HL **ISSN: 2231 – 3087(print) / 2230 – 9632 (Online)**
http://heteroletters.org **Vol. 2: (1), 2012, 53-70**

SYNTHESIS, ANTIMICROBIAL AND ANTIOXIDANT ACTIVITIES OF PYRIMIDO [5,4-e]THIAZOLO[3,2-a]PYRIMIDINES LINKED TO INDOLE NUCLEUS

Anand R. Saundane*, Prabhaker Walmik, Katkar Vijaykumar and Yarlakatti Manjunatha

Department of Post-Graduate Studies & Research in Chemistry,
Gulbarga University, GULBARGA-585 106, India
E-mail: arsaundane@rediff.com

Abstract

Indole, Thiazolopyrimidine, thiazolopyrimidopyrimidine analogues were found to possess wide range of biological activities, such as antimicrobial, antioxidant, etc. In the present investigation we have prepared the substituted indole-3-yl thiazolopyrimidopyrimidines in which indole, thiazole and pyrimidine systems are embedded in one molecule so to get the enhanced biological activities. These compounds have been evaluated for their antimicrobial and antioxidant activities. Amongst them compounds **3a**, **4d** and **5g** exhibited promising antibacterial activity, whereas the compounds **4a** and **6a** exhibited promising antifungal activity. Compound **5a** has shown significant radical scavenging activity. It has been found that chloro substituted compounds exhibited good antimicrobial activity, whereas the dithioxopyrimidine system enhanced antioxidant activity.

Keywords: Indole, thiazole, pyrimido[5,4-e]thiazolo[3,2-a]pyrimidine, antimicrobial, antioxidant activities.

Introduction

Antioxidants are extensively studied for their capacity to protect organisms and cells from damage induced by stress. Scientists in various disciplines have become interested in new compounds, either synthesized or obtained from natural sources that could provide active components to prevent or reduce the impact of oxidative stress on cell [1]. Exogenous chemicals and endogenous metabolic process in human body or in food system might produce highly reactive free radicals, especially oxygen derived radicals, which are capable of oxidizing biomolecules, resulting in cell death and tissue damage. Oxidative damage play a significant pathological role in human diseases for example, cancer, emphysema, cirrhosis, atherosclerosis and arthritis have been correlated with oxidative damage. Also, excessive generation of reactive oxygen species (ROS) induced by various stimuli and exceeds the antioxidant capacity of the organisms leading to various pathological processes such as, inflammation, diabetes, genotoxicity and cancer [2].

An antioxidant is a molecule of showing or preventing the oxidation of other molecules. Oxidation process is one of the most important routes for producing free radicals in food, drugs, living system and also participates in numerous pathological processes. A balance between tissue concentration of ROS and anti-oxidative mechanisms in tissue is disturbed under such conditions. This may be either result from an increased local production of ROS or from exhaustion of the oxidant capacity of the tissue. In cellular oxidation reactions, super oxide radical normally is formed first, and its effect magnified because it produces other kinds of cell damaging action of the hydroxyl radical, which is the strongest amongst free radicals [3].

Indole derivatives create a distinct chemical and pharmacological group. Some of them seem to exhibit a promising battery of useful properties. Indole derivative have been reported to posses promising biological activities including antimicrobial [4], antiviral [5], antimalerial [6], antioxidant [7], etc. Pyrimidine derivatives represents the most active class of compounds possessing wide spectrum of biological activities viz., significant *in vitro* activity against unrelated DNA and RNA, viruses including polio herpes viruses, diuretic, antitumor, anti-HIV, cardiovascular [8], anti-inflammatory [9], analgesic [10], antioxidant [11], antimicrobial [12], etc. Whereas thiazolo[3,2-a]pyrimidine is an important heterocyclic analogues of this system have been reported to possess promising biological activities including antimicrobial [13, 14], analgesic [15], anti-inflammatory [16], hypoglycemic [17], bactericidal [18], antioxidant [19], etc. In view of these observations and in continuation of our research on the synthesis of biologically active molecules [20-24], the objective of this study were to synthesize title compounds in which pyrimido[5,4-e]thiazolo[3,2-a]pyrimidine system linked to position-3 of indole nucleus so as to get a molecule which may exhibit enhanced biological activities.

Results and discussion

Chemistry

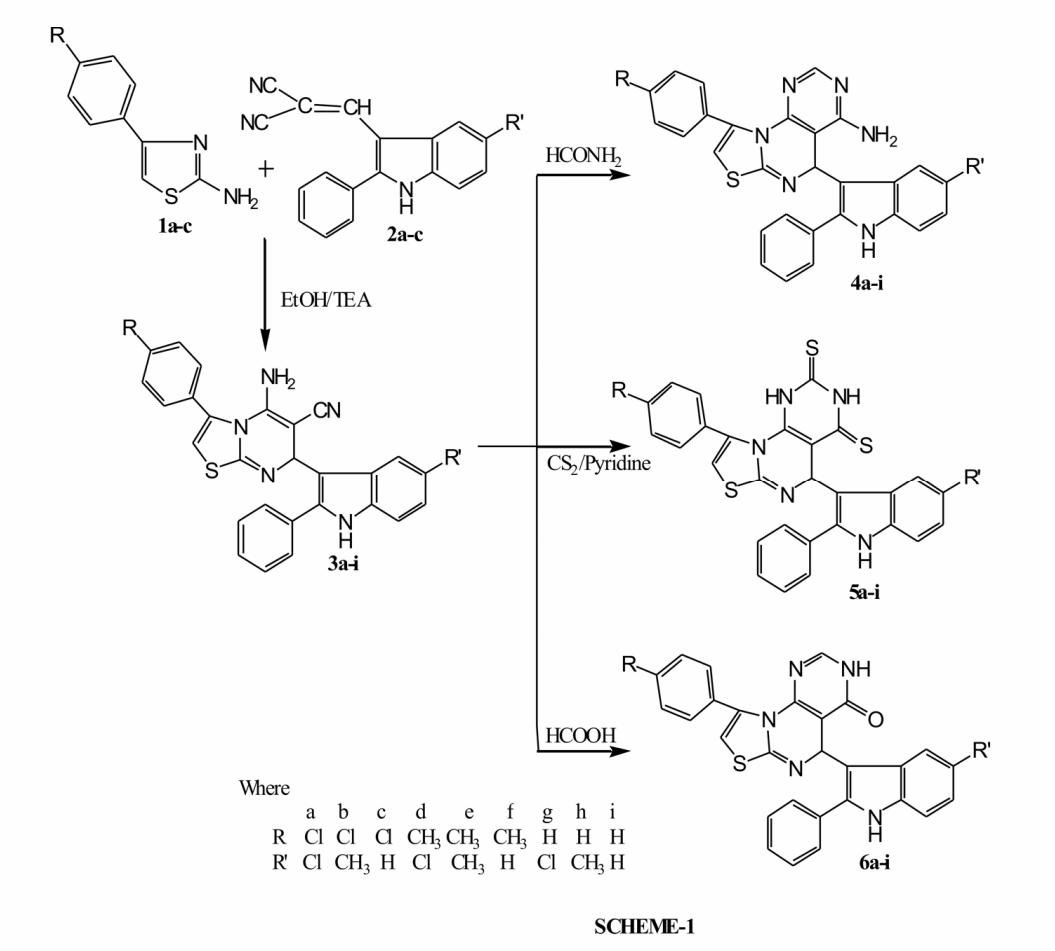
The starting compounds 2-amino-4-(4-substituted phenyl)thiazoles (**1a-c**) [25] and (5'-sustituted 2'-phenyl-1*H*-indol-3'-yl)methylene carbonitriles (**2a-c**) [26] were prepared by literature procedure. The compound (**1a)** on cyclocondensation with (**2a)** in ethanol containing catalytic amount of triethylamine under reflux conditions, afforded the key intermediate 5-amino-3-(4-chlorophenyl)-7-(5'-chloro-2'-phenyl-1*H*-indol-3'-yl)-thiazolo[3,2-a]pyrimidin-6-carbonitrile (**3a**). IR spectrum of **3a** showed absorption peaks at 3435, 3115, 2218 cm^{-1} corresponding to the NH_2, NH and CN functions, respectively. In its ^1H NMR spectrum a singlet at δ 4.53 was resonated due to two protons of amino group of pyrimidine ring. A singlet appeared at δ 6.10 was attributed to the pyrimidine proton. The multiplet resonated between δ 7.20-8.29 for fourteen aromatic protons and a proton of thiazole ring. The down field singlet appeared at δ 12.90 integrating for one proton was assigned to indole NH. The mass spectrum of compound **3a** showed isotopic molecular ion peak M^+ at m/z 513, M^++2 at m/z 515 and M^++4 at m/z 517, conforming the presence of two chlorine atoms in a molecule corresponds to the molecular formula $C_{27}H_{17}N_5SCl_2$. Similarly, other compounds **3b-i** in the series were prepared.

