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Chrysin–benzothiazole conjugates as antioxidant and anticancer agents

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ABSTRACT

7-(4-Bromobutoxy)-5-hydroxy-2-phenyl-4*H*-chromen-4-one, obtained from chrysin with 1,4-dibromobutane, was combined with a wide range of 6-substituted 2-aminobenzthiazoles, which had been prepared from the corresponding anilines with potassium thiocyanate. Free radical scavenging efficacies of newer analogues were measured using DPPH and ABTS assays, in addition to the assessment of their anticancer activity against cervical cancer cell lines (HeLa and CaSki) and ovarian cancer cell line (SK-OV-3) implementing the SRB assay. Cytotoxicity of titled compounds was checked using Madin–Darby canine kidney (MDCK) non-cancer cell line. Overall, **6a**–**r** indicated remarkable antioxidant power as $DPPH^{\cdot}$ and $ABTS^{\cdot+}$ scavengers; particularly the presence of halogen(s) (**6g**, **6h**, **6j**–**6l**) was favourable with IC_{50} values comparable to the control ascorbic acid. Unsubstituted benzothiazole ring favored the activity of resultant compounds (**6a** and **6r**) against HeLa cell line, whereas presence of chlorine (**6g**) or a di-fluoro group (**6k**) was a key to exert strong action against CaSki. Moreover, a mono-fluoro (**6j**) and a ketonic functionality (**6o**) were beneficial to display anticipated anticancer effects against ovarian cancer cell line SK-OV-3. The structural assignments of the new products were done on the basis of IR, ^{1}H NMR, ^{13}C NMR spectroscopy and elemental analysis.

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Oxygen centred free radicals (OFR), or more generally, reactive oxygen species (ROS) are endogenous stimuli which mediate complex sequences of cellular and molecular changes interacting with DNA initiating cancer formation.[1] Exposure to different physiochemical conditions or pathological states yields such free radicals, which are molecular species bearing an unpaired electron in an atomic orbital responsible for radical instability and high reactivity. They can either donate an electron to or accept an electron from other molecules, therefore behaving as oxidants or reductants, important in the pathogenesis of many different diseases.[2–5] Free radicals lead to cell damage and homeostatic disruption causing diseases including diabetes, cirrhosis, cancer and cardiovascular diseases.[6,7] Imbalance between antioxidant defenses and free radical production generates oxidative stress which is responsible to damage essential biological entities like nucleic acids, lipids, proteins,[8] producing excess ROS. The pathway of cancer and its treatment options create imbalance between antioxidant defenses and free radical production.

Antioxidants, either naturally generated in situ (endogenous antioxidants), or externally supplied through foods (exogenous antioxidants) are molecules those act against any form of oxidative stress and its associated ill effects on cellular system to contribute to disease prevention. Antioxidants are stable molecules capable of donating an electron to unstable ROS, thus neutralizing them and diminishing their DNA damaging abilities.[9] Antioxidants donate an electron and breaks chain reactions initiated by ROS by choking catalysts of ROS. Thus, Antioxidants act as enzyme inhibitor, radical scavenger, singlet oxygen quencher, hydrogen and electron donor, peroxide decomposer, synergist, and metal-chelating agents.[10,11] These points suggests that compounds exhibiting both antioxidant and anticancer effects are of enormous importance in the current drug discovery progress. Research on natural anticancer providers is an important topic of current research globally, as melanoma is an increasing public health menace and has been a reason for significant mortality in most countries.

Cancer accounts for several annual deaths in almost every country in the world, generally in Asia with highest rate followed by Europe. The World Health Organization revealed that globally complete fatalities and death rate due to cancer was 6.2 million in 1997, 7.4 million in 2004, and 7.6 million in 2008, it means

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13% of all fatalities were due to cancer and that the international cancer occurring rate could improve by 50% (15 million new cases) by 2030. According to WHO, more than 70% of all deaths due to cancer happen in low- and middle-income nations. Cancer of the cervix is the 4th most typical among females globally, with an approximated 527,624 new cases and 265,653 deaths in 2012.[12] Cervical cancer is the 2nd most common female cancer in women aged 15–44 years worldwide.[13]

