Study of Antioxidant, Cytotoxic, and Enzyme Inhibition Activities of Some Symmetrical $N^3,N^{3'}$-Bis(disubstituted)isophthalyl-bis(thioureas) and $N^3,N^3,N^{3'},N^{3'}$-Tetrakis(disubstituted)isophthalyl-bis(thioureas) and Their Cu(II) and Ni(II) Complexes

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Summary: The present study includes the evaluation of antioxidant, cytotoxic, antiureas and some other enzymes like α-chymotrypsin, acetylcholinesterse, butyrylcholinesterase inhibition activity of some synthesized $N^3,N^{3'}$-bis(disubstituted)isophthalyl-bis(thioureas) and $N^3,N^3,N^{3'},N^{3'}$-tetrakis(disubstituted)isophthalyl-bis(thiourea) compounds with general formula $[C_6H_4\{CONHCSNR_1R_2\}_2]$, where R_1 = H (L^1-L^5), C_6H_6(L^6), and R_2 = 4-C_6H_4COOH(L^1), 3-$NO_2C_6H_4$(L^2), 2-$NO_2C_6H_4$(L^3), 4-$NO_2C_6H_4$(L^4), C_5H_4N(L^5) and C_6H_{11}(L^6) and their Cu(II) and Ni(II) complexes. The antioxidant inhibition was measured against standard butylated hydroxyl toluene for all the synthesized compounds. Thiourea used as standard against antiureas inhibition. The enzyme inhibition IC_{50} values were used to describe the results. The results of these studies also show that the metal (II) complexes have more potential activity against different enzymatic inhibitions as compared to uncomplexed ligands. The results reveal that synthesized thioureas and their metal (II) complexes can be used as potential inhibitors against some disease causing enzymes like ureas etc.

Keywords: Antioxidant, Antiureas, Cytotoxic, Bisthioureas, Tetrakisthioureas.

Introduction

The coordination compounds are of considerable interest because metal ions are found in active sites of a number of metalloproteins such as hemocyanin, and also in metalloenzymes like in ureases and tyrosinase [1–2]. These proteins are involved in various biological processes. Starting from the antioxidants, these can be expressed as radical scavengers. They inhibit lipid peroxidation and free radical mediated food processes. Human body and processed foods are protected from oxidative damage which is caused by free radicals. The major sources of free radicals are plants and animals because they produce molecules which intercept oxidizing agents and these are called as antioxidants. It has been observed that synthetic antioxidants are more beneficial as compared with natural products. Urease (urea amidohydrolase EC 3.5.1.5) is a nickel-containing enzyme that catalyzes the rapid hydrolysis of urea to form ammonia and carbon dioxide [1–4]. It participates in environmental nitrogen transformations to supply these organisms with a nitrogen source for growth [3]. On the other hand, the reaction catalyzed by the dinuclear nickel present at active sites of thiourease causes an accumulation of ammonia and an abrupt pH increase, which has negative side effects in agriculture and health. For example urease can severely decrease the efficiency of urea fertilizers to cause the release of large amounts of ammonia and further induce plant damage by ammonia toxicity and soil pH increase [5]. Therefore, the capability to control the rate of the enzymatic urea hydrolysis using urease inhibitors is an important goal to pursue.

α-Glucosidase (α-D-glucoside glucohydrolase, EC 3.2.1.20) comprises a family of enzymes hydrolase, located in the brush-border surface membrane of small intestinal cells [6]. The major function of α-glucosidase is to hydrolyze the 1,4 glycosidic linkage from the non-reducing end of the

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α-glucosides, α-linked oligosaccharide, and *α*-glucans substrates to produce α-D-glucose and other monosaccharide that are utilized as carbon and energy source [7]. *α*-Glucosidase inhibitors are molecules or compounds that are used as oral anti-diabetic drugs for patients with type-2 diabetic mellitus. Postprandial hyperglycemia has a vital role in the development of type-2 diabetes and complications associated with disease such as nephropathy, neuropathy, micoangiopathy and macroangipathy [8]. The inhibitors of enzyme can retard the liberation of D-glucose of oligosaccharides and disaccharides from dietary complex carbohydrates and delay glucose absorption, resulting in reduced postprandial hyperglycemia [9]. Therefore, inhibition of α-glucosidase is considered important in managing type-2 diabetes.

