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SYNTHESIS, MOLECULAR SIMULATION, AND ANTIOXIDANT CAPABILITY OF A NEW CLASS OF 2-PHENYL-1-BENZOPYRAN-4-ONE ON HUMAN MYELOPEROXIDASE ENZYME

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ABSTRACT: The aim of the study was synthesis and characterization of 2-phenyl-1-benzopyran-4-one as a potential myeloperoxidase inhibitor. The oxidative stress was related to the generation of Reactive oxygen species (ROS), which is responsible for the enhancement of several degenerative diseases, such as osteoarthritis, cancer, diabetes, cardiovascular diseases, *etc*. Due to this fact, the study was targeted for the development of myeloperoxidase inhibitor for overcoming the oxidative stress. The molecular docking analysis were afforded that the titled compounds posses noteworthy potency against myeloperoxidase (PDB ID: 1DNU). The docking simulation of twenty hybrid of flavone showed the better binding score ranging between -6.66 to -8.56 kcal/mol. Based on the result, the synthesised compounds were screened for the evaluation of various *in-vitro* antioxidant studies. In which, the hydroxyl substituted flavones (HF1-HF8) were posses significant IC_{50} values compared with their respective standards due to their electron donating property over the radical responsible for oxidative stress. The docking score and *in-vitro* study of antioxidant activity were well correlated, which confirmed the antioxidant property of titled compounds and could act as a promising inhibitor to target the myeloperoxidase enzyme. The study summarised that these scaffolds were plays for free radical scavenging property in the management of many chronic diseases.

INTRODUCTION: Bioflavonoids or flavonoids are polyphenolic secondary metabolites and broadly distributed in plant kingdom have been recognized for their interesting medicinal properties. Among the flavonoids, the natural flavones and as well as some of their synthetic derivatives have an excellent antioxidant property and play an important role in preventing the major diseases such as cardiac disorder, diabetic, cancer and *etc*., which are mainly initiated by the development of oxidative stress [1, 2].

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It was clearly predicted that increasing level of nitrite, malondialdehyde and lipid peroxidation leads to decrease the total antioxidant enzymes [3]. Some flavones interfere in distinct oxidative-stress related events by directly reducing the levels of intracellular free radicals like hydroxyl, superoxide and nitric oxide in addition to of reactive species like hydrogen peroxide, peroxynitrite, and hypochlorous acid, thus preventing their intensification in many degenerative diseases.

The best described possible mechanism of antioxidant property of flavones is to directly scavenge the reactive oxygen species. It also has chelating property, which enabled them to chelate or binds to metal ions by donating a hydrogen atom or by single-electron transfer in the human body to prevent them being accessible for oxidation.

Flavones can also hinder the activity of central free radical-producing enzymes and also act as an intracellular antioxidant through inhibition of free radical generating enzymes such as myelo-peroxidase, xanthine oxidase, lipoxygenase, protein kinase C, cyclo-oxygenase, microsomal mono-oxygenase, mitochondrial succinoxidase, and NADPH oxidase [4]. Induction of internal anti-oxidant enzymes is another possible mechanism through which flavonoids act as an antioxidant.

Naturally available polyphenolic phytochemical compounds had a capability in scavenging and suppression of reactive oxygen species and regulate the antioxidant defence as a safeguard [5, 6]. Furthermore, it plays a vital role in ruling the inflammatory cellular activities and also modulates the enzyme activities in arachidonic acid and arginine metabolism. The molecular mechanisms behind the polyphenols were inhibition of enzyme coupled with COX2, iNOS, NF-κB, activating protein-1 and stimulating the phase II antioxidant detoxifying enzymes, mitogen activated protein kinase (MAPK), protein kinase C and some associated factors [7, 8].

It has been reported that flavonoids have strong antioxidant activities; it shows direct scavenging free radicals, suppression of proinflammatory cytokines through the inhibition of reactive oxygen species and nitric oxide, decreasing inflammatory genes including cyclooxygenases (COXs) and inducible nitric oxide synthase (iNOS), upregulating antioxidant enzymes, modulating transcription factors such as NF-κB and AP-1, and enhancing the Nrf2 signaling pathway [9-12].

By keeping the above discussed points in our mind, we have decided to synthesize the two novel flavones *viz*. 2-phenyl- 4H- chromen - 4- one (flavones) and hydroxy 2-phenyl- 4H- chromen - 4-one (hydroxy flavones) with some activating and deactivating groups to produce the target compounds. To reveal the theoretical binding pattern of these compounds with myeloperoxidase, a molecular simulation was carried out by using Autodock 4.2. software for finding its binding affinity towards the receptor protein myelo-peroxidase. In addition with this, the targeted compounds were subjected for the evaluation of *in-vitro* antioxidant activity, which could provide supportive data between *in-silico* and *in-vitro* antioxidant study.

MATERIALS AND METHODS:

Chemical and Reagents: The substituted acetophenones, aromatic aldehydes, hydrogen peroxide, aspirin, sodium dihydrogen phosphate, dimethyl sulfoxide (DMSO), dimethylformamide (DMF) and methanol were purchased from SRL Pvt. Ltd., Mumbai, Merck India, Hi-media Pvt. Ltd., Mumbai and Loba chemicals, Cochin. All the chemicals in this synthesis were of AR and LR grade.

The melting point of the synthesized compounds was determined by an open capillary method using digital melting point apparatus. The purity and progress of reaction were monitored by thin layer chromatography by using the mobile phase as hexane: ethyl acetate (4:1). The spots were observed by the UV light and iodine fumes. The λmax of the synthesized compounds was recorded on Schimadzu Ultra visible spectrophotometer. FTIR was recorded on Schimadzu Fourier Transform Infrared Spectrophotometer in the range of 4000 cm^{-1} - 400 cm^{-1} using KBr pellet technique. Mass fragmentation for the synthesized compounds was recorded using JEOL GC mate (GC-MS) spectrometer. Proton NMR Spectra was recorded using BRUKER Advance III - 500 MHz FT NMR Spectrometer using the solvent DMSO. Chemical shifts were recorded in parts per million and Trimethylsilane as an internal standard.

