Synthesis and *In Vitro* Anticancer Evaluation of Some Benzimidazolium Salts

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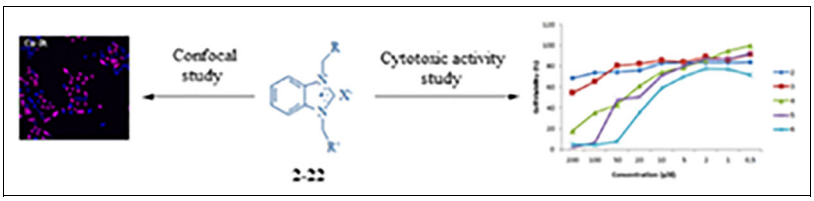

A series of benzimidazolium salts having 2-cyanobenzyl (**2**–**6**), 2-(4-nitrophenyl)ethyl (**7**–**10**), (1,3-dioxoisoindolin-2-yl)butyl (**11**–**17**) or *N*-methylphthalimide (**18**–**22**) substitution were synthesized and characterized by spectroscopic and analytical techniques. The *in vitro* cytotoxic activity of these salts was tested against human colon cancer (DLD-1), human breast cancer (MDA-MB-231), and normal human noncancerous embryonic kidney (HEK-293T) cell lines using the MTT assay method. The viability rates of the cells were determined by confocal microscopy. The majority of the newly synthesized compounds, with the exception of compounds **2**, **3**, **8**, **11**, **12**, and **17**, displayed high cytotoxic activity against cancerous cells. In fact, compounds **6** and **7** exhibited better results than the positive control drugs, cisplatin and busulfan, against the MDA-MB-231 cells. Therefore, compounds **6** and **7** could be considered as anticancer drug candidates for further development.

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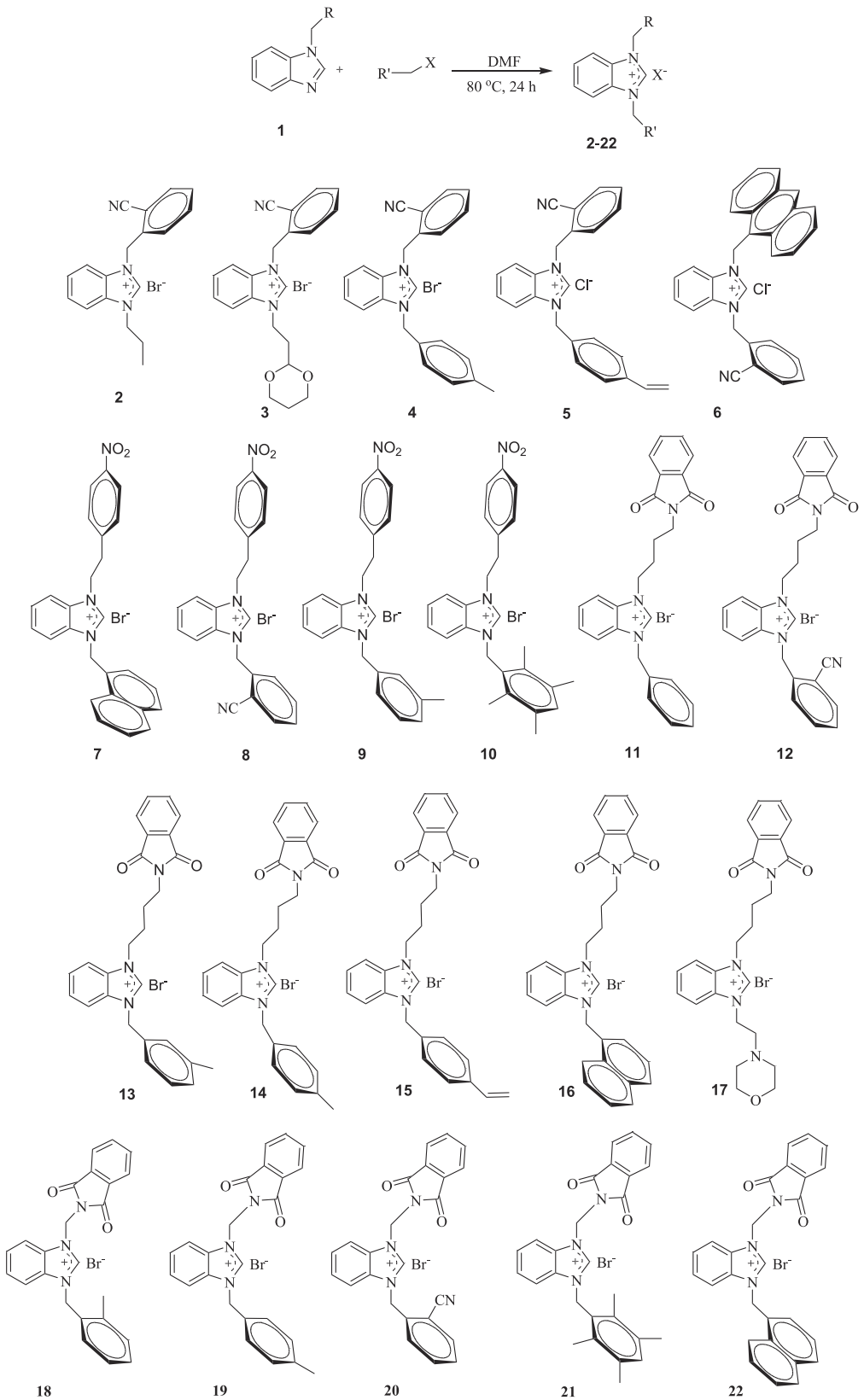
INTRODUCTION

Colon and breast cancers were two of the four major cancers by age and sex group in America for 2016 [1]. The number of deaths from colon cancer is higher than that from breast cancer, and deaths from colon and rectum cancers are almost the same for women (23,170) and men (26,020). Due to the high mortality rates from colon, breast, and other cancer types, there is an ever-increasing need to develop more effective drug therapies, and many different types compounds have been synthesized for this purpose. For instance, Asif et al. [2] prepared two Ag-NHC complexes, which include a heterocyclic group, and screened them against HCT 116, HT-29, MCF-7, MDA-MB-231, PANC-1, SHSY5Y, U-937 human cancer cells, and 3T3 L-1 mouse cancer cells.

Heterocyclic compounds including an imidazole or benzimidazole nucleus have shown different types of biological activity such as antimicrobial, antifungal, anthelmintic, anti-allergic, antihistamine, analgesic, antineoplastic, anti-inflammatory, hypotensive, vasodilator, antipyretic, and antinematodal [3–9]. They also exhibit cytotoxic effects against various cancerous cells including the brain, pancreas, leukemia, ovaries, cervix, lymphocyte, prostate, colon, breast, lung, liver, bone marrow, and peripheral blood [2,10–24]. Due to their anticancer activity, many research groups have been involved in developing new heterocyclic compounds. For example, Shelton's group improved a series of *N*,*N*′-bis(arylmethyl)benzimidazolium salts as antitumor agents against NCl-H460, NCl-H1975, HCC827, and A549 cell lines [25]. In 2015, Haque et al. [11] reported benzimidazole-based *N*-heterocyclic carbene (NHC) proligands and their Ag complexes in human derived colorectal adenocarcinoma (HT29) and colorectal cancer (HCT 116) cell lines. 3,3′-(1,4-phenylenebis(methylene)) bis(1-alkyl-benzimidazolium) salts and their silver complexes were developed by Iqbal et al. and tested their cytotoxicity against HCT 116. The IC_{50} values of compounds for this cell line ranged between 0.03 and 65.9 μM. However, dinuclear Ag(I)-NHC complexes including benzimidazole nucleus was found to be more active against HCT 116 than benzimidazolium salts and 5-fluorouracil, which is standard positive control drug [26].

With the aim of developing effective, safe, and affordable anticancer drug candidates against cancerous colon and breast cell lines, benzimidazolium salts (**2**–**22**) were designed, synthesized, and structurally characterized in this study.

Scheme 1. Synthesis and chemical structures of the benzimidazolium salts.



RESULTS AND DISCUSSION

Synthesis and spectral characterization of benzimidazolium salts. Benzimidazolium salts **2**–**22** were prepared in yields ranging from 35 to 93% by the successive reaction of *N*-alkylbenzimidazole with various alkyl or aryl halides in DMF (Scheme 1). After the recrystallization of products **2**–**22** in suitable solvents, pure forms of the salts were acquired and thoroughly characterized by NMR, Fourier transform infrared, high resolution mass spectrometry (HRMS) (for **8**), and elemental analysis (for **2** and **9**). The NMR (^{1}H and ^{13}C) spectra of the salts were recorded in chloroform ($CDCl_3$) or dimethyl sulfoxide (DMSO-d_6) solvents at room temperature. The compounds were easily soluble in polar organic solvent DMSO; however, they were insoluble in the nonpolar solvent diethyl ether.

