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Phenylsulfonyl piperazine bridged [1,3]dioxolo[4,5-*g*]chromenones as promising antiproliferative and antioxidant agents

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ABSTRACT

Two series of sulfonylpiperazines linked [1,3]dioxolo[4,5-*g*]chromenones were synthesized featuring phenyl (**7a-k**) and chalcone (**12a-k**) bridge representing flavones or homoisoflavonoids core. New molecules are synthesized utilizing aldol condensation to inspect as antioxidants against DPPH$^{\cdot}$ and ABTS$^{\cdot+}$ and antiproliferative agents toward selected human cancer cell lines. Cytotoxicity of new compounds was confirmed using SRB assay against non-cancer MDCK cell line. The results concluded that both individual structures of **7** and **12** were vital for modulating pharmacological potencies and presence of different electron withdrawing and electron donating functional group(s) on the phenylsulfonyl entity yielded varied biological effects. Substituent **h** (OCF_3) and **j**, **k** (OCH_3) were found to play a crucial role scavenging DPPH$^{\cdot}$ and ABTS$^{\cdot+}$ as well as inhibiting cancer cell lines SK-OV-3 and HT-29. Moreover, molecules bearing halogen atom(s) such as substituent **b-g** expressed excellent inhibitory potential against HeLa and A-549 cancerous cell lines. Bioassay data displayed some interesting structure-activity relationships which are discussed in this paper. The results justified that tested derivatives are promising antioxidants and cytotoxic agents and warrant further structural optimization and bioassay studies. Spectroscopic techniques such as FT-IR, ^{1}H NMR, ^{13}C NMR and elemental analysis (CHN) were carried out to confirm the final structures.

1. Introduction

Cancer is a life-threating ailment that is manifested by out-of-control irregular cell development with the chance of invading other cells and/or spread to other organs. Melanoma could impact almost any aspect of the body and has many anatomic and molecular subtypes that each need particular control techniques. It is the major cause of loss of life worldwide and is approximated to account for 9.6 million loss of life in 2018. Cancers such as Liver, prostate, colorectal and lung cancer as well as breast, cervix and thyroid cancer are the most common among men and women, respectively [1]. Despite all the treatment techniques such as radiation, hormone therapy, surgery and chemotherapy, the use of a perfect and outright cure is a major challenge and there is an urgent need to build up more potential anticancer agents [2]. Cancer control can be handled by various systems targeted at different levels of carcinogenesis like initiation, promotion and progression. The desirable options of destroying carcinogenesis include modulation of biotransformation of carcinogen, gene expression manipulation involved in signaling pathways, free radical scavenging as well as cell proliferation and differentiation [3,4]. Moreover, human's exposure to toxins present in the environment as well as their lifestyle factors significantly impact the existing homeostatic balance which results in a diminished antioxidant capacity that triggers production of reactive oxygen species (ROS). This phenomenon is termed as oxidative stress which then causes serious medical conditions like cancer and DNA damage [5,6]. There are enzymatic and non-enzymatic antioxidants those reduce oxidative stress within important biological cells utilizing various mechanisms. In recent years, chemical modification of natural products has attracted great attention of many analysis categories these days, with the aim to improve their unique scientific activities as well as to deliver potencies against one or more targets [7].

Chromanones are the most important heterocyclic substances, which is a frequent and important function of a wide range of natural products, medicinal agents and modification possibilities of their framework provide a high level of benefits to further rationalize potential molecular libraries. They are an essential category of oxygen-containing

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heterocyclic substances with a benzoannelated γ-pyrone ring and are a crucial feature of the flavonoid natural product class [8]. Chromanone and its analogs, abundant in nature, are essential pharmacophores and key structures in drug discovery research and can be seen as the main core in several clinically used drugs [9,10], specifically, as anticancer molecules [11–14]. Furthermore, various chromenes moiety-containing substances which are architectural analogs of chromanones were also revealed with great anticancer action [15,16]. Furthermore, homoisoflavonoids, a flavonoid class, have been identified to possess a broad range of bioactivities like anti-microbial, anti-mutagenic, anti-oxidant, immunomodulatory, anti-diabetic, cytotoxic, anti-angiogenic, vasorelaxant, and anti-inflammatory effects [17,18]. They mainly consist of a chromanone, chromone, or chromane skeleton and are ubiquitous in plants. Therefore, homoisoflavonoids might have a huge prospective for further research of their bioactivities to be able to recognize important leads. Moreover, Chalcones, α-β-unsaturated ketones, are valuable compounds of therapeutic significance due to existence of the reactive double bond in conjugation with carbonyl functionality, reflecting the flavonoid family [19]. Numerous reports highlighting the synthesis and therapeutic importance of chalcone derivatives have already been recorded in the literary concluding them as anti-proliferative, anti-malarial, anti-microbial and anti-HIV agents [20]. In a view of aforementioned points, we have attempted to construct two different types of molecular series featuring chromanone, chalcones and homoisoflavonoid rings. In addition, organic molecules endowed with sulfone entity found to have promising potential as the biologically active agents [21], whereas piperazine analgoues [22] have been proposed to be key factor enhancing biological effects of the bearing molecules. Finally, we have already prepared some flavonoid based therapeutic agents targeting different cancer cell lines (HeLa, CaSki, SK-OV-3) and free radicals (DPPH$^{\cdot}$ and ABTS$^{\cdot+}$) and results obtained in those studies prompted us to further optimize the flavone type ring systems [23,24]. More importantly, compounds holding antiproliferative and antioxidant effects are of key interest and relation of antioxidants with cancer is well studied as antioxidants exercise crucial roles in the maintenance of cellular integrity and therefore are critical in maintaining the homeostasis of the host immune system as it is studied that some flavonoids which exhibit anticarcinogenic properties in part via their antioxidant activities [25]. While such free radicals are utilized as an intrinsic mechanism of host immunity to resist towards extracellular pathogens, an amplified generation outcomes into imbalance in cellular redox potential causes discrepancy in signaling pathways and neocarcinogenesis [26]. When compared to the normal healthy cells, melanoma tissues normally carry higher basal oxidative stress and it is believed that several antioxidant systems play essential positions in counteracting the destructive results of improved ROS [27]. Moreover, one of the repercussions of chemotherapy and radiotherapy treatment is the generation of ROS which then results in aberrations in several cell signaling pathways which lead to the therapy-induced cell death. Thus, new agents with anticancer efficacies are most welcomed which are also helps potentiate chemotherapy/radiotherapy–mediated antitumor reactions required to induce long-term benefits in cancer patients [28]. There are studies suggesting that some antioxidants are found to positively influence cancer treatments and helps inhibiting cancer cells while decreasing cytotoxic impacts on normal cells [29]. Therefore, we have integrated essential entities into the base chromanones in order to obtain new molecules bearing both cytotoxic and free radical scavenging potencies.

