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Original Article

Facile one-pot four-component synthesis of 3,4-dihydro-2-pyridone derivatives: novel urease inhibitor scaffold

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Abstract

In the current study, a series of 3,4-dihydro-2-pyridone derivatives were synthesized in a one-pot four-component reaction of Meldrum's acid, benzaldehyde derivatives, methyl acetoacetate, and ammonium acetate. SiO_2-Pr-SO_3H was used as an efficient catalyst for the synthesis of the target compounds under solvent-free conditions. The most probable mechanism for this reaction has been discussed. The advantages of this methodology are high product yields, being environmentally benign, short reaction times, and easy handling. Eight 2-pyridinone derivatives were evaluated for their inhibitory activities against Jack bean urease. Molecular docking study of the synthesized compounds was also evaluated. All compounds showed good activities against urease and among them, 4-(4-nitrophenyl)-5-methoxycarbonyl-6-methyl-3,4-dihydropyridone (**5a**) showed the most potent activity (IC_{50} = 29.12 μM), more potent than hydroxyurea as the reference drug (IC_{50} = 100.0 μM). Also, the results from docking studies were in good agreement with those obtained with *in vitro* assay. According to the docking studies methionine (Met) 637 and nitro phenyl ring cause n-π interaction between lone pair of sulfur atom and π aromatic ring. Moreover, hydrophobic interactions existed between compound **5a** and alanine (ALA) 636, ALA 440, and isoleucine 411. The results indicated that the inhibitory activities increased with the increase of electron withdrawing ability of the groups despite a less important role of lipophilicity in increasing the inhibitory activity.

Keywords: Multicomponent reaction; Urease inhibitory activity; 3,4-Dihydro-2-pyridone derivatives; SiO_2-Pr-SO_3H

INTRODUCTION

In 1926, crystallized urease from Jack bean urease (EC 3.5.1.5) by James B. Sumner was the first nickel-containing enzyme. The life cycle, pathogenesis of *Helicobacter pylori (H. pylori)* is very appertaining to the presence of nickel in its environment (1).

Urease catalyzes hydrolysis of urea to ammonia and carbon dioxide which neutralizes gastric acid and increases pH in stomach. Urease plays an important role in the nitrogen metabolism, acid resistance, and virulence of *H. pylori* and represented up to 10% of total protein content of the bacteria (2-4).

H. pylori have been colonized in the gastric epithelium of humans for at least 58,000 years. *H. pylori* infection leads to chronic gastritis that most people have no symptoms but is the main risk factor for peptic ulcer, duodenal ulcer, mucosa-associated lymphoid tissue (MALT) lymphoma, and adenocarcinoma (5-7). Existing therapeutic regimens have lost some efficacy due to high level of antibiotic resistance to *H. pylori* and poor patient compliance.

Urease activity control through the use of inhibitors can overcome these shortcomings (8-10). So far, several urease inhibitors are presented; Fig. 1 shows the chemical structure of some of them.

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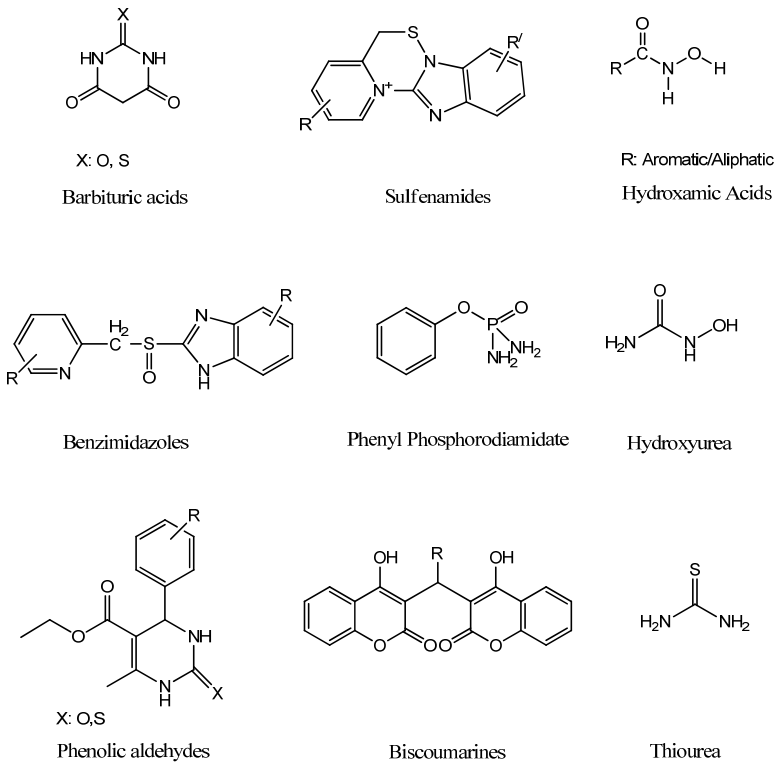