In the ^{1}H-NMR spectra, the resonance of the NC*H*N proton signals belonging to the benzimidazolium salts were found as singlets in the downfield region compared to the other proton signals at δ 9.95, 11.61, 11.80, 10.08, 9.17, 11.23, 11.56, 9.84, 10.23, 11.69, 11.56, 11.54, 11.56, 11.55, 11.50, and 11.10 ppm for **2**–**17**, respectively. The methylene proton resonances belonging to $NCH_2C_6H_4$(CN)-2, which is a linker between the phenyl groups and the nitrogen atoms of the benzimidazole ring, were observed as singlets at δ 6.04, 6.20, 6.17, 5.82, 5.86, 5.99, and 6.17 ppm for **2**–**6**, **8**, and **12**, respectively. Moreover, the resonances of the aromatic protons were determined as multiplets in the low region at δ 7.02–8.92 ppm for **2**–**17**. The important features of **2**–**17** are given in Table 1.

In the ^{13}C-NMR spectra, the characteristic resonance peaks belonging to the C2 (N*C*HN) atom were found in the downfield region at δ 143.60, 143.81, 144.26, 143.46, 142.93, 143.42, 143.03, 142.78, 142.23, 143.15, 143.44, 143.01, 142.77, 142.99, 143.59, and 143.16 ppm for **2**–**17**, respectively. Furthermore, the aromatic carbon peaks were observed in the range δ 98.98–147.15 ppm. The formation of the desired compounds was confirmed by IR, which showed the presence of stretching bands for the C=N vibrations on the benzimidazole ring at 1559.8, 1566.8, 1557.8, 1552.5, 1553.9, 1510.7, 1514.9, 1514.6, 1519.7, 1553.4, 1562.9, 1557.7, 1544.9, 1557.6, 1560.4, and 1564.8 cm^{-1}, for compounds **2**–**17**, respectively. The structure of compound **8** was further characterized by HRMS. The calculated value (383.42 *m/z*) and the found value (383.15 *m/z*) are quite close to each other. Compounds **2** and **9** were also verified by elemental analysis.

Cytotoxic activity of synthesized new compounds. The cytotoxic activity of compounds **2**–**22** was evaluated at different concentrations ranging from 0.5 to 200 μM against DLD-1 and MDA-MB-231 cells and at concentrations ranging from 0.001 to 100 μM against HEK-293T cells by using the MTT assay method. In this work, cisplatin and busulfan were used as positive control drugs for comparison purposes with the drug candidates (**2**–**22**) under the same experimental conditions. After the cells were exposed to compounds **2**–**22** for an incubation time of 72 h (for DLD-1 and MDA-MB-231) or 24 h (for HEK-293T), cytotoxic activity was expressed as IC_{50} values and are given in Table 2.

The variety in the IC_{50} values showed that the substituents at the N^1 and/or N^3 position have a direct impact on the *in vitro* cytotoxic activity. All the synthesized compounds showed evidence of enhanced cytotoxic activity against DLD-1 (except **2**, **3**, **8**, **12**, and **17**) and MDA-MB-231 (except **3**, **11**, **12**, and **17**) cells. Also, compounds **5**, **6**, and **10** showed moderate activity against both cancerous cell lines. Compound **5**,

Table 1

Characterization data of the benzimidazolium salts obtained via ^{1}H-NMR, ^{13}C-NMR, and IR.

Benzimidazolium salt	Solvent	δ_H (N*H*C=N) (ppm)	δ_C (NHC=N) (ppm)	$IR_{C=N}$ (cm^{-1})
2	DMSO-d_6	9.95	143.60	1559.8
3	$CDCl_3$	11.61	143.81	1566.8
4	$CDCl_3$	11.80	144.26	1557.8
5	DMSO-d_6	10.08	143.46	1552.5
6	DMSO-d_6	9.17	142.93	1553.9
7	$CDCl_3$	11.23	143.42	1510.7
8	$CDCl_3$	11.56	143.03	1514.9
9	DMSO-d_6	9.84	142.78	1514.6
10	$CDCl_3$	10.23	142.23	1519.7
11	$CDCl_3$	11.69	143.15	1553.4
12	$CDCl_3$	11.56	143.44	1562.9
13	$CDCl_3$	11.54	143.01	1557.7
14	$CDCl_3$	11.56	142.77	1544.9
15	$CDCl_3$	11.55	142.99	1557.6
16	$CDCl_3$	11.50	143.59	1560.4
17	$CDCl_3$	11.10	143.16	1564.8

Table 2

IC_{50} results for the benzimidazolium salts against the MDA-MB-231 and DLD-1 cancerous cell lines and HEK-293T noncancerous cell line.

Compound	IC_{50} (μM)		
	DLD-1	MDA-MB-231	HEK-293T
2	>200	120.96 ± 5.69	>100
3	196.50 ± 3.48	>200	>100
4	52.47 ± 6.66	44.46 ± 5.73	>100
5	31.01 ± 5.42	27.37 ± 1.62	92.71 ± 3.59
6	10.98 ± 2.33	1.26 ± 0.85	16.22 ± 4.20
7	54.40 ± 4.25	2.01 ± 0.96	N.T.
8	>200	130.16 ± 6.09	>100
9	35.08 ± 0.49	82.17 ± 5.43	N.T.
10	24.98 ± 5.44	31.69 ± 3.69	N.T.
11	142.25 ± 4.94	>200	>100
12	>200	>200	>100
13	74.45 ± 3.59	53.35 ± 2.05	48.48 ± 4.25
14	100.31 ± 2.74	37.43 ± 4.38	N.T.
15	59.37 ± 3.61	105.95 ± 4.84	N.T.
16	53.53 ± 0.43	70.60 ± 4.17	>100
17	>200	>200	>100
18	140.05 ± 3.79	180.05 ± 6.21	N.T.
19	155.60 ± 4.61	133.70 ± 2.48	N.T.
20	41.96 ± 0.35	173.4 ± 3.8	>100
21	95.02 ± 5.06	135.02 ± 2.28	N.T.
22	41.05 ± 1.39	29.83 ± 6.61	87.81
Cisplatin	4.38 ± 0.05	5.77 ± 0.4	33.97 ± 2.44
Busulfan	173.20 ± 5.4	>200	>100

N.T., not tested.

which two aromatic groups, showed an IC_{50} value of 31.01 ± 5.42 μM against DLD-1 and 27.37 ± 1.62 μM against MDA-MB-231 cells. While compounds **2** (which includes one 2-cyanobenzyl and one propyl group) and **8** (which includes one 2-(4-nitrophenyl)ethyl and one 2-cyanobenzyl group) were inactive against DLD-1 with IC_{50} values of >200, they also had a minimal effect against MDA-MB-231 with IC_{50} values of 120.96 ± 5.69 and 130.16 ± 6.09 μM, respectively.

It is noteworthy that **7**, which includes a naphthalen-1-ylmethyl and an 2-(4-nitrophenyl)ethyl substituent, was more effective than the other compounds against MDA-MB-231 with an IC_{50} value of 2.01 ± 0.96 μM. Furthermore, compound **7** showed roughly three times more *in vitro* cytotoxic activity than the well-known chemotherapeutic agent cisplatin against MDA-MB-231 cells. While cisplatin had IC_{50} values of 5.77 ± 0.4 μM (for MDA-MB-231), compound **7** exhibited IC_{50} values of 2.01 ± 0.96 μM (for MDA-MB-231). However, drug candidate **7** was slightly less active against the colon cancer cells compared with the breast cancer cells. Additionally, compound **6** exhibited higher cytotoxic activity against MDA-MB-231 cell line with low IC_{50} values (2.761 ± 0.43 μM) compared to both positive control drugs. The *in vivo* study of these compounds (**6** and **7**) will be performed in the future.

Compounds **11–17** which all include a 4-(1,3-dioxoisoindolin-2-yl)butyl group exhibited lower cytotoxic activity compared to compounds **4–7** and **10**. Particularly, compounds **12** (including 2-cyanobenzyl group) and **17** (including 2-morpholinoethyl group) showed no activity against either cancerous cell lines.

Ghdhayeb et al. [27] have reported that the cytotoxic activity of *n*-butyl and ally substituted benzimidazolium salts and their silver and palladium complexes against human cancerous colon cells (HCT 116). According to their study, benzimidazolium salts exhibited no activity while their silver and palladium complexes showed anticancer activity [27]. However, in this study, almost all the benzimidazolium salts (except **2**, **3**, **8**, **12**, and **17** against DLD-1) had cytotoxic activity against the cancerous cell lines. A plot of drug's effectiveness as a function of concentration on the MDA-MB-231 and DLD-1 cells after 72 h is depicted in Figures1 and 2, respectively.

