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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and antiproliferative activity evaluation of new thiazole–benzimidazole derivatives using real-time cell analysis (RTCA DP)

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Abstract A series of new 2-[(5-substituted-1*H*-benzimidazol-2-yl)thio]-*N*-[4-[2-phenylthiazol-4-yl]phenyl]acetamide derivatives (**4a–p**) were synthesized according to the reported literature, and anticancer activity of the compounds was evaluated. Cytotoxic activity was confirmed by real-time cytotoxicity analysis system determining half maximal inhibitory concentrations (IC_{50}) of the title compounds based on the dose–response curves derived by xCELLigence measurements against NIH/3T3, A549 and Caco2 cell lines for 24, 48 and 72 h exposure. Compound **4c** was found to be as the most efficient molecule that exhibited selective antiproliferative activity against both of the cancer cells.

Keywords Thiazole · Benzimidazole · Cytotoxic activity · Anticancer activity · Antiproliferative activity · Real-time cell analysis (RTCA) · xCELLigence

All cell lines were purchased from ATCC with account number 20033704 on behalf of Miriş Dikmen.

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Introduction

Cytotoxic agents constitute an important class of anticancer drugs. They act by damaging DNA, inhibiting its synthesis or interfering with the mechanics of cell division, for instance, by blocking topoisomerases or binding to microtubules (Chabner and Roberts, 2005; Workman, 2005). Many of the cytotoxic agents were discovered by screening for chemical compounds that were capable to destroy cancer cells, as with a natural product like the microtubule inhibitor paclitaxel (Rowinsky *et al.*, 1992). DNA-alkylating agents, originally based on sulphur and nitrogen mustards, were structurally modified to control their rates of chemical reactivity, leading to drugs like cyclophosphamide and ifosphamide (Colvin, 1999). There have been many remarkable successes with cytotoxic drug treatments for cancer. The cancer exists in a large number of forms, as defined anatomically under the light microscope and at the molecular level. The efficacy of drug treatment varies across these different anatomical, histological and molecular types. Significant progresses have been reached in the treatment of leukaemias, lymphomas, testicular cancer and children's malignancies with cytotoxic drugs, leading to marked increases in survival (Workman and Colins, 2008). The benzimidazole ring system is an essential pharmacophore in medicinal chemistry and modern drug discovery. 2-Substitutedbenzimidazoles have been recognized to act as potential anticancer (Prudhomme, 2006; Kumar *et al.*, 2002; Hranjec *et al.*, 2007; Piskin *et al.*, 2009). For instance, bis-benzimidazole derivatives (Kim *et al.*, 1996; Neidle *et al.*, 1999; Le Sann *et al.*, 2006; Alpan *et al.*, 2009; Huang *et al.*, 2006) were remarkably active compounds in interfering with DNA topoisomerase I (Kim *et al.*, 1996) and were also found to be cytotoxic against breast adenocarcinoma (MCF7) (Le

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Sann *et al.*, 2006) and skin epidermoid carcinoma (A431) (Le Sann *et al.*, 2006; Alpan *et al.,* 2009; Huang *et al.,* 2006). Also, methyl-2-benzimidazole carbamate (carbendazim, FB642) is an anticancer agent that induces apoptosis of cancer cells (Hao *et al.,* 2002; Hammond *et al.,* 2001). The next generation of rationally designed inhibitors, benzimidazole carboxamides, for example, 2-(4-hydroxyphenyl)benzimidazole-4-carboxamide (NU1085) (Bryant and Helleday, 2004) and 2-(4-oxadiazolylphenyl) analogue (Tong *et al.,* 2009), were vastly more potent as poly(ADP-ribose)polymerase inhibitor (PARP inhibitor) and improved the effects of chemotherapy and radiation therapy in vitro (Bryant and Helleday, 2004) and in vivo (Tong *et al.,* 2009). Also, the tricyclic benzimidazole (AG14361), a PARP inhibitor, has been developed and used in vivo at non-toxic doses to augment the effect of the DNA-damaging agents irinotecan (topoisomerase I poisons), g-irradiation or temozolomide (DNA-alkylating agent) (Calabrese *et al.,* 2004).

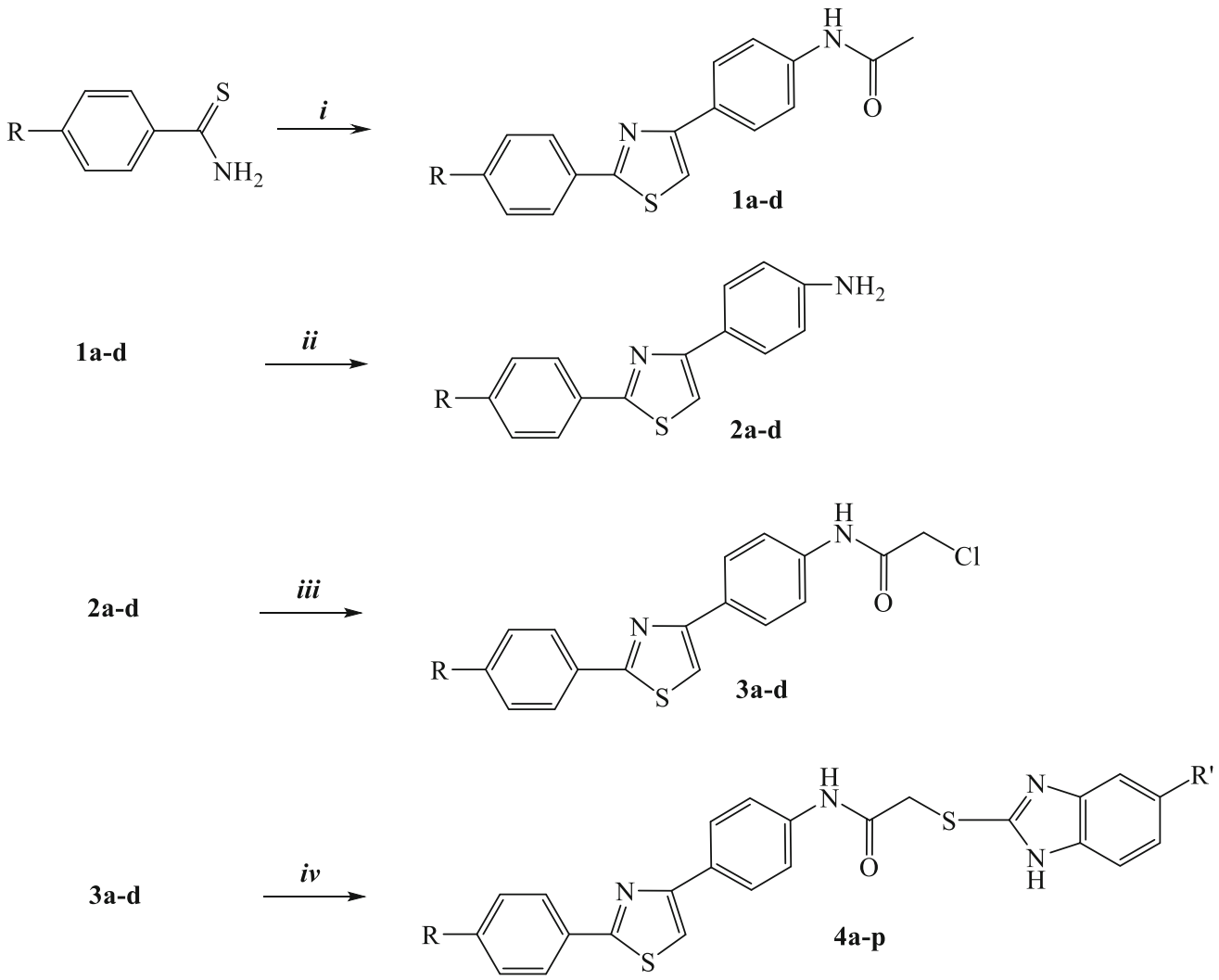
In addition to benzimidazoles, thiazole derivatives concerned the interest of medicinal chemists due to their synthetic feasibility and their incorporation into variety of therapeutically active agents. Meanwhile, DNA is one of the major targets of anticancer drugs since the development of the nitrogen mustards. Targeting DNA of tumour cells has been one of the most effective clinical strategies for many DNA intercalators such as groove binders and anticancer antibiotics (Hurley, 2002; Cai *et al.,* 2009; Bailly *et al.,* 1997; Yamamoto and Kawanishi, 1992). The clinical efficacy of the groove binders tiazofurin, distamycin, netropsin, thia-netropsin and bleomycin pointed out the importance of the 1,3-thiazole moieties and their contribution to enhance the antitumour activity (Chen and Pankiewicz, 2007; Popsavin *et al.*, 2007, Foy *et al.,* 2008; Wolter *et al.,* 2009; Silvermann, 1992; Nelson *et al.,* 2007; Plouvier *et al.,* 1995; Delgado and Remers, 1998; Abraham *et al.,* 2003; Da Rocha *et al.*, 2001). The antitumour activity of 2,4-disubstituted 1,3-thiazole analogues was reported and well documented (Schnur *et al.*, 1991; Kumar *et al.*, 1993; El-Subbagh *et al.,* 1994, 1999, 2001; El-Subbagh and Al-Obaid, 1996; Al-Omary *et al.*, 2012, El-Messery *et al.,* 2012).