General Procedure for the Synthesis of Flavones Derivatives: In the first step, chalcones were synthesized by condensation of an equimolar mixture of substituted acetophenone and substituted aromatic aldehyde in presence of strong base under warm condition. In the second step, chalcones were cyclized to flavone in presence of the oxidising agent, hydrogen peroxide and strong base under the temperature between 50-70 °C [13, 14]. The general scheme of the synthesis of flavones and the substitution patterns for the synthesized compounds F1 to F12 and HF1 to HF8 were mentioned in **Fig. 1** and **Table 1**.

Molecular Modelling Study: AutoDock 4.2 was used to identify the binding modes of synthesized derivatives responsible for the activity to find the

binding energies of these synthesized compounds in the active sites. The ligands were drawn using Chem Draw Ultra 10.0. The mol form of each ligand was converted to PDB format by using Open Babel, prior to the submission for the docking. The preparation of the receptor was processed through downloading the crystal structure of enzyme myeloperoxidase complexed with thiocyanate (Protein Data Bank ID: 1DNU) from Protein Data Bank (http://www. rcsb.org/pdb). The pdb file was imported into Accelrys studio viewer where the receptor preparation module was used to prepare the protein. All the bound water molecules and heteroatom were removed from the complex, both polar and non-polar hydrogens were added and the 3D structure of the protein was corrected. A total of ten conformations were generated for each ligand by this molecular simulation and the top-ranked conformation was considered for the discussion of active flavone compounds.

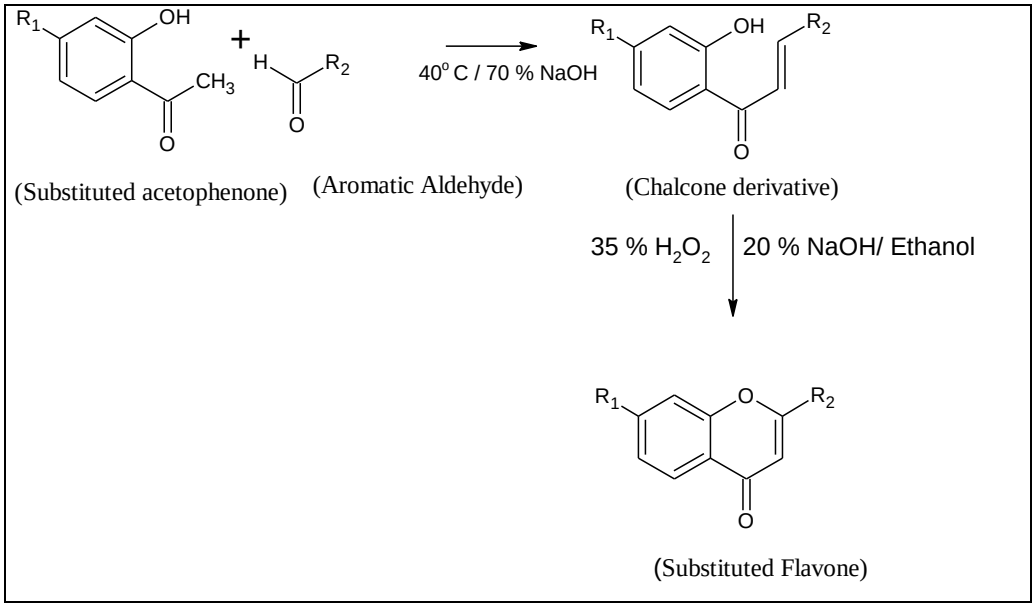

FIG. 1: GENERAL SYNTHETIC SCHEME OF FLAVONES

TABLE 1: SUBSTITUTION PATTERN OF FLAVONES DERIVATIVES

Comp code	R_2	Comp code	R_2	Comp code	R_2	Comp code	R_2
F1	$-C_6H_5$	F6	$-C_6H_4$-2(OH)	F11	$-C_6H_4$-CH=CH	HF4	$-C_6H_4$-4(F)
F2	$-C_6H_4$-2(Cl)	F7	$-C_6H_4$-4(OH)	F12	$-C_4H_3O$	HF5	$-C_6H_4$-4(OH)
F3	$-C_6H_4$-4(Cl)	F8	$-C_6H_4$-4(OCH_3)	HF1	$-C_6H_4$-2(Cl)	HF6	$-C_6H_4$-4(OCH_3)
F4	$-C_6H_4$-4(F)	F9	$-C_6H_3$-2,4(OCH_3)	HF2	$-C_6H_4$-4(Cl)	HF7	$-C_6H_3$-2,4(OCH_3)
F5	$-C_6H_4$-2(NO_2)	F10	$-C_6H_4-N(CH_3)_2$	HF3	$-C_6H_3$-2,4(Cl)	HF8	$-C_4H_3S$

F1-F12: R1 = H; HF1-HF8: R1 = OH

Evaluation of *in-vitro* Antioxidant Activity: All the twenty synthesised flavones (F1 to F12 and HF1 to HF8) and the standard drugs (ascorbic acid and gallic acid) were prepared in the dose of 10, 20, 40, 80 and 160 µg/ml respectively for performing the in-vitro antioxidant activity.

