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SYNTHESIS OF NOVEL HESPERETIN OXIME ESTERS: A NEW DISCERNMENT IN TO THEIR ANTIOXIDANT POTENTIAL

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ABSTRACT: A series of novel hesperetin oxime esters **(3a-l)** were synthesized and there *in vitro* antioxidant potential was examined. Hesperetin oxime **2** was furnished by oximation of hesperetin then subsequently upon esterification with substituted benzoyl chlorides to obtain hesperetin oxime esters **(3a-l)** in good yields. The structure of compounds was elucidated by elemental analysis, IR, NMR (^{1}H, ^{13}C) and mass spectral studies. Among, the synthesized derivatives compounds (**3i-l**) showed pronounced antioxidant activity indeed higher than standard butylated hydroxyl anisole (BHA) and ascorbic acid (AA). Compounds with electronegative groups **3a** and **3b** demonstrated least activity compared to other analogues.

INTRODUCTION: Flavonoids are part of a family of naturally occurring polyphenolic compounds present in a wide variety of fruits and vegetables regularly consumed by humans; they exhibit a broad range of biological and pharmacological activities, such as antiviral, anti-inflammatory, antioxidant, anti-allergic, hepato-protective activities as well as anti-tumoral properties [1].

Flavonoids, known as nature's tender drugs, possess various biological/pharmacological activities including antioxidant, anti-inflammatory, anticancer, antimicrobial, and antiviral. The anti-oxidant activity exhibited by several flavonoids seems to be related with the number of hydroxyl groups in the B ring (**Fig. 1**), responsible for part of the anti-inflammatory properties of these compounds.

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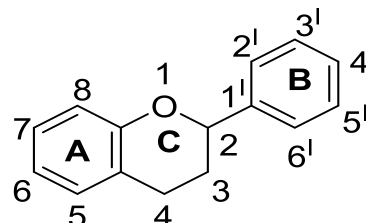


FIG. 1: NUCLEAR STRUCTURE OF FLAVONOID

Besides being related with free radicals scavenging and inhibition of lipid peroxidation, anti-inflammatory activity of flavonoids is also associated with the inhibition of cyclooxygenase and 5-lipooxygenase pathways involved in the arachidonate metabolism [2, 3].

Hesperetin (HTN) (5,7,3-trihydroxy-4-methoxyl flavanone),one of the most abundant flavonoids found in citrus fruits [4]. HTN shows a wide spectrum of pharmacological effects such as anti-inflammatory, anti-carcinogenic, anti-hypertensive and anti-atherogenic effects [4, 5], including the antioxidant properties [6]. The daily intake of citrus juices like orange and grape juices contains of HTN (200–590 mg/L) this is more beneficial for health [7]. HTN, an aglycon of hesperidin is actually a bioactive molecule.

The *in vitro* studies suggest that HTN is a powerful radical scavenger that promotes cellular antioxidant defense related enzyme activity [8, 9].

Oximes have important pharmaceutical and synthetic applications, and are generally used as chemical building blocks for the synthesis of agrochemicals and pharmaceuticals [10]. The oxime and oxime ether functional groups are incorporated into many organic medicinal agents, including some antibiotics, such as gemifloxacin mesylate, pralidoxime chloride, and obidoxime chloride used in the treatment of poisoning by organophosphate insecticides: malathion and diazinon.

Flavanone oxime derivatives (ethers) have been shown to modulate the growth of Yoshida Sarcoma cells *in vivo* and to induce apoptosis, but compared to anticancer drugs (doxorubicin, aclarubicin and mitoxantrone), flavanone oximes displayed cytotoxicity at considerably higher concentrations [11]. The antiradical and antioxidant activities of four biologically active N,N-diethyloaminoethyl ethers of flavanone oximes were investigated, and these compounds were shown to bepromising antioxidants and radio protectors comparable to rutin activities, rendering them useful under oxidative stress conditions [12]. There are no reports available on the synthesis and antioxidant activity of hesperetin oxime esters.

In the light of above information and in continuation of our research interest on functionalization of new tricyclic and heterocyclic compounds [13-17], the present study was carried out to investigate the protective effect of hesperetin oxime esters against radical scavenging activity.

EXPERIMENTAL SECTION:

Materials and Methods: All the reagents used were purchased from Merck (Darmstadt, Germany) chemicals are of AR grade and were used without further purification. Melting points were determined by using an open capillary method and are uncorrected. Thin layer chromatography (TLC) was performed with aluminium sheets–Silica gel 60 F254 purchased from Merck. The synthesized compounds were purified by using column chromatography with silica gel (60–120 mesh) using hexane:ethylacetate (8:2) as eluent. IR Nicolet 5700 FT–IR spectrophotometer, ^{1}H NMR and ^{13}C NMR spectra were recorded at 400 and 100 MHz respectively, in Bruker spectrometer by using DMSO-*d6* as a solvent for all the compounds. Micro analytical data were obtained by Elemental–Vario EL–III and mass spectra were recorded from Waters–Q–TOF ultima spectrometer.