2. Materials and methods

All commercial chemicals and solvents are of reagent grade and were used without further purification. Melting points are uncorrected and recorded on Stuart SMP3 melting point apparatus. The thin layer chromatography was performed on Merck pre-coated silica gel 60 F_{254} plates, with visualization under UV light. IR spectra (KBr) were recorded on an FT-IR 200 spectrophotometer (ύ, cm^{-1}). ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AVANCE III 400 instrument spectrometer and *J* values are in Hertz, and chemical shifts (d) are reported in ppm relative to internal tetramethylsilane. Elemental analysis was carried out using C,H,N,S analyzer.

2.1. Synthesis of 3-(benzo[d][1,3]dioxol-5-yl)-1-(4-fluoro-2-hydroxyphenyl) prop-2-en-1-one (3)

To a solution of piperonal (**1**, mmol) in 20 mL of ethanol with 4′-Fluoro-2′-hydroxyacetophenone (**2**, 10 mmol), 10 mL of 20% KOH was added and the reaction mixture was allowed to react at room temperature for 24 h. After the completion of the reaction monitored by TLC, the reaction mass was quenched by 30 mL of water and extracted with 3 × 30 mL of chloroform followed by drying of the organic layer using anhydrous sodium sulfate. The mass was then concentrated in vacuum and purified by column chromatography with petroleum ether:ethyl acetate to give **3** in 69% of yield. Yellowish brown solid, m.p. 167–169 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 12.71 (s, 1H), 7.83 (d, J = 15.1 Hz, 1H), 7.67–7.44 (m, 6H), 7.39 (d, J = 15.3 Hz, 1H), 6.05 (s, 2H, CH_2).

2.2. Synthesis of 6-(4-fluorophenyl)-8H-[1,3]dioxolo[4,5-g]chromen-8-one (4)

Catalytic amount of I_2 (0.1 equiv) was added to a solution of **3** in DMSO and the mass was refluxed for more than 10 h while monitoring reaction with TLC. After the completion of the reaction, the resulting reaction mass was cooled to room temperature and poured onto saturated aqueous sodium thiosulfate solution and resulting precipitates were filtered, washed with chilled water and recrystallization from dichloromethane-methanol yielded crystalline **4** in 62%. Yellow solid, m.p. 198–200 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.68–7.47 (m, 4H), 7.39 (s, 1H, chroman), 7.11 (s, 1H, chroman), 6.48 (s, 1H, chroman), 5.96 (s, 2H, CH_2).

2.3. Synthesis of 6-(4-(piperazin-1-yl)phenyl)-8H-[1,3]dioxolo[4,5-g] chromen-8-one (5)

To a solution of **4** in DMF (35 mL), equivalent ratio of piperazine hexahydrate was added and reaction was allowed to heat at 110 °C for 12 h in the presence of Cs2CO3. After the completion of the reaction as monitored by the TLC, the reaction mass was poured onto crushed ice, filtered, dried in vacuum and recrystallized from dichloromethane-methanol to result **5** in 72%. Yellow solid, m.p. 198–200 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.74–7.49 (m, 4H), 7.33 (s, 1H, chroman), 7.02 (s, 1H, chroman), 6.42 (s, 1H, chroman), 6.04 (s, 2H, CH_2), 3.45 (t, J = 4.9 Hz, 4H, CH_2), 2.71 (t, J = 4.9 Hz, 4H, CH_2).

2.4. General procedure for the preparation of sulfonylpiperazine based chrysin derivatives (7a-k)

To a solution of 0.5 mL of pyridine and 0.5 mmol of intermediate **5**, 20 mL of dried dichloromethane was added followed by 1 mmol of selected substituted sulfonyl chlorides (**a-k**). The resulting reaction mixture was refluxed for 12–17 h and reaction was monitored by TLC. After completion, the reaction mass was quenched using 20 mL of 10% NaOH followed by the extraction with chloroform with quantities 3 × 15 mL. The organic portion was then passed through anhydrous sodium sulfate, concentrated in vacuo and purified by column chromatography (DCM) to afford **7a-k**. For example, ***6-(4-(4-(phenylsulfonyl)piperazin-1-yl) phenyl)-8H-[1,3]dioxolo[4,5-g]chromen-8-one (7a)***: Yield: 52%. m.p. 247–249 °C; IR (KBr) cm^{-1}: 3066, 3034, 2933, 2855, 1669, 1613, 1590, 1479, 1379, 1344, 1266, 1154, 1039; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.81–7.70 (m, 4H), 7.52–7.38 (m, 5H), 7.34 (s, 1H, chroman), 7.03 (s, 1H, chroman), 6.44 (s, 1H, chroman), 6.05 (s, 2H, CH_2), 3.53 (t,

J = 4.87 Hz, 4H, CH_2), 2.78 (t, J = 4.85 Hz, 4H, CH_2). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 181.1, 156.2, 150.9, 144.7, 139.1–118.0, 112.6, 105.6, 102.1, 97.5, 52.8, 44.4. Anal. Calcd. for $C_{26}H_{22}N_2O_6S$: C, 63.66; H, 4.52; N, 5.71. Found: C, 63.55; H, 4.72; N, 5.89.

*2.5. 3-(Benzo[d][1,3]dioxol-5-yloxy)propanoic acid 9 and 6,7-dihydro-8H-[1,3]dioxolo[4,5-g]chromen-8-one 10 were prepared following the literature procedure [30]. Compounds of **12a-k** were synthesized as per the procedure described for **7a-k**. For example, **7-((4-(phenylsulfonyl) piperazin-1-yl)methylene)-6H-[1,3]dioxolo[4,5-g]chromen-8(7H)-one (12a)***

Yield: 49%. m.p. 222–224 °C; IR (KBr) cm^{-1}: 3066, 3034, 2933, 2855, 1669, 1613, 1590, 1479, 1371, 1344, 1266, 1154, 1039; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.81 (s, 1H, benzylidene), 7.63–7.41 (m, 5H, ArH), 7.31 (s, 1H, chroman), 7.09 (s, 1H, chroman), 6.05 (s, 2H, CH_2), 5.41 (d, 2H, chroman, J = 1.9 Hz), 3.55 (t, J = 4.87 Hz, 4H, CH_2), 2.71 (t, J = 4.85 Hz, 4H, CH_2). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 179.1, 154.5, 152.4, 142.9, 142.2, 138.4–116.5, 110.6, 101.3, 96.3, 64.5, 51.1, 44.2. Anal. Calcd. for $C_{21}H_{20}N_2O_6S$: C, 58.87; H, 4.70; N, 6.54. Found: C, 58.77; H, 4.91; N, 6.67.