Fig. 1. Chemical structures of urease inhibitors

Multicomponent reactions (MCR) have become popular in organic, medicinal, and combinatorial chemistry because they address both diversity and complexity in organic synthesis. MCR is defined as a process in which three or more different components are combined to yield ideally a single product. Such procedures reduce time and save both energy and starting materials (11-13).

The current literature reveals that 1,4-dihydropyridine derivatives (1,4-DHP) exhibit interesting biological activities such as anti-inflammatory (14), antitubercular (15), antiatherosclerotic (16), and anticancer activities (17). 1,4-DHP derivatives are also a class of heterocyclic compounds well-known as Ca^{2+} channel blockers (18). 2-Pyridones are structurally very similar to 1,4-DHP. Compounds with such structures are found to possess various biological and pharmacological properties (19-21). Although several different methods have been reported for the preparation of 2-pyridone derivatives, development of new synthetic methods for efficient synthesis of this class of compounds is still an interesting challenge.

Recently, the emphasis on green chemical principles introduced some significant advances in organic synthesis (22,23). In this regard, heterogeneous catalysts have found considerable interest in organic reactions, since these catalysts can be recovered and reused several times after the reaction without significant loss of reactivity. Reactions with these catalysts are generally clean and selective and give high yields of products.

In continuation of our research on the multicomponent synthesis of heterocyclic compounds of biological importance (24-27), herein we wish to report a green and efficient procedure for the synthesis of 3,4-dihydro-2-pyridone derivatives in the presence of SiO_2-Pr-SO_3H as a heterogeneous acid catalyst under solvent-free conditions with potential urease inhibitory activity.

MATERIALS AND METHODS

Materials

All commercially available chemicals were purchased from Merck Company (Germany) and used without further purification. IR spectra were recorded from KBr disk using a FT-IR Bruker Tensor 27 instrument (USA). Melting points measured by using the capillary tube method with an electro thermal

9200 apparatus (Bibby Scientific Limited, Staffordshire, UK) are uncorrected. The ^{1}H NMR (250 MHz) and ^{13}C NMR (125 MHz) were run on a Bruker DPX (USA) at 250 MHz in $CDCl_3$ and 125 MHz in D_2O using tetramethylsilane as internal standard. GC-Mass analysis was performed on a GC-Mass model: 5973 network mass selective detector, GC 6890 Agilent Technologies (USA).

Preparation of catalyst (SiO_2-Pr-SO_3H)

SiO_2-Pr-SO_3H was prepared according to our previous report (28) and was used as a solid acid catalyst in the following reaction.

General procedure for the synthesis of 2-pyridone derivatives 5a-5h

The SiO_2-Pr-SO_3H (0.02 g) was activated in vacuum at 100 °C and after cooling the catalyst to room temperature, Meldrum's acid **1** (0.43 g, 3 mmol), methyl acetoacetate **2** (0.32 mL, 3 mmol), aromatic aldehyde **3** (3 mmol), and ammonium acetate **4** (0.38 g, 5 mmol) were added. The mixture was heated under solvent-free condition at 140 °C for the time reported in Table 1. The progress of reaction was monitored by thin layer chromatography. The generated solid product was dissolved in hot ethanol, filtered for removing the catalyst, and then the filtrate was cooled to afford the pure product.