Synthesis of hesperetin oxime (2): To the ethanolic solution of hesperetin (**1**) (2 mmol) in a two neck round-bottomed flask fitted with a reflux condenser, was added sodium acetate trihydrate (6 mmol). The mixture was heated to boil for 15 min. Then, hydroxylamine hydrochloride (3 mmol) was added to the above mixture and was heated to reflux for further 2 h. After cooling, the mixture was slowly poured into ice-cold water (50 mL) with constant stirring; the crude product was precipitated and crystallized from absolute ethanol yielded the compound **2** as white soild.

Yield:89 % , m.p. 195-197 ^{o}C; IR (KBr)ν_{max}(cm^{-1}): 3737 (OH), 3058-2959 (Ar-CH) 1676 (C=N), 885 (N-O); ^{1}H NMR (400 MHz, DMSO-*d6*) δ ppm: 7.24-6.04 (m, 5H, ArH), 12.0 (s, 1H, OH), 10.5 (s, 1H, OH), 9.1 (s, 1H, OH), 5.4 (t, 1H, CH), 3.81 (s, 3H, OCH_3) 8.11 (s, 1H, N-OH); ^{13}C NMR (100 MHz, DMSO-*d6*) δ ppm: 163.2, 160.5, 153.4, 151.3, 147.4, 130.6, 119.7, 113.5, 102.4, 95.5, 94.54, 85.6, 56.2, 36.5; MS (m/z): (M^+): 317.17; Anal.calcd. for $C_{16}H_{15}NO_6$, C, 60.57; H, 4.77; N, 4.41 found: C, 60.61; H, 4.80; N, 4.45%.

Synthesis of hesperetin oxime esters (3a-l): To a well stirred solution of hesperetin oxime (**2**) (1mmol) in dry tetehydrofuran (THF) and triethyl amine (TEA) (1.2 mmol) was added and stirred for 15 minutes. Substituted benzoyl chlorides were added and refluxed for 4 hr. Progress of the reaction was monitored by TLC using hexane: ethylacetate (8:2) as mobile phase.

After completion the reaction mixture was quenched with ice cold water and the product was extracted in dichloromethane (DCM). The organics were washed with sodium bicarbonate ($NaHCO_3$) and dried over anhydrous sodium sulphate (Na_2SO_4) and concentrated under rota evaporator to get desired products **(3a-l)**.

(E)-5,7-dihydroxy-2-(3-hydroxy-4-methoxyphenyl)chroman-4-one O-(4-fluoro benzoyl) oxime (3a): White solid, yield: 85 %: IR (KBr)ν_{max}(cm^{-1}): 3725 (OH), 3058-2959 (Ar-CH) 1687 (C=N), 891(N-O); ^{1}H NMR (400 MHz, DMSO-*d6*) δ ppm: 7.94-6.00 (m, 9H, ArH), 12.0 (s, 1H, OH), 10.2 (s, 1H, OH), 8.9 (s, 1H, OH), 5.4 (t, 1H, CH), 3.83 (s, 3H, OCH_3); ^{13}C NMR (100 MHz, DMSO-*d6*) δ ppm: 168.3, 163.2, 153.4, 151.3,149.2, 147.4, 131.6, 119.7, 113.5, 102.4, 95.5, 94.54, 85.3, 56.8, 39.5; MS (m/z): (M^+): 439.32; Anal.calcd. for $C_{23}H_{18}FNO_7$, C, 62.87; H, 4.13; F, 4.32; N, 3.19 found: C, 62.84; H, 4.16; F, 4.34; N, 3.21%.

(E)-5,7-dihydroxy-2-(3-hydroxy-4-methoxyphenyl)chroman-4-one O-(4-chloro benzoyl) oxime (3b): White solid, yield: 84 % R (KBr)ν_{max}(cm^{-1}): 3721 (OH), 3182-2961 (Ar-CH) 1698(C=N), 921 (N-O); ^{1}H NMR (400 MHz, DMSO-*d6*) δ ppm: 7.96-6.14 (m, 9H, ArH), 12.01 (s, 1H, OH), 9.92 (s, 1H, OH), 9.04 (s, 1H, OH), 4.87 (t, 1H, CH), 3.82 (s, 3H, OCH_3; ^{13}C NMR (100 MHz, DMSO-*d6*) δ ppm: 168.8, 161.2, 153.7, 151.3,149.2, 148.4, 131.6, 119.7, 113.5, 102.4, 95.5, 94.54, 85.3, 56.8, 40.5; MS (m/z): (M^+): 455.12; Anal.calcd. for $C_{23}H_{18}ClNO_7$, C, 60.60; H, 3.98; Cl, 7.78; N, 3.07found: C, 60.58; H, 3.95; Cl, 7.80; N, 3.09%.

