

SYNTHESIS, CHARACTERIZATION AND UREASE INHIBITORY ACTIVITIES OF Zn(II) COMPLEXES BEARING C¹-SYMMETRIC LIGANDS DERIVED FROM (R)-PHENYLETHANAMINE

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ABSTRACT. A series of Zn(II) complexes, supported with *N*-substituted phenylethanamine derivatives, [L_nZnCl₂] (where L_n = L_A ((*R*)-1-phenyl-*N*-(thiophene-2-ylmethyl)ethanamine; L_B (*R*)-*N*-(5-methylthiophene-2-yl)methyl-1-phenylethanamine; L_C ((*R*)-*N*-(furan-2-ylmethyl)-1-phenylethanamine and L_D (*R*)-*N*-(5-methylfuran-2-yl)methyl-1-phenylethanamine) were synthesized and characterized. The urease inhibitory activities of these complexes were determined against selected urease inhibitors where [L_BZnCl₂] was found to be the most prominent inhibitor of *Jack bean urease* (*J. B. urease*) (IC₅₀ = 10.39±0.78 μM), whereas the activity of *Bacillus pasteurii urease* (*B. P. urease*) was predominantly inhibited by [L_AZnCl₂] (IC₅₀ = 8.68±0.7 μM). Additionally, MOE-Dock program was used to affirm the probable binding modes of these complexes into the crystal structure of *J. B. urease* which certainly verified the inhibitory mechanism of these novel complexes.

KEY WORDS: Zn(II) complexes, (*R*)-Phenylethanamine, Urease inhibition, Molecular docking

INTRODUCTION

The worldwide increase in gastro-intestinal diseases, i.e. peptic ulcers, kidney stones, urinary tract infections and hepatic coma often lead to the worst cases of liver cirrhosis and kidney cancers [1, 2]. *Helicobacter pylori* (*H. pylori*), a pathogenic bacterium that resides in stomach [3], has been considered to be the main factor responsible for these infections. *H. pylori* releases high amount of its antigen, urease which cause an imbalance in overall ammonia level, thus causing severe infections, soil pH destabilization, ammonia volatilization and damaging of the plant roots [4]. Urease, a nickel containing metallo-enzyme, catalyzes the hydrolysis of urea into ammonia and carbamic acid which spontaneously decompose to yield a second molecule of ammonia and carbon dioxide thus resulting of elevation in pH and enabling *H. pylori* to survive in the extreme acidic conditions of stomach [5-8]. This bacterium is known to have caused infection in almost 50% of the population in developing countries [9-11]. Proton pump inhibitors (PPIs) are the urease inhibitors, including organic compounds, synthetic complexes/metal salts and plant extracts, usually used to control the activity of *H. pylori* by inhibiting the excess urease secreted [12, 13]. In spite of the successful triple therapy (PPIs with two of the antibiotics) in the treatment of *H. pylori* infections, the excessive and inappropriate uses of these drugs have been encountering bacterial resistance problems and severe side effects [14]. Moreover, a recent study suggests that most of the PPIs used in the treatment of stomach ulcers including esomeprazole, omeprazole, rabeprazole etc. have been risked with severe kidney and liver diseases [15, 16]. In this regard, there is a need of more effective PPIs/urease inhibitors with enhanced bio-compatibility and lesser cytotoxicity to human cells [13].

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The use of both inner and outer transition metal complexes has gained attention recently as potential urease inhibitors [17]. A variety of metal based urease inhibitors including, zinc(II) [18-20], vanadium(IV) [21], tin(IV) [22], bismuth(III) [23], cadmium(II) [24] and copper(II) [25] complexes have been reported and proved to be effective urease inhibitors. However, the use of biocompatible metal based-urease inhibitors is much preferred. In recent years, Zn(II) based urease inhibitors play an important role in the treatment of the infections caused by urease producing bacteria Zn(II) complexes [19, 20, 26-28]. The important fact for targeting Zn(II) system is its biocompatibility and less toxic nature compared to other heavy metals. Additionally, Zn(II) is abundantly found trace element in biological system together with iron and are vital component of most of the metallo-proteins [29-32]. Particularly, Zn(II) complexes bearing nitrogen containing ligands, with various functionalities, are considered to be privileged systems owing to their versatility and promising urease inhibitory potential [33-41]. Keeping in view the versatility of amine based ligands and biocompatible importance of zinc complexes, we herein reported the synthesis, characterization Zn(II) complexes bearing thiophenyl and furyl based chiral amines and their *in vitro* urease inhibitory effects. The molecular docking studies of synthesized complexes are also discussed.

EXPERIMENTAL

Standard procedures were carried out for the synthesis of ligands and their Zn(II) complexes, unless otherwise specified. The starting materials included: zinc chloride (ZnCl_2), sodium borohydride (NaBH_4), thiophene-2-carbaldehyde, furan-2-carbaldehyde, 5-methylthiophene-2-carbaldehyde, 5-methyl-furan-2-carbaldehyde and (*R*)-methylbenzylamine were purchased from Aldrich. Solvents, such as EtOH, MeOH and CH_2Cl_2 used in this study were obtained from Aldrich and used without further purification. MgSO_4 was obtained from Merck. The Fourier transform infra-red (FTIR) spectral measurements were obtained through Jasco FT-IR-620 spectrophotometer where bands were recorded in cm^{-1} while bands intensity were termed as w = weak, m = medium, s = strong. NMR data was obtained by Avance digital NMR spectrometer (^1H NMR = 400 MHz, δ = ppm). Elemental analysis was performed on elemental analyser (EA 1108) for C, H and N. Melting point has been determined using Dynalox DMP100 Digital Melting Point apparatus.

