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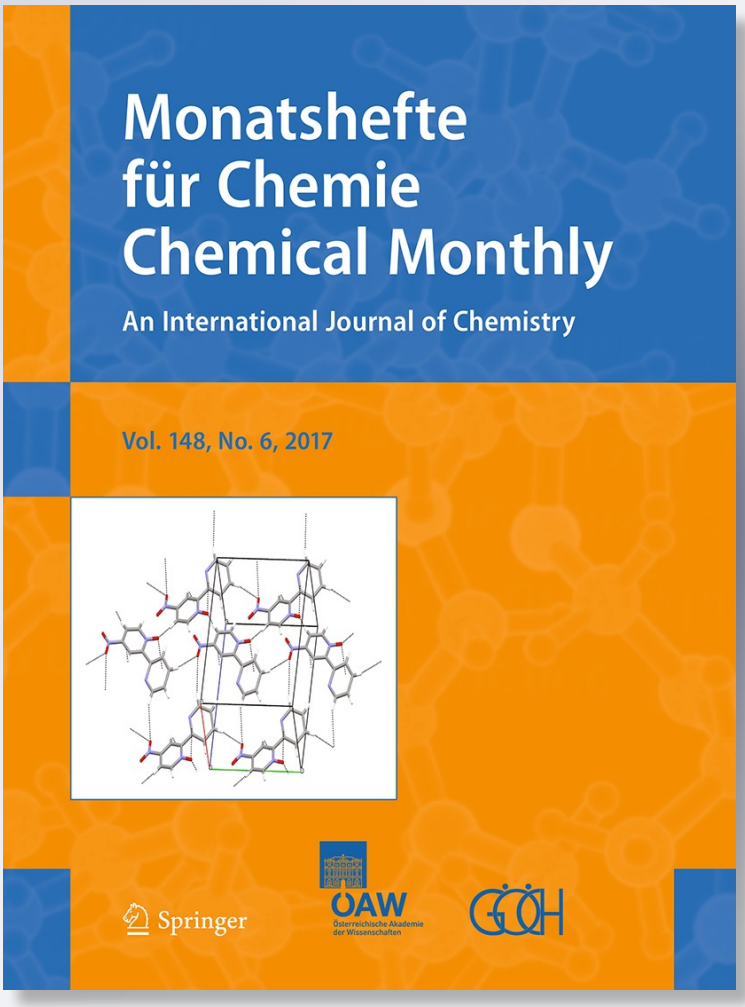

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ORIGINAL PAPER

Synthesis, anti-microbial activity, and cytotoxicity of novel 1-[5-[6-[(2-benzoylbenzofuran-5-yl)methyl]-2-oxo-2*H*-chromen-3-yl]thiazol-2-yl]urea derivatives

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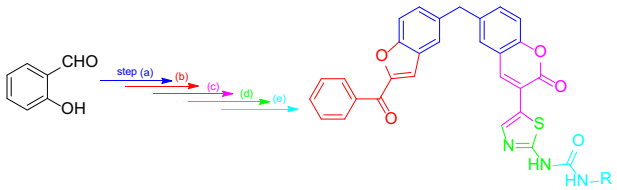
Abstract A series of novel 1-[5-[6-[(2-benzoylbenzofuran-5-yl)methyl]-2-oxo-2*H*-chromen-3-yl]thiazol-2-yl]urea derivatives were synthesized by the reaction of 3-(2-aminothiazol-5-yl)-6-[(2-benzoylbenzofuran-5-yl)methyl]-2*H*-chromen-2-one with various substituted amines and triphosgene, in the presence of a base. The chemical structures of newly synthesized compounds were elucidated by ^{1}H NMR, ^{13}C NMR, IR, MS, and HRMS spectral data. The synthesized compounds evaluated for their inhibitory effects as anti-microbial activity and cytotoxicity. Most of the compounds exhibited a promising anti-microbial activity against the selected Gram-positive and Gram-negative bacterial strains at MIC values ranging from 0.071 to 0.199 μM and fungal pathogen was moderate to be good. The in vitro cytotoxicity testing of the title compounds was performed against cervical cancer (HeLa) cell lines. In the preliminary MTT cytotoxicity studies, the results have shown that there are a few of the synthesized compounds which exhibit a significant cytotoxicity at microliter concentration were found to non-toxic, their IC_{50} values ranging from 49.322/15 to 52.715/15 mm^3.

Graphical abstract



Keywords Coumarylthiazole · Urea · Anti-microbial activity · Cytotoxicity

Introduction

The discovery and the development of new anti-microbial and anticancer inhibitory agents are the essential goals in medicinal chemistry. Cancer is a main cause of health concern with an alarming raise in the number of patients throughout the world, in which the relative death rate is caused by cancer which is very high in the third world countries [1]. Chemotherapy is the most effective tool for curing the disease, but the use of chemotherapeutics is restricted due to their undesirable affects, and it also grows the resistance to chemotherapeutic agents [2]. Cytotoxicity and genotoxicity of anticancer drugs to the normal cells are the key problems in cancer therapy and produce the risk of inducing secondary malignancy [3]. A dosage of anticancer drug is enough to kill tumor cells which is often toxic to the normal tissue and leads to many undesirable affects, which, in turn, limits its treatment efficacy. In modern years, several researchers have concentrated in searching of the discovery and the development of novel selective

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antitumor agents, devoid of a lot of the unpleasant side effects of the conventional antitumor agents.

Coumarin heterocyclic pharmacophore is considered privileged nucleus, which is found in several natural and synthetic biologically active molecules [4, 5]. The coumarylthiazole derivatives were found to exhibit potential anti-microbial properties [6]. Several coumarylthiazole containing pyrazole derivatives and coumarylthiazole they exhibited a very good anti-microbial activity [7, 8]. Recently, Siddiqui and co-workers reported that some novel molecules which containing thiazole and coumarin moieties in the single frame work showed the promising activities, such as analgesic, anti-inflammatory, and anticonvulsant [9, 10]. Belma et al. reported coumarylthiazole attached aryl urea and thiourea groups shown as antioxidant and anti-cholinesterase activities [11]. Moreover, the slight modification of sulfonamide-substituted coumarylthiazole derivatives revealed that it increased inhibitory activities of antioxidant and carbonic anhydrase I and II [12]. Several researchers put their efforts for the development process in anti-microbial and cytotoxicity activities of coumarylthiazole (**A** and **B**), and unsymmetrical urea (**C** and **D**) derivatives, [13–16] as shown in Fig. 1.

In view of the above significant derivatives with various pharmacological activities, it was encouraged by the derivatives and in continuation of the present research, it designed the synthesis of a series of substituted benzofuranyl chromenyl thiazolyl urea (BCTU) derivatives and assayed their anti-microbial activity and cytotoxicity.

Results and discussion

Chemistry

The synthesis of the BCTU derivatives is illustrated in Scheme 1. The reported compound 5,5′-methylenebis(2-hydroxybenzaldehyde) (**2**) was prepared in good yield by the electrophilic substitution reaction of salicylaldehyde with trioxane in the presence of glacial acetic acid and concentrated sulfuric acid [17]. To build up of benzofuran nucleus, the compound **2** was allowed to condense cyclization with the phenacyl bromide at a normal temperature in the presence of K_2CO_3 to obtain the corresponding 5-[(2-benzoylbenzofuran-5-yl)methyl]-2-hydroxybenzaldehyde (**3**) in excellent yield [18]. The structures of compound **3** were confirmed by their spectroscopic data (^{1}H NMR, ^{13}C NMR, IR, MS, and HRMS) which were provided in the experimental section. The IR spectra of the compound **3** showed the absorption bands of C=C, C=O, and –OH in the region 1650, 1720, and 3550 cm^{-1}, respectively. The ^{1}H NMR spectrum of compound **3** showed two singlet signals at 9.85 and 10.98 ppm corresponding to aldehyde and hydroxyl groups, respectively. A singlet signal assigned to the bridged methylene protons at 3.98 ppm in addition to down-field singlet signal assigned to benzofuran proton and aromatic protons in the region at 8.03–6.95 ppm. The ^{13}C spectrum of the compound **3** showed aldehydic and keto signals at 191.8 and 182.5 ppm, respectively. The two carbons of the –COC– linkage in benzofuran nucleus exhibited the absorption peaks at 159.7 and 151.9 ppm, respectively. The carbon of aromatic ring was observed at 154.5–112.7 ppm. The bridged methylene group between two aromatic rings was observed at 49.0 ppm. Further conformation HRMS spectra showed peak obtained at $m/z = 357.11204$ ([M+H]$^+$), corresponding to a molecular formula $C_{23}H_{16}O_4$.

