WALAILAK JOURNAL
http://wjst.wu.ac.th Agriculture Technology and Biological Sciences

# Discovery of Substituted Hydroxylphenyl Pyrimidine-2(1H) Thione as a New Series of Antioxidant using AAU and AAI Method

**Rupesh DUDHE[1, 2,*], Pramod Kumar SHARMA[2] and Prabhakar Kumar VERMA[3]**

[1]*Uttarakhand Technical University, Uttarakhand, Dehradun 284007, India*
[2]*Department of Pharmacy, Galgotias University, Uttar Pradesh 201306, India*
[3]*Maharshi Dayanand University, Haryana 124001, India*

**([*]Corresponding author's e-mail: rdudhe121@rediffmail.com)**

*Received: 8 September 2012, Revised: 1 April 2014, Accepted: 9 May 2014*

**Abstract**

A series of substituted pyrimidine has been discovered as a new class of potent antioxidant. 6-(Substituted aldehyde)- 3,4-dihydro-1-(tetrahydro-3,4-dihydroxy-5-hydroxymethyl)furan-2-yl)-4-(4-hydroxyphenyl)pyrimidine-2(1H)-thione derivative (XVII-XXIV) was synthesized from the 6-(Substituted aldehyde) -4-phenylpyrimidine-2(1H)-thione derivative (IX-XVI) through the reaction mechanisms of Claisen-Schmidt, Cyclization and Satos fusion. The structures of the synthesized compounds were elucidated by I.R., $^1$H-NMR, elemental analysis and mass spectroscopic techniques. The synthesized compounds were screened for *in vitro* antioxidant activity using the DPPH assay, based on the AAI and AAU, using a combination relation between DPPH concentration and absorbance. The antioxidant strength of compounds was compared against ascorbic acid. Among them, compounds XVII, XX, XIX and XXIV exhibited significant antioxidant activity, as seen by using the free radical scavenging (DPPH) method.

**Keywords:** Pyrimidine, antioxidant, DPPH, AAU

## Introduction

Free radicals are well known for playing a dual role in our body, deleterious as well as beneficial. They uses the metabolic pathways for their generation [1]. The literature show mainly the damage caused by free radicals in a biological system. Oxidative stress in our body occurs due to the excessive generation of free radicals and reduced levels of antioxidants but, at low concentrations, these radicals perform normal physiological functions of the body. Scientific evidence suggests that antioxidants reduce the risk of chronic diseases, including cancers and heart disease [2].

Free radicals may be defined as the atoms, molecules or ions with unpaired electrons in an open shell configuration. Sometimes free radicals may contain a positive, negative or zero charge [3]. Free radicals play an important role in combustion, atmospheric chemistry, polymerization, plasma chemistry, and many other chemical processes [4]. The large generation of free radicals, particularly reactive oxygen species, and their high activity plays an important role in the progression of a great number of pathological disturbances, such as inflammation [5], atherosclerosis [6], cancer [7], parkinsonism [8], and Alzheimer's disease [9].

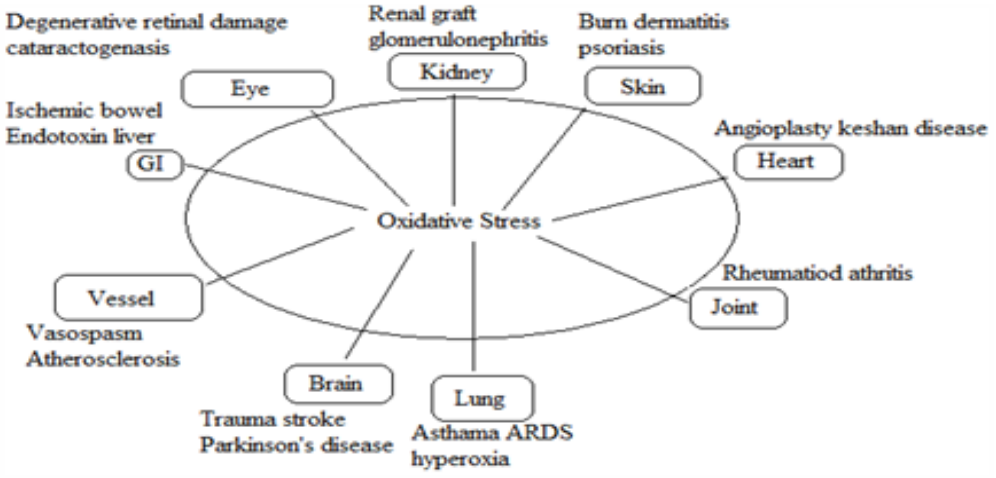



**Figure 1** Effect of oxidative stress on different parts of Body.

Inflammation is mostly caused through excessive generation of free radicals in the body. Pyrimidine is a 6 member heterocyclic compound that contains 2 nitrogen atoms at the 1st and 3rd positions. Pyrimidine, being an integral part of DNA and RNA, has diverse pharmacological properties, such as an effective bactericide and a fungicide [10], Nitrogen containing heterocyclic rings, such as pyrimidine, are a promising structural moiety for drug design. Pyrimidine derivatives form a component in a number of useful drugs, and are associated with many biological and therapeutic activities [11]. Condensed furanose pyrimidine derivatives have been reported as being antioxidant [12], anti-microbial [13], analgesic [14], anti-viral [15], anti-inflammatory [16], anti-HIV [17] anti-tubercular [18], anti-tumor [19], anti-neoplastic [20], and anti-malarial [21]. The condensed furanose pyrimidine derivative has the power to accept free radicals during the different above-mentioned diseases due to the presence of NH and OH molecules in the ring.

## Materials and methods

### Experimental

#### Chemistry

All the reagents and solvents used were of laboratory grade, and obtained from the supplier (Sigma-Aldrich, CDH and Rankem) or recrystallized/ redistilled as necessary. Monitoring of the purity of the compounds synthesized, the commercial reagents used, of the reaction was done by use of thin layer chromatography (TLC) plates (Silica gel G). Two solvent systems: Toluene: Ethyl acetate: Formic acid (5:4:1) and Ethyl acetate: n-Hexane (3:7) were used to run TLC. The spots were located under iodine vapors and UV light. The melting points of the products were determined by the open capillaries method and were uncorrected. IR spectra (KBr) were recorded on an FTIR spectrophotometer (Shimadzu FTIR 8400s, 4000 - 400 $cm^{-1}$). The elemental analysis was carried out using a Heraus CHN rapid analyzer. 1H-NMR spectra were recorded on a JEOL AL300 FTNMR 300 MHz spectrometer in DMSO/$CDCl_3$ using TMS as an internal standard, with $^1H$ resonance frequency of 300 MHz; chemical shift values were expressed in δ ppm. The activity was performed using a UV Visible Spectrophotometer UV-1700 Pharmaspec Shimadzu at M.I.E.T., Meerut, India.

### General procedures for the synthesis of compounds

### Synthesis of compounds (I-VIII)

Equimolar portions of the appropriately substituted aromatic aldehyde (10 mmol) and acetophenone (10 mmol) were dissolved in approximately in 15 ml of ethanol. The mixture was stirred for several

minutes at 5 - 10 °C. Then 10 ml of 40 % aq. NaOH solution was added drop wise to the reaction mixture in the conical flask. The reaction mixture was then stirred at room temperature for 4 h using a stirrer and the precipitate allowed to stand overnight in a refrigerator. Precipitate was formed, which was collected by filtration and repeatedly washed with distilled water and finally recrystallized in ethanol. The solvent system was used for the TLC Ethyl acetate: n-Hexane (3:7).

