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Chrysin-piperazine conjugates as antioxidant and anticancer agents

Rahul V. Patel[a]*, Riyaz Syed[c], Anuj K. Rathi[d], Yoo-Jung Lee[e], Jung-Suk Sung[e] and Young-Soo Keum[b*]

[a] *Laboratory of Growth Regulators, Centre of the Region Haná for Biotechnological and Agricultural Research, Institute of Experimental Botany ASCR & Palacky´ University, Šlechtitelu˚ 27, 783 71 Olomouc, Czech Republic*

[b] *Organic Research Laboratory, Department of Bioresources and Food Sciences, College of Life and Environmental Sciences, Konkuk University, Seoul, Republic of Korea*

[c] *Department of Chemistry, J.N.T.University, Kukatpally, Hyderabad, 500 085, India.*

[d] *Regional Centre of Advanced Technologies and Materials, Department of Physical Chemistry, Faculty of Science, Palacky University, Šlechtitelů 11, Olomouc, Czech Republic*

[e] *Department of Life Science, Dongguk University, Goyang, Gyeonggi-do 10326, Republic of Korea*

*Correspondence to: Rahul V. Patel, email: rahul.svnit11@gmail.com and Young-Soo Keum, email: rational@konkuk.ac.kr

Abstract

Synthesis of 7-(4-bromobutoxy)-5-hydroxy-2-phenyl-4H-chromen-4-one intermediate treating chrysin with 1,4-dibromobutane facilitated combination of chrysin with a wide range of piperazine moieties which were equipped via reacting the corresponding amines with bis(2-chloroethyl)amine hydrochloride in diethylene glycol monomethyl ether solvent. Free radical scavenging potential of prepared products was analyzed *in vitro* adopting DPPH and ABTS

bioassay in addition to the evaluation of *in vitro* anticancer efficacies against cervical cancer cell lines (HeLa and CaSki) and an ovarian cancer cell line SK-OV-3 using SRB assay. Bearable toxicity of **7a-w** was examined employing Madin-Darby canine kidney (MDCK) cell line. In addition, cytotoxic nature of the presented compounds was inspected utilizing Human bone marrow derived mesenchymal stem cells (hBM-MSCs). Overall, **7a-w** indicated remarkable antioxidant power in scavenging $DPPH^{\cdot}$ and $ABTS^{\cdot+}$, particularly analogues **7f**, **7j**, **7k**, **7l**, **7n**, **7q**, **7v**, **7w** have shown promising free radical scavenging activity. Analogues **7j** and **7o** are identified to be highly active candidates against HeLa and CaSki cell lines, whereas **7h** and **7l** along with **7j** proved to be very sensitive towards ovarian cancer cell line SKOV-3. None of the newly prepared scaffolds showed cytotoxic nature toward hBM-MSCs cells. From the structure–activity point of view, nature and position of the electron withdrawing and electron donating functional groups on the piperazine core may contribute to the anticipated antioxidant and anticancer action. Different spectroscopic techniques (FT-IR, ^{1}H NMR, ^{13}C NMR, Mass) and elemental analysis (CHN) were utilized to confirm the desired structure of final compounds.

Keywords: Chrysin, piperazines, antioxidant, anticancer, flavones

1. Introduction

Reactive oxygen species (ROS) are free radicals carrying unpaired electron in their outer orbit and typically produced during normal aerobic cellular metabolism. ROS play a key role in biological evolution and the origin of life (McCord, 2000), capable of reacting with essential macromolecules thereby introduce damage to vital cell particles, DNA, proteins and lipids and

lift the chances of disease processes. There are numbers of ROS, such as superoxide; hydrogen peroxide; a hydroxyl radical; hydroxyl ion; and nitric oxide, which are formed via the addition of electrons implying a sequential reduction of oxygen (Hancock et al., 2001). The move instability between oxidant/antioxidant in support of oxidants is known as oxidative stress, which plays a role in many pathological conditions, including cancer, neurological disorders, atherosclerosis, hypertension, ischemia/perfusion, diabetes, acute respiratory distress syndrome, idiopathic pulmonary fibrosis, chronic obstructive pulmonary disease and asthma (Valko et al., 2007). Natural or synthetic endogenous compounds, responsible for destroying ROS, inhibiting their formation, scavenging their precursors and capable of ROS formation upon binding metal ions are termed as antioxidant (Gilgun-Sherkie et al., 2001). Studies support the statement that several antioxidant molecules acquire antitumor, antimicrobial, antiviral and anti-inflammatory effects (Mitscher et al., 1996).

Malignancies figure among the main reasons for deaths and loss of life rate worldwide, with roughly 14 million new cases and 8.2 million cancer-related fatalities in 2012 (WHO, 2015). Over the next two decades, the number of new situations is predicted to rise by about 70% as from 14 million in 2012 to 22 million (WHO, 2015). Among all objectives of cancer research, sensitive oxygen varieties (ROS) play a crucial role in anticancer agent discovery. Since, the creation of extreme ROS outcomes in the launch of cytochrome c from mitochondria (via perturbation of the mitochondrial membrane potential) into the cytosol and inevitably triggers caspase-9 expression followed by initial of executioner caspases including caspases-3 and -7 which induce execution phase of apoptosis (Simon et al., 2000). Furthermore, initial of caspase-8 is closely engaged in the extrinsic signaling pathway of apoptosis (Jin et al., 2009) which

associated with the destruction of NF-κB translocation (Yang et al., 2004). If the action of this aspect is obstructed, tumor cells can go through apoptosis (Dalen and Neuzil, 2003).

Natural products have traditionally been an extremely effective resource for new medicines in all cultures and continue to provide a huge variety of structural layouts for drug discovery and development. Before the post-genomic era and the emergence of high throughput screening, nearly 80% of drug-like molecules rose from natural products or their semisynthetic analogs generated through synthetic modification in the core of natural compounds (Harvey, 2008; Katiyar et al., 2012). Among the extensive variety of natural products, flavonoids are a broad class of polyphenolic secondary metabolites abundant in plants and various common foods. Chrysin, a naturally wide distributed flavonoid, has been revealed to have a plenty of pharmacological actions such as antioxidant (Fonseca et al., 2015; Wang et al., 2014) and anticancer agents (Zhu et al., 2014; Liu et al., 2014). It has a precautionary effect on cancer caused chemically (DMBA-induced hamster buccal pouch carcinomas) as well as on xenograft tumor models by inducing the activity of antioxidant and detoxification enzymes (glutathione peroxidase, glutathione, glutathione reductase, glutathione S-transferase and quinone Reductase), decreasing the actions of cytochrome P450 (CytP450)-dependent monooxygenases, suppressing cellular proliferation, invasion, angiogenesis and inducing apoptosis (Karthikeyan et al., 2013). To be able to provide semi-synthetic derivatives of chrysin, we have chosen piperazine skeletons to link with this flavone moiety because we have successful encounter in previous research with piperazines delivering significant important medicinal results (Patel et al., 2013).

2. Results and discussion

2.1 Chemistry

The scaffolds rationalized in the present study were prepared to adopt chemical strategies described in Scheme 1. An efficient procedure to prepare *N*-aryl or *N*-heteroaryl piperazines was adopted reacting corresponding anilines (**1a-w**) with bis(2-chloroethyl)amine hydrochloride (**2**) in the presence of diethylene glycol monomethyl ether as described in the literature (Liu et al., 2005). The analytical data of the synthesized piperazines were of adequate accordance with those reported in the literature (Liu et al., 2005; Igor et al., 2011). For example, FT-IR data shows aromatic C-H and C=C stretching bands at 3077 cm^{-1} and 1584 cm^{-1}, respectively for the compound **3w**. In addition, C-N band corresponding to the piperazine ring appeared at 1305 cm^{-1}. In further analysis, ^{1}H NMR spectrum of **3w** displayed characteristic signals for the proton atoms of the piperazine ring at 3.52 ppm and 3.27 ppm in the form of multiplets. The types of analysis data were consistent for all other piperazine moieties explored and were further treated in the reaction sequence. In an another step of synthetic transformation, intermediate 7-(4-bromobutoxy)-5-hydroxy-2-phenyl-4H-chromen-4-one (**6**) was delivered in 83% of yield reacting readily available chrysin (**4**) with 1,4-dibromobutane (**5**) under an N_2 atmosphere in the presence of a base (Hu et al., 2011). The OH group observed at 3073 cm^{-1} in the FT-IT spectrum of **6**, whereas carbonyl functionality confirmed upon observing its characteristic band at 1642 cm^{-1}. In the ^{1}H NMR spectrum of **6**, proton atoms present on the phenyl ring of chrysin core, resonated as multiplet signals in the range 7.88-7.47 ppm, however, the proton of hydroxyl group showed its signal as a singlet at 12.64 ppm. Furthermore, three proton atom signals in the form of the doublet, singlet and doublet at 6.42 ppm, 6.62 ppm and 6.33 ppm attributed to the chromane

ring. At last, methylene proton atoms of the aliphatic chain appeared to have their characteristic signals in the form of triplet and multiplet in the range between 4.11-1.91 ppm. ^{13}C NMR spectrum further confirmed the presence of the attachment of aliphatic chain with their characteristic signals in the range 67.7 ppm to 28.7 ppm. All spectroscopic data of compound **6** were inaccurate accordance with those reported earlier in the literature (Hu et al., 2011). In the continue reaction sequences, piperazines **3a-w** were allowed to connect with chrysin core utilizing Intermediate **6** under reflux conditions in acetonitrile solvent to furnish titled compounds **7a-w** in 44-67% of yields. Compound **7j** exhibited aromatic C-H and C-C stretching frequencies at 3075 cm^{-1} and 2926 cm^{-1}. Moreover, ^{1}H NMR signals (**7j**) for the chromane rings and its phenyl moiety as well as aliphatic chain were in accordance with those observed for intermediate **6** in addition to the presence of two multiplet signals at 3.51 ppm and 3.38 ppm for the proton atoms of piperazine core. In addition, proton atoms present in the form of two methyl groups on the phenyl ring of piperazine entity appeared to have their signals at 1.92 ppm as a singlet. The ^{13}C NMR data observed for compound **7j** further confirmed the correct formation of the desired structure of the compounds. Mass spectrometric data were inaccurate accordance as observed from the M^{+} ion values for the final compounds **7a-w**. All of the novel compounds gave C, H and N analyses within 0.4 percent points from the theoretical values, i.e. in an acceptable range.

2.2 Pharmacology

2.2.1 Antioxidant activities

To evaluate the free radical scavenging activity of flavonoids, a DPPH (2,2-diphenyl-1-picryhydrazyl) assay was performed, and the results are expressed in terms of IC_{50} value (concentration required to inhibit 50% of the radicals) as summarized in **Table 1**. Researchers have demonstrated that chrysin bears low level of antioxidant power in DPPH and ABTS (2,2'-azinobis (3-ethylbenzthiazoline-6-acid)) assay (Fonseca et al., 2015; Sim et al., 2007) and hence in the present study, coupling of pharmaceutically diverse piperazines, morpholine and piperidine rings to the chrysin core has been carried out and the resulting scaffolds **7a-w** possessed 20.30 ± 0.476 - 34.06 ± 0.913 μg/mL and 5.569 ± 0.025 - 8.971 ± 0.881 μg/mL of IC_{50}s in DPPH and ABTS bioassay, respectively and can be comparable to that of control ascorbic acid with 12.72 ± 0.274 μg/mL (DPPH) and 5.0925 ± 0.2090 μg/mL (ABTS). From the bioassay data observed in both of these bioassay evaluations, it can be stated that presence of a variety of piperazine derivatives as linkers to the chrysin core has significant effects on radical scavenging capabilities of the resultant molecules. Electron withdrawing and electron donating functional groups influenced the antioxidant power of corresponding titled analogues as well. Outputs of DPPH bioassay revealed that the bulk of piperazine, morpholine and piperidine substituents connected to the chrysin core are essential to trigger the DPPH radical scavenging potencies, as those with very low bulk and high bulk did not present a significant level of IC_{50s} because they were not able to scavenge 50% of the DPPH radical. For example, molecule **7a** and **7b** with low bulk showed >28 μg/mL of IC_{50}s as well as scaffolds **7d**, and **7e** exerted >28 μg/mL of IC_{50}s. In addition, unsubstituted piperazine analogues with a single phenyl ring were inactive

