HL **ISSN: 2231 – 3087(print) / 2230 – 9632 (Online)**
http://heteroletters.org **Vol. 2: (3), 2012, 333-348**

SYNTHESIS, ANTIMICROBIAL AND ANTIOXIDANT ACTIVITIES OF SOME NEW 1'-(10-SUBSTITUTED 5*H*, 6*H*, *7H*-INDOLO[2,3-c]ISOQUINOLIN-5YLTHIO)FORMYL-3',5'-DISUBSTITUTED PYRAZOLES, -3'-METHYLPYRAZOL-5'-ONES AND -1',3',4' OXIDIAZOL-2'-THIONES[#]

Saundane Anand R[a]*, Vaijinath A.Verma[b] and Katkar Vijaykumar[a]

[a]*Department of Post-Graduate Studies and Research in Chemistry, Gulbarga University, Gulbarga -585 106, Karnataka, INDIA*
[b]Shri Prabhu Arts, Science & J M. Bohra Commerce College, Shorapur-585 224.
E-Mail:arsaundane@rediff.com

Abstract
The precursor ethyl(10-substituted *7H*-indolo[2,3-c]isoquinolin-5-ylthio)formates (**2a-c**) were synthesized by reacting compounds (**1a-c**) with ethyl chloroformate. Compounds (**2a-c**) on treatment with hydrazine hydrate afforded (10-substituted *7H*-indolo[2,3-c]isoquinolin-5-ylthio)carbohydrazides (**3a-c**). These hydrazides on cyclocodensation with acetyl acetone, dibenzoyl methane, ethyl acetoacetate and carbon disulphide afforded (10-substituted *7H*-indolo[2,3-c]isoquinolin-5-ylthio)formyl-, -3',5'-dimethyl/phenylpyrozoles (**4a-f**), -3'-methylpyrozol-5'-ones (**5a-c**) and 5'-(10-substituted *7H*-indolo[2,3-c]isoquinolin-5-ylthio) -1',3',4'-oxidiazol-2'-thiones (**6a-c**), respectively. The structures of these newly synthesized compounds were confirmed by their spectral studies and elemental analysis. These compounds were screened for their antimicrobial and antioxidant activities. Compounds **2a**, **3a**, **4a**, **3e**, **4b**, **5c**, **6b** and **6c** showed good antibacterial activity, whereas compounds **2c**, **3a**, **4a**, **4c**, **4e**, **4f**, **5a**, **5b**, **6a**, **6b** and **6c** exhibited maximum zone of inhibition in case of antifungal activity. Compounds **2a**, **3a**, **3c**, **4a**, **4c**, **4d**, **4e**, **5a**, **5c**, **6a** and **6c** showed good radical scavenging activity. Compounds **2a**, **3c**, **4a** and **5a** exhibited good reducing power activity, whereas the compounds **2a**, **3a**, **4a**, **4c**, **4f**, **5a** and **5b** showed promising metal chelating activity when compared with standards.

Keywords: Indolo[2,3-c]isoquinoline, pyrozole, pyrazolone, oxadizole, antimicrobial, antioxidant.

---

* To whom correspondence should be addressed.

presented at the "Indian Council of Chemists XXIX Annual Conference-2010" organized by Department of Chemistry, Punjab University, Chandigarh during December 19th-21st, 2010.

Introduction

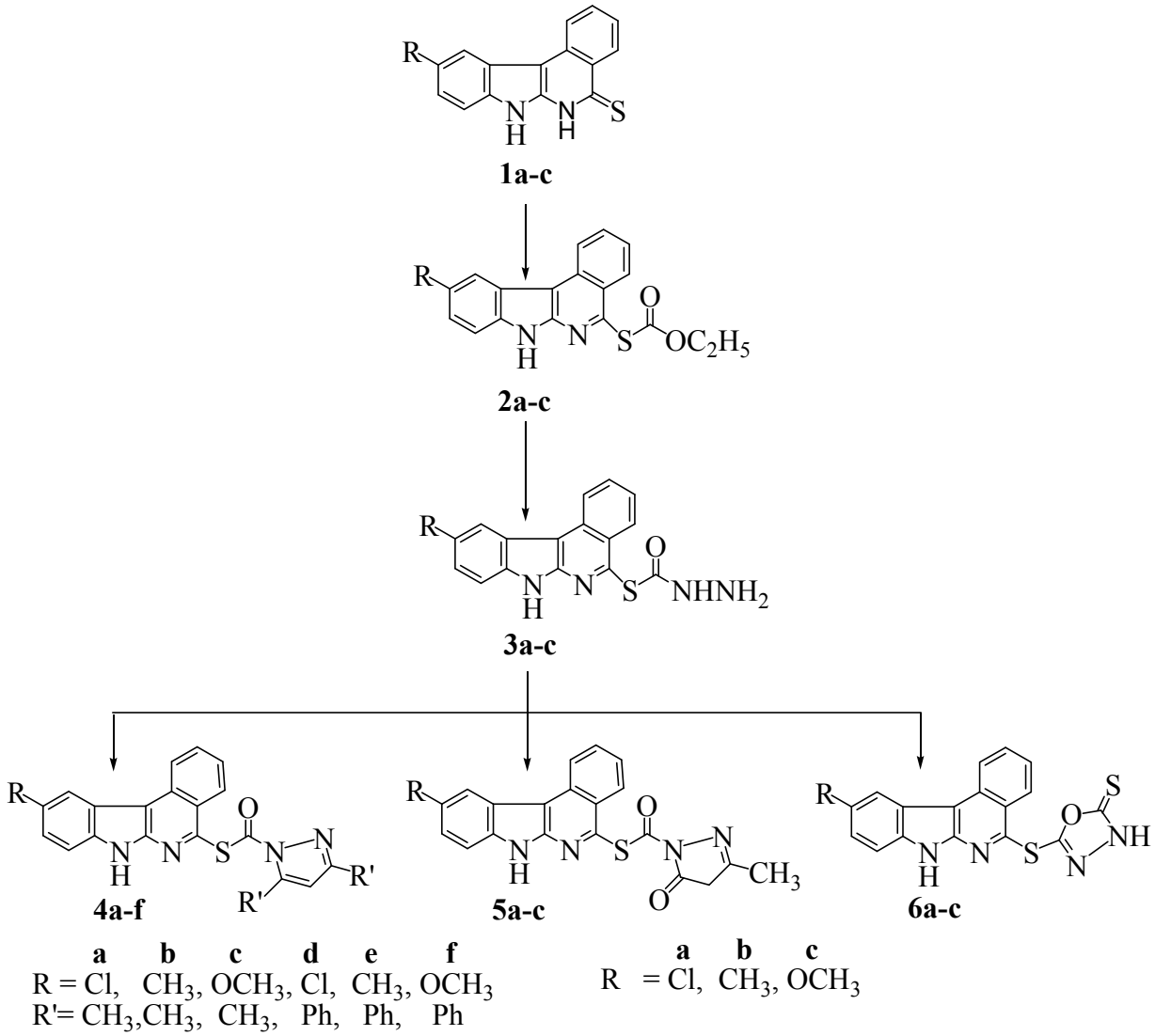
A large number of drugs and biologically relevant molecules contains heterocyclic systems. Often the presence of hetero atoms or groupings imparts preferential specificities in their biological response. The chemistry and biological study of heterocyclic compounds has been interesting field for a long time due to medicinal and agricultural applications. Indoloisoquinolines are known for their bactericidal[1], fungicidal[2], anticancer[3], antihistaminic[4] activities. Some of indolyl pyrozole derivatives are the most active classes of compounds possessing wide spectrum of biological importance such as anti-inflammatory, anti-pyretic and analgesic properties.[5-7] Also some of 3,5-dimethyl pyrazoles and 3-methyl pyrazol-5-ones compounds showed anti-infalammatory, analgesic, ulcerogenic and lipid peroxidation activities[8]. On the same line, 1,3,4-oxdiazoles compounds exhibited anti-cancer[9], anti-convulsant[10] and antidiabetic[11] activities. Some of the 6*H*, 11*H*-indolo[3,2-c]isoquinolin-5-ones/thiones have been reported from this laboratory found to possess potent antibacterial and antifungal activities[12,13]. In view of these findings and in continuation of our ongoing search for new heterocyclics[14-16] of biological importance, we have synthesized the title compounds and screened them for their antimicrobial and antioxidant activities.

