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FULL PAPER

Sulfonylpiperazines based on a flavone as antioxidant and cytotoxic agents

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Abstract

Chrysin-based sulfonylpiperazines **7a–k** were synthesized and investigated for their *in vitro* free radical scavenging potential as well as cytotoxic efficacies against selected cancer cell lines. Cytotoxicity of the new compounds toward noncancer cells was confirmed using the SRB assay against Madin–Darby Canine Kidney cells. Reaction of piperazine with different substituted benzenesulfonyl chlorides in triethylamine furnished sulfonylpiperazines (**3a–k**), which were then allowed to react with 7-(4-bromobutoxy)-5-hydroxy-2-phenyl-4*H*-chromen-4-one (**6**) prepared reacting chrysin with 1,4-dibromobutane to give the final derivatives **7a–k**. The results concluded that chrysin-sulfonylpiperazines exerted better antioxidant and anticancer efficacies than previously studied chrysin-piperazine precursors. For example, compounds **7h**, **7j**, and **7k** with 4-OCF_3, 4-OCH_3, and 2,4-diOCH_3 groups exhibited the best antioxidant potential against 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radicals. Moreover, halogenated analogues (**7b**, **7c**, **7g**, and **7h**) demonstrated promising anticancer potential against SK-OV3, HeLa, and HT-29 cell lines, whereas those bearing a methoxy functional group (**7j** and **7k**) had beneficial effects against the cell lines A-549 and HT-29. Thus, it can be confirmed from the bioassay results that the overall structural design as well as proper substitution is crucial to deliver the anticipated biological effects. Spectroscopic techniques such as FT-IR, ^{1}H NMR, ^{13}C NMR, mass and elemental analysis (CHN) were carried out to confirm the final structures.

KEYWORDS
anticancer, antioxidant, chrysin, flavone, sulfonylpiperazine

1 | INTRODUCTION

Flavonoids as plant specialized metabolites are prominent in fruits, nuts, and vegetables bearing excellent therapeutic safety profiles and bearable toxicity. Flavonoids have obtained significant attention in the area of chemoprevention as presence of oxygenated groups and aromatic moieties as well as optimum placement of varied pharmacophores on flavonoids' structure (Figure 1) attributed to the higher biological efficacies those are preferred for human wellness.[1,2] These phytochemicals possess significant qualities in chemical defense and nitrogen fixation in addition to wide range of postulated health benefits such as antioxidant, anticancer, antiviral, and anti-inflammatory.[3] A phytochemical, chrysin (5,7-dihydroxyflavone), is a naturally active compound of the flavone group and can be extracted from honey, propolis, and plants. It has been a hot spot as a prospective chemo-preventive compound and served biological

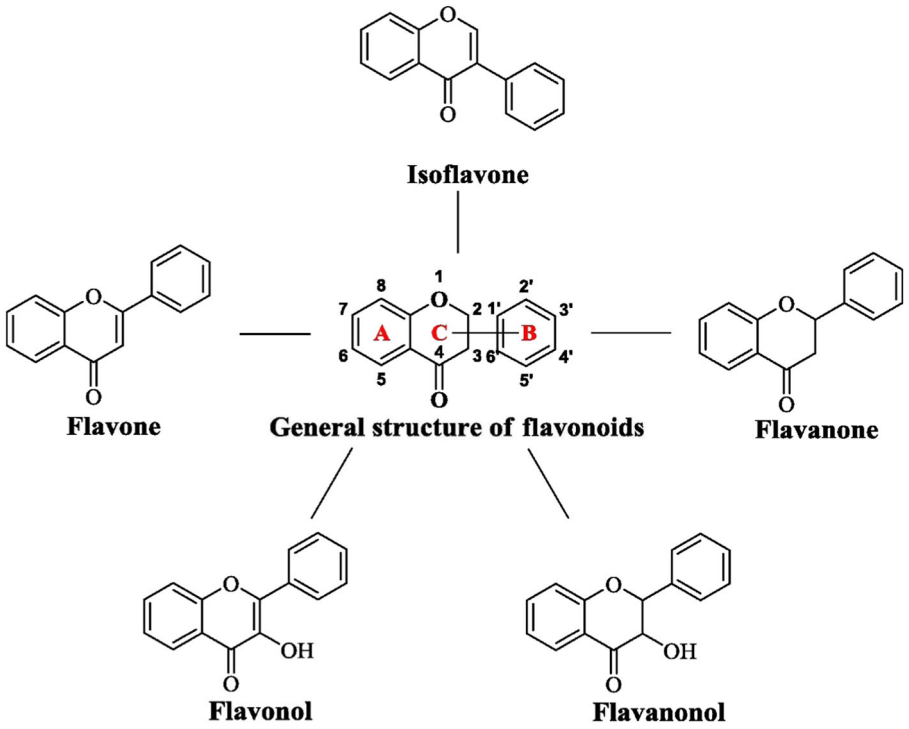


FIGURE 1 General chemical structure of flavonoids and their different classes. Reused with permission from Zhao et al.[2]

potencies as antimicrobial, antiviral, anticancer, antioxidant and so on,[4] due to which it has become the foremost contender among flavonoids in drug discovery research. There are improving proofs of the prospective advantages of chrysin as a medicinal agent.[5,6] For example, chrysin-benzimidazoles were reported to have strong anti-proliferative activity against MFC cells and flow cytometry results displayed that they induced apoptosis of MFC cells in a dose-dependent manner and caused the cell cycle to be arrested in the G0/G1 phase.[7] Furthermore, flavonoid salicylates were also found potent against MCF-7 cells, HepG2 cells, MGC-803cells, and MFC cells.[8,9]

Cancer, the second leading death cause globally, is the most significant concerns that the current healthcare system faces. Diverse components may add to this encumbrance of malignancy including environmental factors, genetics, and lifestyles. It has recorded 9.6 million loss of life in 2018. All inclusive, around one out of six deaths is because of this malignancy. Regardless of numerous endeavors in cancer research to identify and treat malignancy, disclosure of optimal cancer treatments is yet troublesome because of the serious adverse reactions related with chemotherapeutics, like tumor resistance. Malignancies, for example, liver, prostate, colorectal, and lung cancer as well as breast, cervix, and thyroid cancer are the most common among men and women, respectively.[10,11] Initiation, promotion, and progression are the basic steps of carcinogenesis, which remain as the main target to be focused in the cancer control strategies. Be that as it may, over the most recent couple of years, treatment conventions have fundamentally been improved; gratitude to a superior comprehension of the key oncogenes and signaling pathways associated with its pathogenesis and progression. Anticancer treatment would either execute tumor cells by activating apoptosis or permanently arrest them in cell cycle's G1 phase.[12,13] Human's existing homeostatic balance can be compromised through the exposure toward environmental toxins and undesirable lifestyle outcomes into the occurrence of reactive oxygen species (ROS). Because of their high level of reactivity, ROS are generally thought to solely intervene the oxygen toxicity. Oxidative stress caused by ROS can structurally and/or functionally compromise proteins, nucleic acids, and lipids causing serious medical conditions like cancer.[14,15] As of late, chemical modification of natural products has pulled in extraordinary consideration of numerous strategies nowadays, which are expected to improve their pharmacological efficacies against multiple biological targets.[16]

