*Available online at* www.derpharmachemica.com

**Scholars Research Library**

**Der Pharma Chemica, 2013, 5(4):213-222**
*(http://derpharmachemica.com/archive.html)*

**ISSN 0975-413X**
**CODEN (USA): PCHHAX**

# Antioxidant activity of novel coumarin substituted benzothiazole derivatives

**Shivani Choudhary, Suvarna G. Kini* and Muhammad Mubeen**

*Department of Pharmaceutical Chemistry, Manipal College of Pharmaceutical Sciences, Manipal University, Manipal, Karnataka, India*

---

## ABSTRACT

*An attempt was made to synthesize newer coumarin substituted derivatives of benzothiazole and they were evaluated for antioxidant activity by DPPH radical scavenging activity. The test compound SC-7 showed good in-vitro antioxidant activity. The same compound was selected for in-vivo antioxidant studies by administering different doses in Ehrlich Ascites Carcinoma (EAC) treated mice. It reduced the levels of lipid peroxidation and increased the GST contents as well as CAT levels in EAC induced mice. The present study revealed that newly synthesized coumarin substituted benzothiazole derivatives showed significant antioxidant activity in EAC bearing mice.*

**Key words:** Coumarin, benzothiazole, antioxidant, DPPH radical scavenging, lipid peroxidation

---

## INTRODUCTION

Antioxidants in food play an important role as a health-protecting factor. Scientific evidence suggests that antioxidants reduce the risk for chronic diseases including cancer and heart disease. Primary sources of naturally occurring antioxidants are whole grains, fruits and vegetables. Plant sourced food antioxidants like vitamin C, vitamin E, carotenes, phenolic acids, phytate and phytoestrogens have been recognized as having the potential to reduce disease risk. Most of the antioxidant compounds in a typical diet are derived from plant sources and belongs to various classes of compounds with a wide variety of physical and chemical properties. Some compounds, such as gallates have strong antioxidant activity, while others, such as the mono-phenols are weak antioxidants.

The main characteristic of an antioxidant is its ability to trap free radicals. Highly reactive free radicals and oxygen species are present in biological systems from a wide variety of sources. These free radicals may oxidize nucleic acids, proteins, lipids or DNA and can initiate degenerative disease. Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases. There are a number of clinical studies suggesting that the antioxidants in fruits, vegetables, tea and red wine are the main factors for the observed efficacy of these foods in reducing the incidence of chronic diseases including heart disease and some cancers. The free radical scavenging activity of antioxidants in foods has been substantially investigated and reported in the literature by Miller and Rigelhof et al [1]. A wide range of antioxidants both synthetic and natural have been proposed in the treatment of human diseases. Hence, considerable attention has been devoted for the development of techniques for measurement of antioxidant activity.

Benzothiazoles comprise a novel class of therapeutic compounds shown to exert a wide range of biological activities. Most of the compounds that exhibited both high levels of fluorescence properties and a capacity to bind

with cellular structures were extensively used as fluorochromes. Since the 1990s, various pharmacological investigations of newly synthesized benzothiazole derivatives demonstrated interesting pharmacological activities. Biologist's attention was drawn to this series when the pharmacological profile of Riluzol [2] was discovered. Riluzole (6-trifluoromethoxy-2-benzothiazolamine, PK-26124, RP-25279, Rilutek) was found to interfere with glutamate neurotransmission in biochemical, electrophysiological and behavioral experiments.

Although they have been known from long ago to be biologically active [3, 4, 5] their varied biological features are still of great scientific interest. Benzothiazole shows antioxidant, antitumor [6], antiviral [7], anti HIV [8] and antimicrobial [9] activity. In the present study a brief account of various alterations on the coumarin moiety which is attached to the benzothiazole ring and their associated biological activities. Coumarins (known as 1, 2-benzopyrones or *o*-hydroxycinnamic acid-8-lactones) comprise a very large class of phenolic derivatives found in plants and consist of fused benzene and α-pyrone rings [10]. Coumarins have attracted intense interest in recent years because of their diverse pharmacological properties. Owing to their importance in many fields of everyday life, such as pharmaceutical, cosmetic, perfume, and nutrition, their chemistry has been widely investigated and many natural and non natural coumarins have been synthesized. In the recent years, one method which has been vastly investigated for the synthesis of coumarins is the Pechmann reaction, which starts from phenols. In the conventional production of coumarins by Pechmann, concentrated sulfuric acid is used as the catalyst [11].

During the last twenty years, the study of the biological activities of coumarin derivatives has been the aim of many researchers [12]. Based on these findings, we describe the synthesis of some compounds featuring heterocyclic ring (benzothiazole) fused onto the coumarin moiety with the aim of obtaining more potent pharmacologically active compounds.

## MATERIALS AND METHODS

All the chemicals and solvents used were of AR-grade and LR-grade and obtained from Sigma-Aldrich, Sisco Research Laboratories, Qualingens, Hi-media, Nice chemicals and Spectrochem.

Melting points were measured on an electrothermal melting point apparatus (Shital scientific Industries, Mangalore, India). Infrared (IR) spectra were recorded as KBr pellets with FTIR- 8310 spectrophotometer (Shimadzu, Japan). Proton magnetic resonance (1H NMR) spectra were recorded in DMSO-d6 (Merck) on an AMX-400 NMR Spectrometer (IISc, Bangalore, India). Mass spectra were recorded on a GCMS (QP 5050A, Shimadzu Corporation, Japan). Absorption Maxima were taken on a UV-Visible spectrophotometer-1650 (Shimadzu, Japan). Thin-layer chromatography (TLC) was performed on pre-coated aluminium plates (silica gel 60 F254, Merck). Plates were visualized by UV light and iodine vapor.

