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ORIGINAL PAPER

Silica-supported heterogeneous catalysts-mediated synthesis of chalcones as potent urease inhibitors: in vitro and molecular docking studies

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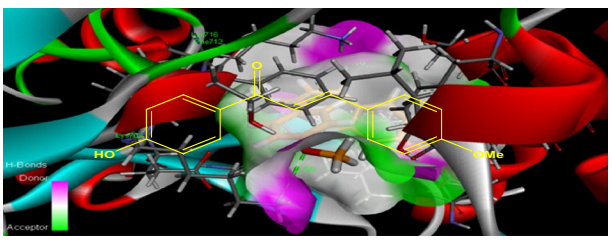
Abstract

We herein report a facile and high yielding protocol for silica-supported heterogeneous catalysts-mediated synthesis of chalcones. A comparison of results of our synthesis with conventional synthetic protocols is also being offered to assess the efficiency of the prepared catalysts. Biological evaluation of the newly synthesized compounds as urease inhibitors was performed. Most of the compounds were found to have potent urease inhibition activity. The chalcone 3-(3-hydroxyphenyl)-1-phenylpropenone was found to be the most potent with percentage inhibition 86.17 ± 0.89 and half maximal inhibitory concentration (IC_{50}) value 11.51 ± 0.03 µM. The molecular docking study emphasized that the same congeners 3-(furan-2-yl)-1-(4-hydroxyphenyl)propenone, 3-(4-hydroxyphenyl)-1-(4-methoxyphenyl)propanone, and 3-[4-(dimethylamino)phenyl]-1-(*p*-tolyl)propenone showed very good inhibitory potential against urease and show a higher docking scores 5718, 5940, 5596 and an ACE of − 246.66, − 244.79, and − 243.06 kJ/mol, respectively than the control ligand.

Graphic abstract



Keywords Heterogeneous catalyst · Ligand · Docking · Urease · Chalcones

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Introduction

Heterogeneous catalysts have been prepared by immobilization of transition metal complexes on various supports and the nature of action and mechanism of catalysis has been studied/developed [1]. The development of first mesoporous materials as heterogeneous catalysts in early 90 s led to extensive research which also triggered exploration of silica-supported catalysts. Silica (SiO_2) whether found naturally or synthesized artificially exists in variety of forms i.e., gels, crystalline, and amorphous. Each form exhibits different physicochemical properties. Because of its greater surface area, synthetic silica is extensively used as adsorbing material and as catalyst support. Silica has a tetrahedral geometry with each silicon atom bonded to four oxygen atoms and each oxygen atom being bound to two silicon atoms. Two types of functional group: silanol groups (Si–O–H) and siloxane groups (Si–O–Si) are present on the silica surface. All the chemical processes and even the physical processes like adsorption takes place on the silanol sites; the siloxane sites, which form the backbone of silica, remain inert to most of the activities [2]. The innate combination of inertness of siloxane group and activity of silanol groups in silica offers the advantages of chemical inertness and ease of its modification in the presence/influence of metals and organic substances. These properties make silica an attractive support for heterogeneous catalysis [3].

Chalcones (1,3-diaryl-2-propenones) and their derivatives have a wide range of biological activities, which include (but not limited to) anti-inflammatory [4, 5], antifungal [6], antioxidant [7, 8], antimalarial [9], antituberculosis [10], analgesic [11, 12], anti-HIV [13, 14], anticancer [15-17], antitumor [18] activities. These also act as inhibitors of α-amylase [19], acetylcholinesterase, butyrylcholinesterase, and lipoxygenase [20]. These secondary metabolites, along with their physiological importance, also serve as precursor/intermediates in organic synthesis of compounds of medicinal importance. Some examples of biologically active chalcones are shown in Fig. 1 [21–24].

Urease is a member of superfamily of enzymes amidohydrolases and phosphotriesterases. These are high molecular weight nickel-based metalloenzymes, which can be found in bacteria, fungi, algae, plants, certain invertebrates, as well as in soil where these biological catalysts mediate the hydrolysis of urea into CO_2 and NH_3. The presence of ammonia in animals and human is considered harmful, because of its toxic effect. Ureases, because of their role in the production of ammonia, have been found to be associated with the development of a number of physiological disturbances such as pyelonephritis, urolithiasis, hepatic coma, hepatic encephalopathy, and urinary catheter encrustation [25].

The use of metals in heterogenous catalysis has been widely reported. The supported metal based heterogeneous catalysts, owning to their selectivity and higher turnover rates, hold special place in the field of heterogenous catalysts. The catalytic activity of these supported catalysts is dependent upon sizes of metallic species, interaction of the metallic species with supports, substrates, and solvents [26–30]. In continuation with our previous studies toward exploration of silica-supported heterogeneous catalysts [31], the presented work is concerned with preparation of various silica-supported transition metals based heterogeneous catalysts and evaluation of their effectiveness as catalyst for synthesis of chalcones. Since urease inhibitory activity of chalcones is still a neglected field, the synthesized compounds were evaluated for their in vivo and in silico anti-urease potential.