Spectral data

*4-(4-Nitrophenyl)-5-methoxycarbonyl-6-methyl-3,4-dihydropyridone (**5a**)*

Mp 210-212 °C; IR (KBr): 3375, 1697, 1638, 1517, 1349 cm^{-1}; ^{1}H NMR (500 MHz, D_2O): δ 8.14-8.16 (^{2}H, m, ArH), 7.56 (^{1}H, s, NH), 7.25-7.35 (^{2}H, m, ArH), 4.35 (^{1}H, d, J = 8.0 Hz), 3.66 (^{3}H, s, OCH_3), 3.01 (^{1}H, dd, J = 16.5 Hz, 8.0 Hz), 2.67 (^{1}H, dd, J = 16.5 Hz, 8.0 Hz), 2.45 (^{3}H, s, CH_3) ppm; ^{13}C NMR (125 MHz, D_2O): δ_C 19.3, 37.5, 37.9, 51.6, 105.8, 124.1, 127.6, 136.5, 140.2, 143.3, 147.2, 149.4, 166.7, 169.7; MS (EI, *m/z*): 290 (M^+).

*4-(3-Nitrophenyl)-5-methoxycarbonyl-6-methyl-3,4-dihydropyridone (**5b**)*

Mp 204-206 °C; IR (KBr): 3351, 1704, 1648, 1528, 1348 cm^{-1}; ^{1}H NMR (250 MHz, $CDCl_3$): δ 8.03-8.35 (^{3}H, m, ArH, NH), 7.24-7.73 (^{2}H, m, ArH), 4.34 (^{1}H, dd, J = 8.0 Hz, 1.5 Hz), 3.66 (^{3}H, s, OCH_3), 3.01 (^{1}H, dd, J = 16.4 Hz, 8.0 Hz), 2.66 (^{1}H, dd, J = 16.4 Hz, 1.5 Hz), 2.45 (^{3}H, s, CH_3) ppm; MS (EI, *m/z*): 290 (M^+).

*4-(4-Methoxyphenyl)-5-methoxycarbonyl-6-methyl-3,4-dihydropyridone (**5c**)*

Mp 188-190 °C; IR (KBr): 3217, 1691, 1631, 1249, 1085 cm^{-1}; MS (EI, *m/z*): 275 (M^+).

*4-Phenyl-5-methoxycarbonyl-6-methyl-3,4-dihydropyridone (**5d**)*

Mp 193-194 °C; IR (KBr): 3218, 1696, 1638, 1282, 1086 cm^{-1}; MS (EI, *m/z*): 245 (M^+).

*4-(4-Chlorophenyl)-5-methoxycarbonyl-6-methyl-3,4-dihydropyridone (**5e**)*

Mp 195-198 °C; IR (KBr): 3218, 1694, 1633, 1281, 1082 cm^{-1}; MS (EI, *m/z*): 279 (M^+).

Table 1. Synthesis of 3,4-dihydro-2-pyridones **5a-5h** under optimized conditions.

Entry	Aldehyde	Product	Time (min)	Yield (%)[a]	M.p.[b] (°C)	M.p.[c]
1	4-$NO_2C_6H_4$	**5a**	45	89	210 - 212	210 - 211 (34)
2	3-$NO_2C_6H_4$	**5b**	40	90	204 - 206	205 - 206 (35)
3	4-$OCH_3C_6H_4$	**5c**	60	78	188 - 190	187 - 188 (33)
4	Ph	**5d**	32	93	193 - 194	197 - 198 (33)
5	4-ClC_6H_4	**5e**	25	93	195 - 198	198 - 200 (34)
6	2,4-$(OCH_3)_2C_6H_3$	**5f**	30	89	140 - 141	136 - 139 (35)
7	2,4-$Cl_2C_6H_3$	**5g**	25	90	203 - 206	204 - 206 (35)
8	2-$OCH_3C_6H_4$	**5h**	35	88	202 - 205	206 - 208 (35)

(a), Isolated yields; (b), melting point; (c), as reported in the literature.