Result and discussion

The title compounds were synthesized as outlined in **Scheme-1**. The starting material 10-substituted *5H*, *6H*, *7H*-indolo[2,3-c]isoquinolin-5-thiones (**1a-c**) were synthesized using reported procedure[17]. The precursor ethyl(10-substituted *7H*-indolo[2,3-c]isoquinolin-5-ylthio)formates (**2a-c**) were synthesized by reacting compounds (**1a-c**) with ethyl chloroformate in dry acetone at refluxed temperature. Compounds (**2a-c**) on reaction with hydrazine hydrate in refluxing ethanol afforded (10-substituted 7*H*-indolo[2,3-c]isoquinolin-5-ylthio)carbohydrazide (**3a-c).**

The hydrazide (**3a**) was subjected to cyclocondensation with acetyl acetone, dibenzoyl methane, ethyl acetoacetate in dry methanol containing catalytic amount of conc. hydrochloric acid to yield 1'-(10-chloro-7*H*-indolo[2,3-c]isoquinolin-5-ylthio)formyl-3',5'-dimethylpyrozole (**4a**), 1'-(10-chloro-7*H*-indolo[2,3-c]isoquinolin-5-ylthio)formyl-3',5'-diphenylpyrozole (**4d)** and 1'-(10-chloro-7*H*-indolo[2,3-c]isoquinolin-5-ylthio)formyl-3'-methylpyrozol-5'-one (**5a)**, respectively.

The compound (**3a**) on reaction with carbon disulphide and potassium hydroxide in dry methanol under reflux conditions afforded 5'-(10-chloro-7*H*-indolo[2,3-c]isoquinolin-5-ylthio) - 1',3',4'-oxidiazol-2'-thione (**6a).** Similarly other derivatives in the series were prepared and structures of these compounds were confirmed by their spectral studies and elemental analysis.



Scheme -1

Biological results

Antimicrobial activity

The newly synthesized compounds (**2-6)** were evaluated for their antibacterial activity against *Escherichia coli, Bacillus subtilis, Pseudomonas putida* and *Klebsiella pneumonia* and antifungal activity against *Asperigillus niger, Asperigillus flavus, Asperigillus fumigates* and *Trichophyton tonsurans* by cup-plate method at a concentration of 1mg/ml following reported procedure[18]. The zones of inhibition were compared with the standards streptomycin and flucanazole for antibacterial and antifungal activity, respectively. These results are reported in **Table-1**.

The investigation of antibacterial screening revealed that, compounds **3a**, **4a**, **4e** and **5c** exhibited maximum zone of inhibition against *E. coli.* Compounds **2a**, **3a**, **4b** and **6c** showed maximum zone of inhibition against *B. subtilis.* Compounds **4a** and **4b** exhibited maximum zone of inhibition against *P. putida* and compounds **3a**, **4b**, **4e** and **6b** exhibited the maximum zone of inhibitory against *K. pneumonia.*

In case of antifungal screening, the compounds **2c**, **4f**, **5a**, **6b** and **6c** exhibited promising activity against *A. niger,* whereas compounds **3a**, **4c**, **4e**, **4f**, **5b** and **6b** exhibited maximum zone of inhibition against *A. flavus.* Compounds **5a** and **6b** showed maximum zone of inhibition against *A. fumigatus.* The compounds **3a**, **4a**, **4c**, **4f** and **6a** exhibited maximum zone of inhibition against *T. tonsurans.*

From the results of antimicrobial activities, it could be assumed that, the majority of synthesized compounds having chloro or methoxy substitutents exhibited maximum growth inhibitory activity. The electronegative nature of the chloro or electron donor methoxy group may be responsible to inhibit the growth of the microbes.

Table-1 Antimicrobial activity results of compounds (2-6)

Comp No	Antibacterial activity (zone of inhibition in mm)				Antifungal activity (zone of inhibition in mm)			
	E. Coli	*B. Subtilis*	*P. Putida*	*K. Pneumonia*	*A. Niger*	*A. Flavus*	*A. Fumigatus*	*T. Tousurans*.
2a	10	14	13	14	05	10	13	10
2b	10	11	08	08	10	01	06	11
2c	06	09	10	02	16	11	02	08
3a	13	14	6	19	05	15	13	18
3b	5	7	3	10	11	10	10	09
3c	11	12	6	4	14	03	07	07
4a	12	9	16	12	10	8	5	18
4b	06	14	17	17	08	07	11	11
4c	09	08	06	02	13	15	13	17
4d	01	11	07	03	2	11	10	14
4e	13	03	07	17	14	14	09	11
4f	09	05	08	11	16	14	10	18
5a	04	08	01	14	16	10	16	09
5b	08	04	02	11	6	15	11	11
5c	13	08	08	05	4	5	13	13
6a	06	06	05	02	09	07	02	17
6b	01	07	12	18	16	14	15	10
6c	11	14	08	05	17	01	04	14
Std_1	-	-	-	-	18	16	18	19
Std_2	`14	16	18	20	-	-	-	-

Where, Std_1= Streptomycin, Std_2= Flucanazole.

