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*Original Article*

# Synthesis and evaluation of the complex-forming ability of hydroxypyranones and hydroxypyridinones with Ni (II) as possible inhibitors for urease enzyme in *Helicobacter pylori*

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## *Abstract*

The complex-forming ability of 2-methyl-3-hydroxypyran-4-one (**1a**), 2-ethyl-3-hydroxypyran-4-one (**1b**), 1,2-dimethyl-3-hydroxypyridin-4-one (**4a**) and 1-ethyl-2-methyl-3-hydroxypyridin-4-one (**4b**) with nickel(Ni(II)) were characterized by infrared, ultraviolet, proton nuclear magnetic resonance spectroscopy and melting point. The mole-ratio of nickel:ligands was analyzed by atomic-absorption-spectrometry. The partition-coefficients ($K_{OW}$) of the compounds were also determined. The binding of ligands with Ni(II) are through deprotonated hydroxyl group ($-O^-$, disapeared at 3259 $cm^{-1}$) and ioan-pairs of carbonyl group ($=CO^-$, shifted from 1650 to 1510-1515 $cm^{-1}$). The characterization of complex geometry for bis-(2-methyl-3-hydroxypyranonato)Ni(II) (**5a**) and bis-(2-ethyl-3-hydroxypyranonato)Ni(II) (**5b**) predicted to be square-planer while for bis-(1,2-dimethyl-3-hydroxypyridinonato)Ni(II) (**5c**) and bis-(1-ethyl-2-methyl-3-hydroxypyridinonato)Ni(II) (**5d**) distorted to tetrahedral-geometry. Inhibitors of *Helicobacter pylori* urease are nickel chelators. The compounds **1a**, **4a** and **4b** are likely suitable ligands with complex forming-ability to make complexes of **5a**, **5c** and **5d** with nickel. The $K_{OW}$ values show the compound **5c** with low partition-coefficient is more suitable ligand with lower penetration from GI lumen. Future studies demand to find out the biological activity of developed compounds on *H. pylori*.

***Keywords:*** 3-Hydroxypyran-4-one; 3-Hydroxypyridin-4-one; Nickel(II) complexes; *Helicobacter pylori*.

## INTRODUCTION

Nickel is one of the transition elements and could has a role in health and disease (1,2). It is not clear if it is essential, although a nickel deficiency in animals could delay growth, cause anemia, and decrease enzyme activities. In humans, there is some evidence to recommend that nickel ion has a vital role in hematopoiesis together with vitamin B12 (3,4). Long term exposure to nickel could develop lung fibrosis, skin allergies, kidney and cardiovascular system poisoning and cancer (5). Nickel-containing proteins or compounds have also been described in different microorganisms and also in humans. Though, only ultratrace amounts of nickel are needed, conditions associated with nickel deficiency is not yet known (6). The nickel level in unexposed individuals is as low as serum/plasma: < 19 nmol/L and urine: < 102 nmol/L (7-10). Ingestion of nickel salts causes gastrointestinal symptoms (nausea, diarrhea), neurological symptoms (headache, lassitude) and mild nephrotoxicity (11,12). It is also carcinogenesis and air pollution containing nickel increases the risk of cancer of the respiratory system (13,14).

There is evidence that T-cells maintain immune tolerance to nickel in healthy individuals (15). Despite the fact that the very low level of metal nickel, more than 250 µg/day, is toxic for human (8,9), it is an essential element and critical for the pathogenicity of *Helicobacter pylori*. Indeed the element is necessary for the activity of

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urease and hydrogenase enzymes. Several studies revealed that these enzymes are important for *in vivo* colonization of the host gastric mucosa (16,17).

Urease, as a virulence factor for pathogens, is a nickel-dependent metalloenzyme catalyzes the hydrolysis of urea to form carbon dioxide and ammonia. This phenomenon causes to promote bacterial host colonization by neutralizing the low pH in the stomach (18). Urease accounts for up to 10% of the total cellular *H. pylori* protein content, and therefore the bacteria nickel demand is very high (19,20).

There is a biochemical network for the adaptation of *H. pylori* to life in the gastric mucosa. When the environment around the bacteria is more acidic (low pH) the nickel influx network motivates to supply nickel ion to inactive urease for activation. The resultant of the process increases the amount of ammonia and exits from the bacteria to neutralize the acidic environment (21). The major nickel-transporter of *H. pylori* is a 37-kDa NixA protein (22). The urease inhibitors have been investigated for the treatment of *H. pylori* infections. The structural studies on the urease, which is similar to *H. pylori* urease, have revealed that the enzyme contains a di-nuclear active site with nickel ion at the center with an amino acid side (23-25). The crystal structure shows that bacterial urease has an active center, which contains two simple coordinated water molecules and a bridging OH group. The specificity of the enzyme is closely related to the shape of its active center (26). Chelating ligands have the potential of removing nutrient nickel from the bacterial network inhibiting survival of the *H. pylori*. These types of compound have been considered recently (19-23).

As shown in Fig. 1 the pathogenesis of helicobacter pylori life cycle is extremely depend on the presence of nickel in its enviroment. Chelation of nickel (Ni)(II) by specific ligands could remove the Ni(II) ions reaching the bacteria and then inhibits the urease and prevent the bacterial survival in the stomach.