The need for anticancer agents that selectively kill or inhibit the growth of neoplastic cells without affecting non-cancerous host tissues is high and persistent. Thus, the aim of the current study was the synthesis of novel benzimidazole derivatives that incorporated thiazole ring system, which have been expected to exhibit potent cytotoxic activity against cancer cells.

Results and discussion

Chemistry

The synthesis of 2-[(5-substituted-1*H*-benzimidazol-2-yl)thio]-*N*-[4-[2-(4-substituted-phenyl)thiazol-4-yl]phenyl] acetamide derivatives (**4a–p**) was carried out as shown in Scheme 1. All reactions were carried out according to our previous study (Yurttas *et al.* 2014) starting from 4-(2-bromoacetyl)acetanilide which was prepared from 4-aminoacetophenone via acetylation and bromination reactions. Compounds **1a–d** were gained from the reaction of the obtained α-halo ketone and thioacetamide derivatives to get 4-(2-aryl-4-thiazolyl)acetanilides. The hydrolysation reaction of the acetamide group of compounds **1a–d** provided 4-(2-aryl-4-thiazolyl)aniline derivatives (**2a–d**). After acetylation reaction with chloroacetyl chloride, the obtained amide compounds (**3a–d**) were reacted to acquire final compounds (**4a–p**) in yields ranging from 66 to 81 %. The structures of all final compounds were demonstrated using IR, ^{1}H-NMR, ^{13}C-NMR and MS spectral data. The characteristic stretching bands in the IR spectra of the compounds were determined at 3276–3264, 1662–1649, 1568–1279 and 1266–972 cm^{-1} that belong to N–H; C=O; C=C; C=N double bonds; and C–O, C–N single bond. In the 500-MHz ^{1}H-NMR of the compounds, singlet peaks at about 8.08–8.12 ppm and 12.52–12.89 ppm were assigned which belong to C_5 proton of thiazole ring and nitrogen proton of the benzimidazole ring in suitable compounds. Aromatic ring protons, belonging to phenyls at second and fourth positions of thiazole and the protons of benzimidazole, overlapped due to the multiplicity and were assigned between 6.76 and 8.10 ppm. The CH_2 and NH protons of acetamide residue were observed at about 4.25–4.37 and 10.61–10.81 ppm, respectively. The aliphatic protons of CH_3 and OCH_3 groups were observed at 2.38–2.39 ppm and 3.75–3.84, respectively. In the 100 MHz ^{13}C-NMR, peaks at about 36.03–36.71 ppm were assigned for the acetyl carbon bonded to sulphur atom and the signals which were seen in the down field of the spectrum over 149.26 ppm were defined for carbonyl carbons and vicinal aromatic carbons to heteroatoms as expected. Mass spectra of the compounds M + 1 peaks agreed well with the calculated molecular weight of the target compounds, and elemental analysis results were found within the calculated values for the C, H, N elements of the compounds (Figs. 1, 2, 3).



4	R	R'	4	R	R'
a	H	H	**i**	Cl	H
b	H	CH_3	**j**	Cl	CH_3
c	H	OCH_3	**k**	Cl	OCH_3
d	H	Cl	**l**	Cl	Cl
e	OCH_3	H	**m**	F	H
f	OCH_3	CH_3	**n**	F	CH_3
g	OCH_3	OCH_3	**o**	F	OCH_3
h	OCH_3	Cl	**p**	F	Cl

Scheme 1 Synthesis of the compounds (**4a–p**). Reactions and conditions: *i* 4-(2-bromoacetyl)acetanilide, EtOH, r.t., 8 h; *ii* 10 % HCl, EtOH, reflux; *iii* chloroacetyl chloride, TEA, THF, r.t.; *iv* 2-mercaptobenzimidazole derivatives, K_2CO_3, acetone, reflux, 2 h

Cytotoxic properties of the compounds

The xCELLigence RTCA DP system was used for real-time and time-dependent analysis of the cellular response of NIH/3T3, A549 and Caco2 cells. Conventional methods (MTT) were used to select appropriate concentrations of the compounds to test in xCELLigence system. The indicated method provides dynamic cell response profiles and temporal IC_{50} histograms, through cell index (CI) term which is based on the changes of measured electrical impedance of viable cells depending on applied test compounds at various concentrations (12.5, 50 and 200 μM) in

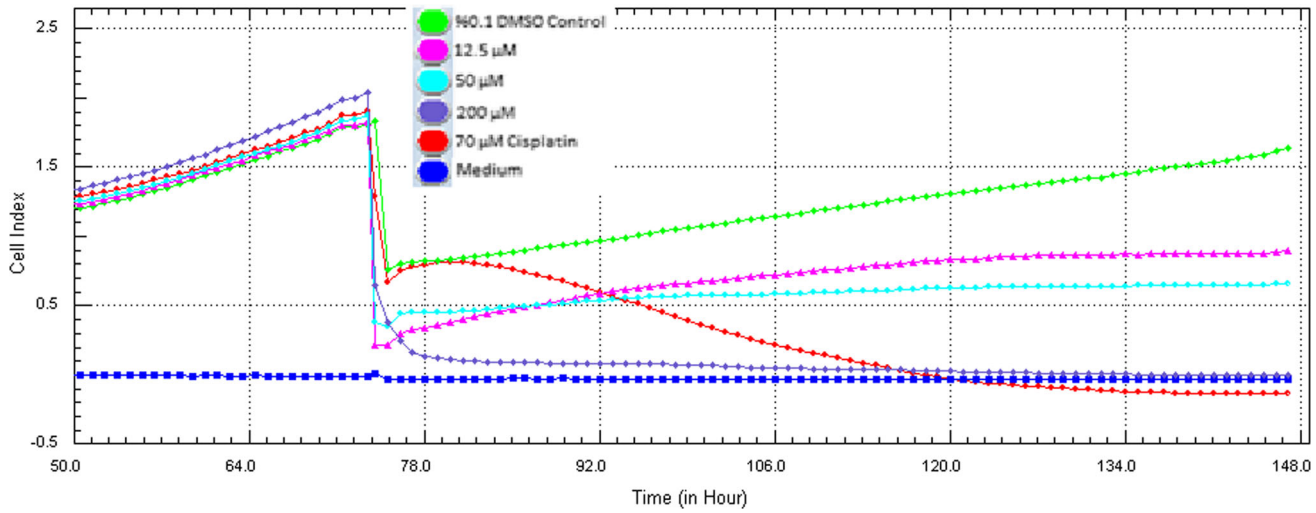


Fig. 1 Real-time monitoring of cell proliferative effects of compound **4c** at 12.5, 50 and 20 µM, cisplatin at 70 µM concentrations, test medium and 0.1 % solution of control group in DMSO on NIH/3T3 cells using RTCA DP System ($n = 6$)