α-Chymotrypsin (EC 3.4.21.1), a protease, which is secreted from pancreas, catalyzes the breakdown of polypeptide and proteins. If the precursor of chymotrypsin, the chymotrypsinogens is cleaved to form active enzyme before the target side, then it digest the tissues inside body such as in cases of pancreatitis [10]. α- Chymotrypsin has been found to be involved in clearance of ulcer, digesting damaged tissue and debris in the infected site [11-13]. Selective urease inhibitors which are also not involved in inhibiting α-chymotrypsin, may enhance the rate of healing of peptic ulcer. Due to this unique feature of action in treating peptic ulcers both urease and α-chymotrypsin were selected for their inhibitory activities of the synthesized compounds.

Inhibition of acetylcholinesterase (AChE) is considered as a promising approach for the treatment of Alzheimer's disease (AD) and for possible therapeutic applications in the treatment of Parkinson's disease, aging, and myasthenia gravis [14–19]. Meanwhile, butyrylcholinesterase (BChE) has been considered to be directly associated with the side effects of the acetylcholinesterase (AChE) inhibitors and the existing drugs for Alzheimer disease (AD). Recent studies have shown that (BChE) is found in significantly higher quantities in AD plaques than in the plaques of age related non-demented brains. Other relevant studies have also reported that the unfavorable side effect profiles of AChE inhibitors are not associated with their poor selectivity toward AChE [20]. To overcome AD, drugs have been developed which prevent the hydrolysis of the acetylcholine by blocking the acetylcholinesterse (AChE).

Thiourea derivatives possess wide spread applications as analgesic, herbicidal [21, 22], plant growth regulating [23], antiaggregating [24], antiarrythmic, potent antitumor [25], local anesthetic [26], antihyperlipidemic [27], and cytotoxic [28]. In view of these observations we became interested in the synthesis of some new bis(thiourea) derivatives and their Cu(II) and Ni(II) complexes. In present work these compounds were screened for their antioxidant, and cytotoxic inhibitions and their effect on the inhibition of enzymes like urease, α-glucosidase, α-chymotrypsin, acetylcholinesterse, butyrylcholinesterase.

Results and Discussions

Synthesis and Characterization

The detailed composition structure and synthetic route of ligands (L^1-L^6) is given in Scheme-I. Their Cu (II) and Ni (II) complexes were also synthesized. These ligands (L^1-L^6) and their metal (II) complexes were synthesized according to literature [29-34]. The synthesized compounds were also characterized by IR, ^{1}H-NMR, elemental analysis, electronic spectral data and magnetic moment values.

Antioxidant Activity

Table-1 depicts the antioxidant inhibitory effect of the synthesized compounds. The antioxidant activities of these compounds are expressed as 50% inhibitory concentration (IC_{50} in μM). The compound C^1 and C^7 shows better inhibitory effect with IC_{50} values 6.11±0.15 μM and 7.71±0.67 μM. IC_{50} values of compounds C^2, C^3, C^8 and C^9 shows that these compounds have better antioxidant inhibition as compared with other compounds. It can also be noted that the metal (II) complexes show better activity as compared to the ligands.

Cytotoxic Activity

Cytotoxicity (brine shrimp bioassay) was determined for all the compounds and their metal (II) complexes. The cytotoxicity is expressed as LD_{50}, i.e. concentration, at which 50% of the viable cells were killed under the assay conditions. From the data recorded in Table-2, it is evident that compounds **L^1**, C^1, C^7 and C^{10} displayed potent cytotoxic activity (LD_{50} = 3.32 x 10^{-4}, 3.12 x 10^{-4}, 3.65 x 10^{-4} and 1.32 x 10^{-4} moles/mL) against *Artemia Salina***,** while the other synthesized compounds were almost inactive in this assay. It was interesting to note that complexation with copper increased cytotoxicity. These findings may help to serve as a basis for future direction towards the development of bacteriostatic agents of lower cytotoxicity.

Table-1: Antioxidant activity of ligands (L^1-L^6) and their metal (II) complexes (C^1-C^{12}).

Compounds	Antioxidant (IC_{50})µg/mL	Compounds	Antioxidant (IC_{50})µg/mL	Compounds	Antioxidant (IC_{50})µg/mL
L^1	17.21±0.11	C^1	6.11±0.15	C^7	7.71±0.67
L^2	12.32±0.32	C^2	8.23±0.42	C^8	9.83±0.25
L^3	13.27±0.38	C^3	9.25±0.16	C^9	8.15±0.65
L^4	14.45±0.18	C^4	11.51±0.34	C^{10}	13.23±0.71
L^5	15.71±0.01	C^5	9.31±0.56	C^{11}	10.33±0.14
L^6	31.39±0.08	C^6	14.33±0.32	C^{12}	16.27±0.21
BHT	10.06± 0.09[f]	-	-	-	-

BHT = Butylated hydroxyl toluene

Table-2: Brine shrimp bioassay data of ligands (L^1-L^6) and their metal (II) complexes (C^1-C^{12}).