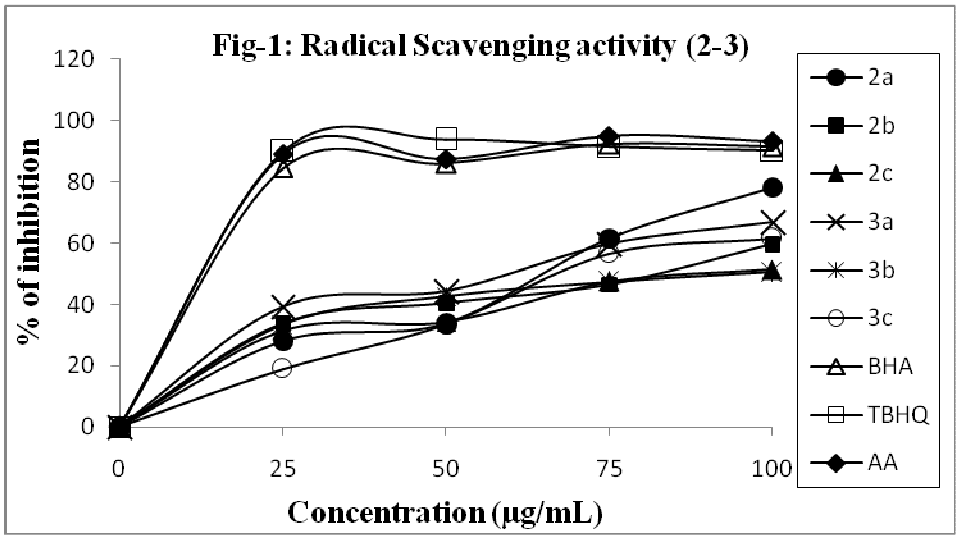
Antioxidant activities

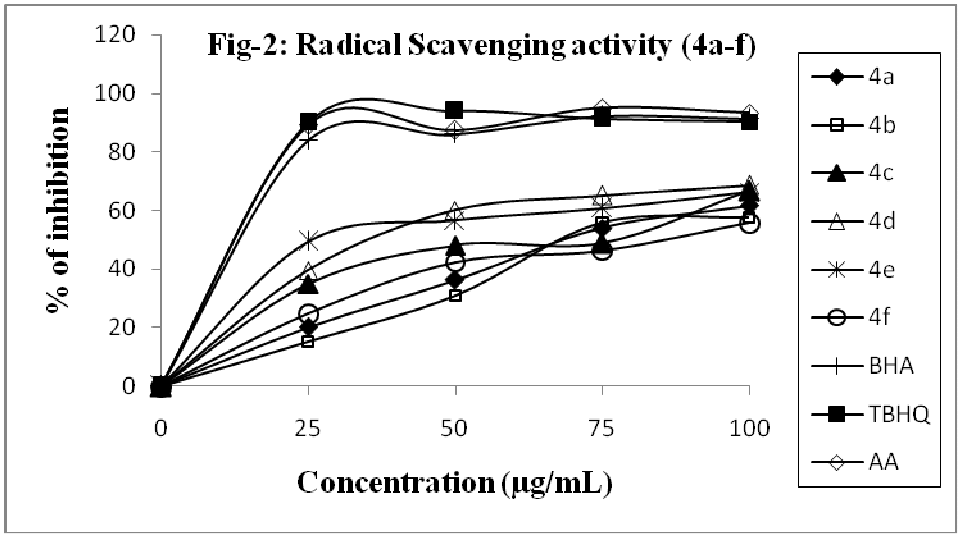
I) 1, 1-diphenyl-2-picryl hydrazyl (DPPH) radical scavenging activity (RSA)

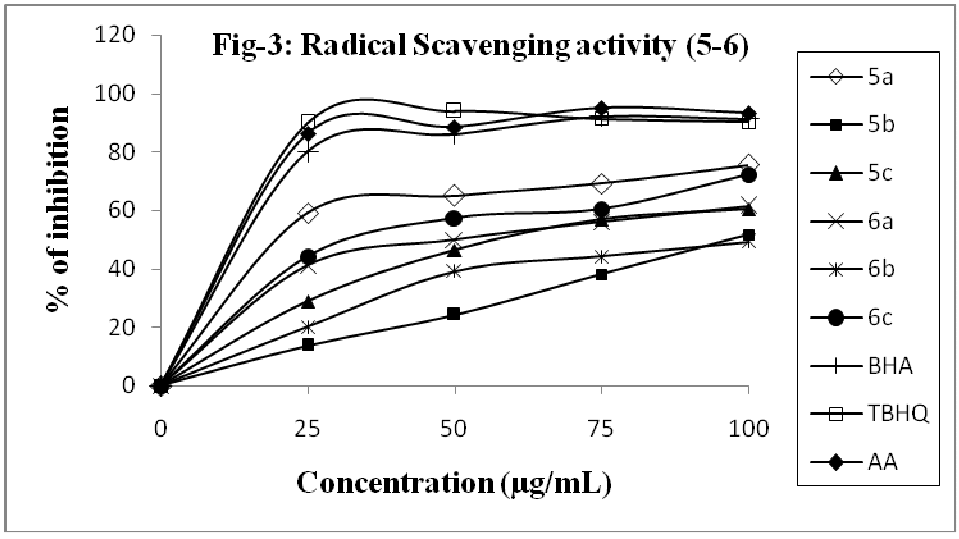
Free radicals are atomic or molecular species with unpaired electrons that are highly reactive. They take part in chemical reactions and play an important role in many chemical processes. The RSA of synthesized compounds was compared with the standards 2-tert-butyl-4-methoxy phenol (butylated hydroxyl anisole, BHA), 2-(1, 1-dimethylethyl)-1, 4-benzenediol (tertiary butylated hydroquinone, TBHQ) and Ascorbic acid (AA) by using Hatano's method[19]. The results are shown in the figs 1-3.

The analysis of results indicated that, compounds **4d** and **5a** exhibited good radical scavenging activity of 60.09 and 65.09%, respectively at conc. of 50 µg/ml. Compounds 2**a**, **4d**, **4e**, **5a** and **6c** showed radical scavenging ability of 61.55, 65.09, 60.69, 69.18 and 60.45% at conc. of 75 µg/ml, respectively. Whereas, **2a**, **3a**, **3c**, **4a**, **4c**, **4d**, **4e**, **5a**, **5c**, **6a** and **6c** exhibited good radical

scavenging activity of 78.33, 66.85, 61.55, 61.64, 66.60, 68.55, 66.28, 75.54, 60.42, 61.56 and 72.14 %, respectively at 100 µg/ml.







II) Iron Metal ions Chelating Activity

Extensive experience demonstrates that acute and chronic human intoxications with a wide range of metals can be treated with considerable efficiency by the administration of a relevant chelating agent. Development of effective chelating agent is based on combinations of chemical consideration and whole animal experimentation on the toxicokinetics and toxicodynamic of metal and chelating agents, followed by clinical experience, with regard to monitoring metal excretion and status of tissue damage[20]. Metal chelating capacity was significant, since it reduces the concentration of the catalysing transition metal in lipid peroxidation. It was reported that chelating agents, which form σ-bonds with a metal, are effective as secondary antioxidants because they reduce the redox potential therapy stabilizing the oxidized form of metal ion[21]. Transition metals (iron, copper, chromium, cobalt, vanadium, cadmium and nickel) can also be mediators in the formation of free radicals[22]. Among the transition metal ions, iron is known as the most important lipid oxidation pro-oxidant due to its high reactivity. Iron can stimulate lipid peroxidation by the Fenton reaction and also accelerates peroxidation by decomposing lipid hydroperoxides into peroxy and alkoxy radicals that can themselves abstract hydrogen and perpetuate the chain reaction of lipid peroxidation[23, 24].

$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + OH^- + OH^{\cdot}$$

Fe^{3+} ion also produces radical from peroxides, although the rate is ten-fold less than that of Fe^{+2} ion, which is the most powerful pro-oxidant among the various types of metal ions[25].

A) Ferric ions (Fe^{+3}) reducing power

The ferric ion (Fe^{3+}) is the relatively biological inactive form of iron. However, it can be reduced to the active Fe^{2+}, depending on condition, particularly pH[26] and oxidized back through Fenton type reaction with production of hydroxyl radical or Haber-Weiss reaction with superoxide anions. Reducing power is to measure the reductive ability of antioxidant and it is evaluated by the transformation of Fe^{3+} to Fe^{2+} by donation of an electron, in the presence of test compounds. Therefore, the Fe^{2+} can be monitored by measuring the formation of Perl's Prussian blue at 700 nm.

The FRAP of synthesized compounds (**2-6**) was determined at concentrations (25, 50, 75 and 100 µg/mL) at pH 6.6 by Oyaizu method[27] using BHA, TBHQ and AA as standards. Thus, higher absorbance of the reaction mixture indicated greater reducing power of the test compounds. The results are shown in figs 4-6. The analysis of results indicated that, compounds **2a**, **4a** and **5a** exhibited good reducing activity at 75 µg/ml. Compounds **2a**, **3c**, **4a** and **5a** showed reducing power at 100 µg/ml.