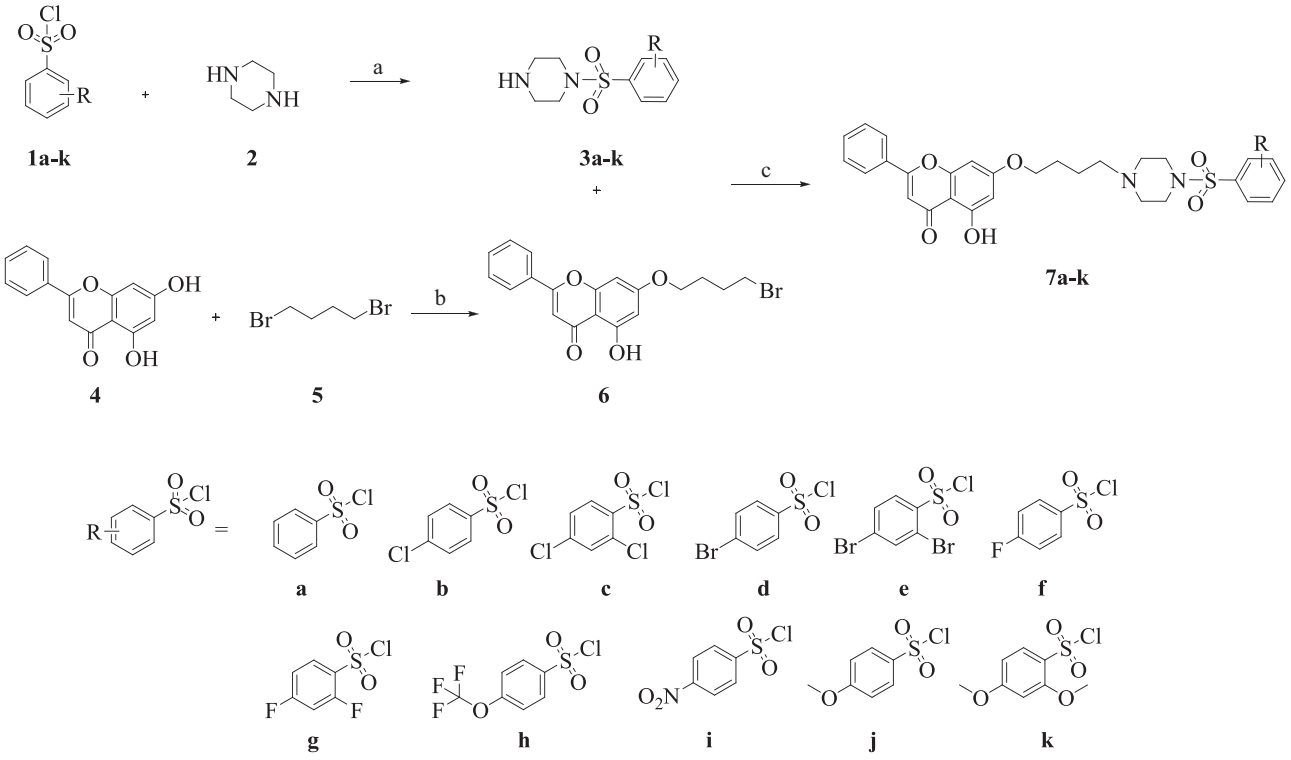
In this context, it has been revealed that chrysin and its derivatives prevent cancer growth and cause apoptosis in melanoma tissues,[17] and also act as a strong antioxidant to scavenge harmful free radicals.[18] It has been exposed that chrysin has anticancer action by promoting the cell death caused by tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) and enhancing TRAIL-induced degradation of caspases 3 and 8.[19,20] By such means, chrysin is able to destroy melanoma cells of lung, breast, cervical, liver, leukemia, colon, nasopharyngeal, prostate, glioblastoma, thyroid, and pancreatic cancer.[21] Previous attempts to derivatise chrysin core revealed that such modifications includes reaction with the one of the OH groups of chrysin. In addition, it is affirmed that OH group of the chrysin is crucial to furnish anticipated biological potencies as it interacts with ROS and protects against oxidative stress as well as DNA damage.[22] In a light of aforementioned facts, we have designed chrysin-based piperazine molecules, which includes the presence of one OH group in chyrsin core as well as substituting another OH group by desired active pharmacophores[23] and these analogues appeared to have significant antioxidant and anticancer effects. Moreover, organic and inorganic precursors holding sulfone entity

were found to have promising potential as biological active agents,[24] whereas piperazine analgoues[25] have been proposed to be a key factor enhancing biological effects of the bearing molecules. Most important, the piperazine-based anticancer agents include abemaciclib, bosutinib, brigatinib, dexrazoxane, dosatinib, imatinib, leucovorin, olaparib, palbociclib, ponatinib, rociletinib, venetoclax, and trabectidin, and so forth.[26] Thus, we have aimed to prepare chrysin-sulfonylpiperazine derivatives in the present research to gain enhanced biological significance.

2 | RESULTS AND DISCUSSION

2.1 | Chemistry

Synthetic steps adopted to furnish final chrysin-sulfonylpiperazines **7a–k** are drawn in Scheme 1. Reaction of piperazine in the presence of triethylamine with selected different substituted benzenesulfonyl chlorides gave intermediates **3a–k**. Correct structure of these derivatives were checked observing physical parameters as well as spectral data. For example, intermediate **3e** was obtained as yellow powder with excellent 76% of product yield, in which, aromatic proton showed multiplet signals in the range 7.89–7.69 parts per million (ppm), whereas protons belong to the piperazine ring resonated as triplet signals at 3.44 and 2.87 ppm. Finally, an –NH signal was observed at 1.18 ppm as singlet. Second, most important intermediate 7-(4-bromobutoxy)-5-hydroxy-2-phenyl-4*H*-chromen-4-one (**6**) was successfully synthesized using method previously reported by us with the use of chrysin (**4**) with 1,4-dibromobutane (**5**). In the continuous reaction sequences, at the final stage, sulfonylpiperazines **3a–k** were allowed to react with 7-(4-bromobutoxy)-5-hydroxy-2-phenyl-4*H*-chromen-4-one (**6**) in acetonitrile under reflux conditions to give final analogues **7a–k** in reasonable yields. Compound **7b** as a representative compound, showed aromatic C-H and C-C stretching frequencies at 3,083 and 2,925 cm^{-1}. In addition, C-N band corresponding to the piperazine ring appeared at 1,339 cm^{-1}, whereas SO_2 group revealed its characteristic signals at 1,384 and 1,259 cm^{-1} and carbonyl of chromane moiety appeared at 1,655 cm^{-1}. In addition, ^1H NMR spectrum of **7b** displayed characteristic signals for the proton atoms of the piperazine ring at 3.51 and 2.88 ppm in the form of triplets, whereas doublet, singlet and doublet noticed at 6.42, 6.75, and 6.12 ppm was attributed to the proton atoms of a chromane ring. In this context, hydroxyl proton was found present at 12.69 ppm as a singlet and other multiplets in the range 7.86–7.12 assigned to aromatic protons. Proton present in the aliphatic chain resonated in the form of triplet and multiplets in the range between 4.25 and 1.95 ppm. The presence of chain further confirmed taking ^{13}C NMR data of **7b** where these proton expressed their signals at around 69.1 and 28.8 ppm. Moreover, piperazine carbon atoms were found to be present at 54.5 and 34.1 ppm. Other chromane ring-based carbon atoms along with aromatic carbons resonated above 158 ppm level. All compounds gave C, H, and N analyses within acceptable limits from the theoretical values.



