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# Synthesis of 3-methyl-1phenyl-4-(thiazol-2-yl)-1*H*-pyrazol-5(4*H*)-one *via* Sandmeyer Reaction and their Transition Metal Complexes; Spectral, XRD, Cytotoxicity, Molecular Docking and Biological Evaluation

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## ABSTRACT

*The ligand 5-Methyl-2-phenyl-4-(1,3-thiazol-2-yl)-2,4-dihydro-3H-pyrazol-3-one (MPP) has been synthesized via Sandmeyer reaction of MPP with 2-aminothaizole. A series of complexes of the ligand with Co(II), Ni(II), Cu(II) and Zn(II) ions are synthesized and structurally characterized by Proton Nuclear Magnetic Resonance ($^1$H-NMR), Infra-Red (IR), UV-Visible and Powder X-ray Diffraction (PXRD) spectral techniques. The powder XRD studies reveal that all complexes are in crystalline nature. The cytotoxic activity of the complexes and the uncoordinated ligand against human breast cancer (MCF-7) and chronic myelogenous leukemia cell line (K-562) exhibits good viability in the range of 50.16-55.16% at a concentration of >100-110 µg/ml as compared to the inhibition in the untreated cells. Further, the metal complexes and the ligand were screened for the antibacterial activity, the cytotoxicity studies are correlated with computational docking analysis.*

**Keywords**: Sandmeyer reaction, Catalytic reaction, Antioxidant, Antimicrobial activity

## INTRODUCTION

The Sandmeyer reaction is very important to convert any aromatic amino group to various functional groups some of the group are halogens, hydroxy, cyano boryl [1-4] azide [5] and olefins [6]. More recently Sandmeyer-type trifluoromethylation [7-10] and trifluoromethylthiolation [11-13] of aryl diazonium salts have also been reported. From the past 150 years, arenediazonium salts plays a vital role to delivered practically beneficial organic reactions such as Meerwein [14] Pschorr [15] Gomberg-Bachmann [16] the Japp-Klingemann transformations [17-19] cross coupling reaction [20] and some conversion of Pinacol Boronates [21]. Although the mechanism of the Sandmayer reaction was generally believed to be initiated with a Single Electron Transfer (SET) *in situ* with various diazotization reagents and in presence of catalyst. Transitional metal-catalyzed Sandmeyer reaction in which copper salts as catalyst and sodium nitrite, Silver Nitrite ($AgNO_2$) as nitro sating reagents are used [22,23]. Apart from this iron catalyze *via* radical (2,2,6,6-tetramethylpiperidine Noxyl TEMPO) supported conversion [24,25] reactions also been carryout, Titanium(III) Chloride ($TiCl_3$), Iron(II) Sulfate ($FeSO_4$), isoamyl nitrite and hydrochloric acid method [26]. Palladium-catalyzed [20], well soluble copper complex like N,N,N',N'-tetramethylethylenediamine [27], $Pd(OAc)_2$ and trifluoroborate [28] and iron salts catalyzed with yield oriented [29]. In addition metal-free methods such as; Borylation by 2,2'-azobisisobutyronitrile and benzoyl peroxide [21], 2,2,6,6-tetramethyl-1-piperidin-1-oxyl, benzoyl peroxide, diboron pinacol as borylating reagent [30] and recently Ascorbic acid initiated and tertbutyl nitrite as Nitrosating reagent were also reported [31].

Many researchers worked on 5-methyl-2-phenyl-2,4-dihydro-3H-pyrazol-3-one and their metal chelates are biologically potent organic moiety [32-37]. To the best of our knowledge, this type of transformation has not been reported to date. The aim of the present study was to synthesize the MPP via the Sandmeyer reaction and their 3d metal complexes were prepared. The *in silico* molecular docking studies for cytotoxic activities are correlated.

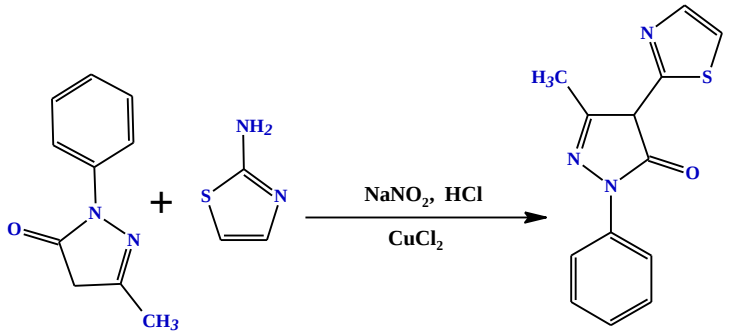
## MATERIALS AND METHODS

All the chemicals used in this study were pure and AR grade. 2-Aminothiazole, ethanol, Dimethyl Sulfoxide (DMSO), were procured from Sigma–Aldrich and all the metal salts were procured from obtained Hi-Media and the solvents used after purification by distillation [37].