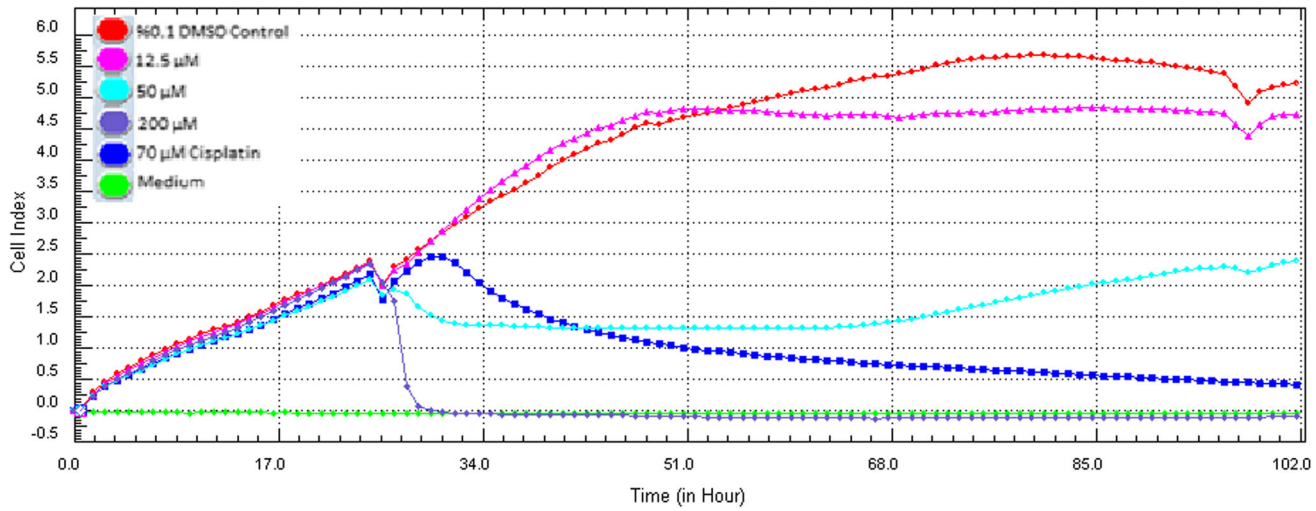


Fig. 2 Real-time monitoring of cell proliferative effects of compound **4c** at 12.5, 50 and 20 µM, cisplatin at 70 µM concentrations, test medium and 0.1 % solution of control group in DMSO on A549 cells using RTCA DP System ($n = 6$)

a certain period of time (Moe *et al.,* 2013). The starting CI values, as a measure of cell proliferation, were determined as 2.0, 2.5 and 1.0 on NIH/3T3, A549 and Caco2 cells, respectively, and the values decreased in a time- and concentration-dependent manner after the application of test compounds, while the CI values of the controls in 0.1 % dimethylsulphoxide kept rising to the end of the test except a little decrease, initially. The test compounds did not show any significant changes in the cell index during the first 5 h of their addition on NIH/3T3 cells. After this time, there was a slight increase or a prominent decrease in the CI values of treated cells due to concentration increasing. In order to compare the cytotoxic effects of the sixteen thiazole compounds (**4a**–**p**), their IC_{50} values were calculated after 24, 48 and 72 h exposure. Compounds **4c**, **4d**, **4f**, **4g**, **4h**, **4j**, **4k**, **4l**, **4n**, **4o** and **4p** caused A549 cell death at the highest concentration (200 µM) due to viewing the cell index value as zero in various time periods to the end of test time (102 h) in contrast to standard drug, cisplatin. This situation was also observed only in compounds **4b** and **4l** at same concentration against Caco2 cell line although most of IC_{50} values were found lower than cisplatin's IC_{50} values, especially after 48-h incubation. Therefore, this finding can be claimed that tested two lower doses of the compounds were found to be cytostatic not cytotoxic.

As shown in Tables 1, 2 and 3, most of the compounds exhibited significant cytotoxicity against A549 and Caco2 tumour cell lines at lower doses. Among them, compound **4c** caused antiproliferation more efficiently than the other

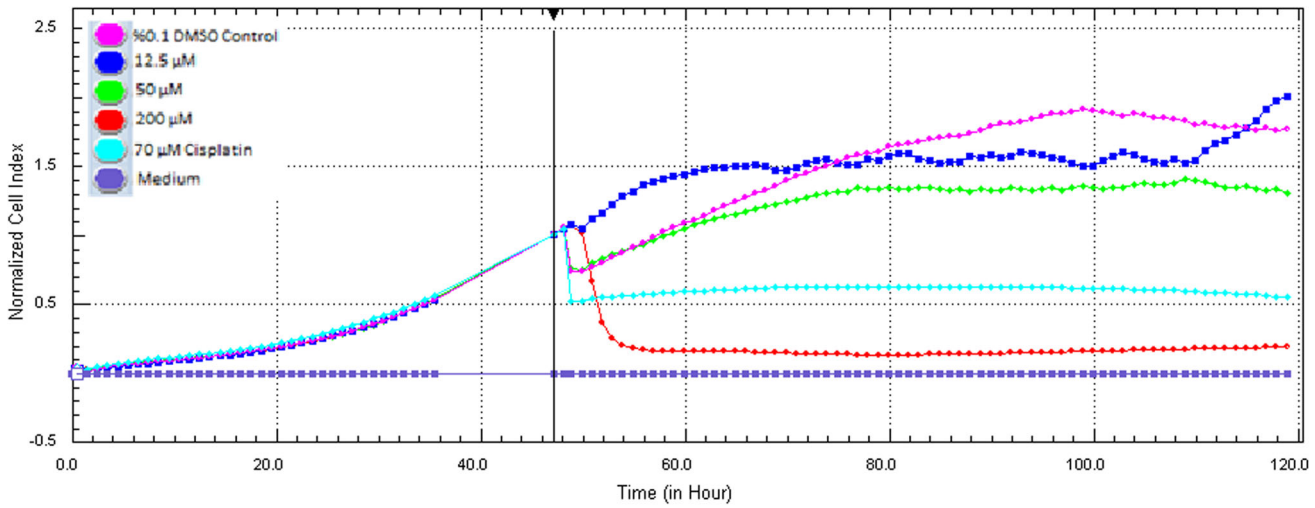


Fig. 3 Real-time monitoring of cell proliferative effects of compound **4c** at 12.5, 50 and 20 µM, cisplatin at 70 µM concentrations, test medium and 0.1 % solution of control group in DMSO on Caco2 cells as a function of time 72 h using RTCA DP System ($n = 6$). In the graph the *black line* shows the time of drug addition at which the CI values were normalized

Table 1 IC_{50} values (µM) of the compounds **4a–p** against NIH/3T3, A549 and Caco2 cell lines calculated via xCELLigence analysis after 24-h incubation

Compound	NIH/3T3	A549	Caco2
4a	33.45	42.26	103.75
4b	33.87	38.84	164.59
4c	74.56	27.90	65.21
4d	66.94	18.69	68.83
4e	35.29	38.05	70.38
4f	37.86	35.16	57.30
4g	37.47	44.14	71.58
4h	186.04	42.29	105.14
4i	57.37	25.54	71.35
4j	30.33	35.09	86.20
4k	36.93	36.57	60.56
4l	48.26	18.22	68.89
4m	44.98	50.17	33.06
4n	43.50	16.79	76.39
4o	71.00	36.67	113.17
4p	23.13	32.75	75.61
Cisplatin	95.42	20.33	69.10

compounds against both of cancer cell lines and in three measurement periods. The IC_{50} values were found to be 74.56, 82.24 and 128.96 µM against NIH/3T3 for compound **4c** which were higher than the inhibition values against the tumour cell lines of the same compound; therefore, it can be claimed that the compound **4c** showed selectivity against tumour cells and it also exhibited more similar responses to cisplatin.

The structures of the 2-[(5-substituted-1*H*-benzimidazol-2-yl)thio]-*N*-[4-[2-(4-substituted-phenyl)thiazol-4-yl]phenyl]acetamide derivatives (**4a–p**) differ from each other with substituents methyl, methoxy and chloro at the fifth position of the benzimidazole ring and the substituents methoxy, chloro and fluoro at *para* position of phenyl residue. Compound **4c** is the most active compound that possesses methoxy substitution on benzimidazole ring. In evaluating substituent effect of the title compounds, methoxy and chloro substituents cause higher increase in activity than the other substituents.

Conclusion

New 2-[(5-substituted-1*H*-benzimidazol-2-yl)thio]-*N*-[4-[2-phenylthiazol-4-yl]phenyl]acetamide (**4a–p**) derivatives were synthesized, and their antiproliferative activities were investigated using RTCA system on NIH/3T3, A549 and Caco2 cell lines. The measurements were carried out via xCELLigence system which is a reliable and accurate method. Most of the compounds showed significant cytotoxic activity with low IC_{50} values. However, compound **4c** exhibited selective cytotoxicity against both of the cancer cells and determined as the most efficient molecule.