The cytotoxicity of the new compounds on the MDA-MB-231 (Fig. 1) and DLD-1 cell lines (Fig. 2) was seen to change depending on the dose. For example, MDA-MB-231 cell viability was shown to be around 5% when using 200 μM of compound **7** while it was around 75% when using 0.5 μM of salt **7**. The big differences in cell viability due to concentration were also valid when other sample doses were used.

Apohan et al. have evaluated the cytotoxic activity of 14 zinc (II) and cobalt (II) complexes including those that contain a benzimidazole nucleus against a lung cancer cell line (A549) and a BEAS-2B cell line over various time periods (24, 48, and 72 h). They reported the IC_{50} values for three complexes ranging between 1.87 and 1.97 μM at 72 h against A549 cell line [28]. In other words, these benzimidazole-based compounds exhibited better cytotoxic activity than cisplatin. However, they were less cytotoxic against BEAS-2B cells than cisplatin.

In another study, Cheong et al. [22] evaluated the anticancer activity of water soluble benzimidazole-based organic compounds against the following three cancer cell lines: PC3MLN4, PC3, and A549. They reported that nearly all of the compounds exhibited low cytotoxicity against the cell lines.

Confocal microscopy images. The photographs of the DLD-1 and MDA-MB-231 cells, and cells treated with test samples and positive control drugs (cisplatin and busulfan), are depicted in Figure 3A and 3B, respectively.

The control group images of the DLD-1 and MDA-MB-231 cells demonstrate completely confluent growth. However, the cellular images show that the normal morphology of the cells treated with benzimidazolium salts changed, and the cells lost their viability compared to the control. Therefore, the benzimidazolium salts have a noteworthy effect on the viability of cells.

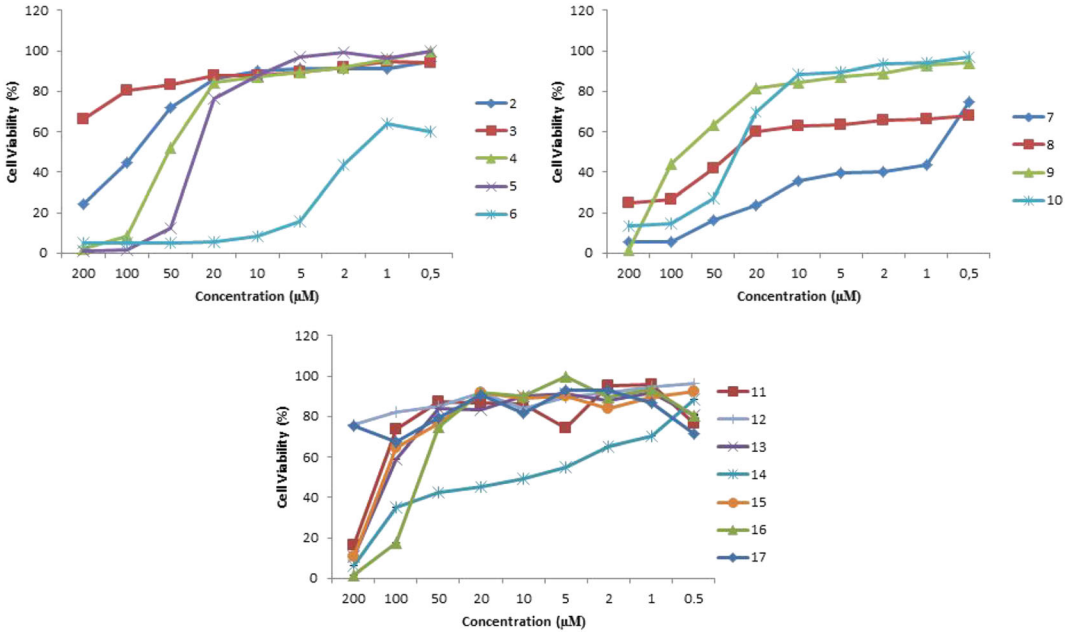


Figure 1. Cell viability of MDA-MB-231 cells treated with the drug candidates. [Color figure can be viewed at wileyonlinelibrary.com]

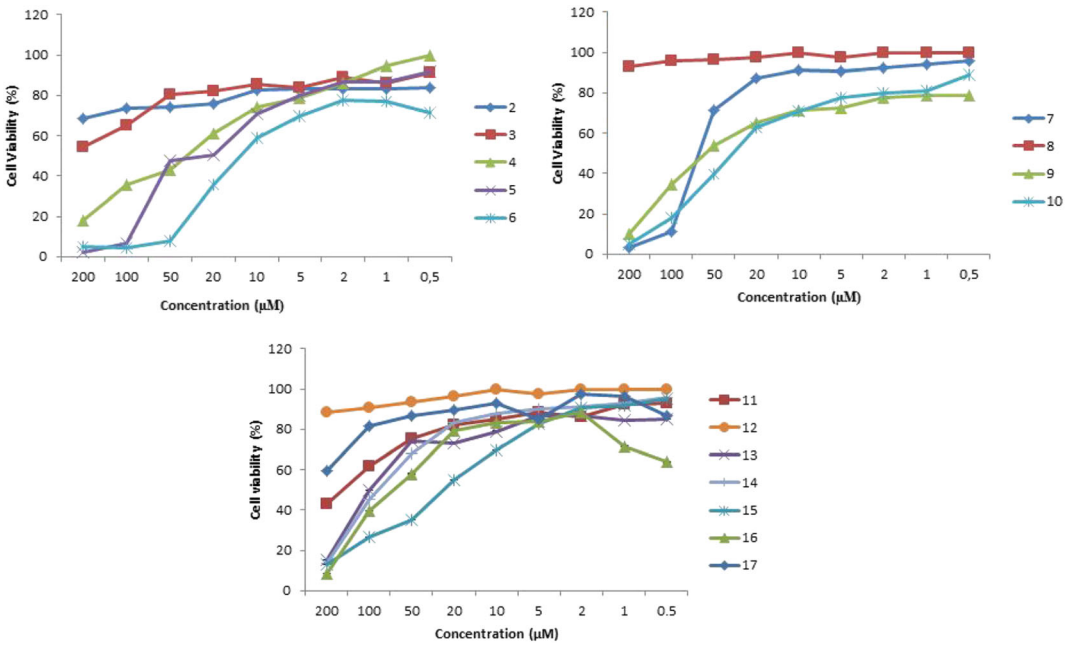


Figure 2. Cell viability of DLD-1 cells treated with the drug candidates. [Color figure can be viewed at wileyonlinelibrary.com]

Also, the positive control drug (cisplatin) affected the population of the DLD-1 cells, and the viability of cells treated with cisplatin decreased significantly. However, the morphology of both kinds of cell types treated with busulfan protected their cell viability. The images of the DLD-1 cells treated with **10** showed that more cells lost their viability compared with drug candidates **4** and **5**. The images of the MDA-MB-231 cells after exposure to **9** at 20 μM show that these cells did not survive. The anticancer potential of this compound was more potent against breast cancer than compound **2**.

MATERIALS AND METHODS

Reagents and solvents for the synthesis of new compounds. The chemicals 1,2-phenylenediamine,