The hydroxypyranons parents are 3-hydroxy-2-methyl-4-pyranon (Maltol) and 3-hydroxy-2-ethyl-4-pyranon (Ethyl-maltol). These compounds usually behave as potential bidentate ligands and chelate divalent metal ions with variable affinity and selectivity. Therefore, the complex formation of hydroxypyranones and hydroxypyridinones with Ni(II) ion was investigated.

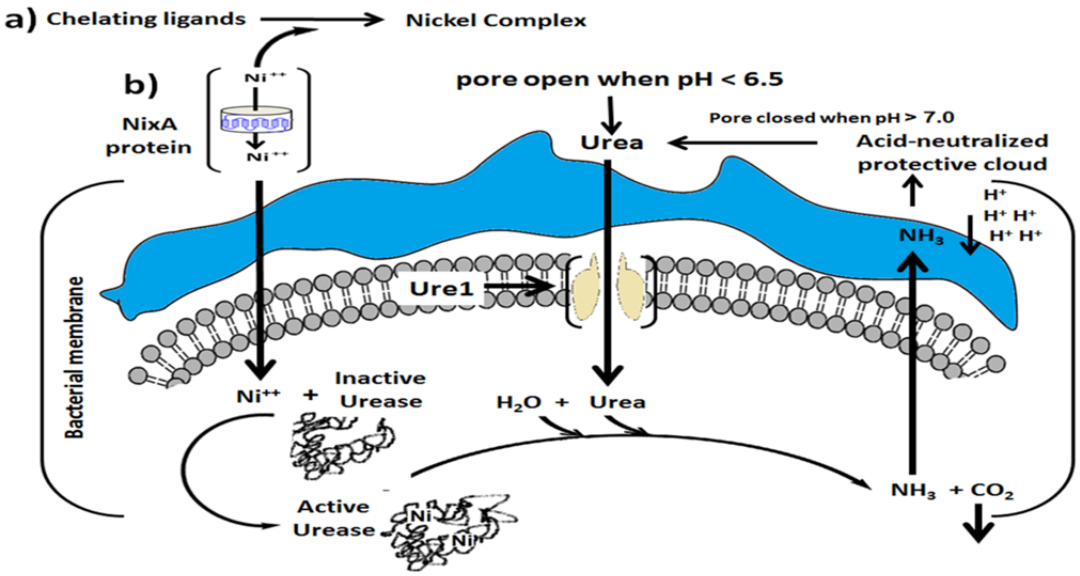


**Fig. 1.** Representation of urease activity. (a) The pathway for removing the nutrient nickel ion from reaching the bacteria. (b) NixA is a nickel transporter protein entering the bacteria to activate the urease.

## MATERIALS AND METHODS

All chemicals used in this study were purchased from Sigma-Aldrich (UK) except otherwise mentioned. Infrared (IR) spectrometry of compounds was analyzed by a Perkin-Elmer 1420 instrument (Perkin-Elmer, USA). proton nuclear magnetic resonance ($^1$H NMR) spectra were determined with a 80 MHZ NMR (Bruker Corporation, Germany). Elemental analyses of nickel complexes and the mole ratio variability of complex formation were performed using a Flame Atomic Absorption Spectrometry Perkin-Elmer instrument (USA). The partition coefficients ($K_{OW}$) values were calculated using UV/Vis spectrophotometer.

### *Synthesis of 3-Hydroxypyridin-4-one ligands*

3-Hydroxypyridin-4-one ligands, such as 1,2-dimethyl-3-hydroxypyridin-4-one (**4a**) and 1-ethyl-2-methyl-3-hydroxypyridin-4-one (**4b**) were not commercially available and WERE synthesized in our laboratory based on previously developed procedure (27,28). The reaction steps are shown in Fig. 2.

### *Synthesis of 2-methyl-3-benzyloxypran-4-one (Benzyl maltol or Benzyle Ethyl-maltol ethyl derivatives (Compounds 2a and 2b)*

As outlined previously (27,28), to 100 mL methanol solution of 2-methyl-3-hydroxypyran-4-one, 2-methyl-3-hydroxypyran-4-one (**1a**) or 2-ethyl-3-hydroxypyran-4-one (**1b**) (1 mol/L), was added 10 mL alkaline solution of sodium hydroxide in distilled water (11 mol/L). Then, benzyl chloride (0.11 mol/L) was added to the mixture and finally left with refluxing for 6 h. After end of the reaction, solvent was removed by a rotary evaporation. The residue was mixed with 50 mL water and the compound was extracted into dichloromethane (3 × 50 mL). The pooled extracts were washed with 5% solution of sodium hydroxide (3 × 150 mL) and finally washed with distilled water (2 × 150 mL). The organic fraction was dried over anhydrous sodium sulfate. The anhydrous organic phase filtered and the solvent removed by rotary evaporator (Heidolph, Germany) to yield orange oil. The product was purified by recrystallization in diethyl ether to form colorless needles.