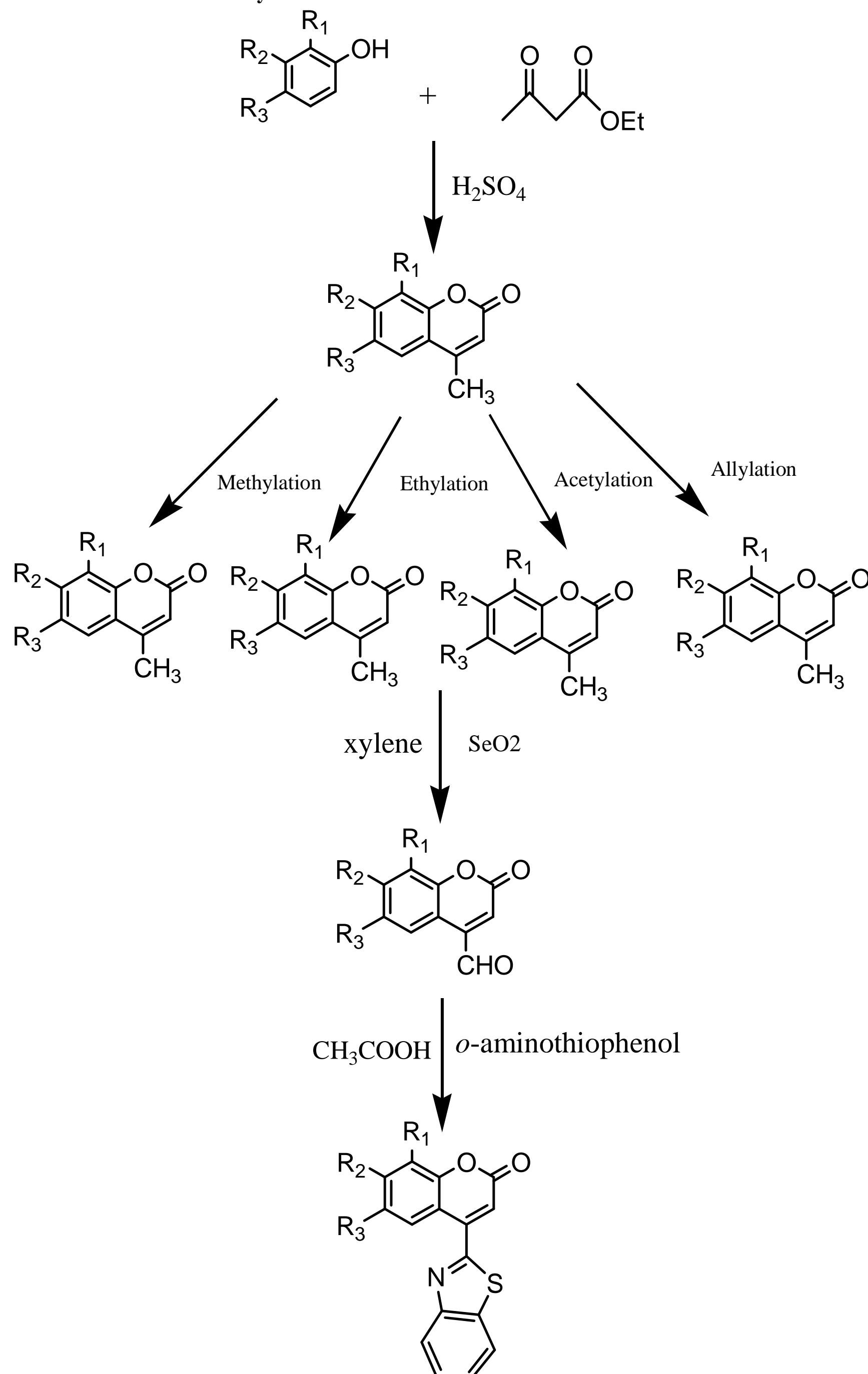
**Synthesis of substituted coumarin** [13, 14]: A mixture of the substituted resorcinol (0.01mol) and concentrated sulphuric acid (10ml) was taken in a flask. To this ethylacetoacetate (0.01mol) was added dropwise by maintaining the temperature below $100^0$C. Kept aside the reaction mixture at room temperature for 18h and then poured into a mixture of crushed ice (20g) and water (30ml). The precipitate was collected, washed with water. This solid was dissolved in 5% sodium hydroxide solution (15ml), filtered and 2M sulphuric acid was added with stirring until the solution was acid to litmus. Substituted coumarin was collected, washed with water and dried.

**Methylation** [15]: A mixture of the coumarin (0.01mol), dimethyl sulphate (0.15g), anhydrous potassium carbonate (0.4g) and acetone (0.7ml) was taken in a microwave flask. The flask was placed in microwave oven for 10 min. The contents of the flask were poured into ice water and neutralized using 10% hydrochloric acid and then extracted with ether. The organic layer was washed with aqueous bicarbonate solution and brine respectively and dried over anhydrous sodium sulphate. Ether was removed to get o-methylated product.

**Ethylation** [16]: A mixture of the substituted coumarin (0.002mol), ethyl iodide (0.022mol), anhydrous potassium carbonate (0.005mol) and acetone (50ml) was refluxed for 24h. The reaction mixture was poured into water. The precipitate was collected, washed with water and dried.

**Acetylation** [17]: A mixture of substituted coumarin (0.01mol), anhydrous sodium acetate (0.049mol) and acetic anhydride (0.06mol) was heated on a water bath for 8h and poured into water. The precipitate was collected, washed with 1% aqueous sodium hydroxide solution and dried.

**Scheme for the synthesis of coumarin substituted benzothiazole derivatives**




**Allylation** [18]: A mixture of substituted coumarin (40g), allyl bromide (2.1ml) and anhydrous potassium carbonate (8g) in acetone (100ml) was refluxed on a water bath for 10h and poured into water. The precipitate was collected, washed with aqueous sodium hydroxide.

**Formylation** [19]: Substituted coumarin (1g) was dissolved in hot xylene (50ml). The solution was cooled and selenium dioxide (1g) was added. The mixture was refluxed for 12h and filtered in hot condition. Solvent was removed to get desired product.

**Synthesis of coumarin substituted benzothiazole** [20]: The coumarin-4-carboxaldehyde (0.055mol) and o-aminothiophenol (0.05mol) was refluxed in acetic acid (7ml) for 5h. The solution was cooled and the product precipitated. This solid was collected, washed with water and recrystallized from methanol. Yields and physical characteristics are listed in Table 1.