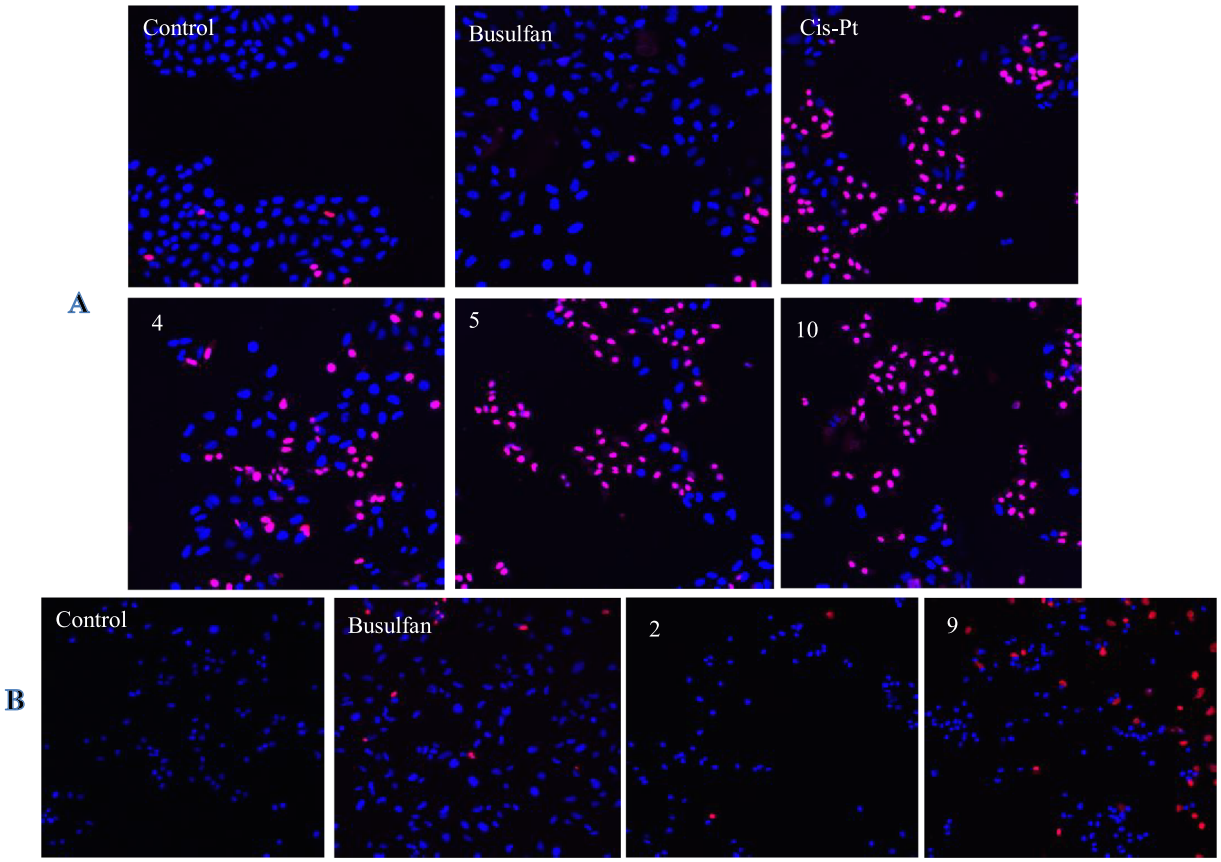


Figure 3. Confocal images of DLD-1 (A) and MDA-MB-231 (B) cells, (A) after treatment with the synthesized compounds (20 μM) for 24 h incubation, and (B) the cells stained with Hoechst (blue) and PI (red). [Color figure can be viewed at wileyonlinelibrary.com]

1-bromopropan, 2-(bromomethyl)benzonitrile, 10-(chloro methyl)anthracene, 2-(2-bromoethyl)-1,3-dioxane, benzyl chloride, 3-methylbenzylchloride, 4-methylbenzylchlo ride, 4-vinylbenzyl chloride, 1-(chloromethyl)naphtha lene, 1-(2-bromoethyl)-4-nitrobenzene, 2,3,5,6-tetramethy lbenzylchloride, *N*-(bromomethyl)phthalimide, *N*-(4-bro mobutyl)phthalimide, *N*-(2-chloroethyl)-morpholinium chloride, and *N*,*N*-dimethylformamide (DMF), were bought from chemical companies such as Sigma-Aldrich (Interlab A.S., USA), Merck (Darmstadt, Germany), or Scharlau (Barcelona, Spain).

Cell culture. For the cell culture studies Dulbecco's modified Eagle's medium, fetal bovine serum, GlutaMAX, trypsin-EDTA, phosphate-buffered saline, busulfan, cis-diammineplatinum (II) dichloride, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), Hoechst 33258, pentahydrate (bis-benzimide), propidium iodide (PI), and laboratory materials such as tissue culture flasks and 96-well plates were purchased from Gibco (Life Technologies, USA), Medicago (Astral Scientific, Sweden), Lonza (Arch Chemicals, Inc., North Sydney, Australia), Thermo Fisher Scientific (Molecular Probes by Life Technologies, Australia), Jet Biofil (Pathtech, China), Fluka, Nalgene, or Sigma (Sigma-Aldrich, USA). Cell lines were donated by Dr Fanfan Zhou and Dr Binh Pham of the University of Sydney.

Instrumentation. The following instrumentation was used in this study: a Bruker 300 or 400 MHz Ultra Shield NMR, an electrothermal-9200 melting point apparatus, a Shimadzu Fourier transform infrared 8400 spectrophotometer, a HRMS device, an Invitrogen Countess automated cell counter, and an Olympus FV1000 confocal microscope [12,18].

CONCLUSION

Sixteen new and five known benzimidazolium salts were synthesized and extensively characterized using various spectroscopic techniques. The anticancer properties of the salts **2–22** were tested against DLD-1, MDA-MB-231, and HEK-293T cell lines by the MTT assay method and compared with the known anticancer drugs cisplatin and busulfan. Our results demonstrate that benzimidazolium salts had *in vitro* anticancer activity. Specifically, compounds **6** and **7** exhibited promising results against MDA-MB-231 cell line that warrant further investigation as a potential anticancer agents, even for other cell lines, such as Hela, PC-3,

BEL-7404, A549, and HL7702. Furthermore, the low IC_{50} value of compound **6** indicates its potential as a drug candidate for DLD-1. In conclusion, our results may be useful in designing new compounds as anticancer agents in the future.

EXPERIMENTAL

Synthesis of new benzimidazolium salts. Compounds **2**–**22** were prepared according to previously published procedures [29–31]. Benzimidazole (1 mmol) was dissolved in 20 mL of ethanol. KOH (1.5 mmol) was added, and the mixture was stirred for 1 h at room temperature. Alkyl halide (1 mmol) was added to this mixture and was refluxed for 6 h. Subsequently, it was filtered to remove the potassium chloride that was formed, and pure *N*-alkylbenzimidazole was obtained. The solvent was removed under vacuum. This intermediate product was washed several times with diethyl ether. Alkyl halide (1 mmol) and *N*-alkylbenzimidazole (1 mmol) were then added to a new 100 mL of Schlenk tube with 4 mL of dried DMF. The mixture was allowed to react further for 24 h at 80°C, before the final product was purified by crystallization with suitable solvent or solvent mixtures.

1-(2-Cyanobenzyl)-3-propyl-1H-benzo[d]imidazol-3-ium bromide (2). 1-Bromopropan (0.46 g, 1 mmol) was slowly added to the DMF solution of synthesized 2-((1*H*-benzo[*d*] imidazol-1-yl)methyl)benzonitrile (0.868 g, 1 mmol) in the previous step 2.4 and was stirred for 24 h at 80°C. DMF was aspirated from the reaction medium under vacuum after the reaction was completed. With this method, compound **2** was synthesized in a moderate yield, and the final product was purified by crystallization in a mixture of ethyl alcohol-diethyl ether (2:1). Yield: 51%, m.p.: 146–147°C, color: white. IR: 1559.8 (C═N); 2967.6 and 3356.9 cm^{-1} (C-H). ^{1}H-NMR (400.13 MHz, DMSO-d_6, 298 K), δ: 0.94 [t, *J*: 4.0 Hz, 3 H, $NCH_2CH_2CH_3$]; 1.95 [h, *J*: 8.0 Hz, 2 H, $NCH_2CH_2CH_3$]; 4.54 [t, *J*: 12.0 Hz, 2 H, $NCH_2CH_2CH_3$]; 6.04 [s, 2 H, $NCH_2C_6H_4$(CN)-2]; 7.49–8.19 (m, 8 H, Ar-*H*); 9.95 (s, 1 H, NC*H*N). ^{13}C-NMR (100.13 MHz, DMSO-d_6, 298 K), δ: 11.1 ($NCH_2CH_2CH_3$); 22.5 ($NCH_2CH_2CH_3$); 48.7 ($NCH_2CH_2CH_3$); 48.9 [$NCH_2C_6H_4$(CN)-2]; 111.2, 114.1, 114.4, 114.5, 117.3, 127.2, 127.4, 129.3, 129.6, 130.0, 131.5, 131.7, 134.3, 134.4, and 137.4 (Ar-*C*; *C*≡N); 143.6 (N*C*HN). Elemental analysis $C_{18}H_{18}N_3Br$ (356.26 g/mol) (%): Found C: 60.54; H: 4.93; N: 11.37. *Anal*. Calcd C: 60.68; H: 5.09; N: 11.79.

1-(2-Cyanobenzyl)-3-(1,3-dioxane-2-yl)ethyl-1H-benzo[d]imidazol-3-ium bromide (3). Compound **3** (428.32 g/mol) was synthesized from 2-((1*H*-benzo[*d*]imidazol-1-yl) methyl)benzonitrile (1 mmol) and 2-(2-bromoethyl)-1,3-dioxane (1 mmol) under the same conditions and procedure as for **2**. The final product was crystallized in ethyl alcohol. Yield: 45%, color: white. IR: 1140.0 (C-O); 1566.8 (C═N); 2218.9 (C≡N); 2854.7, 2931.6 and 3008.5 cm^{-1} (C-H). ^{1}H-NMR (400.13 MHz, $CDCl_3$, 298 K), δ: 1.92 (p, *J*: 4.0 Hz, 2 H, $OCH_2CH_2CH_2O$); 2.35 (q, *J*: 4.0 Hz, 2 H, $NCH_2CH_2CHO_2$); 3.76 and 3.95 (dd, *J*: 8.0 Hz, *J*: 12.0 Hz, 4 H, $OCH_2CH_2CH_2O$); 4.78 (t, *J*: 8.0 Hz, 2 H, $NCH_2CH_2CHO_2$); 6.20 [s, 2 H, $NCH_2C_6H_4$(CN)-2]; 7.28–8.17 (m, 8 H, Ar-*H*); 11.61 (s, 1 H, NC*H*N). ^{13}C-NMR (100.13 MHz, $CDCl_3$, 298 K), δ: 20.40 ($OCH_2CH_2CH_2O$); 25.35 ($NCH_2CH_2CHO_2$); 33.83 ($OCH_2CH_2CH_2O$); 42.69 ($NCH_2CH_2CHO_2$); 48.40 ($NCH_2CH_2CHO_2$); 66.79 [$NCH_2C_6H_4$(CN)-2]; 98.98, 112.09, 113.25, 113.43, 117.26, 127.21, 127.43, 129.95, 130.93, 131.12, 131.32, 133.19, 134.36 and 136.16 (Ar-*C*; *C*≡N); 143.81 (N*C*HN).