Yield 17.6 g (81%). mp 52-53 °C. IR (KBr): 1656 (C=O) cm$^{-1}$. $^1$H NMR (DMSO-$d_6$): δ 2.10 (s, 3H, 2-$CH_3$), 5.10 (s, 2H, O-$CH_2$-Ph), 7.93 (d, 1H, 6-H).

### *Synthesis of 1, 2-dimethyl-3-benzyloxy-pyridin-4-one (Compound 3a)*

To 200 mL solution of 2-methyl-3-benzyloxypyran-4-one **(**compound **2a,** methly derivative**)** in ethanol:water (50:50) (0.3 mol/L) was added 40% aqueous methylamine (0.9 mol). Then, 5 mL sodium hydroxide solution (2N) was added and finally the mixture left with refluxing for 12 h. The pH was adjusted to 7.0 with HCl (0.1 N) and the solvent removed by a rotary evaporator to reduce volume to 100 mL. To the mixture, 100 mL distilled water was added and then washed with 200 mL diethyl ether. The anhydrous organic phase was filtered and the solvent removed by rotary evaporator to yield orange oil. This oil was dissolved in ethanol/hydrochloric acid and the solvent evaporated to get white powder. The product was purified by recrystallization in ethanol/diethyl ether to form white powder.

Yield 12.2 g, (78%). mp 205-207 °C. IR (KBr): 1655(C=O) cm$^{-1}$. $^1$H NMR (DMSO-$d_6$): δ 2.19 (s, 3H, 2-$CH_3$), 3.93 (s, 3H, N-$CH_3$), 5.04 (s, 2H, O-$CH_2$-Ph), 6.19 (d, j = 6.9, 1H, 5-H) 7.25-7.51 (m, 5H, Ph), 7.56 (d, 1H, 6-H).

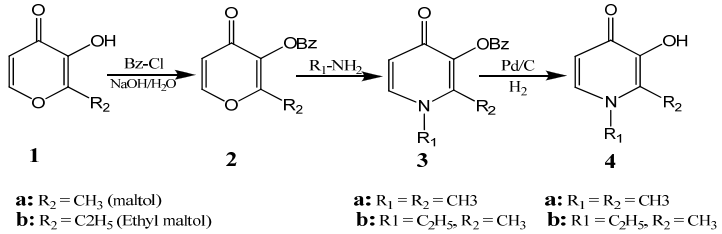


**Fig. 2.** Synthesis of 3- hydroxypyridin-4-ones. Three step-reaction to synthesis the entire ligands (27,28).

***Synthesis of 1-ethyl-2-methyl-3-benzyloxy-pyridin-4-one (Compound 3b)***

The procedure for synthesis of the compound **3b** is similar to that of the synthesis of 1, 2-dimethyl-3-benzyloxypyridin-4-one hydrochloride, the compound **3a.** In this reaction, methylamine was replaced with ethylamine.

Yield 28.1 g (82% yield), mp 177-178 °C. IR (KBr): 1656(C=O) $cm^{-1}$. $^1H$ NMR (DMSO-$d_6$): δ 1.18 (t, j = 6.4, 3H, N-$CH_2CH_3$), 2.1 (s, 3H, 2-$CH_3$), 4.2 (q, 2H, N-$CH_2CH_3$), 5.0 (s, 2H, O-$CH_2$-Ph), 7.1 (d, 1H, 5-H), 7.4-7.5 (m, 5H, Ph), 8.0 (d, 1H, 6-H).

***Synthesis of 1, 2-dimethyl-3-hydroxypyridin-4-one (Compound 4a)***

The compound **3a** (0.075 mol) was dissolved in 270 mL ethanol plus 30 mL water and exposed to hydrogenolysis in the presence of Pd:C catalyst.

The product was separated by filtration and then the solvent evaporated by a rotary evaporator to form white powder. The product was re-crystallized in ethanol/diethyl ether to yield extra pure white powder.

Yield 11.5 g (87%). mp 190-191 °C. IR (KBr): 3259 (OH), 1653 (C=O), for free base) $cm^{-1}$. $^1H$ NMR (DMSO-$d_6$): δ 2.45 (s, 3H, 2-$CH_3$), 4.15 (s, 3H, N-$CH_3$), 7.5(d, 1H, 5-H) 8.3 (d, 1H, 6-H).

***Synthesis of 1-ethyl-2-methyl-3-hydroxy-pyridin-4-one (Compound 4b)***

The synthesis procedure is similar to the one described above for the synthesis of 1-ethyl - 2 - dimethyl - 3 - benzyloxypyridin - 4 - one hydrochloride (**4a**) except that in the reaction mixture the compound 1, 2-dimethyl-3-benzyloxypyridin-4-one hydrochloride (**3a**) was replaced with 1-ethyl-2-methyl-3-benzyloxypyridin-4-one (**3b**). A pure white powder was formed by re-crystallization in ethanol/diethyl ether.

Yield 10.9 g, (77.1%), mp 206-207 °C. IR (KBR): 3259 (OH), 1657 (C=O) $cm^{-1}$. $^1H$ NMR (DMSO-$d_6$): δ 1.5 (t, 3H, N-$CH_2CH_3$), 2.7 (s, 3H, 2-$CH_3$), 4.5 (q, 2H, N-$CH_2CH_3$), 7.5 (d, 1H, 5-H), 8.6 (d, 1H, 6-H).