Compounds	LD_{50} (M)	Compounds	LD_{50} (M)	Compounds	LD_{50} (M)
L^1	3.32×10^{-4}	C^1	3.12×10^{-4}	C^7	3.65×10^{-4}
L^2	$>1.15 \times 10^{-3}$	C^2	$>2.15 \times 10^{-4}$	C^8	$>2.28 \times 10^{-3}$
L^3	$>1.13 \times 10^{-3}$	C^3	$>2.32 \times 10^{-3}$	C^9	$>2.98 \times 10^{-3}$
L^4	$>1.17 \times 10^{-3}$	C^4	$>1.43 \times 10^{-3}$	C^{10}	$>1.32 \times 10^{-4}$
L^5	$>2.31 \times 10^{-3}$	C^5	$>1.76 \times 10^{-3}$	C^{11}	$>2.45 \times 10^{-3}$
L^6	$>1.40 \times 10^{-3}$	C^6	$>2.30 \times 10^{-3}$	C^{12}	$>2.58 \times 10^{-3}$

Enzyme Inhibitory Assays

Antiurease Activity

The urease inhibition activity was carried out in accordance with the literature protocol [26] using thiourea as the standard inhibitor with an IC_{50} value of 21.0 ± 0.1 µM. The urease inhibition activity of all the synthesized compounds is given in Table-3. All the scanned compounds showed moderate to good urease inhibition activity. Compounds C^3, C^4, C^5, C^7, C^8, C^9 and C^{10} showed the potent urease inhibition with IC_{50} values of 16.7±0.7, 17.4±0.4, 20±0.8, 18.3±0.1, 20.6±0.9, 17.2±0.2 and 16.4±0.5 µM respectively. The least activity is shown by the compound L^5 showing an IC_{50} value of 29.32±0.32 µM. It can be noted that the Cu (II) complexes showed more activity as compared to Ni (II) complexes.

α-Glucosidase Activity

α-Glucosidase activity of all the synthesized compounds was performed against acarbose as standard with IC_{50} value of 36.20±0.05. Table-4 represents the α-glucosidase activits of all the synthesized materials. The different compounds show potential activity while some show moderate activity. The maximum potential activity with IC_{50} value 22.42 ± 0.43 µM is for the compound C^4. Most of the metal complexes exhibit better activity as compared to the standard material. It can also be noted that the complexed compounds have better activity as compared with the uncomplexed compounds. The IC_{50} values of compounds C^1- C^8 suggest that these compounds are better α-glucosidase inhibitors. The uncomplexed thiourea derivatives with some modifications seem to be potential material for future studies.

α- Chymotrypsin Activity

Table-5 shows the *α*-chymotrypsin inhibiton of all the synthesized compounds. In *α*-chymotrypsin inhibitory assay, only compounds C^1 and C^2 showed better inhibition against the enzyme with IC_{50} values of 4.13±0.20 µM and 4.27±0.16 µM respectively. Chymostatin was used as standard inhibitor in this assay (IC_{50} = 5.7±0.1 µM). Most of the compounds C^{11} and C^{12} also showed potential activity with IC_{50} values of 6.30±0.31 µM and 6.20±0.40 µM which are comparable with the standard. These results show that these compounds especially the Cu (II) complexes can be used as potential *α*-chymotrypsin inhibitors.

AChE, BChE and LOX Activity

The inhibitory activity of our synthetic derivatives was evaluated against AChE, BChE and LOX enzymes. Their IC_{50} values and selectivity index for the inhibition of AChE and BChE are summarized in Table-6 and 7. All these compounds were tested in five concentrations limited up to 100 µM, resulting in ranged from 20% to 80% enzyme inhibition. For comparison, AChE and BChE were studied against eserine while the LOX activity was studied against baicalein. All synthetic derivatives showed weak inhibitory activity selectivity against AChE over BChE. The maximum BChE inhibition was shown compounds C^7 with an IC_{50} value of 9.13±0.81 µM. The maximum AChE inhibition was shown by Compounds C^1 with an IC_{50} value of 3.04±0.12 µM. These results show that these compounds do not have better activity as compared with the standard material. While on the other hand the LOX inhibition studies of the synthesized compounds show that these compounds can be used for LOX inhibition. Most of the compounds show better inhibition as compared with the standard material. It can also be noted that the LOX inhibition increased on complexation as shown in Table-8.

