Bioorganic & Medicinal Chemistry Letters 22 (2012) 933–936

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis of substituted benzimidazolyl curcumin mimics and their anticancer activity

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ARTICLE INFO

Article history:
Received 3 October 2011
Revised 17 November 2011
Accepted 3 December 2011
Available online 20 December 2011

Keywords:
Synthesis
Curcumin mimics
Natural product
Anticancer
Aldol reaction

ABSTRACT

A novel curcumin mimic library (**14a**–**14h** and **15a**–**15h**) possessing variously substituted benzimidazole groups was synthesized through the aldol reaction of (*E*)-4-(4-hydroxy-3-methoxyphenyl)but-3-en-2-one (**7**) or (*E*)-4-(3-hydroxy-4-methoxyphenyl)but-3-en-2-one (**13**) with diversely substituted benzimidazolyl-2-carbaldehyde (**12a**–**12h**). The MTT assay of the cancer cells MCF-7, SH-SY5Y, HEP-G2, and H460 showed that compound **14c** with IC_{50} of 1.0 and 1.9 μM has a strong inhibitory effect on the growth of SH-SY5Y and Hep-G2 cells, respectively, and that compound **15h** with IC_{50} of 1.9 μM has a strong inhibitory effect on the growth of MCF-7 cancer cells.

Curcumin (diferuloyl methane, **1**), a prominent constituent of the root of *Curcuma longa* L., has versatile biological properties; it is antiinflammatory,[1] antioxidant,[2] and antiviral,[3] and it shows chemopreventive effects,[4] anti-infective activity,[5] and wound-healing properties.[6] We have previously reported that various curcumin mimics (**2**) possessing alkyl amide and aryl amide functional groups have an angiogenesis inhibition effect[7] and show multidrug resistance (MDR) reversal activity.[8,9] Additionally, sulfonyl amide-linked curcumin derivatives (**3**) exhibit a vasodilatation effect on the basilar artery induced by high K^+ ion.[10] Very recently, we discovered that substituted triazolyl curcumin mimics (**4**) synthesized through Cu(I)-catalyzed Huisgen 1,3-cycloaddition as a key reaction exhibit moderate to strong inhibitory activity against the osteoclastogenesis induced by the receptor activator of NF-κB ligand (RANKL)[11] (Fig. 1). The diverse biological properties of curcumin mimics are mainly dependent on the additional functional groups attached to the feruloyl structure, which is evident from our previous researches on the synthesis of curcumin mimics with various functional groups and the biological activities of these mimics. All curcumin mimics in our in-house library show mild cytotoxicity, and this can be used to develop a drug that does not show toxicity.[9] Therefore, we designed a novel curcumin mimic library by dramatically varying the attached functional groups on the feruloyl structure to obtain an unexpected biological activity and expand the utility of the curcumin library. Specifically, as in the case of compound **5** shown in Figure 1, we introduced variously substituted benzimidazole groups (**6**) to the feruloyl scaffold through aldol condensation as a key reaction and tested the resulting compound for unexpected biological properties. Because benzimidazole molecules exhibit a variety of biological properties that enable anticancer,[12] antiviral,[13] and anti-hypertensive activity,[14] we can expect the resulting compound to exhibit novel bioactivity owing to the benzimidazole group and will additionally investigate the variation in biological activity with the variation of the attached functional groups.

We attempted the retrosynthetic analysis shown in Scheme 1 to efficiently synthesize benzimidazolyl curcumin mimic library (**5**). We can obtain the required curcumin mimics through an aldol reaction between (*E*)-4-(4-hydroxy-3-methoxyphenyl)but-3-en-2-one (**7**) and the substituted benzimidazolyl-2-carbaldehyde (**8**). As shown in Scheme 2, the reaction of 1,2-phenylenediamine (**9a**) with glycolic acid (3 equiv) in hydrochloric acid (4 N concentration) under reflux for 6 h produced benzimidazolyl methanol (**10a**),[15] which subsequently reacted with iodomethane (3 equiv) in the presence of tetrabutylammonium bromide (TBAB, 0.2 equiv) and potassium hydroxide (KOH, 1.1 equiv) in tetrahydrofuran