(E)-5,7-dihydroxy-2-(3-hydroxy-4-methoxyphenyl)chroman-4-one O-(4-nitro benzoyl) oxime (3c): Yellow solid: yield: 76 % IR (KBr)ν_{max}(cm^{-1}): 3734 (OH), 3058-2959 (Ar-CH) 1676(C=N), 891 (N-O); ^{1}H NMR (400 MHz, DMSO-*d6*) δ ppm: 7.81-6.13 (m, 9H, ArH), 11.7(s, 1H, OH), 10.21 (s, 1H, OH), 9.12 (s, 1H, OH), 4.88 (t, 1H, CH), 3.82 (s, 3H, OCH_3); ^{13}C NMR (100 MHz, DMSO-*d6*) δ ppm: 165.3, 163.5, 162.6, 160.3, 153.1, 149.5, 147.6, 131.2, 130.5, 123.4, 119.7, 113.7, 102.0, 95.4, 94.5, 85.4, 56.3, 40.8; MS (m/z): (M^+): 466.31; Anal.calcd. for $C_{23}H_{18}N_2O_9$, C, 59.23; H, 3.89; N, 6.01 found: C, 60.23; H, 3.87; N, 6.11%.

(E)-5,7-dihydroxy-2-(3-hydroxy-4-methoxyphenyl)chroman-4-one O-(4-methyl benzoyl) oxime (3d): Brown solid: yield: 68 % IR (KBr)ν_{max}(cm^{-1}): 3718 (OH), 3058-2959 (Ar-CH) 1681(C=N), 919 (N-O); ^{1}H NMR (400 MHz, DMSO-*d6*) δ ppm: 7.79-6.03 (m, 9H, ArH), 12.00 (s, 1H, OH), 10.03 (s, 1H, OH), 9.08 (s, 1H, OH), 5.23 (t, 1H, CH), 3.81 (s, 3H, OCH_3), 2.30 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-*d6*) δ ppm: 163.2, 162.3, 153.1, 149.5, 147.6, 131.2, 130.5, 128.4, 123.4, 119.7, 113.7, 102.0, 95.4, 94.5, 85.4, 56.3, 40.8, 21.65; MS (m/z): (M^+): 435.13, ; Anal.calcd. for $C_{24}H_{21}NO_7$, C, 66.20; H, 4.86; N, 3.22; found: C, 66.22; H, 4.83; N, 3.20%.

(E)-5,7-dihydroxy-2-(3-hydroxy-4-methoxyphenyl)chroman-4-one O-(4-methoxy benzoyl) oxime (3e): Off white solid: yield: 87 % IR (KBr)ν_{max}(cm^{-1}): 3729 (OH), 3171-2956 (Ar-CH) 1691 (C=N), 964 (N-O); ^{1}H NMR (400 MHz, DMSO-*d6*) δ ppm: 7.98-6.03 (m, 9H, ArH), 12.32 (s, 1H, OH), 10.05 (s, 1H, OH), 9.20 (s, 1H, OH), 4.85 (t, 1H, CH), 3.85 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3); ^{13}C NMR (100 MHz, DMSO-*d6*) δ ppm: 165.3, 163.5, 160.3, 153.1, 149.5, 147.6, 131.2, 130.5, 123.4, 119.7, 113.7, 102.0, 95.4, 94.5, 85.4, 55.3, 40.8; MS (m/z): (M^+): 451.98; Anal.calcd. for $C_{24}H_{21}NO_8$, C, 63.85; H, 4.69; N, 3.10 found: C, 63.87; H, 4.59; N, 3.20%.

(E)-5,7-dihydroxy-2-(3-hydroxy-4-methoxy phenyl)chroman-4-one O-(4-hydroxy-2-methoxy benzoyl) oxime (3f): Brown solid: yield: 89 % IR (KBr)ν_{max}(cm^{-1}): 3722 (OH), 3027-2945 (Ar-CH) 1666(C=N), 971 (N-O); ^{1}H NMR (400 MHz, DMSO-*d6*) δ ppm: 7.91-6.03 (m, 8H, ArH), 12.10 (s, 1H, OH), 10.51 (s, 1H, OH), 9.2 (s, 1H, OH), 6.3 (s, 1H, OH), 4.88 (t, 1H, CH), 3.84 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3); ^{13}C NMR (100 MHz, DMSO-*d6*) δ ppm: 165.8, 162.5, 160.3, 153.5, 148.5, 147.6, 131.2, 130.5, 123.6, 119.5, 113.7, 102.0, 95.4, 94.5, 85.4, 56.3, 40.4; MS (m/z): (M^+): 467.65; Anal.calcd. for $C_{24}H_{21}NO_9$, C, 61.67; H, 4.53; N, 3.00 found: C, 61.65; H, 4.56; N, 3.08%.