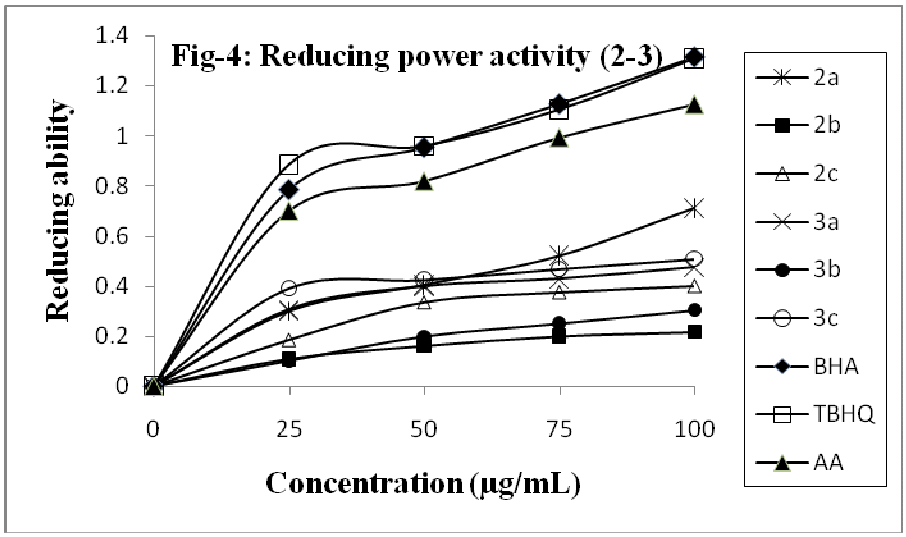


Fig-4: Reducing power activity (2-3)
Reducing ability
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
25
50
75
100
Concentration (µg/mL)
2a
2b
2c
3a
3b
3c
BHA
TBHQ
AA

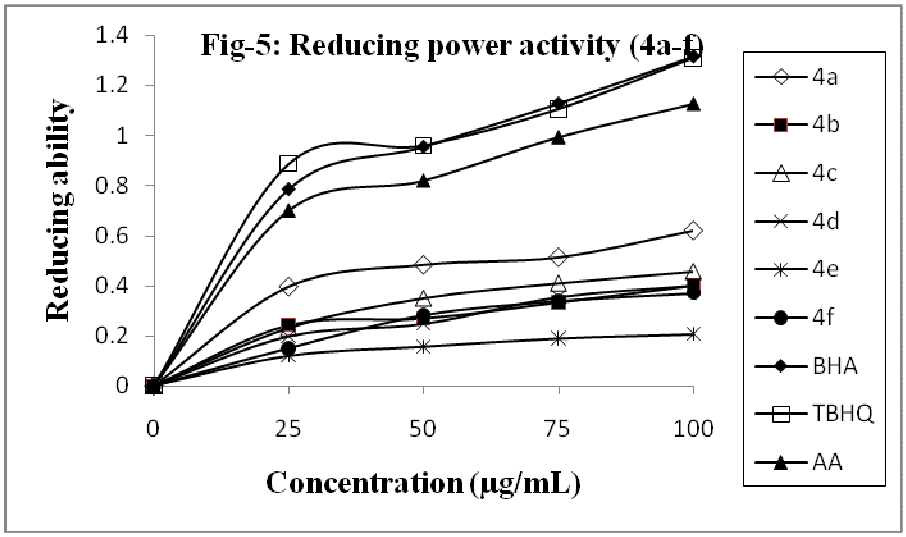


Fig-5: Reducing power activity (4a-f)
Reducing ability
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
25
50
75
100
Concentration (µg/mL)
4a
4b
4c
4d
4e
4f
BHA
TBHQ
AA

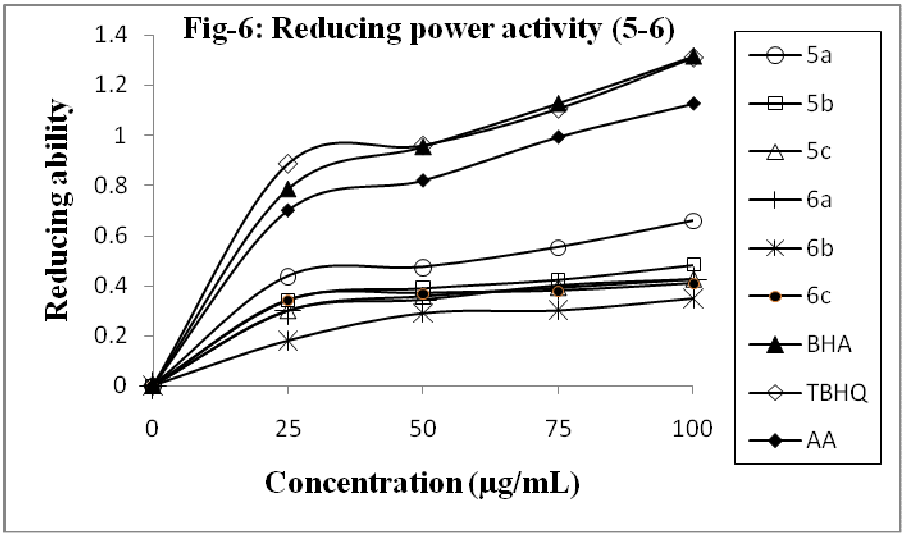
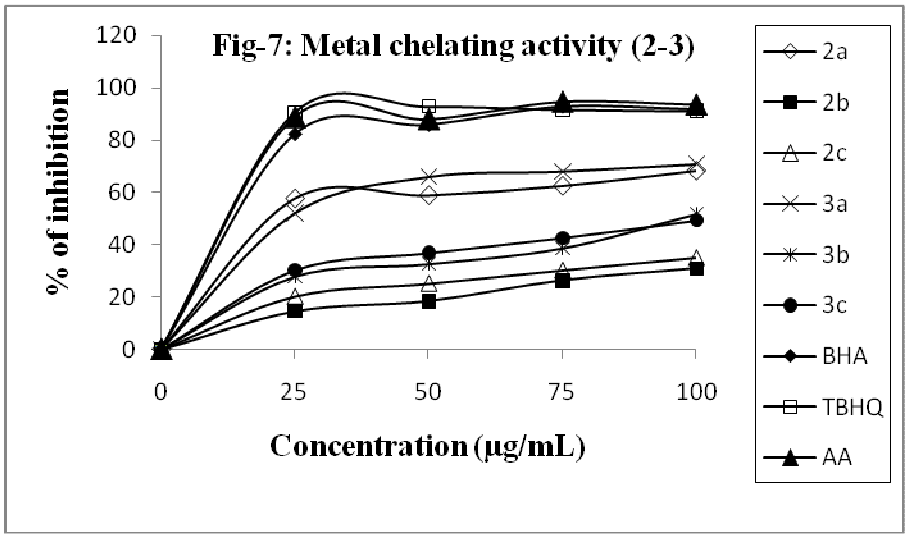


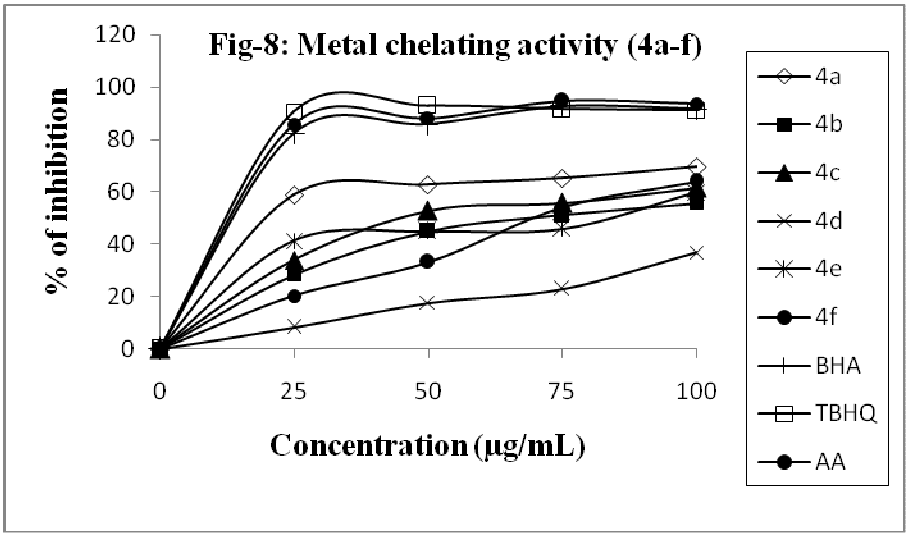
Fig-6: Reducing power activity (5-6)
Reducing ability
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
25
50
75
100
Concentration (µg/mL)
5a
5b
5c
6a
6b
6c
BHA
TBHQ
AA