Materials and methods

Chemistry

All chemicals were purchased from Sigma-Aldrich Chemical Co., (Sigma-Aldrich Corp., St. Louis, MO, USA) and Merck (Merck, Darmstadt, Germany). All melting points (m.p.) were determined by Electrothermal 9300

Table 2 IC_{50} values (μM) of the compounds **4a–p** against NIH/3T3, A549 and Caco2 cell lines calculated via xCELLigence analysis after 48-h incubation

Compound	NIH/3T3	A549	Caco2
4a	44.79	58.82	68.12
4b	43.68	35.94	78.21
4c	82.24	45.27	77.05
4d	34.57	29.30	108.74
4e	45.25	45.63	42.46
4f	44.41	33.17	42.14
4g	43.49	52.26	82.36
4h	50.63	38.55	86.88
4i	39.99	20.00	63.30
4j	41.98	20.02	90.31
4k	44.53	18.48	49.95
4l	39.59	22.60	81.60
4m	40.62	73.04	43.53
4n	27.91	36.57	93.00
4o	60.32	45.39	71.85
4p	40.31	41.68	76.20
Cisplatin	106.90	13.60	86.72

Table 3 IC_{50} values (μM) of the compounds **4a–p** against NIH/3T3, A549 and Caco2 cell lines calculated via xCELLigence analysis after 72-h incubation

Compound	NIH/3T3	A549	Caco2
4a	26.27	93.87	48.50
4b	37.68	32.94	54.95
4c	128.96	50.18	85.84
4d	30.80	30.59	65.69
4e	35.35	62.56	96.87
4f	34.98	30.99	44.96
4g	16.92	97.95	143.14
4h	32.01	41.68	51.75
4i	45.15	31.21	123.02
4j	30.68	19.64	71.16
4k	44.75	35.29	53.77
4l	40.94	36.58	54.74
4m	41.87	72.09	32.90
4n	36.52	36.57	55.33
4o	55.39	44.27	71.55
4p	43.58	42.41	90.68
Cisplatin	139.06	14.17	45.14

digital melting point apparatus (Electrothermal, Essex, UK) and are uncorrected. All the reactions were monitored by thin-layer chromatography (TLC) using Silica gel 60 F254 TLC plates (Merck, Darmstadt, Germany). Spectroscopic data were recorded with the following instruments: IR, Shimadzu 8400S spectrophotometer (Shimadzu, Tokyo, Japan); ^{1}H-NMR, Bruker 500 MHz spectrometer (Bruker Bioscience, Billerica, MA, USA); ^{13}C-NMR, VARIAN Mercury 100 FT spectrometer (Varian Inc., Palo Alto, CA, USA); VG Quattro mass spectrometer (VG BioTech, Altrincham, UK), and elemental analyses were performed on a Perkin Elmer EAL 240 elemental analyser (Perkin Elmer, Norwalk, CT, USA).

General procedure for the synthesis 2-[(5-substituted-1H-benzimidazol-2-yl)thio]-*N*-[4-[2-(4-substituted-phenyl)thiazol-4-yl]phenyl]acetamide derivatives (4a–p)

A mixture of compound **3a–d** (1 mmol), the appropriate mercapto-benzimidazole derivative (1 mmol) and K_2CO_3 (1 mmol) in acetone was refluxed for 2 h. After the solvent was evaporated, the obtained residue was treated with water, filtered, dried and then recrystallized from ethanol to afford target compounds **4a–p**.

*2-[(1H-benzimidazol-2-yl)thio]-N-[4-[2-phenylthiazol-4-yl]phenyl]acetamide **(4a)*** It was obtained as brown powder, yield 71 %; m.p. 233–234 °C; IR (KBr, cm^{-1}): ν_{max} 3269 (amide N–H), 1659 (C=O), 1568–1279 (C=C, C=N), 1228–975 (C–O, C–N); ^{1}H-NMR (500 MHz, DMSO-d_6, ppm): δ 4.32 (s, 2H, $COCH_2$), 7.13–7.16 (m, 2H, Ar–H), 7.47–7.56 (m, 5H, Ar–H), 7.71 (d, 2H, *J*: 8.50 Hz, Ar–H), 8.01–8.04 (m, 4H, Ar–H), 8.12 (s, 1H, thiazole C_5–H), 10.68 (s, 1H, CONH), 12.67 (s, 1H, benzimidazole N–H); ^{13}C NMR (100 MHz, DMSO-d_6, ppm): δ 36.71 (CH_2, $COCH_2$), 114.02 (CH, thiazole C_5), 117.54 (2 × CH, benzimidazole $C_4 + C_7$), 119.66 (2 × CH, benzene $C_2 + C_6$), 122.00 (2 × CH, benzimidazole $C_5 + C_6$), 126.67 (2 × CH, benzene $C_3 + C_5$), 127.21 (C, benzene C_4), 129.74 (CH, benzene C_4'), 129.78 (2 × CH, benzene $C_3' + C_5'$), 130.82 (2 × CH, benzene $C_2' + C_6'$), 133.50 (2 × C, benzimidazole $C_{3a} + C_{7a}$), 139.34 (C, benzene C_1), 143.82 (C, benzene C_1'), 150.31 (C, benzimidazole C_2), 155.39 (C, thiazole C_4), 166.72 (C=O, $COCH_2$), 167.33 (C, thiazole C_2); ESMS *m/z* 442 $[M]^+$ (100); Anal. Calcd. for $C_{24}H_{18}N_4OS_2$: C, 65.13; H, 4.10; N, 12.66. Found: C, 65.14; H, 4.14; N, 12.71.

*2-[(5-Methyl-1H-benzimidazol-2-yl)thio]-N-[4-[2-phenylthiazol-4-yl]phenyl]acetamide **(4b)*** It was obtained as brown powder, yield 75 %; m.p. 189–192 °C; IR (KBr, cm^{-1}): *v* 3269 (amide N–H), 1662 (C=O), 1554–1302 (C=C, C=N), 1264–975 (C–O, C–N); ^{1}H-NMR (500 MHz, DMSO-d_6, ppm): δ 2.38 (s, 3H, CH_3), 4.37 (s, 2H, $COCH_2$), 6.95 (d, 1H, *J*: 8.0 Hz, Ar–H), 7.24 (brs, 1H, Ar–H), 7.34 (brs, 1H, Ar–H), 7.51–7.57 (m, 3H, Ar–H), 7.71 (d, 2H, *J*: 8.5 Hz, Ar–H), 7.98–8.05 (m, 4H, Ar–H), 8.09 (s, 1H, thiazole C_5–H), 10.71 (s, 1H, CONH), 12.65 (s, 1H, benzimidazole N–H); ^{13}C NMR (100 MHz, DMSO-d_6,

ppm): δ 21.10 (CH_3), 36.17 (CH_2, $COCH_2$), 113.44 (CH, thiazole C_5), 117.29 (CH, benzimidazole C_7), 117.98 (CH, benzimidazole C_4), 119.06 (2 × CH, benzene $C_2 + C_6$), 122.73 (CH, benzimidazole C_6), 126.09 (2 × CH, benzene $C_3 + C_5$), 126.62 (C, benzene C_4), 129.17 (CH, benzene C_4'), 130.24 (2 × CH, benzene $C_3' + C_5'$), 132.93 (2 × CH, benzene $C_2' + C_6'$), 133.45 (C, benzimidazole C_5), 135.29 (C, benzimidazole C_{7a}), 138.77 (C, benzimidazole C_{3a}), 138.96 (C, benzene C_1), 143.56 (C, benzene C_1'), 149.89 (C, benzimidazole C_2), 154.81 (C, thiazole C_4), 166.20 (C=O, $COCH_2$), 166.74. (C, thiazole C_2); ESMS *m/z* 456 $[M]^+$ (100); Anal. Calcd. for $C_{25}H_{20}N_4OS_2$: C, 65.76; H, 4.42; N, 12.27. Found: C, 65.71; H, 4.54; N, 12.35.

2-[(5-Methoxy-1H-benzimidazol-2-yl)thio]-N-[4-[2-phenyl thiazol-4-yl]phenyl]acetamide ***(4c)*** It was obtained as brown powder, yield 70 %; m.p. 182–184 °C; IR (KBr, cm^{-1}): ν_{max} 3267 (amide N–H), 1654 (C=O), 1568–1288 (C=C, C=N), 1234–975 (C–O, C–N); ^{1}H-NMR (500 MHz, DMSO-d_6, ppm): δ 3.76 (s, 3H, OCH_3), 4.25 (s, 2H, $COCH_2$), 6.76 (d, 1H, *J*: 8.5 Hz, Ar–H), 6.98 (brs, 1H, Ar–H), 7.34 (brs, 1H, Ar–H), 7.49–7.56 (m, 3H, Ar–H), 7.70 (d, 2H, *J*: 9 Hz, Ar–H), 8.01–8.03 (m, 4H, Ar–H), 8.08 (s, 1H, thiazole C_5–H), 10.67 (s, 1H, CONH), 12.55 (s, 1H, benzimidazole N–H); ^{13}C NMR (100 MHz, DMSO-d_6, ppm): δ 36.26 (CH_2, $COCH_2$), 55.38 (OCH_3), 101.70 (CH, benzimidazole C_4), 110.39 (CH, benzimidazole C_6), 113.44 (CH, thiazole C_5), 117.52 (CH, benzimidazole C_7), 119.09 (2 × CH, benzene $C_2 + C_6$), 126.10 (2 × CH, benzene $C_3 + C_5$), 126.63 (C, benzene C_4), 129.17 (CH, benzene C_4'), 129.20 (2 × CH, benzene $C_3' + C_5'$), 130.25 (2 × CH, benzene $C_2' + C_6'$), 132.93 (C, benzimidazole C_{7a}), 138.79 (C, benzimidazole C_{3a}), 138.99 (C, benzene C_1), 143.07 (C, benzene C_1'), 148.68 (C, benzimidazole C_2), 154.83 (C, thiazole C_4), 155.27 (C, benzimidazole C_5), 166.25 (C=O, $COCH_2$), 166.77 (C, thiazole C_2); ESMS *m/z* 472 $[M]^+$: (100); Anal. Calcd. for $C_{25}H_{20}N_4O_2S_2$: C, 63.54; H, 4.27; N, 11.86. Found: C, 63.59; H, 4.24; N, 11.75.