Synthesis of ligands

(*R*)-1-Phenyl-*N*-(thiophen-2-ylmethyl)ethanamine, (*L*₁). CH_2Cl_2 solution of (*R*)-methyl benzyl amine (3.00 g, 24.75 mmol) was added drop wise to CH_2Cl_2 solution of thiophene-2-carbaldehyde (2.77 g, 24.75 mmol). The reaction mixture was dried over MgSO_4 after being stirred at ambient temperature for 48 h. The progress of the reaction was monitored by TLC. The solvent was removed under reduced pressure to yield light yellow residue (4.50 g, 85%). Further reduction of imine moiety product was carried out by dissolving the resultant residue in MeOH (4.00 g, 17.30 mmol) and was treated with NaBH_4 (0.97 g, 25.95 mmol) at 0 °C. The solvent was removed under reduced pressure after stirring the reaction mixture overnight. The residue obtained was suspended in 40 mL distilled water and the organic oil was extracted with CH_2Cl_2 (3 × 30 mL). The CH_2Cl_2 solution was dried over anhydrous MgSO_4 . The solvent was evaporated to get lemon color oil as a final product (3.50 g, 93%) based on the imine product. FTIR (oily liquid neat; cm^{-1}): $\nu(\text{N-H})$ 3195 w; $\nu(\text{C-H})$ 2928 w; $\nu(\text{C=C})$ 1671 s; $\delta(\text{-C-H } \text{sp}^3)$ 1488 m; $\nu(\text{N-C})$ 1359 w; $\delta(\text{C-H } \text{sp}^2)$ 878 m, $\nu(\text{C-S})$ 852 m; ^1H NMR (400 MHz, CDCl_3 , ppm): δ = 7.35-7.28 (4H, m, ArH), 7.18 (1H, m, ArH), 7.05 (s, 2H, ArH), 6.96-6.77 (1H, d, J = 4.40 Hz, ArH), 3.73 (1H, q, J = 8.40 Hz, J = 3.68 Hz, Ar-NH- CH_2), 3.62 (2H, s, -(NH-CH)- CH_3), 1.50 (1H, br s, -(NH-CH)- CH_3), 1.34 (3H, d, J = 6.4 Hz, -(NH-CH)- CH_3). Analysis calculated for $\text{C}_{13}\text{H}_{15}\text{NS}$ (%): C, 71.84; H, 6.96; N, 6.44. Found: C, 71.80; H, 6.99; N, 6.47.

(*R*)-*N*-((5-Methylthiophen-2-yl)methyl)-1-phenylethanamine, (L_B). The synthesis of L_B was carried out according to procedure described for L_A , except utilizing 5-methylthiophene-2-carbaldehyde (2.08 g, 16.50 mmol) to yield an imine residue (3.50 g, 93%). Further reduction was carried out with an analogues procedure as stated for L_A except utilizing *N*-((5-methylthiophen-2-yl)methylene)-1-phenylethanamine (3.50 g, 14.04 mmol) and NaBH_4 (0.8 g, 21.07 mmol) to get L_B (3.23 g, 92%). FTIR (oily liquid neat; cm^{-1}): $\nu(\text{N-H})$ 3311 w; $\nu(\text{C-H})$ 2961 w; $\nu(\text{C=C})$ 1492 s; $\delta(\text{C-H sp}^3)$ 1450 m; $\nu(\text{N-C})$ 1369 w; $\delta(\text{C-H sp}^2)$ 886 m; $\nu(\text{C-S})$ 840 m; $^1\text{H NMR}$ (400 MHz, CDCl_3 , ppm): $\delta = 7.27$ (2H, br s, ArH), 7.26 (2H, m, ArH), 7.19-7.17 (1H, m, ArH), 6.53 (1H, d, $J = 3.28$ Hz, ArH), 6.49-6.48 (1H, m, ArH), 3.78 (1H, q, $J = 13.38$ Hz, $J = 6.82$ Hz, $-(\text{NH-CH})\text{-CH}_3$), 3.65 (2H, s, Ar-NH- CH_2), 2.38 (3H, br s, Ar- (CH_3)), 1.52 (1H, br s, $-(\text{NH-CH})\text{-CH}_3$), 1.28 (3H, d, $J = 6.56$ Hz, $-(\text{NH-CH})\text{-CH}_3$). Analysis calculated for $\text{C}_{14}\text{H}_{17}\text{NS}$ (%): C, 72.68; H, 7.41; N, 6.05. Found: C, 72.70; H, 7.40; N, 6.10.

(*R*)-*N*-((Furan-2-ylmethyl)-1-phenylethanamine, (L_C). The synthesis of L_C was carried out according to procedure described for L_A , except utilizing furan-2-carbaldehyde (1.58 g, 16.50 mmol) to yield an imine residue (3.10 g, 94%). Further reduction was carried out with an analogues procedure as stated for L_A except utilizing *N*-((furan-2-ylmethylene)-1-phenylethanamine (3.10 g, 15.55 mmol) and NaBH_4 (0.88 g, 23.32 mmol) to get L_C (3.00 g, 96%). FTIR (oily liquid neat; cm^{-1}): $\nu(\text{N-H})$ 3188 w; $\nu(\text{C-H})$ 2931 w; $\nu(\text{C=C})$ 1506 s; $\delta(\text{C-H sp}^3)$ 1452 m; $\nu(\text{N-C})$ 1348 w; $\nu(\text{C-O})$ 1021s; $\delta(\text{C-H sp}^2)$ 700s. $^1\text{H NMR}$ (400 MHz, CDCl_3 , ppm): $\delta = 7.40$ (1H, m, ArH), 7.28 (2H, br s, ArH), 7.20 (1H, m, ArH), 7.19-7.17 (2H, m, ArH), 6.65-6.63 (1H, m, ArH), 6.06-6.04 (1H, m, ArH), 3.71 (1H, q, $J = 13.13$ Hz, $J = 6.56$ Hz, $-(\text{NH-CH})\text{-CH}_3$), 3.55 (2H, s, Ar-NH- CH_2), 1.52 (1H, br s, $-(\text{NH-CH})\text{-CH}_3$), 1.29 (3H, d, $J = 6.56$ Hz, $-(\text{NH-CH})\text{-CH}_3$). Analysis calculated for $\text{C}_{13}\text{H}_{15}\text{NO}$ (%): C, 77.58; H, 7.51; N, 6.96. Found: C, 77.59; H, 7.52; N, 6.97.