Table-3: Antiureas activity of ligands (L^1-L^6) and their metal (II) complexes (C^1-C^{12}).

Compounds	Antiureas (IC_{50}) µg/mL	Compounds	Antiureas (IC_{50}) µg/mL	Compounds	Antiureas (IC_{50}) µg/mL
L^1	28.4 ± 0.3	C^1	13.4 ± 0.13	C^7	18.3 ± 0.1
L^2	26.3 ± 0.5	C^2	19.3 ± 0.2	C^8	20.6 ± 0.9
L^3	26.7 ± 0.5	C^3	16.7 ± 0.7	C^9	17.2 ± 0.2
L^4	27.4 ± 0.2	C^4	17.4 ± 0.4	C^{10}	16.4 ± 0.5
L^5	29.32 ± 0.32	C^5	20 ± 0.8	C^{11}	20.21 ± 0.51
L^6	50.1 ± 0.5	C^6	32.5 ± 0.1	C^{12}	34.12 ± 0.3
Thiourea	21.0 ± 0.1	-	-	-	-

Table-4: α-glucosidase activity of ligands (L^1-L^6) and their metal (II) complexes (C^1-C^{12}).

Compounds	α-glucosidase (IC_{50}) µg/mL	Compounds	α-glucosidase (IC_{50}) µg/mL	Compounds	α-glucosidase (IC_{50}) µg/mL
L^1	37.41 ± 0.32	C^1	20.14 ± 0.11	C^7	28.53 ± 0.15
L^2	48.3 ± 0.15	C^2	24.13 ± 0.52	C^8	27.61 ± 0.19
L^3	56.7 ± 0.52	C^3	23.47 ± 0.71	C^9	37.32 ± 0.28
L^4	52.4 ± 0.22	C^4	22.42 ± 0.43	C^{10}	35.42 ± 0.56
L^5	49.32 ± 0.31	C^5	38.73 ± 0.28	C^{11}	27.26 ± 0.21
L^6	50.1 ± 0.57	C^6	29.5 ± 0.41	C^{12}	42.23 ± 0.32
acarbose	36.20±0.05	-	-	-	-

Table-5: α-chymotrypsin activity of ligands (L^1-L^6) and their metal (II) complexes (C^1-C^{12}),

Compounds	α-chymotrypsin (IC_{50}) µg/Ml	Compounds	α-chymotrypsin (IC_{50}) µg/Ml	Compounds	α-chymotrypsin (IC_{50}) µg/Ml
L^1	19.11±0.12	C^1	4.13±0.20	C^7	16.81±0.04
L^2	21.53±0.19	C^2	4.27±0.16	C^8	19.03±0.12
L^3	23.27±0.18	C^3	10.21±0.13	C^9	18.15±0.14
L^4	24.15±0.53	C^4	13.52±0.10	C^{10}	13.02±0.24
L^5	55.17±0.21	C^5	17.21±0.42	C^{11}	6.30±0.31
L^6	41.41±0.12	C^6	19.13±0.20	C^{12}	6.20±0.40
Chymostatin	5.7±0.1	-	-	-	-

Table-6: AChE activity of ligands (L^1-L^6) and their metal (II) complexes (C^1-C^{12}).

Compounds	AChE (IC_{50}) µg/mL	Compounds	AChE (IC_{50}) µg/mL	Compounds	AChE (IC_{50}) µg/mL
L^1	12.32±0.43	C^1	3.04±0.12	C^7	5.01±0.21
L^2	13.11±0.31	C^2	6.24±0.02	C^8	7.12±0.32
L^3	17.23±0.35	C^3	8.21±0.30	C^9	9.31±0.50
L^4	14.13±0.32	C^4	10.04±0.06	C^{10}	12.14±0.03
L^5	36.63±0.81	C^5	12.60±0.03	C^{11}	18.10±0.05
L^6	25.22±0.22	C^6	9.21±0.15	C^{12}	10.22±0.1
Eserine	0.04±0.001	-	-	-	-

Table-7: BChE activity of ligands (L^1-L^6) and their metal (II) complexes (C^1-C^{12}).