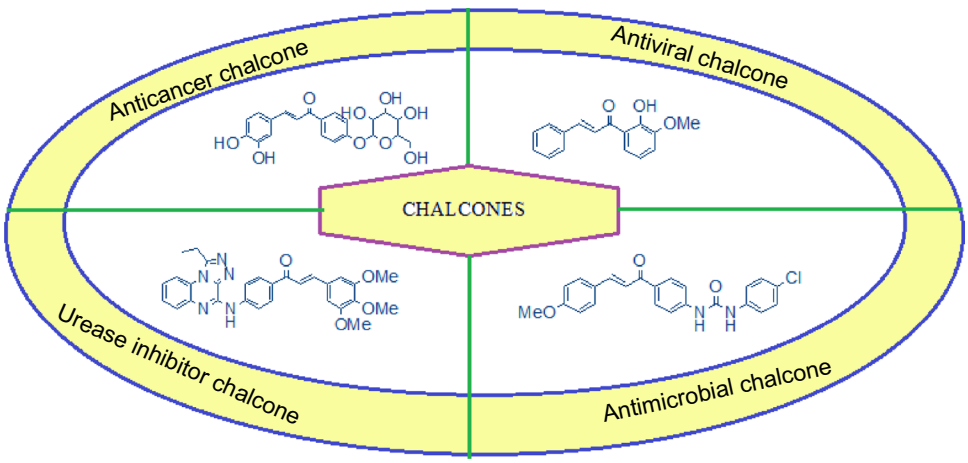


Fig. 1 Some examples of biologically active chalcones

Results and discussion

Synthesis

The metal immobilized over silica was prepared by stirring metal halides ($NiCl_2$, $FeCl_3$, $ZnCl_2$, $CuCl_2$) with silica using dry ethanol as solvent at room temperature for 4 h followed by evaporating the solvent to dryness under reduced pressure and the dispersed solid solution was then heated at 100 °C for 1 h under reduced pressure to afford active silica-supported metal catalysts [32]. The effectiveness of these silica-supported transition metal catalysts was evaluated by carrying out a model reaction of condensation of benzaldehyde and acetophenone under solvent-free conditions. To confirm the role of catalyst in this condensation reaction, the same model/control experiment was carried out in the absence of any catalyst and using H_2SO_4 as a catalyst as well. The findings of model reaction are summarized in Table 1, which clearly indicate that catalysts do have a role in the reaction. Absence of any catalyst does not lead to formation of product in significant yield.

The conventional H_2SO_4 used in the condensation reaction did not yield product in reasonable amount. The same model reaction when tried with SiO_2–H_2SO_4 yielded desired chalcone **3** in excellent yield (94%). Metal based catalysts also afforded better yields as compared with H_2SO_4; however, their yields were lower than that for SiO_2–H_2SO_4. All heterogeneous catalysts (SiO_2–H_2SO_4 and metal based catalysts) could be recycled by filtering them from the reaction mixture and then washing the solid reagent by ethyl acetate followed by drying in an oven at 100 °C for 30 min. We believe that catalytic effect of SiO_2–MCl_x catalysts (M=Fe, Ni, Cu, Zn; $x = 2, 3$) is due to their ability to act as Brønsted acid (due to presence of Si–OH groups), as well as Lewis acid (due to presence of empty π orbital of transition metals), whereby these polarizes the C=O of aldehyde via an acid–base interaction.

Table 1 Evaluation of effectiveness of catalysts for synthesis of chalcone **3**

Entry	Reagent/catalyst	Solvent	Time/min	Product (yield/%)
1	None	–	210	**3** (< 10)
2	None	Dry EtOH	180	**3** (15)
3	H_2SO_4	Dry EtOH	150	**3** (45)
4	SiO_2–H_2SO_4	–	30	**3** (92)
5	SiO_2–$FeCl_3$ (10 mol%)	–	30	**3** (96)
6	SiO_2–$NiCl_2$ (10 mol%)	–	45	**3** (88)
7	SiO_2–$CuCl_2$ (10 mol%)	–	50	**3** (72)
8	SiO_2–$ZnCl_2$ (10 mol%)	–	45	**3** (78)

Recycling test

We have studied the recycling efficiency of the catalysts, i.e. whether the catalysts can be reused further for several cycles. To test the catalytic efficiency for further cycles, a control experiment was performed using benzaldehyde and acetophenone. After each reaction cycle, the catalysts were recovered by filtration, washed thoroughly with acetonitrile, and then treated with 0.1 M HCl solution in ethanol at 338 K for 8 h for regeneration and finally dried at 373 K for 2 h. The catalytic reactions have been carried out following the same experimental procedure as that with the original catalysts. The catalytic activity decreased very slightly in the successive catalytic cycles until six control reactions. After six cycles a very pronounced decreased in catalytic activity was observed.

After successful model studies, the silica-supported heterogeneous catalysts were then evaluated for their effectiveness toward different substituted acetophenones (PhAc **1**) and aldehydes (ArCHO **2**). Significantly lower yields were observed for H_2SO_4 mediated reaction of substituted PhAc **1** and ArCHO **2**. Although SiO_2-MCl_x mediated reactions afforded products with significant yields; however, the yields were quite lower for chalcones bearing nitro, hydroxy, and/or amino groups. In case of silica-sulfuric acid, the yields were quite high even for compounds with aforementioned substituents (Table 2).