The human breast cancer (MCF-7) cell line was obtained from the National Center for Cell Science (NCCS), Pune, India. IR spectra were recorded as KBr pellets in the Bruker Alpha FT-IR Spectrometer, $^1$H-NMR were recorded on a Bruker DPX 400, δ values relative to the Deuterated DMSO-$d_6$. Magnetic susceptibilities were measured at room temperature by the Gouy method. The molar conductance measurements were measured in a solution of the metal complexes in Dimethylformamide (DMF) ($10^{-3}$ M) using Equip-Tronics EQ-660A conductivity meter, mass spectra are recorded in a Quattro LC, Micro Mass spectrometry. Elemental analysis is obtained from a Vario-Micro Qub elemental analyzer. Absorbance is measured using Systronics UV-VIS spectrometer-119, X-ray diffractometer (PHILIPSPW3710) using CuKa (1.5418 A˚) radiation operated at 45 kV and 25 mA is used in X-ray investigation.

**Synthesis of 5-methyl-2-phenyl-4-(1,3-thiazol-2-yl)-2,4-dihydro-3H-pyrazol-3-one (MPP)**

The ligand was synthesized according to the procedure [38,39]. 2-aminothiazole (0.01 mol) dissolved in 5 ml (15% HCl) this solution was stirred to make it a clear solution, after cooled to 0-5°C, 3 ml (30% $NaNO_2$) solution was added dropwise with continuous stirring for about 30 min. The product was separated by filtration and MPP 1.3 g (0.007 mol), $CuCl_2.2H_2O$ (0.3 g in 5 ml $H_2O$) (Sandmayer catalyst) added at the temperature of about 0-5°C. The reaction mixture was slowly stirred at this temperature range. The precipitate was filtered, washed with several times with water and neutralized with 5% sodium hydrogen carbonate and final products were dried and recrystallized from ethanol (Scheme 1).



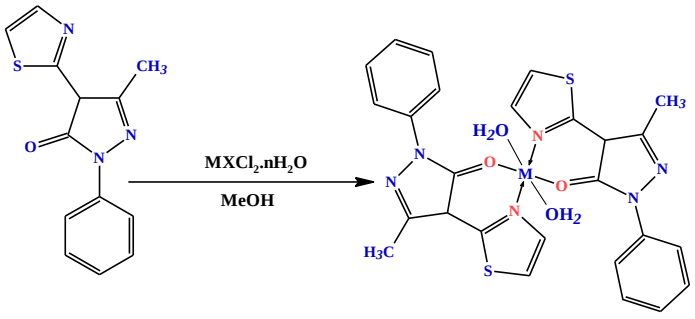

**Scheme 1: Synthesis of 5-methyl-2-phenyl-4-(1,3-thiazol-2-yl)-2,4-dihydro-3H-pyrazol-3-one**

**MPP**

It was obtained as black solid in 79% yield; mp. 168-172°C; IR (KBr, $cm^{-1}$): 1668 (C=O), 1574 (C=N), 759 (C-S), $^1$H-NMR: (400 Mz, DMSO-$d_6$, ppm): δ=2.50 (s, 3H, CH3), 3.34 (s, 1H, CH), 8.41-7.30 (m, 7H, Ar-H), MS (m/z): 257.0.

**Synthesis of metal complexes (1-4)**

A methanolic solution of metal chlorides (0.461 g, 0.015 mol) in 10 ml was added dropwise to the methanolic solution (10 ml) of ligand (1.044 g, 0.03 mol) with continuous stirring (Scheme 2). The resulting solution was refluxed for 4-6 h and the solution was reduced to half of its initial volume. It was then allowed to stand overnight in a refrigerator. A colored complex was precipitated, which was separated by filtration under vacuum. It was washed thoroughly with distilled water then with cold methanol and dried *in vacuo* over fused $CaCl_2$ and was recrystallized from methanol [40].




**Scheme 2: Synthesis of metal complexes (M=Cu, Co, Ni and Zn)**

$[CuL_2(H_2O)_2]Cl_2$ (1): Light brown; yield 64%; m.p. >256-258°C; Molecular weight (614.19 g/mol); found for $C_{26}H_{26}CuN_6O_4S_2$: C: 56.17; H: 3.98; N: 15.09; M: 11.51, Calc. for C: 56.07; H: 3.58; N: 15.12, M: 11.58. UV-Vis (DMF): $\lambda_{max}$ (logε)=20,000. FT-IR (KBr/($cm^{-1}$): ν(C=O) 1603, υ(C=N) thiazole 1550, ν(C-S) 757, ν(M-O) 509, ν(M-N) 477. ΛM=130 $\Omega^{-1}.cm^2.mol^{-1}$.