(**7c** and **7d**), whereas compound with two phenyl rings in the form of 1-naphthyl piperazine entity (**7f**) expressed excellent DPPH radical scavenging efficacy at 20.30 ± 0.476 μg/mL of IC_{50}. In the case of nitrogen heterocycle containing piperazine moieties, that with pyrimidyl ring bearing two nitrogen atom (**7h**) was more powerful against DPPH radical than that of pyridine bearing piperazine molecule (**7g**) with IC_{50}s, 22.32 ± 1.042 μg/mL and 34.06 ± 0.913 μg/mL, respectively. Furthermore, substitution pattern of EWD or ED functional groups on the aryls ring of the piperazine moiety was the key to exercise positively influenced DPPH radical scavenging potencies, as those scaffolds with *p*-substituted aryl piperazines were more active than their *ortho*-, *meta*- and di-substituted congeners. For instance, compound **7k** with *p*-alkoxy functionality featured 23.09 ± 0.351 μg/mL of IC_{50} followed by the compound with 2,4-disubstituted alkoxy phenylpiperazine with 25.04 ± 0.668 μg/mL of $IC_{50.}$ The same pattern was seen in the case of compound incorporating methyl- (**7i** and **7j**) and nitro- (**7m** and **7n**) phenylpiperazines. However, it was observed that compounds with di-substituted functional groups (one group should be present at *para*-position) were more active in DPPH assay than their similar functionality congeners with *ortho*-substitution, as compound **7t** with p-fluorophenyl piperazine ring showed 24.24 ± 1.382 μg/mL of IC_{50} followed by **7u** with 2,4-difluorophenyl piperazine ring with 26.97 ± 1.159 μg/mL of IC_{50} and **7s** with ortho-fluorophenyl piperazine moiety with 30.10 ± 0.938 μg/mL of $IC_{50.}$ At last, analogues with *p*-trifluoromethyl (**7v**) and *p*-trifluoromethoxy (**7w**) functional groups showed tremendous antioxidant power in scavenging $DPPH^{\cdot}$ with 20.62 ± 1.237 μg/mL and 21.56 ± 1.027 μg/mL of $IC_{50,}$ respectively.

<**Table 1**>

The $ABTS^{\cdot+}$ assay is based on a single electron transfer, the ABTS radical cation decolorization, which is based on the reduction of $ABTS^{\cdot+}$ radicals by antioxidants. To evaluate the free radical scavenging activity of flavonoids, ABTS assay was performed, and the results are expressed regarding IC_{50} value (concentration required to inhibit 50% of the radicals) as summarized in **Table 1**. It was observed that performance of titled compounds as antioxidant agents was better in ABTS assay compared to DPPH assay, as four among twenty-three tested analogues expressed around 5 μg/mL of $IC_{50s,}$ comparable to that of the control drug ascorbic acid with 5.0925 ± 0.2090 μg/mL. Opposite to DPPH assay, herein, no direct relation between the position of the EWD or ED substituent on the aryl ring of piperazine entity and corresponding ABTS radical scavenging capacities of resultant compounds has been shown. For example, compound **7p** with meta-chlorophenyl piperazine has 7.707 ± 0.034 μg/mL of TC50 when compared to that (8.049 ± 0.034 μg/mL) of **7q** holding para-chloro moiety. In fact, nature of the substituent played a key role in delivering the $ABTS^{\cdot+}$ scavenging potential for the resultant scaffolds. For example, similar to DPPH assay, compound **7f** with 1-naphthyl piperazine entity exercised potent antioxidant power with 5.625 ± 0.035 μg/mL of $IC_{50.}$ Furthermore, titled analogues with EWD alkyl (**7j**) and alkoxy (**7k** and **7l**) functional groups demonstrated 5.570 ± 0.084 μg/mL, 5.569 ± 0.025 μg/mL and 5.733 ± 0.005 μg/mL of $IC_{50s,}$ respectively, comparable to ascorbic acid. All analogues with halogen atoms (**7o-7w**) observed to have almost similar level of $ABTS^{\cdot+}$ scavenging activity with IC_{50}s in the range 7.012 ± 0.192 - 7.971 ± 0.881 μg/mL, whereas, analogues with nitro substituents (**7m** and **7n**), heterocyclic rings (**7g** and **7h**), unsubstituted aryl rings (**7c-7e**) as well as with morpholine (**7a**) and piperidine (**7b**)

entities showed least radical scavenging effects with IC_{50}s in the range 8.048 ± 0.104 - 8.608 ± 0.199 μg/mL and can be considered to have moderately active antioxidant agents. Hence, from the ABTS bioassay data, it is worth to state that compounds with ED groups were more active than those bearing EWD groups. Overall, all the scaffolds generated in the present study displayed anticipated antioxidant power as compared to the control.

2.2.2 Anticancer activities

Analogues **7a-w** were evaluated in the 3-cell line panel consisting of HeLa (cervical) CaSki (cervical) and SK-OV-3(ovarian). Endpoint determinations were made with a protein binding dye, sulforhodamine B (SRB) called SRB assay. Literature revealed that parent compound chrysin exerts 10.93 μg/mL of IC_{50}s against HeLa cell line. In fact, substitution of piperazines constructed in the present study to the chrysin core via a butyl chain revealed a constant activity profile for the resultant analogues as anticancer agents than those described in the literature with ethylene linker substituting aliphatic amines and smaller bulky piperazines (Hu et al., 2011). Hence with the aim of obtaining more potent chrysin analogues, the results of anticancer screening tests for **7a-w** against cervical cancer cell lines are summarized in **Table 2**. Similar to antioxidant assay results, in the case of anticancer potential of titled analogues, nature of the substituent present on the aryl ring of the piperazine moiety as well as its position was essential. Scaffolds **7a-w** presented an appreciable overall level of potencies against HeLa and CaSki cell lines with 5.044 ± 0.423 - 9.914 ± 0.445 and 4.213 ± 0.202 - 9.127 ± 0.153 μg/mL of IC_{50}s, respectively. In addition, it can be stated that analogies **7a-w** were more active against CaSki cell lines than HeLa. Moreover, cytotoxic concentration (CC_{50s}) of presented molecules against hBM-MSCs cells ranged from 4.809 ± 0.050 mg/mL to 23.59 ± 0.061 mg/mL presenting

them very selective drug-like molecules. As stated in the antioxidant assay, analogue with a bulky substituent (**7d**) was found in active too in the anticancer assay with >8 μg/mL of IC_{50} against both cervical cancer cell lines. Furthermore, incorporation of unsubstituted phenyl ring on the piperazine entity (**7c** and **7f**) as well as heterocyclic ring systems as pyridyl (**7g**) and pirimidyl (**7h**) entities exerted significant anticancer efficacies against HeLa cell line with 5.562 ± 0.282 μg/mL, 5.051 ± 0.170 μg/mL, 5.104 ± 0.164 μg/mL and 5.643 ± 0.199 μg/mL of IC_{50}s, respectively. Bearable toxicity of **7a-w** towards healthier tissues was examined employing Madin-Darby canine kidney (MDCK) cell lines, and scaffolds **7f** showed 284.5 ± 3.422 μg/mL of CC_{50}, thereby exhibited potent selectivity index (SI) of 56.33. In addition, **7h** had also exercised bearable level of toxicity and strong SI of 48.27. In the case of effects of EWD and ED functional groups present on the aryl rings of piperazine core, those with di-substitution pattern revealed good potency against HeLa than those with mono-substituted functional groups. For example, analogues **7j** with 2,4-dimethylphenyl piperazine, **7r** with 2,3-dichlorophenyl piperazine and **7u** with 2,3-difluorophenyl piperazine moieties presented 5.643 ± 0.199 μg/mL, 5.499 ± 0.088 μg/mL and 5.044 ± 0.423 μg/mL of IC_{50}s, respectively than their corresponding mono-substituted molecules (**7i**, **7o-7q** and **7s-7t**, IC_{50}s: >6 μg/mL). Analogue **7j** found to exercise desirable level of cytotoxicity at 323.1± 3.772 μg/mL of CC_{50} and exhibited 57.26 of SI with 10.26 ± 0.062 mg/mL of CC_{50} displayed against hBM-MSCs cells. Furthermore, an exceptional compound (**7o**) with mono-substituted functional group in the form of chlorine atom on the *ortho*-position of phenyl ring of piperazine entity expressed 6.361 ± 0.255 μg/mL of IC_{50}**,** 371.1 ± 1.856 μg/mL of CC_{50} and excellent SI of 58.34, which was the most potent analogue against HeLa cell lines among all tested. In the case of inhibition potencies of **7a-w** against

CaSki cell line, titled compounds with 1-benzoylpiperazine (**7c**) and 1-furoylpiperazine core (**7e**) presented an excellent level of IC_{50}s, 5.116 ± 0.321 μg/mL and 5.367 ± 0.222 μg/mL, respectively. Compound **7e** had cytotoxicity level of 264.6 ± 6.571 μg/mL and exerted moderate selectivity index of 49.30. This compound had succeeded to show highly tolerant cytotoxic nature as evaluated in the form of CC_{50} at 15.43 ± 0.080 mg/mL against hBM-MSCs cells in addition to the similar nature of **7c** with 18.46 ± 0.058 mg/mL of CC_{50} in the same experiment. Among those compounds with electron releasing alkyl substituents, compound with di-substitution (**7j**, IC_{50}: 4.872 ± 0.134 μg/mL) was active than mono-substituted one (**7i**, IC_{50}: 5.134 ± 0.096 μg/mL) as well as in case of alkoxy functional groups, analogue with mon-substitution was (**7k**, IC_{50}: 6.227 ± 0.097 μg/mL) favorable than di-substituted analogue (**7l**, IC_{50}: 7.628 ± 0.146 μg/mL). It is worth to state that molecules are bearing methyl functional group(s) namely **7i** and **7j** had desirable cytotoxicity as 298.1 ± 1.207 μg/mL and 323.1± 3.772 μg/mL, thereby furnishing strong selectivity indices of 58.05 and 66.32, respectively. Scaffolds with a nitro functional group (**7m** and **7n**), those with morpholine (**7a**), piperidine (**7b**) as well as heterocyclic-piperazine resides (**7g** and **7h**) were found inactive against CaSki with <30 of TI. Compound **7n** demonstrated 18.89 ± 0.041 mg/mL of CC_{50} against hBM-MSCs cells and found to have very tolerable cytotoxicity towards human non-cancer cells. Moreover, among the final compounds, analogues with *ortho*-substituted phenylpiperazine rings were concluded to have better anticancer effects against CaSki when compared to their meta- or di-substituted structural analogues, for instance, analogue **7o** (Cl) and **7s** (F) displayed 4.650 ± 0.078 μg/mL and 4.764 ± 0.251 μg/mL of IC_{50}s, 371.1 ± 1.856 μg/mL and 277.7 ± 0.162 μg/mL of CC_{50}s as well as 79.81 and 59.29 SIs, respectively. Analogue **7o** was proved to be the most potent analogue among all

tested against both HeLa and CaSki cell lines. Compounds **7r** with 2,3-dichlorophenyl piperazine and **7u** with 2,4-difluorophenyl piperazine ring appeared to have 4.222 ± 0.129 and 4.213 ± 0.202 μg/mL of IC_{50}s, 212.3 ± 1.436 μg/mL and 203.5 ± 2.429 μg/mL of CC_{50}s as well as 50.28 and 48.30 of SIs, respectively. Moreover, final two compounds **7v** and **7w** with *p*-trifluoromethyl and the *p*-trifluoromethoxy functional group were inactive with SIs of 34.49 and 27.45, respectively, where analogue **7v** indicated 23.59 ± 0.061 mg/mL of CC_{50} level against hBM-MSCs cells and was the scaffold with most tolerable cytotoxicity level toward non-cancer human cell line. These facts reveal that compounds with *p*-halo substituted phenylpiperazine rings were inactive against CaSki cell line.