(E)-5,7-dihydroxy-2-(3-hydroxy-4-methoxy phenyl)chroman-4-one O-(2,4-dimethoxy benzoyl) oxime (3g): Off white solid: yield: 86 % IR (KBr)ν_{max}(cm^{-1}): 3715 (OH), 3046-2955(Ar-CH) 1676 (C=N), 948(N-O); ^{1}H NMR (400 MHz, DMSO-*d6*) δ ppm: 7.99-6.13 (m, 8H, ArH), 12.02 (s, 1H, OH), 10.40 (s, 1H, OH), 9.32 (s, 1H, OH), 4.95 (t, 1H, CH), 3.85 (s, 3H, OCH_3), 3.83 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3); ^{13}C NMR (100 MHz, DMSO-*d6*) δ ppm: 165.3, 163.5, 160.3, 153.1, 149.5, 147.6, 131.2, 130.5, 123.4, 119.7, 113.7, 102.0, 94.5, 85.4, 55.3, 40.8; MS (m/z): (M^+): 481.34; Anal.calcd. for $C_{25}H_{23}NO_9$, C, 62.37; H, 4.82; N, 2.91 found: C, 62.36; H, 4.80; N, 2.89 %.

(E)-5,7-dihydroxy-2-(3-hydroxy-4-methoxy phenyl)chroman-4-one O-(2-hydroxybenzoyl) oxime (3h): Off white solid:yield: 81 % IR (KBr)ν_{max}(cm^{-1}): 3718 (OH), 3171-2959 (Ar-CH) 1663 (C=N), 951 (N-O); ^{1}H NMR (400 MHz, DMSO-*d6*) δ ppm: 8.0-6.03 (m, 9H, ArH), 11.92 (s, 1H, OH),10.45 (s, 1H, OH), 9.37 (s, 1H, OH), 5.83(s, 1H, OH), 4.89 (t, 1H, CH), 3.83 (s, 3H, OCH_3); ^{13}C NMR (100 MHz, DMSO-*d6*) δ ppm: 165.8, 163.1, 160.2, 154.5, 148.5, 131.2, 130.5, 124.6, 119.5, 113.7, 102.6, 94.4, 85.4, 56.3, 41.3; MS (m/z): (M^+): 437.65; Anal.calcd. for $C_{23}H_{19}NO_8$, C, 63.16; H, 4.38; N, 3.20; found: C, 63.10; H, 4.36; N, 3.18; %.

(E)-5,7-dihydroxy-2-(3-hydroxy-4-methoxy phenyl)chroman-4-one O-(3-hydroxy benzoyl) oxime (3i): Off white solid: yield: 73 % IR (KBr)ν_{max}(cm^{-1}): 3736 (OH), 3143-2851 (Ar-CH) 1638 (C=N), 942 (N-O); ^{1}H NMR (400 MHz, DMSO-*d6*) δ ppm: 8.04-6.06 (m, 9H, ArH), 12.07 (s, 1H, OH), 10.31 (s, 1H, OH), 9.21 (s, 1H, OH), 5.74 (s, 1H, OH), 4.89 (t, 1H, CH), 3.81 (s, 3H, OCH_3); ^{13}C NMR (100 MHz, DMSO-*d6*) δ ppm: 165.2, 163.0, 160.5, 153.5, 148.5, 131.2, 130.5, 124.6, 119.5, 113.7, 102.6, 94.7, 85.4, 56.3, 40.3; MS (m/z): (M^+): 437.65; Anal.calcd. for $C_{23}H_{19}NO_8$, C, 63.16; H, 4.38; N, 3.20; found: C, 63.10; H, 4.36; N, 3.18; %.

(E)-5,7-dihydroxy-2-(3-hydroxy-4-methoxy phenyl)chroman-4-oneO-(4-hydroxy benzoyl) oxime (3j): Off white solid: yield: 71 % IR (KBr)ν_{max}(cm^{-1}): 3741 (OH), 3079-2977 (Ar-CH) 1691 (C=N), 892 (N-O); ^{1}H NMR (400 MHz, DMSO-*d6*) δ ppm: 8.05-6.03 (m, 9H, ArH),11.91 (s, 1H, OH), 10.57 (s, 1H, OH), 9.09 (s, 1H, OH), 9.09 (s, 1H, OH), 4.89 (t, 1H, CH), 3.81 (s, 3H, OCH_3); ^{13}C NMR (100 MHz, DMSO-*d6*) δ ppm: 165.2, 163.0, 160.5, 153.5, 148.5, 131.2, 130.5, 124.6, 119.5, 113.7, 102.6, 94.7, 85.4, 56.3, 40.3; MS (m/z): (M^+): 437.65; Anal.calcd. for $C_{23}H_{19}NO_8$, C, 63.16; H, 4.38; N, 3.20; found: C, 63.10; H, 4.36; N, 3.18; %.