2-[(5-Chloro-1H-benzimidazol-2-yl)thio]-N-[4-[2-phenylth iazol-4-yl]phenyl]acetamide ***(4d)*** It was obtained as white powder, yield 75 %; m.p. 241–243 °C; IR (KBr, cm^{-1}): ν_{max} 3269 (amide N–H), 1656 (C=O), 1568–1305 (C=C, C=N), 1264–975 (C–O, C–N); ^{1}H-NMR (500 MHz, DMSO-d_6, ppm): δ 4.32 (s, 2H, $COCH_2$), 7.15 (d, 1H, *J*: 8.5 Hz, Ar–H), 7.45 (d, 1H, *J*: 7.50 Hz, Ar–H), 7.51–7.55 (m, 4H, Ar–H), 7.69 (d, 2H, *J*: 8.0 Hz, Ar–H), 8.0–8.03 (m, 4H, Ar–H), 8.08 (s, 1H, thiazole C_5–H), 10.62 (s, 1H, CONH), 12.75 (s, 1H, benzimidazole N–H); ^{13}C NMR (100 MHz, DMSO-d_6, ppm): δ 36.14 (CH_2, $COCH_2$), 113.46 (CH, thiazole C_5), 115.62 (CH, benzimidazole C_4), 116.54 (CH, benzimidazole C_7), 119.11 (2 × CH, benzene $C_2 + C_6$), 121.53 (CH, benzimidazole C_6), 125.80 (C, benzimidazole C_5), 126.09 (2 × CH, benzene $C_3 + C_5$), 126.62 (C, benzene C_4), 129.17 (CH, benzene C_4'), 129.23 (2 × CH, benzene $C_3' + C_5'$), 130.25 (2 × CH, benzene $C_2' + C_6'$), 132.93 (C, benzimidazole C_{7a}), 138.73 (C, benzimidazole C_{3a}), 138.98 (C, benzene C_1), 143.11 (C, benzene C_1'), 151.65 (C, benzimidazole C_2), 154.81 (C, thiazole C_4), 165.9 1(C=O, $COCH_2$), 166.75 (C, thiazole C_2); ESMS *m/z* 476 $[M]^+$: (100); Anal. Calcd. for $C_{24}H_{17}ClN_4OS_2$: C, 60.43; H, 3.59; N, 11.75. Found: C, 60.55; H, 3.51; N, 11.63.

2-[(1H-Benzimidazol-2-yl)thio]-N-[4-[2-(4-methoxyphenyl) thiazol-4-yl]phenyl]acetamide ***(4e)*** It was obtained as cream powder, yield 68 %; m.p. 238–239 °C; IR (KBr, cm^{-1}): ν_{max} 3275 (amide N–H), 1651 (C=O), 1541–1296 (C=C, C=N), 1256–972 (C–O, C–N); ^{1}H-NMR (500 MHz, DMSO-d_6, ppm): δ 3.84 (s, 3H, OCH_3), 4.30 (s, 2H, $COCH_2$), 7.08 (d, 2H, *J*: 8.50 Hz, Ar–H), 7.13–7.15 (m, 2H, Ar–H), 7.46 (brs, 1H, Ar–H), 7.69 (d, 2H, *J*: 9 Hz, Ar–H), 7.95–7.99 (m, 6H, Ar–H), 10.68 (s, 1H, CONH), 12.66 (s, 1H, benzimidazole N–H); ^{13}C NMR (100 MHz, DMSO-d_6, ppm): δ 36.03 (CH_2, $COCH_2$), 55.19 (OCH_3), 112.24 (CH, thiazole C_5), 114.38 (2 × CH, benzene $C_3' + C_5'$), 118.94 (2 × CH, benzimidazole $C_4 + C_7$), 121.34 (2 × CH, benzene $C_2 + C_6$), 125.67 (2 × CH, benzimidazole $C_5 + C_6$), 126.46 (2 × CH, benzene $C_3 + C_5$), 127.56 (C, benzene C_4), 129.22 (2 × CH, benzene $C_2' + C_6'$), 132.27 (2 × C, benzimidazole $C_{3a} + C_{7a}$), 136.75 (C, benzene C_1'), 138.55 (C, benzene C_1), 149.62 (C, benzimidazole C_2), 154.40 (C, thiazole C_4) 160.72 (C, benzene C_4'), 165.99 (C=O, $COCH_2$), 166.54 (C, thiazole C_2); ESMS *m/z* 472 $[M]^+$: (100); Anal. Calcd. for $C_{25}H_{20}N_4O_2S_2$: C, 63.54; H, 4.27; N, 11.86. Found: C, 63.61; H, 4.30; N, 11.71.

2-[(5-Methyl-1H-benzimidazol-2-yl)thio]-N-[4-[2-(4-meth oxyphenyl)thiazol-4-yl]phenyl]acetamide ***(4f)*** It was obtained as brown powder, yield 66 %; m.p. 201–202 °C. IR (KBr, cm^{-1}): ν_{max} 3268 (amide N–H), 1661 (C=O), 1567–1312 (C=C, C=N), 1254–975 (C–O, C–N); ^{1}H-NMR (500 MHz, DMSO-d_6, ppm): δ 2.39 (s, 3H, CH_3), 3.84 (s, 3H, OCH_3), 4.28 (s, 2H, $COCH_2$), 6.95 (d, 1H, *J*: 8.5 Hz, Ar–H), 7.08 (d, 2H, *J*: 8.5 Hz, Ar–H), 7.22 (brs, 1H, Ar–H), 7.33 (brs, 1H, Ar–H), 7.69 (d, 2H, *J*: 9 Hz, Ar–H), 7.95–7.99 (m, 5H, Ar–H), 10.68 (s, 1H, CONH), 12.65 (s, 1H, benzimidazole N–H); ^{13}C NMR (100 MHz, DMSO-d_6, ppm): δ 21.17 (CH_3), 36.23 (CH_2, $COCH_2$), 55.38 (OCH_3), 112.43 (CH, thiazole C_5), 114.56 (2 × CH, benzene $C_3' + C_5'$), 117.26 (CH, benzimidazole C_7), 117.92 (CH, benzimidazole C_4), 119.11 (2 × CH, benzene $C_2 + C_6$), 125.85 (CH, benzimidazole C_6), 126.65 (2 × CH, benzene $C_3 + C_5$), 127.75 (C, benzene C_4), 129.38 (2 × CH, benzene $C_2' + C_6'$), 133.41 (C, benzimidazole C_5), 134.93 (C,

benzene C_1'), 135.32 (C, benzimidazole C_{7a}), 138.74 (C, benzimidazole C_{3a}), 138.97 (C, benzene C_1), 149.81 (C, benzimidazole C_2), 154.58 (C, thiazole C_4), 160.91 (C, benzene C_4'), 166.24 (C=O, $COCH_2$), 166.73. (C, thiazole C_2); ESMS *m/z* 486 $[M]^+$: (100); Anal. Calcd. for $C_{26}H_{22}N_4O_2S_2$: C, 64.17; H, 4.56; N, 11.51. Found: C, 64.11; H, 4.39; N, 11.61.