<**Table 2**>

Chrysin has no activity against ovarian cancer cell line SK-OV-3 as concluded in a previous research study (Sak, 2014). In fact, it has a moderate level of cancerous cell inhibitory profile (IC_{50}s) of several types of cancer cell lines (Kasala et al., 2015). Hence, analogues **7a-w** were tested to inspect their *In vitro* inhibitory efficacies against ovarian cancer cell line SK-OV-3with an aim to observe the influence of piperazine substitution on the activity profiles and increase the anticancer effects of chrysin core. Although, the activity profiles were seen for **7a-w** against SK-OV-3were poor when compared to those observed in cervical cancer cell lines, still the activity against SK-OV-3 was appreciable as compared to parent chrysin. Titled compounds provided 12.876 ± 0.411 - 63.210 ± 0.158 μg/mL of IC_{50}s, 163.1 ± 5.741 - 371.1 ± 1.856 μg/mL of CC_{50}s as well as 3.22-21.24 of SIs. To reveal desirable SK-OV-3inhibitory effects, presence

of EWD or ED substituent as well as a heterocyclic core was essential, as scaffolds with unsubstituted phenylpiperazine rings (**7a-7f**) were found inactive with IC_{50}s ranging from 23.234 ± 0.169 - 63.210 ± 0.158 μg/mL and SIs of 3.22-6.74. Moreover, the presence of a heterocyclic ring (**7g** and **7h**) in the piperazine core enhances the potency of resultant molecules against SKOV-3. Though, a molecule with pyrimidine ring (**7h**, IC_{50}: 12.876 ± 0.411 μg/mL) was more active than that with pyridine ring (**7g**, IC_{50}: 17.324 ±0.322 μg/mL) with SI of 19.11 and 12.68, respectively. However, cytotoxicity levels of **7g** and **7h** ranged around 8 mg/mL as tested against hBM-MSCs cells. In the case of substitution pattern for EWD and ED, those with di-substituted functional groups were found excellently active when compared to their mono-substituted analogues. For instance, analogue **7j** with dimethyl, **7l** with dimethoxy, **7r** with dichloro and **7u** with difluoro substituents were reasonable more active with IC_{50}s, 15.213 ± 0.252 μg/mL, 14.213 ± 0.068 μg/mL, 16.983 ± 0.210 μg/mL and 14.332 ± 0.248 μg/mL, respectively than their mono-substituted congeners with IC_{50}s, >20 μg/mL. In fact, among EWD and ED substituted ones, those bearing EWD alkyl and alkoxy functional group were more potent than those carrying halo substituents. For example, **7j** and **7l** had very low cytotoxicity towards MDCK cell lines with 323.1± 3.772 μg/mL and 286.3 ± 4.250 μg/mL of CC_{50}s as well as 21.24 and 20.14 of SIs, respectively when compared to SIs of halo-substituted phenylpiperazine based compounds **7o-7w** with 4.55-12.50 SIs. Thus, analogues **7j** and **7l** were considered to have highest anticancer effects against SK-OV-3cell lines among all tested. Furthermore, *para*-substituted compounds (**7n**, **7q**, **7t**) were reasonable with IC_{50}s than those with *ortho*-substituted (**7m**, **7o**, **7p**, **7s**) ED or EWD groups, but their cytotoxicity was higher than those with *ortho*-substituted ones, resulting in overall better anticancer potencies of *ortho*-substituted analogues. For example, **7q** has IC_{50} of

31.289 ± 0.159 μg/mL and **7o** has IC_{50} of 36.321 ± 0.142 μg/mL, whereas their SI were observed at 6.63 and 10.22, respectively. Compound **7q** exhibited excellent CC_{50} against hBM-MSCs cells at 16.59 ± 0.044 mg/mL. Final two compounds with trifluoromethyl (**7v**) or trifluoromethoxy (**7w**) group indicated strong inhibition of SK-OV-3with IC_{50}s 22.654 ± 0.22 and 26.219 ± 0.133, CC_{50}s 280.3 ± 0.627 and 215.9 ± 3.231 as well as SIs of 12.37 and 8.23, respectively. Overall titled compounds **7a-w** presented an anticipated level of anticancer potential against ovarian cancer cell line SK-OV-3when compared to the inactive profile of parent chrysin compound.

<Table 3>

2.3 Quantitative structure-activity relationships (QSARs) of chrysin derivatives.

Regression coefficients (r^2) of QSAR equations were 0.4842-0.7636, indicating reasonable correlations with activities and molecular descriptors (**Table 4**). Combinations of some topological indices and 3D-MoRSE descriptors were generally included in the equations. In some cytotoxicity studies (Caski and MDCK) and antioxidant properties (DPPH), LUMO and molecular length gave positive correlation with activities (**Table 4**). For the cytotoxicities, other variables generally have shown negative relationships with IC_{50}s – negative sign of variables (**Table 4**). For example, increasing the atomic polarizability (Mor17p) and electronegativity (Mor22e) may decrease the IC_{50}s against SKOV cell lines (**Table 4**). However, there were more complex relationships between antioxidant activities (DPPH and ABTS) and molecular descriptors-positive correlations. In general, the selected descriptors for different activity test set were largely different from another set. It can be suggested that the major molecular properties

for each activity set are different may be a different requirement of proper ADME-properties (or reactivity).

<Table 4>

2.4 Correlations between different activities

Cytotoxicity of chrysin derivatives was tested against several cancer cell lines (HeLa, Caski, and SKOV) and normal kidney cell (MDCK). Statistical analysis of their IC_{50} indicated that there were weak (e.g., HeLa vs. Caski and SKOV) or no correlations (e.g., MDCK vs. cancer cells) (**Table 5**). Interestingly, ABTS-based antioxidant activity showed a reasonable correlation with the cytotoxicity of derivatives on MDCK cells, while the results from DPPH tests did not give appreciable relationships with the cytotoxicity (**Table 5**). It can be suggested that there are large biochemical differences between cell lines, and radical-related oxidative stress plays a minor role in chemical cytotoxicity.

<Table 5>

3. Conclusion

In summary, a new class of chrysin derivatives linked to a variety of substituted piperazines, morpholine and piperidine via a butyl chain was produced and analyzed for their antioxidant and anticancer action in vitro. The products were easily obtained implementing facile synthetic transformations. Overall, in the evaluation of the activity of identical kinds of scaffolds, it is worth to state that butyl chain and types of piperazine rings were optimal to furnish constant pharmacological actions as antioxidant and anticancer agents of resultant scaffolds. Bioassay outcomes recommended that analogues **7a-w** be the effective scavengers of free radicals DPPH$^{\cdot}$

and $ABTS^{\cdot+}$, proving themselves as a tool for the further antioxidant drug discovery process. To obtain DPPH scavenging efficiency, the bulk of the substituent connected to the chrysin core performed a crucial part. *Para*-substituted piperazines worked out better antioxidant potencies in DPPH assay as well as against CaSki (cervical cancer) and SK-OV-3 (ovarian cancer) cell lines. The existence of heterocycle in the piperazine moiety placed an essential activity profile against $DPPH^{\cdot}$ as well as against HeLa and SK-OV-3 cell lines. Titled compounds possessing ED functional groups were discovered extremely effective in scavenging $ABTS^{\cdot+}$ than those holding EWD groups, whereas, against HeLa cell line, di-substituted phenylpiperazine rings were valuable than their mono-substituted identical. Analogues **7f**, **7n**, **7q**, **7v**, **7w** were potent scavengers of free radical $DPPH^{\cdot}$, able to rapid donate a hydrogen atom to the radicals. Moreover, **7f**, **7j**, **7k** and **7l** were found excellent scavengers of $ABTS^{\cdot+}$ and observed as highly active antioxidant agents when compared to control ascorbic acid. Three scaffolds with 1-naphthylpipearzine (**7f**), 2,4-dimethylphenyl piperazine (**7j**) as well as 2-chlorophenyl piperazine (**7o**) entities showed promising action against HeLa cell line, whereas **7j**, **7i** with 4-methylphenyl piperazine, **7o** and **7s** with 2-fluorophenyl piperazine rings expressed strong inhibition of CaSki cell line. Analogues **7h** with 1-pyrimidyl piperazine, **7j**, **7l** with 2.4-dimethoxyphenyl piperazine entity indicated significant anticancer perspective against ovarian cancer cell line SKOV-3. All the final compounds presented highly tolerant cytotoxic nature towards human bone marrow derived mesenchymal stem cells (hBM-MSCs) and thus can be presented as an important class of anticancer drug-like molecules. Lastly, the high degree of sensitivity and selectivity indicated by **7a-w** against two types of free radicals and three types of cancer cell lines enhances the possibility of their upcoming derivatization to find out a different class of flavone derivatives.

4. Experimental

All chemicals and solvents are commercially available used without purification or after distillation and treatment with drying agents. Reactions were monitored by thin-layer chromatography (TLC) on pre-coated silica gel plates (Kieselgel 60 F254, Merck) and visualized by UV254 light. Shimadzu 8400-S FT-IR spectrophotometer was used to obtain FT-IR spectra of the title compounds. NMR spectra were taken using a Bruker AVANCE III 400 instrument (^{1}H NMR, 400 MHz; ^{13}C NMR, 100 MHz). ^{1}H NMR spectra are represented as follows: chemical shift, multiplicity (s= singlet, d= doublet, t= triplet, q= quartet, m= multiplet), integration, and coupling constant (*J*) in Hertz (Hz). ^{1}H NMR and ^{13}C NMR chemical shifts are reported relative to $CDCl_3$ as an internal standard. The purity of compounds was determined by elemental analyses performed using CHN analyzer.

4.1 General Procedure for the Synthesis of Piperazine derivatives 3a-w

In a flask charged with diethylene glycol monomethyl ether (8 mL) added equimolar quantities (25 mmol) of appropriate amine (**1a-w**) and bis(2-chloroethyl)amine hydrochloride (**2**). The resulting mixture was heated for 150 ^{o}C for 16–48 h under N_2 atmosphere in the presence of catalytic amount of KI (3 mol%). The progress of the reaction was monitored using TLC with the mobile phase toluene: acetone (8:2). After the completion of the reaction, the mixture was cooled to room temperature and dissolved in methanol (25 mL), and ethyl acetate (100 mL) was added. The precipitate thus obtained were filtered, washed with ethyl acetate and the resulting salt was then converted to free amines upon treating with Na_2CO_3 and extracted with ethyl acetate (2 x 25 mL). The combined organic phase was dried over $MgSO_4$ and evaporated to give

analytically pure piperazine derivatives **3a-w** in reasonably good yields. For example, **3w**: Yield: 72%, FT-IR (KBr, cm^{-1}): 3077 (aromatic C-H stretching), 1584 (aromatic C=C), 1305 (C-N stretching); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 9.57 (br s, NH, piperazine ring), 7.32 (d, *J* = 6.5 Hz, 2H, Ar-H), 7.11 (d, *J* = 6.5 Hz, 2H, Ar-H), 3.52 (m, 4H, piperazine), 3.27 (m, 4H, piperazine).

4.2 Synthesis of 7-(4-bromobutoxy)-5-hydroxy-2-phenyl-4H-chromen-4-one (6)

In a flask equipped with N_2 atmosphere and charged with 400 mL of acetone, added 118 mmol of Chrysin (**4**) and 1 eq. of 1,4-dibromobutane (**5**) in the presence of 1.05 eq. of potassium carbonate. The reaction mixture was refluxed for 24 h until no starting material left as monitored by TLC. After the reaction completion, the reaction mixture was concentrated, cooled at room temperature and diluted with ethyl acetate (100 mL) and washed with water (2 x 75 mL). The organic phase was separated and treated with $MgSO_4$ and then concentrated under vacuum to furnish a yellowish white colored compound **6** (Mistry et al., 2015a), Yield: 83%, IR (KBr) cm^{-1}: 3072, 2954, 2855, 1642, 1604, 1589; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 12.64 (s, 1H, OH), 7.88-7.81 (m, 2H, Ar-H), 7.61–7.47 (m, 3H, Ar-H), 6.62 (s, 1H, chromen-4-one ring), 6.42 (d, *J* = 2.2 Hz, 1H, chromen-4-one ring), 6.33 (d, *J* = 2.2 Hz, 1H, chromen-4-one ring), 4.11 (t, *J* = 6.0 Hz, 2H, CH_2, aliphatic chain), 3.45 (t, *J* = 6.5 Hz, 2H, CH_2, aliphatic chain), 2.19–2.09 (m, 2H, CH_2, aliphatic chain), 1.99–1.91 (m, 2H, CH_2, aliphatic chain); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 181.7 (OH), 165.6, 164.8, 163.0, 158.2, 133.2, 131.7, 129.6, 127.1, 106.1, 105.4, 98.5, 95.4, 67.7, 32.8, 29.9, 28.7; EMI–MS (m/z): 390.43 (M^+).