It is quite clear from Table 2 that SiO_2–$FeCl_3$ is the most efficient and effective catalyst for synthesis of chalcones with different substituent. SiO_2–H_2SO_4 is quite effective catalyst, but low yields are obtained with this catalyst when OH and or NMe_2 substituent are present. SiO_2–$NiCl_2$, SiO_2–$CuCl_2$, and SiO_2–$ZnCl_2$ also gave reasonable yields, however, with OH and NMe_2 substituent, yields were significantly lower. We suggest that lower yields with these substituents are due to possible complexation of catalysts with O and N acting as strong donors.

Urease inhibition study

The biological activity of the chalcones **3a**–**3i** toward urease inhibition was determined using Berthelot assay [33]. Thiourea was used as positive control. In the present study, a series of synthesized chalcones **3a**–**3i** have been tested for urease inhibition activity (Table 3). The inhibition pattern of this series found to be **3f > 3e > 3i > 3 g > 3b > 3a > 3h > 3c > 3 d** and according to substituents 4′-OH–, 4-OCH_3–> 4′-OH–, 2-furyl–> 4′-CH_3–, 4-$(CH_3)_2$NPh–> 4′-CH_3–, Ph–> 3′-OH–, Ph–> H–, Ph–> 4′-CH_3–, 2-furyl–> 3′-OH–, 2-furyl–> 4′-OH–, Ph–. The studies showed that 4′-OH–,

Table 2 Comparison of effect of catalysts/reagents on yield of different substituted chalcones **3a**–**3i**

Compound	R–	Ar–	Yield/%					
			H_2SO_4	SiO_2–H_2SO_4#	SiO_2–$FeCl_3$^	SiO_2–$NiCl_2$^	SiO_2–$CuCl_2$^	SiO_2–$ZnCl_2$^
3a	H–	Ph-	45	92	96	88	72	78
3b	3′-OH-	Ph-	21	90	92	80	56	59
3c	3′-OH-	2-furyl-	34	79	94	72	46	44
3d	4′-OH-	Ph-	47	86	93	75	48	52
3e	4′-OH-	2-furyl-	52	84	97	82	43	57
3f	4′-OH-	4-OMe-	58	90	95	86	52	61
3g	4′-Me-	Ph-	56	86	98	91	68	72
3h	4′-Me-	2-furyl-	52	97	96	95	72	76
3i	4′-Me-	4-Me_2NPh-	< 10	84	88	82	34	45

1.5 equivalent to acetophenone, 5 h reflux in methanol; #SSA heating at 65 °C for 1.5 h under solvent-free conditions; ^rt, 70–110 min, solvent-free conditions

4-OCH_3– (**3f**, IC_{50}, 11.51 ± 0.03 µM) and 4′-OH–, 2-furyl– (**3e**, IC_{50}, 14.62 ± 0.01 µM) are the most potent compounds against urease even better than the standard thiourea. The four compounds 4′-CH_3–, 4-$(CH_3)_2$NPh– (**3i**), 4′-CH_3–, Ph– (**3g**), 3′-OH–, Ph– (**3b**) and H–, Ph– (**3a**) have shown moderate enzyme inhibitions with IC_{50} values ≤ 28 µM with the following order of decreasing activity as 4′-CH_3–, 4-$(CH_3)_2$NPh– > 4′-CH_3–, Ph– > 3′-OH–, Ph– > H–, Ph–. However, compounds 4′-CH_3–, 2-furyl– (**3h**), 3′-OH–, 2-furyl– (**3c**) and 4′-OH–, Ph– (**3d**) have exhibited weak inhibitions.

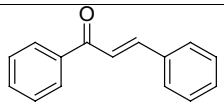
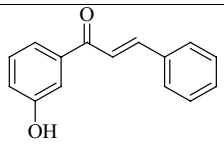
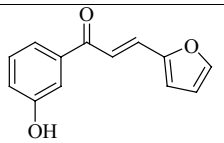
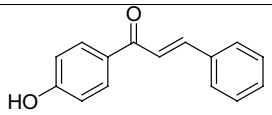
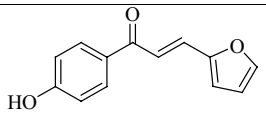
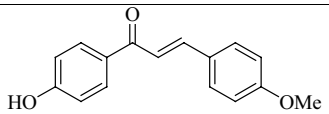
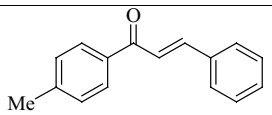
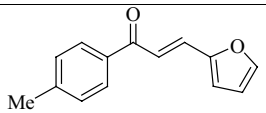
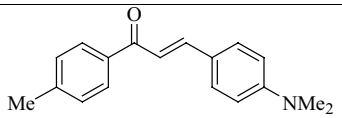
It is important to note that mild electron donating groups increase the urease inhibition. However, the size and the position of the substituents also matter. In *para* substituted ligands, the observed order of their activity has been found as follows 4′-OH–, 4-OCH_3– > 4′-OH–, 2-furyl– > 4′- CH_3–, 4-$(CH_3)_2$NPh– > 4′-CH_3–, Ph– > 4′-CH_3–, 2-furyl– > 4′-OH–, Ph–. Similarly, among the *meta* substituted ligands, 3′-OH–, Ph– (**3b**) has shown more inhibition of urease as compared to 3′-OH–, 2-furyl– (**3c**). On the other hand, electron withdrawing groups e.g., OH- and CH_3-groups, especially, at *meta* positions were found to reduce the urease inhibition. Though, it is critical to establish structure–activity relationship of the studied chalcones **3a**–**3i** possibly, owing to the series of factors, i.e., size, shape, polarizability, and electronegativity of a ligand playing essential function in enzyme inhibition.