(E)-5,7-dihydroxy-2-(3-hydroxy-4-methoxy phenyl)chroman-4-one O-(2,4-dihydroxy benzoyl) oxime (3k): Brown solid: yield: 73% IR (KBr)ν_{max}(cm^{-1}): 3735 (OH), 3037-2968 (Ar-CH) 1678 (C=N), 939 (N-O); ^{1}H NMR (400 MHz, DMSO-*d6*) δ ppm: 7.84-6.12 (m, 8H, ArH), 12.10 (s, 1H, OH), 10.03 (s, 1H, OH), 9.41 (s, 1H, OH), 6.80 (s, 1H, OH), 5.75 (s, 1H, OH), 5.29 (s, 1H, OH),4.89 (t, 1H, CH), 3.81 (s, 3H, OCH_3); ^{13}C NMR (100 MHz, DMSO-*d6*) δ ppm: 163.2, 162.0, 160.5, 153.5, 147.5, 133.2, 131.5, 124.6, 119.5, 113.7, 108.6, 94.7, 85.6, 56.0, 40.1; MS (m/z): (M^+): 453.34; Anal.calcd. for $C_{23}H_{19}NO_9$, C, 60.93; H, 4.22; N, 3.09; found: C, 60.93; H, 4.22; N, 3.09; %.

(E)-5,7-dihydroxy-2-(3-hydroxy-4-methoxy phenyl)chroman-4-one O-(3,4,5-trihydroxy benzoyl) oxime (3l): Brown solid:yield: 82 % IR (KBr)ν_{max}(cm^{-1}): 3728 (OH), 3045-2950 (Ar-CH) 1665 (C=N), 952 (N-O); ^{1}H NMR (400 MHz, DMSO-*d6*) δ ppm: 7.10-6.02 (m, 7H, ArH), 12.3 (s, 2H, OH), 9.87 (s, 2H, OH), 9.30 (s, 1H, OH), 6.29 (s, 1H, OH), 5.87 (s, 2H, OH), 5.27 (s, 1H, OH,)4.94 (t, 1H, CH), 3.83 (s, 3H, OCH_3); ^{13}C NMR (100 MHz, DMSO-*d6*) δ ppm: 165.2, 163.0, 161.5, 153.5, 147.5, 133.2, 131.5,119.5, 113.7, 109.6, 94.7, 85.6, 56.0, 40.6; MS (m/z): (M^+): 469.49; Anal.calcd. for $C_{23}H_{19}NO_{10}$, C, 58.85; H, 4.08; N, 2.98; found: C, 58.83; H, 4.09; N, 2.95; %.

Antioxidant evaluation:

2,2'–diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity: The evaluation of antioxidant activity of newly synthesized compounds was done by DPPH radical scavenging assay.[18] Internal standards BHA, AA and the synthesized compounds of different concentrations were prepared in distilled ethanol, 1 mL of each compound solutions having different concentrations (10 µM, 25 µM, 50 µM, 100 µM, 200 µM and 500 µM) were taken in different test tubes, 4 mL of 0.1 mM ethanol solution of DPPH was added and shaken vigorously. The tubes were then incubated in the dark room at RT for 20 min.

A DPPH blank was prepared without compound and ethanol was used for the baseline correction. Changes (decrease) in the absorbance at 517 nm were measured using a UV-visible Spectrophotometer and the remaining DPPH was calculated. The percent decrease in the absorbance was recorded for each concentration, and percent quenching of DPPH was calculated on the basis of the observed decreased in absorbance of the radical. The radical scavenging activity was expressed as the inhibition percentage and was calculated using the formula:

Radical scavenging activity (%) = $[(A_o-A_1)/A_o X 100]$

Where A_o is the absorbance of the control (blank, without compound) and A_1 is the absorbance of the compound.

2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid ($ABTS^{\bullet+}$) radical scavenging assay: The $ABTS^{\bullet+}$ cation was produced by the reaction between 7 mmol ABTS in H_2O and 2.45 mmol potassium persulfate, stored in the dark at room temperature for 12 h.[19] Before the usage, the $ABTS^{\bullet+}$ solution was diluted to get an absorbance of 0.700±0.025 at 734 nm with phosphate buffer (0.1M, pH 7.4). Then, 1 mL of $ABTS^{\bullet+}$ solution was added to the compounds solution in ethanol at different concentrations (10, 25, 50, 100, 200, 500 μM/mL). After 30min, the percentage inhibition at 734 nm was calculated for each concentration relative to a blank absorbance (ethanol). The scavenging capability of $ABTS^{\bullet+}$ radical was calculated using the following equation:

$ABTS^{\bullet+}$ scavenging effect (%) $=[(A_c-A_s) / A_c] \times 100$

Where, A_c is the absorbance of initial concentration of the $ABTS^{\bullet+}$ and A_s is the absorbance of the remaining concentration of $ABTS^{\bullet+}$ in the presence of the compounds.