4.3 General procedure for the preparation of 5-hydroxy-2-phenyl-7-(4-(4-substituted piperazinyl/piperidinyl/morpholinyl)butoxy)-4H-chromen-4-ones (7a-w)

In a flask charged with 50 mL of CH_3CN was added 2.5 mmol of compound **6** and appropriate piperazine derivatives (**3a-w**, 1.5 eq.) and the reaction mixture was refluxed for 10-38 h until the complete consumption of starting material as detected by TLC. After the completion of the reaction, the reaction mixture was treated with ice and the resulting solid was filtered and washed with water (2 x 25 mL). The residue was purified with a silica gel column chromatography and was eluted with dichloromethane: methanol (40:1) to afford corresponding products **7a-w** in 49-82% of yields.

4.3.1 5-hydroxy-7-(4-morpholinobutoxy)-2-phenyl-4H-chromen-4-one (**7a**)

Yield: 63%. m.p. 253-255°C; IR (KBr) cm^{-1}: 3072, 2955, 2924, 2862, 1660, 1620, 1576, 1343, 1250, 1162, 1043; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 12.81 (s, 1H), 7.90–7.87 (m, 2H), 7.58–7.50 (m, 3H), 6.60 (s, 1H), 6.36 (d, *J* = 2.3 Hz, 1H,), 6.18 (d, *J* = 2.2 Hz, 1H,), 4.12 (t, *J* = 6.0 Hz, 2H), 3.61 (t, *J* = 6.5 Hz, 2H), 2.58-2.47 (m, 4H, CH_2, morpholine), 2.13–2.05 (m, 2H), 1.97–1.89 (m, 2H), 1.77-1.71 (m, 2H, CH_2, morpholine), 1.34-1.29 (m, 2H, CH_2, morpholine). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 181.8, 166.1, 163.7, 161.2, 157.4, 135.6-125.5, 108.6, 103.8, 98.3, 92.9, 68.6, 58.7, 53.1, 33.3, 28.1, 27.2. EMI–MS (m/z): 396.60 (M^+). Anal. Calcd. for $C_{23}H_{25}NO_5$: C, 69.86; H, 6.37; N, 3.54. Found: C, 69.98; H, 6.51; N, 3.63.

4.3.2 5-hydroxy-2-phenyl-7-(4-(piperidin-1-yl)butoxy)-4H-chromen-4-one (**7b**)

Yield: 67%. m.p. 235-237°C; IR (KBr) cm^{-1}: 3062, 2964, 2933, 2875, 1652, 1612, 1588, 1332, 1263, 1173, 1028; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 12.79 (s, 1H), 7.95–7.89 (m, 2H), 7.51–7.44 (m, 3H), 6.73 (s, 1H), 6.42 (d, *J* = 2.2 Hz, 1H), 6.10 (d, *J* = 2.3 Hz, 1H), 4.19 (t, J = 5.9 Hz, 2H), 3.86 (t, *J* = 5.3 Hz, 4H, piperidine), 3.71 (t, *J* = 5.7 Hz, 4H, piperidine), 3.44 (t, J = 6.4 Hz, 2H), 2.15–2.08 (m, 2H), 2.02–1.95 (m, 2H), 1.65-1.59 (m, 2H, piperidine). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 182.4, 164.8, 162.9, 159.6, 158.1, 139.3-127.4, 106.3, 105.4, 97.9, 93.3, 67.5, 55.4, 52.6, 44.1, 32.8, 30.2, 25.5. EMI–MS (m/z): 394.63 (M^+). Anal. Calcd. for $C_{24}H_{27}NO_4$: C, 73.26; H, 6.92; N, 3.56. Found: C, 73.41; H, 6.78; N, 3.68.

4.3.3. 7-(4-(4-benzoylpiperazin-1-yl)butoxy)-5-hydroxy-2-phenyl-4H-chromen-4-one (**7c**)

Yield: 49%. m.p. 265-267°C; IR (KBr) cm^{-1}: 3065, 2961, 2932, 2877, 1641, 1613, 1583, 1331, 1262, 1174, 1031; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 12.75 (s, 1H), 7.91–7.84 (m, 2H), 7.54–7.48 (m, 3H), 7.40–7.15 (m, 5H), 6.74 (s, 1H), 6.43 (d, *J* = 2.4 Hz, 1H), 6.11 (d, *J* = 2.4 Hz, 1H), 4.20 (t, *J* = 6.1 Hz, 2H), 3.57 (br s, 4H), 3.45 (t, *J* = 6.6 Hz, 2H), 3.21 (br s, 4H), 2.17–2.104 (m, 2H), 1.96–1.90 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 183.7, 165.9, 164.4, 161.7, 160.9, 156.7, 146.1, 144.6-126.7, 107.7, 104.7, 96.7, 92.3, 66.2, 51.8, 43.1, 34.6, 29.8, 26.9. EMI–MS (m/z): 499.71 (M^+). Anal. Calcd. for $C_{30}H_{30}N_2O_5$: C, 72.27; H, 6.06; N, 5.62. Found: C, 72.39; H, 6.14; N, 5.76.

4.3.4 7-(4-(4-benzhydrylpiperazin-1-yl)butoxy)-5-hydroxy-2-phenyl-4H-chromen-4-one (**7d**)

Yield: 44%. m.p. 285-287°C; IR (KBr) cm^{-1}: 3056, 2971, 2935, 2856, 1656, 1605, 1594; 1351, 1265, 1157, 1024; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 12.77 (s, 1H), 7.93–7.88 (m, 2H), 7.57–7.51

(m, 3H), 7.46–7.14 (m, 10H), 6.69 (s, 1H), 6.53 (d, *J* = 2.3 Hz, 1H), 6.28 (d, *J* = 2.4 Hz, 1H), 5.36 (s, 1H), 4.06 (t, *J* = 6.0 Hz, 2H), 3.59 (br s, 4H), 3.55 (t, *J* = 6.5 Hz, 2H), 3.31 (br s, 4H), 2.13–2.07 (m, 2H), 1.99–1.91 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 181.5, 165.2, 162.4, 159.3, 156.8, 154.7, 152.2, 149.7, 145.6-125.8, 108.5, 103.3, 98.7, 93.2, 68.8, 50.2, 42.3, 33.7, 28.4, 27.5. EMI–MS (m/z): 561.82 (M^+). Anal. Calcd. for $C_{36}H_{36}N_2O_4$: C, 77.12; H, 6.47; N, 5.00. Found: C, 77.21; H, 6.34; N, 5.12.

4.3.5 7-(4-(4-(cyclopenta-1,3-dienecarbonyl)piperazin-1-yl)butoxy)-5-hydroxy-2-phenyl-4H-chromen-4-one (**7e**)

Yield: 66%. m.p. 247-249°C; IR (KBr) cm^{-1}: 3059, 2972, 2928, 2853, 1628, 1615, 1571, 1338, 1256, 1158, 1022; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 12.74 (s, 1H), 7.89–7.85 (m, 2H), 7.52–7.46 (m, 3H), 7.37–6.97 (m, 5H), 6.65 (s, 1H), 6.50 (d, *J* = 2.3 Hz, 1H), 6.25 (d, *J* = 2.4 Hz, 1H), 4.03 (t, *J* = 6.0 Hz, 2H), 3.56 (br s, 4H), 3.52 (t, *J* = 6.5 Hz, 2H), 3.28 (br s, 4H), 2.17–2.10 (m, 2H), 2.03–1.93 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 183.7, 165.5, 164.0, 162.7, 160.5, 157.7, 153.2, 144.3-125.3, 108.7, 103.6, 98.4, 92.7, 68.5, 50.5, 42.6, 33.4, 28.5, 27.4. EMI–MS (m/z): 487.68 (M^+). Anal. Calcd. for $C_{29}H_{30}N_2O_5$: C, 71.59; H, 6.21; N, 5.76. Found: C, 71.43; H, 6.36; N, 5.91.

4.3.6 5-hydroxy-7-(4-(4-(naphthalen-2-yl)piperazin-1-yl)butoxy)-2-phenyl-4H-chromen-4-one (**7f**)

Yield: 45%. m.p. 281-283°C; IR (KBr) cm^{-1}: 3071, 2959, 2942, 2869, 1651, 1608, 1591, 1348, 1255, 1161, 1025; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 12.82 (s, 1H), 7.92–7.87 (m, 2H), 7.56–7.50

(m, 3H), 7.41–7.04 (m, 7H), 6.71 (s, 1H), 6.40 (d, *J* = 2.4 Hz, 1H), 6.08 (d, *J* = 2.2 Hz, 1H), 4.17 (t, *J* = 6.1 Hz, 2H), 3.61 (br s, 4H), 3.42 (t, *J* = 6.4 Hz, 2H), 3.18 (br s, 4H), 2.15–2.11 (m, 2H), 2.02–1.98 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 183.2, 165.3, 161.5, 159.0, 156.5, 154.2, 149.7, 137.2-123.9, 107.0, 103.2, 96.3, 92.5, 66.3, 50.1, 42.2, 33.1, 28.9, 25.1. EMI–MS (m/z): 521.53 (M^+). Anal. Calcd. for $C_{33}H_{32}N_2O_4$: C, 76.13; H, 6.20; N, 5.38. Found: C, 76.21; H, 6.38; N, 5.22.

4.3.7 5-hydroxy-2-phenyl-7-(4-(4-(pyridin-2-yl)piperazin-1-yl)butoxy)-4H-chromen-4-one (**7g**)

Yield: 47%. m.p. 245-247°C; IR (KBr) cm^{-1}: 3070, 2956, 2925, 2863, 1638, 1622, 1572, 1345, 1251, 1163, 1032; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 12.76 (s, 1H), 7.94–7.91 (m, 2H), 7.53–7.42 (m, 2H), 7.36–7.01 (m, 5H), 6.61 (s, 1H), 6.37 (d, *J* = 2.2 Hz, 1H), 6.19 (d, *J* = 2.2 Hz, 1H), 4.13 (t, *J* = 5.9 Hz, 2H), 3.63 (br s, 4H), 3.62 (t, *J* = 6.4 Hz, 2H), 3.37 (br s, 4H), 2.16–2.09 (m, 2H), 2.01–1.91 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 183.9, 166.5, 163.9, 160.2, 158.5, 156.3, 153.7, 138.9-127.8, 106.5, 105.7, 97.2, 94.5, 67.9, 52.5, 44.3, 32.1, 30.5, 25.9. EMI–MS (m/z): 472.71 (M^+). Anal. Calcd. for $C_{28}H_{29}N_3O_4$: C, 71.32; H, 6.20; N, 8.91. Found: C, 71.21; H, 6.04; N, 8.98.

4.3.8 5-hydroxy-2-phenyl-7-(4-(4-(pyrimidin-2-yl)piperazin-1-yl)butoxy)-4H-chromen-4-one (**7h**)

Yield: 56%. m.p. 266-268°C; IR (KBr) cm^{-1}: 3064, 2965, 2939, 2877, 1642, 1614, 1582, 1335, 1264, 1172, 1029; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 12.73 (s, 1H), 7.88–7.84 (m, 2H), 7.60–7.54 (m, 3H), 7.45–7.12 (m, 3H), 6.64 (s, 1H), 6.48 (d, *J* = 2.4 Hz, 1H), 6.24 (d, *J* = 2.2 Hz, 1H),

4.02 (t, *J* = 6.1 Hz, 2H), 3.55 (br s, 4H), 3.51 (t, *J* = 6.4 Hz, 2H), 3.27 (br s, 4H), 2.17–2.11 (m, 2H), 1.99–1.89 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 181.6, 164.4, 162.5, 160.3, 157.0, 156.3, 155.2, 143.9-126.3, 106.2, 105.5, 96.1, 93.7, 66.5, 51.4, 43.5, 32.4, 29.4, 26.5. EMI–MS (m/z): 473.39 (M^+). Anal. Calcd. for $C_{27}H_{28}N_4O_4$: C, 68.63; H, 5.97; N, 11.86. Found: C, 68.75; H, 5.82; N, 11.97.