2-[(5-Methoxy-1H-benzimidazol-2-yl)thio]-N-[4-[2-(4-methoxyphenyl)thiazol-4-yl]phenyl]acetamide ***(4g)*** It was obtained as cream powder, yield 69 %; m.p. 191–193 °C. IR (KBr, cm^{-1}): ν_{max} 3269 (amide N–H), 1658 (C=O), 1558–1288 (C=C, C=N), 1245–976 (C–O, C–N); ^{1}H-NMR (500 MHz, DMSO-d_6, ppm): δ 3.79 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 4.25 (s, 2H, $COCH_2$), 6.76 (d, 1H, *J*: 8.50 Hz, Ar–H), 6.98 (brs, 1H, Ar–H), 7.09 (d, 2H, *J*: 8.0 Hz, Ar–H), 7.35 (d, 1H, *J*: 8.5 Hz, Ar–H), 7.69 (d, 2H, *J*: 9.0 Hz, Ar–H), 7.95–8.0 (m, 5H, Ar–H), 10.68 (s, 1H, CONH), 12.65 (s, 1H, benzimidazole N–H); ^{13}C NMR (100 MHz, DMSO-d_6, ppm): δ 36.39 (CH_2, $COCH_2$), 55.46 (OCH_3), 55.54 (OCH_3), 101.73 (CH, benzimidazole C_4), 110.60 (CH, benzimidazole C_6), 112.52 (CH, thiazole C_5), 114.66 (2 × CH, benzene $C_3' + C_5'$), 117.58 (CH, benzimidazole C_7), 119.26 (2 × CH, benzene $C_2 + C_6$), 125.94 (2 × CH, benzene $C_3 + C_5$), 126.74 (C, benzene C_4), 127.85 (2 × CH, benzene $C_2' + C_6'$), 129.51 (C, benzimidazole C_{7a}), 136.12 (C, benzene C_1'), 138.80 (C, benzimidazole C_{3a}), 139.05 (C, benzene C_1), 148.72 (C, benzimidazole C_2), 154.66 (C, thiazole C_4), 155.45 (C, benzimidazole C_5), 161.00 (C, benzene C_4'), 166.40 (C=O, $COCH_2$), 166.86 (C, thiazole C_2); ESMS *m/z* 502 $[M]^+$: (100); Anal. Calcd. for $C_{26}H_{22}N_4O_3S_2$: C, 62.13; H, 4.41; N, 11.15. Found: C, 62.18; H, 4.39; N, 11.21.

2-[(5-Chloro-1H-benzimidazol-2-yl)thio]-N-[4-[2-(4-methoxyphenyl)thiazol-4-yl]phenyl]acetamide ***(4h)*** It was obtained as white powder, yield 77 %; m.p. 238–240 °C; IR (KBr, cm^{-1}): ν_{max} 3267 (amide N–H), 1658 (C=O), 1555–1287 (C=C, C=N), 1231–976 (C–O, C–N); ^{1}H-NMR (500 MHz, DMSO-d_6, ppm): δ 3.84 (s, 3H, OCH_3), 4.33 (s, 2H, $COCH_2$), 7.08 (d, 2H, *J*: 8.5 Hz, Ar–H), 7.15 (d, 1H, *J*: 7.5 Hz, Ar–H), 7.46 (d, 1H, *J*: 8.5 Hz, Ar–H), 7.53 (s, 1H, Ar–H), 7.69 (d, 2H, *J*: 8.0 Hz, Ar–H), 7.93–8.0 (m, 5H, Ar–H), 10.62 (s, 1H, CONH), 12.88 (s, 1H, benzimidazole N–H); ^{13}C NMR (100 MHz, DMSO-d_6, ppm): δ36.22 (CH_2, $COCH_2$), 55.37 (OCH_3), 112.43 (CH, thiazole C_5), 114.56 (2 × CH, benzene $C_3' + C_5'$), 115.58 (CH, benzimidazole C_4), 116.81 (CH, benzimidazole C_7), 119.15 (2 × CH, benzene $C_2 + C_6$), 121.56 (CH, benzimidazole C_6), 125.86 (C, benzimidazole C_5), 126.64 (2 × CH, benzene $C_3 + C_5$), 127.74 (C, benzene C_4), 129.42 (2 × CH, benzene $C_2' + C_6'$), 132.98 (C, benzimidazole C_{7a}), 138.71 (C, benzimidazole C_{3a}), 139.13 (C, benzene C_1), 143.24 (C, benzene C_1'), 151.78 (C, benzimidazole C_2), 154.59 (C, thiazole C_4), 160.91 (C, benzene C_4'), 165.99 (C=O, $COCH_2$), 166.73 (C, thiazole C_2); ESMS *m/z* 506 $[M]^+$: (100); Anal. Calcd. for $C_{25}H_{19}ClN_4O_2S_2$: C, 59.22; H, 3.78; N, 11.05. Found: C, 59.25; H, 3.71; N, 11.13.

2-[(1H-Benzimidazol-2-yl)thio]-N-[4-[2-(4-chlorophenyl)thiazol-4-yl]phenyl]acetamide ***(4i)*** It was obtained as white powder, yield 68 %; m.p. 233–235 °C; IR (KBr, cm^{-1}): ν_{max} 3275 (amide N–H), 1658 (C=O), 1552–1287 (C=C, C=N), 1266–975 (C–O, C–N); ^{1}H-NMR (500 MHz, DMSO-d_6, ppm): δ 4.30 (s, 2H, $COCH_2$), 7.12–7.15 (m, 2H, Ar–H), 7.48 (brs, 2H, Ar–H), 7.61 (d, 2H, *J*: 8 Hz, Ar–H), 7.70 (d, 2H, *J*: 8.5 Hz, Ar–H), 7.97–8.06 (m, 4H, Ar–H), 8.12 (s, 1H, thiazole C_5–H), 10.71 (s, 1H, CONH), 12.69 (s, 1H, benzimidazole N–H); ^{13}C NMR (100 MHz, DMSO-d_6, ppm): δ 36.19 (CH_2, $COCH_2$), 113.99 (CH, thiazole C_5), 117.59 (2 × CH, benzimidazole $C_4 + C_7$), 119.14 (2 × CH, benzene $C_2 + C_6$), 121.46 (2 × CH, benzimidazole $C_5 + C_6$), 126.72 (2 × CH, benzene $C_3 + C_5$), 127.85 (C, benzene C_4), 129.10 (2 × CH, benzene $C_2' + C_6'$), 129.29 (2 × CH, benzene $C_3' + C_5'$), 131.83 (C, benzene C_4'), 134.82 (2 × C, benzimidazole $C_{3a} + C_{7a}$), 138.91 (C, benzene C_1), 143.89 (C, benzene C_1'), 149.83 (C, benzimidazole C_2), 155.00 (C, thiazole C_4), 165.46 (C=O, $COCH_2$), 166.22 (C, thiazole C_2); ESMS *m/z* 476 $[M]^+$: (100); Anal. Calcd. for $C_{24}H_{17}ClN_4OS_2$ calculated: C, 60.43; H, 3.59; N, 11.75. Found: C, 60.45; H, 3.61; N, 11.68.

2-[(5-Methyl-1H-benzimidazol-2-yl)thio]-N-[4-[2-(4-chlorophenyl)thiazol-4-yl]phenyl]acetamide ***(4j)*** It was obtained as white powder, yield 70 %; m.p. 202–204 °C; IR (KBr, cm^{-1}): ν_{max} 3276 (amide N–H), 1659 (C=O), 1548–1299 (C=C, C=N), 1263–978 (C–O, C–N); ^{1}H-NMR (500 MHz, DMSO-d_6, ppm): δ 2.39 (s, 3H, CH_3), 4.28 (s, 2H, $COCH_2$), 7.61 (d, 2H, *J*: 9.0 Hz, Ar–H), 7.71 (d, 2H, *J*: 9.0 Hz, Ar–H), 8.01 (d, 2H, *J*: 8.0 Hz, Ar–H), 8.06 (d, 2H, *J*: 8.50 Hz, Ar–H), 8.12 (s, 1H, thiazole C_5–H), 10.72 (s, 1H, CONH), 12.52 (s, 1H, benzimidazole N–H); ^{13}C NMR (100 MHz, DMSO-d_6, ppm): δ 21.18 (CH_3), 36.24 (CH_2, $COCH_2$), 113.98 (CH, thiazole C_5), 117.12 (CH, benzimidazole C_7), 117.84 (CH, benzimidazole C_4), 119.12 (2 × CH, benzene $C_2 + C_6$), 122.74 (CH, benzimidazole C_6), 126.72 (2 × CH, benzene $C_3 + C_5$), 127.85 (C, benzene C_4), 129.09 (2 × CH, benzene $C_3' + C_5'$), 129.29 (2 × CH, benzene $C_2' + C_6'$), 130.58 (C, benzimidazole C_5), 131.83 (C, benzimidazole C_{7a}), 134.83 (C, benzene C_4'), 138.94 (C, benzimidazole C_{3a}), 139.15 (C, benzene C_1), 144.24 (C, benzene C_1'), 149.26 (C, benzimidazole C_2), 155.02 (C, thiazole C_4), 165.46 (C=O, $COCH_2$), 166.32. (C, thiazole C_2); ESMS *m/z* 490 $[M]^+$: (100); Anal. Calcd. for $C_{25}H_{19}ClN_4OS_2$: C, 61.15; H, 3.90; N, 11.41. Found: C, 61.24; H, 3.78; N, 11.33.