Ferric ion reducing antioxidant power (FRAP) assay: The ferric ion reducing power of synthesized compounds was determined according to the method of Oyaizu [20]. The compounds having 10 μM were mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of 1% potassium ferric cyanide, and then incubated at 50 °C for 20 min. To this mixture 2.5 mL of 10% trichloroacetic acid was added and the mixture was centrifuged at 3000 rpm for 20 min.

The upper layer (2.5 mL) was mixed with 2.5 mL of deionized water and 0.5 mL of 0.1% ferric chloride and the absorbance was measured at 700 nm using a spectrophotometer (Shimadzu 160A). Increases of absorbance of the reaction mixture indicate higher reducing power. Mean values from three independent samples were calculated for each compound and standard deviations were less than 5%

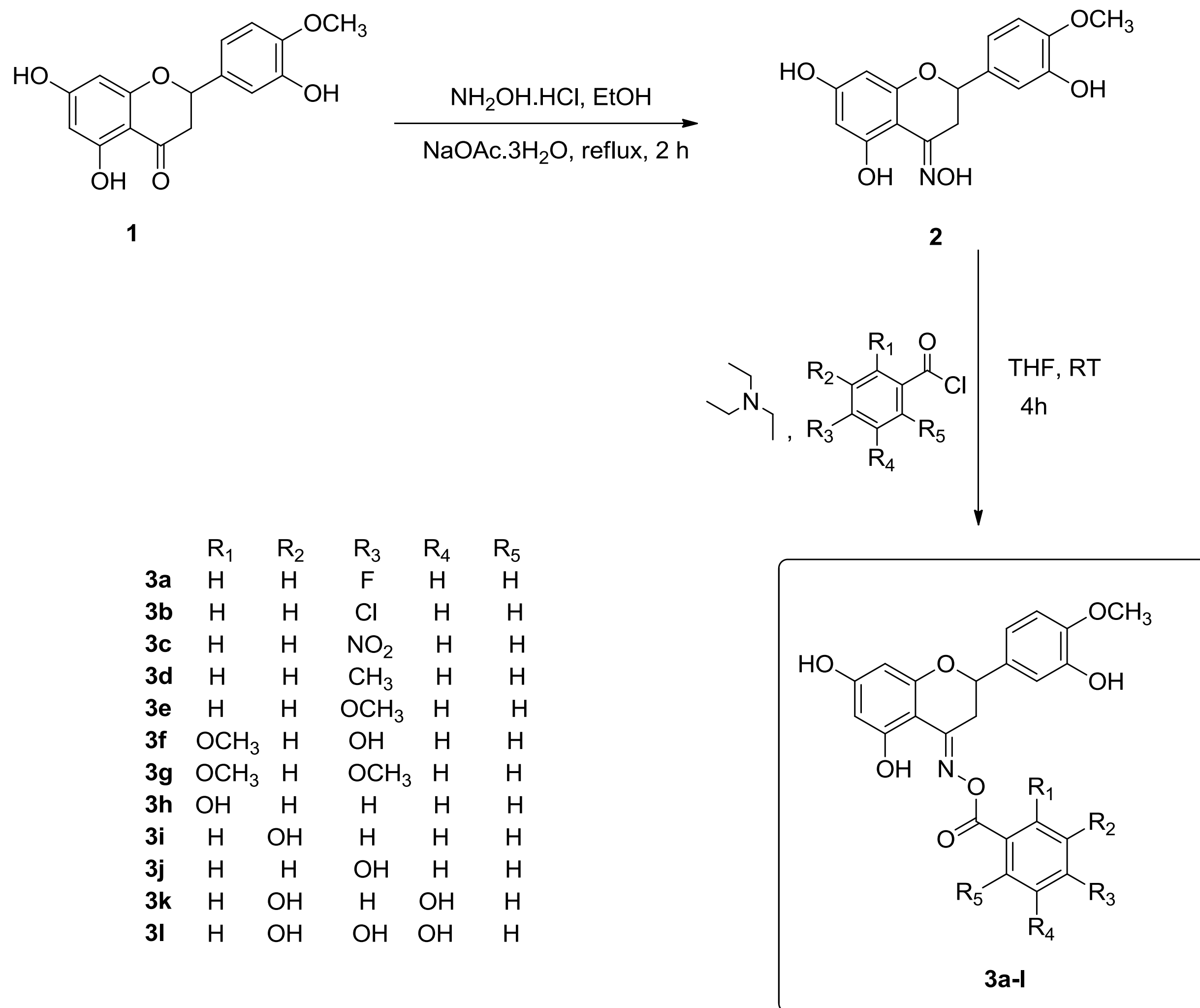
Cupric ion reducing antioxidant capacity (CUPRAC) assay: Cupric ion ($Cu^{2+)}$) reducing ability was determined according to the known method.[21] 1 mL of $CuCl_2$ (0.01 M) solution, ethanolic neocuproine solution (1 mL, 7.5 x 10-3 M) and 1 mL ammonium acetate buffer (1 M, pH 7) were added to a test tube, followed by mixing with 10 μM concentrations of each compounds. Then, the total volume was adjusted to 4.0 mL with distilled water and mixed well. The tubes were kept for incubation at room temperature for 30 min and the absorbance was measured at 450 nm against a reagent blank. Increased absorbance of the reaction mixture indicates increased reduction capability.