4.3.9 5-hydroxy-2-phenyl-7-(4-(4-p-tolylpiperazin-1-yl)butoxy)-4H-chromen-4-one (**7i**)

Yield: 60%. m.p. 275-277°C; IR (KBr) cm^{-1}: 3067, 2966, 2940, 2857, 1644, 1621, 1585, 1334, 1274, 1179, 1020; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 12.78 (s, 1H), 7.90–7.85 (m, 2H), 7.51–7.44 (m, 3H), 7.42–7.08 (m, 4H), 6.76 (s, 1H), 6.45 (d, *J* = 2.2 Hz, 1H), 6.13 (d, *J* = 2.3 Hz, 1H), 4.07 (t, *J* = 5.9 Hz, 2H), 3.52 (br s, 4H), 3.47 (t, *J* = 6.4 Hz, 2H), 3.23 (br s, 4H), 2.23 (s, 3H), 2.14–2.05 (m, 2H), 1.96–1.91 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 182.1, 164.5, 163.4, 160.6, 157.1, 150.6, 141.2-127.1, 106.6, 105.8, 97.6, 92.2, 67.7, 52.4, 44.4, 32.3, 30.4, 26.4, 21.6. EMI–MS (m/z): 485.58 (M^+). Anal. Calcd. for $C_{30}H_{32}N_2O_4$: C, 74.36; H, 6.66; N, 5.78. Found: C, 74.21; H, 6.55; N, 5.86.

4.3.10 7-(4-(4-(2,4-dimethylphenyl)piperazin-1-yl)butoxy)-5-hydroxy-2-phenyl-4H-chromen-4-one (**7j**)

Yield: 56%. m.p. 261-263°C; IR (KBr) cm^{-1}: 3075, 2954, 2926, 2866, 1639, 1618, 1573, 1346, 1253, 1166, 1033; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 12.66 (s, 1H), 7.95–7.88 (m, 2H), 7.61–7.55 (m, 3H), 7.44–7.11 (m, 3H), 6.62 (s, 1H), 6.38 (d, *J* = 2.4 Hz, 1H), 6.20 (d, *J* = 2.3 Hz, 1H), 4.14 (t, *J* = 6.1 Hz, 2H), 3.63 (t, *J* = 6.6 Hz, 2H), 3.51 (br s, 4H), 3.38 (br s, 4H), 2.15–2.06 (m,

2H), 1.97–1.87 (m, 2H), 1.92 (s, 6H, $2CH_3$); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 183.3, 165.4, 163.2, 160.4, 158.9, 151.5, 142.7-126.9, 107.1, 104.0, 96.9, 92.1, 66.6, 51.3, 43.4, 34.5, 29.3, 25.2, 23.6. EMI–MS (m/z): 499.76 (M^+). Anal. Calcd. for $C_{31}H_{34}N_2O_4$: C, 74.67; H, 6.87; N, 5.62. Found: C, 74.79; H, 6.96; N, 5.74.

4.3.11 5-hydroxy-7-(4-(4-(4-methoxyphenyl)piperazin-1-yl)butoxy)-2-phenyl-4H-chromen-4-one (**7k**)

Yield: 57%. m.p. 252-254°C; IR (KBr) cm^{-1}: 3076, 2977, 2946, 2859, 1658, 1624, 1575, 1354, 1269, 1180, 1050; ^1H NMR ($CDCl_3$, 400 MHz): δ 12.69 (s, 1H), 7.91–7.83 (m, 2H), 7.57–7.50 (m, 3H), 7.38–7.02 (m, 4H), 6.78 (s, 1H), 6.47 (d, *J* = 2.3 Hz, 1H), 6.22 (d, *J* = 2.2 Hz, 1H), 4.23 (t, *J* = 6.2 Hz, 2H), 3.95 (s, 3H, OCH_3), 3.67 (br s, 4H), 3.49 (t, *J* = 6.5 Hz, 2H), 3.17 (br s, 4H), 2.17–2.08 (m, 2H), 2.02–1.96 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 183.0, 165.1, 163.8, 159.9, 157.8, 154.2, 143.8-125.2, 106.8, 104.6, 98.0, 94.9, 67.4, 56.7, 50.6, 44.7, 33.9, 29.9, 26.1. EMI–MS (m/z): 501.75 (M^+). Anal. Calcd. for $C_{30}H_{32}N_2O_5$: C, 71.98; H, 6.44; N, 5.60. Found: C, 71.85; H, 6.56; N, 5.74.

4.3.12 7-(4-(4-(2,4-dimethoxyphenyl)piperazin-1-yl)butoxy)-5-hydroxy-2-phenyl-4H-chromen-4-one (**7l**)

Yield: 51%. m.p. 262-264°C; IR (KBr) cm^{-1}: 3066, 2960, 2936, 2874, 1655, 1617, 1590, 1330, 1267, 1175, 1042; ^1H NMR ($CDCl_3$, 400 MHz): δ 12.80 (s, 1H), 7.93–7.87 (m, 2H), 7.52–7.44 (m, 3H), 7.36–7.26 (m, 3H), 6.72 (s, 1H), 6.41 (d, *J* = 2.3 Hz, 1H), 6.09 (d, *J* = 2.4 Hz, 1H), 4.18 (t, *J* = 6.0 Hz, 2H), 3.69 (s, 6H, $2OCH_3$), 3.53 (br s, 4H), 3.43 (t, *J* = 6.5 Hz, 2H), 3.19 (br s,

4H), 2.24–2.18 (m, 2H), 1.96–1.92 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 183.5, 166.4, 163.5, 161.3, 158.2, 155.4, 152.3, 143.8-125.7, 108.8, 103.4, 98.2, 94.4, 68.1, 57.4, 50.3, 42.4, 33.2, 28.7, 27.1. EMI–MS (m/z): 531.48 (M^+). Anal. Calcd. for $C_{31}H_{34}N_2O_6$: C, 70.17; H, 6.46; N, 5.28. Found: C, 70.29; H, 6.54; N, 5.41.

4.3.13 5-hydroxy-7-(4-(4-(2-nitrophenyl)piperazin-1-yl)butoxy)-2-phenyl-4H-chromen-4-one (**7m**)

Yield: 52%. m.p. 272-274°C; IR (KBr) cm^{-1}: 3053, 2953, 2938, 2873, 1645, 1610, 1595, 1355, 1257, 1160, 1047; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 12.70 (s, 1H), 7.89–7.84 (m, 2H), 7.56–7.48 (m, 3H), 7.34–7.04 (m, 4H), 6.56 (s, 1H), 6.46 (d, *J* = 2.2 Hz, 1H), 6.30 (d, *J* = 2.3 Hz, 1H), 4.16 (t, *J* = 5.9 Hz, 2H), 3.62 (br s, 4H), 3.57 (t, *J* = 6.4 Hz, 2H), 3.24 (br s, 4H), 2.16–2.05 (m, 2H), 1.98–1.87 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 182.9, 165.6, 163.6, 159.1, 158.0, 157.6, 152.9, 140.7-126.1, 107.4, 104.8, 97.8, 94.8, 67.6, 51.7, 43.7, 33.8, 29.7, 26.8. EMI–MS (m/z): 516.42 (M^+). Anal. Calcd. for $C_{29}H_{29}N_3O_6$: C, 67.56; H, 5.67; N, 8.15. Found: C, 67.44; H, 5.75; N, 8.26.

4.3.14 5-hydroxy-7-(4-(4-(4-nitrophenyl)piperazin-1-yl)butoxy)-2-phenyl-4H-chromen-4-one (**7n**)

Yield: 59%. m.p. 279-281°C; IR (KBr) cm^{-1}: 3074, 2958, 2945, 2861, 1647, 1604, 1580, 1540, 1347, 1329, 1252, 1168, 1035; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 12.61 (s, 1H), 7.92–7.85 (m, 2H), 7.53–7.43 (m, 3H), 7.40–7.13 (m, 4H), 6.58 (s, 1H), 6.34 (d, *J* = 2.2 Hz, 1H), 6.16 (d, *J* = 2.3 Hz, 1H), 4.10 (t, *J* = 5.9 Hz, 2H), 3.69 (br s, 4H), 3.59 (t, *J* = 6.6 Hz, 2H), 3.34 (br s, 4H), 2.21–2.16 (m, 2H), 1.97–1.88 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 182.5, 166.6, 163.3, 159.2, 158.4,

156.9, 152.9, 141.2-127.2, 107.9, 104.3, 97.7, 94.2, 67.3, 52.8, 44.1, 34.8, 30.1, 25.3. EMI–MS (m/z): 516.73 (M^+). Anal. Calcd. for $C_{29}H_{29}N_3O_6$: C, 67.56; H, 5.67; N, 8.15. Found: C, 67.42; H, 5.54; N, 8.03.

4.3.15 7-(4-(4-(2-chlorophenyl)piperazin-1-yl)butoxy)-5-hydroxy-2-phenyl-4H-chromen-4-one (**7o**)

Yield: 58%. m.p. 238-240°C; IR (KBr) cm^{-1}: 3063, 2962, 2943, 2871, 1643, 1616, 1584, 1333, 1273, 1177, 1045, 776; ^1H NMR ($CDCl_3$, 400 MHz): δ 12.65 (s, 1H), 7.94–7.89 (m, 2H), 7.55–7.45 (m, 3H), 7.35–7.01 (m, 4H), 6.75 (s, 1H), 6.44 (d, *J* = 2.3 Hz, 1H), 6.12 (d, *J* = 2.2 Hz, 1H), 4.21 (t, *J* = 6.0 Hz, 2H), 3.58 (br s, 4H), 3.46 (t, *J* = 6.5 Hz, 2H), 3.22 (br s, 4H), 2.15–2.04 (m, 2H), 2.02–1.97 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 181.1, 164.3, 161.8, 161.1, 157.3, 150.1, 141.2-125.6, 108.4, 103.0, 98.5, 94.7, 68.7, 50.9, 42.1, 33.5, 28.3, 27.3. EMI–MS (m/z): 505.41 (M^+). Anal. Calcd. for $C_{29}H_{29}ClN_2O_4$: C, 68.97; H, 5.79; N, 5.55. Found: C, 68.83; H, 5.87; N, 5.47.

4.3.16 7-(4-(4-(3-chlorophenyl)piperazin-1-yl)butoxy)-5-hydroxy-2-phenyl-4H-chromen-4-one (**7p**)

Yield: 58%. m.p. 233-235°C; IR (KBr) cm^{-1}: 3077, 2975, 2927, 2867, 1640, 1619, 1574, 1349, 1254, 1159, 1034, 748; ^1H NMR ($CDCl_3$, 400 MHz): δ 12.79 (s, 1H), 7.88–7.83 (m, 2H), 7.58–7.49 (m, 3H), 7.46–7.12 (m, 4H), 6.63 (s, 1H), 6.39 (d, *J* = 2.3 Hz, 1H), 6.21 (d, *J* = 2.2 Hz, 1H), 4.08 (t, *J* = 6.0 Hz, 2H), 3.64 (t, *J* = 6.5 Hz, 2H), 3.56 (br s, 4H), 3.39 (br s, 4H), 2.12–2.06 (m, 2H), 2.03–1.92 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 181.2, 166.9, 162.6, 160.8, 158.7, 153.2, 139.7-126.2, 107.2, 103.5, 98.6, 93.4, 68.9, 51.5, 43.6, 33.6, 29.5, 25.9. EMI–MS (m/z): 505.39

(M^+). Anal. Calcd. for $C_{29}H_{29}ClN_2O_4$: C, 68.97; H, 5.79; N, 5.55. Found: C, 68.86; H, 5.66; N, 5.42.