It has been shown that natural products signify the wealthiest source of high substance variety, offering the foundation for recognition of novel scaffolding components that provides starting points for rational drug design.[14] Natural products are small-molecule secondary metabolites that contribute to organism survival. This can be one of the factors that prompted researchers to find appealing anticancer therapeutics from natural resources. According to a latest evaluation, ~49% of cancer medication was either natural products or their derivatives that are used as chemotherapeutic drugs.[15] A latest review states that there are several natural product agents approved for the cancer therapy, for example temsirolimus, everolimus, ixabepilone, vinflunine, romidepsin, trabectedin, cabazitaxel, abiraterone acetate, eribulin mesylate, homoharringtonine, carfilzomib, ingenol mebutate.[16]

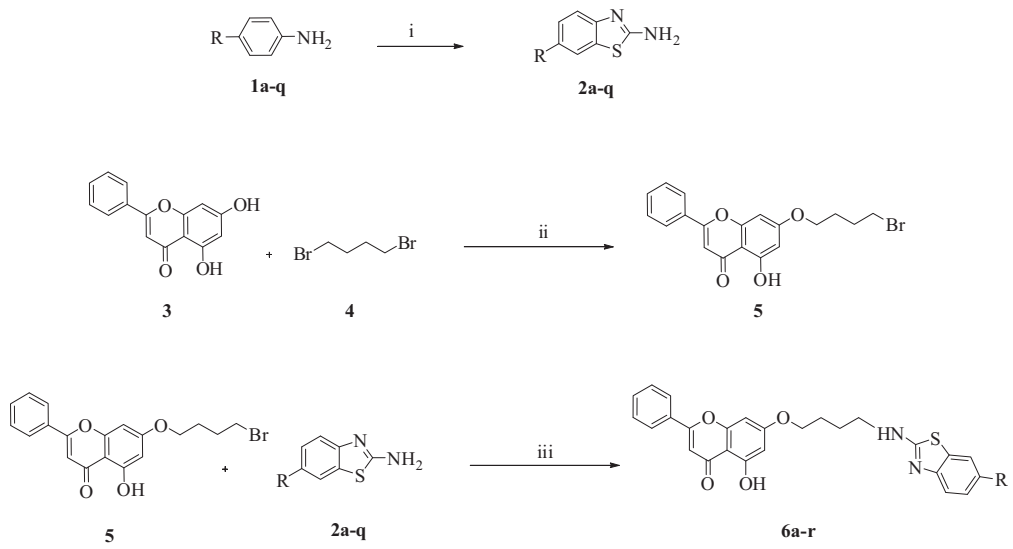
Flavonoids[17] are a broad class of polyphenolic secondary metabolites abundant in plants and in various foods. Chrysin, a naturally wide distributed flavonoid, has been revealed to have a plenty of pharmacological actions such as antioxidant[18,19] and anticancer.[20,21] It might have been engaged in maintaining the oxidant and antioxidant balance during 7,12-dimethylbenza[*a*]anthracene (DMBA)—induced hamster buccal pouch carcinogenesis. Chrysin induces the activity of antioxidant and detoxification enzymes like glutathione peroxidase, glutathione, glutathione reductase, glutathione *S*-transferase and quinone reductase in xenograft tumour models, which diminish the action of cytochrome P450 (CytP450)-dependent monooxygenases thereby suppresses cellular proliferation, invasion and angiogenesis.[22] To be able to provide semi-synthetic derivatives of chrysin, we have chosen benzothiazole skeletons to link with this flavone moiety because we have encountered in previous research that substituted 2-aminobenzothiazoles cause significant pharmacological action.[23,24] The huge selection of biological efficacies[25] associated with benzothiazoles has led to the elements being regarded as a blessed framework. Some opinions on participation of benzothiazoles as antitumour[26] agents are available in the literature. Therefore, depending on the concerns above, and in expansion of our research on the features of benzothiazole centred substances and trying to combine the bioactive functions of chrysin with those of benzothiazoles, we report here our results in the synthesis and bioevaluations of new chrysin–benzothiazole conjugates with the aim to support our speculation that the modification of active natural product skeletons may lead to novel agents delivering anticipated anticancer and antioxidant effects.