*2.6. Synthesis of 7-(piperazin-1-ylmethylene)-6H-[1,3]dioxolo[4,5-g] chromen-8(7H)-one (**11**)*

A solution of 0.7 mmol of 1-formyl piperazine and 6,7-dihydro-8H-[1,3]dioxolo[4,5-*g*]chromen-8-one **10** (0.5 mmol) was ice cooled and dry HCl was passed through it in the presence of 5 mL of absolute ethanol for 5 min. The resulting reaction mass was then allowed to stand for two days at room temperature so that precipitation occurs when were then filtered, dried in vacuum and recrystallized from ethanol and water to yield **11**. Yield: 41%. m.p. 189–191 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.87 (s, 1H, benzylidene), 7.36 (s, 1H, chroman), 7.05 (s, 1H, chroman), 6.01 (s, 2H, CH_2), 5.48 (d, 2H, chroman, J = 1.8 Hz), 3.51(t, J = 4.9 Hz, 4H, CH_2), 2.77 (t, J = 4.9 Hz, 4H, CH_2), 2.14 (s, 1H).

3. Biological screening

3.1. Evaluation of antioxidant capacity by using the DPPH assay

In vitro free radical scavenging potential of the final derivatives was quantitatively measured by DPPH method [31,32]. In brief, 20 µL of tested compounds (0.1, 1, 10, 100 µL) were added to a 96-well microplate, to which 180 µL of DPPH was added. Methanol (20 µL) was used as the blank, and after incubation for 30 min, the optical density at 517 nm was calculated. Ascorbic acid was used as the reference compound and all determinations were carried out in triplicate.

The DPPH$^{\cdot}$ scavenging activity was calculated by using the equation provided by Mensor et al. [33].

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

A plot of concentration of test compounds and % scavenging activity showed half-maximal inhibitory concentrations (IC_{50s}) in the presence of ascorbic acid as the standard.

3.2. Evaluation of antioxidant capacity of the ABTS assay

All the final compounds were screened for ABTS$^{\cdot+}$ radical cation scavenging assay [31,34]. In brief, Different concentrations (0.1, 1, 10, 100 µL) of tested derivatives (20 µL) were added to a 96-well microplate, than added 180 µL of ABTS solution followed by 10 min of incubation under dark condition. The absorbance was read at 734 nm. Ascorbic acid was used as the reference compound and all determinations were carried out in triplicate. ABTS scavenging effect was calculated as percentage of ABTS$^{\cdot+}$ scavenging using the following equation:

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

A plot of concentration of test compounds and % scavenging activity showed half-maximal inhibitory concentrations (IC_{50s}) in the presence of ascorbic acid as the standard.

3.3. In vitro cytotoxicity bioassay

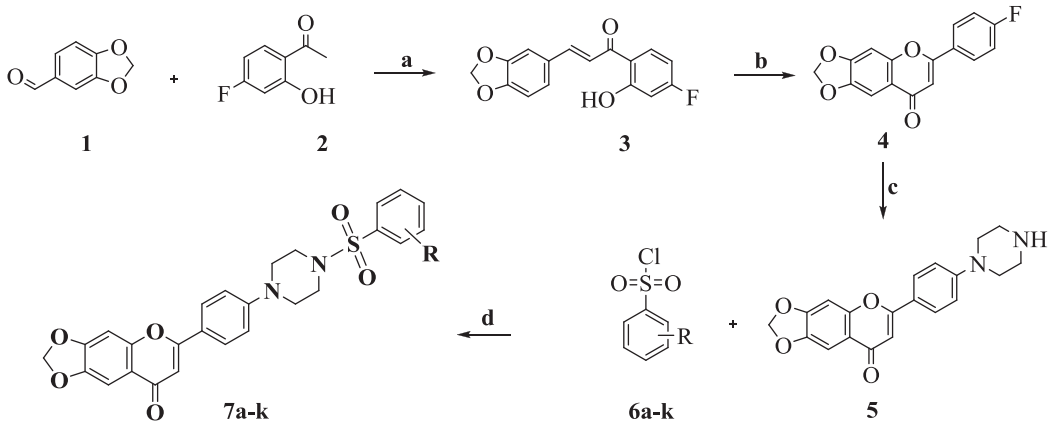
In vitro cytotoxicity bioassay of the synthesized compounds was carried out using the SRB assay method [31,35]. In briefly, all the cell lines were well maintained in Dulbecco's Modified Eagle's Medium (DMEM) and RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS) and 1% antibiotic-antimycotic solution (100X) in a humidified cell culture incubator in the presence of 5% of CO_2 at 37 °C. HeLa, SK-OV-3, A-549, HT-29 and Madin-Darby canine kidney non-cancer (MDCK) cells were seeded into 96-well plates at the density of 2×10^4 cells per well plate. The synthesized compounds were dissolved in DMSO and treated with the cells after 24 h and diluted in RPMI or DMEM medium giving risen to four concentrations comprising 0.1, 1, 10, and 100 µL. The infected plates were then incubated in a CO_2 incubator for 48 h after the addition of the compounds, 100 µL of SRB (0.4 mg/L) was added to each well and incubated for overnight. After that, 70% of cold acetone was added to each well to fix the viable cells washed, dried, and dyed by 100 µL of SRB (0.4 mg/L) followed by SRB removal and three washes with 1% acetic acid. The unbounded dye was separated, while the protein-bound dye was extracted with 10 mM Tris base and incubated overnight. Multi-well spectrophotometric data were recorded at 510 nm to calculate the IC_{50} and the 50% cytotoxic concentration (CC_{50}).