The intermediate 3-acetyl-6-[(2-benzoylbenzofuran-5-yl)methyl]-2*H*-chromen-2-one (**4**) was obtained via the solvent free Knoevenagel reactions of 5-[(2-benzoylbenzofuran-5-yl)methyl]-2-hydroxybenzaldehyde and ethyl acetoacetate in the presence of piperidine [19]. In the IR spectra of compound **4**, the C=O band of the chromenyl moiety was observed at about 1600 cm^{-1}. In the ^{1}H NMR spectra of compound **4** recorded in $CDCl_3$, the signal of the chromenyl protons appeared at 8.51 ppm as a singlet and

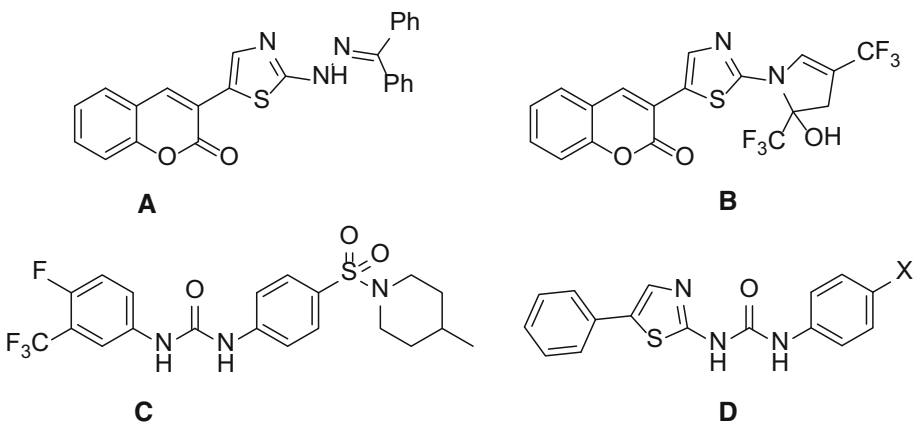
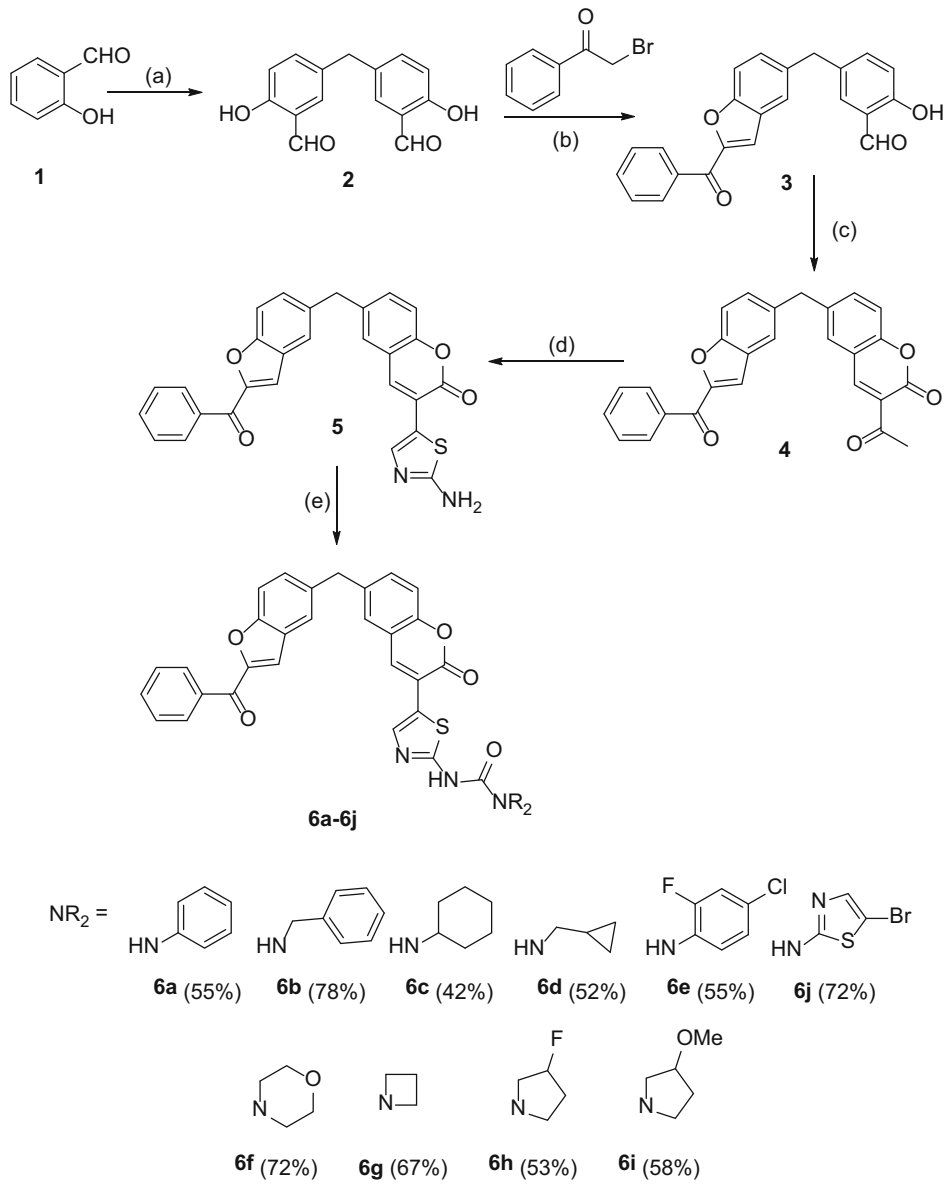


Fig. 1 Anti-microbial and cytotoxicity of coumarylthiazole and unsymmetrical urea

Scheme 1



the CH_3 proton appeared at 2.73 ppm as a singlet. All the other aromatic protons of compound **4** were observed at the expected regions.

The compound 3-(2-aminothiazol-5-yl)-6-[(2-benzoylbenzofuran-5-yl)methyl]-2*H*-chromen-2-one (**5**) was obtained in an excellent yield via the Biginelli reaction of compound **4** and thiourea in the presence of catalytic amount of iodine [20]. The infrared spectrum of compound **5** showed characteristic absorptions corresponding to amine ($–NH_2$) is of 3367 cm^{-1} and C=N is of 2234 cm^{-1} groups, and it was also confirmed by the ^1H NMR which showed broad singlet at 7.16 ppm corresponding to amine protons attached to thiazole ring and singlet at 7.37 ppm corresponding to thiazole ring proton.

Finally, the reaction of the compound **5** with various substituted amines and triphosgene [21], in the presence of Et_3N in DCM to obtain products 1-[5-[6-[(2-benzoylbenzofuran-5-yl)methyl]-2-oxo-2*H*-chromen-3-yl]thiazol-2-yl]-3-phenylurea derivative **6a–6j**. The two carbons of the –COC– linkage in benzofuran nucleus exhibited the absorption peaks at 159.7 and 151.9 ppm, respectively. The **6a-6j** compounds were confirmed by their spectral data included in the experimental section. In the IR spectra of the compounds, it was individually observed the absorptions between 3370 and 3250 cm^{-1} which correspond to -NH stretch of urea moiety, about 1550 cm^{-1} relating to -CN stretch for thiazole, absorptions about 1680 cm^{-1} from coumarin carbonyl moiety stretch and absorptions between

1725 and 1655 cm^{-1} for urea carbonyl moiety. The ^1H NMR spectra of the hydrogen attached to the amide nitrogen was shown between 9.85 and 8.12 ppm. The signals for aromatic hydrogens were observed between 8.0 and 6.50 ppm, and the proton signal of thiazole ring was detected at 7.47 ppm. This is followed by the signal at 154.7 ppm for urea carbonyl group. All the synthesized compounds exhibited satisfactory spectral data consistent with their molecular structures, as shown in Scheme 1.