**Table 1 Physical data of Coumarin substituted benzothiazole derivatives**

| Compound | $R_1$ | $R_2$ | $R_3$ | % yield | Mt. pt. | Rf value |
|---|---|---|---|---|---|---|
| **SC1** | H<br>SHC1 | $OCH_3$ | H | 74 | 152-154<br>152-154 | 0.70 |
| **SC2** | H<br>SC2 | $OC_2H_5$ | H | 74 | 150-155<br>150-155 | 0.71 |
| **SC3** | H<br>SC3 | OAc | H | 68 | 177-180<br>177-180 | 0.68 |
| **SC4** | H<br>SC4 | $OCH_2CH{=}CH_2$ | H | 70 | 180-183<br>180-183 | 0.72 |
| **SC5** | H<br>SC5 | H | $OCH_3$ | 72 | 172-174<br>172-174 | 0.62 |
| **SC6** | H<br>SC6 | H | $OC_2H_5$ | 70 | 170-173<br>170-173 | 0.66 |
| **SC7** | H<br>SC7 | H | OAc | 60 | 170-172<br>170-172 | 0.79 |
| **SC8** | $OCH_3$<br>SC8 | $OCH_3$ | H | 66 | 210-212<br>210-212 | 0.68 |
| **SC9** | OAc<br>SC9 | OAc | H | 62 | 190-193<br>190-193 | 0.64 |
| **SC10** | H | $N(C_2H_5)_2$ | H | 56 | 300-303<br>300-303 | 0.62 |
| **SC11** | $CH_3$<br>SC10 | $OCH_3$ | H | 72 | 212-215<br>212-215 | 0.74 |
| **SC12** | $CH_3$<br>SC11 | OAc | H | 63 | 220-222<br>220-222 | 0.72 |
| **SC13** | H<br>SC13 | $OCH_3$ | Cl | 67 | 200-203<br>200-203 | 0.62 |
| **SC14** | H<br>SC14 | $OCH_3$ | $OCH_3$ | 75 | 200-202<br>200-202 | 0.76 |
| **SC15** | H<br>SC15 | OAc | OAc | 65 | 176-180<br>176-180 | 0.72 |
| **SC16** | H<br>SC16 | $OC_2H_5$ | $OC_2H_5$ | 73 | 178-181<br>178-181 | 0.70 |
| **SC17** | H<br>SC17 | $OCH_2CH{=}CH_2$ | $OCH_2CH{=}CH_2$ | 63 | 212-216<br>212-216 | 0.67 |
| **SC18** | $NO_2$<br>SC18 | $OCH_3$ | H | 68 | 176-180<br>176-180 | 0.64 |
| **SC19** | H<br>SC19 | $SCOCH_3$ | H | 65 | 202-206<br>202-206 | 0.68 |
| **SC20** | H<br>SC20 | $SCH_3$ | H | 72 | 189-192<br>189-192 | 0.72 |

**4-(benzo[d]thiazol-2-yl)-7-methoxy-2H-chromen-2-one (SC1)** IR (KBr): 2922.25(C-H), 1616.40(C=C), 1734.06(C=O), 1546.96(C=N), 1383.01(C-N), 756.12(C-S) cm-1. 1H NMR (DMSO): δ = 6.75(s, 1H, H-3), 8.20(d, 1H, J = 8.22Hz, H-5), 7.07(d, 1H, J = 7.07 Hz, H-6), 7.59(s, 1H, J = 7.62, H-8), 7.04(d, 1H, J = 7.01Hz, H-4'), 7.64(t, 1H, J = 7.64Hz, H-5'), 8.22 (t, 1H, J = 8.22 Hz, H-6'), 8.53(d, 1H, J = 8.50Hz, H-7'), 3.89(s, 3H, CH3). GC-MS: 309(M+).

**4-(benzo[d]thiazol-2-yl)-7-ethoxy-2H-chromen-2-one (SC2)** IR (KBr): 2976.26(C-H), 1606.76(C=C), 1714.77(C=O), 1546.96(C=N), 1383.01(C-N), 765.77(C-S) cm-1. 1H NMR (DMSO): δ = 6.74(s, 1H, H-3), 8.23(d, 1H, J = 8.22 Hz, H-5), 7.03(d, 1H, J = 7.07 Hz, H-6), 7.65(s, 1H, J = 7.62 Hz, H-8), 7.0(d, 1H, J = 7.01 Hz, H-4'), 7.62(t, 1H, J = 7.64 Hz, H-5'), 8.24(t, 1H, J = 8.22 Hz, H-6'), 8.51(d, 1H, J = 8.50 Hz, H-7'), 4.18(s, 2H, CH2), 1.38(s, 3H, CH3). GC-MS: 323(M+).

**4-(benzo[d]thiazol-2-yl)-7-acetoxy-2H-chromen-2-one (SC3)** IR (KBr): 2935.16(C-H), 1609.73(C=C), 1721.25(C=O), 1552.26(C=N), 1379.71(C-N), 782.47(C-S) cm-1. 1H NMR (DMSO): δ = 6.77(s, 1H, H-3), 8.18(d,

1H, J = 8.20 Hz, H-5), 7.11(d, 1H, J = 7.09 Hz, H-6), 7.59(s, 1H, J = 7.59 Hz, H-8), 7.2(d, 1H, J = 7.1 Hz, H-4'), 7.58(t, 1H, J = 7.60 Hz, H-5'), 8.14(t, 1H, J = 8.16 Hz, H-6'), 8.58(d, 1H, J = 8.57 Hz, H-7'), 3.408(s, 3H, CH3). GC-MS: 337(M+).

**4-(benzo[d]thiazol-2-yl)-7-allyloxy-2H-chromen-2-one (SC4)** IR (KBr): 2958.76(C-H), 1625.18(C=C), 1732.57(C=O), 1556.27(C=N), 1367.06(C-N), 749.87(C-S) cm-1. 1H NMR (DMSO): δ = 6.84(s, 1H, H-3), 8.54(d, 1H, J = 8.52 Hz, H-5), 7.13(d, 1H, J = 7.15 Hz, H-6), 7.57(s, 1H, J = 7.57 Hz, H-8), 7.1(d, 1H, J = 7.1 Hz, H-4'), 7.57(t, 1H, J = 7.57 Hz, H-5'), 8.33(t, 1H, J = 8.32 Hz, H-6'), 8.50(d, 1H, J = 8.50 Hz, H-7'), 4.22(s, 2H, CH2), 1.75(s, 3H, CH3). GC-MS: 335(M+).

**4-(benzo[d]thiazol-2-yl)-6-methoxy-2H-chromen-2-one (SC5)** IR (KBr): 2945.40(C-H), 1604.83(C=C), 1716.70(C=O), 1558.45(C=N), 1479.45(C-N), 756.12(C-S) cm-1. 1H NMR (DMSO): δ = 6.86(s, 1H, H-3), 8.60(d, 1H, J = 8.52Hz, H-5), 7.29(d, 1H, J = 7.27 Hz, H-6), 7.59(s, 1H, J = 7.62, H-8), 7.34(d, 1H, J = 7.34Hz, H-4'), 7.64(t, 1H, J = 7.64Hz, H-5'), 8.24 (t, 1H, J = 8.24 Hz, H-6'), 8.62(d, 1H, J = 8.60Hz, H-7'), 3.84(s, 3H, CH3). GC-MS: 309(M+).

**4-(benzo[d]thiazol-2-yl)-6-ethoxy-2H-chromen-2-one (SC6)** IR (KBr): 2972.40(C-H), 1604.83(C=C), 1716.70(C=O), 1558.54(C=N), 1483.31(C-N), 769.62(C-S) cm-1. 1H NMR (DMSO): δ = 6.95(s, 1H, H-3), 8.17(s, 1H, H-5), 7.57(d, 1H, H-7), 7.41(d, 1H, H-8), 7.30(d, 1H, H-4'), 7.42(t, 1H, H-5'), 8.17(t, 1H, H-6'), 8.24(d, 1H, H-7'), ), 4.09(s, 2H, CH2), 1.36(s,3H, CH3). GC-MS: 323(M+).