(*R*)-*N*-((5-Methylfuran-2-yl)methyl)-1-phenylethanamine, (L_D). The synthesis of L_D was carried out according to procedure described for L_A , except utilizing 5-methylfuran-2-carbaldehyde (1.81 g, 16.50 mmol) to yield an imine residue (3.20 g, 96%). Further reduction was carried out with an analogues procedure as stated for L_A except utilizing *N*-((5-methylfuran-2-yl)methylene)-1-phenylethanamine (3.20 g, 15.00 mmol) and NaBH_4 (0.85 g, 22.50 mmol) to get L_D (3.01 g, 93%). FTIR (oily liquid neat; cm^{-1}): $\nu(\text{N-H})$ 3239 w; $\nu(\text{C-H})$ 2975 w; $\nu(\text{C=C})$ 1441 s; $\delta(\text{C-H sp}^3)$ 1382 m; $\nu(\text{N-C})$ 1277 w; $\nu(\text{C-O})$ 1186s; $\delta(\text{C-H sp}^2)$ 764 m. $^1\text{H NMR}$ (400 MHz, CDCl_3 , ppm): $\delta = 7.40$ -7.30 (2H, m, ArH), 7.28-7.27 (2H, m, ArH), 7.18-7.15 (1H, m, ArH), 6.59 (1H, m, ArH), 6.47-6.480 (1H, m, ArH), 4.35 (1H, q, $J = 13.38$ Hz, $J = 6.56$ Hz, $-(\text{NH-CH})\text{-CH}_3$), 3.60 (2H, s, Ar-NH- CH_2), 2.38 (3H, br s, Ar- (CH_3)), 1.56 (1H, br s, $-(\text{NH-CH})\text{-CH}_3$), 1.29 (3H, d, $J = 6.56$ Hz, $-(\text{NH-CH})\text{-CH}_3$). Analysis calculated for $\text{C}_{14}\text{H}_{17}\text{NO}$ (%): C, 78.10; H, 7.96; N, 6.51. Found: C, 78.11; H, 7.95; N, 6.52.

Synthesis of complexes

(*R*)-1-Phenyl-*N*-(thiophen-2-ylmethyl)ethanamine Zn(II) chloride, [$L_A\text{ZnCl}_2$]. A solution of L_A (1.00 g, 4.60 mmol) in EtOH was added to a solution of ZnCl_2 (0.627 g, 4.60 mmol) in EtOH. Precipitation of white solid occurred while stirring at room temperature for 12 h. The precipitate was filtered and washed with cold EtOH (20.0 mL \times 2), followed by washing with Et_2O (20.0 mL \times 2) to yield final product (1.50 g, 92%); mp 171 °C; FTIR (solid neat; cm^{-1}): $\nu(\text{N-H})$ 3222 w; $\nu(\text{C-H})$ 2927 w; $\nu(\text{C=C})$ 1674 s; $\delta(\text{C-H sp}^3)$ 1486 m; $\nu(\text{N-C})$ 1308 w; $\delta(\text{C-H sp}^2)$ 870 m, $\nu(\text{C-S})$ 843 m; $\nu(\text{M-N})$ 567 s; $^1\text{H NMR}$ (CDCl_3 , 400 MHz): $\delta = 7.92$ -7.82 (2H, m, ArH), 7.43 (3H, m, ArH), 6.72 (s, 1H, ArH), 6.92 (s, 1H, ArH), 6.02 (1H, m, ArH), 4.61 (1H, q, $J = 8.72$ Hz, $J = 4.80$ Hz, $-(\text{NH-CH})\text{-CH}_3$), 4.25 (2H, br s, Ar-NH- CH_2), 2.00 (1H, br s, $-(\text{NH-CH})\text{-CH}_3$),

1.90 (3H, d, $J = 6.56$ Hz, $-(NH-CH)-CH_3$). Analysis calculated for $C_{13}H_{15}Cl_2NSZn$ (%): C, 44.15, H, 4.28, N, 3.96. Found: C, 44.14, H, 4.29, N, 3.98.

(R)-N-((5-Methylthiophen-2-yl)methyl)-1-phenylethanamine Zn(II) chloride, [L_BZnCl₂]. [L_BZnCl_2] was prepared according to the similar procedure described for [L_AZnCl_2] except utilizing L_B (1.06 g, 4.60 mmol) to yield white precipitate (1.50 g, 88%); mp 171 °C; FTIR (solid neat; cm^{-1}): $\nu(N-H)$ 3267 w; $\nu(C-H)$ 2951 w; $\nu(C=C)$ 1636 s; $\delta(-C-H\ sp^3)$ 1455 m; $\nu(N-C)$ 1308 w; $\delta(C-H\ sp^2)$ 873 m; $\nu(C-S)$ 850 m; $\nu(M-N)$ 558 s; 1H NMR ($CDCl_3$, 400 MHz): $\delta = 7.90-7.80$ (2H, m, ArH), 7.40 (3H, m, ArH), 6.77 (1H, m, ArH), 6.52 (1H, m, ArH), 4.48 (1H, q, $J = 13.20$ Hz, $J = 3.68$ Hz, $-(NH-CH)-CH_3$), 3.78 (2H, br s, Ar-NH- CH_2), 1.52 (1H, br s, $-(NH-CH)-CH_3$), 1.48 (3H, d, $J = 6.52$ Hz, $-(NH-CH)-CH_3$). Analysis calculated for $C_{14}H_{17}Cl_2NSZn$ (%): C, 45.74, H, 4.66, N, 3.81. Found: C, 45.73, H, 4.68, N, 3.82.

(R)-N-(Furan-2-ylmethyl)-1-phenylethanamine, [L_CZnCl₂]. [L_CZnCl_2] was prepared according to the similar procedure described for [L_AZnCl_2] except utilizing L_C (0.925 g, 4.60 mmol) to yield white precipitate (1.45 g, 93%); mp 165 °C; FTIR (solid neat; cm^{-1}): $\nu(N-H)$ 3350 w; $\nu(C-H)$ 2923 w; $\nu(C=C)$ 1606 s; $\delta(-C-H\ sp^3)$ 1452 m; $\nu(N-C)$ 1360 w; $\nu(C-O)$ 1052s; $\delta(C-H\ sp^2)$ 899 m; $\nu(M-N)$ 578 s; 1H NMR ($CDCl_3$, 400 MHz): $\delta = 7.40-7.35$ (2H, m, ArH), 7.30-7.28 (3H, m, ArH), 6.29 (s, 1H, ArH), 6.44 (s, 1H, ArH), 6.34 (1H, m, ArH), 6.29 (s, 1H, ArH), 4.08 (1H, q, $J = 8.0$ Hz, $J = 4.80$ Hz, $-(NH-CH)-CH_3$), 3.22 (2H, br s, Ar-NH- CH_2), 2.00 (1H, br s, $-(NH-CH)-CH_3$), 1.28 (3H, d, $J = 6.56$ Hz, $-(NH-CH)-CH_3$). Analysis calculated for $C_{13}H_{15}Cl_2NOZn$ (%): C, 46.39, H, 4.19, N, 4.16. Found: C, 46.41, H, 4.20, N, 4.17.