*4-(2,4-Dimethoxyphenyl)-5-methoxycarbonyl-6-methyl-3,4-dihydropyridone (**5f**)*

Mp 140-141 °C; IR (KBr): 3225, 1694, 1611, 1260, 1036 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$): δ 8.20 (^1H, br s, NH), 7.26 (^1H, s, ArH), 7.04 (^1H, d, J = 9.25 Hz, ArH), 6.86 (^1H, d, J = 5.25 Hz, ArH), 4.63 (^1H, dd, J = 8.3 Hz, 1.9 Hz), 3.75 (^3H, s, OCH_3), 3.70 (^3H, s, OCH_3), 3.46 (^3H, s, OCH_3), 2.80 (^1H, dd, J = 16.5 Hz, 8.3 Hz), 2.42 (^1H, dd, J = 16.5 Hz, 1.9 Hz), 1.50 (^3H, s, CH_3) ppm; MS (EI, *m/z*): 305 (M^+).

*4-(2,4-Dichlorophenyl)-5-methoxycarbonyl-6-methyl-3,4-dihydropyridone (**5g**)*

Mp 203-206 °C; IR (KBr): 3229, 1705, 1642, 1591, 800 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$): δ 8.79 (^1H, br s, NH), 7.40 (^1H, s, ArH), 7.14 (^1H, d, J = 7.5 Hz, ArH), 6.96 (^1H, d, J = 8.25 Hz, ArH), 4.64 (^1H, dd, J = 8.3 Hz, 1.9 Hz), 3.60 (^3H, s, OCH_3), 2.94 (^1H, dd, J = 16.5 Hz, 8.3 Hz), 2.78 (^1H, dd, J = 16.5 Hz, 1.9 Hz), 2.45 (^3H, s, CH_3) ppm; MS (EI, *m/z*):313 (M^+).

*4-(2-Methoxyphenyl)-5-methoxycarbonyl-6-methyl-3,4-dihydropyridone (**5h**)*

Mp 202-205 °C; IR (KBr): 3240, 1699, 1634, 1245, 1051 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$): δ 8.15 (^1H, br s, NH), 7.26 (^1H, d, J = 10 Hz, ArH), 7.19 (^1H, d, J = 7.75 Hz, ArH), 6.79-6.96 (^2H, m, ArH), 4.57 (^1H, dd, J = 8.3 Hz, 1.9 Hz), 3.83 (^3H, s, OCH_3), 3.60 (^3H, s, OCH_3), 2.87 (^1H, dd, J = 16.5 Hz, 8.3 Hz), 2.77 (^1H, dd, J = 16.5 Hz, 1.9 Hz), 2.42 (^3H, s, CH_3) ppm; MS (EI, *m/z*): 275 (M^+).

Urease inhibitory assay

Urease (EC 3.5.1.5) from Jack beans and sodium nitroprusside were purchased from Sigma (USA). Ultra-pure water, (HPLC grade, Duksan, Korea) was used throughout the experiments. Measuring the release of ammonia by a modification of the Berthelot reaction determined the urease inhibitory activity of the synthesized compound (29-30). The reaction mixture consisted of urea (850 µL, 25 mM), 15 µL of urease enzyme solution (2 mg/mL) and 135 µL of the test compounds of various concentrations in 100 mM sodium phosphate buffer (pH 7.4). Mixture was pre-incubated for 30 minutes in water bath at 37 °C. After pre-incubation, 100 µL of mixture were mixed with 500 µL of phenol reagent (contained 2.5 mg of sodium nitroprusside and 0.5 g phenol in 50 mL of distilled water) and 500 µL of alkaline reagent containing 820 µL of sodium hypochlorite 5% and 250 mg sodium hydroxide in 50 mL of distilled water. After further 30 min incubation at 37 °C, absorbance was measured at 625 nm.

Data processing

The inhibition percentage (I (%)) was calculated by the following equation:

$$I\ (\%) = 100 - ((A_{INH}/A_B) \times 100)$$

where, A_{INH} is the absorbance of the tested sample and A_B is the absorbance of the solvent in the presence of enzyme. The IC_{50} values were calculated using GraphPad Prism 6 software. All experiments were performed in triplicate and hydroxyurea was used as the standard compound which is already confirmed to have significant inhibitory characteristics for urease.