Anti-microbial activity

The compounds **4**, **5**, and titled BCTU derivatives **6a–6j** were evaluated for their in vitro antibacterial activity against *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Bacillus licheniformis*, *Streptococcus pneumonia*, and *Staphylococcus aureus* by the disc diffusion method [22, 23] and the mean zone of inhibition data are reported in Table 1. The BCTU derivatives **6b**, **6d**, **6e**, and **6f** showed a significant antibacterial activity (>20 mm) against all six bacterial strains compared with the standard drug, rifampicin. From the screened results, it is evident that the presence of phenyl, cyclopropyl, chloro-fluoro, and bromothiazole urea moieties enhanced the antibacterial activity. The compounds **6e** and **6f** were shown maximum activity against the *Escherichia coli* organisms employed. The compounds **6h** and **6i** showed good activity against *Bacillus licheniforms* which is equally compared with the standard drug. The compounds **6a**, **6c**, **6g**, **6i**, and **6j** showed good activity to moderate antibacterial activity (>20 mm) against all six bacterial strains; however, the compound **4** and **5** were shown less activity against all six bacterial strains.

The minimum inhibitory concentrations (MIC) of the screened compounds were revealed in Table 2. According to the observation from Table 2, the minimum inhibitory efficiency of all the BCTU derivatives, **6a–6j**, was higher than intermediate compounds **4** and **5**. It may be attributed that the presence of urea nuclei as a common pharmacophore. Among all the tested compounds, the inhibitory efficiency of compound **6e** against *Escherichia coli,* and the compounds **6b** and **6f** against *Escherichia coli* and *Pseudomonas aeruginosa,* S*treptococcus pneumonia* was close to that of standard (MIC = 0.071–0.098 μM). The inhibitory efficiency of the compounds **6e** and **6i** against *Bacillus subtilis*, the compounds **6e** and **6j** against S*taphylococcus aureus* were close to that of standard (MIC = 0.079–0.083 μM). It was observed that compound **6e** exhibited broad spectrum of antibacterial activity and more potent, as it was observed low MIC value against all the tested strains. All other remaining compounds showed slightly higher MIC values. The antibacterial evaluation study of coumarylthiazole moiety with substituted urea group contained chloro and fluoro groups on phenyl ring showed the promising activity. Other compounds **6a**, **6c**, **6g**, and **6h** showed the moderate inhibitory efficiency

Table 1 Zone of inhibition of newly synthesized BCTU analogues for bacteria and fungus

Organic compound	Zone of inhibition/mm							
	Gram-negative bacteria			Gram-positive bacteria			Fungi	
	E. coli	*K. pneumonia*	*P. aeruginosa*	*B. licheniformis*	*S. pneumoniae*	*S. aureus*	*A. niger*	*C. albicans*
4	10	08	06	10	09	07	08	06
5	14	19	18	21	14	16	10	09
6a	19	19	22	22	17	22	12	13
6b	25	21	22	18	24	23	12	08
6c	20	17	18	18	16	23	12	18
6d	21	26	23	18	22	23	11	18
6e	28	23	26	25	24	26	15	18
6f	26	23	22	22	23	23	15	14
6g	12	24	22	23	12	14	11	13
6h	21	17	13	24	23	23	11	14
6i	17	17	13	24	12	23	11	18
6j	24	17	22	18	18	19	12	18
Rifampicin	25	24	23	24	24	25	–	–
Nystatin	–	–	–	–	–	–	22	22
Control (1% DMSO)	–	–	–	–	–	–	–	–

– No activity

Table 2 Anti-microbial activity (MICs) of newly synthesized BCTU analogues for bacteria and fungus

Organic compound	MIC/μM							
	Gram-negative bacteria			Gram-positive bacteria			Fungi	
	E. coli	*K. pneumonia*	*P. aeruginosa*	*B. licheniformis*	*S. pneumoniae*	*S. aureus*	*A. niger*	*C. albicans*
4	0.210	0.299	0.193	0.245	0.196	0.236	0.451	0.574
5	0.175	0.125	0.121	0.299	0.227	0.129	0.213	0.425
6a	0.110	0.101	0.098	0.095	0.115	0.098	0.628	0.412
6b	0.098	0.095	0.092	0.192	0.078	0.197	0.415	0.216
6c	0.195	0.100	0.127	0.191	0.192	0.184	0.259	0.618
6d	0.089	0.079	0.089	0.111	0.095	0.188	0.413	0.598
6e	0.071	0.081	0.075	0.081	0.084	0.079	0.251	0.614
6f	0.082	0.085	0.092	0.099	0.079	0.095	0.199	0.287
6g	0.118	0.098	0.192	0.089	0.214	0.212	0.611	0.544
6h	0.099	0.105	0.240	0.079	0.097	0.089	0.450	0.689
6i	0.113	0.124	0.174	0.083	0.241	0.092	0.680	0.621
6j	0.098	0.154	0.095	0.174	0.199	0.080	0.541	0.547
Rifampicin	≤0.075	≤0.075	≤0.075	≤0.075	≤0.075	≤0.075	–	–
Nystatin	–	–	–	–	–	–	≤0.075	≤0.075
Control (1% DMSO)	–	–	–	–	–	–	–	–

The MIC values are interpreted as an average of triplets

MIC: minimum inhibitory concentration (the lowest concentration that inhibited the bacterial growth)

– No activity

against all bacterial stains. The BCTU derivatives were also confirmed the antifungal activity against two fungal strains, viz., *Aspergillus niger* and *Candida albicans*. The results were compared with the standard antifungal drug, nystatin. However, all the coumarylthiazole compounds were ineffective of MIC values against all the tested fungal strains up to 0.075–0.650 μM.

In vitro cytotoxicity

The appreciable results obtained from the previous biological studies, namely, anti-microbial activity for the derivatives of the BCTU, encouraged in the present research work to test their cytotoxicity against a human cervical (HeLa) cell lines. These derivatives were dissolved in the DMSO and blank sample containing the same volume of the DMSO was taken as control to identify the activity of the solvent in the experiment. The results were analyzed by means of cell inhibition expressed as IC_{50} values and are shown in Table 3. In all the cases, the activity of the BCTU derivatives was found to be significantly higher than the controller of the DMSO solvent. In vitro cytotoxicity of the synthesized compounds was assessed by a standard 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) bioassay on HeLa cell lines at 24 h of drug exposure. To assess the efficacy and to select the promising compounds, human cancer cells (HeLa) were used. The values of IC_{50} for the BCTU derivatives were found in the range of 27.83–52.71/15 mm^3, as shown in Table 3. The compounds **6c**, **6g**, and **6h** did not show 50% inhibition even at a concentration of 15 mm^3, and hence, they were not evaluated on HeLa cells. This study reveals that the human cancer cell lines are tested, HeLa cells are more sensitive, many anticancer drugs are effective against HeLa cells by causing apoptosis through the expression of caspase-3, generating reactive oxygen species (ROS), and it is damaging DNA [24]. Cisplatin causes cytotoxicity in HeLa cells [25], chemotherapeutic agents, such as doxorubicin, mitoxantrone, and bleomycin, cause cytotoxicity by generating the ROS [26]. Hence, it is similar to other cytotoxic drugs (doxorubicin, mitoxantrone, and cisplatin), the synthesized compounds which possess superior to cytotoxic properties over the ureas moieties **6d**, **6i**, and **6j** which may be attributed to the presence of substituted cyclopropyl, methoxy, and bromo group have been playing vital role for the activity, as shown in Table 3 and Fig. 2. These three compounds display a significant cell death in concentration during dependent manner. It is evident from Fig. 3 that the viability of cell decreases upon increasing the concentration of the compound. Surprisingly, none of the compounds have displayed toxicity against the normal HeLa cancer cells. The selected compounds **6a**, **6b**, **6d**, **6e**, **6f**, **6i**, and **6j** showed concentration-dependent activities. The compounds **6f**, **6g**, and **6h** have showed the lowest viability and cytotoxicity. On the other hand, the remaining compounds **6a**, **6b**, and **6e** showed good activity to moderate activity. From