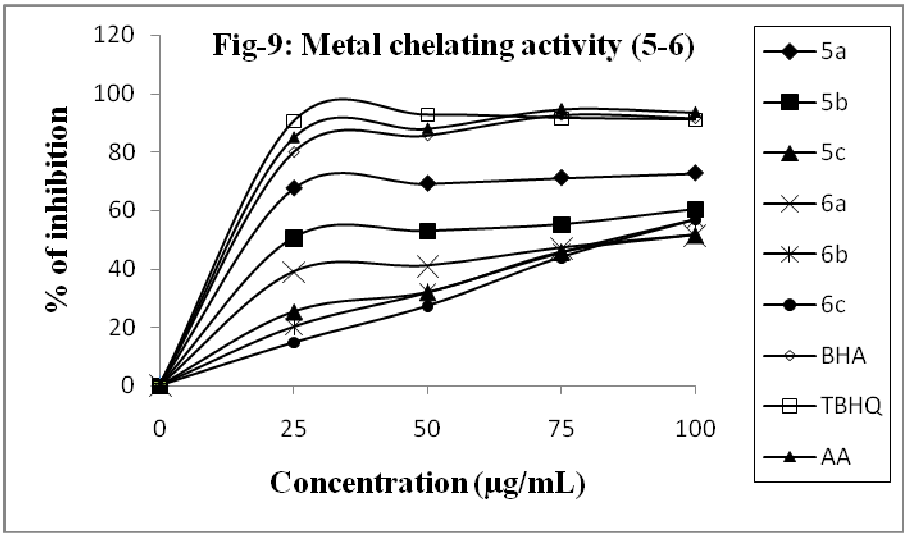
B) Ferrous ions (Fe^{+2}) chelating activity

The chelating effect of ferrous ions (Fe^{2+}) towards the test compounds (**2-6**) and standards was determined by following Dinis method[28] using BHA, TBHQ and AA as standards. Ferrozine can make complexes with ferrous ions. In the presence of chelating agents, complex (red colored) formation is interrupted and as a result, the red color of the complex is decreased. Thus, the chelating effect of the coexisting chelator can be determined by measuring the rate of color reduction. The results are shown in figs **7-9**.

The analysis of results indicated that, compounds **3a**, **4a** and **5a** showed good metal chelating activity (66.06, 62.73 and 69.16 %) at 50 µg/ml concentration, respectively, whereas **2a**, **3a**, **4a** and **5a** exhibited promising metal chelating activity (62.42, 68.18, 65.15 and 71.10 %) at 75 µg/ml concentration. Compounds **2a**, **3a**, **4a**, **4c**, **4f**, **5a**, and **5b** exhibited good metal chelating activity (68.18, 70.941, 69.70, 61.64, 72.73 and 60.39 %) at 100 µg/ml concentration, respectively. Whereas, only compound **5a** exhibited good metal chelating activity (67.53 %) at 25 µg/ml concentration.







Experimental Section

All the reagents were obtained commercially and used by further purification. Melting points were determined by open capillary methods and are uncorrected. Purity of the compounds was checked by TLC using silica gel-G coated aluminimum plates (Merck) and spots were visualized by exposing the dry plates in iodine vapors. The IR (KBr) Spectra were recorded with a Perkin-Elmer Spectrum on FT-IR spectrometer. The ^{1}H NMR (DMSO) spectra recorded with Marcy Plus (varian 400 MHz) and the chemical shifts were expired in ppm (δ scale). Mass spectra were recorded with a ILS-CHU-C-41-VBV4 MS mass spectrometer.

General procedure for the synthesis of 10-substituted *5H*, *6H*, *7H*-indolo[2,3-c]isoquinolin-5-thiones (1a-c)

These compounds were prepared by reported literature[17].

General procedure for the synthesis of ethyl(10-substituted 7*H*-indolo[2,3-c]isoquinolin-5-ylthio)formates (2a-c)

A mixture of compounds (**1a-c**) (0.01 mol), anhydrous potassium carbonate (0.02 mol) and ethylchloroformate (0.01 mol) in super dry acetone (20 ml) was refluxed on steam-bath for 5 hrs. The inorganic solids were filtered while hot and the solvent was removed from the filtrate under reduced pressure. The resulting residue was treated with water to remove water soluble materials, filtered, dried and crystallized from methanol.

Ethyl(10-chloro-7*H*-indolo[2,3-c]isoquinolin-5-ylthio)formate (2a): Yield: 68%, mp 258-59 ºC; Rf, 0.67 ethyl acetate: acetone (6:4) mixture; FTIR (KBr) cm^{-1}: 3282 (Indole NH); 1700 (C=O); 1616 (C=N); ^{1}H NMR (DMSO-d_6, δ, ppm) 11.90 (s, 1H indole NH); 7.20-8.40 (m, 7H, Ar-H); 5.10 (q, 2H, CH_2); 3.50 (t, 3H, CH_3); MS (EI) *m/z* 356 (M^+); 358 (M^++2). Anal. % $C_{18}H_{13}N_2O_2SCl$: C, 60.59; H, 3.67; N, 7.85. Found: C, 60.41; H, 3.72; N, 7.79.

Ethyl(10-methyl-7*H*-indolo[2,3-c]isoquinolin-5-ylthio)formate (2b): Yield: 78%, mp 221-12 ºC; Rf, 0.58 ethyl acetate: acetone (6:4) mixture; FTIR (KBr) cm^{-1}: 3300 (Indole NH); 1705 (C=O); 1622 (C=N); ^{1}H NMR (DMSO-d_6, δ, ppm) 12.00 (s, 1H indole NH); 7.00-8.12 (m, 7H, Ar-H); 5.00 (q, 2H, CH_2); 3.32 (t, 3H, CH_3); 2.32 (s, 3H, CH_3); Anal. % $C_{19}H_{16}N_2O_2S$: C, 67.84; H, 4.79; N, 8.33. Found: C, 68.01; H, 4.72; N, 8.29.

Ethyl(10-methoxy-7*H*-indolo[2,3-c]isoquinolin-5-ylthio)formate (**2c**): Yield: 82%, mp 262-63 ºC; Rf, 0.58 ethyl acetate: acetone (6:4) mixture; FTIR (KBr) cm^{-1}: 3300 (Indole NH); 1700 (C=O); 1625 (C=N); ^{1}H NMR (DMSO-d_6, δ, ppm) 11.95 (s, 1H indole NH); 7.05-8.09 (m, 7H, Ar-H); 4.95 (q, 2H, CH_2); 3.46 (t, 3H, CH_3); 3.32 (s, 3H, OCH_3); Anal. % $C_{19}H_{16}N_2O_3S$: C, 64.76; H, 4.58; N, 7.95. Found: C, 64.71; H, 4.52; N, 7.99.

General procedure for the synthesis of (10-substituted 7*H*-indolo[2,3-c]isoquinolin-5-ylthio)carbohydrazides (3a-c)

The suspension of compounds (**2a-c**) (0.005 mol) and hydrazine hydrate (99-100%) (1.5 ml) in absolute ethanol (15 ml) was refluxed on steam-bath for 4 hrs, excess of ethanol was removed under vaccum to about one third of its original volume. After cooling, the resulting crystallized product separated was filtered, washed with little alcohol, dried and crystallized using benzene.