4.3.17 7-(4-(4-(4-chlorophenyl)piperazin-1-yl)butoxy)-5-hydroxy-2-phenyl-4H-chromen-4-one (**7q**)

Yield: 54%. m.p. 255-256°C; IR (KBr) cm^{-1}: 3068, 2967, 2931, 2876, 1654, 1611, 1577, 1329, 1268, 1176, 1044, 781; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 12.60 (s, 1H), 7.90–7.84 (m, 2H), 7.51–7.41 (m, 3H), 7.37–6.99 (m, 4H), 6.57 (s, 1H), 6.33 (d, *J* = 2.3 Hz, 1H), 6.15 (d, *J* = 2.4 Hz, 1H), 4.09 (t, *J* = 5.9 Hz, 2H), 3.68 (br s, 4H), 3.58 (t, *J* = 6.6 Hz, 2H), 3.33 (br s, 4H), 2.12–2.04 (m, 2H), 2.01–1.90 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 181.4, 164.1, 162.3, 160.1, 157.9, 152.2, 143.6-127.0, 106.1, 105.3, 97.5, 93.9, 67.1, 51.2, 43.9, 32.2, 29.2, 26.3. EMI–MS (m/z): 506.47 (M^+). Anal. Calcd. for $C_{29}H_{29}ClN_2O_4$: C, 68.97; H, 5.79; N, 5.55. Found: C, 68.82; H, 5.93; N, 5.69.

4.3.18 7-(4-(4-(2,3-dichlorophenyl)piperazin-1-yl)butoxy)-5-hydroxy-2-phenyl-4H-chromen-4-one (**7r**)

Yield: 61%. m.p. 239-241°C; IR (KBr) cm^{-1}: 3073, 2957, 2941, 2864, 1637, 1606, 1581, 1342, 1269, 1164, 1037, 756; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 12.62 (s, 1H), 7.95–7.90 (m, 2H), 7.54–7.44 (m, 3H), 7.42–6.97 (m, 3H), 6.59 (s, 1H), 6.35 (d, *J* = 2.4 Hz, 1H), 6.17 (d, *J* = 2.2 Hz, 1H), 4.11 (t, *J* = 6.1 Hz, 2H), 3.70 (br s, 4H), 3.60 (t, *J* = 6.6 Hz, 2H), 3.35 (br s, 4H), 2.12–2.09 (m, 2H), 2.01–1.94 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 181.7, 164.6, 161.6, 159.9, 156.6, 155.0, 154.2, 151.7, 140.4-126.5, 107.5, 104.5, 96.5, 93.5, 66.7, 51.6, 43.3, 34.7, 29.6, 26.7. EMI–MS

(m/z): 539.33 (M^+). Anal. Calcd. for $C_{29}H_{28}Cl_2N_2O_4$: C, 64.57; H, 5.23; N, 5.19. Found: C, 64.44; H, 5.12; N, 5.34.

4.3.19 7-(4-(4-(2-fluorophenyl)piperazin-1-yl)butoxy)-5-hydroxy-2-phenyl-4H-chromen-4-one (**7s**)

Yield: 53%. m.p. 244-246°C; IR (KBr) cm^{-1}: 3069, 2968, 2949, 2858, 1659, 1609, 1587, 1336, 1270, 1154, 1027; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 12.68 (s, 1H), 7.91–7.82 (m, 2H), 7.57–7.48 (m, 3H), 7.33–6.98 (m, 4H), 6.77 (s, 1H), 6.55 (d, *J* = 2.4 Hz, 1H), 6.14 (d, *J* = 2.3 Hz, 1H), 4.22 (t, *J* = 6.1 Hz, 2H), 3.59 (br s, 4H), 3.48 (t, *J* = 6.6 Hz, 2H), 3.25 (br s, 4H), 2.17–2.09 (m, 2H), 2.02–1.94 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 183.1, 164.7, 162.8, 159.7, 156.9, 154.3, 151.7, 139.8-125.4, 108.3, 104.2, 96.2, 93.6, 66.8, 50.4, 42.5, 34.3, 28.6, 27.6. EMI–MS (m/z): 489.53 (M^+). Anal. Calcd. for $C_{29}H_{29}FN_2O_4$: C, 71.29; H, 5.98; N, 5.73. Found: C, 71.42; H, 6.08; N, 5.61.

4.3.20 7-(4-(4-(4-fluorophenyl)piperazin-1-yl)butoxy)-5-hydroxy-2-phenyl-4H-chromen-4-one (**7t**)

Yield: 57%. m.p. 256-258°C; IR (KBr) cm^{-1}: 3058, 2973, 2944, 2854, 1653, 1603, 1593, 1352, 1272, 1156, 1023; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 12.64 (s, 1H), 7.93–7.86 (m, 2H), 7.52–7.45 (m, 3H), 7.42–7.09 (m, 4H), 6.67 (s, 1H), 6.52 (d, *J* = 2.4 Hz, 1H), 6.27 (d, *J* = 2.3 Hz, 1H), 4.05 (t, *J* = 6.1 Hz, 2H), 3.61 (br s, 4H), 3.54 (t, *J* = 6.6 Hz, 2H), 3.30 (br s, 4H), 2.14–2.08 (m, 2H), 2.03–1.96 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 182.2, 164.1, 162.2, 159.8, 158.3, 156.4, 153.3, 144.6, 139.7-126.1, 107.3, 104.9, 96.8, 93.1, 66.4, 51.1, 43.2, 34.4, 29.1, 26.2. EMI–MS

(m/z): 489.26 (M^+). Anal. Calcd. for $C_{29}H_{29}FN_2O_4$: C, 71.29; H, 5.98; N, 5.73. Found: C, 71.16; H, 6.84; N, 5.60.

4.3.21 7-(4-(4-(2,4-difluorophenyl)piperazin-1-yl)butoxy)-5-hydroxy-2-phenyl-4H-chromen-4-one (**7u**)

Yield: 55%. m.p. 242-244°C; IR (KBr) cm^{-1}: 3055, 2974, 2929, 2855, 1636, 1602, 1592, 1350, 1271, 1155, 1021; ^1H NMR ($CDCl_3$, 400 MHz): δ 12.63 (s, 1H), 7.89–7.83 (m, 2H), 7.56–7.47 (m, 3H), 7.38–6.98 (m, 3H), 6.66 (s, 1H), 6.51 (d, *J* = 2.2 Hz, 1H), 6.26 (d, *J* = 2.3 Hz, 1H), 4.04 (t, *J* = 5.9 Hz, 2H), 3.66 (br s, 4H), 3.53 (t, *J* = 6.4 Hz, 2H), 3.29 (br s, 4H), 2.16–2.10 (m, 2H), 1.99–1.90 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 182.6, 15.7, 162.1, 160.7, 157.5, 156.8, 155.3, 152.8, 141.8-125.2, 106.4, 105.6, 97.0, 94.3, 67.9, 52.6, 44.5, 32.6, 30.3, 25.7. EMI–MS (m/z): 507.26 (M^+). Anal. Calcd. for $C_{29}H_{28}F_2N_2O_4$: C, 68.76; H, 5.57; N, 5.53. Found: C, 68.62; H, 5.69; N, 5.28.

4.3.22 5-hydroxy-2-phenyl-7-(4-(4-(4-(trifluoromethyl)phenyl)piperazin-1-yl)butoxy)-4H-chromen-4-one (**7v**)

Yield: 48%. m.p. 274-276°C; IR (KBr) cm^{-1}: 3061, 2963, 2934, 2865, 1649, 1601, 1586, 1344, 1261, 1165, 1030; ^1H NMR ($CDCl_3$, 400 MHz): δ 12.71 (s, 1H), 7.92–7.86 (m, 2H), 7.53–7.45 (m, 3H), 7.43–7.05 (m, 4H), 6.68 (s, 1H), 6.49 (d, *J* = 2.2 Hz, 1H), 6.23 (d, *J* = 2.3 Hz, 1H), 4.01 (t, *J* = 6.0 Hz, 2H), 3.54 (br s, 4H), 3.50 (t, *J* = 6.5 Hz, 2H), 3.26 (br s, 4H), 2.17–2.11 (m, 2H), 2.03–1.91 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 182.7, 166.2, 163.1, 161.4, 158.6, 155.6, 144.8, 136.9-124.9, 108.9, 104.1, 98.1, 94.1, 68.4, 52.9, 44.0, 34.9, 30.0, 27.0. EMI–MS (m/z):

539.85 (M^+). Anal. Calcd. for $C_{30}H_{29}F_3N_2O_4$: C, 66.90; H, 5.43; N, 5.20. Found: C, 66.76; H, 5.28; N, 5.32.

4.3.23 5-hydroxy-2-phenyl-7-(4-(4-(4-(trifluoromethoxy)phenyl)piperazin-1-yl)butoxy)-4H-chromen-4-one (**7w**)

Yield: 55%. m.p. 249-251°C; IR (KBr) cm^{-1}: 3054, 2952, 2937, 2872, 1657, 1607, 1596, 1353, 1266, 1167, 1046; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 12.67 (s, 1H), 7.94–7.87 (m, 2H), 7.58–7.48 (m, 3H), 7.46–7.10 (m, 4H), 6.70 (s, 1H), 6.54 (d, *J* = 2.2 Hz, 1H), 6.29 (d, *J* = 2.4 Hz, 1H), 4.15 (t, *J* = 5.9 Hz, 2H), 3.60 (br s, 4H), 3.56 (t, *J* = 6.4 Hz, 2H), 3.32 (br s, 4H), 2.18–2.12 (m, 2H), 1.98–1.88 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 182.8, 166.3, 161.9, 159.5, 157.6, 155.7, 151.6, 147.9, 139.8-125.7, 106.7, 105.9, 97.4, 94.6, 67.8, 52.2, 44.6, 32.5, 30.6, 26.6. EMI–MS (m/z): 555.63 (M^+). Anal. Calcd. for $C_{30}H_{29}F_3N_2O_5$: C, 64.97; H, 5.27; N, 5.05. Found: C, 64.85; H, 5.38; N, 5.22.

4.4. Biological Assays

4.4.1 DPPH free radical scavenging assay

DPPH$^\bullet$ (2,2-diphenyl-1-picryhydrazyl) is one of the few stable organic nitrogen radicals. A DPPH$^\bullet$ solution in ethanol has an intense strong violet color with a powerful VIS consumption at 515 nm. When it responded with an antioxidant, the DPPH$^\bullet$ radical is transformed into DPPH, and its color modified from violet to yellow-colored. The anti-oxidant impact could be analyzed by monitoring the loss of VIS consumption at 515 nm. The DPPH analysis relies mainly on the electron exchange response, while hydrogen – atom abstraction is a marginal reaction pathway.

The interactions between antioxidants and DPPH$^{\bullet}$ are also driven by the antioxidant's architectural conformation. Some substances respond quickly with DPPH$^{\bullet}$, and they reduce the number of DPPH• elements corresponding to the variety of available hydroxyl categories (Martysiak-Żurowska and Wenta 2012). Free radicals exercise a deleterious role in biological systems and foods, and hence radical scavenging activities are very useful. Various chemical reactions are running in the biological systems usually furnish free radicals which are responsible for causing damage to the building block of biologics like DNA, lipids, etc. Reduction of a stable free radical, 2,2-diphenyl-1-picrylhydrazyl is the base of DPPH antioxidant bioassay. It has an odd electron which exerts a maximum absorption band of 517 nm (deep violet colour) in ethanol. The DPPH bioassay is the widely used and acceptable method for inspecting the free radical scavenging efficacy of the intended compound. Such substances donate a hydrogen atom when it mixes with the DPPH thereby introducing its reduced congener, diphenyl picrylhydrazine (non-radical) with the loss of violet colour.

In the present study, DPPH bioassay was adopted to screen the chrysin based compounds for their *In vitro* antioxidant potencies. The results of this bioassay screenings were presented in the form of the percentage of radical scavenging antioxidant activity (RSA %) of each substance. The investigation of the DPPH radical scavenging activity was operated according to methodology described by Brand-Williams et al. (Brand-Williams et al., 1995) A stable free radical, 2,2-diphenyl-1-picrylhydrazyl was allowed to react with chrysin based scaffolds in methanol solvent as 20 μL quantities of titled compounds were mixed up with 180 μL of DPPH in MeOH. These titled compounds donated hydrogen in this mixing thereby carried out the reduction of DPPH and hence a change in the color was observed from deep violet to light

yellow at 517 nm after 25 min of reaction using a UV-Visible spectrophotometer (Perkin Elmer). The blank reading was also performed using the mixture of methanol (20 µL) and sample (180 µL of DPPH). Ascorbic acid served as a controlled drug in this assay, and its solution was prepared by mixing methanol (20 µL) and DPPH radical solution (180 µL). The results of this bioassay, RSA % (the radical scavenging activity in percentage) was determined according to Mensor et al. (Mensor et al., 2001) as described in below equation and as reported earlier (Mistry et al., 2015b).

$$\%Scavenging = \frac{Absorbance\ of\ \ blank - Absorbance\ of\ \ test}{Absorbance\ of\ \ blank} \times 100$$

A plot of the concentration of test compounds and % scavenging introduced IC_{50}s in the presence of an Ascorbic acid as standard.