Compounds	BChE (IC_{50}) µg/mL	Compounds	BChE (IC_{50}) µg/mL	Compounds	BChE (IC_{50}) µg/mL
L^1	14.32±0.21	C^1	8.12±0.21	C^7	9.13±0.81
L^2	12.16±0.11	C^2	11.23±0.11	C^8	12.08±0.06
L^3	16.28±0.34	C^3	10.21±0.34	C^9	10.01±0.32
L^4	18.13±0.81	C^4	12.04±0.81	C^{10}	12.19±0.11
L^5	40.63±0.86	C^5	16.20±0.86	C^{11}	16.30±0.23
L^6	34.24±0.23	C^6	21.31±0.23	C^{12}	21.21±0.27
Eserine	0.85±0.001	-	-	-	-

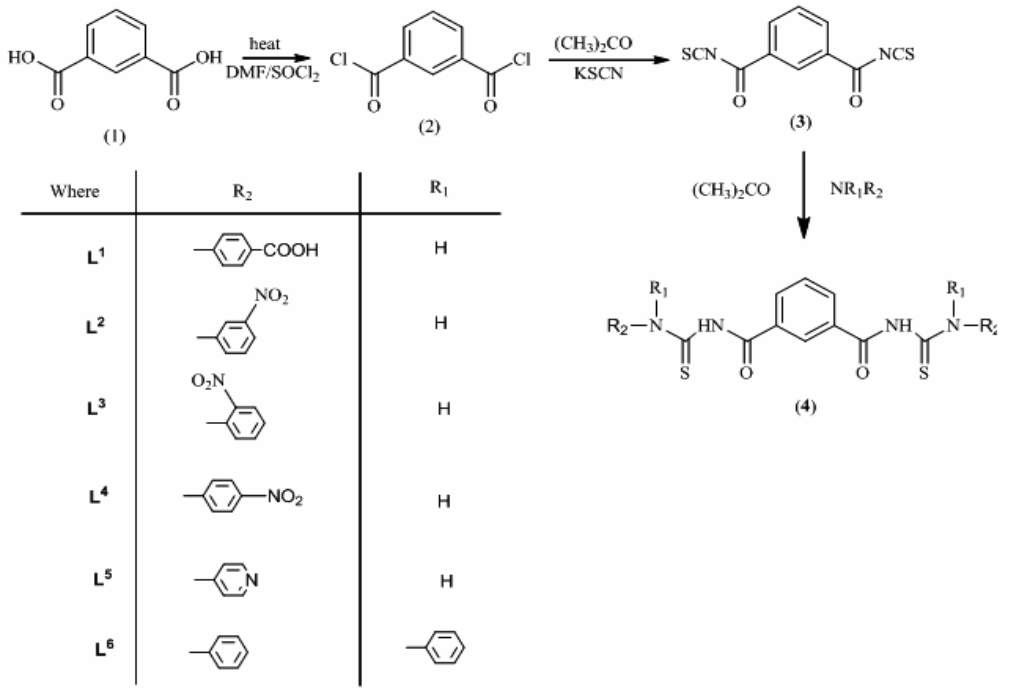
Table-8: LOX activity of ligands (L^1-L^6) and their metal (II) complexes (C^1-C^{12}).

Compounds	LOX (IC_{50}) µg/mL	Compounds	LOX (IC_{50}) µg/mL	Compounds	LOX (IC_{50}) µg/mL
L^1	31.42±0.54	C^1	11.12±0.51	C^7	14.92±0.50
L^2	28.11±0.01	C^2	18.15±0.05	C^8	19.85±0.38
L^3	27.23±0.32	C^3	17.21±0.35	C^9	27.27±0.21
L^4	34.13±0.32	C^4	24.03±0.31	C^{10}	22.23±0.98
L^5	33.71±0.01	C^5	23.43±0.31	C^{11}	21.13±0.32
L^6	40.34±0.12	C^6	20.54±0.21	C^{12}	19.32±0.11
Baicalein	22.4±1.3	-	-	-	-

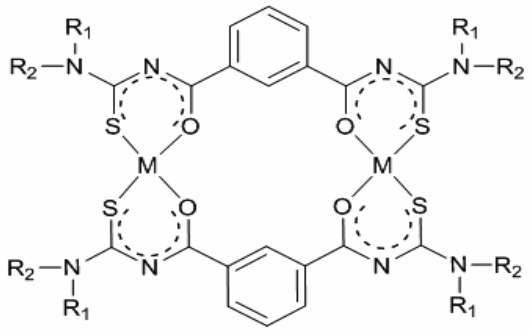
Experimental

Synthesis

The synthetic route for the newly synthesized compounds, N^3, $N^{3'}$-bis(disubstituted) isophthalyl-bis(thioureas) **(L^1-L^6)**, is illustrated in Scheme-1. The proposed structure for metal (II) complexes is presented in Fig. 1. The target compounds **(L^1-L^6)** were purified by recrystallization from DMF and characterized by IR and ^{1}H NMR data. Metal complexation was carried out in DMF solvent. The formation of metal (II) complexes **(**C^1-C^{12}) was confirmed through IR, elemental analysis, magnetic moment values and electronic transition studies.