Molecular docking studies

The X-ray crystallographic structure of jack bean urease protein was retrieved from RCSB Protein Data Bank (PDB ID 4H9M). The preparation of protein structure was done by deletion of water molecules, cofactor, and co-crystallized ligands by utilizing Discovery Studio 4.5 Visualizer. Ligands **3a**–**3i** and standard thiourea were docked with jack bean urease (4H9M) via PatchDock [34]. PatchDock affords the top 20 solutions and "solution 1" was picked as binding pocket for docking analyses, as it is surrounded by most significant residues designated in crystal structure of jack bean urease receptor (4H9M) [35]. The docked structures were inspected using Discovery Studio 4.5 Visualizer.

The binding affinities of the docked ligands were evaluated as scores and ACE (atomic contact energy) of the docked complexes. The hydrogen bonding and hydrophobic interactions of each ligand were evaluated within binding pocket of receptor protein. The conformation of the ligands which illustrated the highest biological activities is showed in Table 4, Figs. 2, 3, 4, 5 with their favorable contacts in the binding pockets of enzyme. To get qualitative evaluation and to recognize molecular basis of the calculated biological activities (IC_{50}), the docked complexes of ligands **3a**–**3i** and standard thiourea were investigated. Standard thiourea was also docked with jack bean urease for comparison and has illustrated score 1607 with an ACE value − 106.77 kJ/mol (Fig. 2). Thiourea has showed hydrophobic contact potential with pocket amino acids Val^{416}, Tyr^{410}, Met^{453}, Ser^{456}, Pro^{413}, Leu^{460}, Thr^{457}, Phe^{464}. In the beginning, assessment of the docked complexes of jack bean urease with **3a**–**3i** disclosed that ligands **3e**, **3f**, and **3i** exhibited important interaction patterns. Visual scrutiny of these complexes predicts major interactions between a binding conformation of ligand **3f** with jack bean urease as compared to the other eight ligands; all ligands showed superior interactions than standard thiourea. Ligand **3f** showed most potent interaction with jack bean urease with a score of 5940 and an ACE of − 244.79 kJ/mol. The interacting residues of this complex are Lys716, Lys709 (Fig. 5, Table 5). Ligand **3f** has shown a potential hydrogen bond between carbonyl directly attached with 4-hydroxyphenyl group and amino of Lys^{716} (1.98 Å), while second hydrogen bond has formed between 4-methoxyphenyl group and amino of Lys^{709} (2.56 Å)

Table 3 Determination of IC_{50} values of urease inhibition of chalcones **3a**–**3i**

S. No.	Comp.	Structure	Conc. /mM	Inhibition /%	IC_{50} /μM[a]
1	**3a**		0.25	81.59±0.91	28.41±0.09
2	**3b**		0.25	81.85±0.86	27.11±0.03
3	**3c**		0.25	61.29±0.72	58.81±0.11
4	**3d**		0.25	58.75±0.36	74.35±0.19
5	**3e**		0.25	83.42±0.92	14.62±0.01
6	**3f**		0.25	86.17±0.89	11.51±0.03
7	**3g**		0.25	84.61±0.51	25.78±0.11
8	**3h**		0.25	56.58±0.41	35.44±0.11
9	**3i**		0.25	81.81±0.81	23.52±0.01
Thiourea	Std.		0.25	98.21±0.18	21.25±0.15

[a]Results are the mean of three independent experiments ($n=3$) ± S.D

indicating important interaction. Similarly, ligand **3f** exhibited hydrophobic contact potential with pocket amino acids Glu^{718}, Asp^{730}, Pro^{717}, Glu^{742}, Val^{36}, Thr^{33} and also depicted π-stacking contact potential with Phe^{712} amino acid.

To explain the structural elements responsible for the indicative inhibitory effect against the binding site of jack bean urease, the most active ligand **3f** bound to protein was subjected to binding affinity assessment using hydrogen bond donor–acceptor surface of Discovery Studio 4.5 Visualizer software. It permits diagrammatic estimation of favorable and unfavorable supports as a result of the structure/bound conformation of inhibitor with the neighboring amino acids. The favorably causative structural elements (atoms and torsions) on the whole binding energy are visually color in green, likewise the structural elements that are not contributing favorably are colored in pink, and neutral elements are in white (Fig. 3). The aromatic phenyl moieties 4-methoxyphenyl and 4-hydroxyphenyl ring are contributing favorably to the binding energy. The only unfavorable structural element is the ethylenic scaffold. This lead to the supposition that if phenyl ring is substituted by some other atoms, i.e., carbon or other heteroatoms, it may be a reason for even better binding affinity and thereby showing increased anti-urease activity.