**4-(benzo[d]thiazol-2-yl)-6-acetoxy-2H-chromen-2-one (SC7)** IR (KBr): 2935.16(C-H), 1609.73(C=C), 1721.25(C=O), 1552.26(C=N), 1379.71(C-N), 782.47(C-S) cm-1. 1H NMR (DMSO): δ = 6.77(s, 1H, H-3), 8.18(d, 1H, J = 8.20 Hz, H-5), 7.11(d, 1H, J = 7.09 Hz, H-6), 7.59(s, 1H, J = 7.59 Hz, H-8), 7.2(d, 1H, J = 7.1 Hz, H-4'), 7.58(t, 1H, J = 7.60 Hz, H-5'), 8.14(t, 1H, J = 8.16 Hz, H-6'), 8.58(d, 1H, J = 8.57 Hz, H-7'), 3.48(s, 3H, CH3). GC-MS: 337(M+).

**4-(benzo[d]thiazol-2-yl)-7, 8-dimethoxy-2H-chromen-2-one (SC8)** IR (KBr): 2931.90(C-H), 1600.93(C=C), 1726.35(C=O), 1552.75(C=N), 1475.59(C-N), 750.33(C-S) cm-1. 1H NMR (DMSO): δ = 6.95(s, 1H, H-3), 8.28(d, 1H, J = 8.28Hz, H-5), 7.36(d, 1H, J = 7.37 Hz, H-6), 7.81(s, 1H, J = 7.84, H-8), 7.24(d, 1H, J = 7.21Hz, H-4'), 7.74(t, 1H, J = 7.74Hz, H-5'), 8.42 (t, 1H, J = 8.42 Hz, H-6'), 8.50(d, 1H, J = 8.54Hz, H-7'), 3.93(s, 3H, CH3). GC-MS: 339(M+).

**4-(benzo[d]thiazol-2-yl)-7, 8-diacetoxy-2H-chromen-2-one (SC9)** IR (KBr): 2990.44(C-H), 1660.12(C=C), 1732.41(C=O), 1559.72(C=N), 1337.11(C-N), 777.56(C-S) cm-1. 1H NMR (DMSO): δ = 6.33(s, 1H, H-3), 8.29(d, 1H, J = 8.28 Hz, H-5), 7.24(d, 1H, J = 7.28 Hz, H-6), 7.84(s, 1H, J = 7.81 Hz, H-8), 7.40(d, 1H, J = 7.31 Hz, H-4'), 7.73(t, 1H, J = 7.75 Hz, H-5'), 8.46(t, 1H, J = 8.44 Hz, H-6'), 8.71(d, 1H, J = 8.70 Hz, H-7'), 4.48(s, 2H, CH2), 1.34(s, 3H, CH3). GC-MS: 395(M+).

**4-(benzo[d]thiazol-2-yl)-7-diethylamino-2H-chromen-2-one (SC10)** IR (KBr): 2918.57(C-H), 1652.34(C=C), 1709.80(C=O), 1527.36(C=N), 1389.31(C-N), 725.47(C-S) cm-1. 1H NMR (DMSO): δ = 6.87(s, 1H, H-3), 8.33(d, 1H, J = 8.29 Hz, H-5), 7.17(d, 1H, J = 7.17 Hz, H-6), 7.22(s, 1H, J = 7.22 Hz, H-8), 7.5(d, 1H, J = 7.51 Hz, H-4'), 7.50(t, 1H, J = 7.54 Hz, H-5'), 8.27(t, 1H, J = 8.30 Hz, H-6'), 8.49(d, 1H, J = 8.48 Hz, H-7'), 4.05(s, 2H, CH2), 1.26(s, 3H, CH3). GC-MS: 350(M+).

**4-(benzo[d]thiazol-2-yl)-7-methoxy-8-methyl-2H-chromen-2-one (SC11)** IR (KBr): 2968.55(C-H), 1600.93(C=C), 1726.35(C=O), 1554.68(C=N), 1475.59(C-N), 752.26(C- S) cm-1. 1H NMR (DMSO): δ = 6.88(s, 1H, H-3), 8.25(d, 1H, J = 8.22Hz, H-5), 7.07(d, 1H, J = 7.07 Hz, H-6), 7.33(s, 1H, J = 7.30, H-8), 7.53(d, 1H, J = 7.51Hz, H-4'), 7.11(t, 1H, J = 7.14Hz, H-5'), 8.32 (t, 1H, J = 8.32 Hz, H-6'), 8.27(d, 1H, J = 8.24Hz, H-7'), 3.78(s, 3H, CH3). GC-MS: 323(M+).

**4-(benzo[d]thiazol-2-yl)-7-acetoxy-8-methyl-2H-chromen-2-one (SC12)** IR (KBr): 2945.85(C-H), 1644.42(C=C), 1732.33(C=O), 1538.21(C=N), 1467.19(C-N), 765.20(C- S) cm-1. 1H NMR (DMSO): δ = 6.74(s, 1H, H-3), 8.55(d, 1H, J = 8.52Hz, H-5), 7.22 (d, 1H, J = 7.22 Hz, H-6), 7.23(s, 1H, J = 7.21, H-8), 7.56(d, 1H, J = 7.55Hz, H-4'), 7.32(t, 1H, J = 7.32Hz, H-5'), 8.32 (t, 1H, J = 8.32 Hz, H-6'), 8.08(d, 1H, J = 8.07Hz, H-7'), 3.35(s, 3H, CH3). GC-MS: 351(M+).

**4-(benzo[d]thiazol-2-yl)-6-chloro-7-methoxy-2H-chromen-2-one (SC13)** IR (KBr): 2985.91(C-H), 1614.69(C=C), 1735.99(C=O), 1546.96(C=N), 1487.17(C-N), 752.26(C-S) cm-1. 1H NMR (DMSO): δ = 6.40(s, 1H, H-3), 8.39(d, 1H, J = 8.28Hz, H-5), 7.81 (d, 1H, J = 7.80 Hz, H-6), 7.30(s, 1H, J = 7.29, H-8), 7.47(d, 1H, J = 7.47Hz, H-4'), 7.12(t, 1H, J = 7.12Hz, H-5'), 8.38 (t, 1H, J = 8.37 Hz, H-6'), 8.11(d, 1H, J = 8.11Hz, H-7'), 3.25(s, 3H, CH3). GC-MS: 344(M+).

**4-(benzo[d]thiazol-2-yl)-6, 7-dimethoxy-2H-chromen-2-one (SC14)** IR (KBr): 2982.91(C-H), 1614.47(C=C), 1728.28(C=O), 1546.96(C=N), 1456.30(C-N), 758.05(C-S) cm-1. 1H NMR (DMSO): δ = 6.44(s, 1H, H-3), 8.22(d, 1H, J = 8.24Hz, H-5), 7.31 (d, 1H, J = 7.31 Hz, H-6), 7.53(s, 1H, J = 7.51, H-8), 7.14(d, 1H, J = 7.14Hz, H-4'), 7.09(t, 1H, J = 7.10Hz, H-5'), 8.28 (t, 1H, J = 8.29 Hz, H-6'), 8.22(d, 1H, J = 8.23Hz, H-7'), 3.17(s, 3H, CH3). GC-MS: 339(M+).