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doi:10.1016/j.bmcl.2011.12.074

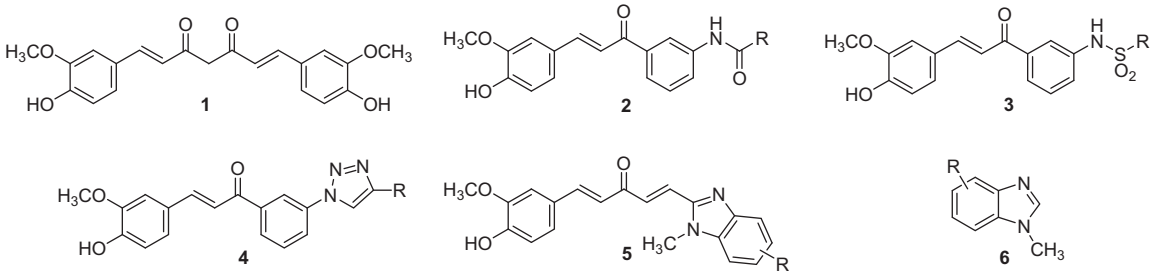
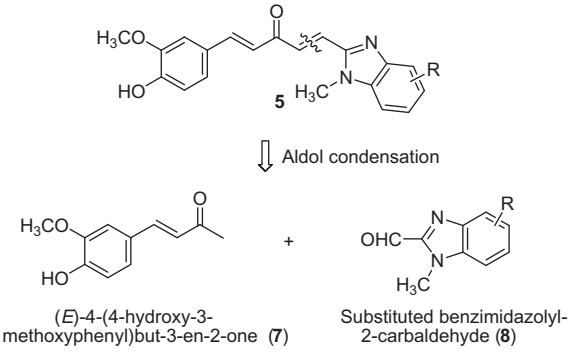


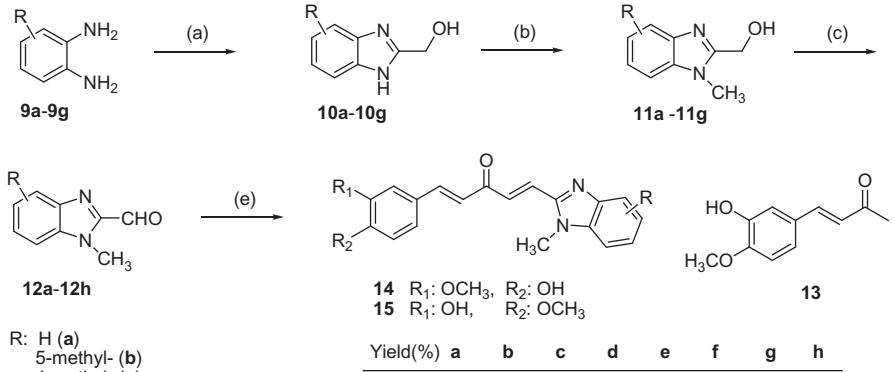
Figure 1. Structures of curcumin and synthetic curcumin mimic derivatives.



Scheme 1. Retrosynthetic analysis of substituted benzimidazolyl curcumin mimics.