Scheme-1: synthesis of disubstituted bisthiourea derivatives (L^1–L^6).



Where M = Cu (II) for C^1-C^6 and Ni (II) for C^7-C^{12}

Fig. 1: Proposed structure for metal (II) complexes.

Pharmacology

Antioxidant Activity

The synthetic organic compounds were dissolved in appropriate solvent to prepare a solution of known concentration and used for the determination of antioxidant activity by the method of Garrat, (1964) [35]. Known volume (20 µl) of sample solution was taken in one properly labeled dry test tube followed by the addition of 200 µl of sodium nitroprusside (10mM solution) and 1780 µl of phosphate buffer (pH = 7.4). The whole experimental set was incubated at 37°C for 2 h. After the completion of incubation period 200 µl Griess reagent (0.3 % sulphanilic acid in glacial acetic acid, 0.1 % naphthyl ethylene diamine (NED): Mixed equal volume of both solutions just before use. Mixture was kept at room temperature for 20 min. The absorbance was measured at 528 nm. Each sample was analyzed in triplicate. Positive control (containing every component in the same proportion as added in the sample reaction mixtures except that the phosphate buffer is added in place of sample; keeping the total volume of reaction mixture same as that of sample reaction mixtures) and negative control (containing all but sodium nitroprusside) were run in parallel. Negative control was used to calibrate the instrument. In case of colored compounds, sample blanks were also prepared. Absorbance of sample blanks was subtracted from the absorbance of sample to get the corrected absorbance of sample. Butylated hydroxyl toluene was used as positive control.

Cytotoxic Activity

In vitro cytotoxic activity of all the synthesized ligands (L^1–L^6) and their metal (II) complexes (C^1–C^{12}) were studied using the protocol of Meyer *et al*. [36] Brine shrimp (Artemia salina Leach) eggs were hatched in a shallow rectangular plastic dish (22×32 cm), filled with artificial sea water, which was prepared with commercial salt mixture and double-distilled water. Data were

analyzed by Finney computer program to determine the LD_{50} values [37].

Enzyme Inhibitory Activities

Antiurease Activity

The reaction mixtures comprising 25 µL of Jack bean Urease solution, 55 µL of buffers and 100 mM urea were incubated with 5 µL (1 mM conc.) of the test compounds at 30 ºC for 15 min in well plates. The measurement of ammonia production (indophenol method) [38] was used to determine the urease activity. The phenol reagent (45 µL, 1 % w/v phenol and 0.005 % w/v sodium nitroprusside) and alkali reagent (70 µL, 0.5 % w/v sodium hydroxide and 0.1 % NaOCl) were added to each well and the increasing absorbance at 630 nm was measured after 50 min, using a microplate reader (Molecular Device, USA). The change in absorbance per minute was noted and the results processed using Soft Max Pro software (Molecular Device, USA). All the reactions were performed in triplicate. All the assays were performed at pH 8.2 (0.01 M $K_2HPO_4.3H_2O$, 1 mM EDTA and 0.01 M $LiCl_2$). Thiourea was used as the standard inhibitor.

α-Glucosidase Activity

The α-glucosidase inhibition activity was performed according to the slightly modified method of Pierre *et al*. [39]. Total volume of the reaction mixture of 100 µL contained 70 µl 50 mM phosphate buffer saline, pH 6.8, 10 µl (0.5 mM) test compound, followed by the addition of 10 µl (0.057 units) enzyme. The contents were mixed, preincubated for 10 min at 37ºC and pre read at 400 nm. The reaction was initiated by the addition of 10 µl of 0.5 mM substrate (p-nitrophenyl glucopyranoside). Acarbose was used as positive control. After 30 min of incubation at 37ºC, absorbance was measured at 400 nm using Synergy HT microplate reader. All experiments were carried out in triplicates.

α- Chymotrypsin Activoty

The- chymotrypsin inhibition activity is performed according to slightly modified method of A.U. Rehman *et al*., [40]. Total volume of the reaction mixture of 100 µl contained 60 µl 50 mM Tris-HCl, buffer, pH 7.6, 10µl (0.5mM) by the addition of 15µl(0.9units) enzyme. The contents were mixed, preincubated for 15 min at 37ºC and pre read at 410nm. The reaction was initiated by the addition of 15µl of 1mM substrate (N-succinyl phenyl-alanine-P-nitroanilide).70µl of buffer was used as a control. After 60 min of incubation at 37ºC, absorbance was measured at 410 nm using synergy HT microplate reader. All experiments were carried out in triplicate.