Table 3 Synthesis of BCTU derivatives different concentrations for the evaluation of viability and cytotoxicity

Compound	% of viability (2 mm^3)	(5 mm^3)	(10 mm^3)	(15 mm^3)	% of cytotoxicity (2 mm^3)	(5 mm^3)	(10 mm^3)	(15 mm^3)
6a	97.511	77.375	61.31	55.429	2.489	22.625	38.69	44.571
6b	92.805	72.85	68.099	65.837	7.195	27.15	31.901	34.163
6d	89.864	70.588	52.262	47.285	10.136	29.412	47.738	52.715
6e	88.235	77.171	65.837	52.036	11.765	22.829	34.163	47.964
6f	63.8	50	52.714	72.171	36.2	50	47.286	27.829
6i	89.366	72.624	54.524	47.285	10.634	27.376	45.476	52.715
6j	87.33	77.375	64.479	50.678	12.67	22.625	35.521	49.322
Control cells	100	0						
Cisplatinum (30 nM/2 mm^3)	46.725	53.274						
Doxorubicin (25 nM/2 mm^3)	50.388	49.611						

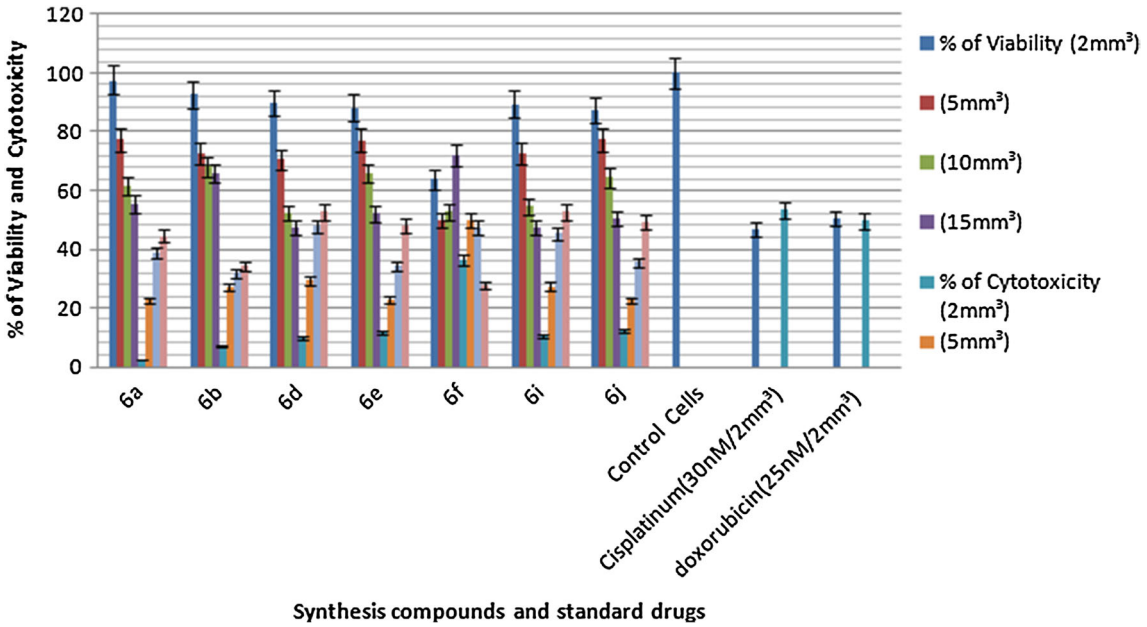


Fig. 2 Cytotoxcity of some of the synthesized BCTU derivatives on HeLa cancer cell lines

the screening results, one may conclude that the presence of phenyl, methyl phenyl, chloro, and fluoro-substituted phenyl ring enhances the cytotoxicity. The IC_{50} values of promising compounds at 48 h were significantly reduced as compared with 24 h values, as shown in Table 4.

Conclusion

In conclusion, a new class of 1-[5-[6-[(2-benzoylbenzofuran-5-yl)methyl]-2-oxo-2*H*-chromen-3-yl]thiazol-2-yl]urea derivatives (**6a**–**6j**) has been synthesized from 3-(2-aminothiazol-5-yl)-6-[(2-benzoylbenzofuran-5-yl)methyl]-2*H*-chromen-2-one (**5**). The anti-microbial activities of these compounds were evaluated against various bacterial and fungal strains. The synthesized compounds **6d**, **6e**, and **6f** showed good activity against the tested bacteria and it was emerged as potential molecules for further development. Moreover, in vitro cytotoxicity studies revealed that the compounds **6d**, **6i**, and **6j** were found to non-toxic compared, similar, with the standard drugs. They also provided an opportunity of laying the foundation for the further development of more promising molecules of anti-microbial and cytotoxicity potency.

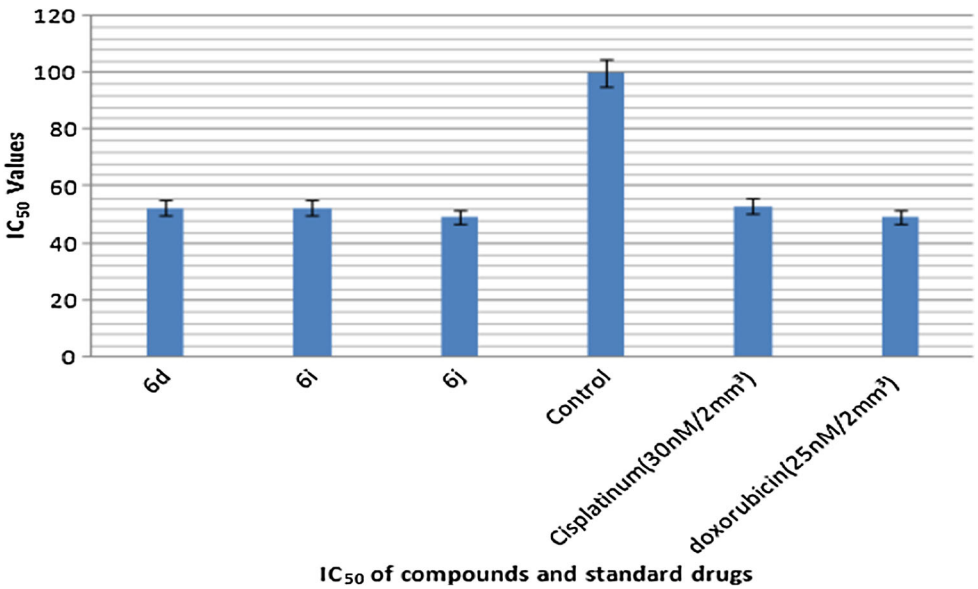


Fig. 3 BCTU derivatives in vitro cytotoxic activity (IC_{50}) compared the standard drugs

Table 4 In vitro cytotoxic activity (IC_{50}) of synthesis BCTU analogues against HeLa cancer cell lines

Compound	IC_{50} value
6d	52.715
6i	52.715
6j	49.322
Control	100
Cisplatinum (30 nM/2 mm^3)	53.274
Doxorubicin (25 nM/2 mm^3)	49.611

Experimental

All starting materials, reagents, and solvents were commercially available and used after purification. All the melting points were determined in open capillary tubes using sulfuric acid bath. IR spectra were recorded on Perkin-Elmer 1000 instrument using KBr pellet. ^1H and ^{13}C NMR spectra were obtained in DMSO-d_6 and $CDCl_3$ calibrated solvents on a VARIAN spectrometer at 500 and 125 MHz, respectively. Chemical shifts signal are given in δ (ppm) relative to TMS, and coupling constants (J) are expressed in hertz (Hz). The combinations of the following abbreviations are used to describe NMR spectra: s (singlet), d (doublet), t (triplet), q (quartet), and m (multiplet). Flash column chromatography was performed using silica gel (Merck, 60–120 mesh). Commercially available reagents were used as supplied and some of them were distilled before use. All reactions were performed in oven dried glassware. Electron Spray Ionization (ESI) and high-resolution mass spectra were recorded on a QSTARXL hybrid MS/MS system (Applied Bio systems, USA) under electrospray ionization.