Target molecules (**6a**–**r**) were synthesized in three steps as shown in Scheme 1. 2-Amino-6-substituted benzothiazoles were obtained in satisfactory yields by reacting related anilines with potassium thiocyanate in glacial acetic acid. Structural assignments were in agreement with the data of reported analogues.[27] Benzothiazoles were characterized by FT-IR spectra displaying NH_2 band at 3410 cm^{-1} and C=N band at 1589 cm^{-1} for **2h** as a representative example. The ^1H NMR data were also in agreement with the formation of **2h**, as the signal appearing at 6.72 ppm was attributed to the —NH_2 proton. A doublet at 7.72 ppm was assigned to 7-H and a multiplet in the range of 7.39–7.46 ppm represented the vicinal 4-H and 5-H of the benzotriazole ring. In the succeeding step, refluxing chrysin (**3**) with 1,4-dibromobutane (**4**) under an inert atmosphere in the presence of base yielded intermediate 7-(4-bromobutoxy)-5-hydroxy-2-phenyl-4*H*-chromen-4-one (**5**) in 83% of yield.[28] Compound **5** showed compatible IR spectral data as reported earlier.[28] Compound **5** showed the ring C=O and OH stretching characteristic at 1642 cm^{-1} and 3073 cm^{-1}, respectively, whereas it delivered the expected signals corresponding to OH protons at 12.64 ppm; besides, two doublets (6.42 ppm and 6.33 ppm) and a singlet (6.62 ppm) attributed to the chromane ring in the ^1H NMR spectrum. The ^1H NMR spectra of **5** displayed resonances assigned to the phenyl ring of chrysin core in the range 7.88–7.47 ppm. At last, methylene proton atoms of the aliphatic sequence showed up to have their signals by means of triplet and multiplet in the range between 4.11 and 1.91 ppm. The ^{13}C NMR spectrum showed the introduced aliphatic chain with signals in the range of 67.7–28.7 ppm (Supporting information).

Next, benzothiazoles **2a**–**r** were condensed with **5** in refluxing acetonitrile to yield **6a**–**r** in 50–67% yield (Scheme 1). The structure of the prepared compounds was confirmed by the appearance of strong absorption band of (C=O) stretching at 1570–1585 cm^{-1}, C—H and C—C stretching frequencies at 3073 cm^{-1} and 2972 cm^{-1}, respectively as well as and disappearance of that of NH_2 stretching from around 3400 cm^{-1} (**2a**–**q**) and appearance of NH proton at 3310 cm^{-1} for a representative **6b**. Aliphatic chain has its characteristic FT-IR bands below 1200 cm^{-1} in **6b** spectrum. The ^1H NMR spectra of compound **6b** lack the NH_2 proton signal and expressed a singlet at 8.22 ppm for an —NH proton providing confirmatory evidence for the condensation of all **2a**–**q** to the key intermediate **5**. The ^1H NMR spectrum of **6b** showed a multiplet at 7.61–7.51 ppm and 7.64 ppm, respectively, due to the benzothiazole 4-H, 5-H and 7-H, respectively. In addition, singlet peaks at 12.75 ppm and 8.22 ppm were attributed to OH and NH functionalities, whereas other signals for aliphatic chain and chromano-phenyl entity were identical to those assigned in case of intermediate **5**. On the other hand, ^{13}C NMR data observed for compound **6b** further confirmed the correct formation of the desired structure of the compounds. Mass spectrometric data confirmed the molecular weights and empirical formulae of the compounds and fragments as observed from the M^+ ion values for **6a**–**r**. All of the novel compounds gave C, H and N analyses within 0.4 percent points from the theoretical values, that is, in acceptable range.