AChE, BChE and LOX Enzymes Activity

Lipoxygenase (LOX), Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzyme inhibitory assays were performed according to the method described in literature [41-43] with slight modifications. The percentage inhibition (%) was calculated by formula given below:

$$\% \text{ Inhibition} = \frac{\text{Control - Test}}{\text{Control}} \times 100$$

where: Control = Total enzyme activity without inhibitor, Test = Activity in the presence of test compound. In all enzyme inhibitory assays, the IC_{50} values (concentration of substrate at which there is 50% enzyme catalyzed the reaction) were calculated using EZ–Fit Enzyme Kinetics software (Perrella Scientific Inc. Amherst, USA). All the measurements were done in triplicate and statistical analysis was performed by Microsoft Excel 2007. Results are presented as mean ± sem.

Conclusion

Some novel $N^3,N^{3'}$-bis(disubstituted) isophthalyl-bis(thioureas) and $N^3,N^3,N^{3'},N^{3'}$-tetrakis(disubstituted)isophthalyl-bis(thiourea) and their Cu (II) and Ni (II) complexes have been synthesized and characterized by analytical and spectral (IR, ^{1}H-NMR, electronic) techniques. Antioxidant, antiureas, cytotoxic and some other enzyme inhibition activities of these compounds was studied against some standard compounds. Some compounds showed potential activity against some enzyme species. It was concluded that these compounds could be a potential source as active antioxidant and antiureas agents.

References

1. P. A. Karplus, M. A. Pearson and R. P. Hausinger, *Accounts of Chemical Research*, **30,** 330 (1997).
2. R. P. Hausinger, Biochemistry of Nickel, Plenum Press, New York, 23 (1993).
3. H. L. T. Mobley and R. P. Hausinger, *Microbiological Reviews*, **53**, 85 (1989).
4. H. L. T. Mobley, M. D. Island and R. P. Hausinger, *Microbiological Reviews*, **59**, 451 (1995).
5. E. Jabri, M. B. Carr, R. P. Hausinger and P. A. Karplus, *Science*, **268**, 998 (1995).
6. A. J. Hirsh, S. Y. Yao, J. D. Young and C. I. Cheeseman, *Gastroenterology*, **113**, 205 (1997).
7. S. Chiba, *Bioscience Biotechnology and Biochemistry,* **61**, 1233 (1997).