5-[(2-Benzoylbenzofuran-5-yl)methyl]-2-hydroxybenzaldehyde (**3**, $C_{23}H_{16}O_4$)

The mixture of 3 g compound **2** (0.012 mmol), 1.16 g phenacyl bromide (0.005 mmol), and 2.42 g K_2CO_3 (0.017 mmol) was stirred in 15 cm^3 acetone at room temperature for 12 h [18]. The completion of the reaction was monitored by TLC; the product was washed with 15–25 cm^3 water and extracted from ethyl acetate. The pure compound **3** was separated through column chromatography using petroleum ether/ethyl acetate (70:30, v/v) as white solid (2.7 g, 90%). M.p.: 120–125 °C; ^1H NMR (400 MHz, $CDCl_3$): $\delta = 10.95$ (s, 1H, Ar–CHO), 9.85 (s, 1H, OH), 8.03 (d, 2H, $J = 7.93$ Hz, Ar–H), 7.66–7.46 (m, 4H, Ar–H), 7.40–7.30 (m, 5H, Ar–H), 6.95 (d, 2H, $J = 8.39$ Hz, Ar–H), 3.98 (s, 2H, $–CH_2–$) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 191.3$, 182.1, 159.2, 154.0, 151.4, 137.9, 137.3, 136.8, 135.3, 132.3, 132.1, 130.9, 129.8 (2C), 128.7 (2C), 126.9, 123.0, 122.0, 117.4, 117.1, 112.1, 42.0 ppm; IR (KBr): $\bar{\nu} = 3550$, 2800, 1650, 1579, 1720 cm^{-1}; MS (ESI +): $m/z = 357.1$ ($[M+H]^+$); and HRMS: m/z calcd for C_{23} H_{17} O_4 ($[M + H]^+$) 357.11214, found 357.11204.

3-Acetyl-6-[(2-benzoylbenzofuran-5-yl)methyl]-2H-chromen-2-one (**4**, $C_{27}H_{18}O_5$)

A mixture of 2 g compound **3** (5.62 mmol) and 1.46 g ethylacetoacetate (11.24 mmol) was stirred and cooled, then 0.2 g piperidine (0.56 mmol) was added and stirred for 2–3 h at room temperature. The reaction mixture was neutralized with dil. hydrochloric acid, and then, the pure crystalline product was isolated by filtration followed by recrystallization to afford pure compound **4** as yellow solid (1.71 g, 85.5%). M.p.: 225–227 °C; ^1H NMR (400 MHz, $CDCl_3$): $\delta = 8.51$ (s, 1H, chrom-H), 7.65 (m, 2H, Ar–H),

7.60–7.29 (m, 8H, Ar–H), 6.95 (d, 2H, $J = 8.2$ Hz, Ar–H), 3.96 (s, 2H, $–CH_2–$), 2.73 (s, 3H, CH_3) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 191.4$, 183.3, 159.2, 158.3, 154.5, 154.0, 151.6, 137.2, 136.8, 136.7, 134.3, 132.9, 132.1, 131.0, 130.6, 129.1, 128.4, 127.0, 124.8, 124.2, 121.9, 118.0, 117.4, 116.0, 112.1, 40.0, 30.0 ppm; IR (KBr): $\bar{\nu} = 1720$, 1680 cm^{-1}; MS (ESI+): $m/z = 422.9$ ($[M+H]^+$); and HRMS: m/z calcd for $C_{27}H_{19}O_5$ ($[M+H]^+$) 423.11214, found 423.11257.

3-(2-Aminothiazol-5-yl)-6-[(2-benzoylbenzofuran-5-yl)methyl]-2H-chromen-2-one (**5**, $C_{28}H_{18}N_2O_4S$)

A mixture of 1.5 g compound **4** (2 mmol), 1.767 g thiourea (2 mmol), and iodine in 10 cm^3 MeOH then heated under reflux for 12 h [20]. The completion of the reaction was monitored by TLC. The resulted mass was diluted with water and extracted with ethyl acetate, washed the organic layer with water, brine, dried over Na_2SO_4, filtered, and concentrated to yield crude residue. It was further purified by column chromatography eluting with petroleum ether/ethyl acetate (80:20, v/v) on silica gel (60–120 mesh) to afford pure compound **5** as pale yellow solid (1.314 g, 87.6%). M.p.: 250–252 °C; ^1H NMR (400 MHz, DMSO-d_6): $\delta = 8.46$ (s, 1H, chrome-H), 7.98 (d, 3H, $J = 7.5$ Hz, Ar–H), 7.76–7.69 (t, 6H, $J = 11.54$ Hz, Ar–H), 7.61 (t, 2H, $J = 7.2$ Hz, Ar–H), 7.52–7.46 (m, 1H, Ar–H), 7.37 (s, 1H, thiazole-H), 7.16 (s, 2H, NH_2), 4.16 (s, 2H, $–CH_2–$) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 183.4$, 167.3, 158.7, 154.0, 151.6, 150.6, 143.2, 137.9, 137.7, 136.9, 133.0, 131.9, 129.7 (2C), 128.6 (2C), 128.0, 127.0, 123.1, 120.4, 119.1, 116.9, 115.8, 112.2, 108.6, 48.5 ppm; IR (KBr): $\bar{\nu} = 3367$, 2900, 2234, 1720, 1714, 1650, 1604, 1579 cm^{-1}; MS (ESI+): $m/z = 479.1$ ($[M+H]^+$); and HRMS: m/z calcd for $C_{28}H_{19}N_2O_4S$ ($[M+H]^+$) 479.12152, found 479.12182.

General procedure for the synthesis of compounds ***6a–6j***

For the preparation of compounds **6a–6j**, the 0.500 g compound **5** (1.046 mmol) was dissolved in 5 cm^3 DCM and cooled to 0 °C. To this solution, 0.29 g triphosgene (1.046 mmol) and 0.137 cm^3 triethylamine (1.359 mmol) were added. The reaction mixture was stirred at room temperature for 2 h. Then, it was allowed to react with different substituted 0.15 g aniline (2.19 mmol) individually at 0 °C and stir for 5 h at room temperature. The completion of the reaction was monitored by TLC. The resulted mass was diluted with water and extracted with DCM, washed the organic layer with water, brine, dried over Na_2SO_4, filtered, and concentrated to yield crude residue. It was further purified by column chromatography eluting with using petroleum ether/ethyl acetate (80:20, v/v) [21]. The other compounds **6b–6j** were also prepared by the similar procedure.