Molecular docking

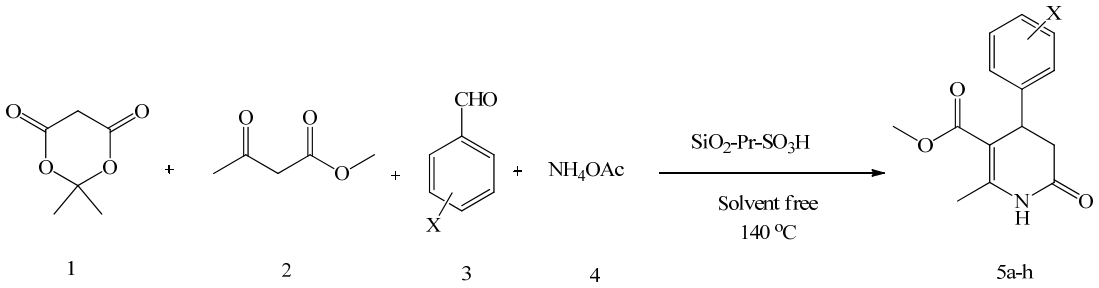
Eight compounds have sketched by Marvin sketch applet (Marvin package, Chemaxon Company, Hungary). Afterward polar hydrogens and rotatable bonds was added with AutoDockTools 1.5.6 (ADT). The crystal structure of *H. pylori* urease enzyme with resolution of 2.05 Å was downloaded from the protein data bank (3LA4, http://www.pdb.org) and was used for docking studies (31,32). In the present study, the metal ions and non-standard protein residues (KCX and CME) were contained within the binding site specification but before initiating the docking simulations, all ligands and all water molecules were removed from urease structure file with ADT. A grid map was used consisted of 70 × 70 × 70 Å points around the active site and calculated by AutoGrid 4.2. The center of the grid was set to the mean coordinates of the two Ni^{2+} ions in the α chain of the urease enzyme. Docking with a maximum number of 25×10^6 energy evaluations were performed by AutoDock 4.2. The other docking parameters were set to default values. Once clustering analysis was performed, selection of the conformation based on the most favorable binding energy was done.

RESULTS

In the present work, we explored the catalytic activity of SiO_2-Pr-SO_3H toward the clean one-pot synthesis of pyridone derivatives (Scheme 1). At first, the effect of different solvents on the reaction times and yields of the product **5d** was examined. For this purpose, the model reaction of Meldrum's acid **1**, methyl acetoacetate **2**, benzaldehyde **3**, and ammonium acetate **4** was investigated in various solvents such as H_2O, MeCN, EtOH, EtOH/H_2O (1:1), and solvent-free system (Table 2). It was found that solvent-free medium was the most effective condition in terms of reaction time (32 min) and yield of compound **5d** (93%). To determine the optimum temperature, we investigated the model reaction at room temperature, 50 ºC, 140 ºC, and the best result was obtained at 140 ºC. Then, in regard to library construction, we extended our study with different substituted aldehydes under solvent-free condition at 140 °C in the presence of a catalytic amount of SiO_2-Pr-SO_3H. The results are summarized in Table 1. A series of pyridine derivatives were successfully synthesized in high yields and appropriate times. It was reported that this reaction could be observed when the aliphatic aldehydes were used as starting materials (33). The structures of some products were characterized by spectral analysis and melting points were compared with values reported in the literature (Table 1).

This reaction may occur via a condensation, addition, cyclization, and elimination mechanism (Scheme 2). After protonation of carbonyl groups of Meldrum's acid and benzaldehyde derivatives by the solid acid catalyst, a Knoevenagel condensation occurs between Meldrum's acid and the corresponding benzaldehyde to afford the intermediate **6.** Then, intermediate **6** undergoes a Michael-type addition with enamino compound **7** (resulting from the reaction of ammonia and methyl acetoacetate). Finally, intramolecular cyclodehydration in intermediate **8** provides the desired product **5**.

The NMR experiment also confirmed the formation of the pyridone rings. The ^{1}H NMR spectrum of compound **5b** shows the two protons on C-3 as a part of an ABX system which was confirmed by a doublet of doublet at δ = 4.34 corresponding to the proton on C-4 due to the splitting by coupling with the protons on C-3 (J = 8.0 Hz and J = 1.5 Hz). Due to the greater acidity of Meldrum's acid (pK_a = 9.97) in comparison with methyl acetoacetate (pK_a = 11.0), we do not obtain 1,4-dihydropyridines.