(S)-N-((5-Methylfuran-2-yl)methyl)-1-phenylethanamine, [L_DZnCl₂]. [L_DZnCl_2] was prepared according to the similar procedure described for [L_AZnCl_2] except utilizing L_D (0.99 g, 4.60 mmol) to yield white precipitate (1.47 g, 90%); mp 150 °C; FTIR (solid neat; cm^{-1}): $\nu(N-H)$ 3278 w; $\nu(C-H)$ 3091 w; $\nu(C=C)$ 1443 s; $\delta(-C-H\ sp^3)$ 1408 m; $\nu(N-C)$ 1345 w; $\nu(C-O)$ 1112s; $\delta(C-H\ sp^2)$ 803 m; $\nu(M-N)$ 583 s; 1H NMR ($CDCl_3$, 400 MHz): $\delta = 7.40$ (2H, m, ArH), 7.29 (3H, m, ArH), 6.14 (s, 1H, ArH), 6.02 (1H, d, $J = 4.00$ Hz, ArH), 4.10 (4H, q, $J = 8.72$ Hz, $J = 4.80$ Hz, $-(NH-CH)-CH_3$), 3.50 (2H, s, Ar-NH- CH_2), 2.15 (3H, s, Ar- CH_3), 1.50 (1H, br s, $-(NH-CH)-CH_3$), 1.28 (3H, d, $J = 6.56$ Hz, $-(NH-CH)-CH_3$). Analysis calculated for $C_{14}H_{17}Cl_2NOZn$ (%): C, 47.83, H, 4.87, N, 3.98. Found: C, 47.81, H, 4.88, N, 3.97.

Urease inhibitory assay

The urease inhibitory effect of the complexes was measured using conventional indophenol's method [43]. Briefly, the assay mixture containing 100 μ L of enzymes (*Jack bean* and *Bacillus pasteurii*) ureases and 400 μ L buffer which contained urea (100 mM) with a pH of 8.2 were mixed with 50 μ L of each sample, incubated at 30 °C for 15 min in 96 well plates. The other reagents included: 200 μ L phenol reagent (1% phenol (w/v) and 0.005% sodium nitroprusside (w/v) and 200 μ L alkali reagent (0.5% NaOH (w/v) and 0.1% NaOCl) were added to each well and the increase in absorbance was measured after 50 min at 630 nm (λ_{max}) using spectrophotometer (Shimadzu, USA). All the reactions were carried out in triplicate to a final volume of 1000 μ L. The results were processed using Soft Max Pro software (change in absorbance per min). The reference inhibitor used was thiourea (standard).

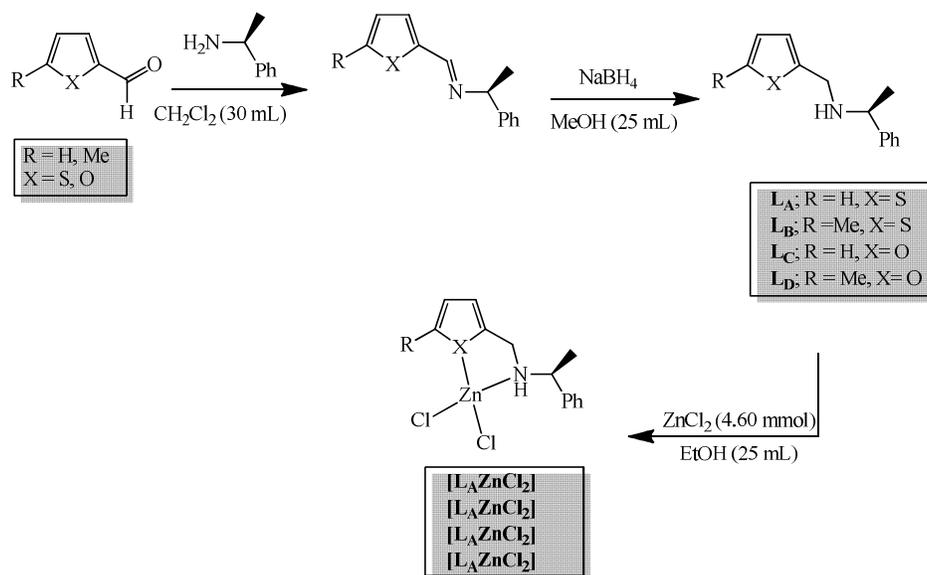
Molecular docking

The complexes were Molecular Operating Environment (MOE) program was used to dock the complexes within binding pockets of urease using default parameters. The 3D structures for complexes were modeled in MOE-2009-2010 program while the energies were minimized by