(10-chloro-7*H*-indolo[2,3-c]isoquinolin-5-ylthio)carbohydrazide (3a): Yield: 88%, mp 272-73 ºC; Rf, 0.66 ethyl acetate: ethanol (8:2) mixture; FTIR (KBr) cm^{-1}: 3325 (Indole NH); 3250/3220 (NH/NH_2); 1692 (C=O); 1630 (C=N); ^{1}H NMR (DMSO-d_6, δ, ppm) 12.12 (s, 1H indole NH); 7.25 (s, 1H, NH); 6.42-7.05 (m, 7H, Ar-H); 5.00 (s, 2H, NH_2); MS (EI) *m/z* 342 (M^+); 344 (M^++2). Anal. % $C_{16}H_{11}N_4OSCl$: C, 56.06; H, 3.23; N, 16.34. Found: C, 56.12; H, 3.22; N, 16.25.

(10-methyl-7*H*-indolo[2,3-c]isoquinolin-5-ylthio)carbohydrazide (3b): Yield: 88%, mp 272-73 ºC; Rf, 0.58 ethyl acetate: ethanol (8:2) mixture; FTIR (KBr) cm^{-1}: 3330 (Indole NH); 3200/3105 (NH/NH_2); 1700 (C=O); 1625 (C=N); ^{1}H NMR (DMSO-d_6, δ, ppm) 12.00 (s, 1H indole NH); 7.41 (s, 1H, NH); 6.59-7.18 (m, 7H, Ar-H); 5.08 (s, 2H, NH_2); 3.30 (s, 3H, CH_3); Anal. % $C_{17}H_{14}N_4OS$: C, 63.33; H, 4.38; N, 17.38. Found: C, 63.12; H, 4.32; N, 17.42.

(10-methoxy-7*H*-indolo[2,3-c]isoquinolin-5-ylthio)carbohydrazide (3c): Yield: 62%, mp 250-51 ºC; Rf, 0.58 ethyl acetate: ethanol (8:2) mixture; FTIR (KBr) cm^{-1}: 3300 (Indole NH); 3210/3000 (NH/NH_2); 1705 (C=O); 1634 (C=N); ^{1}H NMR (DMSO-d_6, δ, ppm) 12.01 (s, 1H indole NH); 7.24 (s, 1H, NH); 6.60-7.13 (m, 7H, Ar-H); 5.01 (s, 2H, NH_2); 3.82 (s, 3H, OCH_3); Anal. % $C_{17}H_{14}N_4O_2S$: C, 60.34; H, 4.17; N, 16.56. Found: C, 60.12; H, 4.22; N, 16.48.

General procedure for the synthesis of 1'-(10-chloro-7*H*-indolo[2,3-c]isoquinolin-5-ylthio)formyl-3',5'-dimethyl/phenylpyrozoles (4a-f) or 1'-(10-chloro-7*H*-indolo[2,3-c]isoquinolin-5-ylthio)formyl-3'-methylpyrozol-5'-ones (5a-c).

A mixture of hydrazides (**2a-c**) (0.001 mol) and acetyl acetone (or dibenzoyl methane or ethyl acetoacetate) in dry methanol (20 ml) containing 4-5 drops of Conc. HCl was refluxed for n4 hrs on steam-bath. The excess of methanol was removed under vacuum. The concentrated reaction mixture was cooled to room temperature. The separated solid was filtered off, washed with little methanol, dried and crystallized using ethanol to furnish pure (**4a-c)**, (**4d-f)** and (**5a-c)**, respectively.

1'-(10-chloro-7*H*-indolo[2,3-c]isoquinolin-5-ylthio)formyl-3',5'-dimethylpyrozole (4a): Yield: 68%, mp 258-59 ºC; Rf, 0.67 ethyl acetate: acetone (6:4) mixture; FTIR (KBr) cm^{-1}: 3344 (Indole NH); 1700 (C=O); ^{1}H NMR (DMSO-d_6, δ, ppm) 11.09 (s, 1H indole NH); 8.10 (s, 1H, pyrazole-H); 7.00-8.06 (m, 7H, Ar-H); 3.31 (s, 3H, CH_3); 2.62 (s, 3H, CH_3); MS (EI) *m/z* 406 (M^+); 408 (M^++2). Anal. % $C_{21}H_{15}N_4O_2SCl$: C, 61.99; H, 3.72; N, 13.77. Found: C, 61.91; H, 3.82; N, 13.69.

1'-(10-methyl-7*H*-indolo[2,3-c]isoquinolin-5-ylthio)formyl-3',5'-dimethylpyrozole (4b): Yield: 68 %, mp > 300 ºC; Rf, 0.71 ethyl acetate: acetone (6:4) mixture; FTIR (KBr) cm^{-1}: 3300 (Indole NH); 1705 (C=O); ^{1}H NMR (DMSO-d_6, δ, ppm) 11.00 (s, 1H indole NH); 8.25 (s, 1H, pyrazole-H); 7.10-8.20 (m, 7H, Ar-H); 3.65 (s, 3H, CH_3); 3.31 (s, 3H, CH_3); 2.62 (s, 3H, CH_3); MS (EI) *m/z* 406 (M^+); 408 (M^++2). Anal. % $C_{22}H_{18}N_4OS$: C, 68.37; H, 4.69; N, 14.50. Found: C, 68.25; H, 4.72; N, 14.41.

1'-(10-methoxy-7*H*-indolo[2,3-c]isoquinolin-5-ylthio)formyl-3',5'-dimethylpyrozole (4c): Yield: 68 %, mp > 300 ºC; Rf, 0.71 ethyl acetate: acetone (6:4) mixture; FTIR (KBr) cm^{-1}: 3318 (Indole NH); 1700 (C=O); ^{1}H NMR (DMSO-d_6, δ, ppm) 11.82 (s, 1H indole NH); 8.52 (s, 1H, pyrazole-H); 7.10-8.25 (m, 7H, Ar-H); 3.98 (s, 3H, OCH_3); 3.15 (s, 3H, CH_3); 2.78 (s, 3H, CH_3); Anal. % $C_{22}H_{18}N_4O_2S$: C, 65.65; H, 4.51; N, 13.92. Found: C, 65.72; H, 4.59; N, 14.00.

1'-(10-chloro-7*H*-indolo[2,3-c]isoquinolin-5-ylthio)formyl-3',5'-diphenylpyrozole (4d): Yield: 71 %, mp >300 ºC; Rf, 0.71 ethyl acetate: benzene (6:4) mixture; FTIR (KBr) cm^{-1}: 3300 (Indole NH); 1700 (C=O); ^{1}H NMR (DMSO-d_6, δ, ppm) 11.51 (s, 1H indole NH); 8.32 (s, 1H, pyrazole-H); 7.00-8.00 (m, 17H, Ar-H); MS (EI) *m/z* 530 (M^+); 532 (M^++2). Anal. % $C_{31}H_{19}N_4OSCl$: C, 70.12; H, 3.61; N, 10.55. Found: C, 70.25; H, 3.72; N, 10.41.

1'-(10-methyl-7*H*-indolo[2,3-c]isoquinolin-5-ylthio)formyl-3',5'-diphenylpyrozole (4e): Yield: 62 %, mp 295-96 ºC; Rf, 0.51 ethyl acetate: benzene (6:4) mixture; FTIR (KBr) cm^{-1}: 3315 (Indole NH); 1689 (C=O); ^{1}H NMR (DMSO-d_6, δ, ppm) 11.95 (s, 1H indole NH); 8.42 (s, 1H, pyrazole-H); 7.11-8.09 (m, 17H, Ar-H); 2.62 (s, 3H, CH_3); Anal. % $C_{32}H_{22}N_4OS$: C, 75.27; H, 4.34; N, 10.97. Found: C, 75.25; H, 4.42; N, 10.91.