***Synthesis of bis-(2-methyl-3-hydroxypyranonato) nickel (II) complex (Compound 5a)***

The Ni(II) sulfate was heated in an oven at 100 °C for 24 h to produce anhydrous salt and then transferred to a glass desiccator (Camlab, UK), cooled and kept until use. 2-Methyl-3-hydroxypyran-4-one (**1a**, 0.02 mol) was dissolved in 12 mL ethanol:2 mL water. This solution was added to the anhydrous Ni(II) sulfate solution (0.01 mol/L) in 4 mL ethanol:2 mL water. The reaction was mixed well at 70 °C for 2 h. The complex mixture was titrated dropwise with a solution of sodium hydroxide (0.06 N) while pH monitored to find a precipitation pH. At the same time the solution was heated at 70 °C for 2 h and mixed gently. The reaction mixture was stored at 4 °C for 24 h to allow precipitation of the nickel complex. The red brick precipitates were recrystallized in 90% ethanol. The resulting solid was heated at 100 °C for 24 h to yield anhydrous bis-(2-methyl-3-hydroxypyranonato) Ni(II) complex (**5a**) and finally transferred to a desiccator, cooled and kept it dry for further analysis.

Yield 1.9 g (62%), Ni 18.8%. IR (KBr): 1612 (C=O) $cm^{-1}$. The carbonyl group is shifted.

***Synthesis of bis-(2-ethyl-3-hydroxypyranonato) Ni(II) complex (Compound 5b)***

The synthesis procedure is similar to the procedure described above except that in the reaction mixture compound **1a** was replaced with 2-ethyl-3-hydroxypyran-4-one (**1b**). The Ni(II) sulfate was heated in an oven at 100 °C for 24 h to produce anhydrous salt and then transferred to a desiccator, cooled and kept it dry. 2-ethyl-3-hydoxypyran-4-one (**1b**, 0.02 mol) was dissolved in 12 mL ethanol:2 mL water. This solution was added to the anhydrous Ni(II) sulfate solution (0.01 mol) made of 4 mL ethanol:2 mL water. The reaction was mixed well at 70 °C. The complex mixture was titrated dropwise with a solution of sodium hydroxide (0.06 N) and the pH was manitored to find the precipitation pH. At the same time the solution heated and mixed carefully. The reaction mixture was stored at 4 °C for 24 h to allow accumulating precipitation of the nickel complex. The red brick precipitates were recrystallized in 90% ethanol and heated at 100 °C for 24 h to yield

anhydrous bis-(2-ethyl-3-hydroxypyranonato) nickel (II) complex (**5b**) and finally transferred to a desiccator, cooled and kept it dry for further analysis.
Yield 2.31 g (68%), Ni 17.31%. IR (KBr): 1610 (C=O) $cm^{-1}$. The carbonyl group is shifted.

***Synthesis of bis-(1,2-dimethyl-3-hydroxy-pyridinonato) nickel (II) complex (Compound 5c)***

The Ni(II) sulfate was heated in an oven at 100 °C for 24 h to produce anhydrous salt and then transferred to a desiccator, cool and keep it dry. The compound **4a,** (0.02 mol) was dissolved in 12 mL ethanol:2 mL water and then the anhydrous Ni(II) sulfate solution (0.01 mol) in 4 mL ethanol:2 mL water was added, mixed well at 70 °C. The complex mixture was titrated drop wise with a solution of sodium hydroxide (0.06 M) and the pH was controlled to find the precipitation pH. At the same time the solution heated and agitated carefully. The reaction mixture was stored at 4 °C for 24 h to allow full precipitation of the nickel complex. The red brick precipitated compound was recrystallized in 90% ethanol and heated at 100 °C for 24h to yield anhydrous bis-(1,2-dimethyl-3-hydroxy-pyridinonato) nickel (II) (**5c**) and finally transferred to a desiccator, cooled and kept it dry for further analysis.
Yield 1.98 g (59%), Ni 17.42%. IR (KBr): 1613 (C=O) $cm^{-1}$. Mole ratio and relative quantities of reactants and products are used to calculate the stoichiometry of complexes.

***Synthesis of bis-(1-ethyl-2-methyl-3-hydroxy-pyridinonato) Ni(II) complex (Compound 5d)***

The synthesis procedure is similar to the one described above for the synthesis of **5c**, except that in the reaction mixture the compound **4a** was replaced with compound **4b**. The Ni(II) sulfate was heated in an oven at 100 °C for 24 h to produce anhydrous salt and then transferred to a desiccator, cool and keep it dry. The compound **4b** (0.02 mol) was dissolved in 12 mL ethanol:2 mL water and then the anhydrous Ni(II) sulfate solution (0.01 mol) in 4 mL ethanol:2 mL water was added and mixed well at 70 °C. The complex mixture was titrated dropwise with a solution of sodium hydroxide (0.06 M) and the pH was controlled until the precipitation occured. At the same time the solution was heated and agitated carefully. The reaction mixture was stored at 4 °C for 24 h to allow accumulation of precipitation of the nickel complex. The red brick precipitate was recrystallized in 90% ethanol and heated at 100 °C for 24 h to yield anhydrous bis-(1-ethyl-2-methyl-3-hydroxy-pyridinonato) Ni(II) complex (**5d**) and finally transferred to a desiccator, cooled and kept it dry for further analysis.
Yield 2.35 g (60%), Ni 14.9%. IR (KBr): 1611 (C=O) $cm^{-1}$.