**Synthesis of 3-(2-fluorophenyl)-1-(2-hydroxyphenyl) prop-2-en-1-one** (I): Obtained by reaction of compound (2) with 2-fluorobenzaldehyde. Molecular formula: $C_{15}H_{10}FO_2$; Molecular weight: 242; m. p.: 56 - 58 °C; $R_f$. value (Ethyl acetate: n-Hexane; 4: 6): 0.94; IR (KBr, $cm^{-1}$): 3446.56 (OH str), 2925.81(C-H Str.), 1608.52 (Ar C=C str), 1384.79 (Ar C-O-H bend), 1265.22 (C-F), 754.88 (Ar C-H bend).

**Synthesis of 3-(4-fluorophenyl)-1-(2-hydroxyphenyl)prop-2-en-1-one** (II): Obtained by reaction of compound (2) with 4-fluorobenzaldehyde. Molecular formula: $C_{15}H_{11}FO_2$; Molecular weight: 242; m. p.: 46 - 48 °C; $R_f$. value (Ethyl acetate: n-Hexane; 4: 6): 0.96; IR (KBr, $cm^{-1}$): 3415.70 (OH str.), 2939.31(C-H Str.), 1602.74 (Ar. C=C), 1348.15 (Ar. C-O), 1108.99 (C-F), 752.19 (Ar. C-H bend).

**Synthesis of 3-(3-chlorophenyl)-1-(2-hydroxyphenyl)prop-2-en-1-one** (III): Obtained by reaction of compound (2) with 3-chlorobenzaldehyde. Molecular formula: $C_{15}H_{11}ClO_2$; Molecular weight: 258.7; m. p.: 76 - 78 °C; $R_f$. value (Ethyl acetate: n-Hexane; 4: 6): 0.78; IR (KBr, $cm^{-1}$): 3442.70 (OH str), 2937.38(C-H Str.), 1610.45 (Ar C=C str), 1384.79 (Ar C-O), 812.23 (Ar C-H bend), 754.12 (C-Cl).

**Synthesis of 3-(4-chlorophenyl)-1-(2-hydroxyphenyl)prop-2-en-1-one** (IV): Obtained by reaction of compound (2) with 4-chlorobenzaldehyde. Molecular formula: $C_{15}H_{11}ClO_2$; Molecular weight: 258.7; m. p.: 94 - 96 °C; $R_f$. value (Ethyl acetate: n-Hexane; 4: 6): 0.82; IR (KBr, $cm^{-1}$): 3442.70 (OH str), 2925.81(C-H Str.), 1670.24 (Ar C=C Str), 1384.79 (Ar C-O), 829.33(Ar C-H bend), 757.97 (C-Cl).

**Synthesis of 3-(2,4-dichlorophenyl)-1-(2-hydroxyphenyl) prop-2-en-1-one** (V): Obtained by reaction of compound (2) with 2,4-dichlorobenzaldehyde. Molecular formula: $C_{15}H_{10}Cl_2O_2$; Molecular weight: 293; m. p.: 58 - 60 °C; $R_f$. value (Ethyl acetate: n-Hexane; 3: 7): 0.92; IR (KBr, $cm^{-1}$): 3425.34 (OH str), 2910.38(C-H Str.), 1610.45 (Ar C=C str), 1352.01 (Ar C-O), 829.33 (Ar C-H bend), 754.12 (C-Cl).

**Synthesis of 3-(2,6-dichlorophenyl)-1-(2-hydroxyphenyl)prop-2-en-1-one** (VI): Obtained by reaction of compound (2) with 2,6-dichlorobenzaldehyde. Molecular formula: $C_{15}H_{10}Cl_2O_2$; Molecular weight: 293; m. p.: 58 - 60 °C; $R_f$. value (Ethyl acetate: n-Hexane; 3: 7): 0.89; IR (KBr, $cm^{-1}$): 3456.20 (OH str), 2925.81(C-H Str.) 1637.45 (Ar C=C str), 1346.22 (Ar C-O), 750.26 (C-Cl), 636.95 (Ar C-H bend).

**Synthesis of 1,3-bis(2-hydroxyphenyl)prop-2-en-1-one** (VII): Obtained by reaction of compound (2) with 2-hydroxybenzaldehyde. Molecular formula: $C_{15}H_{12}O_3$; Molecular weight: 240.25; m. p.: 75 - 76 °C; $R_f$. value (Ethyl acetate: n-Hexane; 3: 7): 0.81; IR (KBr, $cm^{-1}$): 3456.20 (OH str), 2925.81(C-H Str.) 1637.45 (Ar C=C str), 1346.22 (Ar C-O), 636.95 (Ar C-H bend).

**Synthesis of 1-(2-hydroxyphenyl)-3-(3,4,5-trimethoxyphenyl)prop-2-en-1-one** (VIII): Obtained by reaction of compound (2) with 3,4,5 trimethoxy benzaldehyde. Molecular formula: $C_{18}H_{18}NO_5$; Molecular weight: 314.33; m. p.: 88 - 89 °C; $R_f$. value (Ethyl acetate: n-Hexane; 3: 7): 0.72; IR (KBr, $cm^{-1}$): 3398.34 (OH str), 2918.10(C-H Str.), 1685.67 (Ar C=C str), 1384.79 (Ar C-O), 765.69 (Ar C-H bend).

**Synthesis of compounds (IX-XVI): General procedure**: A mixture of a compound, i.e. substituted chalcone (0.01 M) ($R_1$-$R_8$), was added to 0.01 M of NaOH and thiourea (0.01 M) and was refluxed in ethanol for 8 - 10 h. The content was concentrated and poured into cold water. The product so obtained was washed with water repeatedly and recrystallized in ethanol.

**Synthesis of 4-(2-fluorophenyl)-3,4-dihydro-6-(2-hydroxyphenyl)pyrimidine-2(1H)-thione** (IX): Obtained by reaction of compound 3-(2-fluorophenyl)-1-(2-hydroxyphenyl) prop-2-en-1-one (I) (0.01M), added to 0.01 M of NaOH and thiourea (0.01 M) and refluxed in ethanol for 8 - 10 h after completion of the reaction. The content was concentrated and poured into cold water. The product so obtained was washed with water repeatedly and recrystallized in ethanol. Molecular formula: $C_{16}H_{13}FN_2OS$; Molecular weight: 300.35; $R_f$. value: 0.78, (Toluene: Ethyl acetate: Formic acid:-5:4:1); IR (KBr) $cm^{-1}$: 3452.34 (OH str), 3242.41(N-H), 2925.81(Ar C-H), 1629.74(C=O), 1541.02(C=C), 1384.79(C=S str.), 829.33(C-H Bending).