Reagents and conditions: (a) TEA, CH_2Cl_2; (b) K_2CO_3, Reflux, 24 hr; (c) CH_3CN, Reflux, 13–29 hr.

SCHEME 1 Synthesis of the sulfonylpiperazine-based chrysin derivatives

2.2 | Pharmacology

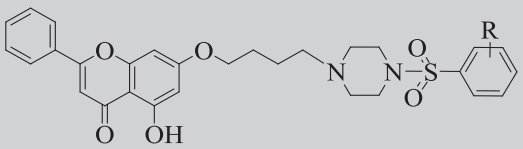
Results of pharmacological screening of newly synthesized sulfonylpiperazine-based chrysin derivatives **7a–k** as antioxidant molecules tested using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) methods as well as their potential as anticancer agents against four different cancerous cell lines namely, human ovarian cancer (SK-OV-3), cervical cancer (HeLa), human colon adenocarcinoma (HT-29), and human non-small-cell lung carcinoma (A-549) cell lines are summarized in Tables 1 and 2 along with bioassay data obtained as cytotoxicity levels of mentioned compounds against Madin–Darby Canine Kidney (MDCK) cell. From the bioassay data obtained in this study, it can be observed that modifying previously studied chrysin-piperazine conjugates to their sulfonylpiperazine precursors, which lead to the substantially enhanced biological efficacies. Our previous research for the similar molecular systems involving chrysin-piperazine analogues revealed 20.30 ± 0.476 to 34.06 ± 0.913 μg/ml and 5.569 ± 0.025 to 8.971 ± 0.881 μg/ml of IC_{50} values in DPPH and ABTS bioassay, respectively, as well as 5.044 ± 0.423 to 9.914 ± 0.445 μg/ml and 12.876 ± 0.411 to 63.210 ± 0.158 μg/ml of IC_{50} values against HeLa and SK-OV-3 cell lines, respectively.[23]

Generally, **7a–k** exerted remarkable free radical scavenging efficacies in the range of 14.67 ± 0.67 μg/ml to 22.12 ± 1.21 μg/ml of IC_{50} against DPPH radical and 4.88 ± 0.93 μg/ml to 11.21 ± 0.59 μg/ml of IC_{50} against ABTS radical, respectively. Further, it seems that compounds bearing electron withdrawing substituents such as methoxy or trifluoromethoxy functionalities as well as unsubstituted derivatives exerted best antioxidant potencies against DPPH and ABTS. In addition, IC_{50} levels of sulfonyl piperazines were found to be lowered significantly against DPPH radical when compared with previously studied chrysin-piperazine derivatives, which showed 20.30 ± 0.476 to 34.06 ± 0.913 μg/ml of IC_{50s}.[23] However, activity against ABTS radical looked similar as previously studied. More important, placing a proper functional group on the piperazine moiety was important to gather anticipated free radical scavenging activities as compounds with methoxy substituent(s) were the most active antioxidants as studied in this research. For example, derivative **7k** with dimethoxy substituent showed 13.92 ± 0.89 μg/ml of IC_{50} and was the most potent one among others in this study against DPPH as compared with the control ascorbic acid with 12.72 ± 0.274 μg/ml of IC_{50}. Furthermore, compound **7j** with *para*-methoxy, **7h** with 4-trifluoromethoxy substitution as well as unsubstituted derivative **7a** demonstrated 14.67 ± 0.67, 14.93 ± 0.83, and 15.03 ± 0.56 μg/ml of IC_{50s}, respectively, against DPPH. Other molecules with halo or nitro substituents were found to have good to moderate potency with 15.33 ± 0.79 to 22.12 ± 1.21 μg/ml of IC_{50s}, though higher than their chrysin-piperazine precursors. During DPPH assay, it was observed that presence of single halogen atom was beneficial to have remarkable antioxidant potential when compared with dihalo derivatives and nitro compound. For instance, **7d** with 4-Br substituent showed 16.33 ± 1.52 μg/ml of IC_{50}, whereas 2,4-diBr substituted derivative (**7e**) exhibited 21.59 ± 0.85 μg/ml of IC_{50} concluding that presence of single halo atom is beneficial for the optimum DPPH scavenging potency.

Bioassay results observed in ABTS assay suggested that both chrysin-piperazine and chrysin-sulfonylpiperazine analogues expressed similar level of efficacies with IC_{50s} in the range 4.88 ± 0.93 to 11.21 ± 0.59 μg/ml. However, the feature of the most potent analogue remained same as above mentioned DPPH assay results, such as compound **7j** with *para*-methoxy substituent revealed 4.88 ± 0.93 μg/ml of IC_{50}, which had even more potent result when compared with control ascorbic acid with 5.0925 ± 0.2090 μg/ml of IC_{50}. Furthermore, **7k** with 2,4-diOCH_3 and **7h** with 4-OCF_3 showed 5.74 ± 0.49 and 5.97 ± 1.23 μg/ml of IC_{50s}, respectively. Hence, it can be stated that compounds with methoxy substituents were the most potent analogues in this study against both DPPH and ABTS radicals. In addition, all halogenated analogues showed almost similar level of IC_{50s} against ABTS with IC_{50s}, approximately ranging 8–9 μg/ml. Overall, when compared with the data recorded for the control in both the antioxidant assay, it can be surely stated that the compounds invented in the present research deliver interesting and promising free radical scavenging potential when compared with the control drug and can be a tool for developing further related molecules with substantially higher effects

Investigation of anticancer potential of **7a–k** analogues against ovarian cancer (SK-OV-3), cervical cancer (HeLa), colon adenocarcinoma

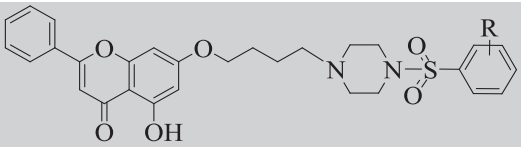
TABLE 1 Screening results for DPPH and ABTS radical scavenging activity of sulfonylpiperazine-based chrysin derivatives (**7a–k**)



No.	R	IC_{50} μg/ml ± SD[a] DPPH	ABTS
7a	H	16.62 ± 0.56	7.03 ± 0.83
7b	4-Cl	15.39 ± 0.42	9.05 ± 1.29
7c	2,4-diCl	20.99 ± 1.09	8.32 ± 0.78
7d	4-Br	16.33 ± 1.52	9.37 ± 0.66
7e	2,4-diBr	21.59 ± 0.85	8.17 ± 0.79
7f	4-F	15.33 ± 0.79	8.35 ± 1.44
7g	2,4-diF	18.92 ± 0.95	7.99 ± 1.03
7h	4-OCF_3	14.93 ± 0.83	5.97 ± 1.23
7i	4-NO_2	22.12 ± 1.21	11.21 ± 0.59
7j	4-OCH_3	14.67 ± 0.67	4.88 ± 0.93
7k	2,4-diOCH_3	13.92 ± 0.89	5.74 ± 0.49
Ascorbic acid		12.72 ± 0.274	5.0925 ± 0.2090

Abbreviations: ABTS, 2,2′-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid; DPPH, 2,2-diphenyl-1-picrylhydrazyl; SD, standard deviation.
[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 ± SD.