***Partition coefficient determination***

The partition coefficient is defined as the ratio of the equilibrium concentrations of a substance distributed between a two-phase system consisting of two immiscible solvents such as n-octanol and water. The $K_{OW}$ of the ligands and their nickel complexes synthesised in this study were determined using the shake-flask method (28,29).

In the method of the shake-flask, a compound is added into an immiscible mixture of n-octanol and water. The mixture is shaken while equilibrium is achieved. The concentrations of the compound in the two liquid phases are measured by a specific method of absorbance.

The two-phase system was Tris-HCl buffer (50 mM, pH 7.4) and n-octanol. The solubility of water in n-octanol is 2.3 M (30). The nickel complex solution ($10^{-4}$ M) was prepared in Tris-HCl buffer and the absorbance of the solution was measured at 305-320 nm and compared with a blank buffer. One solution of each complex ($10^{-4}$ M) was prepared in Tris-HCl buffer (10-50 mL) and mixed with an appropriate volume of n-octanol in a glass vessel. The mixture was vi gorously agitated for 1 h. The two phases were separated by centrifugation for 5 min. An aliquot of the aqueous layers (1-2 mL) was then carefully removed by a glass Pasteur pipette (Camlab, UK), to prevent contamination with n-octanol. The absorbance of each sample was measured and the partition coefficient was calculated using the following equation:

$$K_{part} = \frac{A_1 - A_2}{A_2} \times \frac{V_W}{V_O}$$

$A_1$ and $A_2$, are the absorbance of compounds in the aqueous layer before and after partitioning, respectively. $V_W$ and $V_O$ are the volume of the aqueous and the n-octanol phases used in partitioning, respectively.

## RESULTS

In this study the hydroxypyranones parents are 2-methyl-3-hydroxypyran-4-one (**1a**) and 2-ethyl-3-hydoxypyran-4-one (**1b**). These compounds are known as bidentate ligands could chelate divalent metal ions with variable affinity and selectivity. Maltol and ethyl-maltol or their derivatives were used to evaluate the complex ability by mole ratio of 1 mol metal/2 mol ligand or 1 mol metal/3 mol ligands.

Generally, the complexes were synthesized by solutions of each ligand in ethanol/water (50:50) at pH 7.0 with the Ni(II) ion. The ligand/nickel solutions were mixed well and left the reaction at room temperature for 30 min until completing the precipitation. The re-crystallization of the complexes was achieved in cold ethanol. The anhydrous nickel complexes were obtained by removing water molecules in a vacuum oven at 100 °C for 24 h. Elemental analysis in each complex was consistent with the formulations of Ni(II)$[L]_2$ (L, bidentate ligand).

### *Methods for the synthesis of selected ligands*

The general methodology which adopted for the synthesis of ligands **4a** and **4b**, is summarized in Fig. 2. 2-methyl-3-hydroxypyran-4-one (**1a)** and 2-ethyl-3-hydoxypyran-4-one **(1b)** were purchased. In order to deactivate and protect the hydroxyl group, compound **1a** was benzylated to achieve compound **2**. The products of compound **2** with methylamine or ethylamine are benzylated pyridinenes **3a** and **3b** (27-30). The palladium catalytic hydrogenation reaction removes the benzyl protecting group to yield the hydrochloride salts of bidentate chelators **4a** and **4b**.

### *Methods for the synthesis of the Ni(II) complexes*

Nickel complexes were synthesised at varying pH with mole-ratios of metal:ligand, (M:$[L]_2$ or M:$[L]_3$). The UV and IR spectrometry together with atomic absorption spectrometry were performed to characterize the structure of complexes. The nickel-complexes were designed and synthesized in good yield by a direct reaction of bidentate ligands of **1a**, **1b**, **4a** and **4b** with Ni(II) ion. The pH conditions for the synthesis of complexes were examined and the results of elemental analysis confirmed that a very weak pH close to neutralize pH was favorable to produce a compound with a general formula such as M$[L]_2$. Elemental analysis also confirmed that the complex structure is consistent with the formulations of Ni(II)$[L]_2$.

Mole ratio and relative quantities of reactants and products were used to calculate the stoichiometry of complexes.

The factors that determine the geometry of metal complexes is ligand fields. The nickel ion has $d^8$ electron configuration and usually with bidentat ligands makes complexes with square planer geometry or tetrahedral in the presence of geometric hindrances around metal ion (31). The Interpretation of the results revealed that the nickel complexes of compounds **5a** and **5b** have square planar while the compounds **5c** and **5d** distorted to tetrahedral geometry with a general formula of M[L]. The structures of complexes are shown in Fig. 3.