1-[5-[6-[(2-Benzoylbenzofuran-5-yl)methyl]-2-oxo-2H-chromen-3-yl]thiazol-2-yl]-3-phenylurea

(**6a**, $C_{35}H_{23}N_3O_5S$)

From 0.500 g compound **5** (0.732 mmol), 0.15 g amine (2.19 mmol), and 0.29 g triphosgene (1.046 mmol), the compound **6a** was obtained as yellow solid (0.275 g, 55%) after chromatography on a silica gel column with petroleum ether/ethyl acetate (80:20, v/v). M.p.: 335–336 °C; ^1H NMR (400 MHz, $CDCl_3$): $\delta = 8.71$ (s, 1H, NH), 8.48 (s, 1H, NH), 8.05 (s, 1H, chrome-H), 7.71–7.76 (d, 3H, $J = 7.5$ Hz, Ar–H), 7.63–7.43 (m, 10H, Ar–H), 7.36–7.30 (m, 4H, Ar–H), 7.10 (d, 1H, $J = 8.5$ Hz, Ar–H), 4.02 (s, 2H, $–CH_2–$) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 182.1$, 162.1, 161.0, 159.2, 154.0, 151.4, 150.0, 145.9, 137.9, 137.3, 136.8, 135.3, 132.3, 130.9, 129.8 (2C), 128.7 (2C), 128.4 (2C), 127.5, 126.9, 125.5, 123.0, 122.0, 120.8 (2C), 119.8, 119.3, 117.4, 117.1, 112.1 ppm; IR (KBr): $\bar{\nu} = 3370$, 3250, 1725, 1650, 1528, 1457, 1339, 1239, 1088, 1025, 755, 693, 577 cm^{-1}; MS (ESI+): $m/z = 598.1$ ($[M+H]^+$); and HRMS: m/z calcd for $C_{35}H_{24}N_3O_5S$ ($[M+H]^+$) 598.11314, found 598.11354.

1-[5-[6-[(2-Benzoylbenzofuran-5-yl)methyl]-2-oxo-2H-chromen-3-yl]thiazol-2-yl]-3-benzylurea

(**6b**, $C_{36}H_{25}N_3O_5S$)

From 0.350 g compound **5** (0.732 mmol), 0.117 g amine (1.098 mmol), and 0.202 g triphosgene (0.732 mmol), the compound **6b** was obtained as pale yellow solid (0.27 g, 78%) after chromatography on a silica gel column with petroleum ether/ethyl acetate (80:20, v/v). M.p.: 325–327 °C; ^1H NMR (400 MHz, $CDCl_3$): $\delta = 8.98$ (s, 1H, NH); 8.18 (s, 1H, NH), 7.79 (s, 1H, chrome-H), 7.59–7.35 (m, 4H, Ar–H), 7.33–7.16 (m, 12H, Ar–H), 7.01 (d, 2H, $J = 8.01$ Hz, Ar–H), 4.5 (s, 2H, $–CH_2–$), 4.03 (s, 2H, $–CH_2–$) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 184.3$, 171.3, 161.9, 159.0, 158.3, 156.8, 154.6, 153.6, 150.9, 145.9, 140.2, 138.2, 138.1, 137.5, 137.0, 136.1, 132.2, 129.7 (2C), 129.3, 128.3 (2C), 128.0 (2C), 127.4, 127.0, 126.6 (2C), 126.3, 125.2, 124.3, 122.7, 120.8, 119.0, 117.1, 116.5, 110.9, 45.3, 41.4 ppm; IR (KBr): $\bar{\nu} = 3351$, 3287, 1742, 1671, 1668, 1544, 1509, 1244, 1178, 1108 cm^{-1}; MS (ESI+): $m/z = 612.1$ ($[M+H]^+$); and HRMS: m/z calcd for $C_{36}H_{26}N_3O_5S$ ($[M+H]^+$) 612.21418, found 612.21478.

1-[5-[6-[(2-Benzoylbenzofuran-5-yl)methyl]-2-oxo-2H-chromen-3-yl]thiazol-2-yl]-3-cyclohexylurea

(**6c**, $C_{35}H_{29}N_3O_5S$)

From 0.400 g compound **5** (0.836 mmol), 0.124 g amine (1.254 mmol), and 0.230 g triphosgene (0.836 mmol), the compound **6c** was obtained as yellow solid (0.17 g, 42%) after purified using chromatography on a silica gel column with petroleum ether/ethyl acetate (70:30, v/v). M.p.: 296–298 °C; ^1H NMR (400 MHz, $CDCl_3$): $\delta = 9.85$ (s, 1H, NH), 9.15 (s, 1H, NH), 8.12 (s, 1H, chrome-H), 8.02 (d,

2H, $J = 7.5$ Hz, Ar–H), 7.59–7.49 (m, 6H, Ar–H), 7.47 (s, 1H, Ar–H), 7.40–7.29 (m, 3H, Ar–H), 7.21 (s, 1H, Ar–H), 4.14 (s, 2H, $–CH_2–$), 3.48 (s, 1H, –CH–N), 1.41–1.20 (m, 7H, cyclohex-H), 1.18–1.12 (m, 3H, cyclohex-H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 182.8, 162.9, 156.0, 154.3, 153.0, 148.6, 146.9, 138.2, 137.8, 13.0, 136.0, 132.5, 130.5, 129.6$ (2C), 129.4, 128.2, 127.9, 126.7, 124.2, 122.5, 121.0, 119.1, 117.9, 113.3, 40.7, 31.9 (2C), 26.0, 24.7 (2C) ppm; IR (KBr): $\bar{\nu} = 3361, 1740, 1673, 1604, 1535, 1484, 1373, 1180, 815, 747, 582$ cm^{-1}; MS (ESI+): $m/z = 604.1$ ($[M+H]^+$); and HRMS: m/z calcd for $C_{35}H_{30}N_3O_5S$ ($[M+H]^+$) 604.12164, found 604.12108.

1-[5-[6-[(2-Benzoylbenzofuran-5-yl)methyl]-2-oxo-2H-chromen-3-yl]thiazol-2-yl]-3-(cyclopropylmethyl)urea (**6d**, $C_{33}H_{25}N_3O_5S$)

From 0.500 g compound **5** (1.046 mmol), 0.111 g amine (1.569 mmol), and 0.288 g triphosgene (1.046 mmol), the compound **6d** was obtained as pale yellow solid (0.26 g, 52%) after purified using chromatography on a silica gel column with petroleum ether/ethyl acetate (90:10, v/v). M.p.: 320–324 °C; ^1H NMR (400 MHz, $CDCl_3$): $\delta = 9.08$ (s, 1H, NH), 8.32 (s, 1H, NH), 8.05 (s, 1H, chrome-H), 8.00 (d, 2H, $J = 7.3$ Hz, Ar–H), 7.67–7.50 (m, 7H, Ar–H), 7.47 (s, 1H, thiazole-H), 7.38–7.28 (m, 3H, Ar–H), 7.22 (s, 1H, Ar–H), 4.14 (s, 2H, $–CH_2–$), 3.49 (s, 2H, $–CH_2–$), 0.23 (q, 1H, cycloprop-H), 0.20–0.15 (m, 4H, cycloprop-H) ppm; ^{13}C NMR (400 MHz, $CDCl_3$): $\delta = 184.9, 160.1, 159.5, 157.3, 154.7, 154.5, 151.31, 142.1, 137.7, 137.3, 136.1, 132.9, 131.7, 129.6$ (2C), 129.3, 128.4 (2C), 127.4, 127.2, 122.8, 121.1, 119.5, 116.2, 116.2, 112.5, 45.7, 41.1, 11.1, 3.2 ppm; IR (KBr): $\bar{\nu} = 3350, 3294, 1670, 1665, 1541, 1504, 1290, 1232, 1168, 1035, 1109, 748, 665$ cm^{-1}; MS (ESI+): $m/z = 576.2$ ($[M+H]^+$); and HRMS: m/z calcd for $C_{33}H_{26}N_3O_5S$ ($[M+H]^+$) 576.22108, found 576.22148.