TABLE 2 Cytotoxicity potential of sulfonylpiperazine-based chrysin derivatives (**7a–k**)



No.	R	IC_{50} μg/ml ± SD[a]				CC_{50} μg/ml ± SD[b]
		SK-OV-3	HeLa	A-549	HT-29	MDCK
7a	H	48.34 ± 0.87	59.72 ± 0.48	33.52 ± 1.22	62.19 ± 1.25	324.2 ± 1.74
7b	4-Cl	13.05 ± 0.92	7.33 ± 0.93	29.18 ± 0.56	47.73 ± 0.34	296.8 ± 0.93
7c	2,4-diCl	30.19 ± 1.28	5.02 ± 0.59	25.81 ± 0.71	36.21 ± 0.89	225.7 ± 2.08
7d	4-Br	27.91 ± 0.71	9.12 ± 1.23	27.09 ± 0.90	41.28 ± 1.09	189.3 ± 1.67
7e	2,4-diBr	33.49 ± 1.22	5.89 ± 0.39	26.15 ± 0.47	34.19 ± 0.93	202.4 ± 1.29
7f	4-F	42.14 ± 0.99	8.09 ± 0.68	24.94 ± 0.83	43.55 ± 1.21	303.1 ± 0.79
7g	2,4-diF	12.67 ± 0.74	4.67 ± 1.42	27.63 ± 1.27	31.34 ± 0.77	168.4 ± 2.03
7h	4-OCF_3	34.67 ± 0.71	40.18 ± 1.69	25.44 ± 1.51	21.42 ± 0.51	163.6 ± 2.33
7i	4-NO_2	51.23 ± 0.68	21.34 ± 1.33	28.92 ± 0.77	43.96 ± 0.89	211.5 ± 1.78
7j	4-OCH_3	25.83 ± 1.67	30.99 ± 0.85	25.89 ± 0.79	22.06 ± 1.42	285.4 ± 0.99
7k	2,4-diOCH_3	32.17 ± 1.12	44.57 ± 1.09	24.21 ± 1.14	28.37 ± 0.97	248.5 ± 1.92
Gefitinib[c]	–	12.31 ± 0.33	17.92 ± 1.50	13.75 ± 5.73	23.6 ± 4.1	–

Abbreviations: A-549, human non-small-cell lung carcinoma; HeLa, human cervical cancer; HT-29, human colon adenocarcinoma; MDCK, Madin–Darby Canine Kidney cells; SD, standard deviation; SK-OV-3, human ovarian cancer.

[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 ± SD.

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

[c]Values obtained from the literature.

(HT-29), and non-small-cell lung carcinoma (A-549) cell lines as well as noncancer MDCK cells proved the success of the present research as these derivatives showed positively enhanced IC_{50s} against all of the above cell lines when compared with chrysin-piperazine studied previously.[23]

Overall, presented derivatives expressed significant level of cancerous cell inhibitory potential against all the stated cell lines. For example, analogues **7a–k** showed 12.67 ± 0.74 to 51.23 ± 0.68 μg/ml of IC_{50} against SK-OV-3 ovarian cancer cell line, which were comparable with that of control drug gefitinib with 12.31 ± 0.33 μg/ml of IC_{50}.[27] Derivative **7g** with dual fluorine atom substitution on the sulfonylpiperazine ring exhibited best activity against SK-OV-3 with least 12.67 ± 0.74 μg/ml of IC_{50} and was observed as potent as gefitinib. Furthermore, a compound (**7b**) with single chlorine atom substitution showed 13.05 ± 0.92 μg/ml of IC_{50} against SK-OV-3 cells. It was noted that presence of halogen atom was essential to deliver activity against SK-OV-3 rather than numbers of halogen atoms as 4-Br substituted derivative (**7d**) was found more potent (27.91 ± 0.71 μg/ml of IC_{50}) than its 2,4-diBr precursor (**7e**, IC_{50}: 33.49 ± 1.22 μg/ml). Considering derivatives with electron donating substituents, one with 4-OCH_3 group showed better SK-OV-3 inhibitory effect than that with 2,4-diOCH_3 functionality. At last, derivative with a nitro substituent (**7i**) was found least active against SK-OV-3 with 51.23 ± 0.68 μg/ml of IC_{50}. The bioassay outcome against HeLa cell lines indicated that presence of halo atom(s) was so crucial to deliver potent IC_{50} in the range 4.67 ± 1.42 to 9.12 ± 1.23 μg/ml when compared with control gefitinib at 17.92 ± 1.50 μg/ml.[27] Furthermore, in case of inhibition of HeLa cell line, again derivative with 2,4-difluoro substituent (**7g**) demonstrated remarkable 4.67 ± 1.42 μg/ml of IC_{50} followed by an analogue with 2,4-dichloro functionality (**7c**) with 5.02 ± 0.59 μg/ml of IC_{50}. Bioassay data against HeLa cell line suggested that the presence of halogen atom(s) was a key to exert promising activity as all halogenated derivatives were shown to have lower IC_{50} values when compared with those final derivatives with methoxy, nitro, or trifluoromethoxy groups. In addition, among halogenated derivatives, those with two halo atoms were more active against HeLa than their single halogenated precursors. For example, **7e** with 2,4-diBr has 5.89 ± 0.39 μg/ml of IC_{50} and **7d** with 4-Br revealed 9.12 ± 1.23 μg/ml of IC_{50}. However, within this group, for dual halogen atom analogues, activity order against HeLa cells falls as F > Cl > Br whereas, with respect to the single halo atom, it was found Cl > F > Br. The least active compound against HeLa was **7a** with 59.72 ± 0.48 μg/ml of IC_{50}, which has a feature of unsubstituted sulfonylpiperazine entity. Regarding anticancer activity against lung cancer A-549 cell line, a mixed trend was observed as two compound with different feature was found active, which were **7f** with *para*-fluoro substitution and **7k** with 2,4-diOCH_3 functional group presenting 24.94 ± 0.83 and 24.21 ± 1.14 μg/ml of IC_{50}, respectively. These two derivatives demonstrated half the potential to inhibit A-549 than gefitinib with 13.75 ± 5.73 μg/ml of IC_{50}.[27] It will suffice to mention here that all the other derivatives showed IC_{50} values in the narrow range of 25–29 μg/ml, which suggest that overall chrysin-sulfonylpiperazine system is crucial to furnish activity against A-549 rather than