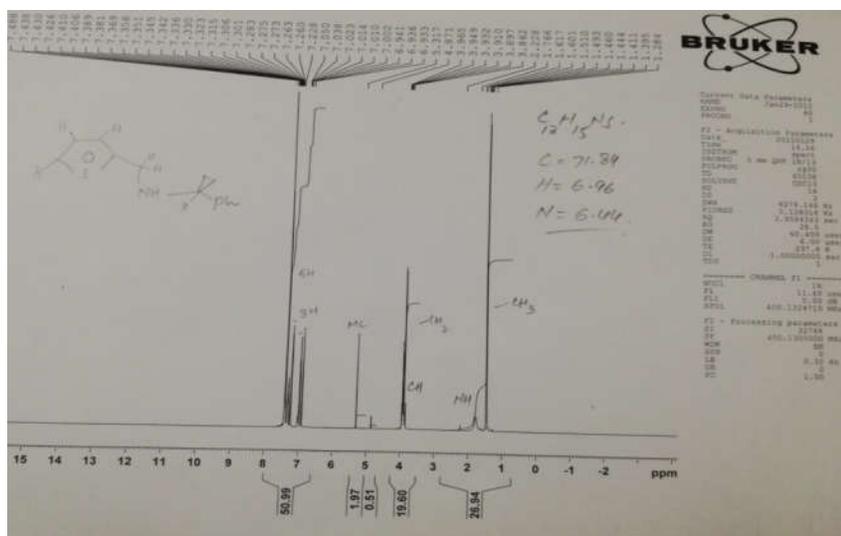
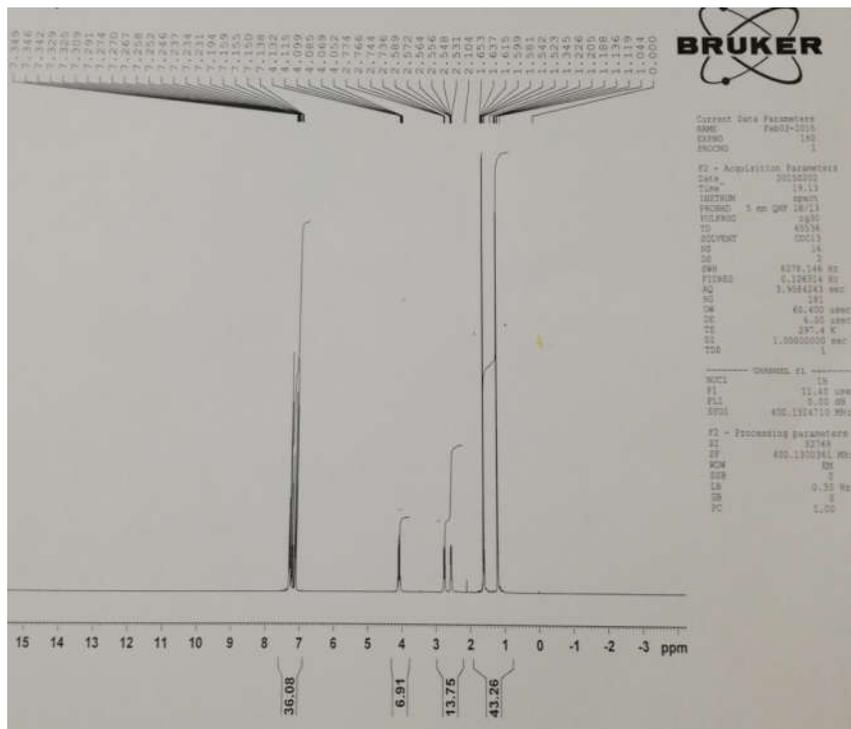
MOE energy minimization algorithm with the parameters: gradient = 0.05; force field = MMFF94X. A database was created where the 3D structures were saved in mdb file format. The crystal structure of receptor protein molecule of urease was retained from protein data bank (PDB code = 4UBP), water molecules were removed by protonate 3D option and the complexes were docked into binding sites of urease. The validity of docking protocols was evaluated with re-docking [42].

RESULTS AND DISCUSSION

Nitrogen containing ligands are important building block and furnished metal complexes of variable geometries owing to their facile modulation of steric and electronic properties. In order to investigate the influence of *N,S,O*-mixed ligands on the urease inhibitory behavior, four *N*-substituted *R*-phenylethanamine derivatives have been selected. For instance, L_A and L_B with an identical framework with the difference of methyl substituent at thiophenyl moiety has been selected to investigate the influence of steric bulk provided by alkyl substituent on the catalytic activities. For comparison, L_C and L_D , which comprise a furyl functionality has been selected, to generate mixed ligand architecture with better δ -donor and π -acceptor properties. The synthetic route of the ligands ($L_A - L_D$) in the current study involved the condensation of corresponding thiophenyl and furylaldehydes with (*R*)-methylbenzyl amine to produce imine product. This is followed by the reduction with mild reducing agent at ambient temperature to obtain the enantiopure amine. The direct coordination of these ligands to $ZnCl_2$ at a 1:1 molar ratio furnished the corresponding dichloro Zn(II) complexes, $[L_nZnCl_2]$ ($L_n = L_A - L_D$); (yield 88-93%). Elemental analysis data for the synthesized Zn(II) complexes were found to be consistent with the structures proposed in Scheme 1. The calculated values based on molecular formula and experimentally determined values agree well with each other which confirm the purity of the synthesized compounds (Table 1). The complexes were stable in the air and could be stored for months at room temperature without appreciable degradation.



Scheme 1. Synthetic route of *N,N*-diamine ligands and their corresponding Zn(II) complexes.

Figure 1. 1H NMR spectrum of L_A .Figure 2. 1H NMR spectrum of L_B .

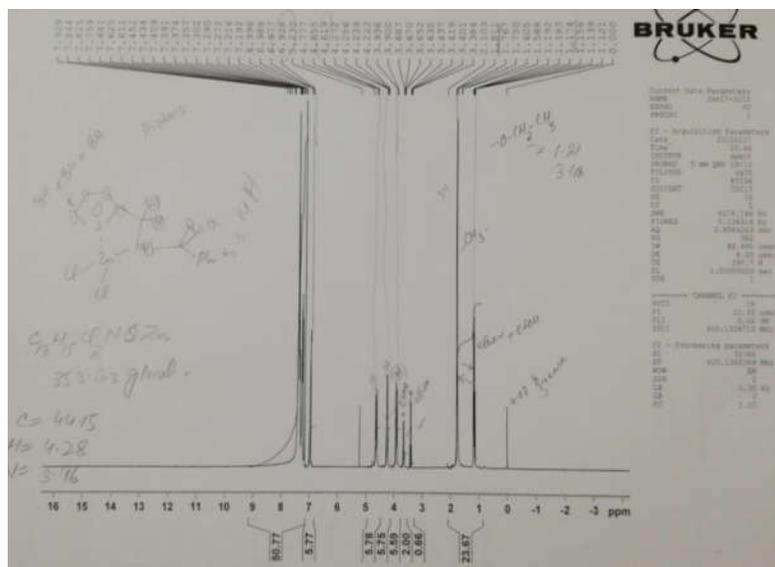


Figure 3. ¹H NMR spectrum of [L_AZnCl₂].