1'-(10-methoxy-7*H*-indolo[2,3-c]isoquinolin-5-ylthio)formyl-3',5'-diphenylpyrozole (4f): Yield: 75 %, mp >300 ºC; Rf, 0.61 ethyl acetate: benzene (6:4) mixture; FTIR (KBr) cm^{-1}: 3305 (Indole NH); 1695 (C=O); ^{1}H NMR (DMSO-d_6, δ, ppm) 11.82 (s, 1H indole NH); 8.35 (s, 1H, pyrazole-H); 7.00-8.15 (m, 17H, Ar-H); 3.82 (s, 3H, OCH_3); Anal. % $C_{32}H_{22}N_4O_2S$: C, 72.98; H, 4.21; N, 10.64. Found: C, 72.88; H, 4.32; N, 10.60.

1'-(10-chloro-7*H*-indolo[2,3-c]isoquinolin-5-ylthio)formyl-3'-methylpyrozol-5'-one (5a): Yield: 66 %, mp >300 ºC; Rf, 0.70 ethyl acetate: methanol (9:1) mixture; FTIR (KBr) cm^{-1}: 3290 (indole-NH); 1700 (C=O); 1660 (C=O); ^{1}H NMR (DMSO-d_6, δ, ppm) 11.00 (s, 1H indole NH); 8.18 (s, 1H, pyrazole-H); 7.01-8.09 (m, 7H, Ar-H); 2.21 (s, 3H, CH_3); MS (EI) *m/z* 408 (M^+); 500 (M^++2). Anal. % $C_{20}H_{13}N_4O_2SCl$: C, 58.75; H, 3.20; N, 13.70. Found: C, 58.81; H, 3.22; N, 13.73.

1'-(10-methyl-7*H*-indolo[2,3-c]isoquinolin-5-ylthio)formyl-3'-methylpyrozol-5'-one (5b): Yield: 78 %, mp >300 ºC; Rf, 0.68 ethyl acetate: methanol (9:1) mixture; FTIR (KBr) cm^{-1}: 3300 (indole-NH); 1705 (C=O); 1654 (C=O); ^{1}H NMR (DMSO-d_6, δ, ppm) 11.42 (s, 1H indole NH); 8.25 (s, 1H, pyrazole-H); 7.11-8.00 (m, 7H, Ar-H); 2.58 (s, 3H, CH_3); 2.31 (s, 3H, CH_3); Anal. % $C_{21}H_{16}N_4O_2S$: C, 64.93; H, 4.15; N, 14.42. Found: C, 64.85; H, 4.22; N, 14.40.

1'-(10-methoxy-7*H*-indolo[2,3-c]isoquinolin-5-ylthio)formyl-3'-methylpyrozol-5'-one (5c): Yield: 70 %, mp 275-76 ºC; Rf, 0.58 ethyl acetate: methanol (9:1) mixture; FTIR (KBr) cm^{-1}: 3315 (indole-NH); 1700 (C=O); 1668 (C=O); ^{1}H NMR (DMSO-d_6, δ, ppm) 11.50 (s, 1H indole NH); 8.38 (s, 1H, pyrazole-H); 7.01-8.18 (m, 7H, Ar-H); 3.88 (s, 3H, OCH_3); 2.45 (s, 3H, CH_3); Anal. % $C_{21}H_{16}N_4O_3S$: C, 62.36; H, 3.99; N, 13.85. Found: C, 62.31; H, 4.02; N, 13.80.

General procedure for the synthesis of 5'-(10-substituted 7*H*-indolo[2,3-c]isoquinolin-5-ylthio)-1',3',4'-oxidiazol-2'-thiones (6a-c).

A mixture of hydrazides (**2a-c**) (0.005 mol), KOH (0.005 mol) and CS_2 (5 ml) in methanol (50 ml) was refluxed on a steam-bath until the evolution of H_2S ceased (45-48 hrs). The solvent was then evaporated and residue dissolved in ice-water. The resulting clear solution was filtered and the filtrate was acidified with dilute HCl. The separated solid was filtered washed with water, dried and crystallized from ethanol to furnish pure **6a-c.**

5'-(10-chloro-7*H*-indolo[2,3-c]isoquinolin-5-ylthio)-1',3',4'-oxidiazol-2'-thione (6a): Yield: 62 %, mp > 300 ºC; Rf, 0.62 ethyl acetate: diethyether (1:1) mixture; FTIR (KBr) cm^{-1}: 3283 (indole-NH); 3054 (NH); 1658 (C=O); 1068 (C=S); ^{1}H NMR (DMSO-d_6, δ, ppm) 11.32 (s, 1H indole NH); 9.21 (s, 1H, NH); 7.23-8.12 (m, 7H, Ar-H); MS (EI) *m/z* 383 (M^+); 385 (M^++2). Anal. % $C_{17}H_9N_4OS_2Cl$: C, 53.05; H, 2.36; N, 14.56. Found: C, 53.00; H, 2.40; N, 14.41.

5'-(10-methyl-7*H*-indolo[2,3-c]isoquinolin-5-ylthio)-1',3',4'-oxidiazol-2'-thione (6b): Yield: 72 %, mp >300 ºC; Rf, 0.62 ethyl acetate: diethyether (1:1) mixture; FTIR (KBr) cm^{-1}: 3300 (indole-NH); 3204 (NH); 1675 (C=O); 1198 (C=S); ^{1}H NMR (DMSO-d_6, δ, ppm) 11.50 (s, 1H indole NH); 9.18 (s, 1H, NH); 7.03-8.00 (m, 7H, Ar-H); 2.25 (s, 3H, CH_3); Anal. % $C_{18}H_{12}N_4OS_2$: C, 59.32; H, 3.32; N, 15.37. Found: C, 59.29; H, 3.40; N, 15.41.

5'-(10-methoxy-7*H*-indolo[2,3-c]isoquinolin-5-ylthio)-1',3',4'-oxidiazol-2'-thione (6c): Yield: 66 %, mp >300 ºC; Rf, 0.58 ethyl acetate: diethyether (1:1) mixture; FTIR (KBr) cm^{-1}: 3342 (indole-NH); 3225 (NH); 1700 (C=O); 1200 (C=S); ^{1}H NMR (DMSO-d_6, δ, ppm) 11.38

(s, 1H indole NH); 9.00 (s, 1H, NH); 7.00-7.89 (m, 7H, Ar-H); 3.92 (s, 3H, OCH_3); Anal. % $C_{18}H_{12}N_4O_2S_2$: C, 59.32; H, 3.32; N, 15.37. Found: C, 59.29; H, 3.40; N, 15.41.

Biological Assay

Antimicrobial activities

The in-vitro biological screening of the synthesized compounds (**2-6**) was carried out against bacteria species, namely *E. Coli*, *S. Aureus*, *K. Penumoniae* and *P. aerginosa* and fungal species, namely, *A. Nizer*, *A. Oryzae*, *A. Terrus* and *A. Flaves* by Cup-plate method[18] using nutrient agar as medium. The holes of 6 mm diameter were punched carefully using a sterile cork borer and these were filled with test solution (1mg/ml in DMF), standards (1mg/ml in DMF) and DMF as control. The plates were incubated at 37^0C for 24 hr and 72 hr in case antibacterial and antifungal activity, respectively. The diameter of the zone of inhibition for all the test compounds was measured (in mm) and the results were compared with the standard drug streptomycin for antibacterial activity and fluconazole for antifungal activity.