$[CoL_2(H_2O)_2]Cl_2$ (2): Black brown; yield 58%; m.p. >252-254°C; Molecular weight (609.58 g/mol); found for $C_{26}H_{26}CoN_6O_4S_2$: C: 54.01; H: 3.84; N: 14.84, M: 11.59. Calc. for C: 54.11; H: 3.68; N: 14.86, M: 11.48. UV-Vis (DMF): $\lambda_{max}$ (logε)=16,675, 14,625. FT-IR (cm-1): ν(C=O) 1630, ν(C=N) thiazole 1545, ν(C-S) 762, ν(M-O) 511, ν(M-N) 473. ΛM=128 $\Omega^{-1}.cm^2.mol^{-1}$.

$[NiL_2(H_2O)_2]Cl_2$ (3): Dark brown; yield 66%; m.p. >249-252°C; Molecular weight (609.34 g/mol); found for $C_{26}H_{26}NiN_6O_4S_2$: C: 56.39; H: 4.01; N: 15.18; M: 11.62, Calc. for C: 55.89; H: 3.98; N: 15.01, M: 11.52. UV-Vis (DMF): $\lambda_{max}$ (logε)=25,435, 20,000, 15,125. FT-IR (KBr/($cm^{-1}$): ν(C=O) 1606, υ(C=N) thiazole 1532, ν(C-S) 757, ν(M-O) 510, ν(M-N) 477. ΛM=132 $\Omega^{-1}.cm^2.mol^{-1}$.

$[ZnL_2(H_2O)_2]Cl_2$ (4): Pale brown; yield 61%; m.p. >260-262°C; Molecular weight (1616.06 g/mol); found for $C_{26}H_{26}N_6O_4S_2Zn$: C: 55.94; H: 4.35; N: 15.06; M: 11.27, Calc. for C: 56.08; H: 4.42; N: 15.10, M: 11.35. IR (KBr/($cm^{-1}$): ν(C=O) 1601, υ(C=N) thiazole 1534, ν(C-S) 757, ν(M-O) 509, ν(M-N) 474. ΛM=125 $\Omega^{-1}.cm^2.mol^{-1}$.

**Molecular docking studies**

Molecular docking of the synthesized compounds has been evaluated as per our previous work [41,42]. For cytotoxic molecular docking study (PDB code: 2A91) as Epidermal Growth Factor Receptor (EGFR) kinase domain were used throughout the work. Followed by actinoin as standards for docking studies. The docking results of the cytotoxic guided for wet analysis.

**Antimicrobial activity**

All the synthesized complexes and ligand have been examined towards three bacterial and fungal strains by using agar well diffusion method [43]. All bacterial traces were maintained on nutrient agar medium at ± 37°C, and fungal strains were maintained on Potato Dextrose Agar (PDA) at ± 25°C. The test compounds had been dissolved in DMSO. Sample-loaded plates were inoculated with the microorganism incubated at 37°C for 24 h, and culture was incubated at 25°C for 60 h. DMSO as control and chloramphenicol and fluconazole is used as standards for bactericide and fungicide. The compounds were also tested for Minimal Inhibitory Concentration (MIC) values [44].

**Antioxidant activity**

Free radical scavenging capacities of the ligand (MPP*)* and their metal complexes were determined using the stable 2,2-Diphenyl-1-Picrylhydrazyl radical (DPPH). An aliquot of 50-100 mg concentrations of synthesized compounds in methanol was added to 3 ml of 0.004% w/v DPPH radical solution and each test tube was made up to the final volume of 4 ml. Butylated Hydroxytoluene (BHT) was used as a standard and dissolved in methanol to get the same concentration as that of synthesized compounds. Each mixture was vortexed for a few seconds and allowed to stand in the dark for 10 min at ambient temperature. The absorbance of each reaction mixture was measured at 517 nm against a blank. The absorbance was measured at 546 nm. The ability to scavenge from both radicals was calculated as:

$$\text{Scavenging ratio (\%)}=[(A_i-A_o)/(A_c-A_o)] \times 100\%$$

Where, $A_i$ is the absorbance in the presence of the test compound, $A_0$ is absorbance of the blank in the absence of the test compound; $A_c$ is the absorbance in the absence of the test solution.