2-[(5-Methoxy-1H-benzimidazol-2-yl)thio]-N-[4-[2-(4-chlorophenyl)thiazol-4-yl]phenyl]acetamide ***(4k)*** It was obtained as brown powder, yield 69 %; m.p. 200–203 °C; IR (KBr, cm^{-1}): ν_{max} 3268 (amide N–H), 1654 (C=O), 1537–1298 (C=C, C=N), 1213–976 (C–O, C–N); ^{1}H-NMR (500 MHz, DMSO-d_6, ppm): δ 3.75 (s, 3H, OCH_3), 4.25 (s, 2H, $COCH_2$), 6.76 (d, 2H, *J*: 8.50 Hz, Ar–H), 6.98 (s, 1H, Ar–H), 7.35 (d, 1H, *J*: 9.0 Hz, Ar–H), 7.60 (d, 2H, *J*: 8.5 Hz, Ar–H), 7.70 (d, 2H, *J*: 8.50 Hz, Ar–H), 8.01 (d, 2H, *J*: 8.5 Hz, Ar–H), 8.4 (d, 2H, *J*: 8.5 Hz, Ar–H), 8.11 (s, 1H, thiazole C_5–H), 10.71 (s, 1H, CONH), 12.88 (s, 1H, benzimidazole N–H); ^{13}C NMR (100 MHz, DMSO-d_6, ppm): δ36.31 (CH_2, $COCH_2$), 55.45 (OCH_3), 101.74 (CH, benzimidazole C_4), 110.41 (CH, benzimidazole C_6), 114.00 (CH, thiazole C_5), 117.61 (CH, benzimidazole C_7), 119.14 (2 × CH, benzene $C_2 + C_6$), 126.72 (2 × CH, benzene $C_3 + C_5$), 127.86 (C, benzene C_4), 129.09 (C, benzene C_4'), 129.30 (2 × CH, benzene $C_3' + C_5'$), 131.83 (2 × CH, benzene $C_2' + C_6'$), 134.83 (C, benzimidazole C_{7a}), 138.93 (C, benzimidazole C_{3a}), 139.07 (C, benzene C_1), 143.16 (C, benzene C_1'), 148.08 (C, benzimidazole C_2), 155.02 (C, thiazole C_4), 155.30 (C, benzimidazole C_5), 165.47 (C=O, $COCH_2$), 166.34 (C, thiazole C_2); ESMS *m/z* 506 $[M]^+$: (100); Anal. Calcd. for $C_{25}H_{19}ClN_4O_2S_2$: C, 59.22; H, 3.78; N, 11.05. Found: C, 59.29; H, 3.72; N, 11.13.

2-[(5-Chloro-1H-benzimidazol-2-yl)thio]-N-[4-[2-(4-chlorophenyl)thiazol-4-yl]phenyl]acetamide ***(4l)*** It was obtained as white powder, yield 72 %; m.p. 210–213 °C; IR (KBr, cm^{-1}): ν_{max} 3266 (amide N–H), 1654 (C=O), 1558–1292 (C=C, C=N), 1264–976 (C–O, C–N); ^{1}H-NMR (500 MHz, DMSO-d_6, ppm): δ 4.32 (s, 2H, $COCH_2$), 7.16 (d, 2H, *J*: 9.0 Hz, Ar–H), 7.49 (brs, 2H, Ar–H), 7.61 (d, 1H, *J*: 8.5 Hz, Ar–H), 7.70 (d, 2H, *J*: 8.5 Hz, Ar–H), 8.01 (d, 2H, *J*: 8.5 Hz, Ar–H), 8.05 (d, 2H, *J*: 8.0 Hz, Ar–H), 8.12 (s, 1H, thiazole C_5–H), 10.61 (s, 1H, CONH), 12.89 (s, 1H, benzimidazole N–H); ^{13}C NMR (100 MHz, DMSO-d_6, ppm): δ 36.20 (CH_2, $COCH_2$), 114.01 (CH, thiazole C_5), 115.08 (CH, benzimidazole C_4), 116.98 (CH, benzimidazole C_7), 119.18 (2 × CH, benzene $C_2 + C_6$), 121.58 (CH, benzimidazole C_6), 125.86 (C, benzimidazole C_5), 126.72 (2 × CH, benzene $C_3 + C_5$), 127.85 (C, benzene C_4), 129.13 (C, benzene C_4'), 129.29 (2 × CH, benzene $C_3' + C_5'$), 131.83 (2 × CH, benzene $C_2' + C_6'$), 134.83 (C, benzimidazole C_{7a}), 138.87 (C, benzimidazole C_{3a}), 139.06 (C, benzene C_1), 144.15 (C, benzene C_1'), 151.73 (C, benzimidazole C_2), 155.00 (C, thiazole C_4), 165.47 (C=O, $COCH_2$), 165.99 (C, thiazole C_2); ESMS *m/z* 510 $[M]^+$: (100); Anal. Calcd. for $C_{24}H_{16}Cl_2N_4OS_2$: C, 56.36; H, 3.15; N, 10.95. Found: C, 56.39; H, 3.02; N, 10.83.

2-[(1H-Benzimidazol-2-yl)thio]-N-[4-[2-(4-fluorophenyl)thiazol-4-yl]phenyl]acetamide ***(4m)*** It was obtained as brown powder, yield 67 %; m.p. 242–245 °C; IR (KBr, cm^{-1}): ν_{max} 3272 (amide N–H), 1657 (C=O), 1531–1296 (C=C, C=N), 1214–975 (C–O, C–N); ^{1}H-NMR (500 MHz, DMSO-d_6, ppm): δ 4.30 (s, 2H, $COCH_2$), 7.12–7.14 (m, 2H, Ar–H), 7.37 (*t*, 2H, *J*: 9 Hz, Ar–H), 7.49 (brs, 2H, Ar–H), 7.69 (d, 2H, *J*: 8.50 Hz, Ar–H), 7.99 (d, 2H, *J*: 8.0 Hz, Ar–H), 8.06–8.09 (m, 3H, Ar–H), 10.67 (s, 1H, CONH), 12.65 (s, 1H, benzimidazole N–H); ^{13}C NMR (100 MHz, DMSO-d_6, ppm): δ 36.20 (CH_2, $COCH_2$), 113.59 (CH, thiazole C_5), 116.26 (2 × CH, *J*: 22.0 Hz, benzene $C_3' + C_5'$), 117.65 (2 × CH, benzimidazole $C_4 + C_7$), 119.14 (2 × CH, benzene $C_2 + C_6$), 121.48 (2 × CH, benzimidazole $C_5 + C_6$), 126.71 (2 × CH, benzene $C_3 + C_5$), 128.44 (C, benzene C_4), 129.19 (2 × C, benzimidazole $C_{3a} + C_{7a}$), 129.69 (2 × CH, *J*: 3.0 Hz benzene $C_2' + C_6'$), 138.86 (C, benzene C_1), 143.67 (C, benzene C_1'), 149.82 (C, benzimidazole C_2), 154.87 (C, thiazole C_4), 163.23 (C, *J*: 247.0 Hz benzene C_4'), 165.64 (C=O, $COCH_2$), 166.22 (C, thiazole C_2); ESMS *m/z* 459 $[M]^+$: (100); Anal. Calcd. for $C_{24}H_{17}FN_4OS_2$: C, 62.59; H, 3.72; N, 12.17. Found: C, 62.68; H, 3.62; N 12.28.

2-[(5-Methyl-1H-benzimidazol-2-yl)thio]-N-[4-[2-(4-fluorophenyl)thiazol-4-yl]phenyl]acetamide ***(4n)*** It was obtained as white powder, yield 71 %; m.p. 204–206 °C; IR (KBr, cm^{-1}): ν_{max} 3267 (amide N–H), 1649 (C=O), 1516–1311 (C=C, C=N), 1232–972 (C–O, C–N); ^{1}H-NMR (500 MHz, DMSO-d_6, ppm): δ 2.38 (s, 3H, CH_3), 4.27 (s, 2H, $COCH_2$), 6.95 (d, 2H, *J*: 8.50 Hz, Ar–H), 7.37 (*t*, 3H, *J*: 8.0 Hz, Ar–H), 7.69 (d, 2H, *J*: 8.0 Hz, Ar–H), 8.0 (d, 2H, *J*: 8.5 Hz, Ar–H), 8.06–8.09 (m, 4H, Ar–H), 10.75 (s, 1H, CONH), 12.64 (s, 1H, benzimidazole N–H); ^{13}C NMR (100 MHz, DMSO-d_6, ppm): δ21.18 (CH_3), 36.25 (CH_2, $COCH_2$), 113.59 (CH, thiazole C_5), 116.27 (2 × CH, *J*: 21.0 Hz, benzene $C_3' + C_5'$), 117.11 (CH, benzimidazole C_7), 117.82 (CH, benzimidazole C_4), 119.13 (2 × CH, benzene $C_2 + C_6$), 122.42 (CH, benzimidazole C_6), 126.71 (2 × CH, benzene $C_3 + C_5$), 128.44 (C, benzene C_4), 129.70 (2 × CH, *J*: 2.0 Hz benzene $C_2' + C_6'$), 132.41 (C, benzimidazole C_5), 135.36 (C, benzimidazole C_{7a}), 138.87 (C, benzimidazole C_{3a}), 139.11 (C, benzene C_1), 143.22 (C, benzene C_1'), 149.91 (C, benzimidazole C_2), 154.88 (C, thiazole C_4), 163.23 (CH, *J*: 247.0 Hz benzene C_4'), 165.65 (C=O, $COCH_2$), 166.28. (C, thiazole C_2); ESMS *m/z* 473 $[M]^+$: (100); Anal. Calcd. for $C_{25}H_{19}FN_4OS_2$: C, 63.27; H, 4.04; N, 11.81. Found: C, 63.20; H, 3.98; N, 11.73.