4. Results and discussion

4.1. Chemistry

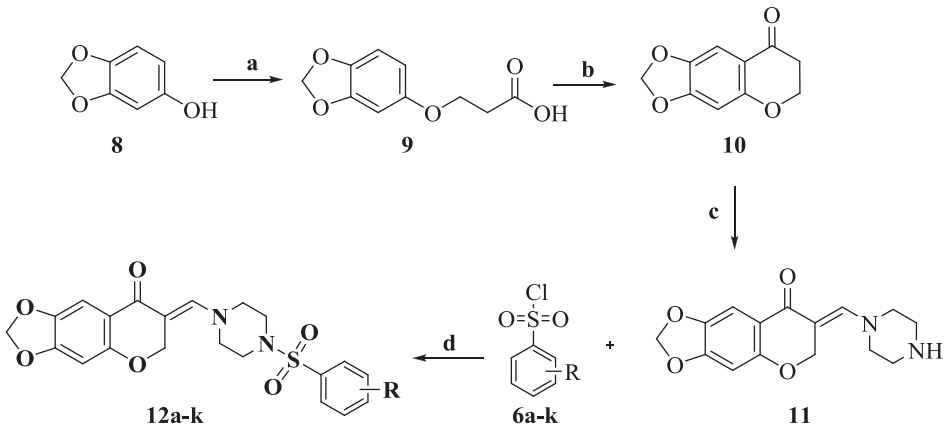
Synthetic steps adopted to furnish final compounds **7a-k** and **12a-k** are drawn in scheme 1. Treatment of commercially available piperonal (**1**) with 4′-Fluoro-2′-hydroxyacetophenone (**2**) gave an intermediate chalcone **3** in the presence of KOH utilizing aldol condensation. Cyclization of **3** in the DMSO using catalytic amount of I_2 followed by treatment with saturated aqueous sodium thiosulfate solution lead to the formation of a key intermediate 6-(4-fluorophenyl)-8H-[1,3]dioxolo [4,5-*g*]chromen-8-one (**4**). Subsequently, the final intermediate (**5**) was prepared by the nucleophilic substitution of fluorine atom of **4** with piperazine following the literature procedure [36,37]. Finally, in the last step, refluxing **5** with different substituted benzenesulfonyl chlorides (**a-k**) in the presence of pyridine in DCM solvent followed by the treatment with 10% NaOH furnished final molecules **7a-k** in reasonable yields. Furthermore, for the initiation of the synthesis of **12a-k**, benzo [d][1,3]dioxol-5-ol (**8**) was allowed to react with 3-bromopropanoic acid in basic conditions using sodium hydroxide produced compound **9** which was then further treated with oxalyl chloride in benzene solvent in the presence of Tin tetrachloride to give intermediate **10**. Chalcone moiety (**11**) was constructed utilizing aldol condensation of **10** with 1-formyl piperazine in the presence of HCl gas. In the last step, final intermediate **11** was refluxed with different substituted benzenesulfonyl chlorides (**a-k**) in the presence of pyridine in DCM solvent followed by the treatment with 10% NaOH furnished final molecules in reasonable yields [38,39]. FT-IR, ^{1}H NMR and ^{13}C NMR data were in the accordance of the proposed structures. All compounds gave C, H and N analyses within limits from the theoretical values.

Series 1



Reagents and conditions: **(a)** 20% KOH, EtOH, rt, 24h; **(b)** I_2, DMSO, reflux, 11 h; **(c)** piperazine hexahydrate, Cs_2CO_3, DMF, 110°C, 12h; **(d)** pyridine, DCM, reflux, 12-17 h;

Series 2



Reagents and conditions: (**a**) NaOH, Na_2CO_3, $Br(CH_2)_2COOH$, H_2O, reflux; (**b**) oxalyl chloride, $SnCl_4$, benzene; (**c**) 1-formyl piperazine, HCl (g), 0°C; (**d**) pyridine, DCM, reflux, 8-21h;

R= **a**: H; **b**: 4-Cl; **c**: 2,4-diCl; **d**: 4-Br; **e**: 2,4-diBr; **f**: 4-F; **g**: 2,4-diF; **h**: 4-OCF_3; **i**: 4-NO_2; **j**: 4-OCH_3; **k**: 2,4-diOCH_3

Scheme 1. Synthesis of phenylsulfonyl piperazine bridged [1,3]dioxolo[4,5-*g*]chromenones **7a-k** and **12a-k**.

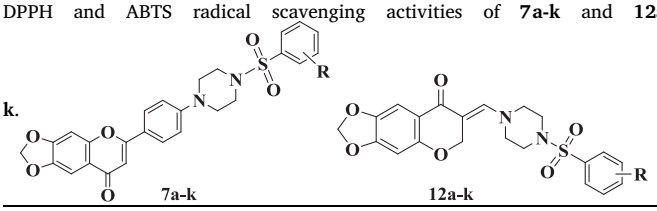
4.2. Pharmacology

4.2.1. Antioxidant activities

Newly synthesized analogues **7a-k** and **12a-k** were subjected to evaluate their *in vitro* antioxidant (DPPH˙ and $ABTS^{\cdot+}$ scavenging) and antiproliferative (human ovarian cancer SK-OV-3, cervical cancer HeLa, human colon adenocarcinoma HT-29, and human non-small-cell lung carcinoma A549) activities. The results of bioassay screenings adopting DPPH, ABTS and SRB assay are furnished in Tables 1 and 2. Along with cytotoxicity of new compounds against cancer cell lines, they were also inspected for their tolerable cytotoxic properties against non-cancer MDCK cells and results are noted down it Table 2. Overall, tested compounds were found to have promising pharmacological potential and results delivered some interesting structure-activity relationships. For example, in some bioassay cases, overall structure found to be responsible to express specific bioactivities, while for the other cases, placing an appropriate substituent was a key to anticipated potency. Moreover, presence of electron withdrawing (EWD) or electron donating (ED) functional group exercised different activity levels against different targets. Likewise, amongst EWD and ED, presence of single or more than one groups had a substantial impact on the biological activities of the resultant molecules.

DPPH antioxidant assay revealed that chalcone hybrids **12a-K** were more powerful in scavenging DPPH˙ than **7a-k**. Hence, it would suffice to mention here that overall structure of the designed molecule was essential to deliver anti- DPPH˙ activity. However, there was an exception in case of an analogue bearing nitro group (**7i**) from **7a-k** family showing 28.79 ± 0.94 μg/mL of IC_{50} when compared to that of **12i** with 33.45 ± 1.09 μg/mL. Amongst the most potent derivatives, those from **12a-k** set with 2,4-diOCH_3 (**12k**) and OCF_3 (**12h**) functionalities showed most potent action against DPPH˙ with 8.21 ± 0.83 μg/mL and 8.98 ± 0.56 μg/mL of IC_{50s}, respectively. Overall, amongst both sets of compounds, those bearing OCF_3 and OCH_3 functional groups were found to have better DPPH˙ scavenging efficacies that those holding halo atom (s). For example, within **7a-k** and **12a-k** groups, derivatives **7j**, **7k**, **7h** and **12j** expressed 9.34–10.39 μg/mL of IC_{50s} in comparison to those

Table 1
DPPH and ABTS radical scavenging activities of **7a-k** and **12a-k**.