1-[5-[6-[(2-Benzoylbenzofuran-5-yl)methyl]-2-oxo-2H-chromen-3-yl]thiazol-2-yl]-3-(4-chloro-2-fluorophenyl)urea (**6e**, $C_{35}H_{21}ClFN_3O_5S$)

From 0.250 g compound **5** (0.523 mmol), 0.113 g amine (1.254 mmol), and 0.144 g triphosgene (0.523 mmol), the compound **6e** was obtained as brick red solid (0.137 g, 55%) after purified using chromatography on a silica gel column with petroleum ether/ethyl acetate (80:20, v/v). M.p.: 313–316 °C; ^1H NMR (400 MHz, DMSO-d_6): $\delta = 9.09$ (s, 1H, NH), 8.25 (s, 1H, NH), 8.10 (s, 1H, chrome-H), 8.02–7.95 (m, 5H, Ar–H), 7.81–7.61 (m, 6H, Ar–H), 7.60 (t, 2H, $J = 7.5$ Hz, Ar–H), 7.55–7.39 (m, 1H, Ar–H), 7.04 (d, 1H, $J = 8.2$ Hz, Ar–H), 4.13 (s, 2H, $–CH_2–$) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 182.1, 164.0, 12.7, 161.8, 159.1, 154.1, 153.1, 151.4, 150.5, 144.2, 137.9, 137.5, 136.7, 135.3, 132.4, 132.0, 131.0, 129.8$ (2C), 129.2, 128.7 (2C), 128.3, 126.9, 124.7 (2C), 124.1, 122.9, 122.0, 118.5, 117.4, 117.3, 117.1, 113.7, 112.1 42.1 ppm; IR (KBr): $\bar{\nu} = 3373, 3231, 1684, 1676, 1526, 1514, 1282, 31251, 1207, 1189, 757, 694$ cm^{-1}; MS (ESI+): $m/z = 650.0$ ($[M+H]^+$); and HRMS: m/z calcd for $C_{35}H_{22}ClFN_3O_5S$ ($[M+H]^+$) 650.22183, found 650.22145.

N-[5-[6-[(2-Benzoylbenzofuran-5-yl)methyl]-2-oxo-2H-chromen-3-yl]thiazol-2-yl]morpholine-4-carboxamide (**6f**, $C_{33}H_{25}N_3O_6S$)

From 0.350 g compound **5** (0.732 mmol), 0.076 g amine (0.878 mmol), and 0.202 g triphosgene (0.732 mmol), the compound **6f** was obtained as pale yellow solid (0.288 g, 72%) after chromatography on a silica gel column with petroleum ether/ethyl acetate (70:30, v/v). M.p.: 322–324 °C; ^1H NMR (400 MHz, $CDCl_3$): $\delta = 9.25$ (s, 1H, NH), 8.35 (s, 1H, chrome-H), 8.03 (d, 2H, $J = 7.01$ Hz, Ar–H), 7.66–7.49 (m, 7H, Ar–H), 7.38–7.27 (m, 4H, Ar–H), 7.22 (s, 1H, Ar–H), 4.60 (s, 1H, NH), 4.15 (s, 2H, $–CH_2–$), 3.30–3.20 (m, 4H, morph-H), 3.13–3.03 (m, 4H, morph-H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 182.7, 162.9, 159.2, 158.0, 156.0, 150.3, 148.6, 138.7, 137.8, 137.4, 136.0, 132.5, 130.5, 129.7$ (2C), 129.4, 128.2 (2C), 127.5, 126.7, 122.4, 122.0, 121.0, 119.0, 117.9, 116.1, 112.2, 62.5, 50.5, 40.7 ppm; IR (KBr): $\bar{\nu} = 3368, 1679, 1678, 1562, 1497, 1332, 1255, 1168, 1103, 688$ cm^{-1}; MS (ESI+): $m/z = 592.1$ ($[M+H]^+$); and HRMS: m/z calcd for $C_{33}H_{26}N_3O_6S$ ($[M+H]^+$) 592.18222, found 592.18148.

N-[5-[6-[(2-Benzoylbenzofuran-5-yl)methyl]-2-oxo-2H-chromen-3-yl]thiazol-2-yl]azetidine-1-carboxamide (**6g**, $C_{32}H_{23}N_3O_5S$)

From 0.200 g compound **5** (0.418 mmol), 0.055 g amine (0.062 mmol), and 0.155 g triphosgene (0.418 mmol), the compound **6g** was obtained as brown solid (0.134 g, 67%) after purified using chromatography on a silica gel column with petroleum ether/ethyl acetate (90:10, v/v). M.p.: 308–310 °C; ^1H NMR (400 MHz, $CDCl_3$): $\delta = 9.50$ (s, 1H, NH), 7.99 (s, 1H, chrome-H), 7.60–7.45 (m, 3H, Ar–H), 7.50–7.14 (m, 7H, Ar–H), 6.36 (s, 2H, Ar–H), 4.04 (s, 2H, $–CH_2–$), 3.69 (m, 4H, azeti-H), 2.06 (m, 2H, azeti-H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 182.9, 160.1, 159.5, 157.3, 154.7, 152.5, 151.3, 146.0, 137.7, 137.3, 136.9, 136.1, 132.9, 131.7, 129.6$ (2C), 129.3, 128.4 (2C), 127.4, 127.2, 124.3, 123.0, 121.1, 119.5, 116.3, 116.2, 112.5, 45.7 (2C), 41.1, 13.7 ppm; IR (KBr): $\bar{\nu} = 3365, 3284, 1704, 1680, 1542, 1487, 1311, 1230, 1150, 993, 855, 654$ cm^{-1}; MS (ESI+): $m/z = 562.0$ ($[M+H]^+$); and HRMS: m/z calcd for $C_{32}H_{24}N_3O_5S$ ($[M+H]^+$) 562.116149, found 562.11696.

N-[5-[6-[(2-Benzoylbenzofuran-5-yl)methyl]-2-oxo-2H-chromen-3-yl]thiazol-2-yl]-3-fluoropyrrolidine-1-carboxamide (**6h**, $C_{33}H_{24}FN_3O_5S$)

From 0.250 g compound **5** (0.523 mmol), 0.069 g amine (0.784 mmol), and 0.144 g triphosgene (0.523 mmol), the

compound **6h** was obtained as yellow solid (0.140 g, 53%) after purified using chromatography on a silica gel column with petroleum ether/ethyl acetate (70:30, v/v). M.p.: 280–283 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 8.85$ (s, 1H, NH), 8.01 (s, 1H, chrome-H), 7.63–7.41 (m, 8H, Ar–H), 7.34–7.29 (m, 2H, Ar–H), 7.09 (d, 1H, $J = 8.4$ Hz, Ar–H), 6.94 (s, 1H, Ar–H), 4.08 (s, 2H, $-CH_2-$), 3.93-3.87 (m, 5H, pyrro-H), 2.06–1.98 (m, 2H, pyrro-H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 182.1$, 161.8, 159.1, 154.1, 153.1, 151.4, 144.2, 137.9, 137.5, 136.7, 135.3, 132.4, 132.0, 131.9, 131.0, 129.8 (2C), 128.7 (2C), 126.9, 126.7, 122.9, 122.0, 118.5, 117.3, 117.1, 112.1, 86.7, 56.2, 47.0, 42.6, 27.3 ppm; IR (KBr): $\bar{\nu} = 3368$, 1740, 1663, 1613, 1465, 1253, 1107, 1150, 984, 842, 754, 667 cm^{-1}; MS (ESI+): *m/z* = 594.1 ($[M+H]^+$); and HRMS: *m/z* calcd for $C_{33}H_{25}FN_3O_5S$ ($[M+H]^+$) 594.15092, found 594.15049.