Compound **3a** when subjected to annulation with formamide under reflux temperature yielded 4-amino-9-(4-chlorophenyl)-5-(5'-chloro-2'-phenyl-1*H*-indol-3'-yl)-5*H*-pyrimido[5,4-e] thiazolo[3,2-a]pyrimidine (**4a**). The IR spectrum of **4a** showed absorption frequencies at 3431, 3303, 1629, 768, and 697 cm^{-1} due to NH_2, NH, CN, Ar-Cl and C-S-C functions, respectively. The structure of this compound was further supported by its ^1H NMR spectrum. The two protons

of amino group of pyrimidine ring resonated as singlet at δ 4.52. The signal appeared at δ 6.10 as singlet integrating for one proton of pyrimidine at C_5. The multiplet resonated between δ 6.99-8.34 was assigned to thirteen aromatic protons and a proton of thiazole ring. The singlet at δ 9.70 integrating for one proton was assigned to the proton of pyrimidine at C_2. The down field singlet at δ 12.01 was assigned to a proton of indole NH. Further the structure of compound **4a** supported by its mass spectrum. In its mass spectrum, isotopic molecular ion peak M^+ was appeared at m/z 540, M^++2 at m/z 542 and M^++4 at m/z 544 corresponds to the molecular formula $C_{28}H_{17}N_5SCl_2$. Similarly, other compounds **4b-i** in this series were prepared (**Scheme-I**).

Compound **3a** on treatment with carbon disulfide in pyridine under reflux condition, gave a cyclocondensation product 9-(4-chlorophenyl)-5-(5'-chloro-2'phenyl-1*H*-indol-3'-yl)-2,4-dithioxo-1,3,4,5-tetrahydro-2*H*-pyrimido[5,4-e]thiazolo[3,2-a]pyrimidine (**5a**). In the IR spectrum of **5a,** disappearance of CN function at 2218 cm^{-1}, and appearance of new absorption peaks at 3302, 3171, 3138, 1299 and 1175 cm^{-1} due to the NH/NH/NH and CS functions, respectively, confirmed the formation of **5a** form **3a**. The structure of this compound was further supported by its ^{1}H NMR spectrum. One proton of pyrimidine C_5 was resonated as singlet at δ 6.00. The clustered of thirteen aromatic protons and a proton of thiazole ring were resonated as multiplet extending form δ 6.88-8.17. The singlet at δ 11.01 integrating for one proton was assigned for pyrimidine NH. The indole NH proton appeared as a singlet at δ 11.88, whereas other pyrimidine-NH proton was resonated as a singlet at δ 12.89. The mass spectrum of compound **5a** showed isotopic molecular ion peak M^+ at m/z 589, M^++2 at m/z 591 and M^++4 at m/z 593 confirms the two chlorine atom in a molecule corresponding to the molecular formula $C_{28}H_{17}N_5S_3Cl_2$. Similarly, other compounds **5b-i** in this series were prepared (**Scheme-I**).

Similarly, compound **3a** when subjected to cyclocondensation with formic acid yielded 9-(4-chlorophenyl)-5-(5'-chloro-2'-phenyl-1*H*-indol-3'-yl)-3,5-dihydropyrimido[5,4-e]thiazolo [3,2-a]pyrimidin-4-one (**6a**). The structure of which was confirmed by its spectral data. In its IR spectrum the absorption peaks at 3435 and 2218 cm^{-1} due to the NH_2 and CN function were missing, whereas the new absorption frequencies due to the cyclic carbonyl and NH/NH functions were appeared at 1674, 3161 and 3056 cm^{-1}, respectively, confirmed the formation of **6a** from **3a**. In the ^{1}H NMR spectrum of **6a**, the pyrimidine C_2 proton resonated as singlet at δ 6.12. The clustered of fourteen aromatic protons and a proton of thiazole ring were resonated as multiplet extending from δ 7.01-8.22. The pyrimidine proton at C_5 was resonated as singlet at δ 9.72. The singlet at δ 10.91 integrating for one proton was attributed to pyrimidine NH, whereas down field singlet at δ 12.20 integrating for one proton was assigned to indole NH. Further the structure of **6a** was supported by its mass spectrum, which exhibited the isotopic molecular ion peaks M^+ at m/z 541, M^++2 at m/z 543 and M^++4 at m/z 545 conforming the presence of two chlorine atoms in a molecule corresponds to the molecular formula $C_{28}H_{17}N_5OSCl_2$. Similarly, other compounds **6b-i** in this series were prepared.



SCHEME-1

Evaluation of Antimicrobial activity

Antibacterial results of the test compounds revealed that (**Table-I**) compounds **3a**, **4d** and **5g** exhibited maximum zone of inhibition against *S. aureus, K. pneumonia*, *P. aeruginosa,* whereas the antifungal activity results (**Table-I**) indicated that the compounds **4a** and **6a** showed maximum zone of inhibition against all four fungi namely, *A. oryzae*, *A. niger, A. flavus* and *A. terreus.* Compounds **3d** showed good zone of inhibition against *A. nizer, A. flavus* and *A. terreus* as compared to the standard drugs Gentamycin and fluconazole. The antimicrobial results suggested that compounds containing chloro substituent's either in phenyl ring or at 5-position of indole exhibited maximum zone of inhibition as compared to the compare to the rest of compounds in the series.

Table-I Antimicrobial activity of synthesized compounds (3-6)

Comp.	Antibacterial activity (zone of inhibition in mm)				Antifungal activity (zone of inhibition in mm)			
	E. C.	***S. A.***	***K. P.***	***P. A.***	***A. O.***	***A. N.***	***A. F.***	***A.T.***
3a	16	12	11	10	14	15	10	15
3b	17	18	20	23	10	13	12	12
3c	14	12	10	13	11	11	14	16
3d	15	16	13	15	15	18	20	21
3e	12	15	10	11	13	16	13	14
3f	09	11	12	14	12	21	15	13
3g	13	08	11	15	11	18	10	16
3h	12	13	14	17	14	11	11	14
3i	14	16	13	12	10	13	15	17
4a	17	09	10	11	19	22	20	21
4b	10	11	13	13	10	13	13	16
4c	09	15	12	18	14	10	11	10
4d	15	18	22	22	11	09	13	15
4e	11	15	12	15	11	12	14	11
4f	12	13	14	09	13	15	10	15
4g	13	11	10	10	16	13	09	14
4h	14	08	11	14	15	10	08	10
4i	13	10	13	08	10	14	12	11
5a	12	10	13	11	09	12	14	15
5b	12	15	10	15	15	11	12	13
5c	15	12	14	12	10	16	16	16
5d	13	13	15	10	13	15	12	14
5e	10	10	14	09	15	12	09	11
5f	14	08	11	12	12	11	11	15
5g	18	19	22	21	14	10	13	14
5h	12	13	19	20	15	09	12	13
5i	10	12	15	12	12	11	15	10
6a	18	17	17	19	20	22	21	23
6b	15	15	16	10	18	17	12	10
6c	12	14	10	08	11	15	16	13
6d	09	11	14	14	12	13	15	11
6e	15	08	11	13	15	12	10	09
6f	12	12	09	11	09	14	12	12
6g	14	15	08	12	14	10	12	13
6h	15	13	12	15	12	16	14	15
6i	13	12	15	10	11	13	12	12
Std-1	26	23	25	26	--	--	--	--
Std-2	--	--	--	--	23	26	24	26

Antioxidant activity

Radical scavenging activity (RSA) assay

All the newly synthesized compounds were tested for their radical scavenging activity (RSA) using stable free radical 1,1-diphenyl-2-picryl hydrazyl (DPPH). The results were compared with the standards 2-tert-butyl -4-methoxy phenol (butylated hydroxyl anisole BHA) and 2-(1,1-dimethyl)-1,4-benzenediol (2-tert–butyl hydroquinone, TBHQ). Fig. (**1-4**). The compounds **3a**, **4g**, **4h**, **5a**, **5e**, **5h**, **6b**, **6f** and **6h** exhibited 65.55, 66.16, 66.77, 68.90, 68.21, 68.60, 65.55, 67.99 and 66.77 % radical scavenging activity at concentration 100 µg/ml, respectively. The radical scavenging activity of these compounds may be due to the presence of amino, cyclic amide, or thioamide groups present in these compounds. The higher the radical scavenging activity of compound **5a** may be due to the presence dithioxopyrimidine system, which may increase the acidity of hydrogen of pyrimidine ring, and enhance the antioxidant activity. However none of the compounds exhibited better radical scavenging activity than the standards BHA and TBHQ.

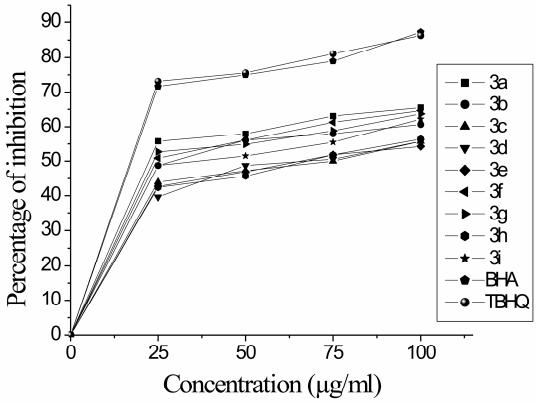


Figure-1 Scavenging effect of compounds **3a-i,** BHA and TBHQ at different.

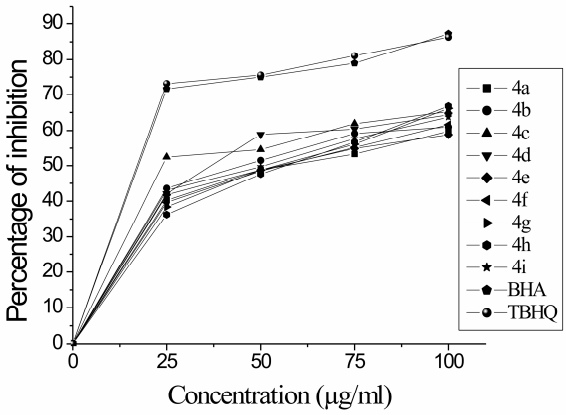


Figure-2 Scavenging effect of compounds **4a-i,** BHA and TBHQ at different.