No.	R	IC_{50}[a] μg/mL ± SD	
		DPPH[b]	ABTS[c]
7a	H,	39.19 ± 0.74	24.25 ± 1.48
7b	4-Cl	23.81 ± 1.15	11.29 ± 1.22
7c	2,4-diCl	21.26 ± 0.51	9.18 ± 1.02
7d	4-Br	25.98 ± 0.88	10.55 ± 0.66
7e	2,4-diBr	25.10 ± 0.96	10.92 ± 0.41
7f	4-F	19.04 ± 1.03	9.11 ± 1.29
7g	2,4-diF	17.91 ± 0.59	8.13 ± 0.19
7h	4-OCF_3	8.98 ± 0.43	5.47 ± 0.93
7i	4-NO_2	28.79 ± 0.94	18.99 ± 1.20
7j	4-OCH_3	10.39 ± 0.79	5.66 ± 1.11
7k	2,4-diOCH_3	9.34 ± 1.03	4.74 ± 0.49
12a	H	37.04 ± 0.90	27.90 ± 0.39
12b	4-Cl	14.57 ± 0.35	7.91 ± 1.09
12c	2,4-diCl	12.59 ± 0.69	6.97 ± 0.33
12d	4-Br	15.10 ± 1.13	10.94 ± 1.17
12e	2,4-diBr	12.99 ± 0.21	11.73 ± 0.59
12f	4-F	12.11 ± 1.09	10.18 ± 0.91
12g	2,4-diF	11.24 ± 0.89	9.01 ± 0.55
12h	4-OCF_3	9.41 ± 0.56	5.22 ± 1.07
12i	4-NO_2	33.45 ± 1.09	22.78 ± 0.79
12j	4-OCH_3	9.82 ± 0.36	6.04 ± 0.41
12k	2,4-diOCH_3	8.21 ± 0.83	4.82 ± 0.77
Ascorbic acid		12.72 ± 0.274	5.0925 ± 0.2090

[a] Antioxidant activities are shown as IC_{50} values in μg/mL. All assays were carried out in triplicate, and the results are expressed as an average ± standard deviation.
[b] DPPH = 2,2-diphenyl-1-picrylhydrazyl.
[c] ABTS = 2,2′-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid.

(**7b-7g** and **12b-12g**) endowed with halogen atom(s) with 11.24 ± 0.89–25.98 ± 0.88 μg/mL of IC_{50s}. These results suggested that although overall structure of chalcones was crucial for anti- $DPPH^{\cdot}$ activity, but amongst the active set of compounds, presence of optimum substituent was an influential factor. The statement was further supported by the activity data observed in case of compounds bearing halo functional groups. For instance, all derivatives with dihalo groups acted as better DPPH radical scavengers than their single halogenated precursors. For example, **12e** with 2,4-diBr group showed 12.99 ± 0.21 μg/mL of IC_{50} and **12d** with 4-Br expressed 15.10 ± 1.13 μg/mL of IC_{50}, whereas **7c** (2,4-diCl) and **7b** (4-Cl) expressed 21.26 ± 0.51 μg/mL and 23.81 ± 1.15 μg/mL of IC_{50s}, respectively. Importantly, within halogenated analogues, those presenting fluorine atom(s) such as **12f** and **12g** showed most potent anti- $DPPH^{\cdot}$ action than others observing 12.11 ± 1.09 μg/mL and 11.24 ± 0.89 μg/mL of IC_{50s}, respectively furnishing activity orders of F > Cl > Br. Absence of any substituent lead to the compound with poorer $DPPH^{\cdot}$ scavenging activity as found from the IC_{50} data of **7a** and **12a** above 37 μg/mL. It is suffice to state that almost all derivatives from **12a-k** set as well as some from **7a-k** group presented better $DPPH^{\cdot}$ scavenging potencies then control drug ascorbic acid with 12.72 ± 0.274 μg/mL of IC_{50}. Thus, the study suggested the importance of bridging suitable pharmacophores to a main core.

Bioassay results observed in ABTS assay suggested that both structure and substituents were crucial to effectively scavenge $ABTS^{\cdot+}$. Regarding bioassay data, however, the mixed trend was noticed for **7a-k** and **12a-k** families but presence of three F atoms as well as more OCH_3 groups demonstrated excellent anti-$ABTS^{\cdot+}$ action. For instance, the most potent compounds in ABTS assay were **7k** and **12k** with 2,4-diOCH_3 group with 4.74 ± 0.49 μg/mL and 4.82 ± 0.77 μg/mL of IC_{50s}, respectively, which were even better than control ascorbic acid with 5.0925 ± 0.2090 μg/mL of IC_{50}. Furthermore, **7h** and **12h** with OCF_3 gave 5.47 ± 0.93 μg/mL and 5.22 ± 1.07 μg/mL of IC_{50s}, respectively, which were comparable to the control ascorbic acid. Amongst halogenated analogues, in case of Cl and F containing molecules (**7c**, **7g**, **12c** and **12g**), those with two atoms showed higher $ABTS^{\cdot+}$ scavenging effects than their single halogenated counterparts (**7b**, **7b**, **12f** and **12f**). To mention, **7c** carrying 2,4-diCl showed 9.18 ± 1.02 μg/mL of IC_{50}, while **7b** (4-Cl) displayed 11.29 ± 1.22 μg/mL of IC_{50}. Opposite t it, in case of brominated compounds, single Br bearing analogues (**7d**: 10.55 ± 0.66 μg/mL, **12d**: 10.94 ± 1.17 μg/mL) showed powerful action in terms of IC_{50s} in scavenging $ABTS^{\cdot+}$ than di-Br analogues (**7e**: 10.92 ± 0.41 μg/mL, **12e**: 11.73 ± 0.59 μg/mL) hence establishing the activity order of Cl > F > Br within halogenated subjects. However, to conclude ABTS assay in terms of halogenated compounds, set of **7a-k** exercised better in case of Cl substitution, while **12a-k** chalcones acted well with Br and F substituents. Overall structure of the rationalized molecules had an influential action in ABTS assay as unsubstituted **7a** had greater anti-$ABTS^{\cdot+}$ impact with 24.25 ± 1.48 μg/mL of IC_{50} than **12a** showing 27.90 ± 0.39 μg/mL of IC_{50}. Moreover, it can be stated that in case of nitro bearing substituents **7a-k** were better antioxidants than chalcones **12a-k**, because in ABTS assay **7i** exerted 18.99 ± 1.20 μg/mL of IC_{50} and **12i** furnished 22.78 ± 0.79 μg/mL of IC_{50}. Most importantly, from the above discussion it can be concluded that both two sets of compounds are more effective in scavenging ABTS radical than DPPH radical as shown from the IC_{50} data. Overall, when compared to the data documented for the control in both the antioxidant assay, it can be surely stated that the substances developed in the present research provides exciting and appealing free radical scavenging potential when compared to the control drug and can a tool for creating further related molecules with considerably higher effects.