presence of a functional group. Also it is worth to note that regarding cytotoxicity of the presented analogues against noncancer MDCK cell lines, derivative **7f** with 4-F group had promising result with 303.1 ± 0.79 μg/ml of CC_{50} along with best CC_{50} for unsubstituted **7a** at 324.2 ± 1.74 μg/ml. Although all the derivative tested showed tremendous level of CC_{50} against MDCK ranging from 163.6 ± 2.33 to 324.2 ± 1.74 μg/ml of CC_{50}, which suggested that all the newly constructed derivatives are safer to be developed as cytotoxic agents against cancer cells. At last, bioassay data against HT-29 cell line showed that the presence of electron donating group such as 4-OCF3 (**7h**) and 4-OCH3 (**7j**) was essential to provide inhibition of HT-29 cells with 21.42 ± 0.51 and 22.06 ± 1.42 μg/ml of CC_{50}, respectively. It can be stated that these two derivatives had exerted similar anti HT-29 efficacies as control drug gefitinib with 23.6 ± 4.1 μg/ml of CC_{50}.[28] These facts suggest that not only EW halogen atoms are important to deliver anticancer effects but presence of ED groups is equally crucial, which justified the rationale of the present work. Moreover, the derivative **7k** with 2,4-diOCH3 group also had promising potential against HT-29 cell line with 28.37 ± 0.97 μg/ml of CC_{50}. More important, it was found that the presence of more than one halo atom was beneficial to inhibit HT-29 as dihalo derivatives **7c**, **7e,** and **7g** showed better IC_{50} levels than single halogenated derivatives **7b**, **7d**, and **7f**. Thus, it can be said that in case of anticancer potency against HeLa and HT-29 cell line, dihalo functionality was crucial. From the overall data, it was observed that substituting sulfonylpiperazine with different functionality expressed better anticancer effects as the compound **7a** with the absence of any functional group showed poor action against all the cell lines tested.

3 | CONCLUSION

In a continuous antioxidant and anticancer drug discovery study, we furnished modified chrysin-sulfonylpiperazine analgoues **7a–k** with the optimum and rationalized structural features. It is believed that mechanisms like oncogenic pathways inhibition and variations in the enzymes activity are responsible factors for the biological properties of mentioned derivatives based on the extensive literature reports. Biological studies were conducted against DPPH and ABTS free radicals in an antioxidant assay and against human ovarian cancer (SK-OV-3), cervical cancer (HeLa), colon adenocarcinoma (HT-29), and non-small-cell lung carcinoma (A-549) cell lines in an anticancer assay. The chemistry of the present research is straightforward, which gives easy access to the potential biologically active drug-like molecules. Bioassay results suggested that the overall design of stated structure as well as presence or absence of electron withdrawing (EWD) or electron donating (ED) functional group on the sulfonylpiperazine entity was crucial to express anticipated biological activities. Data showed that converting simple piperazine moiety to its sulfonylpiperazine form increased the overall potency of the resultant molecules. Such as against DPPH free radical, all the new compounds showed significantly lowered IC_{50} values as all the compounds with single halogen atom expressed better potencies then rest of others. In addition, the presence of halogen atom(s) was found essential to get better potency against SK-OV-3, HeLa, and A-549 cell lines, whereas presence of trifluoromethoxy or dimethoxy functional groups revealed better activity against ABTS free radical as well as HT-29 cancerous cell line. Finally, it is worth to mention that some of the new molecules exhibited better anticancer potential against SK-OV-3 and HeLa and similar to moderate inhibition of A-549 and HT-29 when compared with control drug gefitinib. Results against noncancer MDCK cells suggested that all the stated derivatives are safer to be anticancer drugs and provide a platform for further drug discovery process upon careful optimization.

4 | EXPERIMENTAL

4.1 | Chemistry

4.1.1 | General

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 precoated 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}). ^1H NMR and ^{13}C NMR spectra were recorded on a Bruker AVANCE III 400 instrument spectrometer (^1H NMR, 400 MHz; ^{13}C NMR, 100 MHz) 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.

The InChI codes of the investigated compounds together with some biological activity data are provided as Supporting Information.

4.1.2 | General procedure for the synthesis of substituted sulfonylpiperazines (3a–k)

Different benzenesulfonyl chlorides (**1a–k**, 8 mmol) were reacted with piperazine in CH_2Cl_2 at 0°C in the presence of 24 mmol of triethylamine for 2–6 hr. The reaction was monitored by TLC using 2:1 of petroleum ether and ethyl acetate and after the completion the reaction mass was quenched with water and extracted with CH_2Cl_2, where combined organic layer was dried over anhydrous sodium sulfate and evaporated to give **3a–k**.[28]

*1-(Phenylsulfonyl)piperazine (**3a**)*

White powder, yield: 83%, m.p. 111–113°C. ^1H NMR (DMSO-*d6*, 400 MHz): δ 7.83–7.38 (m, 5H, ArH), 3.57 (t, J = 4.88 Hz, 4H, CH_2), 2.84 (t, J = 4.93 Hz, 4H, CH_2), 1.22 (s, 1H, NH).

*1-(4-Chlorophenylsulfonyl)piperazine (**3b**)*

Yellow powder, yield: 69%, m.p. 99–100°C. ^1H NMR (DMSO-*d6*, 400 MHz): δ 7.64–7.55 (m, 4H, ArH), 3.46 (t, J = 4.80 Hz, 4H, CH_2), 2.71 (t, J = 4.80 Hz, 4H, CH_2), 1.17 (s, 1H, NH).

*1-(2,4-Dichlorophenylsulfonyl)piperazine (**3c**)*

Yellow powder, yield: 71%, m.p. 121–123°C. ^{1}H NMR (DMSO-*d6*, 400 MHz): δ 7.83 (d, J = 1.7 Hz, 1H), 7.68 (d, J = 7.3 Hz, 1H), 7.54 (dd, J = 7.5, 1.5 Hz, 1H), 3.53 (t, J = 4.88 Hz, 4H, CH_2), 2.78 (t, J = 4.93 Hz, 4H, CH_2), 1.20 (s, 1H, NH).

*1-(4-Bromophenylsulfonyl)piperazine (**3d**)*

Yellow powder, yield: 73%, m.p. 112–113°C. ^{1}H NMR (DMSO-*d6*, 400 MHz): δ 7.73–7.59 (m, 4H, ArH), 3.50 (t, J = 4.84 Hz, 4H, CH_2), 2.79 (t, J = 4.91 Hz, 4H, CH2), 1.18 (s, 1H, NH).

*1-(2,4-Dibromophenylsulfonyl)piperazine (**3e**)*

Yellow powder, yield: 76%, m.p. 131–133°C. ^{1}H NMR (DMSO-*d6*, 400 MHz): δ 7.89–7.69 (m, 3H, ArH), 3.44 (t, J = 4.81 Hz, 4H, CH_2), 2.87 (t, J = 4.77 Hz, 4H, CH_2), 1.18 (s, 1H, NH).

*1-(4-Fluorophenylsulfonyl)piperazine (**3f**)*

Yellow powder, yield: 70%, m.p. 109–111°C. ^{1}H NMR (DMSO-*d6*, 400 MHz): δ 7.73–7.64 (m, 2H), 7.43–7.32 (m, 2H), 3.55 (t, J = 4.82 Hz, 4H, CH_2), 2.79 (t, J = 4.90 Hz, 4H, CH_2), 1.19 (s, 1H, NH).