Reducing Power Ability Method: The sample of 2.5 ml of (1% w/v) potassium ferric cyanide and 2.5 ml of 0.2 M Phosphate buffer (pH 6.6) were added to 1ml of various concentrations of the sample and standard (ascorbic acid) respectively [15, 16]. The reaction mixtures were incubated at 50 ºC for 20 min followed by the addition of 2.5 ml of (10% w/v) trichloroacetic acid and centrifuged at 3000 rpm for 10 min, soon after 2.5 ml of the supernatant was separated, mixed with 0.5 ml of (0.1% w/v) of ferric chloride and 2.5 ml of distilled water. The Absorbance of the resulting solutions was measured at 700 nm against the blank.

Hydroxyl Radical Scavenging Method: The 1 ml of Iron-EDTA solution, 0.5 ml of 0.018% EDTA, 1 ml of DMSO (0.85% in 0.1 M/l phosphate buffer pH 7.4) and 0.5 ml of ascorbic acid were added to each sample and standard (gallic acid) respectively. The reaction mixtures were heated for 15 min at 80 – 90 ºC and 1 ml of cold trichloroacetic acid

(17.5%) was added to terminate the reaction. After that 3 ml of Nash reagent (75 gm of ammonium acetate, 2 ml of acetylacetone and 3 ml of glacial acetic acid) was added and incubated for 15 min at room temperature and absorbance was measured at 412 nm by using the blank [17].

Nitric Oxide Scavenging Assay Method: The 1 ml of different concentration of synthesised flavones were added with 0.5 ml of 5 mM of sodium nitroprusside in phosphate buffered saline pH 7.4 and incubated for 180 min at 25 ºC. The reaction mixture was mixed with equal volume of Griess reagent (1% sulphanilamide and 0.1% naphthyl ethylenediamine dihydrochloride in 5% phosphoric acid), which results in color formation by diazotisation of nitrite ions with naphthyl ethylenediamine dihydrochloride and sulphanilamide. The absorbance of chromophore was measured at 540 nm by using blank [18]. The percentage inhibition of radical formation by the above methods was calculated by the following formula.

$$\% \text{ Inhibition} = A_c - A_t / A_c \times 100$$

Where A_t = Absorbance of Test, A_c = Absorbance of Control

Statistical Analysis: Experimental results were expressed as mean ± SEM of three parallel measurements. Differences between control and test groups were tested for significance using one way analysis of variance followed by Dunnett's t-test.

RESULTS AND DISCUSSION:

Synthesis and Characterisation of Flavones: The various derivatives of flavones were synthesized according to the protocol reported in the general scheme of synthesis. The percentage yields of the synthesized flavones were obtained moderately and a melting point of those compounds was also recorded and presented uncorrected. The purity of the each synthesized compounds was determined by thin layer chromatography. All the twenty synthesized compounds were characterized by various spectroscopic techniques such as UV, IR, ^{1}H-NMR and mass spectrometry.

F1: 2-phenyl-4H-chromen-4-one: MP: 130-132 ºC; R_f = 0.56; % yield = 65.3% w/w; UV λmax: $CHCl_3$, nm: 297; IR (KBr cm^{-1}): 1739 (lactone), 1643 (CO str), 1585, 1550 (C=C Arom.str), 1134, 1093 (COC str), 771 (C-C bending); ^{1}H NMR (500 MHZ, DMSO): δ 7.4 - 7.9 (m, 8H, ArH), 6.9 (m, 1H, ArH); m/z: 222(M^+), 120.7($C_7H_6O_2$)$^+$, 105.8 (C_7H_8)$^+$, 92.8 (C_6H_6O)$^+$, 77.9 (C_6H_5)$^+$.

F2: 3-(2-chlorophenyl)-4H-1-benzopyran-4-one: MP: 157-160 ºC; R_f = 0.3; % yield = 42.4% w/w; UV λmax: $CHCl_3$, nm: 241; IR (KBr cm^{-1}): 1797 (lactone), 1687 (CO str), 1593, 1564 (C=C Arom.str), 1124, 1103, 1037 (COC str), 754 (C-C bending) ; ^{1}H NMR (500 MHZ, DMSO): δ 6.6 (m, 1H, ArH), 7.4 - 7.8 (m, 8H, ArH); m/z: 256 (M^+), 138.9 (C_8H_7 Cl)$^+$, 120.9 ($C_7H_6O_2$)$^+$, 91.9 (C_6H_5O), 77 (C_6H_5)$^+$.

F3: 3-(4-chlorophenyl)-4H-1-benzopyran-4-one: MP: 167-170 ºC; R_f = 0.84; % yield = 40.4% w/w; UV λmax: $CHCl_3$, nm: 261; IR (KBr cm^{-1}) 1735 (lactone), 1685 (CO str), 1593, 1573 (C=C Arom.str), 1130, 1091 (COC str), 761 (C-C bending) ; ^{1}H NMR (500 MHZ, DMSO): δ 7.4 - 7.9 (m, 8H, ArH), 6.7 (m, 1H, ArH); m/z: 256(M^+), 121.6($C_7H_6O_2$)$^+$, 139.5 (C_8H_7Cl)$^+$, 76.6 (C_6H_5)$^+$.

F4: 2-(4-fluorophenyl)-4H-chromen-4-one: MP: 241-243 ºC; R_f = 0.85; % yield = 59.3% w/w; UV λmax: $CHCl_3$, nm: 250; IR (KBr cm^{-1}): 1772 (lactone), 1685 (CO str), 1577, 1514 (C=C Arom.str), 1126, 1107, 1024 (COC str), 773 (C-C bending); ^{1}H NMR (500 MHZ, DMSO): δ 6.9 (m, 1H, ArH), 7.6 - 8.6 (m, 8H, ArH); m/z: 240(M^+), 122 ($C_7H_6O_2$)$^+$, 119.5 (C_8H_6 F)$^+$, 75.5 (C_6H_3)$^+$.