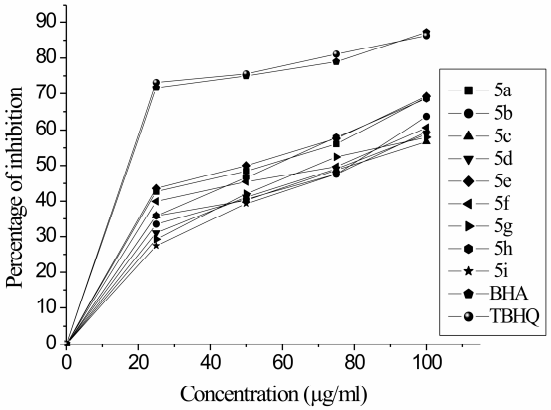


Figure-3 Scavenging effect of compounds **5a-i,** BHA and TBHQ.

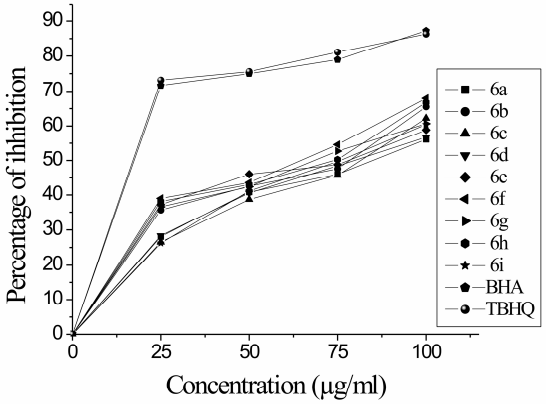


Figure-4 Scavenging effect of compounds **6a-i,** BHA and TBHQ.

Reducing power assay

The reductive capacities of synthesized compounds were assessed by the extent of conversion of Fe^{3+}/ferricyanide complex to the Fe^{2+}/ferrous form. The reductive powers of the compounds were observed at different concentrations and results were compared with standards. The reducing ability of the synthesized compounds augmented with increasing concentration test samples.

As could be seen from Fig. (**5-8**) compounds **3e**, **3f**, **3h**, **4h**, **4i**, **5b**, **5d**, **5g**, **5h**, **6b**, **6e**, **6h** and **6i** reduced metal ion complex to their lower oxidation state or take part in electron transfer reaction. In other words, these compounds showed the ability of electron donor to scavenge free radicals. The rest of the compounds did not showed significant activity as compared to the standards BHA, TBHQ and Ascorbic acid. The higher the absorbance of the compounds indicated the greater reducing power.

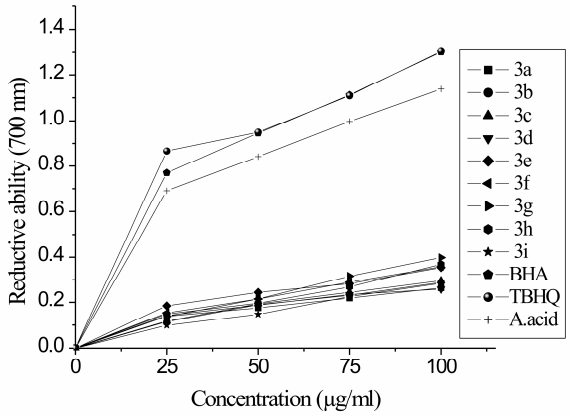


Figure-5 Reducing effect of compounds **3a-i,** BHA, TBHQ and Ascorbic Acid.

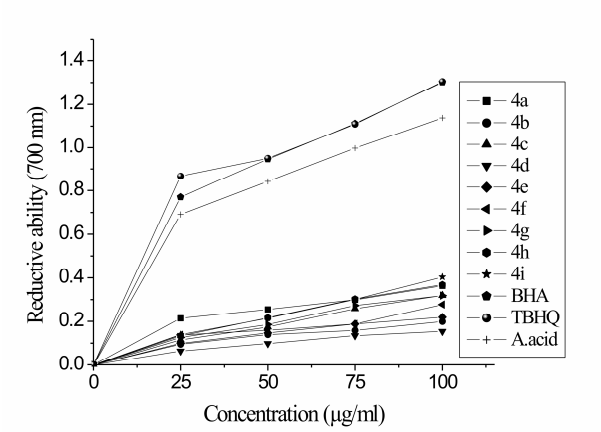


Figure-6 Reducing effect of compounds **4a-i,** BHA, TBHQ and Ascorbic Acid.

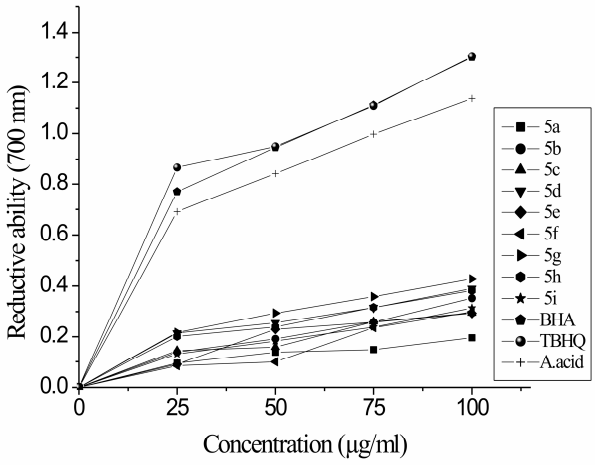


Figure-7 Reducing effect of compounds **5a-i,** BHA, TBHQ and Ascorbic Acid.

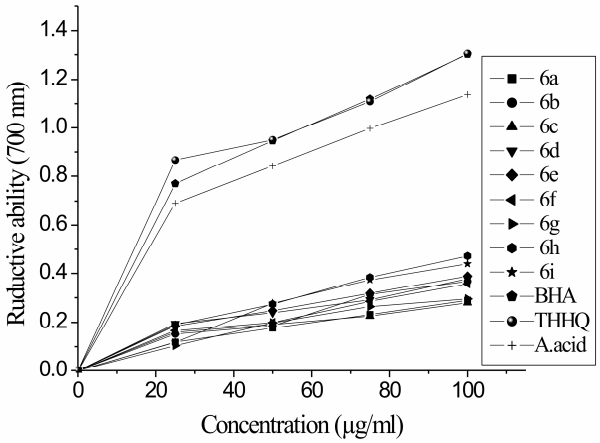


Figure-8 Reducing effect of compounds **6a-i,** BHA, TBHQ and Ascorbic Acid.

Conclusion

The overall results indicated that the compounds possessing chloro substituent exhibited good in-vitro antimicrobial activity. Compound possessing dithioxopyrimidine system exhibited better radical scavenging activity. Obliviously; the comparative evaluation of the compounds requires further studies. The data reported in this article may be helpful guide for the medicinal chemists who are working in the area.

Experimental

All the reagents were obtained commercially and used by further purification. Melting points were determined by an open capillary method and are uncorrected. Purity of the compounds was checked by thin layer chromatography using silica gel-G coated Al plates (Merck) and spots were visualized by exposing the dry plates in iodine vapors, using benzene:ethylacetate (1:1) and/or tolune:ethylacetate (1:1). The IR (KBr pellet) spectra were recorded on a Perkin-Elmer (SPECTRUM ONE) FT-IR Spectrometer. The ^{1}H NMR and ^{13}C NMR (DMSO-d_6) spectra were recorded with a BRUKER NMR 500 MHz and BRUKER NMR 125 MHz spectrometer the chemical shift values are expressed in ppm (δ scale) using tetramethylsilane as an internal standard. The mass spectral measurements were carried out by Electron Impact method on JEOL GC mate spectrometer at 70 eV. Elemental analyses were performed on flash EA 1112 series elemental analyzer.

General procedure for the synthesis of 2-Amino-4-(4-substitutedphenyl)thiazoles 1a-c were prepared by following literature method [25].

General procedure for the synthesis of (5-Substituted 2-phenyl-1H-indol-3-yl)methylene carbonitriles 2a-c were prepared by following literature method [26].

General procedure for the synthesis of 5-Amino-7-(4-aryl)-7-(5'-substituted 2'-phenyl-1H-indol-3'-yl)-thiazolo[3,2-a]pyrimidin-6-carbonitriles (3a-i):

A mixture of compound **1** (0.01 mol) and (5-substituted 2'-phenyl-1*H*-indol-3'-yl)methylene carbonitriles **2** (0.01 mol) in absolute ethanol (50 ml) containing triehylamine (0.5 ml) was refluxed for 7 h, cooled to room temperature and poured into ice-cold water. The solid thus formed was filtered off, washed several times with water, dried and recrystallized from ethanol to give **3a-i**.

5-Amino-3-(4-chlorophenyl)-7-(5'-chloro-2'-phenyl-1H-indol-3'-yl)-thiazolo[3,2-a] pyrimidin- 6-carbonitrile (3a):

Pale yellow crystals, Yield (63 %), m.p.109-110°C; IR (KBr) ν cm^{-1}: 3435 (NH_2), 3115 (NH), 2218 (CN), 1615 (CN), 715 (Ar-Cl), 698 (C-S-C); ^{1}H NMR (500 MHz, DMSO-d_6) δ: 12.90 (s, 1H, indole NH), 7.20-8.29 (m, 14H, 13Ar-H+ thiazole-CH), 6.10 (s, 1H, pyrimidine-CH), 4.53 (s, 2H, NH_2); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 161.57, 150.85, 146.94, 142.45, 133.91, 133.82, 133.16, 132.67, 130.51, 129.44, 128.93, 128.84, 128.66, 127.84, 127.21, 123.61, 120.55, 118.88, , 117.83, 113.61, 111.32, 100.82, 43.68; EI-MS: (m/z, %), 513 (M^+, 34 %), 515 (M^++2, 12 %), 517 (M^++4, 4 %); Anal. Calcd. for $C_{27}H_{17}N_5SCl_2$: C, 63.04; H, 3.33; N, 13.61. Found: C, 63.24; H, 3.41; N, 13.69.