RESULTS AND DISCUSSION: The synthetic approach for novel hesperetin oxime esters is outlined in **Scheme 1**. The key scaffold (**2**) i.e., hesperetin oxime was furnished by oximation of hesperetin with hydroxylamine hydrochloride ($NH_2OH.HCl$) in the presence of sodium acetate trihydrate in absolute alcohol. Finally substituted benzoyl chlorides were esterified with key scaffold **2** in the presence of triethylamine as base to obtain hesperetin oxime esters **(3a-l)** in good yields.

We aimed to widen our knowledge of structure-activity relationship by investigating the possible antioxidant activity of hespertin oxime esters.

DPPH Radical Scavenging assay: DPPH radical scavenging assay results of all synthesized compounds are depicted in the **Table 1**. Most of synthesized compounds exerted a better radical scavenging activity. Initially, the key scaffold **2** also demonstrated good radical scavenging activity. The reason could be the presence of hydroxyl group in the B ring of flavonoid.[2,3] Eventually coupling of substituted benzoyl chlorides to keyscaffold **2** led to significant enhancement of activity.

The considerable increment of radical scavenging activity 2-5 fold was observed in compounds (**3e-g)** having one or more hydrophilic electron donating methoxy group. The improved activity of these analogues in the following order **3g**>**3f**>**3e**.Compound **3g** showed higher potency compared to less substituted ones **3f** and **3e**. The compound **3a** and **3b** with electronegative groups were unfavorable to show improved radical scavenging activity.



	R1	R2	R3	R4	R5
3a	H	H	F	H	H
3b	H	H	Cl	H	H
3c	H	H	NO_2	H	H
3d	H	H	CH_3	H	H
3e	H	H	OCH_3	H	H
3f	OCH_3	H	OH	H	H
3g	OCH_3	H	OCH_3	H	H
3h	OH	H	H	H	H
3i	H	OH	H	H	H
3j	H	H	OH	H	H
3k	H	OH	H	OH	H
3l	H	OH	OH	OH	H

SCHEME 1: REACTION PROTOCOL FOR THE SYNTHESIS OF HESPERETINOXIMEESTERS (3A-L)

Among the synthesized molecules, compounds (**3k, 3l)** having two and three hydroxyl group on phenyl ring were stand out as most efficient radical scavengers up to 12-13 folds more efficient than standards BHA and AA. Placement of hydroxyl group at *ortho* and *meta* position on phenyl ring in compounds (**3i, 3j)** were the next most efficient radical scavengers (11-12 fold increase). The introduction of electron withdrawing nitro group slightly reduces the radical scavenging activity in compounds **3c**. Interestingly placement of electron donating methyl group to *ortho* position and nitro group in compound **3e** results in enhancement of activity compared to **3c** having only methyl group.

ABTS$^{•+}$radical scavenging assay: All synthesized compounds have been assessed for ABTS$^{•+}$ radical scavenging assay. Here the technique is based on the direct production of the blue/green ABTS$^{•+}$chromophore through the reaction between ABTS and potassium persulfate.

The radical scavenging potency was described as the half of maximal inhibitory concentration (IC_{50}). BHA and AA were used as standard.

The obtained results are furnished in **Table-1**. Majority of the synthesized compounds displayed excellent radical scavenging activity. Close surveys of results show that compounds with more than one hydroxyl group (**3k** and **3l**) were found to be almost equipotent and even possess significant activity compared to the standard BHA and AA.

However, the presence of single hydroxyl group in *ortho* and *para* position in compound **3i** and **3j** also exhibits better radical scavenging activity but slightly less than **3k** and **3l**. Besides, moderate radical scavenging activity was observed in rest of the compounds. The order of ABTS$^{•+}$ radical scavenging capacity of the synthesized compounds are as follows **3l**>**3k**>**3i**>**3j**>**3g**>**3h**>**BHA**>**AA**>**3f**>**3d**>**3e**>**3c**>**3b**>**3a**.