**Synthesis of 4-(4-fluorophenyl)-3,4-dihydro-6-(2-hydroxyphenyl)pyrimidine-2(1H)-thione** (X): Obtained by reaction of compound 3-(2-fluorophenyl)-1-(2-hydroxyphenyl) prop-2-en-1-one (II) (0.01 M), added to 0.01 M of NaOH and thiourea (0.01 M) and refluxed in ethanol for 8 - 10 h after completion of the reaction. The content was concentrated and poured into cold water. The product so obtained was washed with water repeatedly and recrystallized in ethanol. Molecular formula: $C_{16}H_{13}FN_2OS$; Molecular weight: 300.35; $R_f$. value:0.61 (Toluene: Ethyl acetate: Formic acid:-5:4:1): I.R. (KBr) $cm^{-1}$: 3438.87 (OH str), 3212.98(N-H), 2918.10(Ar C-H), 1645.98(C=O), 1556.07(C=C), 1363.58(C=S str.), 835.12(C-H bend.).

**Synthesis of 4-(3-chlorophenyl)-3,4-dihydro-6-(2-hydroxyphenyl)pyrimidine-2(1H)-thione** (XI): Obtained by reaction of compound 3-(3-chlorophenyl)-1-(2-hydroxyphenyl)prop-2-en-1-one (III) (0.01 M), added to 0.01 M of NaOH and thiourea (0.01 M) and refluxed in ethanol for 8 - 10 h after completion of the reaction. The content was concentrated and poured into cold water. The product so obtained was washed with water repeatedly and recrystallized in ethanol. Molecular formula: $C_{16}H_{13}ClN_2OS$; Molecular weight: 316.81; $R_f$. value: 0.78 (Toluene: Ethyl acetate: Formic acid: 5:4:1); I.R. $cm^{-1}$: 3406.05 (OH str), 3215.11(N-H), 2918.10(Ar C-H), 1635.52(C=O), 1558.38(C=C), 1380.94(C=S str.), 854.41(C-H Bending).

**Synthesis of 4-(4-chlorophenyl)-6-(2-hydroxyphenyl) pyrimidine-2(1H)-thione** (XII): Obtained by reaction of compound 3-(4-chlorophenyl)-1-(2-hydroxyphenyl)prop-2-en-1-one (IV) (0.01 M), added to 0.01 M of NaOH and thiourea (0.01 M) and refluxed in ethanol for 8 - 10 h after completion of the reaction. The content was concentrated and poured into cold water. The product so obtained was washed with water repeatedly and recrystallized in ethanol. Molecular formula: $C_{16}H_{11}ClN_2OS$; Molecular weight: 314.79; m. p: 110-1120C.; Rf. value:0.78 (Toluene: Ethyl acetate: Formic acid:5:4:1); I.R. (KBr) $cm^{-1}$: 3448.49 (OH str), 3242.12(N-H), 2954.10(Ar C-H), 1668.31(C=O), 1585.38(C=C), 1384.79(C=S str.), 857.41(C-H Bending).

**Synthesis of 4-(2,4-dichlorophenyl)-3,4-dihydro-6-(2-hydroxyphenyl)pyrimidine-2(1H)-thione** (XIII): Obtained by reaction of compound 3-(2,4-dichlorophenyl)-1-(2-hydroxyphenyl) prop-2-en-1-one (V) (0.01 M), added to 0.01 M of NaOH and thiourea (0.01 M) and refluxed in ethanol for 8 - 10 h after completion of the reaction. The content was concentrated and poured into cold water. The product so obtained was washed with water repeatedly and recrystallized in ethanol. Molecular formula: $C_{16}H_{12}Cl_2N_2OS$; Molecular weight: 351.25; m. p:95 °C; Rf. value:0.87 (Toluene: Ethyl acetate: Formic acid: 5:4:1); IR (KBr) $cm^{-1}$: 3444.63 (OH str), 3238.26(N-H), 2916.17(Ar C-H), 1635.52(C=O), 1587.31(C=C), 1382.87(C=S str.), 825.84(C-H bend.).

**Synthesis of 4-(2,6-dichlorophenyl)-3,4-dihydro-6-(2-hydroxyphenyl)pyrimidine-2(1H)-thione** (XIV): Obtained by reaction of compound 3-(2,6-dichlorophenyl)-1-(2-hydroxyphenyl)prop-2-en-1-one (VI) (0.01 M), added to 0.01 M of NaOH and thiourea (0.01 M) and refluxed in ethanol for 8 - 10 h after completion of the reaction. The content was concentrated and poured into cold water. The product so obtained was washed with water repeatedly and recrystallized in ethanol. Molecular formula: $C_{16}H_{12}Cl_2N_2OS$; Molecular weight: 351.25; m. p: 120 - 122 °C.; Rf. value: 0.86, (Toluene: Ethyl acetate:

Formic acid:-5:4:1): 0.78; IR (KBr) cm-1: 3445.59 (OH str), 3234.69(N-H), 2983.40(Ar C-H), 1635.52(C=O), 1575.70(C=C), 1384.70(C=S str.), 838.04(C-H bend.), 759.60(C-Cl).

**Synthesis of 3,4-dihydro-4,6-bis(2-hydroxyphenyl)pyrimidine-2(1H)-thione** (XV): Obtained by reaction of compound 1,3-bis(2-hydroxyphenyl)prop-2-en-1-one (VII) (0.01 M), added to 0.01 M of NaOH and thiourea (0.01 M) and refluxed in ethanol for 8 - 10 h after completion of the reaction. The content was concentrated and poured into cold water. The product so obtained was washed with water repeatedly and recrystallized in ethanol. Molecular formula: $C_{16}H_{13}N_3O_2S$; Molecular weight: 298.36; m. p: 141 - 142 °C.; $R_f$. value: 0.89 (Toluene: Ethyl acetate: Formic acid: 5:4:1), I.R. (KBr) $cm^{-1}$: 3240.19(N-H), 3039.60(Ar C-H), 1517.87(C=C), 1384.79(C=S str.), 831.26(C-H bend.).

**Synthesis of 3,4-dihydro-6-(2-hydroxyphenyl)-4-(3,4,5-trimethoxyphenyl)pyrimidine-2(1H)-thione** (XVI): Obtained by reaction of compound 1-(2-hydroxyphenyl)-3-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (VIII) (0.01 M), added to 0.01 M of NaOH and thiourea (0.01 M) and refluxed in ethanol for 8 - 10 h after completion of the reaction. The content was concentrated and poured into cold water. The product so obtained was washed with water repeatedly and recrystallized in ethanol. Molecular formula: $C_{19}H_{20}N_2O_3S$; Molecular weight: 372.44; m. p: 143 - 144 °C.; Rf. value: 0.69(Toluene: Ethyl acetate: Formic acid: 5:4:1); I.R. (KBr) $cm^{-1}$: 3332.76 (N-H), 3062.60 (C-H, Ar), 1593.09 (C=C), 1716.53 (C=S).

**Synthesis of compounds (XVII-XXIV): General procedure**: To a solution of IX-XVI (0.01M) in ethanol, β-D-ribofuranose-1, 2, 3, 5 tetra, o-acetate (0.01 M) was added in the presence of TsOH, and the contents were refluxed under vacuum with stirring at 155 - 160 °C for 15 - 30 min. The vacuum was removed and the reaction mixture was protected from moisture by fitting of a guard tube. Stirring was further continued for 10 h and vacuum was applied for 10 min at every h. The viscous mass thus obtained was dissolved in sodium containing methanol and boiled for 10 min, then left to stir overnight at room temperature. The reaction mixture was filtered and the filtrate was evaporated to dryness. The viscous residue thus obtained was dissolved in ether, filtered, concentrated, and kept in a refrigerator overnight to get a crystalline product.