5-Amino-3-(4-chlorophenyl)-7-(5'-methyl-2'-phenyl-1H-indol-3'-yl)-thiazolo[3,2-a]pyrimidin-6-carbonitrile (3b):
Pale yellow crystals, Yield (62 %), m.p.114-115°C; IR (KBr) ν cm^{-1}: 3439 (NH_2), 3119 (NH), 2221 (CN), 1617 (CN), 745 (Ar-Cl), 696 (C-S-C); ^{1}H NMR (500 MHz, DMSO-d_6) δ: 12.92 (s, 1H, indole NH), 7.01-8.27 (m, 14H, 13Ar-H+ thiazole-CH), 5.92 (s, 1H, pyrimidine-CH), 4.48 (s, 2H, NH_2); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 161.54, 148.29, 143.63, 142.74, 136.77, 134.89, 134.39, 131.08, 130.82, 130.69, 129.47, 128.21, 128.16, 127.10, 126.17, 125.02, 123.51, 123.43, 117.99, 112.77, 112.58, 101.08, 43.93, 16.72; EI-MS: (m/z, %), 493 (M^+, 66 %), 495 (M^++2, 22 %); Anal. Calcd. for $C_{28}H_{20}N_5SCl$: C, 68.08; H, 4.08; N, 14.18. Found: C, 68.23; H, 4.14; N, 14.28.

5-Amino-3-(4-chlorophenyl)-7-(2'-phenyl-1H-indol-3'-yl)-thiazolo[3,2-a]pyrimidin-6-carbonitrile (3c):
Pale yellow crystals, Yield (67 %), m.p.119-120°C; IR (KBr) ν cm^{-1}: 3435 (NH_2), 3115 (NH), 2218 (CN), 1617 (CN), 735 (Ar-Cl), 690 (C-S-C); ^{1}H NMR (500 MHz, DMSO-d_6) δ: 12.90 (s, 1H, indole NH), 7.02-8.33 (m, 15H, 14Ar-H+ thiazole-CH), 6.11 (s, 1H, pyrimidine-CH), 4.55 (s, 2H, NH_2); EI-MS: (m/z, %), 479 (M^+, 24%), 481 (M^++2, 8 %); Anal. Calcd. for $C_{27}H_{18}N_5SCl$: C, 68.56; H, 3.78; N, 14.59. Found: C, 68.12; H, 3.85; N, 14.68.

5-Amino-3-(4-methylphenyl)-7-(5'-chloro-2'-phenyl-1H-indol-3'-yl)-thiazolo[3,2-a] pyrimidin-6-carbonitrile (3d):
Pale yellow crystals, Yield (60 %), m.p.152-153°C; IR (KBr) ν cm^{-1}: 3436 (NH_2), 3118 (NH), 2214 (CN), 1615 (CN), 695 (C-S-C); ^{1}H NMR (500 MHz, DMSO-d_6) δ: 12.88 (s, 1H, indole NH), 7.17-8.07 (m, 14H, 13Ar-H+ thiazole-CH), 6.13 (s, 1H, pyrimidine-CH), 4.55 (s, 2H, NH_2), 2.55 (s, 3H, CH_3); Anal. Calcd. for $C_{28}H_{20}N_5SCl$: C, 68.08; H, 4.08; N, 14.18. Found: C, 68.25; H, 4.14; N, 14.26.

5-Amino-3-(4-methylphenyl)-7-(5'-methyl-2'-phenyl-1H-indol-3'-yl)-thiazolo[3,2-a] pyrimidin-6-carbonitrile (3e):
Pale yellow crystals, Yield (59 %), m.p.167-168°C; IR (KBr) ν cm^{-1}: 3439 (NH_2), 3118 (NH), 2216 (CN), 1615 (CN), 692 (C-S-C); ^{1}H NMR (500 MHz, DMSO-d_6) δ: 12.93 (s, 1H, indole NH), 7.00-8.30 (m, 14H, 13Ar-H+ thiazole-CH), 6.10 (s, 1H, pyrimidine-CH), 4.58 (s, 2H, NH_2), 2.57 (s, 3H, CH_3), 2.31 (s, 3H, CH_3); Anal. Calcd. for $C_{29}H_{23}N_5S$: C, 73.55; H, 4.90; N, 14.79. Found: C, 73.65; H, 4.94; N, 14.93.

5-Amino-3-(4-methylphenyl)-7-(2'-phenyl-1H-indol-3'-yl)-thiazolo[3,2-a]pyrimidin-6-carbonitrile (3f):
Pale yellow crystals, Yield (56 %), m.p.188-189°C; IR (KBr) ν cm^{-1}: 3438 (NH_2), 3122 (NH), 2223 (CN), 1615 (CN), 695 (C-S-C); ^{1}H NMR (500 MHz, DMSO-d_6) δ: 12.90 (s, 1H, indole NH), 6.98-8.23 (m, 15H, 14Ar-H+ thiazole-CH), 5.88 (s, 1H, pyrimidine-CH), 4.55 (s, 2H, NH_2), 2.31 (s, 3H, CH_3); Anal. Calcd. for $C_{28}H_{21}N_5S$: C, 73.18; H, 4.61; N, 15.24. Found: C, 73.29; H, 4.66; N, 15.36.

5-Amino-3-phenyl-7-(5'-chloro-2'-phenyl-1H-indol-3'-yl)-thiazolo[3,2-a]pyrimidin-6-carbonitrile (3g):
Pale yellow crystals, Yield (63 %), m.p.181-182°C; IR (KBr) ν cm^{-1}: 3439 (NH_2), 3122 (NH), 2219 (CN), 1614 (CN), 696 (C-S-C); ^{1}H NMR (500 MHz, DMSO-d_6) δ: 12.88 (s, 1H, indole NH), 7.20-8.24 (m, 15H, 14Ar-H+ thiazole-CH), 6.11 (s, 1H, pyrimidine-CH), 4.49 (s, 2H, NH_2); Anal. Calcd. for $C_{27}H_{18}N_5SCl$: C, 67.56; H, 3.78; N, 14.59. Found: C, 67.82; H, 3.88; N, 14.68.

5-Amino-3-phenyl-7-(5'-methyl-2'-phenyl-1H-indol-3'-yl)-thiazolo[3,2-a]pyrimidin-6-carbonitrile (3h):
Pale yellow crystals, Yield (64 %), m.p.131-132°C; IR (KBr) ν cm^{-1}: 3431 (NH_2), 3128 (NH), 2224 (CN), 1618 (CN), 697 (C-S-C); ^{1}H NMR (500 MHz, DMSO-d_6) δ: 12.80 (s, 1H, indole NH), 7.02-8.28 (m, 15H, 14Ar-H+ thiazole-CH), 6.11 (s, 1H, pyrimidine-CH), 4.56 (s, 2H, NH_2), 2.33 (s, 3H, CH_3); Anal. Calcd. for $C_{28}H_{21}N_5S$: C, 73.18; H, 4.61; N, 15.24. Found: C, 73.31; H, 4.67; N, 15.36.

5-Amino-3-phenyl-7-(2'-phenyl-1H-indol-3'-yl)-thiazolo[3,2-a]pyrimidin-6-carbonitrile (3i):
Pale yellow crystals, Yield (67 %), m.p.148-149°C; IR (KBr) ν cm^{-1}: 3438 (NH_2), 3115 (NH), 2215 (CN), 1616 (CN), 692 (C-S-C); ^{1}H NMR (500 MHz, DMSO-d_6) δ: 12.9 (s, 1H, indole NH), 7.01-8.31 (m, 16H, 15Ar-H+ thiazole-CH), 6.10 (s, 1H, pyrimidine-CH), 4.55 (s, 2H, NH_2); Anal. Calcd. for $C_{27}H_{19}N_5S$: C, 72.79; H, 4.30 N, 15.72. Found: C, 72.84; H, 4.35; N, 15.81.

General procedure for the synthesis of 4-Amino-9-(4-aryl)-5-(5'-substituted 2'-phenyl-1H-indol-3'-yl)-5H-pyrimido[5,4-e]thiazolo[3,2-a]pyrimidine (4a-i):
A mixture of compound **3** (0.01 mol) and formamide (3 ml) was refluxed for 4 h, cooled to room temperature and poured into ice-cold water. The precipitate thus formed was collected by filtration, washed several times with water, dried and recrystallized from ethanol to give **4a-i**.

