(II) complexes with unusual asymmetrical bridges from a new and versatile dioxime multidentate ligand. Magneto-structural study, Inorg. Chem. 33 (1994) 3914-3924. https://doi.org/10.1021/ic00096a015.
- 39. E. Colacio, J. M. Dominguez-vera, A. Romerosa, R. Kivek, M. Klinga, A. Escuer, A heterodinuclear *anti* oximate-bridged copper(II)-nickel(II) complex: Crystal structure and

magnetic properties, Inorganica Chim. Acta 234 (1995) 61-65. https://doi.org/10.1016/0020-1693 (95)04482-O.

40. E. Colacio, J. M. Dominguez-vera, A. Escuer, M. Klinga, R. Kivekas, A. Romerosa, A mixedbridged Cu<sub>3</sub> cluster with an isosceles-triangular array of copper(II) ions: Synthesis, crystal structure and magnetic properties, J. Chem. Soc. Dalton Trans. 2 (1) (1995) 343–348. https://doi.org/10.1039/DT9950000343.

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# Highlights

- Crystal structure of DANU is studied and molecule is found to be crystalline with one water • molecule attached.
- Solvatochromic and metal ions binding properties are analyzed for both ANU and DANU. ٠
- Benesi-Hilderbrand plot of nitrosouracil ligand with metal ions is calculated for association ٠ constant.
- Job's plot of nitrosouracils with Co<sup>2+</sup>, Cu<sup>2+</sup>, and Ni<sup>2+</sup> ions are studied to understand their • stoichiometric molar ratio for complexation.