Table 4 Docking results for the highest ranked biologically active complexes (urease inhibition)

Compd. Code	IC_{50}/μM	Score	ACE/kJ mol^{-1}	Amino acids show hydrogen bond contacts	Distance/Å	Amino acids show hydrophobic contacts	Amino acids show π-stacking contacts
3a	28.41 ± 0.09	4700	− 684.62	Lys^{716}, Lys^{716}	2.29, 2.49	Ala^{37}, Thr^{33}, Lys^{709}, Glu^{718}, Asp^{730}, Glu^{742}	Phe^{712}
3b	27.11 ± 0.03	4656	− 739.02	Lys^{716}, Glu^{742}	2.93, 3.08, 3.03	Asp^{730}, Glu^{718}, Lys^{709}, Tyr^{32}, Thr^{33}	Phe^{712}
3c	58.81 ± 0.11	4492	− 670.78	–	–	Thr^{33}, Lys^{745}, Val^{744}, Glu^{742}, Pro^{717}, Glu^{718}, Lys^{709}	Phe^{712}
3d	74.35 ± 0.19	4708	− 699.40	Lys^{709}	2.64	Glu^{742}, Thr^{33}, Tyr^{32}, Phe^{712}	–
3e	14.62 ± 0.01	5718	− 1032.02	Lys^{716}, Lys^{716}	2.70, 2.48	Lys^{709}, Glu^{718}, Asp^{730}, Glu^{742}, Thr^{33}	Phe^{712}
3f	11.51 ± 0.03	5940	− 1024.20	Lys^{716}, Lys^{709}	1.98, 2.56	Glu^{718}, Asp^{730}, Pro^{717}, Glu^{742}, Val^{36}, Thr^{33}	Phe^{712}
3g	25.78 ± 0.11	4702	− 594.84	–	–	Glu^{718}, Pro^{717}, Asp^{730}, Lys^{716}, Glu^{742}, Val^{36}, Thr^{33}, Tyr^{32}	Phe^{712}
3h	35.44 ± 0.11	4572	− 647.18	–	–	Met^{746}, Lys^{745}, Tyr^{32}, Glu^{742}, Pro^{743}, Glu^{718}	–
3i	23.52 ± 0.01	5596	− 1016.96	–	–	Leu^{839}, Thr^{33}, Tyr^{32}, Phe^{712}, Lys^{745}, Glu^{742}, Ala^{16}, Pro^{743}, Ala^{37}, Leu^{13}	–
Std Thiourea	21.25 ± 0.15	1600	− 433.92	–	–	Val^{416}, Tyr^{410}, Met^{453}, Ser^{456}, Pro^{413}, Leu^{460}, Thr^{457}, Phe^{464}	–

No. Specific code assigned to compound, *ACE* Atomic contact energy determined by PatchDock (kJ/mol), IC_{50} experimental calculation of inhibitory constant (μM), *Distance* hydrogen bond length analyzed from docked pose via ligand interaction tool of PatchDock

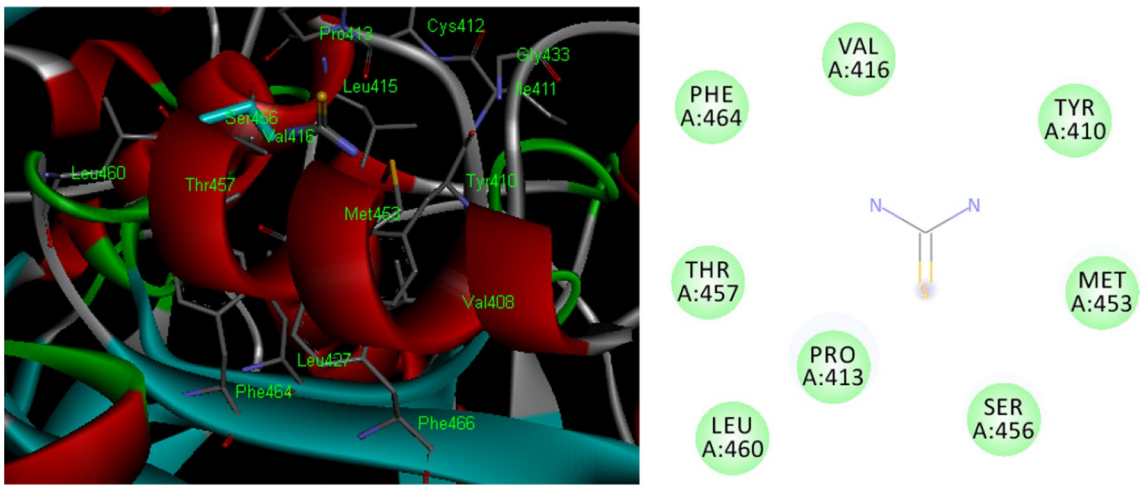


Fig. 2 Binding site interaction of standard thiourea 3D (left) and 2D (right)

Ligand **3e** showed a score of 5718 with jack bean urease and an ACE of − 246.66 kJ/mol. The interacting residues of this complex are Lys^{716}, Lys^{716} (Fig. 5, Table 5). Ligand **3e** has exposed a potential hydrogen bond between carbonyl directly attached with 4-hydroxyphenyl group and amino of Lys^{716} (2.48 Å), while second hydrogen bond has formed between the same carbonyl directly attached with 4-hydroxyphenyl group and hydrogen attached with amino group of Lys^{716} (2.70 Å). Ligand **3e** demonstrated hydrophobic contact potential with pocket amino acids Lys^{709}, Glu^{718}, Asp^{730}, Glu^{742}, and Thr^{33}. This complex also exhibited π-stacking contact potential with Phe^{712} amino acid.