2-[(5-Methoxy-1H-benzimidazol-2-yl)thio]-N-[4-[2-(4-fluorophenyl)thiazol-4-yl]phenyl]acetamide ***(4o)*** It was obtained as white powder, yield 67 %; m.p. 181–185 °C; IR (KBr, cm^{-1}): ν_{max} 3264 (amide N–H), 1662 (C=O), 1515–1282 (C=C, C=N), 1233–975 (C–O, C–N); ^{1}H-NMR (500 MHz, DMSO-d_6, ppm): δ 3.76 (s, 3H, OCH_3), 4.26 (s, 2H, $COCH_2$), 6.75 (d, 1H, *J*: 9.0 Hz, Ar–H), 6.98 (s, 1H,

Ar–H), 7.34–7.37 (m, 3H, Ar–H), 7.71 (d, 2H, *J*: 9.0 Hz, Ar–H), 8.0 (d, 2H, *J*: 9.0 Hz, Ar–H), 8.05–8.07 (m, 3H, Ar–H), 10.81 (s, 1H, CONH), 12.87 (s, 1H, benzimidazole N–H); ^{13}C NMR (100 MHz, DMSO-d_6, ppm): δ 36.35 (CH_2, $COCH_2$), 55.46 (OCH_3), 96.97 (CH, benzimidazole C_4), 110.39 (CH, benzimidazole C_6), 113.57 (CH, thiazole C_5), 114.71 (CH, benzimidazole C_7), 116.26 (2 × CH, *J*: 23.0 Hz, benzene $C_3' + C_5'$), 119.16 (2 × CH, benzene $C_2 + C_6$), 126.72 (2 × CH, benzene $C_3 + C_5$), 128.44 (C, benzene C_4), 129.20 (C, benzimidazole C_{7a}), 129.71 (2 × CH, *J*: 3.0 Hz, benzene $C_2' + C_6'$), 138.51 (C, benzimidazole C_{3a}), 138.92 (C, benzene C_1), 143.47 (C, benzene C_1'), 148.88 (C, benzimidazole C_2), 154.90 (C, thiazole C_4), 155.31 (C, benzimidazole C_5), 162.24 (C, *J*: 247.0 Hz, benzene C_4'), 165.67 (C=O, $COCH_2$), 166.41 (C, thiazole C_2); ESMS *m/z* 489 $[M]^+$: (100); Anal. Calcd. for $C_{25}H_{19}FN_4O_2S_2$: C, 61.21; H, 3.90; N, 11.42. Found: C, 61.28; H, 3.82; N, 11.33.

2-[(5-Chloro-1H-benzimidazol-2-yl)thio]-N-[4-[2-(4-fluorophenyl)thiazol-4-yl]phenyl]acetamide ***(4p)*** It was obtained as brown powder, yield 71 %; m.p. 142–147 °C; IR (KBr, cm^{-1}): ν_{max} 3268 (amide N–H), 1658 (C=O), 1529–1305 (C=C, C=N), 1247–1012 (C–O, C–N); ^{1}H-NMR (500 MHz, DMSO-d_6, ppm): δ 4.32 (s, 2H, $COCH_2$), 7.16 (d, 1H, *J*: 9.0 Hz, Ar–H), 7.38 (*t*, 2H, *J*: 8.50 Hz, Ar–H), 7.46 (d, 1H, *J*: 8.50 Hz, Ar–H), 7.53 (s, 1H, Ar–H), 7.70 (d, 2H, *J*: 8.50 Hz, Ar–H), 8.02 (d, 2H, *J*: 9.0 Hz, Ar–H), 8.07–8.10 (m, 3H, Ar–H), 10.63 (s, 1H, CONH), 12.89 (s, 1H, benzimidazole N–H); ^{13}C NMR (100 MHz, DMSO-d_6, ppm): δ 36.19 (CH_2, $COCH_2$), 113.61 (CH, thiazole C_5), 115.21 (CH, benzimidazole C_4), 116.03 (CH, benzimidazole C_7), 116.26 (2 × CH, *J*: 18.0 Hz benzene $C_3' + C_5'$), 119.18 (2 × CH, benzene $C_2 + C_6$), 121.59 (CH, benzimidazole C_6), 125.87 (C, benzimidazole C_5), 126.70 (2 × CH, benzene $C_3 + C_5$), 128.44 (C, benzene C_4), 129.22 (C, benzimidazole C_{7a}), 129.59(2 × CH, benzene $C_2' + C_6'$), 138.52 (C, benzimidazole C_{3a}), 138.82 (C, benzene C_1), 143.51 (C, benzene C_1'), 151.73 (C, benzimidazole C_2), 154.86 (C, thiazole C_4), 163.22 (C, *J*: 198.0 Hz benzene C_4'), 165.65 (C=O, $COCH_2$), 165.99 (C, thiazole C_2); ESMS *m/z* 493 $[M]^+$: (100); Anal. Calcd. for $C_{24}H_{16}ClFN_4OS_2$: C, 58.23; H, 3.26; N, 11.32. Found: C, 58.29; H, 3.29; N, 11.43.

Cytotoxicity assay

Cell culture and treatment

Mouse embryonic fibroblast cell line (3T3; ATTC Number CRL-1685), human colorectal adenocarcinoma cell line (Caco2; ATCC number: HTB-37), non-small cell lung cancer cell line (A549; ATCC Number: CCL-185) were obtained from American Type Culture Collection. The cells were grown in RPMI 1640 medium supplemented with 2 mM L-glutamine and 10 % foetal bovine serum, 1 % penicillin/streptomycin at a temperature of 37 °C in a humidified incubator with a 5 % CO_2 atmosphere. The cells were harvested at confluence with 0.05 % trypsin—0.02 % EDTA—and plated in tissue culture dishes and grown with fresh medium. Compounds were dissolved in DMSO solution.

Cell growth and proliferation assay using Real-Time Cell Analysis System (RTCA DP)

Kinetics of cell adhesion and spreading were experimented using the Real-Time Cell Analyser (RTCA DP). The growth characteristics following seeding were monitored to determine the optimum seeding density and time to beginning of the death. The system measures electrical impedance across interdigitated microelectrodes incorporated on the bottom of tissue culture e-plates. The impedance measurement, which is indicated as cell index (CI) value, provides quantitative information about the condition of the cells, involving cell number, viability and morphology (Bird and Kirstein, 2009; Solly *et al.,* 2004; Urcan *et al.,* 2010).

Background of the e-plates was measured in 100 µl medium in the Real-Time Cell Analyser (RTCA DP) station (xCELLigence, Roche/ACEA Biosciences, Mannheim, Germany). Afterwards 100 µl of a 3T3, Caco2 and A549 cell suspension was added (10.000 cells/well). Plates were incubated for 30 min at room temperature, e-plates were placed into the Real-Time Cell Analyser, and impedance was measured every hour. When the cells were in the log growth phase, the cells were treated to 100 µl of medium containing different concentrations of compounds **4a**–**p** (12.5, 50 and 200 µM) and 70 µM Cisplatin and impedance monitoring continued for another 72 h. Control was received medium + DMSO with a final concentration of 0.1 %. Impedance was demonstrated as cell index (CI) values. Dose–response curves at 72 h were generated to determine IC_{50} values during the incubation time. The electrical impedance was analysed by the RTCA DP system.

Compliance with ethical standards

Conflict of interests The authors report no conflict of interest.

Ethical statement For this type of study formal consent is not required. Informed consent was obtained from all individual participants included in the study.

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