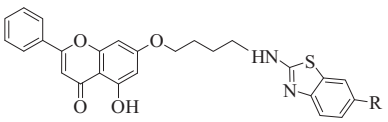
The most common spectrophotometric methods to figure out the antioxidant power of activity of organic compounds are depending on DPPH$^{\cdot}$ and ABTS$^{\cdot+}$, which react directly with the antioxidant species under assessment. In the DPPH analysis, the antioxidants are able to decrease the stable DPPH radical to the yellow coloured diphenylpicrylhydrazine. The method is in accordance with the decrease of an alcoholic DPPH solution in the existence of a hydrogen giving anti-oxidant, due to the development of the nonradical form, DPPH-H, during the reaction. The ABTS$^{\cdot+}$ analysis is depending on a single electron exchange, the ABTS radical-cation decolorization, which is in accordance with the decrease of ABTS$^{\cdot+}$ radicals by antioxidants. To assess the free radical scavenging activity of flavonoids, DPPH and ABTS assays were conducted and the results are indicated with regards to IC_{50} value (concentration required to inhibit 50% of the radicals) as described in Table 1.

Chrysin shows a low level of antioxidant power in DPPH and ABTS assay,[29,30] hence combining of pharmaceutically diverse benzothiazoles to the chrysin core performed and the resulting **6a**–**r** showed 13.16 ± 0.762–38.98 ± 1.141 μg/mL and 4.156 ± 0.095–9.836 ± 0.067 μg/mL of IC_{50} values 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). It was noticed that efficiency of **6a**–**r** as antioxidant agents was better in ABTS assay compared to DPPH assay, as four among entire analogues expressed ⩽5 μg/mL of IC_{50} values, much like that of the control drug ascorbic acid with 5.0925 ± 0.2090 μg/mL of



Scheme 1. Synthesis of piperazine linked chrysin derivatives **6a–r**. Reagents and conditions: (i) KSCN, Br_2, AcOH, rt; (ii) K_2CO_3, reflux, 24 h; (iii) CH_3CN, reflux, 10–38 h.

Table 1
Screening results of DPPH and ABTS radical scavenging activity of **6a–r**



No.	R	IC_{50}[a] μg/mL ± SD	
		DPPH	ABTS
6a	H	26.45 ± 0.458	8.567 ± 0.267
6b	6-CH_3	28.13 ± 1.023	8.785 ± 0.099
6c	6-OCH_3	23.87 ± 0.569	7.356 ± 0.047
6d	6-OC_2H_5	24.41 ± 0.792	8.853 ± 0.348
6e	6-NO_2	30.25 ± 0.349	8.135 ± 0.124
6f	6-CN	15.56 ± 0.229	6.543 ± 0.077
6g	6-Cl	13.16 ± 0.762	4.156 ± 0.095
6h	6-Br	16.09 ± 1.004	6.565 ± 0.210
6i	6-I	25.44 ± 0.471	9.836 ± 0.067
6j	6-F	14.74 ± 0.559	5.246 ± 0.090
6k	4,6-Di F	15.12 ± 0.349	5.764 ± 0.047
6l	6-CF_3	16.27 ± 0.498	4.953 ± 0.022
6m	6-$NHCOCH_3$	29.16 ± 0.892	8.355 ± 0.128
6n	6-COOH	21.66 ± 0.337	6.742 ± 0.113
6o	6-$COCH_3$	30.72 ± 0.097	7.026 ± 0.179
6p	6-COC_2H_5	32.23 ± 1.075	7.157 ± 0.046
6q	6-COC_3H_7	38.98 ± 1.141	8.535 ± 0.059
6r	—	24.34 ± 0.763	8.734 ± 0.751
Ascorbic acid		12.72 ± 0.274	5.0925 ± 0.2090

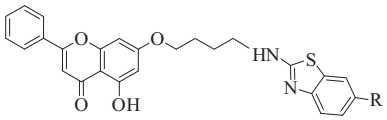
[a] The results are average of triplicate analysis.