**4-(benzo[d]thiazol-2-yl)-6, 7-diacetoxy-2H-chromen-2-one (SC15)** IR (KBr): 2949.58(C-H), 1630.23(C=C), 1745.24(C=O), 1531.13(C=N), 1349.43(C-N), 725.37(C-S) cm-1. 1H NMR (DMSO): δ = 6.46(s, 1H, H-3), 8.29(d, 1H, J = 8.28 Hz, H-5), 7.03(d, 1H, J = 7.07 Hz, H-6), 7.55(s, 1H, J = 7.52 Hz, H-8), 7.0(d, 1H, J = 7.01 Hz, H-4'), 7.64(t, 1H, J = 7.64 Hz, H-5'), 8.42(t, 1H, J = 8.44 Hz, H-6'), 8.63(d, 1H, J = 8.62 Hz, H-7'), 4.23(s, 2H, CH2), 1.29(s, 3H, CH3). GC-MS: 395(M+).

**4-(benzo[d]thiazol-2-yl)-6, 7-diethoxy-2H-chromen-2-one (SC16)** IR (KBr): 2972.38(C-H), 1624.58(C=C), 1719.55(C=O), 1548.24(C=N), 1311.71(C-N), 742.14(C-S) cm-1. 1H NMR (DMSO): δ = 6.14(s, 1H, H-3), 8.22(d, 1H, J = 8.22 Hz, H-5), 7.12(d, 1H, J = 7.11 Hz, H-6), 7.33(s, 1H, J = 7.34 Hz, H-8), 7.20(d, 1H, J = 7.21 Hz, H-4'), 7.19(t, 1H, J = 7.19 Hz, H-5'), 8.33(t, 1H, J = 8.33 Hz, H-6'), 8.42(d, 1H, J = 8.42 Hz, H-7'), 4.11(s, 2H, CH2), 1.42(s, 3H, CH3). GC-MS: 367(M+).

**4-(benzo[d]thiazol-2-yl)-6, 7-allyloxy-2H-chromen-2-one (SC17)** IR (KBr): 2932.27(C-H), 1644.54(C=C), 1765.87(C=O), 1526.57(C=N), 1331.90(C-N), 750.07(C-S) cm-1. 1H NMR (DMSO): δ = 6.77(s, 1H, H-3), 8.08(d, 1H, J = 8.10 Hz, H-5), 7.15(d, 1H, J = 7.15 Hz, H-6), 7.41(s, 1H, J = 7.40 Hz, H-8), 7.09(d, 1H, J = 7.09 Hz, H-4'), 7.38(t, 1H, J = 7.40 Hz, H-5'), 8.49(t, 1H, J = 8.39 Hz, H-6'), 8.47(d, 1H, J = 8.47 Hz, H-7'), 4.41(s, 2H, CH2), 1.39(s, 3H, CH3). GC-MS: 391(M+).

**4-(benzo[d]thiazol-2-yl)-7-methoxy-8-nitro-2H-chromen-2-one (SC18)** IR (KBr): 2919.77(C-H), 1628.27(C=C), 1740.20(C=O), 1555.10(C=N), 1442.11(C-N), 742.33(C- S) cm-1. 1H NMR (DMSO): δ = 6.42(s, 1H, H-3), 8.40(d, 1H, J = 8.42Hz, H-5), 7.30 (d, 1H, J = 7.32 Hz, H-6), 7.11(s, 1H, J = 7.11, H-8), 7.55(d, 1H, J = 7.55Hz, H-4'), 7.26(t, 1H, J = 7.24Hz, H-5'), 8.40 (t, 1H, J = 8.40 Hz, H-6'), 8.16(d, 1H, J = 8.14Hz, H-7'), 3.40(s, 3H, CH3). GC-MS: 354(M+).

**4-(benzo[d]thiazol-2-yl)-2-oxo-2H-chromen-7-yl ethanethioate (SC19)** IR (KBr): 2927.51(C-H), 1639.48(C=C), 1751.10(C=O), 1560.18(C=N), 1436.61(C-N), 766.28(C- S) cm-1. 1H NMR (DMSO): δ = 6.33(s, 1H, H-3), 8.42(d, 1H, J = 8.39Hz, H-5), 7.53 (d, 1H, J = 7.50 Hz, H-6), 7.26(s, 1H, J = 7.26, H-8), 7.49(d, 1H, J = 7.47Hz, H-4'), 7.22(t, 1H, J = 7.22Hz, H-5'), 8.39 (t, 1H, J = 8.40 Hz, H-6'), 8.19(d, 1H, J = 8.19Hz, H-7'), 3.38(s, 3H, CH3). GC-MS: 353(M+).

**4-(benzo[d]thiazol-2-yl)-7-(methylthio)-2H-chromen-2-one (SC20)** IR (KBr): 2925.77(C-H), 1632.27(C=C), 1769.18(C=O), 1560.32(C=N), 1439.12(C-N), 764.29(C- S) cm-1. 1H NMR (DMSO): δ = 6.24(s, 1H, H-3), 8.36(d, 1H, J = 8.40Hz, H-5), 7.46 (d, 1H, J = 7.44 Hz, H-6), 7.27(s, 1H, J = 7.25, H-8), 7.58(d, 1H, J = 7.57Hz, H-4'), 7.34(t, 1H, J = 7.32Hz, H-5'), 8.21 (t, 1H, J = 8.21 Hz, H-6'), 8.27(d, 1H, J = 8.25Hz, H-7'), 3.27(s, 3H, CH3). GC-MS: 325(M+).