4-Amino-9-(4-chlorophenyl)-5-(5'-chloro-2'-phenyl-1H-indol-3'-yl)-5H-pyrimido[5,4-e] thiazolo[3,2-a]pyrimidine (4a):
Brown crystals, Yield (67 %), m.p.148-149°C; IR (KBr) ν cm^{-1}: 3439 (NH_2), 3303 (NH), 1629 (CN), 768 (Ar-Cl), 697 (C-S-C); ^{1}H NMR (500 MHz, DMSO-d_6) δ: 12.01 (s, 1H, indole NH), 9.70 (s, 1H, pyrimidine-CH), 7.03-8.34 (m, 14H, 13Ar-H+ thiazole-CH), 6.10 (s, 1H, pyrimidine-CH), 4.52 (s, 2H, NH_2); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 172.83, 152.54, 149.51, 148.72, 146.72, 142.83, 134.99, 133.61, 133.43, 131.48, 129.89, 129.53, 128.89, 128.32, 128.24, 127.63, 127.46, 127.18, 125.48, 122.39, 115.90, 113.84, 100.83, 44.82; EI-MS: (m/z, %), 540 (M^+, 36 %), 542 (M^++2, 12 %), 544 (M^++4, 4 %); Anal. Calcd. for $C_{28}H_{18}N_6SCl_2$: C, 62.11; H, 3.35; N, 15.52. Found: C, 62.28; H, 3.39; N, 15.64.

4-Amino-9-(4-chlorophenyl)-5-(5'-methyl-2'-phenyl-1H-indol-3'-yl)-5H-pyrimido[5,4-e] thiazolo[3,2-a]pyrimidine (4b):
Brown crystals, Yield (66 %), m.p 172-173°C; IR (KBr) ν cm^{-1}: 3431 (NH_2), 3303 (NH), 1622 (CN), 761 (Ar-Cl), 697 (C-S-C); ^{1}H NMR (500 MHz, DMSO-d_6) δ: 12.01 (s, 1H, indole NH), 9.69 (s, 1H, pyrimidine-CH), 6.95-8.28 (m, 14H, 13Ar-H+ thiazole-CH), 6.14 (s, 1H, pyrimidine-CH), 4.49 (s, 2H, NH_2), 2.38 (s, 3H, CH_3); ^{13}C NMR (125 MHz, DMSO-d_6) δ:

172.12, 152.43, 149.67, 148.84, 146.47, 142.65, 134.99, 133.81, 133.26, 131.48, 130.47, 129.45, 129.32, 129.03, 128.89, 128.17, 127.63, 127.46, 127.27, 125.48, 115.95, 113.87, 100.66, 43.99, 16.45, EI-MS: (m/z, %), 520 (M^+, 15 %), 522 (M^++2, 5 %); Anal. Calcd for $C_{29}H_{21}N_6SCl$: C, 66.85; H, 4.06; N, 16.13. Found: C, 66.97; H, 4.09; N, 16.22.

4-Amino-9-(4-chlorophenyl)-5-(2'-phenyl-1H-indol-3'-yl)-5H-pyrimido[5,4-e]thiazolo[3,2-a] pyrimidine (4c):

Brown crystals, Yield (69 %), m.p.160-161$^{\circ 0}$C; IR (KBr) ν cm^{-1}: 3435 (NH_2), 3306 (NH), 1619 (CN), 764 (Ar-Cl), 695 (C-S-C); ^{1}H NMR (500 MHz, DMSO-d_6) δ: 12.02 (s, 1H, indole NH), 9.69 (s, 1H, pyrimidine-CH), 6.92-8.38 (m, 15H, 14Ar-H+ thiazole-CH), 6.13 (s, 1H, pyrimidine-CH), 4.55 (s, 2H, NH_2); EI-MS: (m/z, %), 506 (M^+, 21 %), 508 (M^++2, 7 %); Anal. Calcd. for $C_{28}H_{19}N_6SCl$: C, 66.38; H, 3.75; N, 16.59. Found: C, 66.44; H, 3.85; N, 16.66.

4-Amino-9-(4-methylphenyl)-5-(5'-chloro-2'-phenyl-1H-indol-3-yl)-5H-pyrimido[5,4-e] thiazolo[3,2-a]pyrimidine (4d):

Brown crystals, Yield (64 %), m.p.189-190°C; IR (KBr) ν cm^{-1}: 3431 (NH_2), 3308 (NH), 1629 (CN), 697 (C-S-C); ^{1}H NMR (500 MHz, DMSO-d_6) δ: 12.03 (s, 1H, indole NH), 9.71 (s, 1H, pyrimidine-CH), 6.96-8.24 (m, 14H, 13Ar-H+ thiazole-CH), 6.13 (s, 1H, pyrimidine-CH), 4.51 (s, 2H, NH_2), 2.51 (s, 3H, CH_3); Anal. Calcd. for $C_{29}H_{21}N_6SCl$: C, 66.85; H, 4.06; N, 16.13. Found: C, 66.89; H, 4.09; N, 16.19.

4-Amino-9-(4-methylphenyl)-5-(5'-methyl-2'-phenyl-1H-indol-3'-yl)-5H-pyrimido[5,4-e] thiazolo[3,2-a]pyrimidine (4e):

Brown crystals, Yield (58 %), m.p.201-202°C; IR (KBr) ν cm^{-1}: 3438 (NH_2), 3305 (NH), 1624 (CN), 696 (C-S-C); ^{1}H NMR (500 MHz, DMSO-d_6) δ: 12.04 (s, 1H, indole NH), 9.73 (s, 1H, pyrimidine-CH), 6.95-8.21 (m, 14H, 13Ar-H+ thiazole-CH), 6.16 (s, 1H, pyrimidine-CH), 4.53 (s, 2H, NH_2), 2.52 (s, 3H, CH_3), 2.35 (s, 3H, CH_3); Anal. Calcd. for $C_{30}H_{24}N_6S$: C, 71.98; H, 4.83; N, 16.79. Found: C, 72.09; H, 4.89; N, 16.88.

4-Amino-9-(4-methylphenyl)-5-(2'-phenyl-1H-indol-3'-yl)-5H-pyrimido[5,4-e]thiazolo[3,2-a] pyrimidine (4f):

Brown crystals, Yield (55 %), m.p.195-196°C; IR (KBr) ν cm^{-1}: 3435 (NH_2), 3309 (NH), 1621 (CN), 696 (C-S-C); ^{1}H NMR (500 MHz, DMSO-d_6) δ: 12.04 (s, 1H, indole NH), 9.70 (s, 1H, pyrimidine-CH), 6.99-8.21 (m, 15H, 14Ar-H+ thiazole-CH), 6.14 (s, 1H, pyrimidine-CH), 4.53 (s, 2H, NH_2), 2.53 (s, 3H, CH_3); Anal. Calcd. for $C_{29}H_{22}N_6S$: C, 71.58; H, 4.56; N, 17.27. Found: C, 71.68; H, 4.52; N, 17.33.

4-Amino-9-phenyl-5-(5'-chloro-2'-phenyl-1H-indol-3'-yl)-5H-pyrimido[5,4-e]thiazolo[3,2-a] pyrimidine (4g):

Brown crystals, Yield (74 %), m.p.143-144°C; IR (KBr) ν cm^{-1}: 3439 (NH_2), 3309 (NH), 1627 (CN), 699 (C-S-C); ^{1}H NMR (500 MHz, DMSO-d_6) δ: 12.01 (s, 1H, indole NH), 9.70 (s, 1H, pyrimidine-CH), 6.98-8.20 (m, 15H, 14Ar-H+ thiazole-CH), 6.12 (s, 1H, pyrimidine-CH), 4.54 (s, 2H, NH_2); Anal. Calcd. for $C_{28}H_{19}N_6SCl$: C, 66.33; H, 3.78; N, 16.99. Found: C, 66.51; H, 3.84; N, 16.75.

4-Amino-9-phenyl-5-(5'-methyl-2'-phenyl-1H-indol-3-yl)-5H-pyrimido[5,4-e]thiazolo[3,2-a] pyrimidine (4h):
Brown crystals, Yield (66 %), m.p.114-115°C; IR (KBr) ν cm^{-1}: 3435 (NH_2), 3306 (NH), 1625 (CN), 695 (C-S-C); ^{1}H NMR (500 MHz, DMSO-d_6) δ: 12.02 (s, 1H, indole NH), 9.71 (s, 1H, pyrimidine-CH), 7.01-8.18 (m, 15H, 14Ar-H+ thiazole-CH), 6.11 (s, 1H, pyrimidine-CH), 4.55 (s, 2H, NH_2), 2.34 (s, 3H, CH_3); Anal. Calcd. for $C_{29}H_{22}N_6S$: C, 71.58; H, 4.56; N, 17.27. Found: C, 71.68; H, 4.61; N, 17.32.

4-Amino-9-phenyl-5-(2'-phenyl-1H-indol-3'-yl)-5H-pyrimido[5,4-e]thiazolo[3,2-a] pyrimidine (4i):
Brown crystals, Yield (68 %), m.p.119-120°C; IR (KBr) ν cm^{-1}: 3436 (NH_2), 3307 (NH), 1624 (CN), 695 (C-S-C); ^{1}H NMR (500 MHz, DMSO-d_6) δ: 12.05 (s, 1H, indole NH), 9.73 (s, 1H, pyrimidine-CH), 7.03-8.19 (m, 16H, 15Ar-H+ thiazole-CH), 6.11 (s, 1H, pyrimidine-CH), 4.53 (s, 2H, NH_2); Anal. Calcd. for $C_{28}H_{20}N_6S$: C, 71.16; H, 4.27; N, 17.78. Found: C, 71.24; H, 4.33; N, 17.85.

General procedure for the synthesis of 9-(4-Aryl)-5H-(5'-substituted 2'phenyl-1H-indol-3'-yl)-2,4-dithioxo-1,3,4,5-tetrahydro-2H-pyrimido[5,4-e]thiazolo[3,2-a]pyrimidines (5a-i):
A mixture of compound **3** (0.01 mol) and carbon disulfide (0.04 mol) in pyridine (15 ml) was refluxed on water bath for 12-15 h. The reaction mixture was cooled to room temperature and poured into ice cold water. The product thus formed was filtered, washed with cold water, saturated sodium bicarbonate solution followed by water, dried and recrystallized from ethanol to give **5a-i**.