However, ligand **3i** depicted score 5596 with an ACE value − 243.06 kJ/mol. Ligand **3i** exhibited no hydrogen

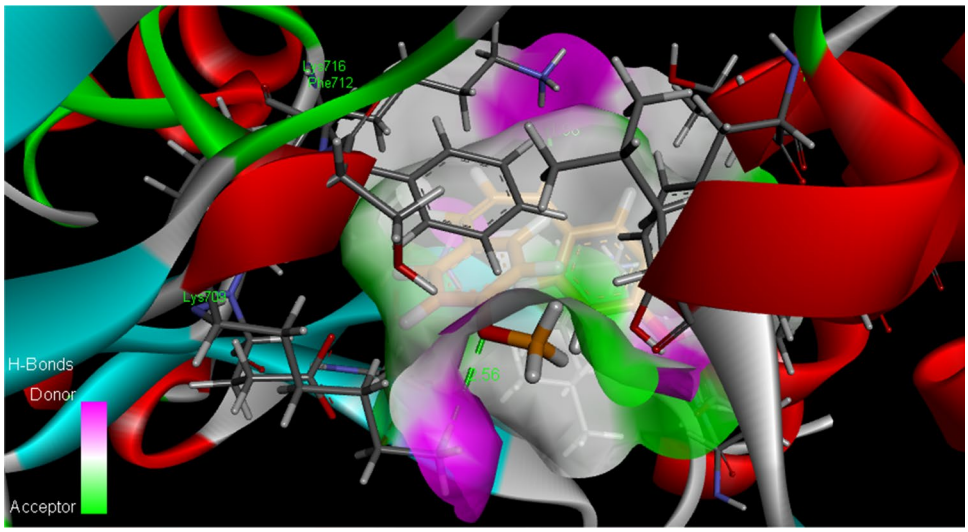


Fig. 3 Hydrogen bond donor–acceptor surface of most active inhibitor ligand **3f**

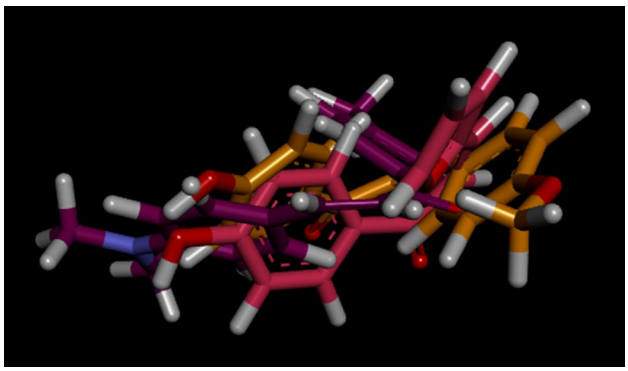


Fig. 4 Overlap of bound conformations of ligands **3e** (pink), **3f** (golden), and **3i** (purple) (color figure online)

bonding with jack bean urease receptor. It revealed potential for hydrophobic contact with pocket amino acids Leu[839], Thr[33], Tyr[32], Phe[712], Lys[745], Glu[742], Ala[16], Pro[743], Ala[37], Leu[13]. Hence, molecular docking studies of ligands **3a**–**3i** exhibited good urease inhibition activity and it is consistent with the in vitro results.

Conclusion

The current work dealt with a comparison of catalytic efficiency of conventional protocols and silica-based heterogenous catalysts for the synthesis of chalcones. A facile and solvent-free synthesis of SiO_2–H_2SO_4 and SiO_2–MCl_x catalysts was carried out and employed for preparation of variety of substituted chalcones. The findings suggest SiO_2–$FeCl_3$ should be further explored for its catalytic activity for broader range of substituent. However, SiO_2–H_2SO_4, SiO_2–$NiCl_2$, and SiO_2–$ZnCl_2$ gave significant lower yields with OH and NMe_2 substituent, while same issue was not observed in case of SiO_2–$FeCl_3$, which resulted in higher product yields as with these substituents.

Biological assessment of the newly synthesized compounds suggested that most of the compounds were found to have potent urease inhibition activity. The ligand **3f** was found to be the most effective ligand with percentage inhibition 86.17 ± 0.89 and IC_{50} value 11.51 ± 0.03 µM. The molecular docking study highlighted that the same ligands **3e**, **3f**, and **3i** illustrated high affinity to urease inhibition with higher docking scores than the control ligand thiourea. Ligands **3e**, **3f**, and **3i** have proved very good inhibitory potential against urease and showed higher docking scores 5718, 5940, 5596 and an ACE of − 246.66, − 244.79, and − 243.06 kJ/mol, respectively, than control ligand thiourea having score 1600 with an ACE value − 103.71 kJ/mol. This is consistent with the urease inhibition values (in vitro) of the ligands **3e**, **3f**, and **3i** even better than standard drug thiourea. In vitro and in silico results suggested that these ligands are excellent urease inhibitors that make them effective anti-urease agents.