F5: 3-(2-nitrophenyl)-4H-1-benzopyran-4-one: MP: 145-148 ºC; R_f = 0.43; % yield = 51.6% w/w; UV λmax: $CHCl_3$, nm: 306; IR (KBr cm^{-1}): 1797 (lactone), 1681 (CO str), 1593, 1573 (C=C Arom.str), 1128, 1091 (COC str), 761 (C-C bending) ; ^{1}H NMR (500 MHZ, DMSO): δ 7.4 - 7.6 (m, 8H, ArH), 6.2 (m, 1H, ArH); m/z: 267 (M^+), 121.1($C_7H_6O_2$)$^+$, 148.9 ($C_8H_7NO_2$)$^+$, 76.9 (C_6H_5)$^+$.

F6: 2-(2-hydroxyphenyl)-4H-chromen-4-one: MP: 185-188 ºC; R_f = 0.58; % yield = 47.2% w/w; UV λmax: $CHCl_3$, nm: 248; IR (KBr cm^{-1}): 1734 (lactone), 1683 (CO str), 1558, 1541 (C=C Arom.str), 1139, 1093 (COC str), 765 (C-C bending) 3566, 3547 (OH str); ^{1}H NMR (500 MHZ, DMSO): δ 6.5 (m, 1H, ArH), 7.1 - 7.9 (m, 8H, ArH); m/z: 238 (M^+), 121 ($C_7H_6O_2$)$^+$, 106 (C_7H_6O)$^+$, 104 (C_8H_8)$^+$, 78 (C_6H_6)$^+$.

F7: 2- (4-hydroxyphenyl)- 4H- chromen- 4- one: MP: 181-183 ºC; R_f = 0.41; % yield = 47.5% w/w; UV λmax: $CHCl_3$, nm: 287; IR (KBr cm^{-1}): 1772 (lactone), 1691 (CO str), 1560, 1543, 1516 (C=C Arom.str), 1047, 1139 (COC str), 748 (C-C bending) 3300, 3545 (OH str); ^{1}H NMR(500 MHZ, DMSO): δ 6.8 (m, 1H, ArH), 7.7 - 7.4 (m, 8H, ArH); m/z: 238 (M^+), 121 $(C_7H_6O_2)^+$, 118 $(C_8H_7O)^+$, 92 $(C_6H_6O)^+$, 76.9 $(C_6H_6)^+$.

F8: 3- (4-methoxyphenyl)- 4H- 1- benzopyran-4-one: MP: 175-177 ºC; R_f = 0.43; % yield = 49.2% w/w; UV λmax: $CHCl_3$, nm: 265; IR (KBr cm^{-1}): 1658 (CO str), 1597, 1550 (C=C Arom.str), 1126, 1064 (COC str), 727 (C-C bending); ^{1}H NMR (500 MHZ, DMSO): δ 3.8 (s, OCH_3, ArH), 6.4 (m, 1H, ArH), 7.0, 7.8 (m, 8H, ArH); m/z: 252 (M^+), 134.9 $(C_9H_{10}O)^+$, 107 $(C_7H_6O)^+$, 120.6 $(C_7H_5O_2)^+$, 77.0 $(C_6H_5)^+$.

F9: 2- (2,4-dimethoxyphenyl)- 4H- chromen- 4-one: MP: 178-180 ºC; R_f = 0.6; % yield = 46.4% w/w; UV λmax: $CHCl_3$, nm: 250; IR (KBr cm^{-1}): 1660 (CO str), 1597, 1550 (C=C Arom.str), 1124, 1066 (COC str), 752 (C-C bending); ^{1}H NMR(500 MHZ, DMSO): δ 3.8 (s, OCH_3, ArH), 6.9 (m, 1H, ArH), 7.0 -7.7 (m, 7H, ArH); m/z: 283 (M^+), 121 $(C_7H_6O_2)^+$, 92.7 $(C_6H_6O)^+$, 164 $(C_{10}H_{11}O_2)^+$, 137.5 $(C_8H_{10}O_2)^+$, 76.7 $(C_6H_5)^+$.

F10: 2-[4-(dimethylamino)phenyl]-4H-chromen-4-one: MP: 169-171 ºC; R_f = 0.71; % yield = 48.4% w/w; UV λmax: $CHCl_3$, nm: 295; IR (KBr cm^{-1}): 1795 (lactone), 1658 (CO str), 1548, 1537 (C=C Arom.str), 1124, 1064 (COC str), 727 (C-C bending); ^{1}H NMR(500 MHZ, DMSO): δ 2.4 (s, 6H, $N(CH_3)_2$), 6.3 - 6.5 (m, 1H, ArH), 7.5 - 7.9 (m, 8H, ArH); m/z: 265 (M^+), 222 $(C_{15}H_{10}O_2)^+$, 121 $(C_7H_6O_2)^+$, 104.9 $(C_8H_8)^+$, 77 $(C_6H_5)^+$.

F11: 2- [(Z)-2-phenylethenyl]- 4H- chromen- 4-one: MP: 152-155 ºC; R_f = 0.76; % yield = 55.3% w/w; UV λmax: $CHCl_3$, nm: 299; IR (KBr cm^{-1}): 1788 (lactone), 1699 (CO str), 1583, 1554 (C=C Arom.str), 1107, 1213 (COC str), 769 (C-C bending), 3093 (C-H str); ^{1}H NMR(500 MHZ, DMSO): δ 3.3 (s, 1H), 6.8 (m, 1H, ArH), 7.3 - 7.8 (m, 9H, ArH); m/z: 248 (M^+), 131 $(C_{10}H_{10}O)^+$, 121 $(C_7H_6O_2)^+$, 106 $(C_8H_9)^+$, 78 $(C_6H_5)^+$.