9-(4-chlorophenyl)-5H-(5'-chloro-2'-phenyl-1H-indol-3'-yl)-2,4-dithioxo-1,3,4,5-tetrahydro-2H-pyrimido[5,4-e]thiazolo[3,2-a]pyrimidine (5a):
Reddish brown crystals, Yield (72 %), m.p.171-172°C; IR (KBr) ν cm^{-1}: 3302 (NH), 3171 (NH), 3138 (NH), 1633 (CN), 1299 (CS), 1175 (CS); ^{1}H NMR (500 MHz, DMSO-d_6) δ: 12.89 (s, 1H, pyrimidine-NH), 11.88 (s, 1H, indole NH), 11.01 (s, 1H pyrimidine-NH), 6.88-8.17 (m, 14H, 13Ar-H+ thiazole-CH), 6.00 (s, 1H, pyrimidine-CH); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 190.73, 177.50, 149.59, 148.21, 148.08, 142.86, 137.56, 137.12, 134.39, 132.77, 129.16. 128.83, 128.66, 127.68, 127.13, 123.96, 123.66, 120.20, 114.36, 113.97, 101.23, 100.61, 43.98; EI-MS: (m/z, %), 589 (M^+, 24 %), 591 (M^++2, 8 %), 593 (M^++4, 4 %); Anal. Calcd. for $C_{28}H_{17}N_5S_3Cl_2$: C, 56.95; H, 2.90; N, 11.86. Found: C, 57.11; H, 3.03; N, 11.94.

9-(4-chlorophenyl)-5H-(5'-methyl-2'-phenyl-1H-indol-3'-yl)-2,4-dithioxo-1,3,4,5-tetrahydro -2H-pyrimido[5,4-e]thiazolo[3,2-a]pyrimidine (5b):
Reddish brown crystals, Yield (66 %), m.p.183-184°C; IR (KBr) ν cm^{-1}: 3304 (NH), 3178 (NH), 3138 (NH), 1630 (CN), 1298 (CS), 1175 (CS); ^{1}H NMR (500 MHz, DMSO-d_6) δ: 12.45 (s, 1H, pyrimidine-NH), 11.67 (s, 1H, indole NH), 10.98 (s, 1H, pyrimidine-NH), 6.80-8.12 (m, 14H, 13Ar-H+ thiazole-CH), 6.11 (s, 1H, pyrimidine-CH), 2.38 (s, 3H, CH_3); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 190.76, 177.08, 148.59, 148.44, 148.16, 143.01, 137.17, 134.53, 134.11, 132.03, 128.76, 127.42, 129.91, 128.69, 128.51. 123.88, 123.42, 120.81, 120.75, 114.51, 113.67, 101.45, 100.77, 45.10, 16.19; EI-MS: (m/z, %), 569 (M^+, 33 %) , 571 (M^++2, 11 %); Anal. Calcd. for $C_{29}H_{20}N_5S_3Cl$: C, 61.09; H, 3.54; N, 12.28. Found: C, 61.25; H, 3.61; N, 12.34.

9-(4-chlorophenyl)-5H-(2'-phenyl-1H-indol-3'-yl)-2,4-dithioxo-1,3,4,5-tetrahydro-2H-pyrimido[5,4-e]thiazolo[3,2-a]pyrimidine (5c):
Yield (66 %), m.p.188-89°C; IR (KBr) ν cm^{-1}: 3304 (NH), 3178 (NH), 3138 (NH), 1630 (CN), 1298 (CS), 1175 (CS); ^{1}H NMR (500 MHz, DMSO-d_6) δ: 12.71 (s, 1H pyrimidine-NH), 11.65 (s, 1H, indole NH), 11.01 (s, 1H, pyrimidine-NH), 6.80-8.21 (m, 15H, 14Ar-H+ thiazole-CH), 5.91 (s, 1H, pyrimidine-CH); EI-MS: (m/z, %), 555 (M^+, 14 %), 557 (M^++2, 5 %); Anal. Calcd. for $C_{28}H_{18}N_5S_3Cl$: C, 60.47; H, 3.26; N, 12.59. Found: C, 60.71; H, 3.29; N, 12.67.

9-(4-methylphenyl)-5H-(5'-chloro-2'-phenyl-1H-indol-3'-yl)-2,4-dithioxo-1,3,4,5-tetrahydro-2H-pyrimido[5,4-e]thiazolo[3,2-a]pyrimidine (5d):
Reddish brown crystals, Yield (58 %), m.p.161-162°C; IR (KBr) ν cm^{-1}: 3308 (NH), 3175 (NH), 3138 (NH), 1633 (CN), 1295 (CS), 1175 (CS); ^{1}H NMR (500 MHz, DMSO-d_6) δ: 12.89 (s, 1H, pyrimidine-NH), 11.88 (s, 1H, indole NH), 11.01 (s, 1H, pyrimidine-NH), 6.88-8.17 (m, 14H, 13Ar-H+ thiazole-CH), 6.00 (s, 1H, pyrimidine-CH), 2.56 (s, 3H, CH_3); Anal. Calcd. for $C_{29}H_{20}N_5S_3Cl$: C, 61.09; H, 3.54; N, 12.28. Found: C, 61.29; H, 3.61; N, 12.40.

9-(4-methyphenyl)-5H-(5'-methyl-2'-phenyl-1H-indol-3'-yl)-2,4-dithioxo-1,3,4,5-tetrahydro-2H-pyrimido[5,4-e]thiazolo[3,2-a]pyrimidine (5e):
Reddish brown crystals, Yield (64 %), m.p.177-178°C; IR (KBr) ν cm^{-1}: 3305 (NH), 3182 (NH), 3138 (NH), 1634 (CN), 1294 (CS), 1173 (CS); ^{1}H NMR (500 MHz, DMSO-d_6) δ: 12.46 (s, 1H, pyrimidine-NH), 11.68 (s, 1H, indole NH), 10.99 (s, 1H, pyrimidine-NH), 6.88-8.11 (m, 14H, 13Ar-H+ thiazole-CH), 6.10 (s, 1H, pyrimidine-CH), 2.52 (s, 3H, CH_3), 2.33 (s, 3H, CH_3); Anal. Calcd. for $C_{30}H_{23}N_5S_3$: C, 65.54; H, 4.22; N, 12.74. Found: C, 65.66; H, 4.32; N, 12.81.

9-(4-methylphenyl)-5H-(2'-phenyl-1H-indol-3'-yl)-2,4-dithioxo-1,3,4,5-tetrahydro-2H-pyrimido[5,4-e]thiazolo[3,2-a]pyrimidine (5f):
Reddish brown crystals, Yield (61 %), m.p.193-194°C; IR (KBr) ν cm^{-1}: 3308 (NH), 3177 (NH), 3139 (NH), 1639 (CN), 1296 (CS), 1174 (CS); ^{1}H NMR (500 MHz, DMSO-d_6) δ: 12.69 (s, 1H, pyrimidine-NH), 11.68 (s, 1H, indole NH), 11.03 (s, 1H, pyrimidine-NH), 6.88-8.14 (m, 16H, 15Ar-H+ thiazole-CH), 6.02 (s, 1H, pyrimidine-CH), 2.58 (s, 3H, CH_3); Anal. Calcd. for $C_{29}H_{21}N_5S_3$: C, 65.02; H, 3.95; N, 13.07. Found: C, 65.11; H, 3.99; N, 13.13.

9-Phenyl-5H-(5'-chloro-2'-phenyl-1H-indol-3'-yl)-2,4-dithioxo-1,3,4,5-tetrahydro-2H-pyrimido[5,4-e]thiazolo[3,2-a]pyrimidine (5g):
Reddish brown crystals, Yield (66 %), m.p.137-138°C; IR (KBr) ν cm^{-1}: 3309 (NH), 3179 (NH), 3138 (NH), 1296 (CS), 1174 (CS); ^{1}H NMR (500 MHz, DMSO-d_6) δ: 12.90 (s, 1H, pyrimidine-NH), 11.90 (s, 1H, indole NH), 11.08 (s, 1H, pyrimidine-NH), 6.88-8.13 (m, 15H, 14Ar-H+ thiazole-CH), 6.11 (s, 1H, pyrimidine-CH); Anal. Calcd. for $C_{28}H_{18}N_5S_3Cl$: C, 60.47; H, 3.26; N, 12.59. Found: C, 60.65; H, 3.31; N, 12.70.

9-Phenyl-5H-(5'-methyl-2'-phenyl-1H-indol-3'-yl)-2,4-dithioxo-1,3,4,5-tetrahydro-2H-pyrimido[5,4-e]thiazolo[3,2-a]pyrimidine (5h):
Reddish brown crystals, Yield (63 %), m.p.135-136°C; IR (KBr) ν cm^{-1}: 3308 (NH), 3182 (NH), 3135 (NH), 1635 (CN), 1296 (CS), 1174 (CS); ^{1}H NMR (500 MHz, DMSO-d_6) δ: 12.48 (s, 1H, pyrimidine-NH), 11.70 (s, 1H, indole NH), 10.98 (s, 1H, pyrimidine-NH), 6.94-8.15 (m, 16H,

15Ar-H+ thiazole-CH), 6.11 (s, 1H, pyrimidin-CH); Anal. Calcd. for $C_{29}H_{21}N_5S_3$: C, 65.02; H, 3.95; N, 13.07. Found: C, 65.11; H, 3.99; N, 13.14.