### *The IR results of stretching frequencies*

The stretching frequency values of C=O bonds ($\nu_{c=o}$ $vm^{-1}$) in ligands of hydroxypyranones (maltol **1a** and ethyl-maltol **1b**) and hydroxypyridinones (**4a and 4b**) and their corresponding nickel complexes **5a, 5b, 5c, and 5d** are summarized in the Table 1 for all compounds. The IR spectra of ligands and complexes are shown in Fig. 2. The absorption band of hydroxyl group at 3259 $cm^{-1}$ was disappeared when deprotonated in complex to make a chemical bond. In complex there is also a 40-50 $cm^{-1}$ shift for carbonyl group in coordinated bond.

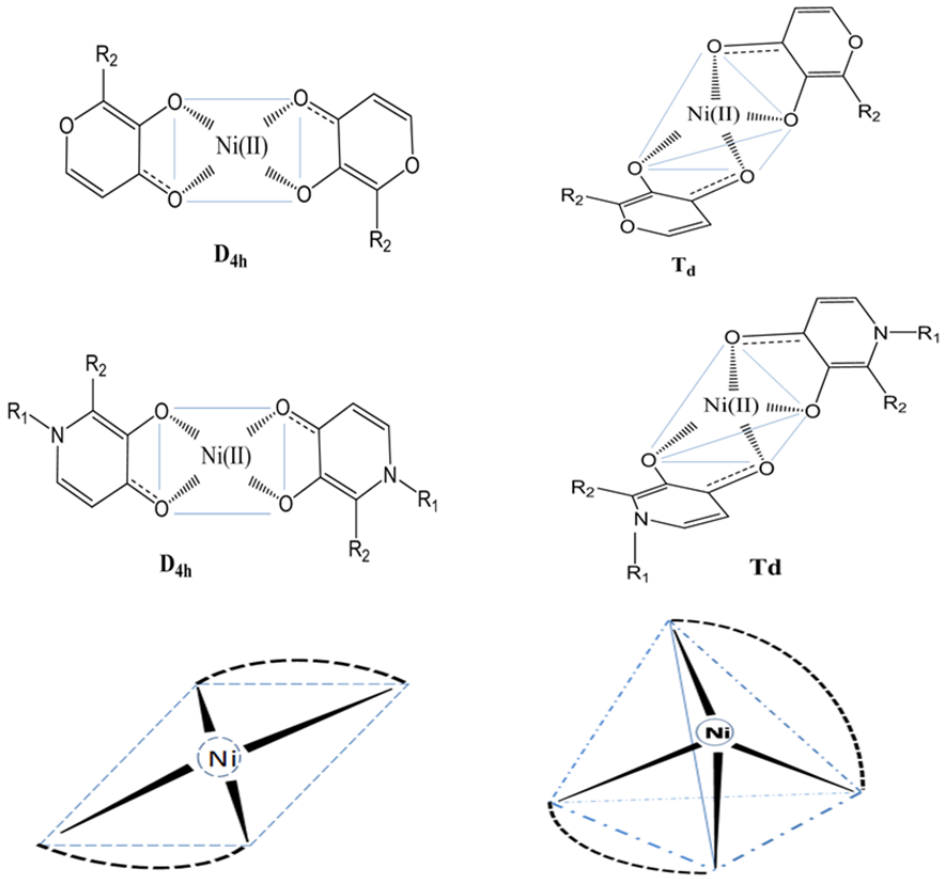


**Fig. 3.** Synthesis of Ni(II)[$L]_2$ complexes. (Left panel) structure for square planner, (right panel) for tetrahedral complexes.

**Table 1.** The stretching frequency values of functional group ($\nu_{C=O}$ $Cm^{-1}$) for C=O and deprotonated hydroxyl groups ($-O^-$) in ligands and their Ni(II) complexes. Variations in C=O and $-O^-$ stretching frequencies are shown as $\Delta\nu_{C=O}$.

| Ligand | $\nu_{C=O}$ ($cm^{-1}$) | $\nu_{OH}$ ($cm^{-1}$) | Complex | $\nu_{C=O}$ ($cm^{-1}$) | $\nu_{OH}$ ($cm^{-1}$) | $\Delta\nu_{C=O}$ ($cm^{-1}$) |
|---|---|---|---|---|---|---|
| $L_1$ (**1a**) | 1655 | 3240-3269 | Ni(II)[$L_1]_2$ (**5a**) | 1612 | Omitted | 44 |
| $L_2$ (**1b**) | 1656 | 3240-3269 | Ni(II)[$L_2]_2$ (**5b**) | 1610 | Omitted | 46 |
| $L_3$ (**4a**) | 1653 | 3240-3269 | Ni(II)[$L_3]_2$ (**5c**) | 1613 | Omitted | 40 |
| $L_4$ (**4b**) | 1657 | 3240-3269 | Ni(II)[$L_4]_2$ (**5d**) | 1611 | Omitted | 46 |

($L_1$) 2-methyl-3-hydroxypyran-4-one, **1a**; ($L_2$) 2-ethyl-3-hydoxypyran-4-one, **1b**; ($L_3$) 1,2-dimethyl-3-hydroxypyridin-4-one,**4a**; ($L_4$) 1-ethyl-2-methyl-3-hydroxypyridin-4-one, **4b**; (Ni(II)[$L_1]_2$) bis-(2-methyl-3-hydroxypyranonato) Ni (II), **5a**; (Ni(II)[$L_2]_2$) bis-(2-ethyl-3-hydroxypyranonato) Ni (II), **5b**, (Ni(II)[$L_3]_2$) bis-(1,2-dimethyl-3- hydroxypyridin-4-one) Ni (II), **5c**; (Ni(II)[$L_4$]) bis-(1-ethyl-2-methyl-3-hydroxypyridin-4-one) Ni (II), **5d**.