*1-(2,4-Difluorophenylsulfonyl)piperazine (**3g**)*

Yellow powder, yield: 68%, m.p. 127–129°C. ^{1}H NMR (DMSO-*d6*, 400 MHz): δ 7.80–7.62 (m, 3H, ArH), 3.49 (t, J = 4.84 Hz, 4H, CH_2), 2.76 (t, J = 4.80 Hz, 4H, CH_2), 1.21 (s, 1H, NH).

*1-(4-(Trifluoromethoxy)phenylsulfonyl)piperazine (**3h**)*

Yellow powder, yield: 59%, m.p. 133–135°C. ^{1}H NMR (DMSO-*d6*, 400 MHz): δ 7.68–7.59 (m, 2H), 7.39–7.27 (m, 2H), 3.48 (t, J = 4.85 Hz, 4H, CH_2), 2.80 (t, J = 4.91 Hz, 4H, CH_2), 1.15 (s, 1H, NH).

*1-(4-Nitrophenylsulfonyl)piperazine (**3i**)*

Yellow powder, yield: 57%, m.p. 95–97°C. ^{1}H NMR (DMSO-*d6*, 400 MHz): δ 7.88–7.51 (m, 4H, ArH), 3.50 (t, J = 4.80 Hz, 4H, CH_2), 2.81 (t, J = 4.83 Hz, 4H, CH_2), 1.21 (s, 1H, NH).

*1-(4-Methoxyphenylsulfonyl)piperazine (**3j**)*

Yellowish brown powder, yield: 73%, m.p. 122–124°C. ^{1}H NMR (DMSO-*d6*, 400 MHz): δ 7.70–7.61 (m, 2H), 7.36–7.22 (m, 2H), 3.87 (s, 3H, OCH_3), 3.55 (t, J = 4.81 Hz, 4H, CH_2), 2.85 (t, J = 4.81 Hz, 4H, CH_2), 1.22 (s, 1H, NH).

*1-(2,4-Dimethoxyphenylsulfonyl)piperazine (**3k**)*

Yellowish brown powder, yield: 67%, m.p. 141–142°C. ^{1}H NMR (DMSO-*d6*, 400 MHz): δ 7.76–7.22 (m, 3H), 3.82 (s, 3H, OCH_3), 3.69 (s, 3H, OCH_3), 3.47 (t, J = 4.83 Hz, 4H, CH_2), 2.77 (t, J = 4.93 Hz, 4H, CH_2), 1.19 (s, 1H, NH).

4.1.3 | Synthesis of 7-(4-bromobutoxy)-5-hydroxy-2-phenyl-4*H*-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 hr until no starting material was left as monitored by TLC. After the reaction completion, the reaction mixture was concentrated, cooled at room temperature, diluted with ethyl acetate (100 ml), and washed with water (2 × 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,[29] Yield: 83%, IR (KBr) cm^{-1}: 3,072; 2,954; 2,855; 1,642; 1,604; 1,589. ^{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), 6.42 (d, J = 2.2 Hz), 6.33 (d, J = 2.2 Hz), 4.11 (t, J = 6.0 Hz, 2H), 3.45 (t, J = 6.5 Hz, 2H), 2.19–2.09 (m, 2H), 1.99–1.91 (m, 2H, CH_2); ^{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^+).

4.1.4 | General procedure for the preparation of 5-sulfonylpiperazine-based chrysin derivatives (7a–k)

In a flask charged with 50 ml of CH_3CN was added 2.5 mmol of compound **6** and appropriate piperazine derivatives (**3a–k**, equiv.) and the reaction mixture was refluxed for 13–29 hr 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 × 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 good yields.[30]

*5-Hydroxy-2-phenyl-7-(4-(4-(phenylsulfonyl)piperazin-1-yl)-butoxy)-4H-chromen-4-one (**7a**)*

Yield: 56%. m.p. 253–255°C; IR (KBr) cm^{-1}: 3,076; 2,943; 2,927; 2,872; 1,664; 1,622; 1,581; 1,380; 1,343; 1,253; 1,161; 1,146; 1,039. ^{1}H NMR (DMSO-*d6*, 400 MHz): δ 12.71 (s, 1H), 7.91–7.86 (m, 2H), 7.57–7.48 (m, 4H), 7.39–7.16 (m, 4H), 6.78 (s, 1H), 6.41 (d, J = 2.52 Hz, 1H), 6.07 (d, J = 2.43 Hz, 1H), 4.27 (t, J = 6.31 Hz, 2H), 3.59 (t, J = 4.80 Hz, 4H, CH_2), 3.41 (t, J = 6.53 Hz, 2H), 2.75 (t, J = 4.91 Hz, 4H, CH_2), 2.18–2.09 (m, 2H), 2.05–1.99 (m, 2H); ^{13}C NMR (DMSO-*d6*, 100 MHz): δ 182.1, 165.9, 162.8, 161.1, 158.1, 139.6–121.3, 109.0, 104.1, 97.5, 91.9, 69.2, 59.3, 55.7, 34.1, 27.9, 27.1. Anal. calcd. for $C_{29}H_{30}N_2O_6S$: C, 65.15; H, 5.66; N, 5.24. Found: C, 65.34; H, 5.51; N, 5.39.

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

Yield: 61%. p. 253–255°C; IR (KBr) cm^{-1}: 3,083; 2,941; 2,925; 2,868; 1,655; 1,619; 1,588; 1,384; 1,339; 1,259; 1,160; 1,136; 1,041. ^{1}H NMR (DMSO-*d6*, 400 MHz): δ 12.69 (s, 1H), 7.86–7.81 (m, 2H), 7.67–7.12 (m, 7H), 6.75 (s, 1H), 6.42 (d, J = 2.31 Hz, 1H), 6.12 (d, J = 2.24 Hz, 1H), 4.25 (t, J = 6.14 Hz, 2H), 3.51 (t, J = 4.82 Hz, 4H, CH_2), 3.46 (t, J = 6.54 Hz, 2H), 2.88 (t, J = 4.91 Hz, 4H, CH_2), 2.17–2.05 (m, 2H), 2.01–1.95 (m, 2H); ^{13}C NMR (DMSO-*d6*, 100 MHz): δ 182.1, 168.7, 163.2, 161.4, 158.1, 141.9–121.8, 107.8,

103.5, 98.7, 91.4, 69.1, 57.2, 54.5, 34.1, 28.8, 26.8. Anal. calcd. for $C_{29}H_{29}ClN_2O_6S$: C, 61.21; H, 5.14; N, 4.92. Found: C, 61.11; H, 5.01; N, 4.99.