1-(2-Cyanobenzyl)-3-(4-methylbenzyl)-1H-benzo[d]imidazol-3-ium bromide (4). Compound **4** (418.33 g/mol) was synthesized from 1-(4-methylbenzyl)benzimidazole (1 g, 1 mmol) and 2-(bromomethyl)benzonitrile (0.88 g, 1 mmol) under the same conditions and procedure as for **2**. The final product was crystallized in ethyl alcohol. Yield: 74%, m.p.: 200–201°C, color: white. IR: 1557.8 (C═N); 2226.4 (C≡N); 2927.7 and 3355.2 cm^{-1} (C-H). ^{1}H-NMR (400.13 MHz, $CDCl_3$, 298 K), δ: 2.31 [s, 3 H, $NCH_2C_6H_4(CH_3)$-4]; 5.82 [s, 2 H, $NCH_2C_6H_4(CH_3)$-4]; 6.17 [s, 2 H, $NCH_2C_6H_4$(CN)-2]; 7.16–8.21 (m, 12 H, Ar-*H*); 11.80 (s, 1 H, NC*H*N). ^{13}C-NMR (100.13 MHz, $CDCl_3$, 298 K), δ: 21.19 [$NCH_2C_6H_4(CH_3)$-4]; 51.78 [$NCH_2C_6H_4$(CN)-2]; 58.32 [$NCH_2C_6H_4(CH_3)$-4]; 111.99, 113.46, 113.92, 117.23, 127.34, 127.81, 128.34, 129.24, 130.01, 130.98, 131.13, 131.22, 133.25, 133.30, 134.50, 135.57, 135.85, 139.41 and 143.59 (Ar-*C*; *C*≡N); 144.26 (N*C*HN).

1-(2-Cyanobenzyl)-3-(4-vinylbenzyl)-1H-benzo[d]imidazol-3-ium chloride (5). Compound **5** (385.89 g/mol) was synthesized from 2-((1*H*-benzo[*d*]imidazol-1-yl)methyl) benzonitrile (1 g, 1 mmol) and 4-vinylbenzyl chloride (0.65 g, 1 mmol) according to the same conditions and procedure as for **2**. The final product was crystallized in ethyl alcohol. Yield: 93%, m.p.: 172–173°C, color: white. IR: 1552.5 (C═N); 2224.1 (C≡N); 2935.4, 3069.2, 3132.8, and 3357.7 cm^{-1} (C-H). ^{1}H-NMR (300.13 MHz, DMSO-d_6, 298 K), δ: 5.82 [s, 2 H, $NCH_2C_6H_4$(CN)-2]; 5.89 (d, *J*: 8.0 Hz, 2 H, $NCH_2C_6H_4CHCH_2$); 6.06 (s, 2 H, $NCH_2C_6H_4CHCH_2$); 6.69–6.78 (m, 1 H, $NCH_2C_6H_4CHCH_2$); 7.53–8.01 (m, 12 H, Ar-*H*); 10.08 (s, 1 H, NC*H*N). ^{13}C-NMR (75.47 MHz, DMSO-d_6, 298 K), δ: 48.58 ($NCH_2C_6H_4CHCH_2$); 49.86 [$NCH_2C_6H_4$(CN)-2]; 110.83, 113.75, 114.17, 115.32, 116.92, 126.62, 126.94, 127.07, 128.72, 129.16, 129.60, 130.91, 131.31, 133.08, 133.83, 134.02, 135.86, 136.83, and 137.55 (Ar-*C*; *C*≡N; *C*H═*C*H_2); 143.46 (N*C*HN).

1-(Anthracen-10-ylmethyl)-3-(2-cyanobenzyl)-1H-benzo[d] imidazol-3-ium chloride (6). Compound **6** (459.97 g/mol) was synthesized from 2-((1*H*-benzo[*d*]imidazol-1-yl) methyl)benzonitrile (0.90 g, 1 mmol) and 10-(chloro methyl)antracene (0.88 g, 1 mmol) according to the same conditions and procedure as for **2**. The final product was crystallized in ethyl alcohol-diethyl ether (2:1) mixture. Yield: 35%, m.p.: 227–228°C, color: yellow. IR: 1553.9 (C═N); 2216.5 (C≡N); 2940.2 and 3133.9 cm^{-1} (C-H). ^{1}H-NMR (400.13 MHz, DMSO-d_6, 298 K), δ: 5.86 [s, 2 H, NCH_2C$_6$H$_4$(CN)-2]; 6.79 (s, 2 H, NCH_2C$_{14}$H$_9$]; 7.07–8.92 (m, 17 H, Ar-*H*); 9.17 (s, 1 H, NC*H*N). ^{13}C-NMR (100.13 MHz, DMSO-d_6, 298 K), δ: 48.67 [N*C*H$_2$C$_6$H$_4$(CN)-2]; 56.47 [N*C*H$_2$C$_{14}$H$_9$]; 110.67, 114.17, 115.06, 117.17, 122.19, 123.94, 126.13, 127.49, 127.81, 127.97, 128.33, 129.62, 129.90, 131.01, 131.50, 131.61, 131.89, 132.32, 134.07, 134.25, and 137.97 (Ar-*C*); 142.93 (N*C*HN).

1-(Naphthalen-1-ylmethyl)-3-[2-(4-nitrophenyl)ethyl]-1H-benzo[d]imidazol-3-ium bromide (7). Compound **7** (488.38 g/mol) was synthesized from 1-(naphthalen-1-ylmethyl)benzimidazole (1.04 g, 1 mmol) and 1-(2-bromoethyl)-4-nitrobenzene (0.93 g, 1 mmol) according to the same conditions and procedure as for **2**. The final product was crystallized in a mixture of ethyl alcohol-diethyl ether (2:1). Yield: 53%, m.p.: 149–150°C, color: cream. IR: 1510.7 (C═N); 1559.1 (NO$_2$); 2980.4, 3350.7, and 3441.9 cm^{-1} (C-H). ^{1}H-NMR (400.13 MHz, CDCl$_3$, 298 K), δ: 3.58 [t, *J*: 8.0 Hz, 2 H, NCH$_2$CH_2C$_6$H$_4$(NO$_2$)-4]; 5.03 [t, *J*: 4.0 Hz, 2 H, NCH_2CH$_2$C$_6$H$_4$(NO$_2$)-4]; 6.19 (s, 2 H, NCH_2C$_{10}$H$_7$); 7.41–8.02 (m, 15 H, Ar-*H*); 11.23 (s, 1 H, NC*H*N). ^{13}C-NMR (100.13 MHz, CDCl$_3$, 298 K), δ: 35.04 [NCH$_2$*C*H$_2$C$_6$H$_4$(NO$_2$)-4]; 47.87 (N*C*H$_2$C$_{10}$H$_7$); 49.38 [N*C*H$_2$CH$_2$C$_6$H$_4$(NO$_2$)-4]; 112.75, 113.79, 122.00, 123.97, 125.39, 126.65, 127.36, 127.48, 127.76, 127.80, 129.41, 129.95, 130.40, 130.56, 131.20, 133.86, 143.24, 143.42, and 147.11 (Ar-*C* and N*C*HN).