**DPPH radical scavenging activity** [21]
Antioxidant activity of the test compounds was determined by diphenylpicrylhydrazyl (DPPH) radical scavenging method. The assay was carried out in a 96 well microtitre plate. 100µl of test sample and standard solution were added to each well separately and in triplicates. 100µl of DPPH solution was added to each well. Control wells were loaded with 100µl each of DMSO and DPPH. Sample blank and control blank were also performed. The plates were incubated at 37$^{o}$C for 30 minutes without exposing to light and the absorbance of each solution was measured with ELISA reader using 540 nm filters. The percentage scavenging of test samples (Table 2 and Figure 1) at each concentration were calculated using the following formula

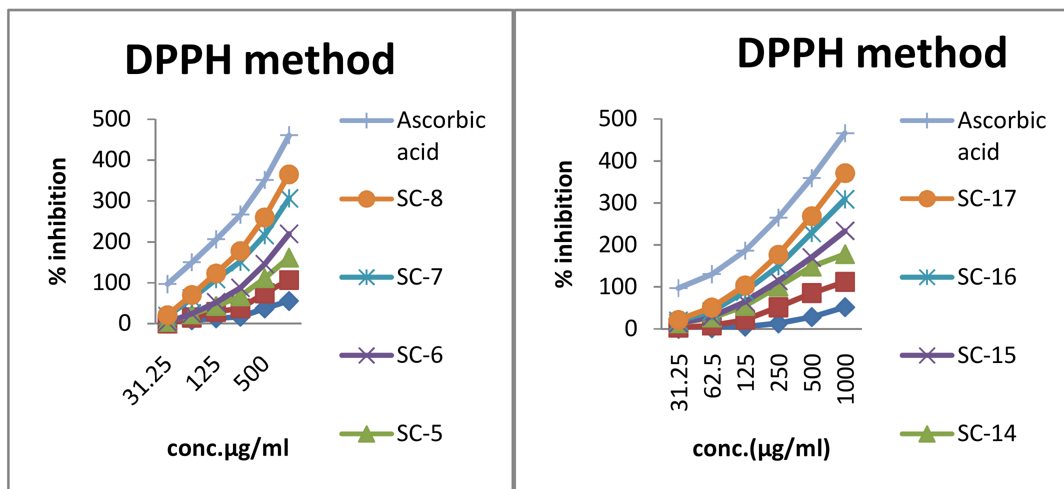
[(Control absorbance – Test absorbance) / Control absorbance] X 100

The IC $_{50}$ was calculated using the Microsoft excel. (Figure 2)

**Table 2 DPPH radical scavenging activity**

| Compound Code | Percentage Scavenging (µg/ml) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1000 | 500 | 250 | 125 | 62.5 | 31.25 | $IC_{50}$(µg/ml) |
| SC-1 | 55.12 | 36.89 | 16.25 | 12.87 | 7.76 | -- | 928.00 |
| SC-2 | 51.12 | 32.89 | 21.00 | 15.65 | 6.78 | -- | 998.00 |
| SC-3 | 37.24 | 30.85 | 22.54 | 10.38 | 4.23 | 1.68 | >1000 |
| SC-4 | 48.28 | 27.89 | 19.68 | 15.21 | 9.26 | -- | >1000 |
| SC-5 | 55.36 | 37.45 | 29.87 | 14.53 | 8.25 | 3.89 | 821.70 |
| SC-6 | 57.83 | 33.78 | 20.65 | 9.05 | 1.98 | -- | 825.84 |
| SC-7 | 86.60 | 70.90 | 63.45 | 56.78 | 3.60 | 14.56 | 246.39 |
| SC-8 | 58.90 | 43.80 | 26.41 | 14.02 | 8.69 | 2.35 | 754.73 |
| SC-9 | 51.40 | 27.80 | 12.90 | 5.24 | 1.25 | -- | 909.12 |
| SC-10 | 60.40 | 57.20 | 38.40 | 15.90 | 6.54 | 2.58 | 654.64 |
| SC-11 | 42.50 | 28.90 | 16.54 | 8.54 | 3.54 | -- | >1000 |
| SC-12 | 38.00 | 16.30 | 6.78 | 1.65 | -- | -- | >1000 |
| SC-13 | 30.00 | 13.50 | 9.10 | 2.50 | -- | -- | >1000 |
| SC-14 | 66.00 | 63.00 | 49.20 | 33.50 | 18.80 | 10.40 | 514.24 |
| SC-15 | 55.20 | 21.80 | 13.25 | 9.40 | 2.54 | -- | 938.70 |
| SC-16 | 75.40 | 58.50 | 35.20 | 24.50 | 11.70 | 5.68 | 540.08 |
| SC-17 | 62.00 | 40.10 | 27.50 | 14.60 | 9.80 | 2.57 | 738.28 |
| SC-18 | 53.20 | 33.50 | 25.30 | 17.70 | 8.65 | 2.50 | 881.70 |
| SC-19 | 31.90 | 16.40 | 11.20 | 8.50 | 3.40 | 1.50 | >1000 |
| SC-20 | 34.40 | 27.80 | 16.20 | 9.50 | 3.20 | 0.50 | >1000 |
| Ascorbic acid | 95.45 | 90.67 | 88.39 | 82.79 | 79.39 | 75.67 | 12.50 |

**Figure 1: Scavenging of DPPH free radical by selected compounds**




***In-vivo* antioxidant studies (Table 3 and Figure 3)**

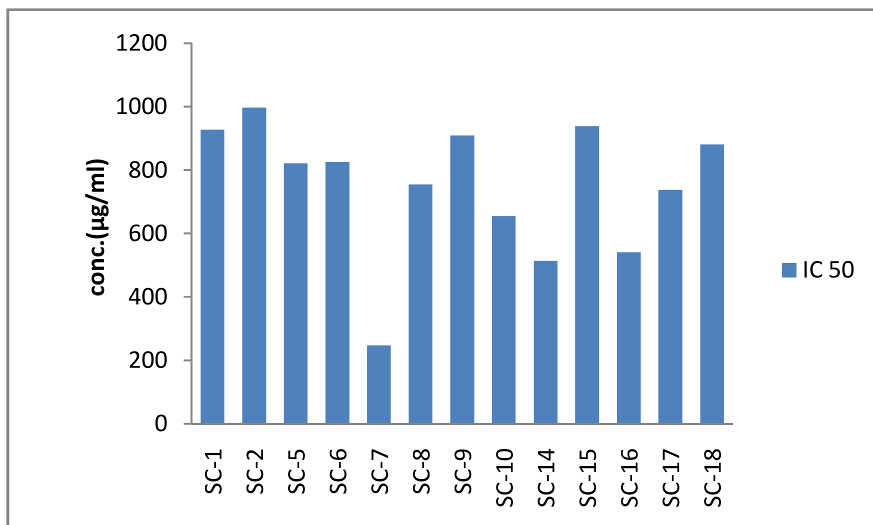
**Total Thiol** [22]: 100µl of liver homogenate was mixed in a 10 ml of centrifuge test tube with 100 µl of the Tris-EDTA buffer followed by addition of 40 µl of 10 mM DTNB [5, 5'-Di thio bis (2-Nitrobenzoic acid)] and 3.16 ml of absolute methanol. The test tubes were capped, and the color was developed for 15-20 mins, followed by centrifugation at 3000 r TMPI for 10 min at ambient temperature. The absorbance of the supernatant was measured at 412 nm (A) DTNB blank without tissue homogenate (B) Tissue blank without DTNB.