9-(Phenyl)-5H-(2'-phenyl-1H-indol-3'-yl)-2,4-dithioxo-1,3,4,5-tetrahydro-2H-pyrimido[5,4-e] thiazolo[3,2-a]pyrimidines (5i):

Reddish brown crystals, Yield (65 %), m.p.182-183°C; IR (KBr) ν cm^{-1}: 3311 (NH), 3181 (NH), 3135 (NH), 1634 (CN), 1295 (CS), 1175 (CS); ^{1}H NMR (500 MHz, DMSO-d_6) δ: 12.71 (s, 1H, pyrimidine-NH), 11.73 (s, 1H, indole NH), 11.04 (s, 1H, pyrimidine-NH), 6.89-8.15 (m, 16H, 15Ar-H+ thiazole-CH), 6.04 (s, 1H, pyrimidine-CH); Anal. Calcd. for $C_{28}H_{19}N_5S_3$: C, 64.46; H, 3.67; N, 13.42. Found: C, 64.55; H, 3.73; N, 13.55.

General procedure for the synthesis of 9-(4-aryl)-5-(5'-substituted 2'-phenyl-1H-indol-3'-yl)-3,5-dihydropyrimido[5,4-e]thiazolo[3,2-a]pyrimidin-4-one (6a-i)

A mixture of compound **3** (0.01 mol) and formic acid (3 ml) was refluxed for 5h. After cooling at room temperature the reaction mixture was poured into ice cold water, the product thus formed was separated, filtered off, dried and recrystallized form benzene to afford pure **6a-i**.

9-(4-chlorophenyl)-5-(5'-chloro-2'-phenyl-1H-indol-3'-yl)-3,5-dihydropyrimido[5,4-e] thiazolo[3,2-a] pyrimidin-4-one (6a):

Yellowish crystals, Yield (68 %), m.p.173-174°C; IR (KBr) ν cm^{-1}: 3161 (NH), 3056 (NH), 1674 (CO), 1620 (CN), 769 (Ar-Cl), 696 (C-S-C); ^{1}H NMR (500 MHz, DMSO-d_6) δ: 12.20 (s, 1H, indole NH), 10.91 (s, 1H, pyrimidine-NH), 9.72 (s, 1H, pyrimidine-CH), 7.01-8.22 (m, 14H, 13Ar-H+ thiazole-CH), 6.12 (s, 1H, pyrimidine-CH); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 166.84, 154.78, 144.30, 143.13, 137.90, 137.87, 135.15, 131.93, 131.48, 130.81, 128.86, 128.49, 127.63, 127.21, 125.19, 124.96, 120.75, 115.94, 114.27, 113.86, 107.06, 100.74, 45.50; EI-MS: (m/z, %), 541 (M^+, 18 %), 543 (M^++2, 6 %), 545 (M^++4, 2 %); Anal. Calcd. for $C_{28}H_{17}N_5OSCl_2$: C, 62.00; H, 3.16; N, 12.91. Found: C, 62.17; H, 3.21; N, 13.04.

9-(4-chlorophenyl)-5-(5'-methyl-2'-phenyl-1H-indol-3'-yl)-3,5-dihydropyrimido[5,4-e] thiazolo[3,2-a]pyrimidin-4-one (6b):

Yellowish crystals, Yield (76 %), m.p.168-169°C; IR (KBr) ν cm^{-1}: 3167 (NH), 3064 (NH), 1673 (CO), 1616 (CN), 768 (Ar-Cl), 695 (C-S-C); ^{1}H NMR (500 MHz, DMSO-d_6) δ: 12.09 (s, 1H, indole NH), 10.98 (s, 1H, pyrimidine-NH), 9.71 (s, 1H, pyrimidine-CH), 6.98-8.20 (m, 14H, 13Ar-H+ thiazole-CH), 6.12 (s, 1H, pyrimidine-CH), 2.41 (s, 3H, CH_3); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 168.94, 154.94, 142.94, 138.91, 137.87, 135.15, 132.84, 131.84, 130.81, 129.53, 128.86, 128.49, 127.86, 127.63, 127.21, 124.96, 120.75, 115.94, 113.86, 107.06, 101.74, 45.51, 16.42; EI-MS: (m/z, %), 521 (M^+, 39 %), 523 (M^++2, 13 %); Anal. Calcd. for $C_{29}H_{20}N_5OSCl$: C, 66.72; H, 3.86; N, 13.42. Found: C, 66.87; H, 3.94; N, 13.50.

9-(4-chlorophenyl)-5-(2'-phenyl-1H-indol-3'-yl)-3,5-dihydropyrimido[5,4-e]thiazolo[3,2-a] pyrimidin-4-one (6c):

Yellowish crystals, Yield (69 %), m.p.162-163°C; IR (KBr) ν cm^{-1}: 3163 (NH), 3059 (NH), 1674 (CO), 1625 (CN), 764 (Ar-Cl), 697 (C-S-C); ^{1}H NMR (500 MHz, DMSO-d_6) δ: 12.10 (s, 1H, indole NH), 11.01 (s, 1H, pyrimidine-NH), 9.80 (s, 1H, pyrimidine-CH), 7.80-8.20 (m, 14H, 13Ar-H+ thiazole-CH), 6.10 (s, 1H, pyrimidine-CH); EI-MS: (m/z, %), 507 (M^+, 39 %), 509

(M^++2, 13 %); Anal. Calcd. for $C_{28}H_{18}N_5OSCl$: C, 66.20; H, 3.57; N, 13.79. Found: C, 66.34; H, 3.65; N, 13.89.

9-(4-methylphenyl)-5-(5'-chloro-2'-phenyl-1H-indol-3'-yl)-3,5-dihydropyrimido[5,4-e] thiazolo[3,2-a]pyrimidin-4-one (6d):
Yellowish crystals, Yield (65 %), m.p.189-190°C; IR (KBr) ν cm^{-1}: 3167 (NH), 3060 (NH), 1678 (CO), 1625 (CN), 694 (C-S-C); ^{1}H NMR (500 MHz, DMSO-d_6) δ: 12.14 (s, 1H, indole NH), 10.94 (s, 1H, pyrimidin-NH), 9.75 (s, 1H, pyrimidine-CH), 7.04-8.19 (m, 14H, 13Ar-H+ thiazole-CH), 6.10 (s, 1H, pyrimidine-CH), 2.58 (s, 3H, CH_3); Anal. Calcd. for $C_{29}H_{20}N_5OSCl$: C, 66.72; H, 3.86; N, 13.42. Found: C, 66.91; H, 3.77; N, 13.67.

9-(4-methylphenyl)-5-(5'-methyl-2'-phenyl-1H-indol-3'-yl)-3,5-dihydropyrimido[5,4-e] thiazolo[3,2-a]pyrimidin-4-one (6e):
Yellowish crystals, Yield (66 %), m.p.164-165°C; IR (KBr) ν cm^{-1}: 3172 (NH), 3058 (NH), 1677 (CO), 1625 (CN), 696 (C-S-C); ^{1}H NMR (500 MHz, DMSO-d_6) δ: 12.16 (s, 1H, indole NH), 10.94 (s, 1H, pyrimidine-NH), 9.75 (s, 1H, pyrimidine-CH), 7.01-8.19 (m, 14H, 13Ar-H+ thiazole-CH), 6.13 (s, 1H, pyrimidine-CH), 2.31(s, 3H, CH_3); Anal. Calcd. for $C_{30}H_{23}N_5OS$: C, 71.83; H, 4.62; N, 13.96. Found: C, 71.94; H, 4.68; N, 14.03.

9-(4-methylphenyl)-5-(2'-phenyl-1H-indol-3'-yl)-3,5-dihydropyrimido[5,4-e]thiazolo[3,2-a] pyrimidin-4-one (6f):
Yellowish crystals, Yield (61 %), m.p.184-185°C; IR (KBr) ν cm^{-1}: 3179 (NH), 3050 (NH), 1681 (CO), 1614 (CN), 696 (C-S-C); ^{1}H NMR (500 MHz, DMSO-d_6) δ: 12.12 (s, 1H, indole NH), 10.99 (s, 1H, pyrimidine-NH), 9.74 (s, 1H, pyrimidine-CH), 6.99-8.14 (m, 15H, 14Ar-H+ thiazole-CH), 6.15 (s, 1H, pyrimidine-CH), 2.34 (s, 3H, CH_3); Anal. Calcd. for $C_{29}H_{21}N_5OS$: C, 71.44; H, 4.34; N, 14.36. Found: C, 71.55; H, 4.42; N, 14.44.

9-Phenyl-5-(5'-chloro-2'-phenyl-1H-indol-3'-yl)-3,5-dihydropyrimido[5,4-e]thiazolo[3,2-a] pyrimidin-4-one (6g):
Yellowish crystals, Yield (70%), m.p.203-204°C; IR (KBr) ν cm^{-1}: 3167 (NH), 3067 (NH), 1678 (CO), 1626 (CN), 696 (C-S-C); ^{1}H NMR (500 MHz, DMSO-d_6) δ: 12.21 (s, 1H, indole NH), 10.99 (s, 1H, pyrimidine-NH), 9.80 (s, 1H, pyrimidine-CH), 6.99-8.14 (m, 15H, 14Ar-H+ thiazole-CH), 6.11 (s, 1H, pyrimidine-CH); Anal. Calcd. for $C_{28}H_{18}N_5OSCl$: C, 66.20; H, 3.57; N, 13.79. Found: C, 66.39; H, 3.64; N, 13.90.