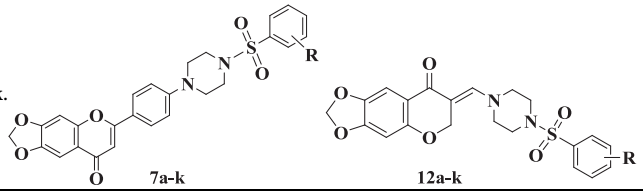
4.2.2. Antiproliferative activities

Cytotoxic potential of **7a-k** and **12a-k** was investigated against four human cancer cell lines namely human ovarian cancer (SK-OV-3), cervical cancer (HeLa), human colon adenocarcinoma (HT-29) and human non-small-cell lung carcinoma (A549) cell lines as well as non-cancer MDCK cells. In general, it can be stated that both sets of compounds were fruitful to deliver anticipated antiproliferative action against all four cancer cell lines and thus, the present work of designing and rationalizing these two different compact systems has been justified. Likewise antioxidant bioassay data, in SRB assay evaluations, structure and selected substituents placed an equal importance while delivering promising structure-activity relationships which will be discussed below.

New derivatives **7a-k** and **12a-k** demonstrated strong inhibitory potential of human ovarian cancer cell line SK-OV-3 with IC_{50s} ranging from 11.35 ± 2.10–58.93 ± 1.18 μg/mL. The mixed IC_{50} trends suggested that both structures were of a great impact on SK-OV-3 with the most influence came from variable substituents. Presence of three F atoms as well as two OCH_3 groups demonstrated excellent anti-ovarian cancer effects as analogues **7h** (4-OCF_3) and **12k** (2,4-diOCH_3) displayed 11.35 ± 2.10 μg/mL and 11.48 ± 0.79 μg/mL of IC_{50s}, respectively followed by 12.68 ± 1.09 and 13.04 ± 0.98 μg/mL of IC_{50s} of compounds **12h** (4-OCF_3) and **7k** (2,4-diOCH_3), respectively. Again, compounds with EWD halo atom(s) revealed interesting structure-activity relationship as in **7a-k** set those with single halo atom inhibited SK-OV-3 well than their dihalo counterparts, whereas, within chalcones family (**12a-k**) a case with fluorine was in line with what observed in **7a-k**, and in case of Cl and Br, those with two atoms were strong inhibitory agents of SK-OV-3 than their single halogenated counterparts. For an example, **7d** with 4-Br, **7e** bearing 2,4-diBr, **12c** holding 2,4-diCl and **12b** carrying 4-Cl had 19.34 ± 1.17 μg/mL, 28.08 ± 0.88 μg/mL, 24.79 ± 0.72 μg/mL and 29.13 ± 0.53 μg/mL of IC_{50s}, respectively.

Table 2

Antiproliferative activities of **7a-k** and **12a-k**.



No.	R	[a]IC_{50} μg/mL ± SD				[b]CC_{50} μg/mL ± SD
		SK-OV-3	HeLa	A-549	HT-29	MDCK
7a	H	58.93 ± 1.18	38.47 ± 1.41	47.11 ± 0.56	56.90 ± 1.09	321.73 ± 1.74
7b	4-Cl	26.10 ± 0.92	7.78 ± 0.91	21.24 ± 0.70	21.23 ± 1.48	289.64 ± 1.42
7c	2,4-diCl	28.90 ± 0.33	7.80 ± 0.59	20.79 ± 1.43	19.12 ± 0.47	277.21 ± 0.64
7d	4-Br	19.34 ± 1.17	8.33 ± 0.99	19.57 ± 1.32	34.08 ± 1.75	265.92 ± 1.25
7e	2,4-diBr	28.08 ± 0.88	7.90 ± 0.46	19.98 ± 0.65	36.72 ± 0.46	247.37 ± 1.01
7f	4-F	23.16 ± 1.01	7.81 ± 0.88	18.33 ± 0.32	15.84 ± 85	272.10 ± 0.53
7g	2,4-diF	34.17 ± 0.62	8.01 ± 1.00	19.56 ± 1.23	16.90 ± 0.56	257.11 ± 1.74
7h	4-OCF_3	11.35 ± 2.10	17.88 ± 1.20	26.47 ± 1.33	17.04 ± 0.37	244.57 ± 0.83
7i	4-NO_2	48.52 ± 0.97	43.19 ± 0.89	36.01 ± 1.11	47.11 ± 0.95	212.90 ± 0.39
7j	4-OCH_3	18.19 ± 0.92	14.88 ± 0.57	29.57 ± 1.13	34.51 ± 1.53	254.12 ± 1.81
7k	2,4-diOCH_3	13.04 ± 0.98	21.22 ± 1.04	33.41 ± 0.43	32.98 ± 1.95	231.19 ± 0.66
12a	H	55.12 ± 0.91	33.10 ± 0.81	56.10 ± 0.85	63.01 ± 0.47	298.81 ± 1.42
12b	4-Cl	29.13 ± 0.53	6.87 ± 1.27	25.19 ± 1.32	24.72 ± 0.76	257.82 ± 0.89
12c	2,4-diCl	24.79 ± 0.72	4.97 ± 0.39	26.22 ± 0.88	22.98 ± 0.35	251.21 ± 0.38
12d	4-Br	33.18 ± 1.24	7.88 ± 0.33	23.04 ± 0.75	27.33 ± 0.54	243.10 ± 0.55
12e	2,4-diBr	31.09 ± 0.67	7.67 ± 1.11	24.81 ± 1.37	36.09 ± 1.86	221.54 ± 0.74
12f	4-F	24.17 ± 0.79	6.98 ± 0.77	22.21 ± 0.84	17.66 ± 0.57	238.76 ± 1.59
12g	2,4-diF	30.44 ± 0.99	4.55 ± 0.44	22.99 ± 1.37	14.43 ± 1.26	231.29 ± 1.84
12h	4-OCF_3	12.68 ± 1.09	16.19 ± 0.36	27.58 ± 0.28	13.39 ± 0.36	247.27 ± 0.36
12i	4-NO_2	47.21 ± 0.82	40.13 ± 0.47	40.19 ± 0.84	50.19 ± 1.90	227.18 ± 0.50
12j	4-OCH_3	17.89 ± 1.01	13.97 ± 0.47	32.67 ± 0.85	32.10 ± 1.75	236.19 ± 1.22
12k	2,4-diOCH_3	11.48 ± 0.79	18.34 ± 0.96	33.13 ± 1.39	29.27 ± 1.65	252.72 ± 0.89

SK-OV-3: human ovarian cancer cell line, HeLa: human cervical cancer cell line, HT-29: human colon adenocarcinoma cell line, A-549: human non-small cell lung carcinoma cell line.