### *The $K_{OW}$ values of nickel complexes*

The $K_{OW}$ of the compounds were measured by the ratio of compound concentrations in n-octanol ($C_O$) and water ($C_W$). Lipophilicity is the permeability capacity of a compound diffusion trough cell membrane via a passive mechanism. It is highly dependent to conformation and polarity of a molecule and traditionally measured in laboratory with a biphasic system of solvents such as n-octanol to water. The relative lipophilicity to the hydrophobicity of each compound was measured by aqueous two-phase partitioning, n-octanol and Tris-HCl buffer at pH 7.4. The mean of partition coefficient constants ($K_{OW}$) are summarized in Table 2.

**Table 2.** The partition coefficients ($pK_a$) values of ligands (**1a**, **1b**, **4a** and **4b**) together with their partition coefficient ($K_{OW}$) values of their nickel complexes (**5a**, **5b**, **5c** and **5d**). Two phases are n-octanol and Tris-HCl buffer at pH 7.4.

| Ligand | $Pk_{a1}$ | $Pk_{a2}$ | $K_{OW}$ of Ligands | Ni(II)[L]2 complexes | $K_{OW}$ of complexes |
|---|---|---|---|---|---|
| **1a** | - | 8.62 | 0.45 ± 0.06 | **5a** | 0.07 ± 0.009 |
| **1b** | - | 8.35 | 0.87 ± 0.08 | **5b** | 0.14 ± 0.060* |
| **4a** | 3.68 | 9.44 | 0.22 ± 0.06 | **5c** | 0.02 ± 0.007 |
| **4b** | 3.81 | 9.71 | 0.55 ± 0.10 | **5d** | 0.04 ± 0.006 |

Data analyzed by excel software to calculate mean and standard deviation (SD), each experiment was performed 4 times. * Ligands and complexes with high permeability.

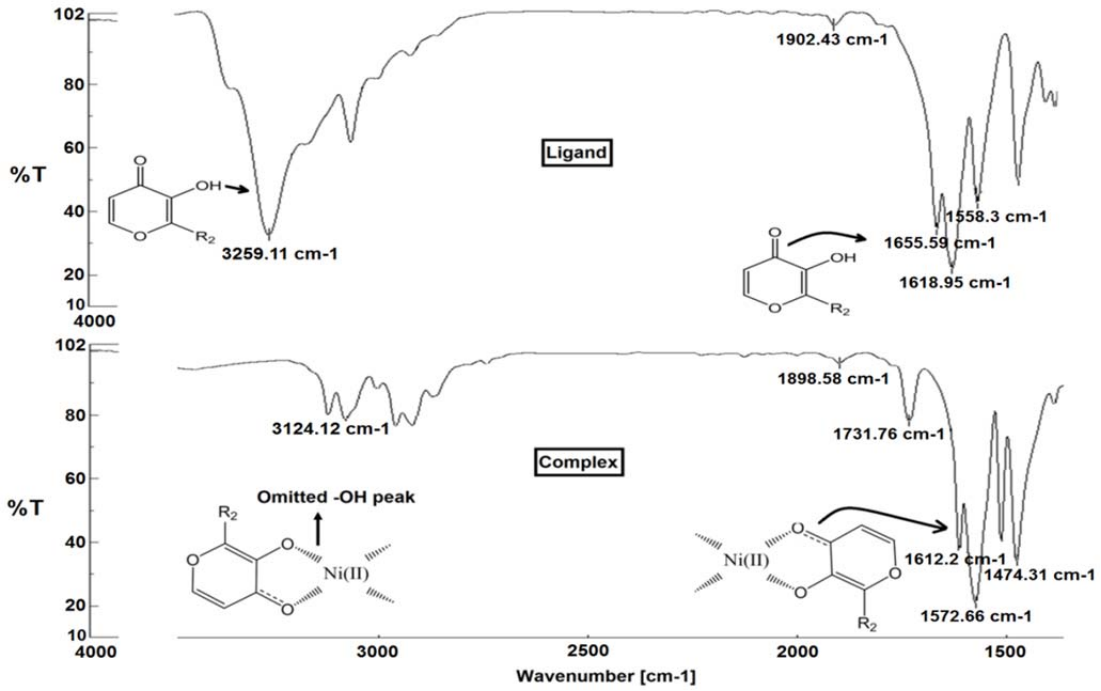


**Fig. 4.** Schematic representation of the infrared spectrum of ligand and complex. The stretching frequencies of hydroxyl ($\nu_{C=O}$ = 3259 $cm^{-1}$) and carbonyl groups ($\nu_{c=o}$ =1655 $cm^{-1}$) for ligand and complex are disappeared or shifted ($\nu_{C=O}$ = 1612 $cm^{-1}$), respectively.