1-(2-Cyanobenzyl)-3-[2-(4-nitrophenyl)ethyl]-1H-benzo[d] imidazol-3-ium bromide (8). Compound **8** (463.33 g/mol) was synthesized from 2-((1*H*-benzo[*d*]imidazol-1-yl) methyl)benzonitrile (1.0 g, 1 mmol) and 1-(2-bromo ethyl)-4-nitrobenzene (0.99 g, 1 mmol) according to the same conditions and procedure as for **2**. The final product was crystallized in dichloromethane. Yield: 87%, m.p.: 237–238°C, color: white. IR: 1514.9 (C═N); 1601.7 (NO$_2$); 2223.9 (C≡N); 2943.2, 3025.6, 3222.7, 3385.9, and 3446.4 cm^{-1} (C-H). ^{1}H-NMR (400.13 MHz, CDCl$_3$, 298 K), δ: 3.62 [t, *J*: 8.0 Hz, 2 H, NCH$_2$CH_2C$_6$H$_4$NO$_2$]; 5.01 [t, *J*: 4.0 Hz, 2 H, NCH_2CH$_2$C$_6$H$_4$NO$_2$]; 5.99 [s, 2 H, NCH_2C$_6$H$_4$(CN)-2]; 7.50–8.11 (m, 12 H, Ar-*H*); 11.56 (s, 1 H, NC*H*N). ^{13}C-NMR (100.13 MHz, CDCl$_3$, 298 K), δ: 35.01 (NCH$_2$*C*H$_2$C$_6$H$_4$NO$_2$); 48.01 [N*C*H$_2$C$_6$H$_4$(CN)-2]; 48.85 (N*C*H$_2$CH$_2$C$_6$H$_4$NO$_2$); 112.04, 112.58, 113.70, 117.07, 124.18, 127.73, 127.96, 130.07, 130.44, 130.92, 131.38, 133.42, 134.59, 135.34, 143.03, and 143.58 (Ar-*C*; *C*≡N; N*C*HN). HRMS [L-Br]$^+$ calcd for C$_{23}$H$_{19}$N$_4$O$_2$: 383.42, found *m/z*: 383.15.

1-(3-Methylbenzyl)-3-[2-(4-nitrophenyl)ethyl]-1H-benzo[d] imidazol-3-ium bromide (9). Compound **9** (452.34 g/mol) was synthesized from 1-(3-methylbenzyl)benzimidazole (1.0 g, 1 mmol) and 1-(2-bromoethyl)-4-nitrobenzene (1.04 g, 1 mmol) according to the same conditions and procedure as for **2**. The final product was crystallized in a mixture of ethyl alcohol-diethyl ether (2:1). Yield: 60%, m.p.: 144–145°C, color: cream. IR: 1514.6 (C═N); 1597.4 (NO$_2$); 2948.0 and 3379.0 cm^{-1} (C-H). ^{1}H-NMR (400.13 MHz, DMSO-d_6, 298 K), δ: 2.27 [s, 3 H, NCH$_2$C$_6$H$_4$(CH_3)-3]; 3.43 (t, *J*: 12.0 Hz, 2 H, NCH$_2$CH_2C$_6$H$_4$NO$_2$); 4.89 (t, *J*: 8.0 Hz, 2 H, NCH_2CH$_2$C$_6$H$_4$NO$_2$); 5.68 [s, 2 H, NCH_2C$_6$H$_4$(CH$_3$)-3]; 7.11–8.16 (m, 12 H, Ar-*H*); 9.84 (s, 1 H, NC*H*N). ^{13}C-NMR (100.13 MHz, DMSO-d_6, 298 K), δ: 21.34 [NCH$_2$C$_6$H$_4$(*C*H$_3$)-3]; 34.50 (NCH$_2$*C*H$_2$C$_6$H$_4$NO$_2$); 47.71 [N*C*H$_2$C$_6$H$_4$(CH$_3$)-3]; 50.18 (N*C*H$_2$CH$_2$C$_6$H$_4$NO$_2$); 114.38, 114.48, 124.08, 125.62, 127.20, 127.28, 129.20, 129.21, 129.83, 130.78, 131.17, 131.47, 134.29, 138.74, 145.64, and 146.93 (Ar-*C*); 142.78 (N*C*HN). Elemental analysis C$_{23}$H$_{22}$N$_3$O$_2$Br (452.34 g/mol) (%): Found C: 59.67; H: 5.24; N: 8.73. *Anal*. Calcd C: 61.07; H: 4.90; N: 9.29.

1-[2-(4-Nitrophenyl)ethyl]-3-(2,3,5,6-tetramethylbenzyl)-1H-benzo[d]imidazol-3-ium bromide (10). Compound **10** (494.42 g/mol) was synthesized from 1-(2,3,5,6-tetramethylbenzyl)benzimidazole (0.9 g, 1 mmol) and 1-(2-bromoethyl)-4-nitrobenzene (0.78 g, 1 mmol) according to the same conditions and procedure as for **2** [32]. The final product was crystallized in ethyl alcohol. Yield: 45%, m.p.: 212–214°C, color: white. IR: 1519.7 (C═N); 1601.1 cm^{-1} (NO$_2$). ^{1}H-NMR (300.13 MHz, CDCl$_3$, 298 K), δ: 2.06 and 2.19 [s, 12 H, NCH$_2$C$_6$H(CH_3)$_4$–2,3,5,6]; 3.44 [t, *J*: 7.2 Hz, 2 H, NCH$_2$CH_2C$_6$H$_4$(NO$_2$)-4]; 5.09 [t, *J*: 7.2 Hz, 2 H, NCH_2CH$_2$C$_6$H$_4$(NO$_2$)-4]; 5.58 [s, 2 H, NCH_2C$_6$H(CH$_3$)$_4$–2,3,5,6]; 7.02–7.98 (m, 9 H, Ar-*H*); 10.23 (s, 1 H, NC*H*N). ^{13}C-NMR (75.47 MHz, CDCl$_3$, 298 K), δ: 16.07 and 20.53 [NCH$_2$C$_6$H(*C*H$_3$)$_4$–2,3,5,6]; 35.07 [NCH$_2$*C*H$_2$C$_6$H$_4$(NO$_2$)-4]; 47.21 [N*C*H$_2$C$_6$H(CH$_3$)$_4$–2,3,5,6]; 47.81 [N*C*H$_2$CH$_2$C$_6$H$_4$(NO$_2$)-4]; 113.09, 113.45, 123.91, 126.97, 127.47, 130.18, 131.20, 131.37, 133.87, 133.92, 135.37, 143.52, and 147.15 (Ar-*C*); 142.23 (N*C*HN).

1-Benzyl-3-[4-(1,3-dioxoisoindolin-2-yl)butyl]-1H-benzo[d] imidazol-3-ium bromide (11). According to the same conditions and procedure as for **2**, compound **11** was synthesized from 1-benzyl-1*H*-benzo[*d*]midazole (0.7 g, 1 mmol) and *N*-(4-bromobutyl)phthalimide (0.95 g, 1 mmol) in DMF for 24 h at 80°C. The product (C$_{26}$H$_{24}$N$_3$O$_2$Br: 490.4 g/mol) was purified by

crystallization in ethyl alcohol. Yield: 51%, m. p.:160–162°C, color: white. IR: 1553.4 (CN); 1698.7 and 1766.4 (C═O); 2920.8, 3065.5, and 3116.8 cm^{-1} (C-H). ^{1}H-NMR (400.13 MHz, $CDCl_3$, 298 K), δ: 1.90 [p, *J*: 8.0 Hz, 2 H, $NCH_2CH_2CH_2CH_2N(C{=}O)_2C_6H_4$]; 2.15 [p, *J*: 8.0 Hz, 2 H, $NCH_2CH_2CH_2CH_2N(C{=}O)_2C_6H_4$]; 3.81 [t, *J*: 8.0 Hz, 2 H, $NCH_2CH_2CH_2CH_2N(C{=}O)_2C_6H_4$]; 4.77 [t, *J*: 8.0 Hz, 2 H, $NCH_2CH_2CH_2CH_2N(C{=}O)_2C_6H_4$]; 5.88 [s, 2 H, $NCH_2C_6H_5$]; 7.27–7.85 (m, 13 H, Ar-*H*); 11.69 (s, 1 H, NC*H*N). ^{13}C-NMR (100.13 MHz, $CDCl_3$, 298 K), δ: 25.61 [$NCH_2CH_2CH_2CH_2N(C{=}O)_2C_6H_4$]; 26.66 [$NCH_2CH_2CH_2CH_2N(C{=}O)_2C_6H_4$]; 36.65 [$NCH_2CH_2CH_2CH_2N(C{=}O)_2C_6H_4$]; 46.96 [$NCH_2CH_2CH_2CH_2N(C{=}O)_2C_6H_4$]; 51.59 [$NCH_2C_6H_5$]; 113.11, 113.77, 123.38, 127.27, 128.37, 129.36, 129.46, 131.45, 131.92, 132.47, and 134.14 (Ar-*C*); 143.15 (N*C*HN); 168.43 [$NCH_2CH_2CH_2CH_2N(C{=}O)_2C_6H_4$].