*7-(4-(4-(2,4-Dichlorophenylsulfonyl)piperazin-1-yl)-butoxy)-5-hydroxy-2-phenyl-4H-chromen-4-one (**7c**)*

Yield: 63%. m.p. 253–255°C; IR (KBr) cm^{-1}: 3,072; 2,933; 2,920; 2,870; 1,661; 1,633; 1,573; 1,372; 1,346; 1,244; 1,166; 1,141; 1,044. ^1H NMR (DMSO-*d6*, 400 MHz): δ 12.75 (s, 1H), 7.91–7.79 (m, 3H), 7.66–7.48 (m, 5H), 6.71 (s, 1H), 6.44 (d, J = 2.35 Hz, 1H), 6.18 (d, J = 2.27 Hz, 1H), 4.27 (t, J = 6.06 Hz, 2H), 3.50 (t, J = 4.88 Hz, 4H, CH_2), 3.42 (t, J = 6.53 Hz, 2H), 2.85 (t, J = 4.89 Hz, 4H, CH_2), 2.19–2.09 (m, 2H), 2.04–1.99 (m, 2H); ^{13}C NMR (DMSO-*d6*, 100 MHz): δ 183.2, 169.7, 164.7, 160.9, 158.2, 137.9–121.7, 107.6, 103.3, 98.9, 93.0, 69.1, 57.5, 52.8, 33.3, 28.6, 27.0. Anal. calcd. for $C_{29}H_{28}Cl_2N_2O_6S$: C, 57.71; H, 4.68; N, 4.64. Found: C, 57.88; H, 4.79; N, 4.50.

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

Yield: 69%. m.p. 253–255°C; IR (KBr) cm^{-1}: 3,076; 2,944; 2,933; 2,881; 1,669; 1,625; 1,586; 1,369; 1,340; 1,250; 1,157; 1,139; 1,036. ^1H NMR (DMSO-*d6*, 400 MHz): δ 12.79 (s, 1H), 7.93–7.87 (m, 2H), 7.75–7.54 (m, 7H), 6.79 (s, 1H), 6.39 (d, J = 2.30 Hz, 1H), 6.10 (d, J = 2.21 Hz, 1H), 4.19 (t, J = 6.13 Hz, 2H), 3.59 (t, J = 4.84 Hz, 4H, CH_2), 3.49 (t, J = 6.55 Hz, 2H), 2.81 (t, J = 4.96 Hz, 4H, CH_2), 2.15–2.08 (m, 2H), 2.03–1.97 (m, 2H); ^{13}C NMR (DMSO-*d6*, 100 MHz): δ 181.0, 166.8, 162.7, 160.7, 159.1, 137.6–126.3, 106.9, 101.3, 99.4, 93.4, 66.8, 58.7, 52.6, 33.8, 28.8, 27.3. Anal. calcd. for $C_{29}H_{29}BrN_2O_6S$: C, 56.77; H, 4.76; N, 4.57. Found: C, 56.71; H, 4.89; N, 4.69.

*7-(4-(4-(2,4-Dibromophenylsulfonyl)piperazin-1-yl)butoxy)-5-hydroxy-2-phenyl-4H-chromen-4-one (**7e**)*

Yield: 57%. m.p. 253–255°C; IR (KBr) cm^{-1}: 3,070; 2,948; 2,921; 2,864; 1,658; 1,617; 1,577; 1,380; 1,343; 1,255; 1,169; 1,152; 1,027. ^1H NMR (DMSO-*d6*, 400 MHz): δ 12.71 (s, 1H), 7.94–7.89 (m, 2H), 7.79–7.68 (m, 6H), 6.70 (s, 1H), 6.48 (d, J = 2.37 Hz, 1H), 6.17 (d, J = 2.28 Hz, 1H), 4.29 (t, J = 6.16 Hz, 2H), 3.55 (t, J = 4.82 Hz, 4H, CH_2), 3.40 (t, J = 6.54 Hz, 2H), 2.87 (t, J = 4.90 Hz, 4H, CH_2), 2.14–2.02 (m, 2H), 2.01–1.96 (m, 2H); ^{13}C NMR (DMSO-*d6*, 100 MHz): δ 182.5, 167.6, 163.1, 162.6, 159.1, 138.9–123.1, 109.6, 104.2, 96.8, 91.9, 68.6, 58.1, 54.4, 31.9, 27.8, 26.3. Anal. calcd. for $C_{29}H_{28}Br_2N_2O_6S$: C, 50.30; H, 4.08; N, 4.05. Found: C, 50.14; H, 4.22; N, 4.34.

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

Yield: 49%. m.p. 253–255°C; IR (KBr) cm^{-1}: 3,084; 2,942; 2,924; 2,874; 1,660; 1,630; 1,581; 1,388; 1,337; 1,242; 1,162; 1,147; 1,033. ^1H NMR (DMSO-*d6*, 400 MHz): δ 12.77 (s, 1H), 7.90–7.82 (m, 2H), 7.70–7.39 (m, 7H), 6.68 (s, 1H), 6.40 (d, J = 2.33 Hz, 1H), 6.12 (d, J = 2.24 Hz, 1H), 4.27 (t, J = 6.11 Hz, 2H), 3.59 (t, J = 4.86 Hz, 4H, CH_2), 3.46 (t, J = 6.56 Hz, 2H), 2.80 (t, J = 4.87 Hz, 4H, CH_2), 2.19–2.08 (m, 2H), 2.04–1.99 (m, 2H); ^{13}C NMR (DMSO-*d6*, 100 MHz): δ 183.3, 166.9, 165.1, 163.7, 158.9, 143.6–127.1, 107.2, 104.3, 99.1, 95.4, 67.2, 57.4, 51.9, 35.6, 29.1, 27.8. Anal. calcd. for $C_{29}H_{29}FN_2O_6S$: C, 63.03; H, 5.29; N, 5.07. Found: C, 63.21; H, 5.13; N, 4.96.

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

Yield: 58%. m.p. 253–255°C; IR (KBr) cm^{-1}: 3,071; 2,937; 2,937; 2,872; 1,655; 1,626; 1,579; 1,377; 1,351; 1,257; 1,159; 1,137; 1,036. ^1H NMR (DMSO-*d6*, 400 MHz): δ 12.74 (s, 1H), 7.92–7.87 (m, 2H), 7.74–7.60 (m, 6H), 6.69 (s, 1H), 6.38 (d, J = 2.32 Hz, 1H), 6.19 (d, J = 2.25 Hz, 1H), 4.22 (t, J = 6.07 Hz, 2H), 3.61 (t, J = 4.83 Hz, 4H, CH_2), 3.41 (t, J = 6.50 Hz, 2H), 2.86 (t, J = 4.96 Hz, 4H, CH_2), 2.15–2.06 (m, 2H), 2.02–1.96 (m, 2H); ^{13}C NMR (DMSO-*d6*, 100 MHz): δ 181.9, 166.1, 162.5, 161.1, 159.3, 139.6–125.9, 108.1, 104.5, 98.9, 93.0, 68.5, 58.5, 53.6, 33.6, 28.9, 26.4. Anal. calcd. for $C_{29}H_{28}F_2N_2O_6S$: C, 61.04; H, 4.95; N, 4.91. Found: C, 61.21; H, 5.12; N, 4.99.