F12: 2- (furan-2-yl)- 4H- chromen- 4- one: MP: 164-168 ºC; R_f = 0.48; % yield = 50.4% w/w; UV λmax: $CHCl_3$, nm: 246; IR (KBr cm^{-1}): 1731 (lactone), 1677 (CO str), 1514, 1541 (C=C Arom.str), 1024, 1137 (COC str), 763 (C-C bending), 3058 (C-H str); ^{1}H NMR(500 MHZ, DMSO): δ 6.8 - 6.9 (m, 1H, ArH), 7.1 - 7.5 (m, 7H, ArH); m/z: 213 (M^+), 92.5 $(C_6H_5O)^+$, 122.4 $(C_7H_6O_2)^+$, 76.6 $(C_6H_5)^+$.

HF1: 2- (2- chlorophenyl)- 7- hydroxy- 4H-chromen-4-one: MP: 172-175 ºC; R_f = 0.68; % yield = 64.4% w/w; UV λmax: $CHCl_3$, nm: 358; IR (KBr cm^{-1}): 1701 (lactone), 1683 (CO str), 1541, 1558 (C=C Arom.str), 1020, 1218 (COC str), 771 (C-C bending), 3033 (C-H str), 3461 (OH str); ^{1}H NMR (500 MHZ, DMSO): δ 6.6 (m, 1H, ArH), 7.4 - 7.9 (m, 7H, ArH), 9.4 (s, 1H, OH); m/z: 272 (M^+), 237.05 $(C_{15}H_{10}O_3)^+$, 138 $(C_7H_6O_3)^+$, 103 $(C_8H_7)^+$, 77.9 $(C_6H_5)^+$.

HF2: 2- (4- chlorophenyl)- 7- hydroxy- 4H-chromen-4-one: MP: 182-184 ºC; R_f = 0.52; % yield = 69.3% w/w; UV λmax: $CHCl_3$, nm: 232; IR (KBr cm^{-1}): 1789 (lactone), 1683 (CO str), 1510, 1542 (C=C Arom.str), 1095, 1130 (COC str), 769 (C-C bending), 3080 (C-H str), 3442 (OH str); ^{1}H NMR (500 MHZ, DMSO): δ 6.5 (m, 1H, ArH), 7.5 - 7.9 (m, 7H, ArH), 9.5 (s, 1H, OH); m/z: 272 (m+1), 137 $(C_7H_5O_3)^+$, 104 $(C_8H_8)^+$, 76.8 $(C_6H_5)^+$.

HF3: 2- (2,4-dichlorophenyl)- 7- hydroxy- 4H-chromen-4-one: MP: 175 - 178 ºC; R_f = 0.81; % yield = 55.6% w/w; UV λmax: $CHCl_3$, nm: 303; IR (KBr cm^{-1}): 1714 (lactone), 1649 (CO str), 1510, 1541 (C=C Arom.str), 1217, 1247 (COC str), 771 (C-C bending), 3033 (C-H str), 3479 (OH str); ^{1}H NMR (500 MHZ, DMSO): δ 6.6 (m, 1H, ArH), 7.3 - 7.9 (m, 6H, ArH), 9.4 (s, 1H, OH); m/z: 307 (M^+), 138.7 $(C_7H_6O_3)^+$, 172.4 $(C_8H_6Cl_2)^+$, 123.6 $(C_7H_6O_2)$, 76.7 $(C_6H_5)^+$.

HF4: 2- (4- fluorophenyl)- 7-hydroxy- 4H-chromen-4-one: MP: 195 - 198 ºC; R_f = 0.72; % yield = 67.2% w/w; UV λmax: $CHCl_3$, nm: 234; IR (KBr cm^{-1}): 1685 (CO str), 1529, 1587 (C=C Arom.str), 1085, 1163, 1242 (COC str), 756 (C-C bending), 3072 (C-H str), 3463 (OH str); ^{1}H NMR (500 MHZ, DMSO): δ 6.3 (m, 1H, ArH), 7.2 - 8 (m, 7H, ArH), 10.7 (s, 1H, OH); m/z: 256 (M^+), 136.5 $(C_7H_6O_3)^+$, 122.6 $(C_8H_7F)^+$, 94.7 (C_6H_6O), 74.8 $(C_6H_5)^+$.

HF5: 7- hydroxy- 2- (4- hydroxyphenyl)- 4H-chromen-4-one: MP: 181-183 ºC; R_f = 0.54; % yield = 57.5% w/w; UV λmax: $CHCl_3$, nm: 315; IR

(KBr cm^{-1}): 1716 (lactone), 1649 (CO str), 1521, 1556 (C=C Arom.str), 1166, 1184, 1218 (COC str), 771 (C-C bending), 3461 (OH str); ^{1}H NMR (500 MHZ, DMSO): δ 6.5 – 6.6 (m, 1H, ArH), 7.3 - 7.8 (m, 7H, ArH), 9.8 (s, 2H, OH); m/z: 254 (M^+), 137.1 $(C_7H_6O_3)^+$, 121.2 $(C_8H_8O)^+$, 93.3 (C_6H_6O), 79 $(C_6H_5)^+$.

HF6: 6- hydroxy- 3- (4- methoxyphenyl)- 4H- 1-benzopyran-4-one: MP: 225-227 ºC; R_f = 0.62; % yield = 62.2% w/w; UV λmax: $CHCl_3$, nm: 253; IR (KBr cm^{-1}): 1712 (lactone), 1635 (CO str), 1556, 1587 (C=C Arom.str), 1078, 1112 (COC str), 757 (C-C bending), 3097 (CH str), 3485 (OH str); ^{1}H NMR (500 MHZ, DMSO): δ 3.8 (s, 3H, OCH_3) 6.6 – 6.9 (m, 1H, ArH), 7.0 - 7.8 (m, 7H, ArH), 9.8 (s, 1H, OH); m/z: 268 (M^+), 136.9 $(C_7H_6O_3)^+$, 134.9 $(C_9H_{10}O)^+$, 110 ($C_6H_6O_2$), 92.9 (C_6H_6O), 78 $(C_6H_5)^+$.