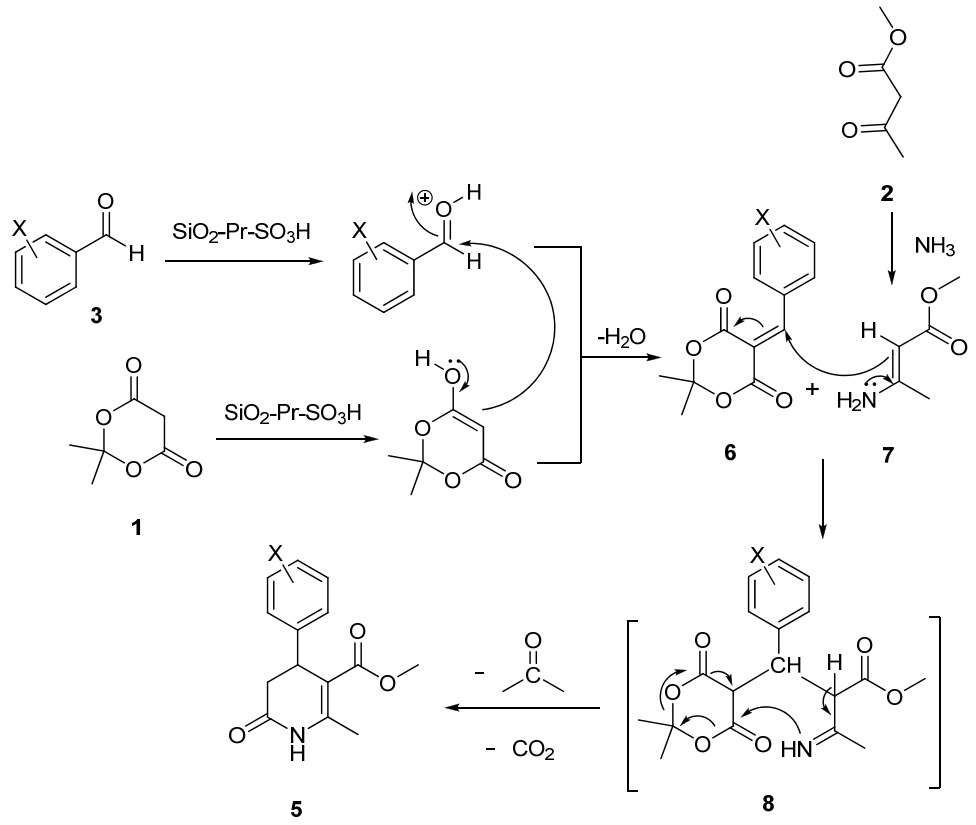
X = H, 4-NO_2, 3-NO_2, 4-OMe, 2-OMe, 4-Cl, 2,4-$(OMe)_2$, 2,4-Cl_2

Scheme 1. Four-component synthesis of 3,4-dihydro-2-pyridone derivatives in the presence of SiO_2-Pr-SO_3H.

Table 2. Effect of different solvents for the yield of compound **5d**.

Entry	Solvent	Temperature (ºC)	Time (min)	Yield (%)[a]
1	H_2O	Reflux	200	85
2	EtOH/H_2O (1:1)	Reflux	230	90
3	MeCN	Reflux	140	75
4	EtOH	Reflux	150	72
5	-	140 °C	32	93

(a) Isolated yields.



Scheme 2. The possible mechanism for the preparation of compounds **5a-5h** in the presence of SiO_2-Pr-SO_3H.

Table 3. Comparison of SiO_2-Pr-SO_3H and various catalysts in the synthesis of **5d**.

Entry	Catalyst	Solvent	Condition	Time (min)	Yield (%)	Year
1	-	EtOH	Reflux	360	26	1990 (36)
2	-	AcOH	Reflux	600	65	1996 (37)
3	-	-	Microwave	15	86	2003 (35)
4	polyphosphoric acid (2-3 drops)	-	Microwave	5	88	2008 (33)
5	-	AcOH	Ultrasound	12	85	2011 (34)
6	SBA-Pr-SO_3H (0.02 g)	-	Heating (140 °C)	40	90	2013 (38)
7	SiO_2-Pr-SO_3H (0.02 g)	-	Heating (140 °C)	32	93	This work

Table 3 illustrates a comparison of the effectiveness of various catalysts used in the synthesis of 2-pyridone derivatives. The results illustrates that SiO_2-Pr-SO_3H provided an efficient route to access these compounds. Because of green chemistry viewpoint, the reusability of the catalyst was investigated under optimized conditions for the synthesis of the model compound **5d**.