**Synthesis of 6-(2-fluorophenyl)-3,4-dihydro-1-(tetrahydro-3,4-dihydroxy-5-hydroxymethyl)furan-2-yl)-4-(4-hydroxyphenyl)pyrimidine-2(1H)-thione** (XVII): Obtained from the reaction of 4-(2-fluorophenyl)-3,4-dihydro-6-(2-hydroxyphenyl)pyrimidine-2(1H)-thione(IX) (0.01 M) in ethanol, β-D-ribofuranose-1, 2, 3, 5 tetra, o-acetate (0.01 M) was added in the presence of TsOH, and the contents were refluxed under vacuum with stirring at 155 - 160 °C for 15 - 30 min. The vacuum was removed and the reaction mixture was protected from moisture by fitting of a guard tube. Stirring was further continued for 10 h. and vacuum was applied for 10 min at every h. The viscous mass thus obtained was dissolved in sodium containing methanol and boiled for 10 min, then left to stir overnight at room temperature. The reaction mixture was filtered and the filtrate was evaporated to dryness. The viscous residue thus obtained was dissolved in ether, filtered, concentrated, and kept in a refrigerator overnight to get a crystalline product. Molecular formula: $C_{21}H_{21}FN_2O_5S$; Molecular weight: 432.47; $R_f$. value. (Ethyl acetate: nHexane: 3:7).0.76 ; IR (KBr) $cm^{-1}$: 3358.77(N-H), 3340.48(O-H str), 3066.61(C-H, Ar.), 1571.88(C=C), 1346.22(C=S), 1166.85(C-O-C), 815.83(C-H bend.). $^1$H-NMR ($CDCl_3$-d, δ, ppm): 2.348 (s, 1H, NH), 2.803(s, 3H, OH), 2.868-3.027(m, 5H, CH,$CH_2$), 3.073-3.620(d, 3H, CH), 5.640(d,1H, CH) 7.068-7.332(m, 8H, Ar-CH), 8.061(s, 1H,Ar-OH). m/e: 413.3, 347.1, 321.0, 298.0, 245.0, 189.0 (100 %), 124.1, 105.0. Elemental analysis calculated: C, 58.3s2; H, 4.89; N, 6.48.

**Synthesis of 6-(4-fluorophenyl)-3,4-dihydro-1-(tetrahydro-3,4-dihydroxy-5-(hydroxymethyl)furan-2-yl)-4-(4-hydroxyphenyl)pyrimidine-2(1H)-thione** (XVIII): Obtained from the reaction of 4-(4-fluorophenyl)-3,4-dihydro-6-(2-hydroxyphenyl)pyrimidine-2(1H)-thione (X) (0.01 M) in ethanol, β-D-ribofuranose-1, 2, 3, 5 tetra, o-acetate (0.01 M) was added in the presence of TsOH, and the contents were refluxed under vacuum with stirring at 155 - 160 °C for 15 - 30 min. The vacuum was removed and the reaction mixture was protected from moisture by fitting of a guard tube. Stirring was further continued for

10 h. and vacuum was applied for 10 min at every h. The viscous mass thus obtained was dissolved in sodium containing methanol and boiled for 10 min, then left to stir overnight at room temperature. The reaction mixture was filtered and the filtrate was evaporated to dryness. The viscous residue thus obtained was dissolved in ether, filtered, concentrated, and kept in a refrigerator overnight to get a crystalline product. Molecular formula: $C_{21}H_{21}FN_2O_5S$; Molecular weight: 432.47; m. p.103 - 105 °C: $R_f$. value. (Ethyl acetate: n Hexane:-3:7) 0.81. IR (KBr $cm^{-1}$): 3446.56(O-H str), 3377.12(N-H), 3066.61(C-H, Ar.), 1569.95(C=C), 1377.08(C=S), 1134.07(C-O-C), 815.83(C-H bend.).; $^1$H-NMR ($CDCl_3$-d, δ, ppm): 2.209 (s, 1H, NH), 2.503(s, 3H, OH), 3.230-3.259(m, 5H, CH, $CH_2$), 3.338-3.414 (d, 2H, CH), 5.499(d,1H, CH) 7.504-7.834(m, 8H, Ar-CH), 8.317(s, 1H, Ar-OH). m/e: 413.3, 374.0, 352.0(100 %), 298.3, 269.0, 245.0, 187.0, 134.1. Elemental analysis calculated: C, 58.32; H, 4.89; N, 6.48.

**Synthesis of 6-(3-chlorophenyl)-3,4-dihydro-1-(tetrahydro-3,4-dihydroxy-5-(hydroxymethyl)furan-2-yl)-4-(4-hydroxyphenyl)pyrimidine-2(1H)-thione** (XIX ): Obtained from the reaction of 4--(3-chlorophenyl)-3,4-dihydro-6-(2-hydroxyphenyl) pyrimidine-2(1H)-thione(XI) ( 0.01 M) in ethanol, β-D-ribofuranose-1, 2, 3, 5 tetra, o-acetate (0.01 M) was added in the presence of TsOH, and the contents were refluxed under vacuum with stirring at 155 - 160 °C for 15 - 30 min. The vacuum was removed and the reaction mixture was protected from moisture by fitting of a guard tube. Stirring was further continued for 10 h and vacuum was applied for 10 min at every h. The viscous mass thus obtained was dissolved in sodium containing methanol and boiled for 10 min, then left to stir over night at room temperature. The reaction mixture was filtered and the filtrate was evaporated to dryness. The viscous residue thus obtained was dissolved in ether, filtered, concentrated, and kept in a refrigerator overnight to get a crystalline product. Molecular formula: $C_{21}H_{21}FN_2O_5S$; Molecular weight: 448.92; m. p.: 132 - 134 °C; $R_f$. value. (Ethyl acetate: n Hexane:-3:7) 0.89. IR (KBr) $cm^{-1}$:3440.77 (O-H str), 3334.69(N-H), 3066.61(C-H, Ar.), 1566.09(C=C), 1375.15(C=S), 1134.07(C-O-C), 815.83(C-H bend.). $^1$H-NMR ($CDCl_3$-d, δ, ppm): 2.52 (s, 1H, NH), 2.98(s, 3H, OH), 3.27-3.26(m, 5H, CH, $CH_2$), 3.34-3.47 (d, 2H, CH), 5.41(d, 1H, CH), 7.504-7.834(m, 8H, Ar-CH), 8.53(s, 1H, Ar-OH). m/e: 448.11; Elemental analysis calculated: C, 58.32; H, 4.89; N, 6.48;