HF7: 2- (2,4-dimethoxyphenyl)- 7- hydroxy- 4H-chromen-4-one: MP: 178-180 ºC; R_f = 0.47; % yield = 58.3% w/w; UV λmax: $CHCl_3$, nm: 293; IR (KBr cm^{-1}): 1735 (lactone), 1683 (CO str), 1523, 1573 (C=C Arom.str), 1091, 1111, 1130 (COC str), 761 (C-C bending), 3093 (CH str), 3424 (OH str); ^{1}H NMR (500 MHZ, DMSO): δ 3.8 (s, 6H, OCH_3), 7.0 (m, 1H, ArH), 7.0 – 7.5 (m, 6H, ArH), 12.6 (s, 1H, OH); m/z: 298 (M^+), 139 $(C_7H_6O_3)^+$, 165.8 $(C_{10}H_{11}O_2)^+$, 149.9 ($C_9H_{11}O_2$), 95.12 (C_6H_6O), 78 $(C_6H_5)^+$.

HF8: 7- hydroxy-2-(thiophen-2-yl)-4H-chromen-4-one: MP: 194-199 ºC; R_f = 0.76; % yield = 45.4% w/w; UV λmax: $CHCl_3$, nm: 326; IR (KBr cm^{-1}): 1772 (lactone), 1681 (CO str), 1541, 1558 (C=C Arom.str), 1043, 1101, 1134 (COC str), 702 (C-C bending), 3089 (CH str), 3444 (OH str); ^{1}H NMR (500 MHZ, DMSO): δ 6.1 (m, 1H, ArH), 7.1 - 7.7 (m, 6H, ArH), 10.5 (s, 1H, OH); m/z: 244 (M^+), 139.2 $(C_7H_6O_3)^+$, 111.3 $(C_6H_6S)^+$, 123.3 $(C_6H_6O_2)^+$, 76.5 $(C_6H_5)^+$.

Molecular Docking Study: The binding interactions of flavones towards the enzyme myeloperoxidase (PDB ID: 1DNU) using docking studies by auto dock 4.2. software [19, 20] were revealed on the basis of structure based design. The most favourable docking poses of the 10 docked conformations for each molecule were analyzed for further investigation of the ligand interactions within the active sites of the receptor. The wonderful number of interactions with the active site residues coupled with minimum favourable binding energy state conditions would help to these compounds which may serve as an effective antioxidant agent for many associated chronic diseases. All the synthesized compounds have a higher binding affinity with the receptors, in the narrow range of binding energy for the protein PDB ID: 1DNU and showed the docking score value in the range of -6.66 to -8.56 kcal/mol **Table 2**.

TABLE 2: BEST DOCKING SCORE OF SYNTHESIZED DERIVATIVES OF FLAVONES WITH MYELOPEROXIDASE (PDB ID: 1DNU)

Comp code	Score	Comp code	Score	Comp code	Score	Comp code	Score
F1	-7.47	F6	-7.56	F11	-8.40	HF4	-7.31
F2	-7.38	F7	-7.60	F12	-6.66	HF5	-7.34
F3	-7.62	F8	-7.32	HF1	-7.82	HF6	-7.49
F4	-7.21	F9	-7.61	HF2	-7.81	HF7	-7.63
F5	-8.56	F10	-7.82	HF3	-8.28	HF8	-7.45

Among these 20 synthesized compounds, the best three compounds *viz*. F5, F11, and HF3 were showed the best docking score and the interaction within the receptor, which was visualized by the discovery studio. Those compounds were studied for their electrostatic, hydrophobic and hydrogen bonding interactions on the receptor site, in which the compound F5, F11, and HF3 were showed their binding score of -8.56, -8.40, -8.28 kcal/mol respectively and present within the binding pocket region forming conventional hydrogen bond with Pro 101 and carbon hydrogen bond with Gln 163 which could favour the antioxidant action. The lipophilic side chain substitution along with the presence of hydroxyl group at position 7 shows stabilised interaction with basic amino acid residues Pro 101 and Gln 163.

***In-vitro* Antioxidant Activity:** The twenty synthesised flavones (F1 to F12 and HF1 to HF8) and its corresponding reference drug were evaluated for *in-vitro* antioxidant studies and the results were shown for reducing power ability method **Table 3**, hydroxyl radical scavenging

method **Table 4** and nitric oxide radical scavenging assay **Table 5** respectively. In reducing power assay method, the antioxidant compound has the ability to donate electron which neutralizes the free radicals. This is shown by conversion of the oxidized form of iron complex (Fe^{3+}) to the reduced form of ferrous complex (Fe^{2+}) which was indicated by Prussian blue colour. Due to reducing power ability of synthesised flavones, the absorbance increases with increasing concentration. Among the all twenty synthesized flavones, the flavone with lipophilic in nature along with the presence of hydroxyl group *viz*. F2, F3, F4, HF1, HF4 and HF6 possess the noteworthy results (0.793, 0.806, 0.789, 0.874, 0.837 and 0.823 respectively) when compared to that of the standard (0.923) **Table 3**.