The process of recycling was completed four times and no significant decrease in activity was observed. The yields for the four runs were found to be 93, 87, 81, and 76%, respectively.

Eight 2-pyridinone derivatives were evaluated for their inhibitory activities against Jack bean urease which is similar to *H. pylori* urease in its structure. All of these compounds showed good activity against urease and four of test compounds (**5a**, **5b**, **5e**, and **5g**) showed potent urease inhibitory activities, in comparison to hydroxyurea as a standard inhibitor with IC_{50} equal to 100 μM (Table 4).

Compound **5a** showed the most potent inhibitory activity against urease (IC_{50} = 29.12 μM). Compounds containing nitro group and chlorine showed greater inhibitory. This result indicated that the inhibitory activities increased with the increase of electron withdrawing ability of the groups despite a less important role of lipophilicity in increasing the inhibitory activity.

Table 4. Inhibitory concentration, binding energy and two-dimensional interaction of 3,4-dihydro-2-pyridone derivatives with urease active site.

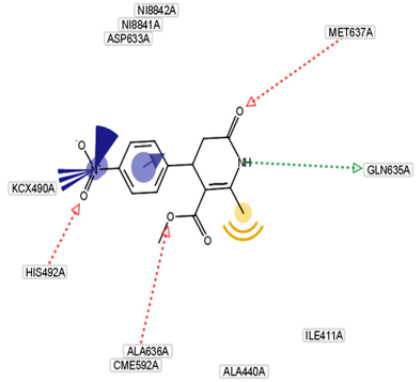
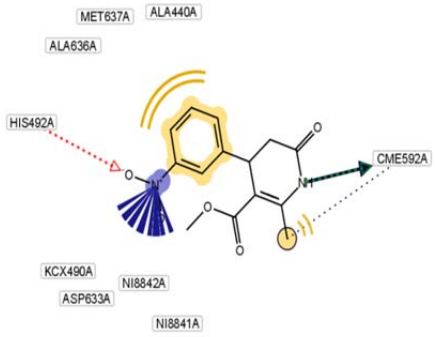
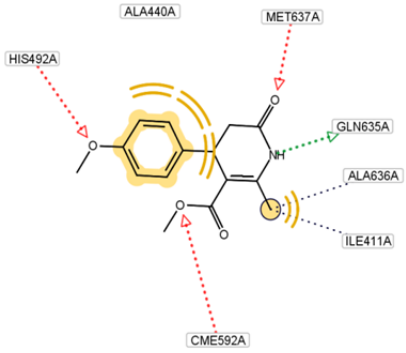
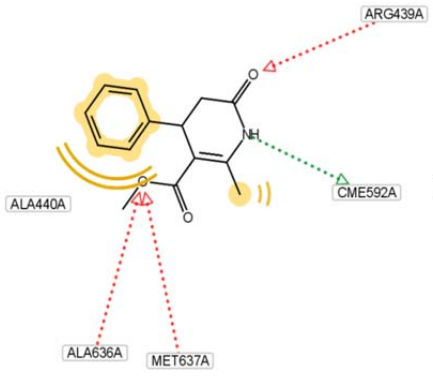
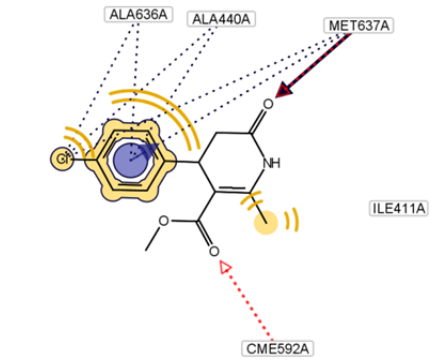
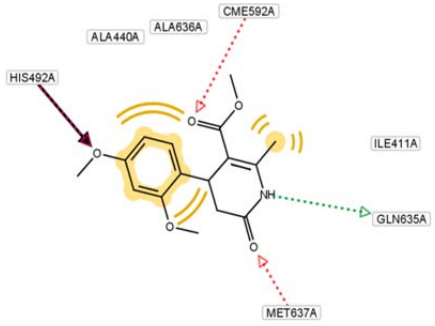
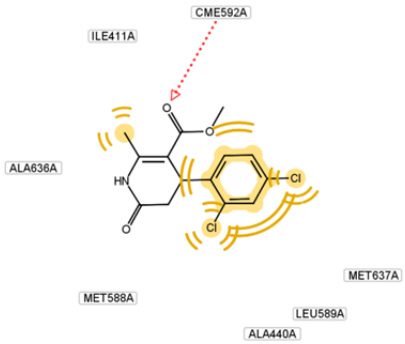
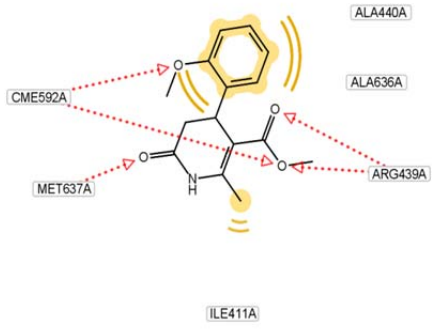
Product	Structure	IC_{50} (µM)	Docking energy (kcal/mol)	KI (µM)
5a		29.12	-6.5	17.17
5b		48.84	-5.97	42.3
5c		119.9	-4.21	455.41
5d		131.3	-4.64	395.83