**Synthesis of 6-(4-chlorophenyl)-3,4-dihydro-1-(tetrahydro-3,4-dihydroxy-5-(hydroxymethyl)furan-2-yl)-4-(4-hydroxyphenyl)pyrimidine-2(1H)-thione** (XX): Obtained from the reaction of 4-(4-chlorophenyl)-6-(2-hydroxyphenyl) pyrimidine-2(1H)-thione (XII)(0.01 M) in ethanol, β-D-ribofuranose-1, 2, 3, 5 tetra, o-acetate (0.01 M) was added in the presence of TsOH, and the contents were refluxed under vacuum with stirring at 155 - 160 °C for 15 - 30 min. The vacuum was removed and the reaction mixture was protected from moisture by fitting of a guard tube. Stirring was further continued for 10 h and vacuum was applied for 10 min at every h. The viscous mass thus obtained was dissolved in sodium containing methanol and boiled for 10 min, then left to stir overnight at room temperature. The reaction mixture was filtered and the filtrate was evaporated to dryness. The viscous residue thus obtained was dissolved in ether, filtered, concentrated, and kept in a refrigerator overnight to get a crystalline product. Molecular formula: $C_{21}H_{21}ClN_2O_5S$; Molecular weight: 448.92; m. p.: 103 - 105 °C; $R_f$. value 0.62. (Toluene: Ethyl acetate: Formic acid: 5:4:1); IR (KBr) $cm^{-1}$: 3444.63 (O-H), 3323.12(N-H, Str), 3060.82(C-H, Ar.), 1589.23(C=C), 1382.87(C=S), 1155.28(C-O-C), 894.91(C-H bend.), 730.97(C-Cl). $^1$H-NMR ($CDCl_3$-d, δ, ppm): 2.51 (s, 1H, NH), 2.97(s, 3H, OH), 3.21-3.33(m, 5H, CH, $CH_2$), 3.39-3.44 (d, 2H, CH), 5.66 (d, 1H, CH), 7.01-7.33(m, 8H, Ar-CH), 8.11(s, 1H, Ar-OH). m/e: 448.09; Elemental analysis calculated: C, 56.18; H, 4.72; Cl, 7.90; N, 6.24.

**Synthesis of 6-(2,4-dichlorophenyl)-3,4-dihydro-1-(tetrahydro-3,4-dihydroxy-5-(hydroxymethyl ) furan-2-yl) -4-(4-hydroxyphenyl)pyrimidine-2(1H)-thione** (XXI): Obtained from the reaction of 4-(2,4-dichlorophenyl)-3,4-dihydro-6-(2-hydroxyphenyl)pyrimidine-2(1H)-thione (0.01 M) in ethanol, β-D-ribofuranose-1, 2, 3, 5 tetra, o-acetate (0.01 M) was added in the presence of TsOH, and the contents were refluxed under vacuum with stirring at 155 - 160 °C for 15 - 30 min. The vacuum was removed and the reaction mixture was protected from moisture by fitting of a guard tube. Stirring was further continued for 10 h and vacuum was applied for 10 min at every h. The viscous mass thus obtained was dissolved in

sodium containing methanol and boiled for 10 min, then left to stir overnight at room temperature. The reaction mixture was filtered and the filtrate was evaporated to dryness. The viscous residue thus obtained was dissolved in ether, filtered, concentrated and kept in a refrigerator overnight to get a crystalline product. Molecular formula: $C_{21}H_{20}Cl_2N_2O_5S$; Molecular weight: 483.36; m. p. 101 - 103 °C; $R_f$. value. (Toluene: Ethyl acetate: Formic acid:-5:4:1)0.78. IR (KBr) $cm^{-1}$: 3452.34(O-H), 3377.12(N-H, str), 3037.68(C-H, Ar.), 1589.23(C=C), 1384.79(C=S), 1153.35(C-O-C), 864.05(C-H bend.), 754.12(C-Cl). $^1$H-NMR ($CDCl_3$-d, δ, ppm): 2.63 (s, 1H, NH), 3.21(s, 3H, OH), 3.33-3.38(m, 5H, CH, $CH_2$), 3.41-3.45 (d, 2H, CH), 5.23-5.24 (d, 1H, CH), 7.11-7.24(m, 7H, Ar-CH), 8.66(s, 1H, Ar-OH). m/e: 483.36; Elemental analysis calculated: C, 52.18; H, 4.17; N, 5.80.

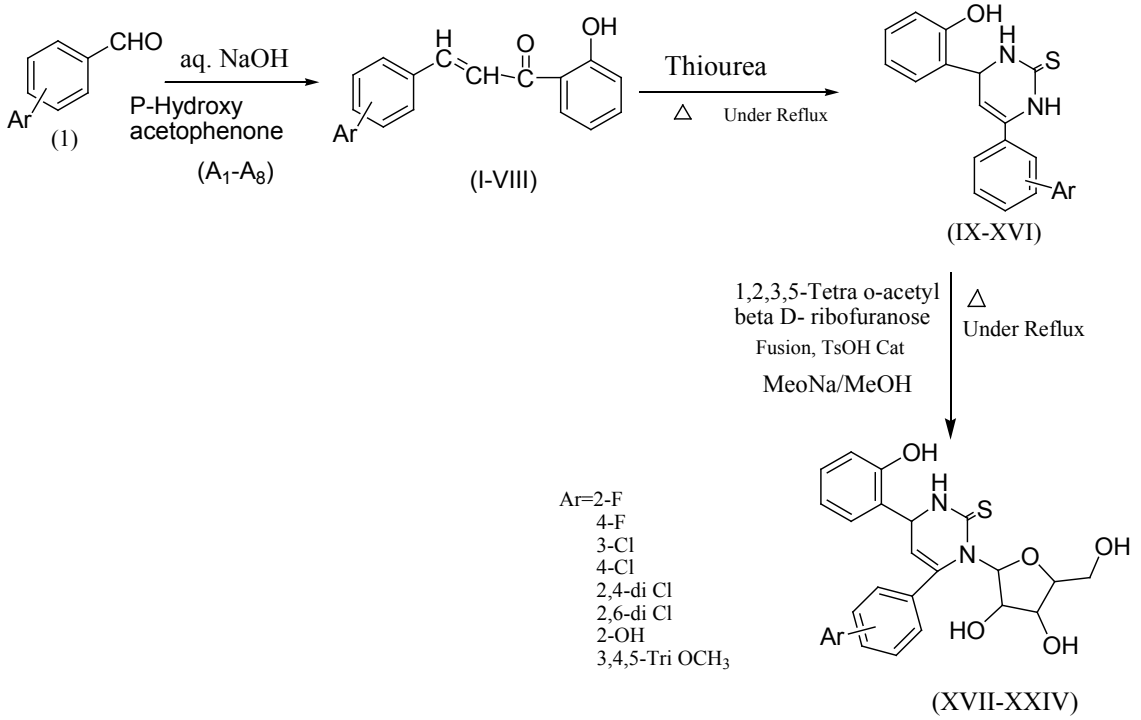
**Synthesis of 6-(2,6-dichlorophenyl)-3,4-dihydro-1-(tetrahydro-3,4-dihydroxy-5-(hydroxymethyl) furan-2-yl)-4-(4-hydroxyphenyl)pyrimidine-2(1H)-thione** (XXII): Obtained from the reaction of 4-(2,6-dichlorophenyl)-3,4-dihydro-6-(2-hydroxyphenyl)pyrimidine-2(1H)-thione (XIV) (0.01 M) in ethanol, β-D-ribofuranose-1, 2, 3, 5 tetra, o-acetate (0.01 M) was added in the presence of TsOH, and the contents were refluxed under vacuum with stirring at 155 - 160 °C for 15 - 30 min. The vacuum was removed and the reaction mixture was protected from moisture by fitting of a guard tube. Stirring was further continued for 10 h and vacuum was applied for 10 min at every h. The viscous mass thus obtained was dissolved in sodium containing methanol and boiled for 10 min, then left to stir overnight at room temperature. The reaction mixture was filtered and the filtrate was evaporated to dryness. The viscous residue thus obtained was dissolved in ether, filtered, concentrated, and kept in a refrigerator overnight to get a crystalline product. Molecular formula: $C_{21}H_{20}Cl_2N_2O_5S$; Molecular weight: 483.36; m. p.: 108 - 110 °C; $R_f$. value.0.75 (Toluene: Ethyl acetate: Formic acid:-5:4:1); IR (KBr) $cm^{-1}$: 3458.13(O-H), 3377.12(N-H, str), 3020.32(C-H, Ar.), 1604.66(C=C), 1384.79 (C=S),1132.14 (C-O-C), 815.83(C-H, bend.), 688.54(C-Cl). $^1$H-NMR ($CDCl_3$-d, δ, ppm): 2.51 (s, 1H, NH), 3.24(s, 3H, OH), 3.35-3.38(m, 5H, CH, $CH_2$), 3.45-3.51 (d, 2H, CH), 5.44-5.54 (d, 1H, CH), 7.06-7.11(m, 7H, Ar-CH), 8.89(s, 1H, Ar-OH). m/e: 482.05: Elemental analysis calculated: C, 52.18; H, 4.17; N, 5.80.