IC_{50}. From the antioxidant inspections, it can be said that the existence of a wide range of electron withdrawing (EWD) and electron donating (ED) functional groups has significant effects in radical scavenging. Particularly, molecules bearing EWD halogen(s) were appeared to have high radical scavenging efficacies as **6g–6l** with chloro, bromo and fluorine atom(s) except **6i** with iodine furnished 13.16 ± 0.762–16.27 ± 0.498 μg/mL of IC_{50} values and were the most potent group of active scaffolds scavenging DPPH·. However, increasing numbers of halogen atom was unfavourable to the antioxidant power of these compounds against DPPH·, for example, **6j** with a single fluorine atom presented 14.74 ± 0.559 μg/mL of IC_{50} followed by **6k** with di-fluoro and **6l** with trifluoromethyl functionalities having 15.12 ± 0.349 and 16.27 ± 0.498 μg/mL of IC_{50} values, respectively. Opposite to it, the same group of molecules (**6g**, **6h**, **6j–6l**; IC_{50} values: 4.156 ± 0.095 μg/mL–6.565 ± 0.210 μg/mL) was sensitive to scavenge $ABTS^{\cdot+}$, but insertion of two or more halogen(s) was found beneficial as **6l** has excellent IC_{50} (4.953 ± 0.022 μg/mL) when compared to **6j** (5.246 ± 0.090 μg/mL). Analogues bearing no substituent on the benzothiazole ring (**6a**, **6r**) as well as **6i** incorporating iodine atom were inactive in both antioxidant assays tested. Among the titled analogues with ED groups, compound (**6c**, DPPH: 23.87 ± 0.569 μg/mL; ABTS: 7.356 ± 0.047 μg/mL) bearing methoxy group was more active than those carrying methyl (**6b**, DPPH: 28.13 ± 1.023 μg/mL; ABTS: 8.785 ± 0.099 μg/mL) and ethoxy (**6d**, DPPH: 24.41 ± 0.792 μg/mL; ABTS: 8.853 ± 0.348 μg/mL) functional groups in terms of IC_{50} values. Analogue **6n** with an acid (COOH) functionality was noticed to exert appreciable activity against DPPH and ABTS radical with 21.66 ± 0.337 μg/mL and 6.742 ± 0.113 μg/mL of IC_{50} values, respectively, however, a sequential conversion of acid to ketones lead to the molecules (**6o–6q**, IC_{50}: DPPH→30 μg/mL; ABTS 7→8 μg/mL) denying further potency. In general, the order of antioxidant power for the substituents can be presented as halogen > acid > alkyl or alkoxy > ketonic > unsubstituted against scavenging DPPH· and $ABTS^{\cdot+}$, respectively.

Analogues **6a–r** were examined for their in vitro antitumour potencies in the 3-cell line panel consisting of HeLa (cervical), CaSki (cervical) and SK-OV-3 (ovarian). End point determinations were made with a protein binding dye, sulforhodamine B (SRB) called SRB assay. Literature works exposed that neat chrysin displays 10.93 μg/mL of IC_{50} values against HeLa cell line. In fact, condensation of benzothiazole with chrysin core through a butyl chain revealed a constant activity profile for the resultant analogues as anticancer agents than those described in the literature works with ethylene linker replacing a variety of anilines or amines.[28] Hence with the aim of acquiring more effective chrysin analogues, the outcomes of anticancer testing assessments for **6a–r** against cervical cancer cell lines have been collected in Table 2.

Similar to antioxidant assay results, nature of the substituent present on the benzothiazole moiety played variable inhibitory effects on the growth of the tested cancerous cell lines. Scaffolds **6a–r** provided overall significant stage of potencies against HeLa and CaSki cell lines with 4.638 ± 0.651–8.533 ± 0.199 μg/mL and 10.824 ± 0.328–18.842 ± 0.190 μg/mL of IC_{50} values, respectively. It can be mentioned that analogies **6a–r** were more sensitive towards HeLa cell line than CaSki. Compounds **6a** and **6r** with unsubstituted benzothiazole ring were the most active analogues against

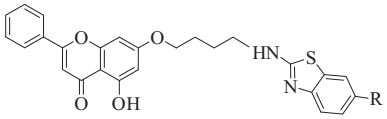
Table 2
Screening results of activity of **6a–r** against cervical cancer cell lines