4.4.2 ABTS radical scavenging assay

In the ABTS assay, 2,2'-azinobis (3-ethylbenzthiazoline-6-acid) (ABTS) is transformed into its radical cation ($ABTS^{\bullet+}$) by addition of potassium persulphate. This blue-green radical cation absorbs light at 734 nm. $ABTS^{\bullet+}$ is sensitive towards most antioxidants. It is not impacted by ionic durability, and it can be used to find out both hydrophilic and hydrophobic anti-oxidant capabilities. During this reaction, the blue-green ABTS radical cation is converted back into its colourless neutral form. The response may be supervised spectrophotometrically. ABTS radical cation ($ABTS^{\bullet+}$) is sensitive towards most antioxidants, and it is dissolvable in both aqueousin and organic solvents. The ABTS•+ technique is a useful device in identifying the antioxidant abilities of both hydrophilic and lipophilic antioxidants in various matrices. ABTS•+ react rapidly with antioxidants, and they can be applied over a wide pH range (Martysiak-Żurowska

and Wenta 2012). The $ABTS^{\bullet+}$ radical cation scavenging efficacies of the test compounds was determined according to the method described earlier (Re et al., 1999) and as reported earlier (Mistry et al., 2015b). Mixing of an equal amount of 7 mM ABTS•+ stock solution with 2.45 mM potassium persulfate stock solution produces The $ABTS^{\bullet+}$ cation. The mixture was kept in dark place at 0^{o}C temperature for 12 h and ABTS solution was diluted with MeOH so that it gives UV absorption value of 0.700 (± 0.200) at the 734 nm. The 1000 µL stock solutions of titled compounds **7a-w** was established upon dissolving them in MeOH and further dilutions furnishes 100 µL, 10 µL, 1 µL, and 0.1 µL of quantities of samples. 180 µL solutions of compounds to be evaluated and 20 µL of the ABTS solution were mixed in 96 well plates in a dark place which were then incubated for 10 min to measure UV absorption at 734 nm. A mixture of 180 µL ABTS and 20 µL mL methanol was used as a control determination, whereas ascorbic acid was used as a reference drug. The UV absorption data represented the radical scavenging rates that give the corresponding IC_{50}s for the test compounds.

The scavenging capability of $ABTS^{\bullet+}$ radical was calculated using the following equation:

$$\%Scavenging = \frac{Absorbance\ of\ \ blank - Absorbance\ of\ \ test}{Absorbance\ of\ \ blank} \times 100$$

4.5. *In vitro* anticancer bioassay

The test compounds **7a-w** were checked for their *In vitro* anticancer potential against cervical cancer cell line HeLa, CaSki, SK-OV-3(Ovarian Cancer Cell Line) and Madin-Darby canine kidney (MDCK) cells were which were purchased from American Type Culture Collection (ATCC). Human bone marrow derived mesenchymal stem cells (hBM-MSCs) were

purchased from Lonza (USA) and cultured in non-hematopoietic (NH) stem cell medium (Miltenyi Biotech, Germany) for 7 days. Cells were detached by using accutase (Millipore, USA) and cells at passage 5 were used for experiment. All the cell lines were well maintained in a humidified cell culture incubator in the presence of 5% of CO_2 at 32 ^{o}C temperature. Dulbecco's Modified Eagle's Medium (DMEM) and RPMI-1640 Medium supplemented with 10% of fetal Bovine Serum (FBS) and 1% of Antibiotic–Antimycotic Solution (100X) were used for HeLa, CaSki, SK-OV-3and MDCK cell growth respectively. DMEM, RPMI-1640, trypsin–EDTA, Antibiotic– Antimycotic Solution 100x and FBS were purchased from Welgene (150-Seongseo Industrial complex Bukro, Dalseogu, Daegu, 704–948 Republic of Korea).

In the 96 well plates, both the cancer cell lines HeLa, CaSki, SK-OV-3and MDCK were seeded, and plates were concentrated as 2×10^4 cells per well plate. Cancerous cells were allowed to grow for 1 day initially and after that the 96 well plates were washed twice with phosphate buffer saline (PBS). DMEM and RPMI-1640 medium contained trypsin–EDTA were used to dilute HeLa, CaSki, SK-OV-3and MDCK cells up to 5×10^3 level which was used for the infection followed by pacing of 10 μL of compound quantities and 90 μL of cell solution onto the 96 well plates in which HeLa, CaSki, SK-OV-3and MDCK cells were grown the previous day. 0.1 μL, 1 μL, 10 μL and 100 μL concentrations of the test compounds were used in 96 well plates for the analysis with three replicates of observations. Infected plates were incubated in a CO_2 incubator for 48 h. After incubation, the medium was removed and washed twice with PBS buffer. After that, 70% of acetone was added to fix the cells and were incubated for 1 h at 4 ^{o}C temperature. After incubation, the solvent was removed, and plates were dried in an oven at 60 ^{o}C temperature. The dried plated were overnight incubated 100 μL of SRB (0.4 mg/L) followed

by SRB removal and washing thrice with 1% of acetic acid and dried again under hot air oven at 60 ^{o}C. Microscopic observation was carried out to determine the morphology of the cells and after this observation the SRB strain was dissolved with 10 mM of Tris base and incubated overnight (Adaramoye et al., 2011). Spectrophotometric data were recorded at 510 nm to calculate the inhibition concentration of 50% (IC_{50}) and cytotoxic concentration of 50% (CC_{50}) as described earlier (Mistry et al., 2015b). hMSCs (6×10^3 cells/well) were seeded in 96 well plates and treated with various concentrations of test compounds for 24 h. Then, cells were washed with PBS and 10 µL EZ-Cytox (Dogen, Korea) was added to each well following incubation at 37 ^{o}C for 2 h. After incubation, absorbance was measured using an enzyme-linked immunosorbent assay reader (Munnyvale, USA) at a wavelength of 450 nm and cytotoxic concentration of 50% (CC_{50}) was calculated.

4.6 Structure-activity relationships of chrysin derivatives

Energy-minimized 3-dimensional structures and partial charges of derivatives were calculated with HyperChem version 8.0 (HyperCube Inc., FL, USA) with AM1 semi-empirical force field. The following properties were obtained with the same software, including logP, length, surface area, dipole moment, highest occupied molecular orbital (HOMO), lowest unoccupied molecular orbital (LUMO), the difference between HOMO and LUMO (GAP). For additional descriptors, the structures were converted to Sybyl Mol2 files and addressed to E-Dragon software (http://www.vcclab.org/lab/edragon/) (Tetko et al., 2005). Among numerous descriptors from the software, two groups of topological indices were selected to construct quantitative structure-activity relationship (QSAR) equations. Those were as follows; topological

charge indices (GGI1-GGI10 and JGI1-JGI10) (Galvez et al., 1994 and 1995), 3D-MoRSE descriptors (Mor01u-Mor32u, Mor01m-Mor32m, Mor01v-Mor32v, Mor01e-Mor32e, and Mor01p-Mor32p) (Gasteiger et al., 1996, Schuur et al, 1996). Multiple regression analyses were performed with STATISTICA ver7.0 (StatSoft Inc., OK, USA). Descriptors with high covariance were eliminated before the regression analysis. Forward stepwise removal selected independent variables in regression analysis.

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Conflict of interest

The authors declare that this article content has no conflict of interest.

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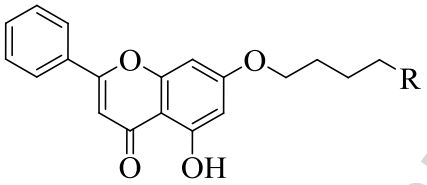
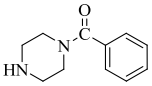
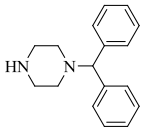
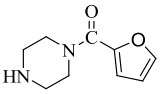
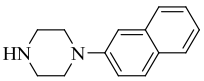
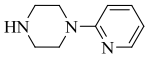
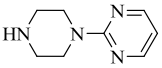
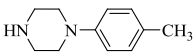
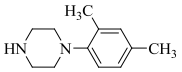
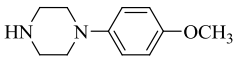
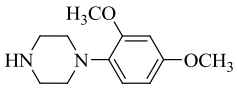
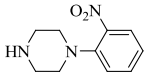
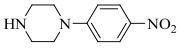
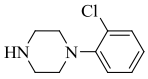
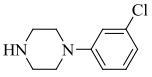
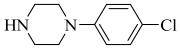
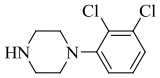
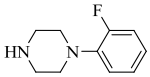
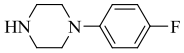
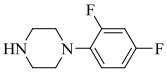
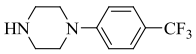
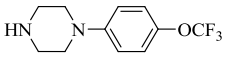
Table 1. Screening results of DPPH and ABTS radical scavenging activity of **7a-w**

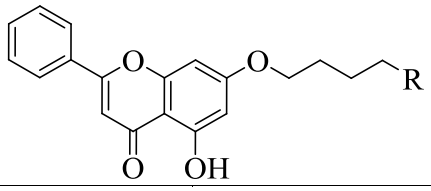


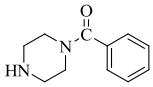
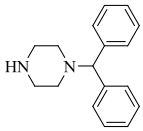
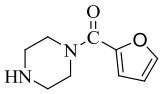
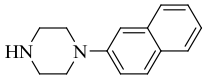
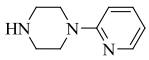
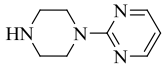
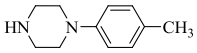
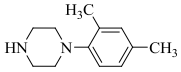
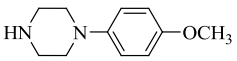
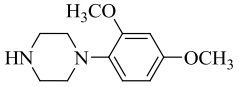
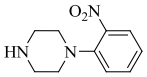
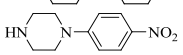
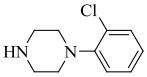
No.	R	IC_{50} µg/ml ± SD[a]	
		DPPH	**ABTS**
7a		30.77 ± 0.653	8.439 ± 0.122
7b		28.30 ± 1.178	8.393 ± 0.048
7c		27.79 ± 0.906	8.196 ± 0.051
7d		31.66 ± 0.829	8.636 ± 0.204
7e		30.22 ± 0.767	8.252 ± 0.015
7f		20.30 ± 0.476	5.625 ± 0.035
7g		34.06 ± 0.913	8.076 ± 0.097
7h		22.32 ± 1.042	8.608 ± 0.199
7i		27.67 ± 0.381	7.474 ± 0.035
7j		30.88 ± 0.571	5.570 ± 0.084
7k		23.09 ± 0.351	5.569 ± 0.025
7l		25.04 ± 0.668	5.733 ± 0.005
7m		29.84 ± 0.555	8.438 ± 0.171
7n		21.78 ± 0.076	8.048 ± 0.104
7o		30.70 ± 0.543	7.012 ± 0.192
7p		32.57 ± 1.178	7.707 ± 0.034
7q		21.07 ± 2.138	8.049 ± 0.034

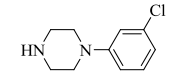
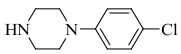
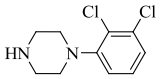
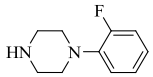
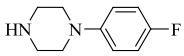
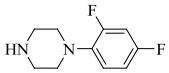
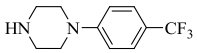
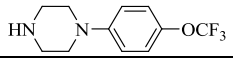
7r		29.68 ± 2.105	8.971 ± 0.881
7s		30.10 ± 0.938	7.919 ± 0.069
7t		24.24 ± 1.382	7.615 ± 0.168
7u		26.97 ± 1.159	7.921 ± 0.057
7v		20.62 ± 1.237	7.119 ± 0.173
7w		21.56 ± 1.027	7.240 ± 0.109
Ascorbic acid		12.72 ± 0.274	5.0925 ± 0.2090

[a]- The results are average of triplicate analysis.