(THF) at 0 °C to afford (1-methyl-1*H*-benzimidazol-2-yl)-methanol (**11a**).[16] Benzimidazolyl 2-carbaldehyde (**12a**) was obtained from the Dess–Martin oxidation of **11a**.[17] To expand the structural diversity of the benzimidazolyl moiety, we synthesized substituted benzimidazoles (**12b**–**12g**) from various phenylene diamine derivatives possessing methyl- (**b**, **c**, and **d**), methoxy- (**e**), chloro- (**f**), and nitro- (**g**) groups using the same procedure for **12a** as shown in Scheme 2. In addition, 5-hydroxy benzimidazolyl 2-carbaldehyde (**12h**) was obtained from the demethylation of **12e** using tribromoborane (BBr_3, 2 equiv) in methylene chloride at −78 °C.[18] The curcumin mimic library with a structure similar to that of (**5**) was obtained from the aldol reaction of the substituted benzimidazoles (**12a**–**12h**) with the commercially available starting material (**7**) in the presence of 40% potassium hydroxide solution in ice-bath.[10] In addition, constitutional isomeric curcumin derivatives (**15a**–**15h**) of **5** were also prepared from the reaction between synthetic (*E*)-4-(3-hydroxy-4-methoxyphenyl)but-3-en-2-one (**13**)[19] and benzimidazoles (**12a**–**12h**) shown in Scheme 2. The structure of the required curcumin library was confirmed through ^{1}H NMR and ^{13}C NMR spectroscopy.[20] The double bonds in the feruloyl and benzimidazolyl structure were determined to be of trans configuration because of a large coupling constant (approximately J = 15 Hz above) between their two protons. The yields of various benzimidazoles (**10a**–**10g**, **11a**–**11g**, and **12a**–**12h**) and the synthetic curcumin mimic library (**14a**–**14h**, and **15a**–**15h**) were summarized in Scheme 2.

To discover novel anticancer agents of small molecular size derived from the curcumin mimics, we conducted a cytotoxicity assay by employing the MTT colorimetric method[21] against cancer cell lines such as MCF-7, SH-SY5Y, HEP-G2, and H460[22]; the inhibitory activity is summarized in Table 1. As shown in Scheme 2, the two groups of curcumin mimics (**14a**–**14h** and **15a**–**15h**) differ only in the position of the methoxy and hydroxyl functionality of feruloyl moiety. A comparison of the growth inhibitory effect of each group of curcumin mimics shows that the positional variation of the methoxy and hydroxyl functionality does not affect the potency of the anticancer activity. However, the inhibitory concentration of the synthetic curcumin mimic library with the benzimidazole group against the growth of cancer cells is dramatically



Yield(%)	a	b	c	d	e	f	g	h
10	77	83	80	60	51	34	90	-
11	92	78	43	83	89	95	90	-
12	67	61	65	49	44	70	51	82
14	77	58	90	88	53	72	40	85
15	48	48	91	92	63	70	45	84

Scheme 2. Reagents and conditions: (a) Glycolic acid (3 equiv), 4*N* HCl, reflux, 6 h; (b) Iodomethane (3 equiv), tetrabutylammonium bromide (0.2 equiv), potassium hydroxide (1.1 equiv), tetrahydrofuran, 0 °C; (c) Dess–Martin periodinane (1.1 equiv), methylene chloride, 0 °C, 6 h; (d) Tribromoborane (2 equiv), methylene chloride, −78 °C; (e) **7** or **13** (each 1 equiv), 40% KOH, Ethanol, ice bath, 10 h.

Table 1
Inhibitory concentration of curcumin mimic library against various cancer cell lines

Entry		Cancer cells (IC_{50} μM)				Entry		Cancer cells (IC_{50} μM)			
		MCF-7	SH-SY5Y	Hep-G2	H460			MCF-7	SH-SY5Y	Hep-G2	H460
14	**a**	3.7	4.7	2.9	7.0	**15**	**a**	3.6	4.8	2.9	6.9
	b	4.5	4.7	4.2	7.7		**b**	3.6	4.8	3.3	7.7
	c	3.5	1.0	1.9	3.0		**c**	7.4	19.1	14.7	25.4
	d	14.1	19.0	8.5	31.9		**d**	3.5	4.8	3.9	7.3
	e	3.9	4.8	3.8	12.7		**e**	3.7	4.8	4.2	11.7
	f	4.4	4.7	4.0	8.9		**f**	3.5	4.8	4.1	7.3
	g	3.7	4.6	3.7	6.7		**g**	4.8	8.1	4.6	10.5
	h	2.9	4.8	3.6	7.6		**h**	1.9	4.6	3.7	7.2
Curcumin		49.8	105.0	87.5	51.3	Taxol		0.003	0.004	0.007	0.008