***In vitro* anticancer activity-cell culture**

The human cancer cells (K-562 ATCC® CCL-243™) and (MCF7-ATCC® HTB 22™) were maintained in Modified Eagles Medium (MEM) supplemented with 10% Fetal Calf Serum (FCS), 2% essential amino acids, 1% each of glutamine, nonessential amino acids, vitamins and 100 U/ml penicillin–streptomycin. Cells were sub-cultured at 80-90% confluence and incubated at 37°C in a humidified incubator supplied with 5% $CO_2$. The stock cells were maintained in 75 $cm^2$ tissue culture flask. The cytotoxicity effect of test samples was performed by 5-Diphenyl-2H-Tetrazolium Bromide (MTT) assay. Briefly, cultured cells ($1 \times 10^{-6}$ cells/ml) were placed in 96 flat-bottom well plates, then cells were exposed to different concentration of prepared samples (1-100 µg/ml) and incubated at 37°C for about 24 h in 5% $CO_2$ atmosphere. After 24 h incubation, MTT (10 µl) was added to the incubated cancer cells and further incubated at 37°C for about 4 h in the same environment. Thereafter, dissolved 200 µl of formazan crystals in of DMSO and monitored the absorbance at 578 nm with reference filter as 630 nm. The cytotoxicity effect was calculated as:

$$\text{Cytotoxicity (\%)}=1\,\frac{\text{Mean absorbance of +ve}}{\text{Mean absorbance of –ve}} \times 100$$

$$\text{Cell viability (\%)} = 100–\text{Cytotoxicity (\%)}$$

## RESULTS AND DISCUSSION

**Mass spectrum**

The mass spectrum of the ligand (Figures 1 and 2) obtained by using of Quattro LC, Micro mass spectrophotometer (ESI) showed the molecular ion peak at m/z 257.0 which matches with formula weight within the precision limit of ±0.02. Metastable ion(s) is not observed represent the proposed fragmentation pattern of the ligand.

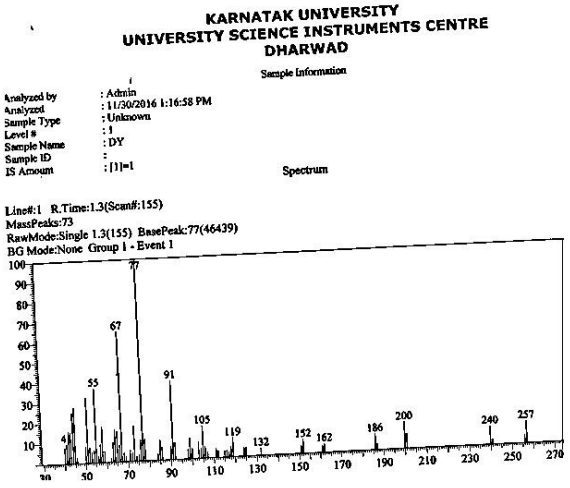



**Figure 1: Mass spectrum of ligand (MPP)**

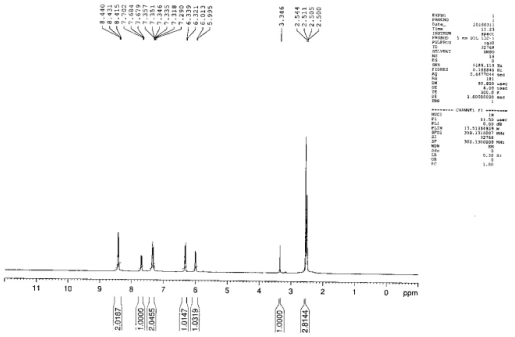


**Figure 2: $^1$H NMR spectrum of MPP**

**IR spectroscopy**

Comparative IR spectral studies of the ligand and its metal complexes have been recorded as KBr pellets. Some significant IR bands for the uncoordinated ligand and their metal complexes have been listed in Table 1. The free ligand showed a strong band at 1668 cm$^{-1}$ and a weak band at 3090 cm$^{-1}$ which are attributed to ν(CO) and ν(C-H) of the aromatic group [45]. The IR spectrum of the ligand displayed a band at 1594 cm$^{-1}$ due to the ν(C=N) of thiazole ring [46]. In the spectrum of the ligand, the hypsochromic shift was observed for the ν(CO) band at 1668 cm$^{-1}$ assigned in the spectrum of the ligand. In the spectra of the complexes, the band due to ν(CO) showed a reduction in intensities between 1668 and 1603 cm$^{-1}$ region observed due to the coordination to central metal ions [47]. In addition, the band due to cyclic (C=N) of thiazole ring was shifted in all complexes. These shifts indicate the involvement of the thiazole nitrogen for the coordination. All the complexes showed broadband in the region 3434-3439 cm$^{-1}$ because of ν(O-H) of coordinated water molecule [48]. This suggests that the sulfur atom of the thiazole ring do not participate in the coordination. This was further supported by the existence of new metal-ligand band in the complex spectrum ranging from 509-511 cm$^{-1}$ for M-O band and 474-477 cm$^{-1}$ for M-N and shown in Table 1.