**Synthesis of 3,4-dihydro-1-(tetrahydro-3,4-dihydroxy-5-(hydroxymethyl)furan-2-yl)-6-(2-hydroxy phenyl) -4-(4-hydroxyphenyl)pyrimidine-2(1H)-thione** (XXIII): Obtained from the reaction of 3,4-dihydro-6-(2-hydroxyphenyl)-4-(4-hydroxyphenyl)pyrimidine-2(1H)-thione (XV) (0.01 M) in ethanol, β-D-ribofuranose-1, 2, 3, 5 tetra, o-acetate (0.01 M) was added in the presence of TsOH, and the contents were refluxed under vacuum with stirring at 155 - 160 °C for 15 - 30 min. The vacuum was removed and the reaction mixture was protected from moisture by fitting of a guard tube. Stirring was further continued for 10 h and vacuum was applied for 10 min at every h. The viscous mass thus obtained was dissolved in sodium containing methanol and boiled for 10 min, then left to stir overnight at room temperature. The reaction mixture was filtered and the filtrate was evaporated to dryness. The viscous residue thus obtained was dissolved in ether, filtered, concentrated, and kept in a refrigerator overnight to get a crystalline product. Molecular formula: $C_{21}H_{22}N_2O_6S$; Molecular weight: 430.47; m. p.: 68 - 70 °C; $R_f$. value. (Ethyl acetate: nHexane, 3:7) 0.53. IR (KBr) $cm^{-1}$: 3494.77(O-H str), 2918.10(N-H, str), 2850.59(C-H, Str), 1508.23(C=C), 1382.87 (C=S), 1134.07 (C-O-C), 759.90(C-H, bend.). $^1$H-NMR ($CDCl_3$-d, δ, ppm): 2.21 (s, 1H, NH), 3.43(s, 3H, OH), 3.45-3.51(m, 5H, CH, $CH_2$), 3.62-3.63 (d, 2H, CH), 5.13-5.15 (d, 1H, CH), 6.93-7.21(m, 8H, Ar-CH), 8.89(s, 2H, Ar-OH). m/e: 430.12; Elemental analysis calculated: C, 58.59; H, 5.15; N, 6.51.

**Synthesis of 3,4-dihydro-1-(tetrahydro-3,4-dihydroxy-5-(hydroxymethyl)furan-2-yl)-4-(4-hydroxy phenyl)-6-(3,4,5-trimethoxyphenyl)pyrimidine-2(1H)-thione** (XXIV): Obtained from the reaction of 4-(3,4,5-trimethoxyphenyl)-3,4-dihydro-6-(2-hydroxyphenyl)pyrimidine-2(1H)-thione (XVI) (0.01 M) in ethanol, β-D-ribofuranose-1, 2, 3, 5 tetra, o-acetate (0.01 M) was added in the presence of TsOH and the contents were refluxed under vacuum with stirring at 155 - 160 °C for 15 - 30 min. The vacuum was removed and the reaction mixture was protected from moisture by fitting of a guard tube. Stirring was further continued for 10 h and vacuum was applied for 10 min at every h. The viscous mass thus obtained was dissolved in sodium containing methanol and boiled for 10 min, then left to stir over night at room

temperature. The reaction mixture was filtered and the filtrate was evaporated to dryness. The viscous residue thus obtained was dissolved in ether, filtered, concentrated, and kept in a refrigerator overnight to get a crystalline product. Molecular formula: $C_{24}H_{28}N_2O_8S$; Molecular weight: 504.55; m. p.: 74 - 76 °C; $R_f$. value. (Ethyl acetate: nHexane: 3:7); 0.67 IR (KBr) $cm^{-1}$: 3350.14(O-H str), 2910.54(N-H, str), 2839.20(C-H, Str), 1595.02(C=C), 1363.58 (C=S), 1126.35 (C-O-C), 752.80(C-H, bending). $^1$H-NMR ($CDCl_3$-d, δ, ppm): 2.40(s, 1H, NH), 3.15(s, 3H, OH), 3.73 (s,9H, $OCH_3$), 3.81-3.95(d, 3H, $CH_2$, CH), 3.99-4.55 (m, 4H, CH), 5.28(d, 1H, CH), 6.41-7.259(m, 6H, Ar-CH), 8.32(s,1H, OH).m/e: 504.55($M^+$) Elemental analysis calculated: C, 57.13; H, 5.59; N, 5.55.

**Reaction scheme**




**Scheme 1** Reaction scheme.

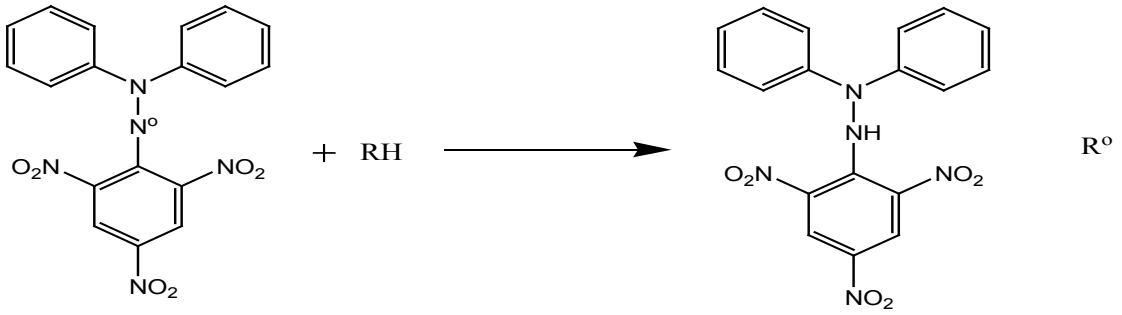
**Pharmacological screening**

**Free radical scavenging method by DPPH assay**

Various concentrations of test compound 10 - 200 µg/ml were prepared in methanol, and 1ml of each concentration was added to 1 ml of 0.1 mM solution of DPPH. The mixture was shaken vigorously and allowed to stand for 30 min in a dark place. Absorbance at 517 nm was determined by UV spectrometer, and the percentage scavenging activity was calculated. A blank solution of DPPH was prepared and ascorbic was used as a reference compound. All the compounds were tested and analyzed by their absorbance. The equation used to measure free radical scavenging was:

A lower value of mean inhibitory concentration shows a higher free radical scavenging activity.

$$\% \text{ scavenging} = \frac{\text{Absorbance of control} - \text{Absorbance of test compound/Std.} \times 100}{\text{Absorbance of control}}$$




**Scheme 2** Reaction of DPPH with compound-having proton.

**Table 1** Percentage inhibition and $IC_{50}$ value of synthesized compounds XVII-XXIV.

| Compounds | % Inhibition at µg/ml | | | | | | $IC_{50}$ value µg/ml |
|---|---|---|---|---|---|---|---|
| | 10 | 25 | 50 | 75 | 100 | 200 | |
| **Standard** | 75.84±2.27 | 79.81±3.47 | 83.79±1.22 | 86.85±1.05 | 88.99±0.8 | 85.41±0.61 | 6.50 |
| XVII | 35.77±2.5 | 49.84±8.31 | 60.85±1.9 | 77.67±7,61 | 81.03±6.91 | 84.09±2.95 | 26.00 |
| XVIII | 33.33±7.42 | 46.18±2.13 | 59.02±2.71 | 59.94±3.72 | 64.53±3.01 | 66.67±2.61 | 41.50 |
| XIX | 16.51±4.23 | 29.35±9.65 | 41.28±2.0 | 56.26±11.31 | 70.33±2.61 | 79.51±3.23 | 70.00 |
| XX | 58.10±2.94 | 62.69±2.51 | 64.53±7.11 | 68.5±4.24 | 68.81±5.43 | 71.25±5.11 | 7.90 |
| XXI | 29.36±4.00 | 47.40±13.6 | 50.76±5.56 | 59.63±4.60 | 62.39±0.81 | 66.36±6.31 | 21.35 |
| XXII | 0.61±0.41 | 4.89±2.81 | 19.27±6.1 | 25.08±4.00 | 31.8±3.75 | 62.39±16.92 | 159.50 |
| XXIII | 0.00±00 | 1.83±1.12 | 23.55±14.1 | 34.56±21.43 | 35.47±13.93 | 37.31±15.32 | 270.00 |
| XXIV | 72.17±1.9 | 77.06±6.24 | 77.68±1.1 | 79.51±1.22 | 84.1±3.36 | 84.71±0.53 | 7.00 |

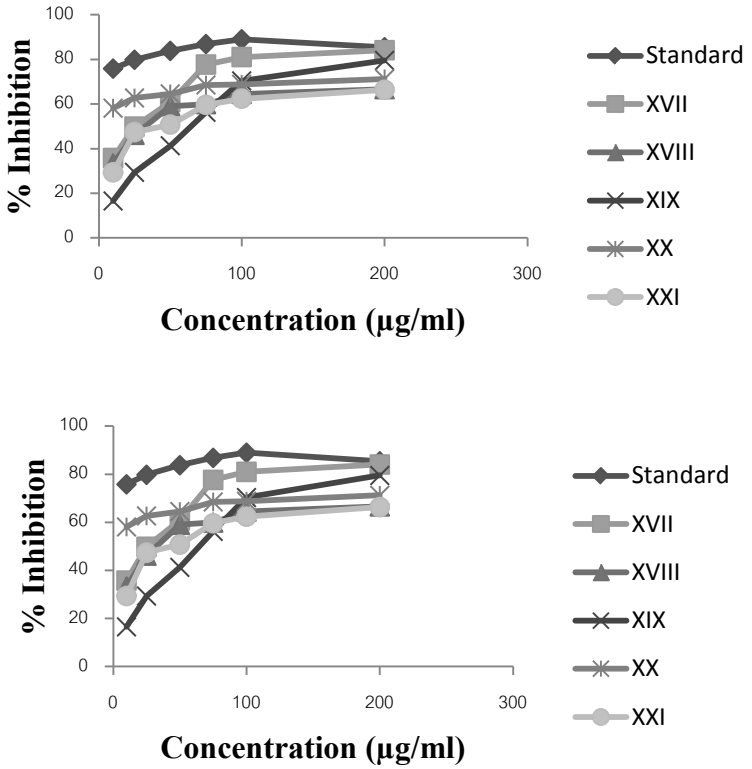






**Figure 2** Percentage inhibition of the synthesized compounds at concentrations of 10 - 200 µg/ml incubated for 30 min with DPPH (0.1 mM) at 517 nm, as compared to standard ascorbic acid.

**AAU equation**

The free radical scavenging fitting curve equation (y = BX + D) when combined with theoretical value of DPPH concentration and absorbance (y = KX) to assume the index antioxidant activity unit (AAU), it is defined as "one mole of DPPH free radical was completely scavenged to consume amount (mole number) of the scavenger". The lower the value of the AAU, the stronger the antioxidant ability of the compound.

where R is the solution volume ratio of the sample to solution volume of DPPH for each sample
B is the slope of the fitting equation of the free radical scavenging ratio
C is the initial concentration of the DPPH solution observed
Mr is the molecular weight of the sample

**Table 2** AAU data of synthesized compound.

| Compound code | Slope | $r^2$ | AAU |
|---|---|---|---|
| XVII | 0.235 | 0.693 | 7.85 |
| XVIII | 0.246 | 0.715 | 9.40 |
| XIX | 0.323 | 0.841 | 6.91 |
| XX | 0.201 | 0.551 | 11.10 |
| XXI | 0.252 | 0.747 | 8.19 |
| XXII | 0.320 | 0.995 | 6.47 |
| XXIII | 0.213 | 0.830 | 10.90 |
| XXIV | 0.231 | 0.528 | 8.59 |

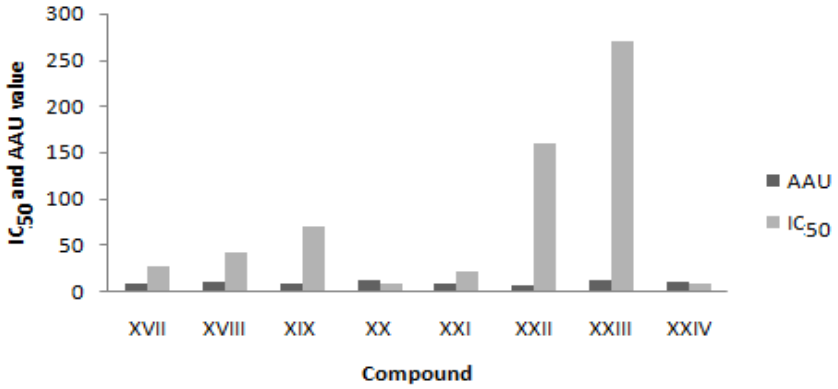



**Figure 3** Comparison of AAU and $IC_{50}$ value of synthesized compounds.

**Results and discussion**

The scavenging effects of the synthesized compounds XVII-XXIV on the DPPH radical was evaluated according to Leong and Shui *et al*. Various concentrations (10, 25, 50, 75, 100 and 200 µg/ml) of the test compounds in methanol were added to a 0.1 mM solution of DPPH radical in methanol. All the tests and analysis were undertaken on 3 replicates and the results averaged. The antioxidant activity of the tested compounds revealed that the reaction with DPPH is a time dependent fashion, and higher concentrations of the tested compounds showed higher radical scavenging activity, as well as % inhibition and AAU. However, compounds XVII, XIX, XX, and XXIV exhibited potent activity, compared by AAU and $IC_{50}$ value. The profiles of the scavenging effect of synthesized compounds are comparable to that of ascorbic acid as a reference compound. Introduction of 2-fluro, 4-chloro, 3-chloro and 3, 4, 5 tri methoxy group showed an almost equivalent antioxidant activity as that of ascorbic acid. Based on the structure activity relationships, it can be concluded that the presence of the halogen group and the methoxy group at the $2^{nd}$, $4^{th}$ and $6^{th}$ positions exhibited potent activity.