increased by about 10–50 times with the IC_{50} of curcumin (**1**), indicating that the increase in potency is caused by the addition of benzimidazole groups to the feruloyl structure. To determine the structure–activity relationship (SAR) with the change of the functional groups of benzimidazole, we synthesized various types of curcumin derivatives possessing benzimidazoles with electron donating groups (methyl-, methoxy-, and hydroxyl-group) and electron withdrawing groups (chloro- and nitro-group). However, there are no substantial differences in the growth inhibitory effects. From the synthetic curcumin library, (1*E*,4*E*)-1-(4-hydroxy-3-methoxyphenyl)-5-(1,4-dimethyl-1*H*-benzo[*d*]imidazol-2-yl) penta-1,4-dien-3-one (**14c**) with the IC_{50} of 1.0 and 1.9 μM strongly inhibited the growth of two cancer cell lines, SH-SY5Y and Hep-G2, respectively. (1*E*,4*E*)-1-(5-Hydroxy-1-methyl-1*H*-benzo[*d*]imidazol-2-yl)-5-(3-hydroxy-4-methoxyphenyl)penta-1,4-dien-3-one (**15h**) (IC_{50}; 1.9 μM) also showed strong inhibitory activity against the MCF-7 cancer cell. Although our in-house curcumin mimic library showed weaker inhibitory activity than the positive control (taxol), the addition of various benzimidazole groups to feruloyl increased cancer cell cytotoxicity. This increase in cytotoxicity due to the addition of benzimidazoles is unexpected, especially when compared to our previous researches that concluded amide (**2**) or sulfonylamide linked curcumin mimics (**3**) and triazole linked curcumin derivatives (**4**) showed no cytotoxicity against various cancer cells at the concentration of 100 μM. Therefore, it is possible to obtain novel drug candidates by adding diverse chemical entities to the feruloyl structure.

In conclusion, we have synthesized a novel curcumin mimics library (**14a**–**14h** and **15a**–**15h**) through the aldol reaction of (*E*)-4-(4-hydroxy-3-methoxyphenyl)but-3-en-2-one (**7**) or (*E*)-4-(3-hydroxy-4-methoxyphenyl)but-3-en-2-one (**13**) with diversely substituted benzimidazolyl-2-cabaldehyde (**12a**–**12h**) with the aim of discovering novel anticancer drug candidates. On the basis of the MTT assay against the cancer cells MCF-7, SH-SY5Y, HEP-G2, and H460, we confirmed that the novel curcumin mimics inhibited the growth of cancer cells more strongly than our previous in-house curcumin mimic libraries and curcumin. Additionally, we hypothesized that the increment in inhibitory potency is caused by the attached benzimidazole functionalities. Among the tested derivatives, **14c** is the strongest candidate against SH-SY5Y and Hep-G2 cells and **15h** effectively inhibited the MCF-7 cancer cells.

Acknowledgment

This work was supported by the Yonsei University Research Fund of 2008.