Table 4. (Continued)

Product	Structure	IC_{50} (µM)	Docking energy (kcal/mol)	KI (µM)
5e		84.08	-4.35	293.22
5f		102.5	-4.13	419.27
5g		81.99	-4.65	348.37
5h		158.5	-4.18	445.04

DISCUSSIONS

To investigate the binding effects between compound **5a** and the *H. pylori* urease the molecular docking study was performed. In the binding model nitro group coordinates with both nickel ions and also asparagine (ASP) 637 and KCX 490. Met 637, CME 592, and histidine (HIS) 492 formed hydrogen bonds and also oxygen of glutamine (GLN) 635 formed a hydrogen bond with pyridine NH.

According to docking studies Met 637 and nitrophenol ring causes π–π interaction between sulfur atom of amino acid and the ring. Moreover, hydrophobic interactions existed between compound **5a** and ALA 636, ALA 440, and isoleucine (ILE) 411.

Shifting of nitro group from para to meta position in compound **5b** caused less hydrogen bonds and therefore less inhibitory activity (IC_{50} = 48.84 μM) compare to compound **5a**. This is probably because of different molecule orientation in active site.

In compound **5e**, which nitro group substituted with Cl result in just two hydrogen bonds with MET 637 and CME 592. Cl and ALA 636 showed hydrophobic interactions and also an n-π interaction between ring and MET 637 (IC_{50} = 84.08 μM). Adding another Cl to ortho position of the ring in compound **5g** slightly improved inhibitory activity due to increase in hydrophobic interactions between two Cl and MET 588, ALA 440, leucine (LEU) 589, Met 637, and CME 592 (IC_{50} = 81.99 μM).

Although compound **5d** did not coordinate with nickel ions formed four hydrogen bonds with ALA 636, MET 637, CME 592, and arginine (ARG) 439 (IC_{50} = 131.3 μM). Methoxy group added to para position of the ring in compound **5c** (IC_{50} = 119.9 μM) and two methoxy group to para and ortho positions of ring in compound **5f** (IC_{50} = 102.5 μM) resulted in increasing hydrophobic interactions between the compounds and amino acids of the *H. pylori* urease.

The confirmation of compound **5h** in the active site of urease led to less inhibition activity (IC_{50} = 158.5 μM) in comparison to other compounds.

CONCLUSIONS

In conclusion, we have demonstrated that SiO_2-Pr-SO_3H is an efficient catalyst for the synthesis of 3,4-dihydro-2-pyridone derivatives under solvent-free conditions. High yields of the products, short reaction times, and simplicity of the system make it an improved protocol in comparison with existing methods. We evaluated inhibitory activity of synthesized compounds through jack bean urease and all of them showed inhibitory activity against urease. Compound **5a** showed good inhibition compared to hydroxyurea.

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