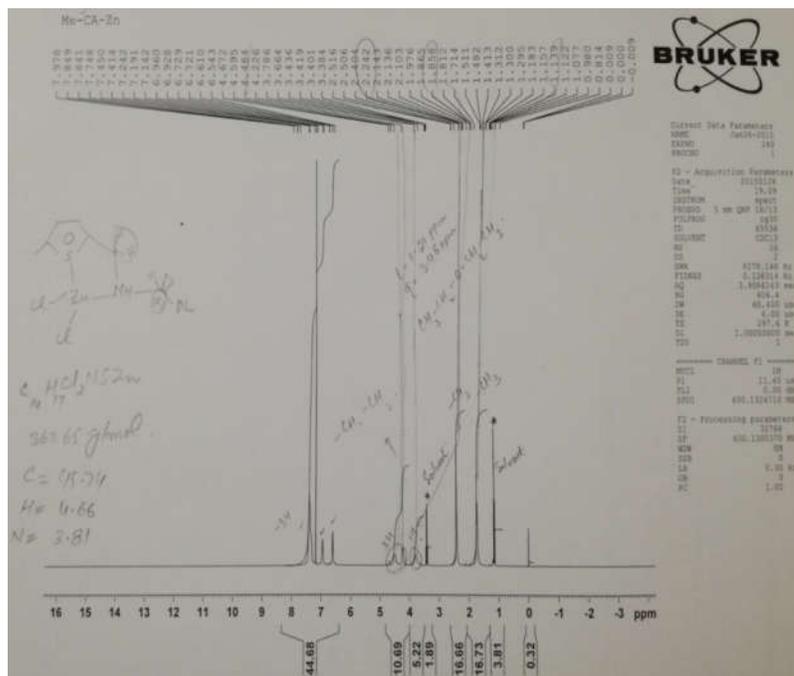


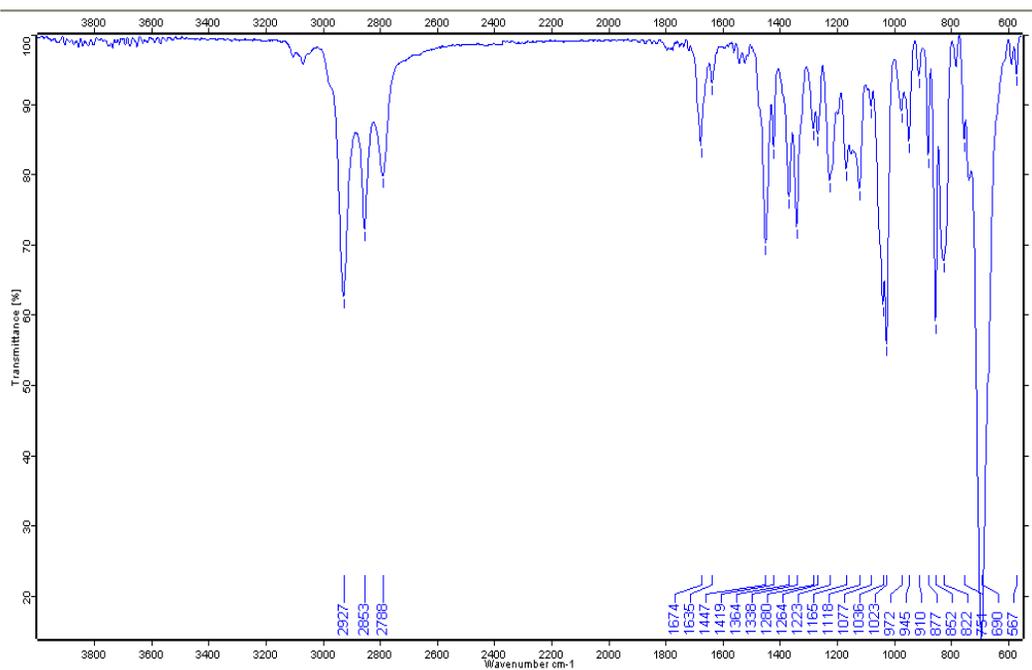
Figure 4. ¹H NMR spectrum of [L_BZnCl₂].

Table 1. Summary of physicochemical parameters and elemental analysis of synthesized ligands and their corresponding Zn(II) complexes.

Compound	Colour	Yields (%)	M.P. (°C)	Elemental analysis found (calcd.) %		
				C	N	H
L _A	Lemon yellow	93	-	71.84 (71.80)	6.44 (6.47)	6.96 (6.99)
L _B	Pale yellow	92	-	72.68 (72.68)	6.05 (6.10)	7.41 (7.40)
L _C	Light brown	96	-	77.58 (77.59)	6.96 (6.97)	7.51 (7.52)
L _D	Light brown	93	-	78.10 (78.11)	6.51 (6.52)	7.96 (7.95)
[L _A ZnCl ₂]	White solid	92	159	44.15 (44.14)	3.96 (5.98)	4.28 (4.29)
[L _B ZnCl ₂]	White solid	88	171	45.74 (45.73)	3.81 (3.98)	4.66 (4.68)
[L _C ZnCl ₂]	White solid	93	165	46.39 (46.41)	4.16 (4.17)	4.19 (4.20)
[L _D ZnCl ₂]	White solid	90	150	47.83 (47.81)	3.98 (3.97)	4.87 (4.88)

The structural studies of all the ligands and their corresponding Zn(II) complexes obtained by FTIR and ¹H NMR spectral studies also remained consistent with their molecular formulas.

A slight change in proton NMR chemical shift values in complexes as compared to the ligands was probably due to the shielding effect caused by Zn(II) center which coordinated with ligands at various sites. For instance, the methine protons of L_A (3.26 ppm) and L_B (3.78) (Figure 1 and 2) are shifted to [L_AZnCl₂] (4.61 ppm) and [L_BZnCl₂] (4.42 ppm) (Figure 3 and 4), respectively. Comparison of an FTIR spectra of ligand with that of synthesized Zn(II) complex was performed, particularly in N-H region. The characteristic N-H peaks for ligands (L_n = L_A–L_D) shifted from lower wavenumbers (3188–3311 cm⁻¹) to higher wavenumbers in their corresponding Zn(II) complexes (3365–3278 cm⁻¹).