**Table 1: IR spectral data (cm$^{-1}$) of the MPP and its metal complexes**

| Ligand/complex | ν(C=O) | ν(C=N) thiazole | ν(C-S) | ν(M-O) | ν(M-N) |
|---|---|---|---|---|---|
| MPP | 1668 | 1574 | 759 | - | - |
| $[CuL_2(H_2O)_2]Cl_2$ (1) | 1603 | 1550 | 757 | 509 | 477 |
| $[CoL_2(H_2O)_2]Cl_2$ (2) | 1630 | 1545 | 762 | 511 | 473 |
| $[NiL_2(H_2O)_2]$ $Cl_2$ (3) | 1606 | 1532 | 757 | 510 | 477 |
| $[ZnL_2(H_2O)_2]Cl_2$ (4) | 1607 | 1534 | 757 | 509 | 474 |

**Electronic spectra and magnetic moment measurements**

The molar conductance value indicates that all metal complexes behave as uni-bivalent nature. The UV-Visible spectrum of the ligand and their metal complexes are recorded in DMF and the values are reported in Table 2. The free ligand showed two recognizable absorption bands at 36,101 and 27,215 cm$^{-1}$ which would be due to the π–π* and n–π* transitions. The spectrum of the Cu(II) complex 1 showed an absorption band at 20,000 cm$^{-1}$ attributed to the transition $^2Eg \rightarrow {}^2T_2g$ due to distorted octahedral geometry [48,49]. Further confirmation was done by magnetic moment values 1.78 B.M. which is consistent with proposed distorted octahedral geometry for Cu(II) complex. The Co(II) complex 2 showed absorption band at 14,625 cm$^{-1}$ and 16,675 cm$^{-1}$ due to the transitions $^4T_{1g}(F) \rightarrow {}^4A_{2g}(F)$ and $^4T_{1g}(F) \rightarrow {}^4A_{2g}(P)$ ($\nu_2$) respectively, the Co(II) complex also showed a magnetic moment at 4.56 B.M. These results suggested the presence of octahedral geometry for Co(II) complex [50,51]. The Ni(II) complex 3 showed three bands at 25,435 cm$^{-1}$,

20,000 cm$^{-1}$ and 15,125 cm$^{-1}$ which are assignable to $^3A_{2g}(F) \rightarrow {}^3T_{2g}(F)$ ($\nu_1$), $^3A_{2g}(F) \rightarrow {}^3T_{1g}(F)$ ($\nu_2$) and $^3A_{2g}(F) \rightarrow {}^3T_{1g}(P)$ ($\nu_3$) transitions respectively. The spectral data indicate that the Ni(II) ion is present in octahedral environment. The magnetic moment of Ni(II) complex is 2.74 B.M [48,52].

**Table 2: Electronic spectral data (cm$^{-1}$) and magnetic values of MPP and its metal complexes**

| Entry | Transitions (cm$^{-1}$) | Transitions | Meff (BM) |
|---|---|---|---|
| MPP | 36,101 | π–π* | - |
| | 27,215 | n–π* | |
| 1 | 20,000 | $^2Eg^{®}{}^2T_2g$ | 1.78 |
| 2 | 16,675 | $^4T1g(F) \rightarrow {}^4A_2g(P)$ ($\nu_2$) | 4.56 |
| | 14,625 | $^4T1g(F) \rightarrow {}^4A_2g(F)$ | |
| 3 | 25,435 | $^3A_{2g}(F) \rightarrow {}^3T_{2g}(F)$ ($\nu_1$) | 2.74 |
| | 20,000 | $^3A_{2g}(F) \rightarrow {}^3T_{1g}(F)$ ($\nu_2$) | |
| | 15,125 | $^3A_{2g}(F) \rightarrow {}^3T_{1g}(P)$ ($\nu_3$) | |
| 4 | Dia | - | Dia |

**Powder X-ray diffraction study of complexes**

Powder X-ray diffract graph of the complexes were recorded over the 2θ=0-80° range. The major peaks of relative intensity greater than 10% were indexed using a computer program. The diffraction data like angle (2h), interplanar spacing (d) and relative intensity (%) have been summarized in Table 3 from the data all compounds show sharp crystalline peaks indicating their crystalline nature. The XRD patterns of all complexes are very similar and suggest that the complexes have identical structure. The average crystallite sizes of the complexes were calculated using the Sherrers formula [53]. 1, 2, 3 and 4 have a crystallite size of 52.95, 52.90, 52.91 and 53.25 nm respectively suggesting that the complexes are in nano crystalline phase (Table 3) and the XRD data of 1 are shown in Table 4.