## Conclusions

A new series of compounds (XVII-XXIV) i.e. pyrimidine analogues were synthesized by thiourea and characterized. The synthesized compounds were screened for their *in vitro* antioxidant activity by calculating percentage scavenging, $IC_{50}$ and AAU values. On the basis of the above study, we conclude that compounds having chloro, methoxy, and chloro substitution having furanose containing pyrimidine derivative compounds, XX and XXIV, showed the most potent antioxidant activity, comparable to ascorbic acid as a standard antioxidant.

## References

[1] AE Amr, MS Nermien and MM Abdulla. Anti-inflammatory activity of heterocyclic systems using abietic acid as starting material. *Monatsh Chem.* 2007; **138**, 699-708.
[2] FW Bell, AS Cantrell, M Hogberg, SR Jaskunas, NG Johansson and CL Jordon. Phenethylthiazolethiourea (PETT) compounds, a new class of HIV-1 reverse transcriptase inhibitors. Synthesis and basic structure-activity relationship studies of PETT analogs. *J. Med. Chem.* 1995; **38**, 4929-36.
[3] A Chaudhary, PK Sharma, P Verma, N Kumar and R Dudhe. Microwave assisted synthesis of novel pyrimidine derivatives and investigation of their analgesic and ulcerogenic activity. *Med. Chem. Res.* 2012; **21**, 3629-45.
[4] L Ballell, RA Field, GAC Chung and RJ Young. New thiopyrazolo[3,4 d] pyrimidine derivatives as antimycobacterial agents. *Bioorg. Med. Chem. Lett.* 2007; **17**, 1736-45.
[5] JK Gupta, PK Sharma, A Chaudhary, PK Verma and R Dudhe. Analgesic study of novel pyrimidine derivatives linked with coumarin moiety. *Med. Chem. Res.* 2012; **21**, 1625-32.
[6] L Cordeu, E Cubedo, E Bandres, A Rebollo, X Saenz and H Choza. Dominguez V. Biological profile of new apoptotic agents based on 2,4-pyrido[2,3-d]pyrimidine derivatives. *Bio. Med. Chem.* 2007; **15**, 1659-69.
[7] K Desai, R Patel and K Chikhalia. Design, synthesis and antimicrobial study of some pyrimidine derivatives. *J. Ind. Chem*. 2006; **45**, 773-9.
[8] N Ergenc, G Capan, NS Gunay, S Ozkirimli, M Gungor, S Ozbey and E Kendi. Synthesis and hypnotic activity of new 4-thiazolidinone and 2-thioxo- 4,5-imidazolidinedione derivatives. *Arch. Pharm. Med. Chem.* 1999; **332**, 343-47.
[9] N Fujiwara, T Nakajima, Y Ueda, H Fujita and H Kawakami. Novel piperidinylpyrimidine derivatives as inhibitors of HIV-1 LTR activation. *Bio. Med. Chem*. 2008; **16**, 9804-14.
[10] E Gursoy and NU Guzeldemirci. Synthesis and primary cytotoxicity evaluation of new imidazo [2,1-b] thiazole derivatives. *Eur. J. Med. Chem*. 2007; **42**, 320-26.
[11] K Gorlitzer, S Herbig and RD Walter. Parallel synthesis of trisubstituted formamidines: a facile and versatile procedure. *Pharmazie* 1997; **52**, 670-9.
[12] KD Hargrave, FK Hess and JT Oliver. N-(4-substitutedthiazolyl) oxamic acid derivatives, new series of potent, orally active antiallergy agents. *J. Med. Chem.* 1983; **26**, 1158-63.
[13] JC Jaen, LD Wise, BW Caprathe, H Tecle, S Bergmeier and CC Humblet. 4-(1, 2, 5, 6-tetrahydro-1-alkyl-3-pyridinyl)-2-thiazolamines: A novel class of compounds with central dopamine agonist properties. *J. Med. Chem.* 1990; **33**, 311-7.
[14] JK Gupta, PK Sharma, R Dudhe, A Chaudhary, SC Mondal and PK Verma. Synthesis and analgesic activity of novel Pyrimidine derivatives of Coumarin moiety. *Acta Poloniae Pharm. Drug Res*. 2011; **68**, 5, 785-93.
[15] M Kurono, M Hayashi, K Miura, Y Isogawa and K Sawai. One pot synthesis of pyrimidine and bispyrimidine derivatives and their evaluation for anti-inflammatory and analgesic activities. *Chem. Abstr*. 1988; **109**, 37832-41.
[16] WC Patt, HW Hamilton, MD Taylor, MJ Ryan, DG Taylor and CJC Connolly. Structure-activity relationships of a series of 2-amino-4-thiazole containing renin inhibitors. *J. Med. Chem*. 1992; **35**, 2562-72.

[17] J Rudolph, H Theis, R Hanke, R Endermann, L Johannsen and FU Geschke. Seco-cyclothialidines: new concise synthesis, inhibitory activity toward bacterial and human DNA topoisomerases, and antibacterial properties. *J. Med. Chem*. 2001; **44**, 619-26.
[18] RN Sharma, FP Xavier, KK Vasu, SC Chaturvedi and SS Pancholi. Synthesis of 4-benzyl-1,3-thiazole derivatives as potential anti-inflammatory agents: an analogue-based drug design approach. *J. Enz. Inhib. Med. Chem*. 2009; **24**, 890-97.
[19] K Tsuji and H Ishikawa. Synthesis and anti-pseudomonal activity of new 2-isocephems with a dihydroxypyridone moiety at C-7. *Bio. Med. Chem. Lett*. 1994; **4**, 1601-6.
[20] IV Ukrainets, IA Tugaibei, NL Bereznykova, VN Karvechenko and AV Turov. Analgesic, anticonvulsant and anti-inflammatory activities of some synthesized benzodiazipine, triazolopyrimidine and bisimide derivatives. *Chem. Heterocycl. Comp*. 2008; **5**, 565-73.
[21] E Wagner, K Al-Kadasi, M Zimecki and W Sawka-Dobrowolska. Synthesis and pharmacological screening of derivatives of isoxazolo[4,5-d]pyrimidine. *Eur. J. Med. Chem.* 2008; **43**, 677-82.