No.	R	IC_{50}[a] μg/mL ± SD HeLa	IC_{50}[a] μg/mL ± SD CaSki	CC_{50}[a] μg/mL ± SD MDCK
6a	H	4.754 ± 0.267	14.643 ± 0.078	347.5 ± 1.426
6b	6-CH_3	6.564 ± 0.341	15.525 ± 0.127	293.6 ± 2.016
6c	6-OCH_3	6.153 ± 0.179	16.124 ± 0.437	284.6 ± 1.156
6d	6-OC_2H_5	5.246 ± 0.551	15.052 ± 0.329	306.5 ± 0.763
6e	6-NO_2	6.436 ± 0.329	13.245 ± 0.177	309.6 ± 2.154
6f	6-CN	8.533 ± 0.199	18.842 ± 0.190	312.5 ± 1.178
6g	6-Cl	5.954 ± 0.129	12.426 ± 0.327	337.6 ± 1.567
6h	6-Br	6.564 ± 0.442	12.425 ± 0.521	289.1 ± 0.098
6i	6-I	8.124 ± 0.671	13.156 ± 0.126	298.1 ± 1.346
6j	6-F	5.032 ± 0.199	13.357 ± 0.226	324.1 ± 2.019
6k	4,6-Di F	5.563 ± 0.193	10.824 ± 0.328	307.3 ± 1.792
6l	6-CF_3	7.842 ± 0.098	11.207 ± 0.119	278.3 ± 1.558
6m	6-$NHCOCH_3$	6.906 ± 0.189	11.357 ± 0.193	291.3 ± 2.259
6n	6-COOH	8.425 ± 0.325	12.074 ± 0.092	324.0 ± 1.826
6o	6-$COCH_3$	7.784 ± 0.519	14.537 ± 0.096	311.1 ± 1.247
6p	6-COC_2H_5	6.257 ± 0.229	17.843 ± 0.341	295.6 ± 1.119
6q	6-COC_3H_7	7.169 ± 0.191	17.753 ± 0.332	277.5 ± 1.909
6r	—	4.638 ± 0.651	14.628 ± 0.172	316.3 ± 1.438
Taxol[b]	—	16.48	2.48	—
Fluorouracil[b]		4.76	—	—

[a] The results are average of triplicate analysis.
[b] IC_{50} values are adopted from literature.[31]

Table 3
Screening results of activity of **6a–r** against ovarian cancer cell line



No.	R	IC_{50}[a] μg/mL ± SD SK-OV-3	CC_{50}[a] μg/mL ± SD MDCK
6a	H	48.533 ± 0.278	347.5 ± 1.426
6b	6-CH_3	48.421 ± 0.315	293.6 ± 2.016
6c	6-OCH_3	38.146 ± 0.169	284.6 ± 1.156
6d	6-OC_2H_5	35.324 ± 0.158	306.5 ± 0.763
6e	6-NO_2	41.434 ± 0.187	309.6 ± 2.154
6f	6-CN	43.731 ± 0.304	312.5 ± 1.178
6g	6-Cl	39.247 ± 0.322	337.6 ± 1.567
6h	6-Br	35.072 ± 0.411	289.1 ± 0.098
6i	6-I	56.434 ± 0.302	298.1 ± 1.346
6j	6-F	33.743 ± 0.252	324.1 ± 2.019
6k	4,6-Di F	36.832 ± 0.110	307.3 ± 1.792
6l	6-CF_3	37.156 ± 0.068	278.3 ± 1.558
6m	6-$NHCOCH_3$	51.254 ± 0.192	291.3 ± 2.259
6n	6-COOH	43.345 ± 0.090	324.0 ± 1.826
6o	6-$COCH_3$	34.257 ± 0.142	311.1 ± 1.247
6p	6-COC_2H_5	35.643 ± 0.096	295.6 ± 1.119
6q	6-COC_3H_7	41.580 ± 0.159	277.5 ± 1.909
6r	—	51.738 ± 0.210	316.3 ± 1.438
Fluorouracil[b]	—	3.93	—

[a] The results are average of triplicate analysis.
[b] IC_{50} values are adopted from literature.[31]