**Table 3: Powder x-ray diffraction spectral data of 1-4**

| Complexes | 2θ | d | Relative intensity | Full width at half maximum |
|---|---|---|---|---|
| 1 | 23.03 | 3.86 | 100 | 0.16 |
| 2 | 22.53 | 3.94 | 100 | 0.16 |
| 3 | 22.63 | 3.92 | 100 | 0.16 |
| 4 | 26.07 | 3.42 | 100 | 0.16 |

**Table 4: X-ray diffraction data of 1**

| Peak No. | 2θ | θ | Sin θ | h k l | d | | Intensity | a in Å |
|---|---|---|---|---|---|---|---|---|
| | | | | | Calculation | Observation | | |
| 1 | 15.59 | 7.795 | 0.9982 | 655 | 5.70165 | 5.7017 | 339.6 | 3.62 |
| 2 | 19.13 | 9.565 | 0.1397 | 757 | 4.63998 | 4.6340 | 451.39 | 3.62 |
| 3 | 20.09 | 10.045 | 0.5812 | 808 | 4.41561 | 4.4155 | 267.93 | 3.62 |
| 4 | 21.14 | 10.570 | 0.9108 | 835 | 4.14681 | 4.1413 | 196.12 | 3.62 |
| 5 | 21.75 | 10.875 | 0.9927 | 929 | 4.08609 | 4.0855 | 336.55 | 3.62 |
| 6 | 22.29 | 11.145 | 0.9888 | 981 | 3.99479 | 3.9951 | 194.86 | 3.62 |
| 7 | 22.75 | 11.375 | 0.9288 | 112 | 3.89165 | 3.8924 | 195.50 | 3.62 |
| 8 | 23.03 | 11.515 | 0.8681 | 116 | 3.86285 | 3.8627 | 382.53 | 3.62 |

**Antimicrobial activity**

All the complexes and ligand showed inhibition property, among them, 3 and 1 showed excellent antibacterial activity when compared to standards (Table 5). The result of MIC values less than 25 µg/ml is presented in Table 6. The antifungal activity results indicate that the MPP and complexes 2, 4 exhibited the least activity and the other two complexes 3, 1 showed promising activity.

**Table 5: Antimicrobial data of 5-methyl-2-phenyl-4-(1,3-thiazol-2-yl)-2,4-dihydro-3H-pyrazol-3-one and their complexes**

| Entry | Antibacterial zone of inhibition in mm (mean ± SD) | | | Antifungal zone of inhibition in mm (mean ± SD) | | |
|---|---|---|---|---|---|---|
| | *Staphylococcus aureus* | *Bacillus subtilis* | *Escherichia coli* | *Streptococcus* | *Candida albicans* | *Aspergillus niger* |
| MPP | 03 ± 0.3 | 05 ± 0.2 | 05 ± 0.7 | 04 ± 0.4 | 03 ± 0.1 | - |
| 1 | 13 ± 0.1 | 15 ± 0.1 | 12 ± 0.3 | 08 ± 0.4 | 10 ± 0.1 | 10 ± 0.3 |
| 2 | 06 ± 0.3 | - | 07 ± 0.3 | 08 ± 0.2 | - | 06 ± 0.3 |
| 3 | 14 ± 0.2 | 12 ± 0.4 | 10 ± 0.2 | 10 ± 0.6 | 09 ± 0.2 | 11 ± 0.3 |
| 4 | 05 ± 0.1 | 10 ± 0.1 | 06 ± 0.1 | 05 ± 0.1 | 03 ± 0.1 | 05 ± 0.1 |
| Chloramphenicol | 15 ± 0.2 | 16 ± 0.3 | 13 ± 0.3 | 12 ± 0.2 | 11 ± 0.4 | 13 ± 0.3 |
| Fluconazole | - | - | - | 12 ± 0.2 | 10 ± 0.1 | 12 ± 0.3 |
| DMSO | 0 | 0 | 0 | 0 | 0 | 0 |

**Table 6 Antimicrobial activity**

| Entry | MIC of the compounds in 25 µg/ml | | | | | |
|---|---|---|---|---|---|---|
| | *Staphylococcus aureus* | *Bacillus subtilis* | *Escherichia coli* | *Streptococcus* | *Candida albicans* | *Aspergillus niger* |
| MPP | 10 | - | 12 | 09 | 08 | - |
| 1 | 19 | 20 | 22 | 17 | 13 | 22 |
| 2 | 11 | 10 | - | 08 | 10 | 11 |
| 3 | 18 | 21 | 17 | 19 | 21 | 15 |
| 4 | 11 | 18 | 15 | 08 | 06 | 09 |

**Antioxidant activity**

DPPH radical scavenging activity data of the MPP and their metal complexes indicate that all the compounds having highly potential activity as resulted (Figure 3). The metal complexes exhibited more radical scavenging activity than that of the MPP. The 1 showed effective antioxidant activity almost closer to the standard BHT, ligand and 4 showed lower antioxidant activity, and the complexes 2 and 3 showed moderate activity when compared with BHT.