## DISCUSSION

Recent studies have shown that the presence of metal ions in the lumen of the stomach, or within host tissues, including nickel and iron can influence regulatory networks for gene expression in *H. pylori* (32). Nickel is an essential element for the pathogenicity of *H. pylori* in human stomach. There is a biochemical network for adaptation of *H. pylori* in the stomach at the low pH of gastric mucosa by neutralizing the acidic environment. As shown in Fig. 1, urease is a nickel-dependent metallo-enzyme which catalyzes the hydrolysis of urea to produce ammonia for neutralizing the acidic environment around the bacterial membrane in the pylori area of the stomach. The urease has di-nuclear active site with two nickel ion at the center. A chelating ligand with high specificity and selectivity for nickel ion could remove the nutrient nickel from the bacteria and subsequently the deactivation of the enzyme. This phenomenon causes the reduction of the *H. pylori* resistance to low pH.

These types of chelating compounds are being vastly investigated. In this study hydroxypyranone and hydroxypridinone compounds (**1a** and **1b)** are also being considered due to their very low toxicity ($LD_{50}$ 1400 mg/Kg), edible with pleasant odors and flavors (29).

Nickel belongs to transition elements with $d^8$ electronic configuration. It produces complexes with octahedral, tetrahedral or square planar configuration. The configurations depend on the amount of energy of the ligand-field splitting and pairing electrons (31). The edible hydroxypyranon parents are 3-hydroxy-2-methyl-4-pyranon (maltol) and 3-hydroxy-2-ethyl-4-pyranon (ethyl-maltol). These compounds usually behave as potential bidentate ligands and chelate divalent metal ions with variable affinity and selectivity which induce a high field and as a result might probably produce the electronic configuration for tetrahedral or square planar geometry for Ni(II). Consequently, the complexation of Ni(II) by specific ligands, chelate the Ni(II) ions to prevent reaching the bacteria and inactivation of the urease enzyme.

Transition metal ions usually behave as Lewis acids acceptor of electron to complete electron configuration of d orbitals while ligands are usually Louis base and donate election. The electron usually transfers from ligands through carboxyl groups, amine groups or via the deprotonated hydroxyl group. The infrared spectra of ligands and complexes were analyzed to evaluate how the groups on the ligands make coordination bonds. The stretching frequencies of C=O bond in ligand and complexes are, 1653-1657 $cm^{-1}$ and 1610-163 $cm^{-1}$, respectively. The shift of 40-50 $cm^{-1}$ for stretching frequencies of C=O bond show that the paring electron on the carbonyl group coordinated to Ni(II)$[L]_2$ (33). To complete the complexation, the other coordination bond comes from de-protonated hydroxyl group ($-O^-$ ). The stretching frequencies of a hydroxyl group (-OH) is a wide peak around 3240-3269 $cm^{-1}$. This stretching frequency of hydroxyl group disappeared as shown in Fig. 4 (34).

The measurement of lipophilicity and hydrophobicity of the synthesized compounds is the early stages of the laboratory tests. Lipophilicity is a measure of the physicochemical behavior of a drug in the body which reflects the absorption, distribution, metabolism and excretion. The relative lipophilicity to the hydrophobicity of each compound was measured for the ligands (**1a, 1b, 4a, and 4b**) and the nickel complexes (**5a, 5b, 5c, and 5d).** The $K_{OW}$ of 2-ethyl-3-hydoxypyran-4-one (**1b**) and its related complexes are higher than the others indicating more lipophilicity and consequently more permeability of the compound through the biological membranes. Therefore, ligand **1b** may not be a suitable candidate. Ligands such as **1a**, **4a,** and **4b** are considered suitable and safe candidate for nickel removal without toxicity due to their low partition coefficients. Elemental analysis for each complex was consistent with the formulations of Ni(II)$[L]_2$. The complex geometry for bis-(2-methyl-3-hydroxypyranonato) Ni(II) (**5a**) and bis-(2-ethyl-3-hydroxypyranonato) Ni(II) (**5b**) might be square planar while for bis-(1,2-dimethyl-3- hydroxypyridinonato) Ni(II) (**5c**) and bis-(1-ethyl- 2 - methyl - 3 - hydroxypyridinonato) Ni(II) (**5d**) distorted to tetrahedral. The geometry of tetrahedral and squire planner for Ni(II) complexes was predicted based on the stoichiometry calculations, the electronic configuration for ***d*** orbitals in nickel ion and the ligand field theory for the ligands. Though, the complex symmetry and geometry was not the major aims of this study and further investigations required to find clear clue.

## CONCLUSION

A nickel-dependent metalloenzyme which catalyzes the hydrolysis of urea to form carbon dioxide and ammonia is urease in *H. pylori*. Ligands that chelates nickel ion could inhibit the urease activity to prevent survival of bacteria in stomach. In this study showed that compounds **1a**, **4a**, and **4b** are suitable ligands with complex ability to make complexes **5a**, **5c**, and **5d** and have good potentials in complexing nickel and its removal from the bacteria *H. pylori* access. Future studies demand to find out the biological activity of compounds in *H. pylori*.

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