[a] Cytotoxicity is shown as IC_{50} values in μg/mL. All assays were carried out in triplicate, and the results are expressed as an average ± standard deviation.

[b] CC_{50} – cytotoxicity concentration of 50%.

Analogues **7j** and **12j** expressed 18.19 ± 0.92 μg/mL and 17.89 ± 1.01 μg/mL of IC_{50s}, respectively, which were better than any other halogenated analogues (19.34 ± 1.17–33.18 ± 1.24 μg/mL) thereby placing an importance of methoxy functional groups to inhibit ovarian cancer cells than halo-substituted compounds. Moreover, it was noticed that presence of appropriate substituent on the phenylpiperazinyl ring is essential to gain activity against SK-OV-3 as all analogues along with those with nitro substituent (**7i**: 48.52 ± 0.97 μg/mL and **12i**: 47.21 ± 0.82 μg/mL) displayed reasonably higher activities than unsubstituted derivatives **7a** (58.93 ± 1.18 μg/mL) and **12a** (55.12 ± 0.91 μg/mL) in terms of IC_{50s}. However, this fact could not discourage the construction of unsubstituted sulfonylpiperazine based molecules because anti-MDCK activity data showed that analogue **7a** and **12a** presented most potent and tolerable behavior with 321.73 ± 1.74 and 298.81 ± 1.42 μg/mL of CC_{50s}, respectively meaning that the presented designs of compounds are safer to use as an anticancer agents with the further development option on the other position of the base core. In case of inhibitory potential of cervical cancer cell line HeLa, the overall structure of the designed compounds was a key as **12a-k** (4.55 ± 0.44–40.13 ± 0.47 μg/mL) were found more potent than **7a-k** (7.78 ± 0.91–43.19 ± 0.89 μg/mL). The most potent analogous were from **12a-k** set as **12g** (2,4-diF) had 4.55 ± 0.44 μg/mL and **12c** (2,4-diCl) showed 4.97 ± 0.39 μg/mL of IC_{50s} while concluding that all the dihalo analogues were more active than their single halogenated counterparts. For example, **12e** and **7e** with 2,4-diBr substituent displayed 7.67 ± 1.11 and 7.90 ± 0.46 μg/mL of IC_{50s}, respectively, while **12d** and **7d** with 4-diBr substituent furnished 7.88 ± 0.33 and 8.33 ± 0.99 μg/mL of IC_{50s}, respectively. Amongst compound bearing halo groups, the overall activity order against HeLa observed Cl > F > Br. Moving forward, observing anti-HeLa activity of molecules with ED groups, those with single methoxy functional group had better activity than dual OCH_3 containing analogues. Such as, **7j** with 4-OCH_3 presented 14.88 ± 0.57 μg/mL of IC_{50} and **7k** with 2,4-diOCH_3 had 21.22 ± 1.04 μg/mL of IC_{50}. Noticing antiproliferative potential of derivatives bearing NO_2 group and derivatives without any substitution, HeLa assay results were an exception because unsubstituted **7a** and **12a** had better IC_{50s} when compared to **7i** and **12i** with NO_2 functionality with 38.47 ± 1.41 μg/mL, 33.10 ± 0.81 μg/mL, 43.19 ± 0.89 μg/mL and 40.13 ± 0.47 μg/mL, respectively. Furthermore, activity data observed against A-549 cell line suggested the importance of the overall structure of **7a-k** analogues and thereby justified the rationale of the current work about optimizing a specific position of a base core for the suitable substitution. As mentioned, **7a-k** were more active against **A-549** than chalcones **12a-k**, wherein, single halo bearing compounds were identified as promisingly active then their dihalo precursors. There was just one exception as **12k** with 2,4-diOCH3 group was slightly more active than its counterpart **7k** having 33.13 ± 1.39 and 33.41 ± 0.43 μg/mL of IC_{50s}, respectively. The most active derivatives noticed were belonged to F and Br group amongst **7a-k**, as **7d** (4-Br), **7e** (2,4-diBr), **7f** (4-F) and **7g** (2,4-diF) showed 18.33 ± 0.32–19.98 ± 0.65 μg/mL of IC_{50s} being 7f as the most active one. The halo and dihalo activity pattern followed in the same manner in **12a-k** set, for example **12b** involving 4-Cl substitution had 25.19 ± 1.32 μg/mL of IC_{50} and **12c** (2,4-diCl) had 26.22 ± 0.88 μg/mL of IC_{50} which showed the activity order for halo compounds as F > Br > Cl. As anticipated from the data set of tested derivatives against A549, those bearing OCF_3 group were more active then molecules endowed with OCH_3 functionality. For an instance, **7h** and **12h** revealed 26.47 ± 1.33 μg/mL and 27.58 ± 0.28 μg/mL of IC_{50s}, respectively, whereas, **7j** and **12j** had 29.57 ± 1.13 μg/mL and 32.67 ± 0.85 μg/mL of IC_{50s}, respectively. In regard with NO_2 bearing analogues versus unsubstituted ones, as usual NO_2 appeared with