Antioxidant activities

I) Radical Scavenging activity (RSA)

The radical scavenging activity (RSA) of test compounds (**2-6**) in methanolic solution at concentrations 25, 50, 75, 100 µg/ml containing freshly prepared DPPH solution (0.004 % w/v) was carried out and compared with those of standards BHA, TBHQ and AA by using Hatano's method[19]. All the test analyses were performed on three replicates were averaged. The results in per cen tag

$$\% \text{ DPPH raddical scavenging} = \frac{\text{Absorbance of control- Absorbance of test sample}}{\text{Absorbance of control}} \times 100$$

e are expressed as the ratio of absorption decrease in the presence of test compounds and absorption of DPPH solution in the absence of test compounds at 517 nm on ELICO SL171 mini spec spectrometer. The percentage scavenging activity of the DPPH free radical was determined using the following equation.

II) Iron Metal ions Chelating Activity

A) Ferric ions (Fe^{3+}) reducing antioxidant power (FRAP)

The reducing power of the synthesized compounds (**2-6**) were determined and compared with BHA, TBHQ and AA as standards by using Oyaizu method[27]. Different concentration of samples (25, 50, 75 and 100 µg/ml) in DMSO (1 ml) were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide (2.5 ml, 1%). The mixture was incubated at 50°C for 20 min. After which a portion of trichloroacetic acid (2.5 ml, 10%) was added to the mixture and centrifuged for 10 min, at 1000 Xg. The upper layer of solution (2.5 ml) mixed with distilled water (2.5 ml) and ferric chloride (0.5 ml, 0.1 %). Then absorbance at 700 nm was measured in spectrophotometer. Higher absorbance of the reaction mixture indicated greater reducing power.

B) Ferrous ions (Fe^{2+}) chelating activity

The chelating activity of ferrous ions was estimated by following Dinis method[28] by using BHA, TBHQ and AA as standards. The test samples (25, 50, 75 and 100 µg/ml) in ethanolic solution (0.4 ml) were added to a solution of $FeCl_2$ (0.05 ml, 2 mM). The reaction was initiated by the addition of ferrozine (0.2 ml, 5 mM) and the total volume was adjusted to 4 ml with ethanol. Ferrozine reacted with the divalent iron to form stable magenta complex species that were very soluble in water. The mixture was shaken vigorously and kept at room temperature for 10 min. Then the absorbance of the solution was measured spectrophotometricaly at 562 nm. All test analyses were run in triplicate and averaged. The percentage of inhibition of the ferrozine Fe^{2+}

$$\text{Ferrous ion chelating effect (\%)} = \frac{\text{Absorbance of control- Absorbance of test sample}}{\text{Absorbance of control}} \text{ x } 100$$

complex formations was calculated using the formula

Conclusion

The present study revealed that, compounds having chloro or methoxy substituent were founds to be good antimicrobial and antioxidant agents.

Acknowledgements

The authors are thankful to the Chairman, Department of Chemistry, Gulbarga University, Gulbarga, for providing laboratory facilities and Chairman, Department of Microbiology, Gulbarga University, Gulbarga for providing facilities to carry out antimicrobial activities. We are also thankful to the Director, Indian Institute of Technology, Madras, Chennai for providing spectral data.

References

1. G. Winter, N. Dimola, M. Berti, V. Ariali. *Farmaco. Ed. Sci.* **34(6)**, 507-517, (1979).
2. K. Ishizumi, J. Katsube.. *Brit. Pat.,* 2, 025, 932*,* (1980), *Chem. Abstr*, 93,186322e, (1980).
3. Sumitomo Chemical Co. Ltd*,* Jpn Kokai Tokkyo Koho, *Jap. Pat*. 5869, 882, (1983), *Chem. Abstr*, **99,** 88182p, (1983).
4. T. Kosuge, H. Zend, J. Torigoe, Japan Kokai , *Jap. Pat.,* 7391,210, (1973), *Chem. Abstr,* **80**, 112616, (1974).
5. F. Mann, F. Chiment, A. Balasco, M. L. Cenicola, M. D. Amico, C. Parrilo, F. Rossi, E. Marmo. *Eur. J. Med. Chem.* **27**, 633-639, (1992).
6. M. Amir and R. Agarwal, *J. Indian. Chem. Soc.* **74**, 154-155, (1997).
7. R. H. Udupi, S. N. Rao, A. A. Bhat. *Indian. J. Heterocycl.* **7**, 217-220, (1998).
8. M. Amir, S. Kumar. *Indian. J. Chem*. **44B,** 2532-2537, (2005).
9. S. A. Ahmed, M. A. Hamdy, M. M. Nadia, A. E. Mahmoud. *Bioorg. Med. Chem.* **14**, 1236-1246, (2006).
10. R. B. Girish, K. Nandakumar, G. N. Pawan, T. Anjali, M. R. Chamallamudi, K. N. Gopalan. *Chemico-Biological. Intractions.* **181**, 377-382, (2009).
11. K. Shaoyong, L. Zhong, Q. Xuhong. *Biorg. Med. Chem*. **16**, 7565-7572, (2008).

12. Saundane A R, Rudresh K, Satyanarayan N D, Hiremath S P. *Indian. J. Pharm. Sci*. **60(6)**, 379-383, (1998).
13. A. R. Saundane, S. H. Ranganath, G. Prayagrai, K. Rudresh, N.D. Satyanayana. *Orient. J. Chem.* **14(2)**, 251-254, (1998).
14. A. R. Saundane, P. M. Veeresh Sharma. *Indian J. Heterocycl. Chem.* **13**, 275-76, (2004).
15. A. R. Saundane, K. Vijaykumar, M. Yarlakatti, W. Prabhaker, V. A. Vaijinath. *Heterocycl. Lett.* **1(4)**, 339-350, (2011).
16. A. R. Saundane, K. Vijaykumar, M. Yarlakatti, V. A. Vaijinath. W. Prabhaker, *Indian J. Heterocycl. Chem.* **20**, 321-324, (2011).
17. S. P. Hiremath, A. R. Saundane, H. K. S. Swamy, B. H. M. Mruthyunjayaswamy. *Indian J. Heterocycl. Chem*. **3**, 37-42, (1993).
18. Indian pharmacopoeia, Government of India 3rd Ed. New Delhi Appendix IV, 90, (1985).
19. T. Hatano, H. Kagawa, T. Yasuhara, T. Okuda. *Chem. Pharm. Bull.* **36(6)**, 2090-2097, (1988).
20. S. J. S. Flora, M. Mittal, A. Mehta. *Indian. J. Med. Res.* **128**, 501-523, (2008).
21. I. Gulcin, M. Elmastas, H. Y. Aboul-Enein. *Phyt. Res.* **21**, 354-361, (2007).
22. Z. K. Marijana, Z. Raji, N. Petri, Z. Branka. *Acta. Pharm.* **59**, 235–242, (2009).
23. I. Gulcin, A. H. Alici, M. Cesur. *Chem. Pharm. Bull*. **53(3)**, 281-285, (2005).
24. B. Halliwell. *Am. J. Med*. **91**, 14-22, (1991).
25. I. Çaliş, M. Hosny, T. Khalifa, S. Nishibe. *Phytochemistry*. **33**, 1453-1456, (1993).
26. M. Strlic, T. Radovic, J. Kolar, B. Pihlar. *J. Agri. Food. Chem*. **50**, 6313-6317, (2002).
27. M. Oyaizu, *Jpn. J. Nutr.* **44**, 307-315, (1986).
28. T. C. P. Dinis, V. M. C Madeira, L. M. Almeida. *Arch. Biochem. Biophys.* **315**, 161-169, (1994).

Received on April 9, 2012.