1-(2-Cyanobenzyl)-3-[4-(1,3-dioxoisoindolin-2-yl)butyl]-1H-benzo[d]imidazol-3-ium bromide (12). According to the same conditions and procedure as for **2**, compound **12** was synthesized from 2-((1*H*-benzo[*d*]imidazol-1-yl)methyl) benzonitrile (1.0 g, 1 mmol) and *N*-(4-bromobutyl) phthalimide (1.21 g, 1 mmol) in DMF for 24 h at 80°C. The product ($C_{27}H_{23}N_4O_2Br$: 515.4 g/mol) was purified by crystallization in a mixture of ethyl alcohol-diethyl ether (2:1). Yield: 58%, m.p.: 186–189°C, color: white. IR: 1562.9 (CN); 1704.4 (C═O); 2943.9 cm^{-1} (C-H). ^{1}H-NMR (400.13 MHz, $CDCl_3$, 298 K), δ: 1.89 [p, *J*: 8.0 Hz, 2 H, $NCH_2CH_2CH_2CH_2N(C{=}O)_2C_6H_4$]; 2.16 [p, *J*: 8.0 Hz, 2 H, $NCH_2CH_2CH_2CH_2N(C{=}O)_2C_6H_4$]; 3.80 [t, *J*: 8.0 Hz, 2 H, $NCH_2CH_2CH_2CH_2N(C{=}O)_2C_6H_4$]; 4.78 [t, *J*: 8.0 Hz, 2 H, $NCH_2CH_2CH_2CH_2N(C{=}O)_2C_6H_4$]; 6.17 [s, 2 H, $NCH_2C_6H_4(CN)$-2]; 7.31–8.18 (m, 12 H, Ar-*H*); 11.56 (s, 1 H, NC*H*N). ^{13}C-NMR (100.13 MHz, $CDCl_3$, 298 K), δ: 25.57 [$NCH_2CH_2CH_2CH_2N(C{=}O)_2C_6H_4$]; 26.63 [$NCH_2CH_2CH_2CH_2N(C{=}O)_2C_6H_4$]; 36.62 [$NCH_2CH_2CH_2CH_2N(C{=}O)_2C_6H_4$]; 47.22 [$NCH_2CH_2CH_2CH_2N(C{=}O)_2C_6H_4$]; 48.92 [$NCH_2C_6H_4(CN)$-2]; 112.03, 113.18, 113.59, 117.26, 123.36, 123.38, 127.27, 127.56, 127.72, 130.09, 131.04, 131.29, 131.91, 133.34, 134.11, 134.15, 134.59, and 135.77 (Ar-*C*; *C*≡N); 143.44 (N*C*HN); 168.43 [$NCH_2CH_2CH_2CH_2N(C{=}O)_2C_6H_4$].

1-[4-(1,3-Dioxoisoindolin-2-yl)butyl]-3-(3-methylbenzyl)-1H-benzo[d]imidazol-3-ium bromide (13). According to the same conditions and procedure as for **2**, compound **13** was synthesized from 1-(3-methylbenzyl)benzimidazole (0.82 g, 1 mmol) and *N*-(4-bromobutyl)phthalimide (1.04 g, 1 mmol) in DMF for 24 h at 80°C. The product ($C_{27}H_{26}N_3O_2Br$: 504.42 g/mol) was purified by crystallization in a mixture of ethyl alcohol-diethyl ether (2:1). Yield: 78%, m.p.: 219–221°C, color: white. IR: 1557.7 (CN); 1699.5 (C═O); 2955.1 cm^{-1} (C-H). ^{1}H-NMR (400.13 MHz, $CDCl_3$, 298 K), δ: 1.90 [p, *J*: 8.0 Hz, 2 H, $NCH_2CH_2CH_2CH_2N(C{=}O)_2C_6H_4$]; 2.14 [p, *J*: 4.0 Hz, 2 H, $NCH_2CH_2CH_2CH_2N(C{=}O)_2C_6H_4$]; 2.34 [s, 3 H, $NCH_2C_6H_4(CH_3)$-3]; 3.79 [t, *J*: 8.0 Hz, 2 H, $NCH_2CH_2CH_2CH_2N(C{=}O)_2C_6H_4$]; 4.77 [t, *J*: 8.0 Hz, 2 H, $NCH_2CH_2CH_2CH_2N(C{=}O)_2C_6H_4$]; 5.82 [s, 2 H, $NCH_2C_6H_4(CH_3)$-3]; 7.15–7.83 (m, 12 H, Ar-*H*); 11.54 (s, 1 H, NC*H*N). ^{13}C-NMR (100.13 MHz, $CDCl_3$, 298 K), δ: 21.36 [$NCH_2CH_2CH_2CH_2N(C{=}O)_2C_6H_4$]; 25.59 [$NCH_2C_6H_4(CH_3)$-3]; 26.65 [$NCH_2CH_2CH_2CH_2N(C{=}O)_2C_6H_4$]; 36.69 [$NCH_2CH_2CH_2CH_2N(C{=}O)_2C_6H_4$]; 46.95 [$NCH_2CH_2CH_2CH_2N(C{=}O)_2C_6H_4$]; 51.60 [$NCH_2C_6H_4(CH_3)$-3]; 113.12, 113.85, 123.36, 125.35, 127.22, 128.93, 129.26, 130.10, 131.22, 131.46, 131.91, 132.41, 134.13, and 139.39 (Ar-*C*); 143.01 (N*C*HN); 168.43 [$NCH_2CH_2CH_2CH_2N(C{=}O)_2C_6H_4$].

1-[4-(1,3-Dioxoisoindolin-2-yl)butyl]-3-(4-methylbenzyl) benzimidazolium bromide (14). According to the same conditions and procedure as for **2**, compound **14** was synthesized from 1-(4-methylbenzyl)benzimidazole (1.3 g, 1 mmol) and *N*-(4-bromobutyl)phthalimide (1.65 g, 1 mmol) in DMF for 24 h at 80°C. The resulting compound ($C_{27}H_{26}N_3O_2Br$: 504.42 g/mol) was purified by crystallization in isopropyl alcohol. Yield: 33%, m.p.: 263–266°C, color: white. IR: 1544.9 (CN); 1737.7 cm^{-1} (C═O). ^{1}H-NMR (400.13 MHz, $CDCl_3$, 298 K), δ: 1.88 [s, 3 H, $NCH_2C_6H_4(CH_3)$-4]; 2.16–2.40 [m, 4 H, $NCH_2CH_2CH_2CH_2N(C{=}O)_2C_6H_4$]; 2.89 [t, *J*: 8.0 Hz, 2 H, $NCH_2CH_2CH_2CH_2N(C{=}O)_2C_6H_4$]; 5.83 (t, *J*: 12.0 Hz, 2 H, $NCH_2CH_2CH_2CH_2N(C{=}O)_2C_6H_4$]; 6.01 [s, 2 H, $NCH_2C_6H_4(CH_3)$-4]; 7.14–7.86 (m, 12 H, Ar-*H*); 11.56 (s, 1 H, NC*H*N). ^{13}C-NMR (100.13 MHz, $CDCl_3$, 298 K), δ: 21.19 [$NCH_2C_6H_4(CH_3)$-4]; 21.20 [$NCH_2CH_2CH_2CH_2N(C{=}O)_2C_6H_4$]; 21.25 [$NCH_2CH_2CH_2CH_2N(C{=}O)_2C_6H_4$]; 34.56 [$NCH_2CH_2CH_2CH_2N(C{=}O)_2C_6H_4$]; 51.52 [$NCH_2CH_2CH_2CH_2N(C{=}O)_2C_6H_4$]; 51.55 [$NCH_2C_6H_4(CH_3)$-4[; 113.81, 127.08, 128.29, 129.50, 130.07, 131.25, 131.41, 131.97, 132.18, 134.19, and 139.32 (Ar-*C*); 142.77 (N*C*HN); 167.70 [$NCH_2CH_2CH_2CH_2N(C{=}O)_2C_6H_4$].