*5-Hydroxy-2-phenyl-7-(4-(4-(4-(trifluoromethoxy)phenylsulfonyl)-piperazin-1-yl)butoxy)-4H-chromen-4-one (**7h**)*

Yield: 62%. m.p. 253–255°C; IR (KBr) cm^{-1}: 3,079; 2,940; 2,934; 2,869; 1,659; 1,617; 1,573; 1,371; 1,348; 1,249; 1,167; 1,144; 1,041. ^1H NMR (DMSO-*d6*, 400 MHz): δ 12.70 (s, 1H), 7.89–7.83 (m, 2H), 7.69–7.37 (m, 7H), 6.78 (s, 1H), 6.47 (d, J = 2.39 Hz, 1H), 6.09 (d, J = 2.22 Hz, 1H), 4.21 (t, J = 6.15 Hz, 2H), 3.55 (t, J = 4.89 Hz, 4H, CH_2), 3.45 (t, J = 6.57 Hz, 2H), 2.82 (t, J = 4.93 Hz, 4H, CH_2), 2.16–2.05 (m, 2H), 2.03–1.99 (m, 2H); ^{13}C NMR (DMSO-*d6*, 100 MHz): δ 182.6, 168.2, 164.7, 163.1, 158.9, 136.6–124.5, 106.2, 103.3, 97.2, 94.5, 70.2, 59.9, 55.2, 35.7, 29.5, 27.2. Anal. calcd. for $C_{30}H_{29}F_3N_2O_7S$: C, 58.25; H, 4.73; N, 4.53. Found: C, 58.13; H, 4.90; N, 4.66.

*5-Hydroxy-7-(4-(4-(4-nitrophenylsulfonyl)piperazin-1-yl)-butoxy)-2-phenyl-4H-chromen-4-one (**7i**)*

Yield: 65%. m.p. 253–255°C; IR (KBr) cm^{-1}: 3,080; 2,933; 2,922; 2,878; 1,669; 1,629; 1,589; 1,379; 1,335; 1,254; 1,171; 1,150; 1,029. ^1H NMR (DMSO-*d6*, 400 MHz): δ 12.73 (s, 1H), 7.93–7.87 (m, 2H), 7.79–7.53 (m, 7H), 6.72 (s, 1H), 6.40 (d, J = 2.35 Hz, 1H), 6.17 (d, J = 2.26 Hz, 1H), 4.25 (t, J = 6.15 Hz, 2H), 3.57 (t, J = 4.80 Hz, 4H, CH_2), 3.39 (t, J = 6.51 Hz, 2H), 2.84 (t, J = 4.90 Hz, 4H, CH_2), 2.17–2.08 (m, 2H), 2.00–1.95 (m, 2H); ^{13}C NMR (DMSO-*d6*, 100 MHz): δ 180.9, 165.7, 162.1, 161.6, 159.2, 138.0–125.2, 109.2, 104.2, 98.9, 92.9, 68.2, 56.8, 51.9, 33.3, 28.1, 27.2. Anal. calcd. for $C_{29}H_{29}N_3O_8S$: C, 60.09; H, 5.04; N, 7.25. Found: C, 60.19; H, 5.23; N, 7.14.

*5-Hydroxy-7-(4-(4-(4-methoxyphenylsulfonyl)piperazin-1-yl)butoxy)-2-phenyl-4H-chromen-4-one (**7j**)*

Yield: 71%. m.p. 253–255°C; IR (KBr) cm^{-1}: 3,069; 2,944; 2,929; 2,872; 1,671; 1,622; 1,574; 1,381; 1,346; 1,244; 1,166; 1,145; 1,031. ^1H NMR (DMSO-*d6*, 400 MHz): δ 12.78 (s, 1H), 7.91–7.85 (m, 2H), 7.68–7.36 (m, 7H), 6.68 (s, 1H), 6.37 (d, J = 2.31 Hz, 1H), 6.15 (d, J = 2.26 Hz, 1H), 4.20

(t, J = 6.09 Hz, 2H), 3.81 (s, 3H, OCH_3), 3.51 (t, J = 4.81 Hz, 4H, CH_2), 3.47 (t, J = 6.57 Hz, 2H), 2.89 (t, J = 4.89 Hz, 4H, CH_2), 2.15–2.03 (m, 2H), 2.01–1.98 (m, 2H); ^{13}C NMR (DMSO-*d6*, 100 MHz): δ 181.2, 166.8, 162.2, 160.7, 157.8, 136.4–122.1, 108.1, 103.2, 96.8, 93.6, 68.9, 57.7, 54.6, 32.9, 29.2, 27.8. Anal. calcd. for $C_{30}H_{32}N_2O_7S$: C, 63.81; H, 5.71; N, 4.96. Found: C, 63.68; H, 5.80; N, 4.83.

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

Yield: 59%. m.p. 253–255°C; IR (KBr) cm^{-1}: 3,077; 2,941; 2,922; 2,866; 1,664; 1,625; 1,586; 1,375; 1,340; 1,259; 1,171; 1,151; 1,039. ^{1}H NMR (DMSO-*d6*, 400 MHz): δ 12.77 (s, 1H), 7.89–7.75 (m, 3H), 7.64-7.33 (m, 5H), 6.74 (s, 1H), 6.41 (d, J = 2.39 Hz, 1H), 6.10 (d, J = 2.22 Hz, 1H), 4.28 (t, J = 6.12 Hz, 2H), 3.85 (s, 3H, OCH_3), 3.73 (s, 3H, OCH_3), 3.60 (t, J = 4.85 Hz, 4H, CH_2), 3.46 (t, J = 6.51 Hz, 2H), 2.81 (t, J = 4.95 Hz, 4H, CH_2), 2.12–2.05 (m, 2H), 2.01–1.96 (m, 2H); ^{13}C NMR (DMSO-*d6*, 100 MHz): δ 183.5, 168.2, 164.8, 163.4, 156.9, 138.3–125.5, 107.6, 102.9, 99.3, 92.6, 69.1, 58.0, 53.0, 32.5, 26.9, 26.1. Anal. calcd. for $C_{31}H_{34}N_2O_8S$: C, 62.61; H, 5.76; N, 4.71. Found: C, 62.47; H, 5.61; N, 4.91.

4.2 | Biological screening

4.2.1 | *In vitro* antioxidant DPPH assay

In vitro free radical scavenging potential of the berberine derivatives **7a–k** was quantitatively measured by the DPPH method.[31] In brief, 20 µl of tested compounds (0.1, 1, 10, and 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 scavenging activity was calculated by using the equation provided by Mensor et al.[32]

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

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

4.2.2 | *In vitro* antioxidant ABTS assay

All the final compounds were screened for ABTS$^{\bullet+}$ radical cation scavenging assay.[31,33] In brief, different concentrations (0.1, 1, 10, and 100 µl) of tested derivatives (20 µl) were added to a 96-well microplate. Then, 180 µl of ABTS solution was added 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 scavenging using the following equation:

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

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

4.2.3 | *In vitro* cytotoxicity SRB assay

In vitro cytotoxicity bioassay of the synthesized compounds was carried out using the SRB assay method.[34–36] In brief, 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 and 1% antibiotic-antimycotic solution (100×) 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 MDCK cells were seeded into 96-well plates at the density of 2×10^4 cells/well plate. The synthesized compounds were dissolved in DMSO and treated with the cells after 24 hr 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 hr after the addition of the compounds, 100 µl of SRB (0.4 mg/L) was added to each well and incubated overnight. After that, 70% of cold acetone was added to each well to fix the viable cells; and 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}).

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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