N-[5-[6-[(2-Benzoylbenzofuran-5-yl)methyl]-2-oxo-2H-chromen-3-yl]thiazol-2-yl]-3-methoxypyrrolidine-1-carboxamide (**6i**, $C_{34}H_{27}N_3O_6S$)

From 0.250 g compound **5** (0.523 mmol), 0.079 g amine (0.784 mmol), and 0.144 g triphosgene (0.523 mmol), the compound **6i** was obtained as yellow solid (0.145 g, 58%) after purified using chromatography on a silica gel column with petroleum ether/ethyl acetate (70:30, v/v). M.p.: 275–278 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 8.98$ (s, 1H, NH), 8.01 (s, 1H, chrome-H), 7.63–7.57 (m, 2H, Ar–H), 7.54–7.42 (m, 5H, Ar–H), 7.33–7.27 (d, 1H, $J = 8.5$ Hz, Ar–H), 6.93 (s, 2H, Ar–H), 4.07 (s, 2H, $-CH_2-$), 3.93 (m, 4H, pyrro-H), 3.89 (m, 2H, pyrro-H), 3.25 (s, 3H, CH_3), 1.73–1.54 (m, 2H, pyrro-H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 185.6$, 162.5, 159.1, 158.8, 155.8, 153.9, 153.1, 148.6, 138.5, 137.8, 135.9, 134.3, 132.5, 130.5, 129.6 (2C), 129.4, 128.2 (2C), 126.7, 122.6, 121.2, 121.0, 119.7, 117.6, 116.7, 112.2, 98.5, 61.0, 56.4, 47.9, 40.7, 33.8 ppm; IR (KBr): $\bar{\nu} = 3358$, 1749, 1673, 1617, 1463, 1257, 1107, 1150, 994, 845, 754 cm^{-1}; MS (ESI+): *m/z* = 606.1 ($[M+H]^+$); and HRMS: *m/z* calcd for $C_{34}H_{28}N_3O_6S$ ($[M+H]^+$) 606.20335, found 606.20388.

1-[5-[6-[(2-Benzoylbenzofuran-5-yl)methyl]-2-oxo-2H-chromen-3-yl]thiazol-2-yl]-3-(5-bromothiazol-2-yl)urea (**6j**, $C_{32}H_{19}BrN_4O_5S_2$)

From 0.250 g compound **5** (0.523 mmol), 0.113 g amine (1.254 mmol), and 0.144 g triphosgene (0.523 mmol), the compound **6j** was obtained as light yellow solid (0.18 g, 72%) after purified using chromatography on a silica gel column with petroleum ether/ethyl acetate (90:10, v/v) (70:30, v/v). M.p.: 338–340 °C; ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 9.6$ (s, 1H, NH), 8.7 (s, 1H, NH), 8.02 (s, 1H, chrome-H), 7.87 (d, 2H, $J = 7.01$ Hz, Ar–H), 7.71 (s, 1H, thiaz-H), 7.55–7.31 (m, 5H, Ar–H), 7.22–6.98 (m, 4H, Ar–H), 6.87 (d, 1H, $J = 8.30$ Hz, Ar–H), 3.99 (s, 2H, $-CH_2-$) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 184.2$, 167.2, 162.2, 161.0, 157.0, 154.8, 152.4, 150.2, 146.0, 140.1, 138.4, 136.9, 137.0, 135.1, 133.0, 132.0, 129.5 (2C), 129.3, 128.4 (2C), 128.2, 127.2, 123.5, 122.8, 121.6, 119.1, 117.2, 116.4, 112.5, 92.7, 41.0 ppm; IR (KBr): $\bar{\nu} = 3350$, 3280, 1745, 1664, 1670, 1487, 1292, 1215, 1176, 1103, 983, 854, 644 cm^{-1}; MS (ESI+): *m/z* = 683.2 ($[M+H]^+$); and HRMS: *m/z* calcd for $C_{32}H_{20}BrN_4O_5S_2$ ($[M+H]^+$) 683.33144, found 683.33105.

Anti-microbial assay

The preparation of microorganism suspension

All the urea derivatives were tested towards anti-microbial activity against *Escherichia colI* MTCC 586, *Klebsiella pneumoniae* MTCC 3384, *Pseudomonas aeruginosa* MTCC 1034, *Bacillus licheniformis* MTCC 1483, *Streptococcus pneumonia* MTCC 1935, *Staphylococcus aureus* MTCC 7443, *Aspergillus niger* MTCC 872, and *Candida albicans* MTCC 854 were obtained from the Microbial Type Culture Collection (MTCC). Nutrient agar (NA) and potato dextrose agar (PDA) were used to culture the test bacteria and fungi, respectively. Each strain was transferred from stored slants at 5 °C to 10 cm^3 of nutrient broth (NB) or potato dextrose broth (PDB) tube and those are cultivated overnight at 37 °C. The bacterial cultures were then diluted in sterile 0.8% saline solution and adjusted to a cell suspension of 10^8 colony forming unit (cfu)/cm^3 using a UV spectrophotometer and digital colony counter. Similarly, for fungi, inoculums of viable spores or mycelia fragments were prepared.

Determination of zone of inhibition

A well-diffusion method was used to evaluate the antibacterial and antifungal activities against test strains on Mueller–Hinton Agar and the PDA plates, respectively. A total 100 mm^3 of diluted inoculum (10^8 cfu/cm^3) from organism suspensions was spread on the surface of the plates and allowable to solidify. Three wells were cut out with the help of a well borer under aseptic conditions on the agar medium. They were filled with 1000 µg of test compounds solution and the DMSO as the control. The plates were incubated for 24 h at 38 °C for test bacteria and for 5 days at 25 °C for fungi. The anti-microbial activity was assayed by measuring the diameter zone of transparent inhibition against test microorganisms.

Determination of MIC

The broth dilution method was used to find out the MIC of the test agents [27], test agents were added as serial dilutions to a series of tubes containing the DMSO and the dissolved mixture is added to Mueller–Hinton broth, so that the concentrations ranged from 0.075 to 0.650 µM. The bacterial concentration in each tube was 5×10^7 cfu/cm^3.

Three kinds of controls, related to the above described, were also prepared. After incubation (37 °C for 24 h for bacteria and 30 °C for 72 h for fungi), the lowest concentration of the agent that led to inhibit the growth of microorganism, was considered the MIC. Whereas the MIC values, where determined with a modified method of Sherris et al., the dilution representing the MIC and at least two lower dilutions of the test product are plated and enumerated to determine viable cfu/cm^3 [28, 29]. The difference of the MIC values of synthesized coumarylthiazole derivatives was presented in Table 1.

MTT cytotoxicity assay

The synthesized BCTU derivatives were studied used for short term in vitro cytotoxicity using HeLa cancer cells gym the method of viability staining by trypan blue dye exclusion. The cells were seeded in 96-well plates. Ten BCTU analogue compounds well for each concentration were seeded and triplicate plates were used the cell line. Then, the cells were incubated at 37 °C and CO_2 incubator. After 24 h, the medium was replaced by fresh medium containing different concentrations of the synthesized compounds used for study. The percent viability and cytotoxicity were calculated using the following formulae:

$$\% \text{ Cell viability} = \text{Absorbance (test sample)} / \text{Absorbance (control)} \times 100$$

$$\% \text{ Cell cytotoxicity} = 100 - \% \text{ Cell viability}.$$

Cytotoxicity of sample on cancer cells was measured by micro-culture tetrazolium (MTT) assay [30]. For the assays, 96-well micro plates were seeded with 100 mm^3 medium containing 5000 cells. After 24 h of incubation and attachment, the cells were treated with different dilution of tested compound. BCTU derivatives from the stock solution (20 mg/1.5 cm^3), each BCTU derivatives' sample was applied in a series of dilutions (final concentrations ranging from 1.3 µg/2 mm^3, 3.2 µg/5 mm^3, 6.4 µg/10 mm^3, 7.7 µg/15 mm^3) with a final DMSO concentration of 0.1% and was tested in quadruplicate and CO_2 incubator. After 48 h incubation, cell viability was determined by adding (Sigma) tetrazolium salt as cytotoxicity indicator, so after 24 h of incubation, 10 mm^3 of MTT (5 mg/cm^3) in phosphate buffered saline (PBS) was added to each well and incubated at 37 °C for 4 h. The medium with MTT was then flicked off, and the formed formazan crystals were solubilized in 100 mm^3 of DMSO and then measured the absorbance at 570 nm using micro-plate reader. Tetrazolium salts are cleaved to formazan dye by cellular enzymes (only in the viable cells).

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