higher activity (IC_{50s}: 36.01 ± 1.11–40.19 ± 0.84 μg/mL) then the later ones (47.11 ± 0.56–56.10 ± 0.85 μg/mL). It seemed that presence of a single functional group was the key to receive good anti-A549 potency as molecules with single OCH_3 groups showed better IC_{50s} than those with 2,4-diOCH_3 functionality. For example, **7j** having 4-OCH_3 showed 29.57 ± 1.13 μg/mL of IC_{50}, while **7k** (2,4-diOCH_3) presented 33.41 ± 0.43 μg/mL of IC_{50} and the similar trend has been recorded in case of **12j** and **12k**. The optimized insertion of fluorine atom(s) into a rationalized molecules can uniquely alter the membrane permeability, pK_a, metabolic pathways, binding interactions, intrinsic potency, selective reactivities, pharmacokinetic properties and molecular conformation [40,41]. The stated importance of F has been proved from the data obtained against HT-29 cell lines as all the fluorinated analogues were of high potency than others. For example, compound **12h** bearing 4-OCF_3 group exhibited 13.39 ± 0.36 μg/mL of IC_{50} and was the most potent one amongst others. However, there was an interesting SAR observed within two sets as single F bearing analogue (**7f**, 15.84 ± 85 μg/mL) from **7a-k** was more active than **12f** with 17.66 ± 0.57 μg/mL of IC_{50}, whereas in terms of two or more F bearing analogues those from **12a-k** set were more efficacious than their **7a-k** counterparts. For example, **12g** having 2,4-diF showed 14.43 ± 1.26 μg/mL of IC_{50} and **7g** holding 2,4-diF expressed 16.90 ± 0.56 μg/mL of IC_{50}. These facts, places an equal importance of deriving two different schemes whereas special emphasis was found placed on the fluorine substitution within both the sets. Interestingly, amongst halogenated analogues within both sets, all the Cl bearing molecules from **7a-k** were more active than chlorinated **12a-k**, where diCl bearing derivatives found to have greater inhibitory effects against HT-29 than single Cl holding compounds within both sets. For example, **7b** (4-Cl) and **7c** (2,4-diCl) showed 21.23 ± 1.48 μg/mL and 19.12 ± 0.47 μg/mL of IC_{50s}, respectively, and **12b** (4-Cl) and **12c** (2,4-diCl) exerted 24.72 ± 0.76 μg/mL and 22.98 ± 0.35 μg/mL of IC_{50s}, respectively. Opposite to it, in regard with brominated compounds, chalcones **12a-k** were effective against HT-29 than **7a-k**. Again, on the contrary to the chlorinated compounds, single Br bearing analogues were active than double Br containing molecules. For example, **12d** (4-Br), **12e** (2,4-diBr), **7d** (4-Br) and and **7e** (2,4-diBr) indicated 27.33 ± 0.54 μg/mL, 36.09 ± 1.86 μg/mL, 34.08 ± 1.75 μg/mL and 36.72 ± 0.46 μg/mL of IC_{50s}, respectively. Clearly, methoxy functionality seemed unsuitable to inhibit HT-29 as all the molecules bearing OCH_3 group appeared to have at least more than 29.27 ± 1.65 μg/mL of IC_{50}, where in fact, **12a-k** worked well than **7a-k** such as, **12j** and **7j** with 4-OCH_3 had 32.10 ± 1.75 μg/mL and 34.51 ± 1.5365 μg/mL of IC_{50s}, respectively. In addition, it was observed that placing of two methoxy groups was beneficial to inhibit HT-29 as IC_{50s} of **12k** and **12j** were 29.27 ± 1.65 μg/mL and 32.10 ± 1.75 μg/mL respectively the same was observed in case of **7j** and **7k**. As observed from the other data, again the substitution of at least one functional group on the piperazinylphenyl entity was crucial as analogues with NO_2 group had higher inhibitory effects against HT-29 than unsubstituted ones. The data facts, revealed the key features of the present study comparing two different heterocyclic systems and linked pharmacophores as a sulfonylpiperazine moiety featuring single and di substitution of the specific functional group(s). As data suggested that all the tested derivatives were fruitful in case of inhibiting cancer cell lines, however, their cytotoxic nature towards non-cancer or healthy cells should be investigated to conclude them as drug-like candidates. Therefore, we have screened all the final compounds against non-cancer MDCK cells where surprisingly, unsubstituted analogues had the highest CC_{50s} followed by those involving substitution of Cl atom(s) as the CC_{50s} order falls as Cl > OCF_3 > Br > F amongst halogenated molecules. Where, single halo bearing compounds were more tolerable by MDCK than their dihalo counterparts. In fact, halogenated molecules from **7a-k** set showed higher CC_{50s} than their **12a-k** counterparts. Finally, methoxy holding molecules revealed mixed trend in terms of CC_{50s} concluding that they were reasonably tolerable too. In addition, chalcones bearing OCH3 functionality had greater CC_{50s} observed than their **7a-k** counterparts. Overall, all the tested derivatives from both the sets demonstrated tremendous inhibitory potential against all the four cancer cell line studied and can be of a great deal of attraction for future optimization.

5. Conclusion

Two series of [1,3]dioxolo[4,5-*g*]chromenones featuring phenylsulfonylpiperazines liked to the chroman core through phenyl (**7a-k**) and chalcone (**12-k**) function has been rationalized, synthesized and investigated adopting antioxidant and anticancer assays. Free radicals ($DPPH^{\cdot}$ and $ABTS^{\cdot+}$) scavenging potencies were inspected along with cancerous cell inhibitory efficacies against human ovarian cancer (SK-OV-3), cervical cancer (HeLa), colon adenocarcinoma (HT-29) and non-small-cell lung carcinoma (A549) cell lines. Results from bioassay investigations concluded that selected molecules provided appealing medicinal potential and exciting structure-activity connections. For example, in some bioassay situations, overall framework discovered to be crucial to convey specific bioactivities, while for the other situations, putting an appropriate substituent(s) was a key to expected efficiency. Moreover, existence of EWD and ED group worked out different action levels against different objectives. Furthermore, amongst EWD and ED, presence of single or more than one groups had significant effect on the scientific activities of the resulting elements. For example, in regard with the importance of the overall structure, analgoues from **7a-k** group presented promising potential against non-small-cell lung carcinoma (A549) cell line, whereas molecules from chalcone series **12a-k** worked well with HeLa, HT-29 and $DPPH^{\cdot}$. Moreover, both sets were equally importance to receive anticipated potencies against $ABTS^{\cdot+}$ and SK-OV-3. Furthermore, single halogenated compounds from both the sets showed promising pharmacological potential against $ABTS^{\cdot+}$, A-549 whereas, dihalo substitution was a key to inhibit $DPPH^{\cdot}$, SK-OV-3 and HeLa. In regard with the OCF_3 and OCH_3 substitutions, all derivatives bearing them showed tremendous potential against both $DPPH^{\cdot}$ and $ABTS^{\cdot+}$ as well as against SK-OV-3 and HT-29. Double OCH_3 substitution was beneficial to inhibit both free radicals as well as SK-OV-3 and HT-29, whereas, single OCH_3 carrying analogues showed higher efficacies against HeLa and A-549. Lowest IC50s observed in case of unsubstituted derivatives from both the set suggested that placing a suitable functional group would worth the research attempt against free radicals and cancer cell lines, but higher CC_{50s} observed in case of these unsubstituted analogues denotes further structural optimizations in the future studies. Thus, the results suggested that there was an equal importance of both features, as a structure as well as placement of functional group(s) to gain enhanced and anticipated potencies.

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Conflict of interest

The authors report no conflicts of interest.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioorg.2019.03.002.

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