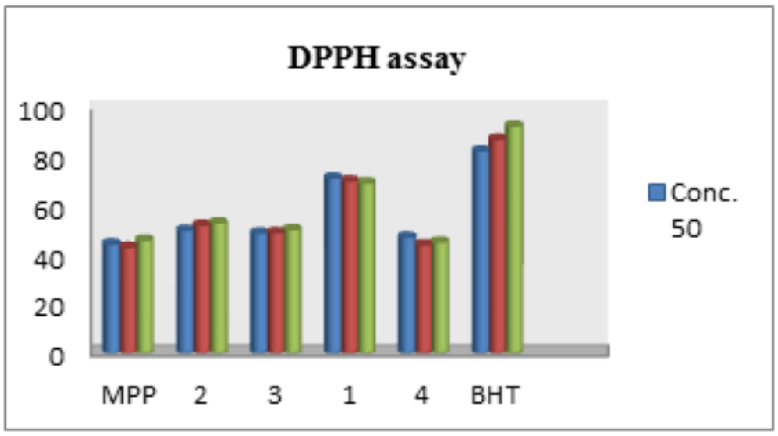



**Figure 3: Antioxidant activity DPPH method**

**Molecular docking study**

The EGFR tyrosine kinase was reported several times as target for the inhibition of cancer cells. Therefore all the four complexes used to study the effect docking inside the active site of EGFR kinase domain with a binding energy values in the range of -290 to -319 kcal/mol$^{-1}$ as tabulated in Table 7. The molecular docking results revealed that 1 has the best binding energy values -319 kcal/mol$^{-1}$ for the EGFR kinase domain inhibited complex formation by forming a five hydrogen bond with THR8 with bond distances 1.10 Å, 1.26 Å, 2.16 Å, 2.83 Å and 3.18 Å and the other interacting amino acids were LEU415, ALA419, CYS5, ASN38, ASP9, LEU39, TYR29, THR8, GLY418 as shown in Figure 4. The 2 formed each single hydrogen bonds with amino acids are ASP9, THR8 and ALA419 with bonds distances are 1.70 Å, 2.69 Å and 2.98 Å respectively. The other interacting amino acids were LY7, GLN36, ASP9, ASN417, MET10, GLU40, TYR29, ASN38, GLN36, CYS5, ALA419 as shown in Figure 5. Even though the 3 (-300 kcal/mol$^{-1}$) and 4 (-290 kcal/mol$^{-1}$) are having good docking energy values than the standard actinoin (-260.51 kcal mol$^{-1}$) but these complexes were not forming having hydrogen bonding interactions with EGFR tyrosine kinase receptor than 1 and 2. The 3 and 4 interact with active sites of EGFR tyrosine kinase with amino acid residues are ALA419, TYR420, LEU39, TYR29, THR8, ASN38, GLU40, LEU415, ASN38, LEU28, CYS5. The binding value of 1 and 2 showed the better cytotoxicity property than the reaming of complexes. The obtained results provide a sufficient explanation and good compromise between docking scores and *in-vitro* results of antibacterial and cytotoxicity activity.

**Table 7: Cytotoxic docking scores**

| Entry | Receptor PDB code | ΔG (Kcal/mol) with MurB |
|---|---|---|
| MPP | 2A91 | -209.39 |
| 1 | 2A91 | -319.73 |
| 2 | 2A91 | -314.82 |
| 3 | 2A91 | -300.48 |
| 4 | 2A91 | -290.84 |
| Actinoin (STD) | 2A91 | -235.99 |