Experimental

All chemicals used were purchase from Sigma, Pakistan. All solid reactants were recrystallized prior use. Purity of compounds was confirmed by thin layer chromatography (TLC) using Al-backed pre-coated silica gel with fluorescent indicator (0.25 mm thick layer). The spots were visualized under UV lamp ($\lambda = 365$ and 254 nm) of 8 W power as well as using $KMnO_4$ dip as universal locating agent. All synthesized products were purified either by column chromatography using silica gel as stationary phase (0.6–0.2 mm, 60 Å mesh size, Merck) or by crystallization. Melting points

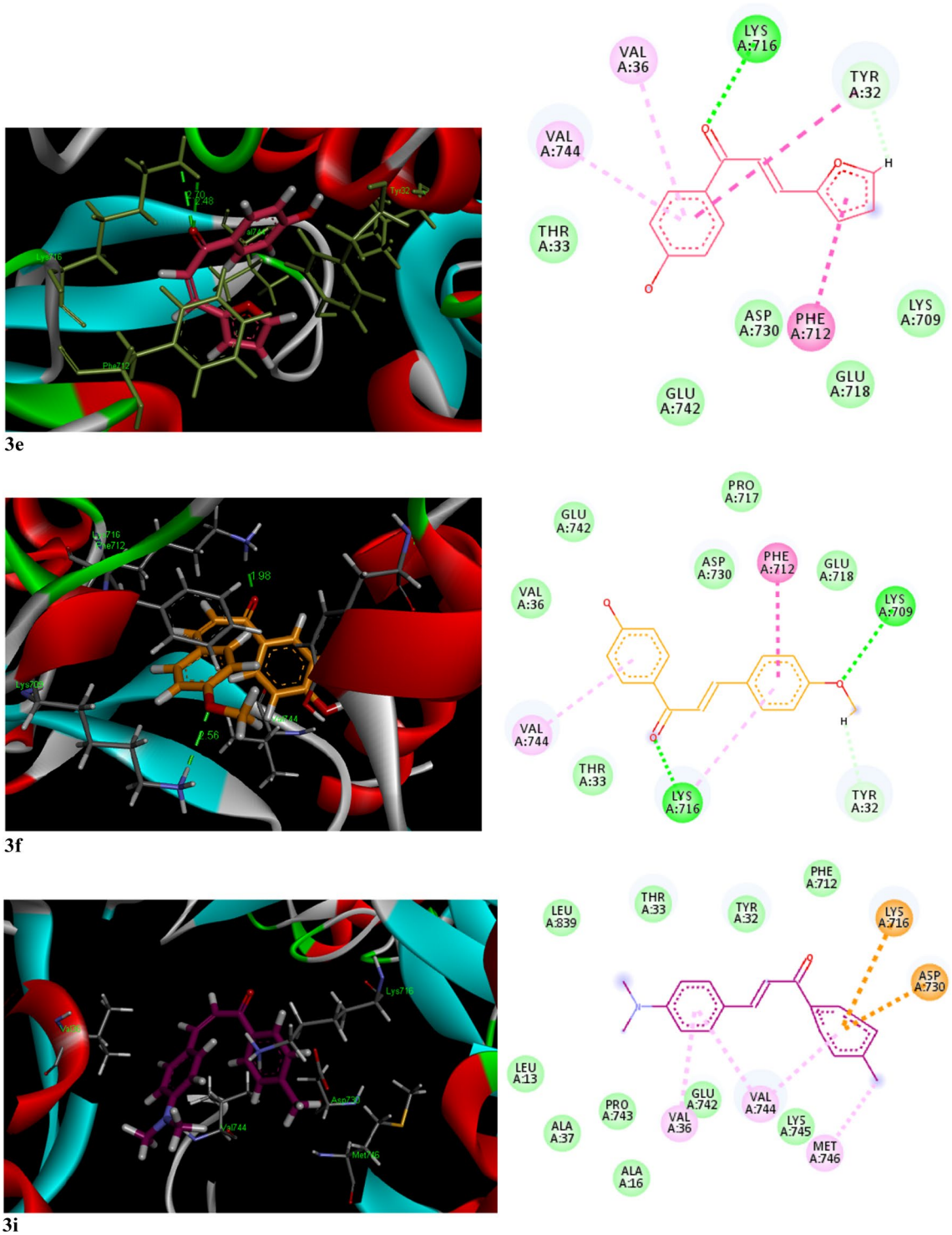


Fig. 5 2D representation of the docked complexes **3e**, **3f**, and **3i** within jack bean urease receptor pocket showing their polar interactions with the enzyme on right hand side and 3D poses of docked ligands **3e**, **3f**, and **3i** on left-hand side with PatchDock, showing unfavorable bump (red), carbon hydrogen bond (light green), π-alkyl (light pink), π-cation (brown), conventional hydrogen bond (green), and amide π-stack (pink) interactions (color figure online)

Table 5 Evaluation of reversibility of silica-supported catalysts

Entry	Catalyst	Cycle (yield/%)					
		Fresh	1	2	3	4	5
1	SiO_2–H_2SO_4	92	88	83	76	71	54
2	SiO_2–$FeCl_3$	96	91	83	78	73	64
3	SiO_2–$NiCl_2$	88	82	75	70	64	45
4	SiO_2–$CuCl_2$	72	66	60	53	48	36
5	SiO_2–$ZnCl_2$	78	72	67	58	51	48

were determined using Gallenkamp melting point apparatus (MF-8, Burladingen, Germany). The IR-spectra are recorded on Prestige 21 spectrophotometer (Shimadzu, Japan) in KBr discs. The LR-EIMS are carried out on a Fisons Autospec Mass Spectrometer (VG, New Jersey, USA). The ^{1}H NMR (400 MHz) and ^{13}C NMR (75 MHz) are recorded on Bruker, Massachusetts, USA in $CDCl_3$ using tetramethylsilane (TMS) as internal standard.