TABLE 1: CONCENTRATION REQUIRED FOR 50% INHIBITION (IC_{50}) OF DPPH$^{\cdot}$ AND ABTS$^{\cdot+}$ RADICALS BY THE COMPOUNDS (3a-l) AND THE STANDARD ANTIOXIDANT COMPOUNDS BHA AND AA

Compound	Scavenging activity (IC_{50})*	
	DPPH$^{\cdot}$	ABTS$^{\cdot+}$
2	97±0.2	201±0.1
3a	98±0.1	192±0.1
3b	92±0.2	186±0.5
3c	75±0.3	151±0.6
3d	48±0.6	95±0.3
3e	69±0.7	120±0.8
3f	21±0.3	44±0.4
3g	11±0.2	28±0.5
3h	9.0±0.4	22±0.6
3i	8.0±0.8	17±0.6
3j	8.4±0.3	20±0.3
3k	7.6±0.4	16±0.8
3l	6.8±.5	14±0.5
BHA	12±0.4	22.6±0.6
AA	14±0.1	23±0.2

*The values are expressed as µm concentration. Lower IC_{50} values indicate higher radical scavenging activity.

Ferric ion reducing antioxidant power (FRAP) assay: The results in **Table 2** reveals the reducing power of hesperetin oxime esters (**3a–l**) examined as a function of their concentration. In this assay, the yellow colour of the test solution change tovarious shades of green and blue depending upon the reducing power of each compound. The presence of reducers (i.e., antioxidants) causes the reaction of the Fe^{3+}/ferri cyanide complex to the ferrous form giving, after the addition of trichloroacetic acid and ferric chloride, the Perl's Prussion blue that can be monitored at 700 nm.

The reducing power of the standards BHA and AA at various concentrations showed higher absorbance value that of newly synthesized compounds. The reducing power of newly synthesized compound solutions in ethanol increases with increase in concentration. Higher absorbance of **3k**, **3l**, **3i** and **3j** in contrast to standard is related to high reactivity of hydroxyl substituents and the electronic effects of hydroxyl substituent in C_2, C_3 and C_4 terminals of phenyl ring.

Rest of the derivatives (**3c-h**) displayed better reducing capacity except **3a** and **3b**. The reason would be the presence of electronegative fluoro and chloro substituent which is unfavorable for antioxidant activity.

Cupric ion reducing antioxidant capacity (CUPRAC) assay: CUPRAC assay is based on the reduction of Cu^{2+} to Cu^{1+} by antioxidants. The method is comprised of mixing the antioxidant solution with aqueous copper (II) chloride, ethanolic neocuproine, and ammonium acetate aqueous buffer at pH 7 and subsequently measuring the developed absorbance at 450 nm after 30 min. Initially key scaffold possess good absorbance value. For further enhancement of cupric ion reducing ability key scaffold **2** was incorporated with substituted benzoyl chlorides. Eventually all the hesperetin oxime derivatives exhibited remarkable enhancement in reducing ability. Electronic effects of substituents on phenyl ring may play a vital role in enhancement of reducing capacity. Introduction of electron donating hydroxyl groups on phenyl ring in compounds (**3i-l**) best suits for excellent cupric ion reducing capacity indeed greater than standard. Placement of methoxy substituent in compounds (**3f-h**) also exhibits better reducing capacity but slightly less than standard. Compounds (**3a-c**) with electron withdrawing groups like floro, chloro and nitro respectively showed weaker reducing capacity compared to other analogues. The methyl group substituted compound demonstrated moderate reducing capacity. Introduction of nitro group in *para* position and methyl group in *ortho* position does not favors appreciable reducing capacity.

TABLE 2: ANTIOXIDANT CAPACITY OF COMPOUNDS (3a-l) AND STANDARDS (BHA AND AA) IN FRAP AND CUPRAC AT THE CONCENTRATION OF 10 µM

Compound	FRAP	CUPRAC
2	0.2910	0.18899
3a	0.2927	0.2015
3b	0.3121	0.2281
3c	0.3472	0.2434
3d	0.4022	0.2952
3e	0.3825	0.2561
3f	0.4310	0.3121
3g	0.5018	0.3301
3h	0.5219	0.3408
3i	0.5629	0.3501
3j	0.5451	0.3482
3k	0.5875	0.3612
3l	0.5991	0.3872
BHA	0.5317	0.3351
AA	0.5413	0.3410

*The values are expressed as absorbance. Maximum absorbance indicates high reducing power.

CONCLUSION: In summary, a simple and convenient method of synthesis of novel hesperetin oxime esters **(3a-l)** is reported. The results of antioxidant assays reveals that coupling of substituted benzoyl chlorides to the key scaffold **2** plays a crucial role in enhancement of antioxidant activity. Majority of the synthesized compounds exhibited better antioxidant activity. Among the tested derivatives analogues (**3h-l)** holding one or more electron donating hydroxyl groups on phenyl ring were exhibited higher antioxidant potential than the standard BHA and AA. On the other hand compounds with electronegative groups **3a** and **3b** demonstrated least activity compared to other analogues.

Thus, an electronic effect of substituents on the phenyl ring has a remarkable contribution towards antioxidant activity. The results indicate that a series of novel analogues may provide good template for the development of valuable synthon for construction of more complex heterocycles of biological importance.

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