TABLE 3: ANTIOXIDANT EFFECT OF HYBRIDISED FLAVONES BY REDUCING POWER ABILITY METHOD

Compounds	Absorbance				
	10 µg/ml	20 µg/ml	40 µg/ml	80 µg/ml	160 µg/ml
F1	0.034±0.001	0.046±0.002	0.075±0.001	0.126±0.007	0.188±0.010
F2	0.143±0.011	0.354±0.016	0.518±0.032	0.671±0.012	0.793±0.003
F3	0.128±0.013	0.342±0.011	0.552±0.028	0.695±0.018	0.806±0.014
F4	0.239±0.010	0.388±0.021	0.505±0.002	0.631±0.005	0.789±0.008
F5	0.086±0.002	0.126±0.009	0.182±0.002	0.258±0.012	0.311±0.020
F6	0.248±0.009	0.368±0.012	0.492±0.015	0.613±0.006	0.756±0.025
F7	0.262±0.010	0.383±0.018	0.471±0.009	0.592±0.002	0.709±0.021
F8	0.121±0.006	0.242±0.016	0.317±0.017	0.404±0.026	0.555±0.028
F9	0.132±0.007	0.214±0.014	0.385±0.019	0.492±0.022	0.589±0.024
F10	0.061±0.005	0.148±0.019	0.201±0.012	0.291±0.018	0.378±0.022
F11	0.038±0.002	0.096±0.002	0.159±0.007	0.208±0.019	0.275±0.020
F12	0.072±0.004	0.155±0.008	0.201±0.015	0.289±0.014	0.342±0.017
HF1	0.278±0.014	0.412±0.028	0.596±0.014	0.729±0.021	0.874±0.014
HF2	0.263±0.020	0.390±0.014	0.503±0.019	0.624±0.012	0.710±0.016
HF3	0.321±0.016	0.439±0.026	0.561±0.011	0.648±0.016	0.733±0.025
HF4	0.358±0.009	0.472±0.009	0.602±0.014	0.720±0.025	0.837±0.026
HF5	0.231±0.008	0.305±0.012	0.458±0.020	0.592±0.023	0.674±0.029
HF6	0.248±0.002	0.385±0.010	0.554±0.016	0.703±0.020	0.823±0.023
HF7	0.105±0.004	0.234±0.006	0.325±0.015	0.478±0.021	0.605±0.027
HF8	0.187±0.006	0.268±0.016	0.394±0.014	0.517±0.028	0.632±0.025
Ascorbic acid	0.361±0.010	0.522±0.014	0.669±0.021	0.792±0.024	0.923±0.022

All values are Mean ± SEM, n = 3. One way Analysis of Variance (ANOVA) followed by Dunnett's test was performed as the test of significance.

Hydroxyl radical is one of the free radical existed in our body, which are highly reactive oxygen species responsible for attacking some of the substrates in our biological system such as carbohydrates, proteins, DNA and polyunsaturated fatty acids. The ascorbic acid - Iron EDTA were responsible for the generation of hydroxyl radical in presence of DMSO to form formaldehyde, which further converted into hydroxyl radicals. These radicals formation was identified by a nash reagent by the yellow colored complex formation. The optical density of this yellow color formation was decreased while increasing in the concentration of compounds. Due to the ability to donate the proton towards the hydroxyl radical were retarded the formation of yellow color. By compared with the standard drug, the synthesised flavones with lipophilic side chain substitution along with hydroxyl substitution at their basic analogue *viz*. F6 and HF2-HF8 have significantly inhibited the color formation by acting as an antioxidant **Table 4**.

TABLE 4: ANTIOXIDANT EFFECT OF HYBRIDISED FLAVONES BY HYDROXYL RADICAL SCAVENGING ASSAY (HRSA)

Compounds	Percentage Inhibition of HRSA					
	10 µg/ml	20 µg/ml	40 µg/ml	80 µg/ml	160 µg/ml	IC_{50}
F1	9.87±1.39	18.45±1.85	30.55±1.18	48.16±1.41	65.62±1.8	105.4
F2	14.86±0.94	25.67±1.56	39.63±1.74	65.77±1.22	74.05±1.62	77.6
F3	9.01±0.72	16.29±1.69	29.51±1.72	45.48±1.65	62.31±2.39	112.8

F4	15.49±0.62	29.43±0.87	43.86±1.61	61.29±1.27	79.01±2.02	72.7
F5	6.89±0.44	10.94±0.63	16.4±1.55	25.34±1.52	32.12±1.91	-
F6	17.23±1.11	29.43±1.56	42.62±1.53	60.62±1.54	79.86±1.88	72.3
F7	18.80±1.86	28.68±1.78	40.37±2.38	58.91±1.18	78.72±1.26	75.2
F8	9.49±1.58	17.72±1.38	29.15±1.47	38.02±1.37	48.18±2.10	-
F9	10.82±1.44	18.23±1.85	26.93±1.58	37.4±1.89	46.63±2.23	-
F10	11.43±1.59	19.9±1.24	27.73±1.14	36.96±1.22	47.1±2.32	-
F11	6.93±0.36	9.52±0.67	16.58±1.18	24.29±2.16	36.3±1.44	-
F12	7.82±0.88	10.45±0.74	17.02±1.25	23.45±1.98	30.85±1.98	-
HF1	14.02±1.86	24.53±2.03	38.79±2.19	55.01±1.18	79.19±2.27	80.5
HF2	13.22±1.09	22.12±1.65	48.67±2.98	69.18±1.96	88.74±2.18	65.4
HF3	16.45±1.14	30.75±2.12	47.1±2.46	68.85±2.41	84.04±2.72	63.4
HF4	20.07±1.63	33.57±2.26	48.32±1.86	70.23±1.51	89.59±1.96	56.7
HF5	18.85±1.48	31.47±1.59	46.86±1.98	69.29±2.56	91.3±1.22	58.6
HF6	20.76±1.78	32.35±1.89	47.17±2.52	71.21±1.58	93.22±2.51	55.7
HF7	18.6±1.12	33.93±2.09	48.65±2.34	71.05±1.92	95.81±1.87	54.5
HF8	19.56±1.64	31.84±1.61	48.32±2.62	70.72±2.35	89.41±1.25	57.6
Gallic acid	23.96±1.63	42.27±2.18	58.69±1.54	77.21±1.72	98.57±1.67	39.6

All values are Mean ± SEM, n = 3. One way Analysis of Variance (ANOVA) followed by Dunnett's test was performed as the test of significance.