Silica-sulfuric acid

Sulfuric acid was added drop-wise to the well-stirred silica gel (SiO_2) and diethylether (Et_2O) suspension. The resulting reaction mixture was stirred for an hour followed by removal of solvent in vacuo. The solid thus obtained was heated in oven at 120 °C for 3 h to yield silica-sulfuric acid as a white solid [31].

Silica-supported metal catalysts

The silica-supported transition metal catalysts were prepared by a modified reported protocol [36]. In a 50 cm^3 round-bottomed (RB) flask, 1.0 g silica and metal chloride (1.0 mmol) were mixed in 5.0 cm^3 of dry ethanol. The mixture was stirred for 4 h under nitrogen atmosphere at room temperature followed by removal of solvent under reduced pressure at room temperature. The resulting solids were then heated for 1 h at 100 °C to yield active metal chlorides dispersed over silica.

Generalized protocol for H_2SO_4 mediated preparation of chalcones under refluxing conditions

Benzaldehyde (1.15 eq) was added to a stirred acidified methanolic solution (1.15 eq of H_2SO_4) of acetophenone (1 eq.). The obtained reaction solution was heated under reflux for 3 h. Then, the solvent was removed under reduced pressure and the reaction solution was sequentially neutralized with $NaHCO_3$:H_2O (9:1) solution and fractioned between H_2O and EtOAc. The formed organic layers were separated and dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure to afford product as white amorphous solid. Pure product was obtained as colorless needles upon crystallization with dichloromethane.

Generalized protocol for SiO_2–H_2SO_4 mediated preparation of chalcones

To a well-stirred homogeneous solution of acetophenone (1 eq) and benzaldehyde (1.15 eq) was added 0.02 g SiO_2–H_2SO_4. The resulting suspension was stirred and heated at 65 °C for 1.5 h. After completion of reaction, the reaction mixture was cooled to ambient temperature and fractioned between brine and CH_2Cl_2; the solid SSA was filtered off. The SSA was washed with acetone to ensure desorption of product from surface of catalyst. The organic layer was dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure to afford the chalcone as colorless solid [31].

Generalized protocol for the SiO_2–MCl_x mediated preparation of chalcones

The heterogeneous mixture comprising of SiO_2–MCl_x (10 mol%), acetophenone (1 eq.), and benzaldehyde (1.15 eq.) was stirred at room temperature for 7–110 min. Progress of reaction was monitored by means of TLC. After completion of reaction, the reaction mixture was fractioned between brine and CH_2Cl_2 and catalyst (in solid state) was filtered. To ensure desorption of product from the catalyst surface, the SSA was washed with 25 cm^3 acetonitrile. The organic layer, upon drying over anhydrous Na_2SO_4 followed by filtration and concentration under reduced pressure, afforded chalcone as colorless solid. The characterization data of substituted chalcones **3a**–**3i** are given in Table 6.

Determination of urease activity

Urease activity was determined using Berthelot assay. The basic solution phenol and hypochlorite constitute the Berthelot's reagent, which develops blue complex with ammonia. Ammonia is a product of urea hydrolysis [34]. The assay solution contained phosphate buffer (i.e. pH = 7, 10, 50 mM), sample solution and enzyme solution (25 cm^3, 0.015 unit) in each well of the 96-well plate. The solutions

Table 6 Physical characterization of different substituted chalcones **3a**–**3i**

Compound	R–	Ar–	R_f*	M.p./°C	Lit. m.p./°C	References
3a	H	Ph	0.58	57	56–57	[37]
3b	3′-OH–	Ph-	0.45	120–121	120.2–120.6	[31]
3c	3′-OH–	2-furyl-	0.48	140–142	141	[38]
3d	4′-OH–	Ph-	0.48	180	179–180	[39]
3e	4′-OH–	2-furyl-	0.62	142	142	[39, 40]
3f	4′-OH–	4-OMe-	0.52	158	158	[41]
3g	4′-Me–	Ph-	0.58	108	108–110	[42]
3h	4′-Me–	2-furyl-	0.53	64–67	62–64	[43]
3i	4′-Me–	4-Me_2NPh-	0.46	107–111	110	[44]

*Solvent system: EtOAc/*n*-hexane 1:3

were incubated at 37 °C for 10 min. Then, 40 cm^3 of 20 mM urea was incorporated to each well to reach the total volume of 85 cm^3. Contents were incubated at 37 °C for 10 min and absorbance was measured at 625 nm using 96-well plate reader Synergy HT, BioTek, USA. After that, 115 cm^3 Berthelot's reagent was added to each well and color intensity was measured after 10 min. The following was employed for the calculation of percentage inhibition:

$$\text{Percentage inhibition} = 100 - \left[(\text{Abs. of test sample/Abs. of control}) \times 100\right]. \quad (1)$$

IC_{50} values of active compounds were determined from EZ-Fit Enzyme kinetics software, Perrella International, USA.

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