**Lipid peroxidation** [23] **(LPO)**: 0.5 ml of liver homogenate was combined with 2.5 ml of TCA-TBA-HCl reagent and mixed thoroughly. The solution was heated for 15 min in a boiling water bath. After cooling, the flocculent precipitate was removed by centrifugation at 1000 rpm for 10 min. The absorbance of the supernatant was measured

at 532 nm against a blank that contains all the reagents minus the liver homogenate. The malondialdehyde concentration of the sample can be calculated using an extinction coefficient of 1.56 x $10^5$ $M^{-1}cm^{-1}$

Malondialdehyde concentration (M)=Absorbance at 532nm /$^{1.56}$ $10^5$

**Figure 2: $IC_{50}$ of selected conpounds**




**Catalase (CAT) activity** [24]: The catalase activity was determined spectrophotometrically according to the protocol of Claiborne (1985). The reaction mixture (3.05 ml) contained 3 ml 10 mM $H_2O_2$ in 60 mM phosphate buffer (pH 7.0). The reaction was started by adding 0.05 ml supernatant and the absorbance was followed for 1 min at 240 nm. Phosphate buffer (60 mM, pH 7.0) was used as a reference. The extinction coefficient of 0.04 $mM^{-1}$ $cm^{-1}$ was used to determine the specific activity of catalase. A unit of catalase is defined as the quantity, which decomposes 1.0 µmole of $H_2O_2$ per min at pH=7.0 at 25 $^0$C, while this $H_2O_2$ concentration falls from 10.3 to 9.2 mM.

**Superoxide dismutase (SOD) activity assay** [25]: The entire supernatant 1.85 ml was taken in 0.1 M carbonate buffer (pH 10.2). After addition of 0.1ml Adrenaline (Bitartarate), the increase in absorbance was measured at 480 nm using a UV–Visible double beam spectrophotometer. The activity of the enzyme has been expressed as U/mg protein, where 1U of the enzyme is defined as the amount of enzyme required to inhibit the rate of epinephrine auto-oxidation by 50% under the conditions of the assay.

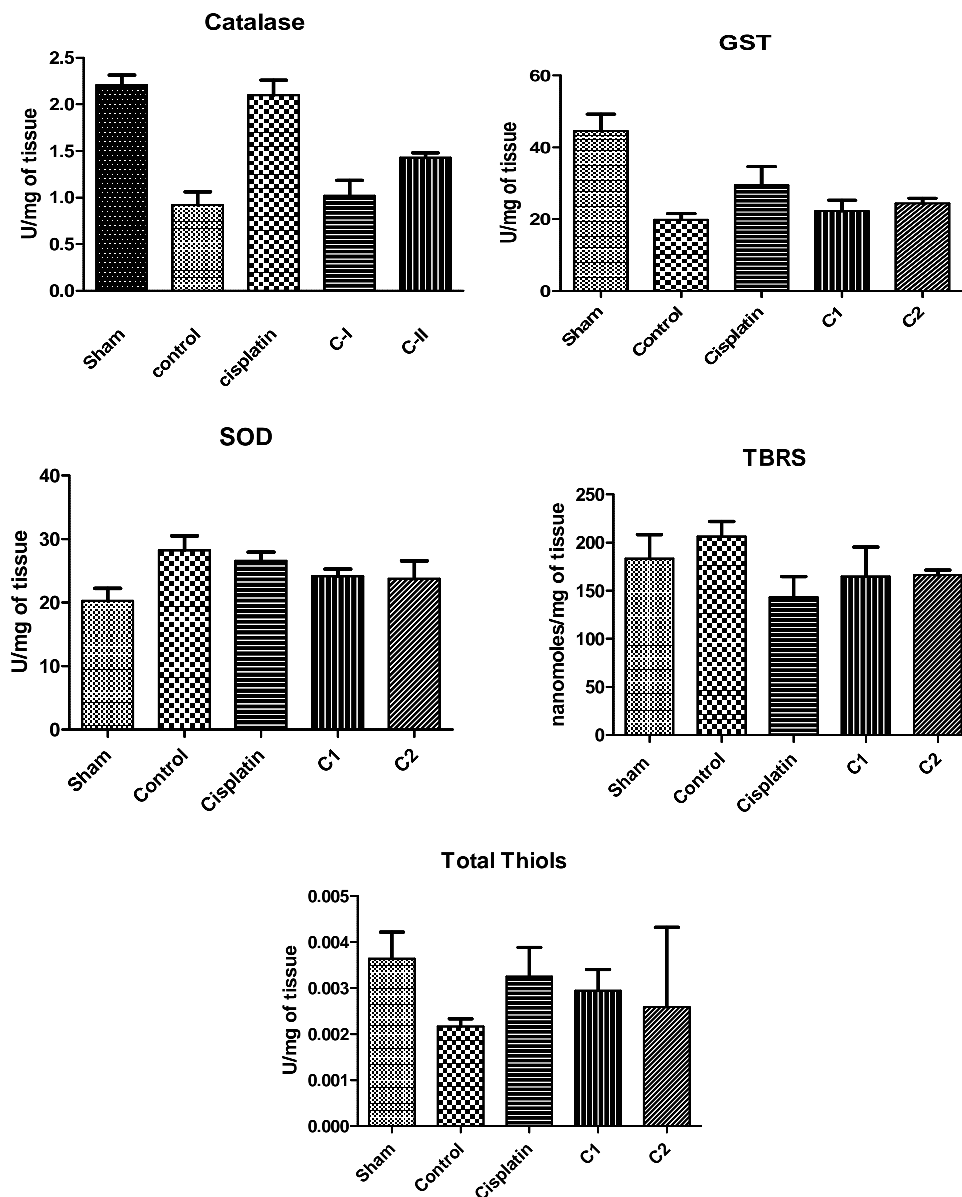
**Glutathione-S-Transferase** [26] **(GST)**: 0.05 ml CDNB (1-chloro-2, 4, dinitrobenzene) was added to 0.1 ml supernatant of liver homogenate and 1.75 ml phosphate buffer pH 7. Incubated at 37°C for 5 minutes and added 0.1 ml 20mM GSH. Absorbance was recorded at 340 nm at intervals of 1 minute. Blank was carried was out in the same manner without homogenate. 1U GST= Activity of enzyme catalyzing the formation of 1µmole of GST-CDNB conjugate per min under the specific assay conditions.