Figure 5. FTIR spectrum of [L_AZnCl₂].

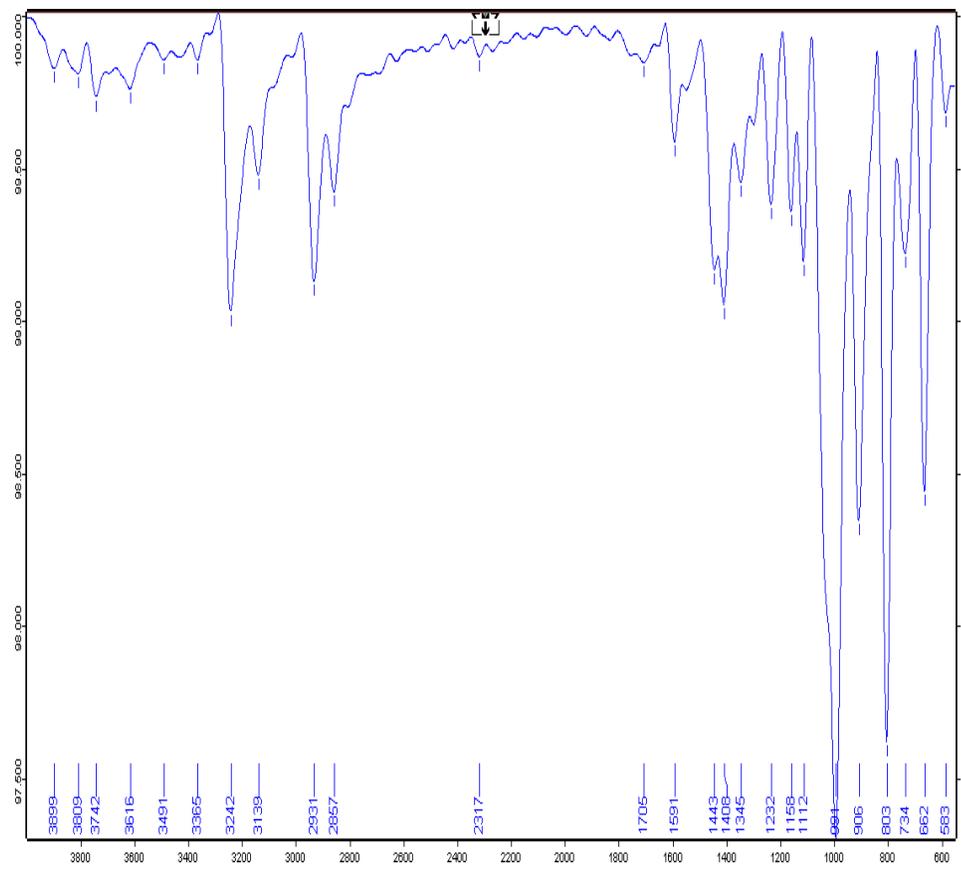


Figure 6. FTIR spectrum of $[L_DZnCl_2]$.

The characteristic bands assigned to the $sp^3 \nu(C-H)$ and $\nu(C=C)$ bonds can be confirmed at $3081-2991 \text{ cm}^{-1}$ and $1671-1441 \text{ cm}^{-1}$ ranges, respectively in $[L_nZnCl_2]$ ($L_n = L_A - L_D$) (see Figure 5 for FTIR spectrum of $[L_AZnCl_2]$). Additionally, a new absorption band at $583-558 \text{ cm}^{-1}$ appeared were assigned to $\nu(M-N)$ bond stretching ($M = Zn$) [44]. The presence of these absorption bands confirmed the involvement of amine nitrogen in the chelation of the Zn(II) center. In addition, the band in $1000-1200 \text{ cm}^{-1}$ range observed for $[L_CZnCl_2]$ and $[L_DZnCl_2]$ can be attributed to the C-O bond stretch (for instance see Figure 6 for FTIR spectrum of $[L_DZnCl_2]$). In the present study the stretching of the C-S bond in the thiophene ring is identified at $850-840 \text{ cm}^{-1}$. These values are in agreement with the reported data [45, 46].

Urease inhibition studies

Urease enzyme is believed to be a cause of various gastro-intestinal disorders including hepatic coma, encephalopathy, ulcer, urinary catheter encrustation [47]. The inhibition of urease activity could prevent the infections caused by pathogenic bacteria *H. pylori* and may be considered as a hopeful remedy for peptic ulcer. Also, urease inhibitors play a major role in decreasing ecological problems related to elevated pH due to urease [48].

In this study, all the synthesized complexes were screened for their anti-urease properties against *J.B. urease* and *B.P. urease* (Table 2). IC₅₀ values were obtained for the samples showing inhibition greater than 50%. All the complexes successfully inhibited the tested enzymes. [L_BZnCl₂] showed the lowest IC₅₀ concentrations (8.68±0.7 and 17.98±0.17), much closer to the IC₅₀ of standard, thiourea (8.7±0.19 and 12.11±0.42) against *J.B. urease* and *B.P. urease*. For [L_AZnCl₂], the IC₅₀ were obtained as 12.01±0.2 and 10.39±0.78, for [L_CZnCl₂], 15.04±0.01 and 18.02±0.14 and for [L_DZnCl₂], 14.02±0.03 and 17.03±0.04, respectively. Our findings are promising towards formulation of new antiulcer agents. The structural features indeed impart these catalytic activities as also indicated from the molecular docking studies. The ligand-complex backbone greatly contributes towards the inhibition of ureases especially where halogen or any other electron contributor groups are attached at phenyl ring. The existence of 2 and 3-chloro functionalized has been reported to inhibit urease strongly in various zinc complexes of isatin-3-thiosemicarbozones [35]. Similarly, various zinc complexes having imino-piperazine moiety showed inhibitory effect however, the presence of ethyl substituents showed much potent effect [37]. In contrast, various benzoates having electron withdrawing groups (methoxy) were also effective inhibitors which suggest that the structural diversity could alter the catalytic mode [36]. Our study reveals that [L_BZnCl₂] and [L_DZnCl₂] bearing methyl substituents on phenyl rings are much potent inhibitors probably due to ring activation or steric effect which render the complexes more suitable in binding to the active pockets of ureases.