HeLa with 4.754 ± 0.267 μg/mL and 4.638 ± 0.651 μg/mL of IC_{50} values, respectively. Cytotoxicities of both compounds towards MDCK normal cell line were in bearable level at 347.5 ± 1.426 μg/mL and 316.3 ± 1.438 μg/mL, respectively. Although, substitution of further functional groups was observed unfavourable, the most beneficial was a fluorine atom (analogue **6j**) with 5.032 ± 0.199 μg/mL and 324.1 ± 2.019 μg/mL of IC_{50} and CC_{50}, respectively. Moreover, compound **6d** with ethoxy and **6k** with di-fluoro functionalities appeared to have promising anticancer effects against HeLa cell line with 5.246 ± 0.551 μg/mL and 5.563 ± 0.193 μg/mL of IC_{50} values, 306.5 ± 0.763 μg/mL and 307.3 ± 1.792 μg/mL of CC_{50} levels, respectively. Substitution of di-fluoro functional group was again beneficial to contribute potent cancerous cell inhibitory effects against CaSki cell lines as **6k** exhibited 10.824 ± 0.328 μg/mL of IC_{50}, 307.3 ± 1.792 μg/mL of CC_{50}, respectively. In case of activity of compounds **6n** with acidic group to **6q** with ketonic functionality, presence of 6-COC_2H_5 group (**6p**) was found optimum to express desirable potency against HeLa. Among analogues carrying halogen atom, activity followed the order of electronegativity (F > Cl > Br > I) against HeLa cell line as **6j** with F was most active and **6i** with I was least, however, insertion of multiple halogens diminished the activity. Final compound with a nitro, a cyano, alkyl and ketonic groups delivered good to moderate activity against HeLa cell lines. Furthermore, a scaffold bearing single chlorine atom (**6g**) showed 5.954 ± 0.129 μg/mL of IC_{50} and carried acceptable CC_{50} at 337.6 ± 1.567 μg/mL. Unlike activity of halo-based compounds against HeLa, no relationship between electronegativity and activity was observed against CaSki as all mono-halogenated analogues (**6g–6j**) furnished almost equal inhibitory effects against CaSki. In fact, analogue bearing trifluoromethyl (**6l**, IC_{50}: 11.207 ± 0.119 μg/mL), an acid group (**6n**, IC_{50}: 12.074 ± 0.092 μg/mL) and an acetamido functionality (**6m**, IC_{50}: 11.357 ± 0.193 μg/mL) demonstrated promising anticancer action. Further extension of an acid to the ketonic chain was not found acceptable as **6q** with 6-COC_3H_7 group presented 17.753 ± 0.332 μg/mL of IC_{50} and can be considered as inactive against CaSki. Unsubstituted scaffolds **6a** (IC_{50}: 14.643 ± 0.078 μg/mL) and **6r** (IC_{50}: 14.628 ± 0.172 μg/mL) as well as that with a nitro group (**6e**, IC_{50}: 13.245 ± 0.177 μg/mL) made up interesting efficacy against CaSki, respectively when compared to those existing alkyl or alkoxy (**6b–6d**) and ketonic groups (**6o–6q**) with almost >15 μg/mL of IC_{50} levels. Overall, titled analogues presented variable potencies against both the cervical cancer cell lines and can be considered of anticipated levels.

Chrysin has no activity against ovarian cancer cell line SK-OV-3 as noticed in a previous research attempts.[32] In fact, it has moderate level of cancerous cell inhibitory profile (IC_{50} values) against several types of cancer cell lines.[33] Hence, analogues **6a–r** were tested to inspect their in vitro inhibitory efficacies against ovarian cancer cell line SK-OV-3 (Table 3) with an aim to observe the positive influence of benzothiazole substitution on the activity profiles of chrysin core.