**Table 3 Effect of SC-7 on liver antioxidants enzymes in EAC inoculated mice**

| Group | Catalase (U/mg of tissue) | GST (U/mg of tissue) | SOD (U/mg of tissue) | Total thiol (U/mg of tissue) | LPO (nm/mg of tissue) |
|---|---|---|---|---|---|
| **Sham** | 2.21±0.18 | 19.83±2.99 | 20.28±3.41 | 0.003±0.0001 | 183.2±43.5 |
| **Control** | 0.92±0.25 | 44.58±8.11 | 28.21±4.00 | 0.002±0.0002 | 206.3±27.2 |
| **Cisplatin** | 2.10±0.28 | 29.46±8.95 | 26.59±2.32 | 0.003±0.001 | 143.1±37.6 |
| **C-I** | 1.02±0.29 | 22.25±5.24 | 24.19±1.89 | 0.002±0.0008 | 164.9±52.7 |
| **C-II** | 1.43±0.09 | 24.37±2.54 | 23.80±4.81 | 0.002±0.003 | 166.5±8.4 |

**All the values are mean ± SEM of three samples.*

**Figure 3: Effect of SC-7 on liver antioxidants enzymes in EAC inoculated mice**




## RESULTS AND DISCUSSION

Several concentrations ranging from 31.25-1000µg/ml of the synthesized compounds were tested for their antioxidant activity by using DPPH scavenging method. Ascorbic acid was used as the standard. The antioxidant activity was estimated by $IC_{50}$ value and the values are shown in the table 2. Compounds such as SC-1, SC-2, SC-5 to SC-10 and SC-14 to SC-18 have $IC_{50}$ values ranging from 246-1000 µg/ml. The $IC_{50}$ of all other synthesized compounds were above 1000µg/ml. The result showed that antioxidant activities of none of the synthesized compounds were comparable with that of $IC_{50}$ of ascorbic acid (12.5µg/ml).

The level of lipid peroxidation was increased in EAC control group as compared to normal group as shown in table 3. After administration of different doses of SC-7 in EAC treated mice the lipid peroxidation decreased as compared to control. Tumor inoculation of EAC increased GST level in the control as compared to sham. CAT level was reduced in control but it was increased in the treated group in a dose dependent manner. Both the doses increase the catalase level.

## CONCLUSION

Since oxidative/electrophilic stress is generally perceived as one of the major cause for the accumulation of mutations in the genome, antioxidants are believed to provide protection against cancer. As SC-7 showed good *in-vitro* antioxidant activity, it must prevent the mutation and exhibit good anticancer activity. Excessive production of free radicals resulted in oxidative stress, which leads to damage of macromolecules such as lipids that can induce lipid peroxidation *in-vivo*. MDA (malondialdehyde) the end product of lipid peroxidation was reported to be higher in carcinomatous tissue than in non-diseased organs. SC-7 reduced the elevated levels of lipid peroxidation and increased the GST contents in EAC induced mice. The administration of SC-7 increased the CAT level.

**Acknowledgements**
We are thankful to Head, Dept. of Biotechnology, Manipal Life Science Centre, Manipal as well as Head, Dept. of Biotechnology, MCOPS, Manipal for helping and providing materials in performing *in-vitro* and *in-vivo* antioxidant activity, we are also thankful to IISc, Bangalore for providing us with the NMR and Mass spectra for our compounds in time.

## REFERENCES

[1] H.E. Miller, F. Rigelhof, L. Marquart, A. Prakash and M. Kanter, *Cereal Foods World*. **2000**, 45(2), 59-63.
[2] Florence Delmas, Carole Di Giorgio, Maxime Robin, Nadine Azas, Monique Gasquet, Claire Detang, Muriel Costa, Pierre Timon-David and Jean-Pierre, *Antimicrobial Agents and Chemotherapy*. **2002**, 8, 2588–2594.
[3] M. Lacova, J. Chovancova, O. Hyblova and S. Varkonda, *Chem. Pap*. **1991**, 45, 411.
[4] I. Chulak, V. Sutorius and V. Sekerka, *Chem. Pap*. **1990**, 44, 131.
[5] T. Papenfuhs, *Ger. Offen. De*. **1987**, 3, 528.
[6] T.D. Bradshaw, M.C. Bibby, J.A. Double, I. Fichtner, P.A. Cooper, M.C. Alley, S. Donohue, S.F. Stinson, J.E. Tomaszewjski, E.A. Sausville and M.F.G. Stevens, *Mol. Cancer. Therapeutics*. **2002**, 1, 239.
[7] M.A. El-Sherbeny, *Arzeneim-Forsch.,* **2000**, 50, 843.
[8] L. Racane, V. Tralic-Kulenovic, L. Fiser-Jakic, D.W. Boykin, and G. Karminski-Zamola, *Heterocycles*. **2001**, 55, 2085.
[9] Mahmood-ul-Hasan, Z.H. Chohan and C.T. Supuran, *Main Group Met.Chem*. **2002**, 25, 291.
[10] Shashwat Malhotra, Gaurav Shakya, Ajit Kumar, W. Barbara, Vanhoecke, L. Ashok, Cholli, Hanumantharao G Raj, Luciano Saso, Balaram Ghosh, Marc E Bracke, Ashok K Prasad, Shyam Biswal and Virinder S Parmar, *ARKIVOC*. **2008**, 119-139.
[11] Lacy, Aoife O Kennedy and Richard, *Applied Biochemistry Group, Current Pharmaceutical Design.* **2004**, 10(30), 3797-3811.
[12] Edmont V Stoyanov and Jochen Mezger, *Molecules*. **2005**, 10, 762–766.
[13] Vogel's Textbook of Practical Organic Chemistry. 5, 1193.
[14] Suvarna G. Kini, Shivani Choudhary and Muhammad Mubeen, *Journal of Computational Methods in Molecular Design*. **2012,** 2 (1), 51-60.
[15] Alok kumar Mitra, Aparna De and Nilay Karchaudhuri, *Indian Journal of Chem.* **2000**, 39-B, 387-389.
[16] C.H. Rao, Prakash and Prashant, *Indian Journal of Heterocyclic Chem*. **1995**, 5(1), 23-28.
[17] Shubhangi S Soman, *Indian Journal of Chem.*, **1999**, 38(B), 542-544.
[18] S.R. Ghanwal and S.D. Samant, *Indian Journal of Chem.* **1999**, 38(B), 1242-1247.
[19] S.D Joshi and R.N. Usgaonkar, *Indian Journal of Chem.* **1982**, 21(B), 399-402.
[20] Kenny, S. Rajesh Mashelkar and C.J. Uday, *Heterocyclic Chem.* **2006**, 43(5), 1367-1369.
[21] R. Wang, L. Lai and S. Wang, *Journal of Computer Aided Drug Molecular Des*ign. **2002**, 16, 11-26.
[22] J. Sedlak and R.H. Lindsey, *Anal. Biochemistry*. **1968**, 25, 192-205.
[23] W.G. Niehaus and B. Samuelsson, *European Journal of Biochem.* **1968**, 6, 126-130.
[24]R.A. Greenwald, *Handbook of Methods for Oxygen Free Radical Research*. CRC Press, Boca Raton, **1985**.
[25] H.P. Mishra and I. Fridovich, *Ochem. Biophysics.* **1977**, 183, 511-513.
[26] W.H. Habig, M.J. Pabst and W.B. Takoby, *Journal of Biochem*. **1974**, 299, 7130-7139.