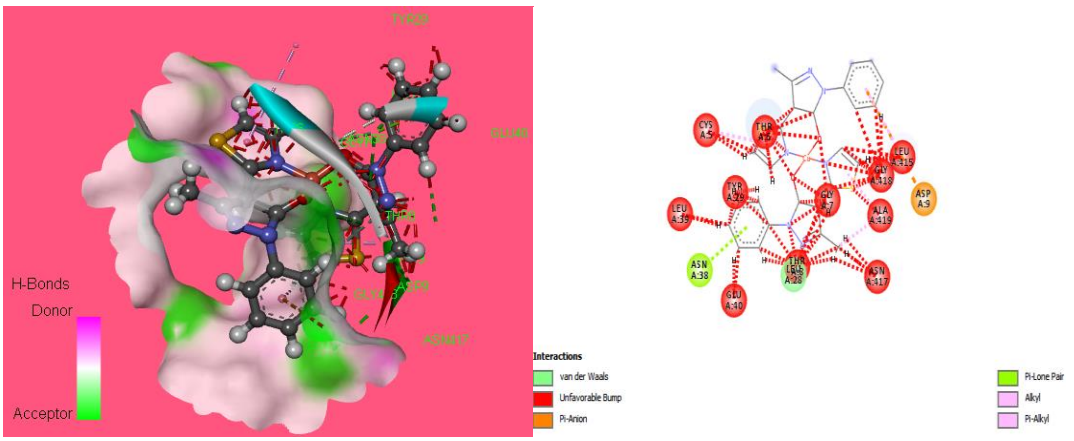



**Figure 4: Interaction of 1 with amino acids of 2A91 (a) 3D-structure with hydrogen bonding of 1 (ball and stick model Oxygen-red, Nitrogen-blue) protein receptor (stick model) (b) 2D-structure of the complex**

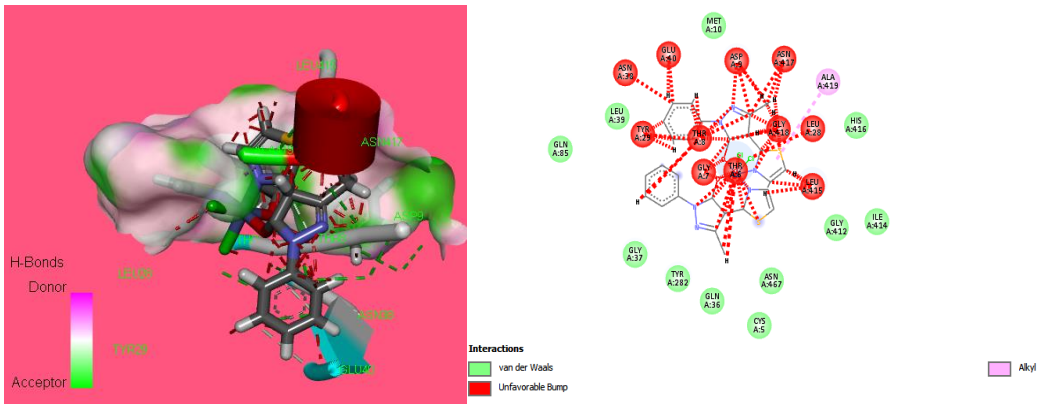



**Figure 5: Interaction of 1 with amino acids of 2A91 (a) 3D-structure with hydrogen bonding of 1 (ball and stick model Oxygen-red, Nitrogen-blue) protein receptor (stick model) (b) 2D-structure of the complex**

### Cytotoxicity

The results of cytotoxic activity are tabulated in Table 8, the reliable criteria for judging the efficacy of any anticancer drug are the prolongation of lifespan, improving the clinical, hematological, biochemical profile and reduction in viable tumor cell count in the host. In order to evaluate the biological effects of the ligand, and its 1, 2, 3 and 4 on cancer cells to treat human breast cancer (MCF7) and K-562 cell lines at the concentrations of 80, 90, 100, 110, 120, >130 and >200 µg/ml for 48 h. The untreated cells were used as a control. The cell growth inhibition was analyzed by the MTT assay and the results showed that the complexes and the ligand exhibited an inhibitory effect on the proliferation of MCF-7 and K-562 cell lines in a dose-dependent manner. Among them, 1 and 2 showed the most potent inhibitory effect on the growth of both the MCF-7 and K-562 cells lines compared to the uncoordinated ligand.

**Table 8: Cytotoxicity activity of ligand and complexes**

| Tested samples | Inhibitory effect on cell lines ($IC_{50}$) | |
|---|---|---|
| | MCF-7 | K-562 |
| MPP | >120 µg/ml | >120 µg/ml |
| | -50.38% | -51.02% |
| 1 | 110 µg/ml | 100 µg/ml |
| | -55.16% | -53.42% |
| 2 | 120 µg/ml | 110 µg/ml |
| | -54.79% | (53.23%) |
| 3 | 100 µg/ml | 90 µg/ml |
| | -51.19% | -53.36% |
| 4 | 80 µg/ml | 90 µg/ml |
| | -50.16% | -51.12% |

## CONCLUSION

The ligand MPP synthesized by Sandmayer type reaction and biologically active Cu(II), Co(II), Ni(II) and Zn(II) ion complexes have been prepared and structurally characterized. The newly synthesized Co(II) and Cu(II) metal complexes are potent antibacterial and antifungal agents against bacteria's and fungi's. The uncoordinated ligand and metal complexes are screened for antioxidant activity, among them, Cu(II) Co(II) and Ni(II) complex showed fairly good activity in DPPH scavenging. The results of computational docking studies promote to evaluate *in vitro* cytotoxic activity, The Cu(II), Co(II) and Ni(II), complex exhibited more *in vitro* cytotoxic activity than unbound ligand and all complexes are crystalline nature.

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