Although, the activity profiles seen for **6a–r** against SK-OV-3 were poor when compared to those observed against cervical cancer cell lines, still the activity against SK-OV-3 was appreciable as compared to parent chrysin. Titled compounds provided 33.743 ± 0.252–56.434 ± 0.302 μg/mL of IC_{50} values and 277.5 ± 1.909–347.5 ± 1.426 μg/mL of CC_{50} levels. Unlike the activity of **6a–r** against cervical cancer cell line, in case of ovarian cancer cell line, no specific trend of the presence of the type of substituent was observed as compounds were variedly active. Compounds **6a** (IC_{50}: 48.533 ± 0.278 μg/mL) and **6r** (IC_{50}: 51.738 ± 0.210 μg/mL) with no substituent were found inactive. Moreover, analogue with acetamido group (**6m**) had failed to present an activity against SK-OV-3 with 51.254 ± 0.192 μg/mL of IC_{50}. Interestingly, likewise efficacies against HeLa and CaSki, scaffold holding fluorine atom (**6j**) expressed excellent IC_{50} of 33.743 ± 0.252 μg/mL, CC_{50} of 324.1 ± 2.019 μg/mL, and was noticed to have high sensitivity towards SK-OV-3. Furthermore, increasing numbers of halogen was not desirable to present an increasing action against SK-OV-3 as analogue **6k** with di-fluoro and **6l** with trifluoro functionalities

exerted 36.832 ± 0.110 μg/mL and 37.156 ± 0.068 μg/mL of IC_{50} values, respectively. In addition, halogenated scaffolds **6g** (Cl) and **6h** (Br) had succeeded to exercise noticeable IC_{50} levels of 39.247 ± 0.322 μg/mL and 35.072 ± 0.411 μg/mL, respectively. Presence of EWD groups was observed to play key role in delivering anti-SK-OV-3 potential as compound **6d** with ethoxy and **6o** with 6-$COCH_3$ group indicated 35.324 ± 0.158 μg/mL and 34.257 ± 0.142 μg/mL of IC_{50} values, 306.5 ± 0.763 μg/mL and 311.1 ± 1.247 μg/mL of CC_{50} levels, respectively. It was noticed that conversion of COOH (**6n**) to the ketonic $COCH_3$ (**6o**) was beneficial to increase the activity of resultant scaffolds against ovarian cancer cell line, however, further extension of the ketonic chain (**6p** and **6q**) lead to the compounds showing diminished effects. Overall, titled analogues had expressed remarkable SAR and activity prospects against SK-OV-3 cell line of ovarian cancer.

The DPPH bioassay is the widely used and acceptable method for inspecting the free radical scavenging efficacy of the intended compound. The investigation of the DPPH radical scavenging activity was operated according to methodology described by Brand–Williams et al.[34,35] The $ABTS^{\cdot+}$ radical cation scavenging efficacies of the test compounds was determined according to the method described earlier.[36] The test compounds **6a–r** were checked for their In vitro anticancer potential against cervical cancer cell line HeLa, CaSki and ovarian cancer cell line SK-OV-3, and Madin Darby canine kidney (MDCK) cells were which were purchased from American Type Culture Collection (ATCC) using Sulforhodamine B colorimetric (SRB) assay.[37]

In summary, we have efficiently produced novel chrysin–benzothiazole conjugates using uncomplicated and well recognized synthetic protocols. To the best of our information, this is the first report where such a comprehensive testing for this type of benzothiazole based chrysin scaffolds has been done on cervical and ovarian cancer cell lines. These preliminary results show the prospective of this type of substance towards anticancer and antioxidant drug discovery program. Bioassay results suggested that analogues **6a–r** are the efficient scavengers of $DPPH^{\cdot}$ and $ABTS^{\cdot+}$ radicals, showing themselves as device for discovering the further antioxidant molecules. In addition, **6a–r** indicated promising anticancer potential against cervical cancer cell line HeLa when compared to the control dugs. It was observed that length of the aliphatic side sequence linking two different pharmacophores as chrysin and benzothiazole was essential in providing reliable biological profiles. From the structure–activity perspective, characteristics and position of the electron withdrawing and electron donating functional groups on the benzothiazole core may promote the expected antioxidant and anticancer action. Lastly, it is possible that further derivatization of such substances will be of attention with desire to get more selective agents.

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Supplementary data

Supplementary data (experimental procedures, spectral data, detailed biological assays) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2015.10.052.

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