Generally, nitric oxide radicals were generated in our body from the amino acid (L – arginine) which are present on our endothelial cells and phagocytes. Being a free radical which contains unpaired electron which reacts with superoxide anion to form peroxynitrite ($ONOO^{\cdot}$) [21]. These radicals were highly toxic and produce inflammation due to cellular damage results in juvenile diabetes, various sclerosis, ulcerative, arthritis and so on [22]. Due to this reason, the present study deals with *in-vitro* scavenging of nitric oxide assay of synthesised flavones in different concentrations. In this method, the sodium nitroprusside acts as a source for the generation of nitric oxide. This nitric oxide further reacts with the oxygen to form nitrite ions. Griess reagent contains sulphanilamide, which undergoes diazotisation with nitrite ions and forms diazonium salt, which couples with the naphthyl ethylene diamine to form a pink color complex. The scavenging of nitric oxide was directly related with decreasing of the optical density of pink color complex. Due to antioxidant property of synthesised compounds, which compete with oxygen interaction on nitric oxide to form the nitrite radicals, by which it retards the reaction along with a Griess reagent to form the pink color. This clearly indicates that the concentration of the drug is inversely proportional to the absorbance [23]. As like the above methods, the percentage inhibition of the synthesised flavones with hydroxyl substitution (F6, F7, and HF1-HF8) were posses the significant result in scavenging of nitric oxide radicals by compared with the reference drug (gallic acid). The IC_{50} values of HF1, HF2 and HF3 showed significantly 38.0, 29.6 and 28.8 µg/ml respectively **Table 5**.

TABLE 5: ANTIOXIDANT EFFECT OF SYNTHESISED FLAVONES BY NITRIC OXIDE SCAVENGING ASSAY

Compounds	% Inhibition of Nitric Oxide Scavenging					
	10 µg/ml	20 µg/ml	40 µg/ml	80 µg/ml	160 µg/ml	IC_{50}
F1	17.45±0.57	23.57±0.49	35.75±2.14	42.24±2.96	49.84±2.57	-
F2	13.79±0.49	24.65±1.29	36.83±0.95	48.81±2.41	57.98±2.92	112.8
F3	4.27±0.21	9.96±0.58	18.98±0.44	25.94±1.36	34.96±1.29	-
F4	14.63±0.54	25.17±1.45	37.03±1.52	49.84±2.41	64.01±2.63	101.2
F5	3.32±0.19	8.54±0.33	18.51±0.63	24.36±1.35	33.86±1.56	-
F6	27.11±1.34	42.53±2.77	58.07±1.13	74.62±2.36	89.7±1.33	39.9
F7	27.64±2.21	44.09±2.75	59.34±1.49	71.88±2.96	87.12±2.10	39.2
F8	5.53±0.27	18.67±1.18	24.36±1.12	32.91±1.64	40.66±2.43	-
F9	2.84±0.14	8.38±0.42	18.03±0.47	22.94±1.14	33.69±1.84	-
F10	3.79±0.22	10.6±0.53	18.67±0.38	25.94±1.68	30.06±1.80	-
F11	4.52±0.27	7.59±0.36	15.18±1.29	22.15±1.32	29.11±1.74	-
F12	4.27±0.29	9.96±0.69	16.93±1.53	22.62±1.38	31.01±2.17	-
HF1	30.53±1.83	43.98±2.63	57.43±2.39	72.46±1.34	87.02±2.22	38.0
HF2	28.48±1.70	43.03±2.16	59.33±1.86	74.05±2.44	87.65±2.25	38.2

HF3	26.4±1.55	44.24±1.92	59.8±2.45	74.36±2.29	86.52±2.73	38.7
HF4	28.48±1.36	42.56±2.41	60.44±1.84	74.05±2.42	87.5±5.25	37.9
HF5	30.37±1.82	45.72±2.74	58.06±2.36	72.15±2.73	87.02±4.09	36.5
HF6	26.6±1.22	44.8±1.83	59.6±1.57	75.4±2.65	91.6±2.16	37.2
HF7	28.32±1.41	46.87±1.89	60.01±1.78	73.57±2.41	88.13±2.96	35.2
HF8	30.69±1.72	43.19±2.23	61.55±1.41	75.15±2.50	89.87±2.34	34.2
Gallic acid	32.27±1.93	45.25±2.46	62.34±1.74	80.22±2.56	98.1±2.88	28.8

All values are Mean ± SEM, n = 3. One way Analysis of Variance (ANOVA) followed by Dunnett's test was performed as the test of significance.

CONCLUSION: The study deals with the synthesis and characterization of novel flavone derivatives by various spectroscopic techniques. An *insilico* docking studies of the twenty target molecules with myeloperoxidase enzyme results showed the best docking score were obtained for the target molecules are in the range of -6.66 to -8.56 kcal/mol. This effect is payable to the presence of hydroxyl group in 2-phenyl-1-benzopyran-4-one moiety with its structure. Further, these synthetic compounds were subjected for *in-vitro* antioxidant activity. Based on the results, the synthesised flavones with electron donor substitution over the ring C and hydroxyl group substitution at position 7 of ring A *viz*. HF1-HF8 were showed a significant scavenging effect on free radicals inhibition when compared with their respective standard drug. Upon docking study results it was quite understood that all the basic analogue of this synthesized compounds showed a best target- protein interaction, which was further confirmed by its least binding score. Thus, this study concluded that the synthesised flavones, with hydroxyl substitution, were posses a great efficacy in antioxidant activity.

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