Table 2. Urease inhibition activities of compounds [L_nZnCl₂] (L_n = L_A–L_D).

Samples	Enzyme (± SEM), IC ₅₀ in μm	
	J.B. Urease	B.P. Urease
[L _A ZnCl ₂]	12.01±0.2	17.98±0.7
[L _B ZnCl ₂]	8.68±0.7	10.39±0.78
[L _C ZnCl ₂]	15.04±0.01	18.02±0.14
[L _D ZnCl ₂]	14.02±0.03	17.03±0.04
Thiourea	8.7±0.19	12.11±0.42

Molecular docking studies

Molecular docking studies were carried out for all the Zn(II) complexes against *J.B. urease*. For [L_AZnCl₂], strong interaction was observed between Zn atom and Ala 170 (bond length = 2.76 Å) as well as His222. The ligand showed relatively weak interactions with Asp224, His324 and 323 Figure 7A(2D). In case of [L_BZnCl₂], two types of strong electrostatic interactions were observed between the thiophenyl moiety of L_B with the amino acid residues, Asp224 (bond distance of 2.88 Å) and His323 (bond distance of 3.86 Å) (Figure 7B(2D)). The strong interaction of His323 with L_B may be attributed to the arene-cation interactions at the active site of enzyme [49, 50]. Experimentally, the most significance inhibition of both the ureases may be due to the stronger interactions between [L_BZnCl₂] with the active sites of ureases. Moreover, the presence of methyl substituents enables the π cloud of thiophenyl moiety to interact closely with the amino acid residues at the active sites of enzymes [51].

Similarly, two interactions have been observed for [L_CZnCl₂] as illustrated in Figure 7C(2D). First interaction observed was between Zn(II) center and carboxylic oxygen of Ala170 with a bond distance of 2.97 Å while second arene-cation interaction was found between Arg339 and furyl moiety of the complex with a bond distance of 3.10 Å. Both the Ni⁺³ ions of the urease occurred at a shorter distance thus confirming the interactions of the complex with the active pocket amino acid residues. [L_DZnCl₂] showed good binding with the enzyme active site by making two prominent interactions with the same single Asp224 amino acid. First metal ion interaction was made with the Zn(II) and second with furyl oxygen of the complex resulting in bond distances of 2.53 and 2.59 Å, respectively. The shorter bond lengths may be attributed

towards the lower IC_{50} values of $[L_DZnCl_2]$ as compared to $[L_CZnCl_2]$ in urease inhibition, Figure 7D (2D). In the inhibitor-urease complex conformation, $[L_AZnCl_2]$ and $[L_BZnCl_2]$ showed a stabilized structure through various interactions with carboxylate group and amino group of the amino acid residues. The results of the molecular docking indicated that both $[L_AZnCl_2]$ and $[L_BZnCl_2]$ were well bounded in the active pocket of *J.B.* urease.

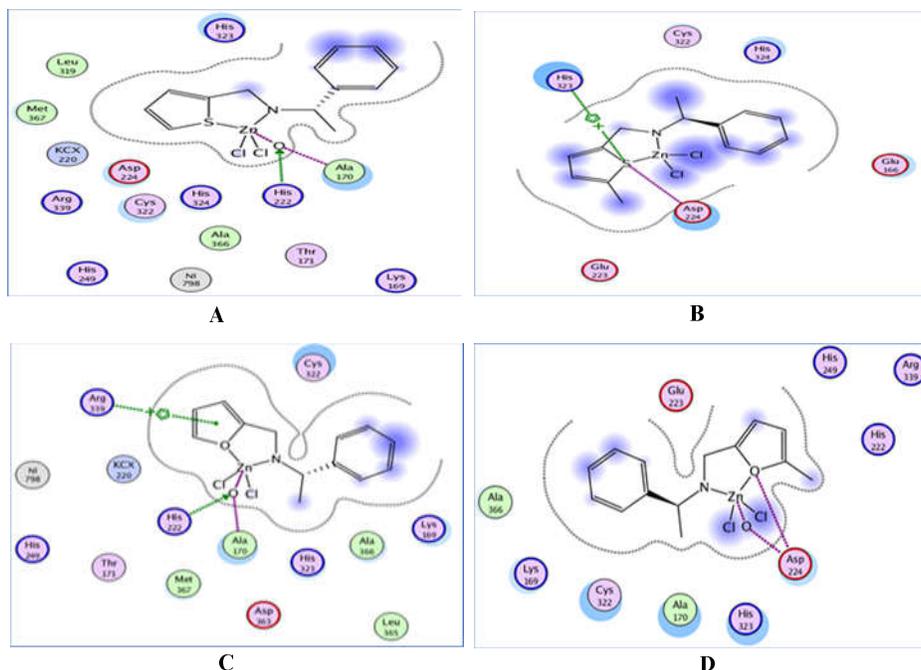


Figure 7. (2D) Molecular docking of **A** = $[L_AZnCl_2]$; **B** = $[L_BZnCl_2]$; **C** = $[L_CZnCl_2]$ and **D** = $[L_DZnCl_2]$ into the active pocket of *J.B.* urease.

CONCLUSION

Novel Zn(II) complexes chelated to *N*-substituted phenylethanamine derivatives have been synthesized and characterized. Investigation of catalytic efficacy of the synthesized complexes in urease inhibition revealed that $[L_BZnCl_2]$ displayed prominent inhibition with IC_{50} values of 8.68 ± 0.7 and 17.98 ± 0.17 against *J.B. urease* and *B.P. urease*. Notably, the thiophenyl containing complexes exhibited better activities compared to furyl containing counterparts. These Zn(II) complexes noticeably displayed their potential to inhibit urease and are likely candidates for the advancement of new drug formulations but detailed *in vivo* studies are required to understand their mechanism of action. Currently, a detailed *in vivo* investigation is underway in our laboratory that is aimed at improving the complexes inhibitory action through modification of the ligand framework.

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