1-[4-(1,3-Dioxoisoindolin-2-yl)butyl]-3-(4-vinylbenzyl)-1H-benzo[d]imidazol-3-ium bromide (15). According to the same conditions and procedure as for **2**, compound **15** was synthesized from 1-(4-vinylbenzyl)-1*H*-benzo[*d*]imidazole (1.4 g, 1 mmol) and *N*-(4-bromobutyl)phthalimide (1.69 g, 1 mmol) in DMF for 24 h at 80°C. The resulting white compound was washed with diethyl ether and then purified by crystallization in a mixture of ethyl alcohol-diethyl ether (2:1). Yield: 60%, m.p.: 110–112°C, color: cream. IR: 1557.6 (CN); 1699.3 (C═O); 2931.3 and 3069.8 cm^{-1} (C-H). ^{1}H-NMR (400.13 MHz, $CDCl_3$, 298 K), δ: 1.87 [p, *J*: 8.0 Hz, 2 H, $NCH_2CH_2CH_2CH_2N(C{=}O)_2C_6H_4$]; 2.13 [p, *J*: 8.0 Hz, 2 H, $NCH_2CH_2CH_2CH_2N(C{=}O)_2C_6H_4$]; 3.78 [t, *J*: 8.0 Hz, 2 H, NCH_2CH_2

$CH_2CH_2N(C{=}O)_2C_6H_4$]; 4.75 [t, *J*: 8.0 Hz, 2 H, $NCH_2CH_2CH_2CH_2N(C{=}O)_2C_6H_4$]; 5.28 (d, *J*: 12 Hz, 1 H, $CH{=}CH_2$); 5.75 (d, *J*: 12 Hz, 1 H, $CH{=}CH_2$); 5.88 [s, 2 H, $NCH_2C_6H_4(CH{=}CH_2)$-4]; 6.66 (dd, *J*: 8 Hz, 1 H, $CH{=}CH_2$); 7.38–7.83 (m, 12 H, Ar-*H*); 11.55 (s, 1 H, NC*H*N). ^{13}C-NMR (100.13 MHz, $CDCl_3$, 298 K), δ: 25.57 [$NCH_2CH_2CH_2CH_2N(C{=}O)_2C_6H_4$]; 26.62 [$NCH_2CH_2CH_2CH_2N(C{=}O)_2C_6H_4$]; 36.69 [$NCH_2CH_2CH_2CH_2N(C{=}O)_2C_6H_4$]; 46.93 [$NCH_2CH_2CH_2CH_2N(C{=}O)_2C_6H_4$]; 51.27 [$NCH_2C_6H_4(CH{=}CH_2)$-4]; 113.13, 113.86, 115.24, 123.36, 127.10, 127.23, 128.66, 131.13, 131.44, 132.89, 131.93, 134.14, 135.82, and 138.48 (Ar-*C*; $CH{=}CH_2$); 142.99 (NCHN); 168.43 [$NCH_2CH_2CH_2CH_2N(C{=}O)_2C_6H_4$].

1-[4-(1,3-Dioxoisoindolin-2-yl)butyl]-3-(naphthalen-1-ylmethyl)-1H-benzo[d]imidazol-3-ium bromide (16). According to the same conditions and procedure as for **2**, compound **16** was synthesized from 1-(naphthalen-1-ylmethyl)-1*H*-benzo[*d*]imidazole (1.30 g, 1 mmol) and *N*-(4-bromobutyl)phthalimide (1.42 g, 1 mmol) in DMF for 24 h at 80°C. The resulting white compound ($C_{30}H_{26}N_3O_2Br$: 540.45 g/mol) was washed with diethyl ether and then purified by crystallization in a mixture of ethyl alcohol-diethyl ether (2:1). Yield: 90%, m.p.: 198–200°C, color: white. IR: 1560.4 (CN); 1710.1 (C=O); 2901.6 cm^{-1} (C-H). ^{1}H-NMR (400.13 MHz, $CDCl_3$, 298 K), δ: 1.88 [p, 2 H, *J*: 8 Hz, $NCH_2CH_2CH_2CH_2N(C{=}O)_2C_6H_4$]; 2.14 [p, 2 H, *J*: 8.0 Hz, $NCH_2CH_2CH_2CH_2N(C{=}O)_2C_6H_4$]; 3.80 [t, 2 H, *J*: 8.0 Hz, $NCH_2CH_2CH_2CH_2N(C{=}O)_2C_6H_4$]; 4.78 [t, 2 H, *J*: 8.0 Hz, $NCH_2CH_2CH_2CH_2N(C{=}O)_2C_6H_4$]; 6.34 (s, 2 H, $NCH_2C_{10}H_7$); 7.47–8.20 (m, 15 H, Ar-*H*); 11.50 (s, 1 H, NC*H*N). ^{13}C-NMR (100.13 MHz, $CDCl_3$, 298 K), δ: 25.58 [$NCH_2CH_2CH_2CH_2N(C{=}O)_2C_6H_4$]; 26.61 [$NCH_2CH_2CH_2CH_2N(C{=}O)_2C_6H_4$]; 36.63 [$NCH_2CH_2CH_2CH_2N(C{=}O)_2C_6H_4$]; 47.04 [$NCH_2CH_2CH_2CH_2N(C{=}O)_2C_6H_4$]; 49.64 ($NCH_2C_{10}H_7$); 113.14, 113.95, 122.40, 123.37, 125.37, 126.63, 127.28, 127.51, 127.68, 127.83, 129.25, 130.36, 130.65, 131.44, 131.93, 133.92, and 134.12 (Ar-*C*); 143.59 (NCHN); 168.40 [$NCH_2CH_2CH_2CH_2N(C{=}O)_2C_6H_4$].

1-[4-(1,3-Dioxoisoindolin-2-yl)butyl]-3-(2-morpholinoethyl)-1H-benzo[d]imidazol-3-ium bromide (17). According to the same conditions and procedure as for **2**, compound **17** was synthesized from 1-(2-morpholinoethyl)-1*H*-benzo[*d*]imidazole (1.0 g, 1 mmol) and *N*-(4-bromobutyl)phthalimide (1.22 g, 1 mmol) in DMF for 24 h at 80°C. The resulting off-white compound ($C_{25}H_{29}N_4O_3Br$: 513.43 g/mol) was first washed with diethyl ether and then purified by crystallization from isopropyl alcohol. Yield: 79%, color: white, m.p.: 142–144°C. IR: 1564.8 (CN); 1705.7 (C=O); 2942.4 cm^{-1} (C-H). ^{1}H-NMR (400.13 MHz, $CDCl_3$, 298 K), δ: 1.87 [p, *J*: 8.0 Hz, 2 H, $NCH_2CH_2CH_2CH_2N(C{=}O)_2C_6H_4$]; 2.13 [p, *J*: 8.0 Hz, 2 H, $NCH_2CH_2CH_2CH_2N(C{=}O)_2C_6H_4$]; 2.97 [t, *J*: 8.0 Hz, 4 H, $NCH_2CH_2N(CH_2CH_2)_2O$]; 3.37 [t, *J*: 8.0 Hz, 2 H, $NCH_2CH_2N(CH_2CH_2)_2O$]; 3.75–3.81 [m, 6 H, $NCH_2CH_2CH_2CH_2N(C{=}O)_2C_6H_4$; $NCH_2CH_2N(CH_2CH_2)_2O$]; 4.72 [tt, *J*: 8.0 Hz, *J*: 8.0 Hz, 4 H, $NCH_2CH_2N(CH_2CH_2)_2O$]; 7.62–7.83 (m, 8 H, Ar-*H*); 11.10 (s, 1 H, NC*H*N). ^{13}C-NMR (100.13 MHz, $CDCl_3$, 298 K), δ: 25.54 [$NCH_2CH_2CH_2CH_2N(C{=}O)_2C_6H_4$]; 26.47 [$NCH_2CH_2CH_2CH_2N(C{=}O)_2C_6H_4$]; 36.68, 43.07, 46.87, 46.97, and 52.95 [$NCH_2CH_2CH_2CH_2N(C{=}O)_2C_6H_4$; $NCH_2CH_2N(CH_2CH_2)_2O$]; 65.65 [$NCH_2CH_2N(CH_2CH_2)_2O$]; 113.02, 113.60, 123.37, 127.24, 127.51, 131.02, 131.25, 131.29, 131.83, 131.88, 134.12, and 134.18 (Ar-*C*); 143.16 (NCHN); 168.42 [$NCH_2CH_2CH_2CH_2N(C{=}O)_2C_6H_4$].

The results of spectroscopic data for compounds **18**–**22** were given in previously published paper [29].

Cytotoxic activity studies. The cytotoxicity activity of the compounds was determined according to our previously established procedure [12,18,33–35]. The DLD-1, MDA-MB-231, and HEK 293T cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum and 1% GlutaMAX. The cells were then seeded into sterile 96-well plates at a density of 1×10^3 cells/well and maintained at 37°C for 24 h. Cancerous cells were exposed to **2**–**22** at concentrations of 0.5, 1, 2, 5, 10, 20, 50, 100, and 200 μM for 72 h. The normal human cells were exposed to **2**, **4**, **5**, **8**, **11**–**13**, **16**, **17**, **20**, and **22** in the 0.001–100 μM range different concentrations for 24 h. The plates were incubated for more than 4 h after adding MTT stock solution (5 mg/mL) to each well. Subsequently, the sample was dried under vacuum, and DMSO (200 μL) was added to each well. The plate was then shaken for 30 min on a plate rocker. GraphPad Prism software program was then used to calculate the IC_{50} values. Two independent studies with triplicate data points were conducted for all tested compounds.

Methods of confocal microscopy. The confocal (Olympus FV1000 confocal microscope with an UPLAPO 20× objective [NA 0.7]) was performed according to the published procedure [12,18].

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

AUTHOR CONTRIBUTIONS

S. Akkoç conducted all experiments and data analysis under the supervision of V. Kayser and İ. Ö. İlhan and wrote the paper. V. Kayser designed the study, analyzed the data, and wrote the manuscript. İ. Ö. İlhan helped wrote the paper.

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