8. A. D. Baron, *Diabetes Research and Clinical Practice*, **40**, 51 (1998).
9. H. E. Lebovitz, *Endocrinology and Metabolism Clinics of North America*, **26**, 539 (1997).
10. A. Coblentz, *Journal of the American Geriatrics Society*, **16**, 1039 (1968).
11. M. Arfan, M. Ali, H. Ahmad, I. Anis, A. Khan, M. I. Choudhary and M. R. Shah, *Journal of Enzyme Inhibition and Medicinal Chemistry*, **25**, 296 (2010).
12. R. J. P. Cannell, S. J. Kellam, A. M. Owsianka and J. M. Walker, *Planta Medica*, **54**, 10 (1988).
13. L. Zhang, S. B. Mulrooney, A. F. K. Leung, Y. Zeng, B. C. K. Ben, R. P. Hausinger and H. Sun, *Biometals*, **19**, 503 (2006).
14. H. Sugimoto, H. Ogura, Y. Arai, Y. Iimura and Y. Yamanishi, *Japanese Journal of Pharmacology*, **89**, 7, (2002).
15. J. L. Sussman, M. Harel, F. Frolow, C. Oefner, A. Goldman, L. Toker and I. Silman, *Science*, **253,** 872 (1991).
16. G. M. Shankar, S. Li, T. H. Mehta, G. A. arcia-Munoz, N. E. Shepardson, I. Smith, F. M. Brett, F. M. A. arrell, M. J. Rowan, C. A. Lemere, C. M. Regan, D. M. Walsh, B. L. Sabatini and D. J. Selkoe, *Nature Medicine*, **14**, 837 (2008).
17. S. Nochi, N. Asakawa and T. Sato, *Biological and Pharmaceutical Bulletin*, **18**, 1145 (1995).
18. W. Tong, E. R. Collantes, Y. Chen and W. J. Welsh, *Journal of Medicinal Chemistry*, **39**, 380 (1996).
19. Q. Yu, H. W. Holloway, T. Utsuki, A. Brossi and N. H. Greig, *Journal of Medicinal Chemistry*, **42**, 1855 (1999).
20. N. H. Greig, T. Utsuki, Q. Yu, X. Zhu, H. W. Holloway, T. Perry, B. Lee, D. K. Ingram and D. K. Lahiri, *Current Medical Research Opinion*, **17**, 159 (2001).
21. L. Xiao, C. J. Liu and Y. P. Li, *Molecules,* **14**, 1423 (2009).
22. J. H. Hua, L. C. Wang, H. Liu and T. B. Wei, *Phosphorus Sulfur*, **181**, 2691 (2006).
23. A. Ranise, F. Bondavalli, O. Bruno, S. Schenone, D. Donnoli, C. Parrillo, M. L. Cenicolaa and F. Rossi, *Farmaco*, **46**, 1203 (1991).
24. A. Ranise, A. Spallarossa, O. Bruno, S. Schenone, P. Fossa, G. Menozzi and F. Bondavalli, *Farmaco*, **58**, 765 (2003).
25. R. Vig, C. Mao, T. K. Venkatachalam, L. Tuel-Ahlgren, E. A. Sudbeck and F. M. Uckun, *Bioorganic and Medicinal Chemistry*, **6**, 1789 (1998).
26. S. Claridge, F. Raeppel, M. C. Granger, N. Bernstein, O. Saavedra, L. Zhan, D. Llewellyn, A. Wahhab, R. Deziel, J. Rahil, N. Beaulieu, H. Nguyen, I. Dupont, A. Barsalou, C. Beaulieu, I. Chute, S. Gravel, M. F. Robert, S. Lefebvre, M. Dubay, R. Pascal, J. Gillespie, Z. Jin, J. Wang, J. M. Besterman, A. R. MacLeod and A. Vaisburg, *Bioorganic and Medicinal Chemistry Letters*, **18**, 2793 (2008).
27. S. N. Manjula, N. M. Noolvi, K. V. Parihar, S. A. M. Reddy, V. Ramani, A. K. Gadad, G. S. Singh, N. G. Kutty and C. M. Rao, *European Journal of Medicinal Chemistry,* **44**, 2923 (2009).
28. Y. M. Zhang, T. B. Wei and L. M. Gao, *Synthetic Communications,* **31**, 3099 (2001).
29. H. Arslan, N. Kulcu and U. Florke, *Transition Metal Chemistry,* **28**, 816 (2003).
30. M. Jamil, M. Zubair, A. A. Altaf, M. A. Farid, M. T. Hussain, N. Rasool, I. H. Bukhari and V. U. Ahmad. *Journal of the Chemical Society of Pakistan*, **35**, 737 (2013).
31. D. S. Mansuroglu, H. Arslan, U. Florke and N. Kulcu, *Journal of Coordination Chemistry,* **61**, 3134 (2008).
32. M. Jamil, M. Zubair, M. A. Farid, U. Rashid, N. Rasool, S. Islam, *Journal of Chemistry* 2013**,** 8, (2013).
33. E. Khan, A. Badshah and R. Kempe, *Journal of the Chemical Society of Pakistan*, **32**, 349 (2010).
34. M. Jamil, M. Zubair, M. A. Farid, A. A. Altaf, N. Rasool, I. H. Bukhari, K. Rizwan and V. U. Ahmad. *Journal of the Chemical Society of Pakistan*, **35**, 834 (2013).
35. B. N. Meyer, N. R. Ferrigni, J. E. Putnam, L. B. Jacobsen, D. E. Nichols and J. L. McLaughlin, *Planta Medica*, **45**, 31 (1982).
36. D. J. Finney, Probit Analysis, 3rd edn, Cambridge University Press, Cambridge, UK (1971).
37. M. W. Weatherburn, *Analytical Chemistry*, **39**, 971 (1967).
38. C. Pierre, R. Roland, Tremblay and J. Y. Dube, *Clinical Chemistry*, **24**, 208 (1978).
39. A. M. Khan, S. Anjum, M. I. Choudhary and A. Rahman, *World Journal of Chemistry* **1**, 30 (2006).
40. G. L. Ellman, K. D. Courtney, V. Andres and R. M. Featherstone, *Biochemical Pharmacology,* **7**, 88, (1961).
41. A. L. Tappel, *Archives of Biochemistry Biophysics,* **44**, 378 (1953).
42. A. T. Evans, *Biochemical Pharmacology,* **36**, 2035 (1987).
43. S. Baylac and P. Racine, *International Journal of Aromatherapy,* **13**, 138 (2003).