9-Phenyl-5-(5'-methyl-2'-phenyl-1H-indol-3'-yl)-3,5-dihydropyrimido[5,4-e]thiazolo[3,2-a] pyrimidin-4-one (6h):
Yellowish crystals, Yield (56 %), m.p.214-215°C; IR (KBr) ν cm^{-1}: 3161 (NH), 3050 (NH), 1681 (CO), 1619 (CN), 694 (C-S-C); ^{1}H NMR (500 MHz, DMSO-d_6) δ: 12.11 (s, 1H, indole NH), 11.01 (s, 1H, pyrimidine-NH), 9.79 (s, 1H, pyrimidine-CH), 7.01-8.15 (m, 15H, 14Ar-H+ thiazole-CH), 6.11 (s, 1H, pyrimidine-CH), 2.34 (s, 3H, CH_3); Anal. Calcd. for $C_{29}H_{21}N_5OS$: C, 71.44; H, 4.34; N, 14.36. Found: C, 71.55; H, 4.45; N, 14.40.

9-Phenyl-5-(2'-phenyl-1H-indol-3'-yl)-3,5-dihydropyrimido[5,4-e]thiazolo[3,2-a]pyrimidin-4-one (6i):

Yellowish crystals, Yield (66 %), m.p.208-209°C; IR (KBr) ν cm^{-1}: 3172 (NH), 3061 (NH), 1680 (CO), 1613 (CN), 697 (C-S-C); ^{1}H NMR (500 MHz, DMSO-d_6) δ: 12.11 (s, 1H, indole NH), 10.98 (s, 1H, pyrimidine-NH), 9.75 (s, 1H, pyrimidine-CH), 6.89-8.11 (m, 16H, 15Ar-H+ thiazole-CH), 6.12 (s, 1H, pyrimidine-CH); Anal. Calcd. for $C_{28}H_{19}N_5OS$: C, 71.02; H, 4.04; N, 14.79. Found: C, 71.15; H, 4.11; N, 14.87.

Biological activities

Antimicrobial activity

The *in-vitro* antimicrobial screening of the synthesized compounds was carried out at Department of Microbiology, Gulbarga University, Gulbarga. Against bacteria *Escherichia Coli (E.C.)* (MTCC-723), *Staphylococcus aureus (S.A.)* (ATCC-29513), *Klebsiella pneumonia (K.P.)* (NCTC-13368) and *Pseudomonas aeruginosa (P.A.) (MTCC-1688)* and fungal species, *Aspergillus oryzae (A.O.)* (MTCC-3567^T), *Aspergillus niger (A.N.)* (MTCC-281), *Aspergillus flavus (A.F.)* (MTCC-1973) and *Aspegillus terreus (A.T.)* (MTCC-1782) by cup-plate method [27] 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 (1000 µg/ml in DMF) and DMF used as control. The plates incubated at 37^{0}C for 24 h and 72 h in case antibacterial and antifungal activity, respectively. The diameter of the zone of inhibition for all the test compounds was measured and the results were compared with the standard drug Gentamycin (Std-1) for bacterial activity and Fluconazole (Std-2) for antifungal activity (**Table-I**).

Antioxidant activity assay

1, 1-Diphenyl-2-Picryl Hydrazyl (DPPH) Radical Scavenging Activity (RSA): The free radical scavenging activity (RSA) of compounds **3**, **4, 5** and **6** at concentration 25, 50, 75, 100 µg/ml was carried out in the presence of freshly prepared solution of stable free radical DPPH (0.04% w/v) following Hatano's method [28], using 2-tert-butyl-4-methoxyphenol (butylated hydroxy anisole, BHA) and 2-(1,1-dimethylethyl)-1,4-benzenediol (2-tert. butyl hydroquinone, TBHQ) as standards. All the test analyses were performed on three replicates and results are averaged. The results in percentage are expressed as the ratio of absorption decrease of DPPH in the presence test compounds and absorption of DPPH in the absence of test compounds at 517 nm on ELICO SL 171 Mini Spec spectrophotometer. The percentage scavenging activity of the DPPH free radical was measured using the following equation.

$$\% \text{ DPPH Radical Scavenging} = \frac{(\text{Absorbance of control- Absorbance of test sample})}{(\text{Absorbance of control})} \times 100$$

The results are shown in the Fig**. (1-4).**

Reducing power assay

The reducing power of the synthesized compounds **3**, **4, 5** and **6** was determined according to the literature method [29] at concentrations 25, 50, 75 and 100 µg/ml in DMSO. Each concentration of sample (1 ml) was 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^{0}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) diluted with distilled water (2.5 ml) and ferric chloride (0.5 ml, 0.1 %) was added. Then absorbance at 700 nm was measured in a

spectrophotometer. Higher absorbance of the reaction mixture indicated greater reducing power. The results are shown in the Fig. (**5-8**).

Acknowledgement

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 activity, to the Director of Indian Institute of Technology, Chennai for providing ^{1}H NMR, ^{13}C NMR and Mass spectra. One of us (P.W.) is thankful to University Grant Commission, New Delhi for providing the financial support through Research Fellowship in Science for Meritorious Students (RFSMS).

References

1. H. H. Hussain, G. Babic, T. Drust, J .Wright, M. Flueraru, A. Chichirau, L. L. Chapeley, *J. Org. Chem.,* **68**, 7023-7032 (2003).
2. J. Clements. MC, E. A. Decker, *J. Food Sci*., **65**, 1270-1408 (2000).
3. F. Liu, T. B. Ng, Z. T. Wang, *Life Sci.,* **66**(8), 709-804 (2000).
4. P. Moreau, *Eur. J. Med. Chem.,* **43**, 2316-2322 (2008).
5. F. D. Popp, H. Pojouhesh. *J. Pharm. Sci*., **72**, 318-322 (1983).
6. T. Michel Monique, A. Luc, *Trans Roy. Soc. Trop. Med. and Hyg*., **102**, 11-17 (2008).
7. X. Quaoje, G. Zhaobing, W. Wei, L. Min, *Trend. Pharmaco. Sci*., **29**(2), 99-107 (2008).
8. J. Birgit, P. Jetiana, C. O. Kappe, *Molecules,* **5**, 227-243 (2005).
9. C. O. Kappe, G. Farber, *J. Chem. Soc. Perkin Trans.,* **1**, 1342-1344 (1991).
10. Y. S. Sadanandam, M. M. Shetty, P. V Diwan, *Eur. J. Med. Chem*., **27**, 87-92 (1992).
11. S. S. Panda, P. V. Chowdary, *Indian J. Pharm. Sci*., **70**(2), 208-215 (2008).
12. T. I. El-Emary, Sh. A. Abdel-Mosen, *Phosphorus Sulfur Silicon Relat. Elem*., **181**, 2459-2474 (2006).
13. A. E. Rashad, H. H. Sayed, A. H Shamroukh, H. M. Awad, *Phosphorus Sulfur Silicon Relat. Elem.,* **180**, 2767-2777 (2005).
14. M. F. M. E. Abdel, N. A. Hassan, M. A. Zahran, A. E. Rashad, *Sulfur Lett.,* **21**, 269-284 (1998).
15. M. I. Hagab, N. A. A. E. Rashad, A. A. Fahmy, M. F. Abdel, *Phosphorus Sulfur Silicon Relat. Elem*., **182**, 1535-1556 (2007).
16. N. P. Peet, *J. Heterocycl. Chem*., **23**, 193-194 (1986).
17. H. W. Lee, B. Y. Khim, J. B. Ahn, S. K. Kang, J. H. Lee, J. S. Shin, S. K. Ahn, S. J. Lee, S. S. Yoon, *Eur. J. Med. Chem.,* **40**, 862-874 (2005).
18. N. C. Desai, *Indian J. Chem.,* **32B**, 343-346 (1993).
19. S. J. Kashyap, P. K. Sharma, V. K. Garg, R. Dudhe, N. Kumar, *J. Adv. Sci. Res*., **2**(3), 18-23 (2011).
20. A. R. Saundane, M. Yarlakatti, Prabhaker. Walmik, *Heterocycl. Commn*., **15,** 303-310. (2009).
21. A. R. Saundane, M. Yarlakatti, *J. Chem. Chemical Engg*., **3**(12), 54-59 (2009).
22. A. R. Saundane, V. Katkar, M. Yarlakatti, A. V. Vaijinath, Prabhaker. Walmik, *Indian J. Heterocycl. Chem.,* **20**, 321-324 (2011).
23. A. R. Saundane, V. Katkar, M. Yarlakatti, Prabhaker. Walmik, A. V. Vaijinath, *Heterocycl. Lett*., **1**(4), 339-350 (2011).

24. A. R. Saundane, M. Yarlakatti, *Arbian J. Chem.*, doi. 10.1016/ j. arabjc.2011.06.011 (2011).
25. R. M. Dodson, K. L. Carroll, *J. Am. Chem. Soc*., **67**, 2242-2243 (1945).
26. S. P. Hiremath, A. Ullagadi, R. K. Sekhar, M. G. Purohit, *Indian J. Heterocycl. Chem*., **27**, 758-762 (1988).
27. Indian Pharmacopeia, Government of India, 3rd Ed. New Delhi Appendix IV**,** 90 (1985).
28. T. Hatano, H. Kangawa, T. Yasuhara, T. Okuda, *Chem. Pharm. Bull.*, **36**, 2090-2097 (1988).
29. M. Oyaizu, *Japan Nutri*., **44**, 307-316 (1986).

Received on November 13, 2011.