Table 2. Screening results of activity of **7a-w** against cervical cancer cell lines

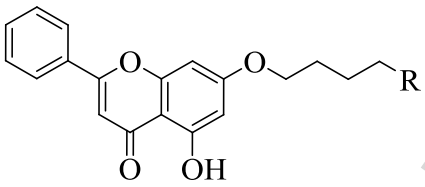


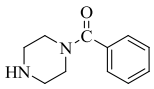
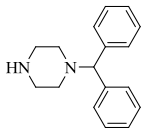
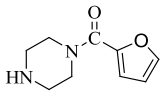
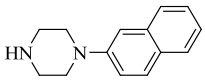
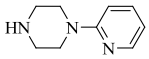
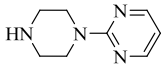
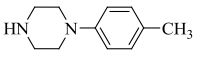
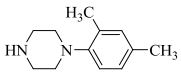
No.	R	IC_{50} µg/ml ± SD[a]	IC_{50} µg/ml ± SD[a]	CC_{50} µg/ml ± SD[a]	SI Ratio- CC_{50}/IC_{50}
		HeLa	CaSki	MDCK	HeLa/ CaSki
7a		8.267 ± 0.123	8.156 ± 0.088	200.5 ± 1.348	24.25/24.58
7b		9.136 ± 0.262	7.986 ± 0.159	222.6 ± 1.573	24.37/27.88
7c		5.562 ± 0.282	5.116 ± 0.321	147.6 ± 2.907	26.54/28.85
7d		8.787 ± 0.487	9.127 ± 0.153	203.5 ± 0.394	23.16/22.30
7e		6.578 ± 0.228	5.367 ± 0.222	264.6 ± 6.571	40.22/49.30
7f		5.051 ± 0.170	6.245 ± 0.130	284.5 ± 3.422	56.33/45.56
7g		5.104 ± 0.164	7.980 ± 0.245	219.6 ± 3.575	43.03/27.52
7h		5.098 ± 0.364	8.119 ± 0.256	246.1 ± 3.700	48.27/30.31
7i		6.789 ± 0.493	5.134 ± 0.096	298.1 ± 1.207	43.91/58.05
7j		5.643 ± 0.199	4.872 ± 0.134	323.1± 3.772	57.26/66.32
7k		8.128 ± 0.106	6.227 ± 0.097	299.3 ± 1.792	36.82/48.06
7l		7.770 ± 0.137	7.628 ± 0.146	286.3 ± 4.250	32.85/37.53
7m		9.914 ± 0.445	9.051 ± 0.189	266.3 ± 2.415	26.86/29.42
7n		7.088 ± 0.287	9.024 ± 0.097	176.0 ± 3.634	24.83/19.50
7o		6.361 ± 0.255	4.650 ± 0.078	371.1 ± 1.856	58.34/79.81

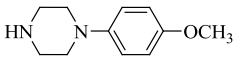
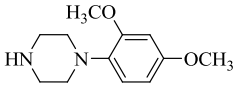
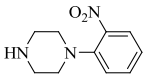
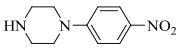
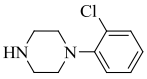
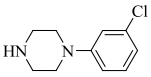
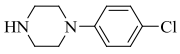
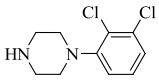
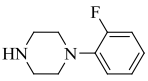
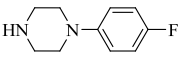
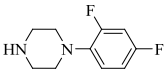
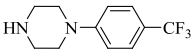
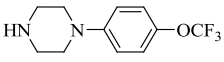
7p		7.046 ± 0.149	6.721 ± 0.269	181.6 ± 0.980	25.77/27.01
7q		6.488 ± 0.171	7.245 ± 0.267	207.5 ± 1.931	31.98/28.64
7r		5.499 ± 0.088	4.222 ± 0.129	212.3 ± 1.436	38.61/50.28
7s		7.390 ± 0.127	4.764 ± 0.251	277.7 ± 0.162	37.58/59.29
7t		6.497 ± 0.091	6.349 ± 0.401	163.1 ± 5.741	25.10/25.69
7u		5.044 ± 0.423	4.213 ± 0.202	203.5 ± 2.429	40.34/48.30
7v		7.552 ± 0.318	8.126 ± 0.420	280.3 ± 0.627	37.12/34.49
7w		6.939 ± 0.223	7.864 ± 0.198	215.9 ± 3.231	31.11/27.45

[a]- The results are average of triplicate analysis.

Table 3. Screening results of activity of **7a-w** against ovarian cancer cell line



No.	R	IC_{50} µg/ml ± SD[a]	CC_{50} µg/ml ± SD[a]	CC_{50} mg/mL ± SD[a]	SI
		SK-OV-3	MDCK	hBM-MSCs	Ratio-$CC_{50\text{-}(MDCK)}/IC_{50}$
7a		56.324 ± 0.278	200.5 ± 1.348	5.393 ± 0.060	3.56
7b		45.567 ± 0.315	222.6 ± 1.573	6.279 ± 0.079	4.89
7c		23.234 ± 0.169	147.6 ± 2.907	18.46 ± 0.058	6.35
7d		63.210 ± 0.158	203.5 ± 0.394	9.764 ± 0.045	3.22
7e		46.908 ± 0.187	264.6 ± 6.571	15.43 ± 0.080	5.64
7f		42.198 ± 0.304	284.5 ± 3.422	9.528 ± 0.053	6.74
7g		17.324 ±0.322	219.6 ± 3.575	8.669 ± 0.055	12.68
7h		12.876 ± 0.411	246.1 ± 3.700	8.681 ± 0.052	19.11
7i		44.432 ± 0.302	298.1 ± 1.207	6.219 ± 0.064	6.71
7j		15.213 ± 0.252	323.1± 3.772	10.26 ± 0.062	21.24

7k		22.965 ± 0.110	299.3 ± 1.792	-nt-	13.03
7l		14.213 ± 0.068	286.3 ± 4.250	11.56 ± 0.034	20.14
7m		51.241 ± 0.192	266.3 ± 2.415	4.809 ± 0.050	5.20
7n		36.213 ±0.090	176.0 ± 3.634	18.89 ± 0.041	4.86
7o		36.321 ± 0.142	371.1 ± 1.856	11.99 ± 0.054	10.22
7p		39.871 ± 0.096	181.6 ± 0.980	17.15 ± 0.058	4.55
7q		31.289 ± 0.159	207.5 ± 1.931	16.59 ± 0.044	6.63
7r		16.983 ± 0.210	212.3 ± 1.436	8.343 ± 0.051	12.50
7s		37.198 ± 0.146	277.7 ± 0.162	5.019 ± 0.071	7.47
7t		33.214 ± 0.155	163.1 ± 5.741	12.66 ± 0.055	4.91
7u		14.332 ± 0.248	203.5 ± 2.429	6.296 ± 0.072	14.20
7v		22.654 ± 0.223	280.3 ± 0.627	23.59 ± 0.061	12.37
7w		26.219 ± 0.133	215.9 ± 3.231	6.194 ± 0.058	8.23

a- The results are average of triplicate analysis.
-nt-: not tested.

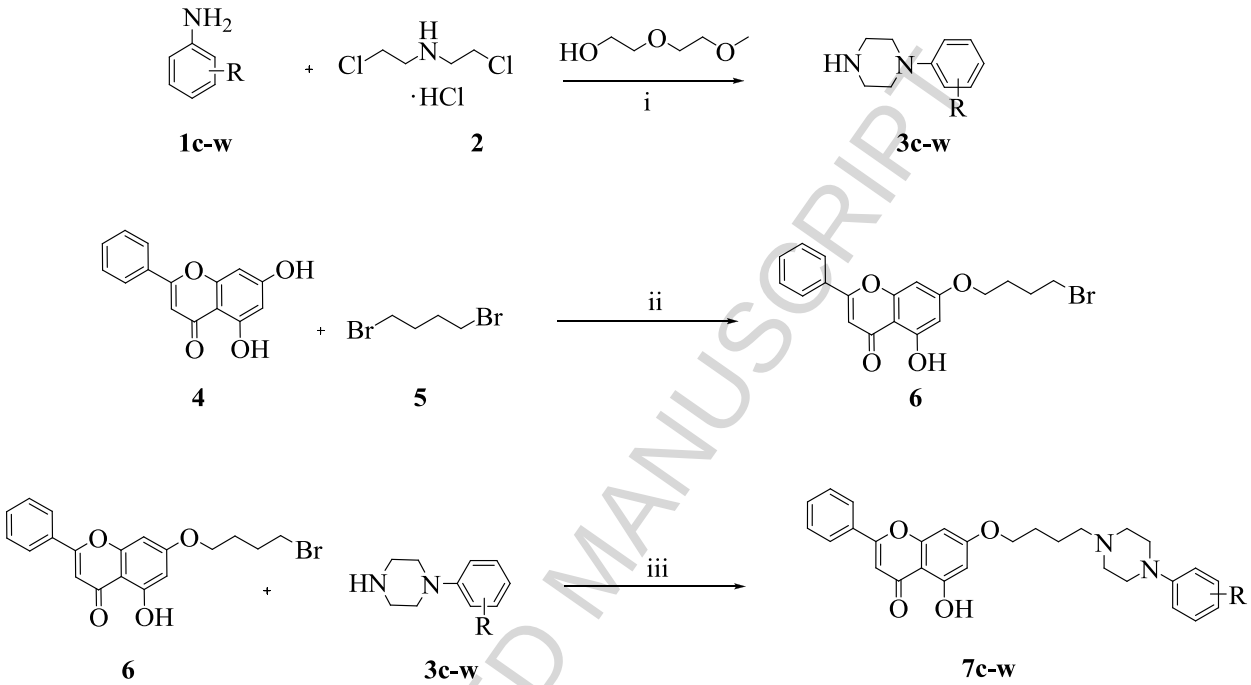
Table 4 Representative structure-activity relationship equations of chrysin derivatives

Activity	Equations and regression coefficients
Cytotoxicity	
HeLa	$LogIC_{50}$ = 1.24 - 0.13JGI9 - 0.87Mor12m - 0.54Mor28m - 0.81Mor31m
	(*n* = 23, r^2 = 0.7484)
Caski	$LogIC_{50}$ = 1.00 + 0.04Length - 0.65JGI2 - 0.41Mor03e
	(*n* = 23, r^2 = 0.4842)
SKOV	$LogIC_{50}$ = 1.59 – 0.54GGI5 – 0.41Mor32u – 0.49Mor22e – 0.44Mor17p
	(*n* = 23, r^2 = 0.7253)
MDCK	$LogIC_{50}$ = 6.55 + 0.88LUMO + 0.81Mor4u – 0.42Mor09v – 0.55Mor19v
	(*n* = 23, r^2 = 0.7609)
Antioxidant	
DPPH	$LogIC_{50}$ = 3.41 + 0.68LUMO + 0.69JGI7 – 0.48Mor30p + 0.04Mor31p
	(*n* = 23, r = 0.7636)
ABTS	$LogIC_{50}$ = 1.35 + 0.49Mor17m + 0.35Mor16v + 0.31Mor21e
	(*n* = 23, r = 0.6083)

Table 5 Correlation matrix of cytotoxicity and antioxidant activities of chrysin derivatives.

	Cytotoxicity				**Antioxidant**	
	HeLa	**Caski**	**SKOV**	**MDCK**	**DPPH**	**ABTS**
HeLa	1.00	0.45	0.56	0.05	0.07	0.01
Caski	0.45	1.00	0.19	-0.20	-0.26	0.08
SKOV	0.56	0.19	1.00	-0.08	0.21	0.23
MDCK	0.05	-0.20	-0.08	1.00	-0.09	-0.59
DPPH	0.07	-0.26	0.21	-0.09	1.00	0.35
ABTS	0.01	0.08	0.23	-0.59	0.35	1.00

Scheme 1 Synthesis of piperazine linked chrysin derivatives **7a-w**



Reagents & Conditions: **i**. KI (3 mol%), Heat, N_2, 150 ^oC, 16-48 h; **ii**. K_2CO_3, reflux, 24 h; **iii**. CH_3CN, reflux, 10-38 h.

Graphical Abstract