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20. Spectral data for **14a**: ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.86 (3H, s, OCH_3), 3.99 (3H, s, NCH_3), 6.85 (1H, d, J = 8.0 Hz, $CH_3OC_6H_3$), 7.30 (1H, d, J = 20.4 Hz, C*H*=CHAr), 7.27–7.34 (1H, m, $CH_3OC_6H_3$), 7.27–7.34 (2H, m, benzimidazole-C_6H_4), 7.46 (1H, s, $CH_3OC_6H_3$), 7.65 (1H, d, J = 20.4 Hz, benzimidazole-CH=C*H*), 7.67 (1H, d, J = 20.4 Hz, CH=C*H*Ar), 7.77 (1H, d, J = 20.4 Hz, benzimidazole-C*H*=CH), 7.79 (2H, d, J = 4.4 Hz, benzimidazole-C_6H_4), 9.76 (1H, s, OH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6) δ 29.9, 55.7, 110.8, 111.9, 115.7, 119.3, 122.8, 122.9, 123.3, 123.8, 126.1, 126.9, 131.2, 136.4, 142.7, 144.5, 147.9, 148.7, 149.8, 187.6 ppm; **14b**: ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.50 (3H, s, CH_3), 3.91 (3H, s, OCH_3), 3.95 (3H, s, NCH_3), 6.85 (1H, d, J = 16.1 Hz, C*H*=CHAr), 6.96 (1H, d, J = 8.1 Hz, $CH_3OC_6H_3$), 7.13 (1H, d, J = 1.7 Hz, $CH_3OC_6H_3$), 7.15–7.19 (2H, m, $CH_3OC_6H_3$, benzimidazole-C_6H_3), 7.27 (1H, d, J = 7.4 Hz, benzimidazole-C_6H_3), 7.60 (1H, s, benzimidazole-C_6H_3), 7.75 (1H, d, J = 15.1 Hz, benzimidazole-CH=C*H*), 7.79 (1H, d, J = 15.5 Hz, CH=C*H*Ar), 7.98 (1H, d, J = 15.1 Hz, benzimidazole-C*H*=CH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6) δ 21.7, 30.1, 56.0, 109.3, 109.8, 115.0, 119.7, 123.9, 124.9, 125.8, 126.1, 127.2, 130.0, 133.4, 134.5, 143.3, 145.0, 147.0, 148.6, 148.8, 188.2 ppm; **14c**: ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.58 (3H, s, CH_3), 3.86 (3H, s, OCH_3), 3.96 (3H, s, NCH_3), 6.85 (1H, d, J = 8.4 Hz, $CH_3OC_6H_3$), 7.08 (1H, d, J = 7.2 Hz, $CH_3C_6H_3$), 7.20 (1H, t, J = 7.2 Hz, $CH_3C_6H_3$), 7.28 (1H, d, J = 7.3 Hz, benzimidazole-C_6H_3), 7.37 (1H, d, J = 16.0 Hz, C*H*=CHAr), 7.43 (1H, d, J = 8.0 Hz, benzimidazole-C_6H_3), 7.48 (1H, s, benzimidazole-C_6H_3), 7.71 (1H, d, J = 15.2 Hz, benzimidazole-CH=C*H*), 7.72 (1H, d, J = 15.6 Hz, CH=C*H*Ar), 7.81 (1H, d, J = 15.2 Hz, benzimidazole-C*H*=CH), 9.76 (1H, s, OH)ppm; ^{13}C NMR (100 MHz, DMSO-d_6) δ 16.5, 30.0, 55.7, 108.2, 111.7, 115.7, 122.8, 122.9, 123.4, 124.0, 126.2, 127.1, 129.1, 131.3, 136.1, 142.2, 144.2, 147.9, 148.0, 149.9, 187.5 ppm; **14d**: ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.40 (3H, s, CH_3), 2.42 (3H, s, CH_3), 3.89 (3H, s, OCH_3), 3.96 (3H, s, NCH_3), 6.85 (1H, d, J = 16.1 Hz, C*H*=CHAr), 6.97 (1H, d, J = 8.0 Hz, $CH_3OC_6H_3$), 7.13–7.16 (2H, m, $CH_3OC_6H_3$), 7.13–7.26 (2H, m, benzimidazole-C_6H_2), 7.57 (1H, s, benzimidazole-C_6H_2), 7.74 (1H, d, J = 15.0 Hz, benzimidazole-CH=C*H*), 7.79 (1H, d, J = 15.8 Hz, CH=C*H*Ar), 7.97 (1H, d, J = 15.0 Hz, benzimidazole-C*H*=CH)ppm; ^{13}C NMR (100 MHz, DMSO-d_6) δ 20.0, 20.2, 29.8, 55.7, 110.6, 111.9, 115.7, 119.2, 123.1, 123.7, 126.2, 127.1, 130.3, 131.5, 132.6, 135.1, 141.5, 144.2, 147.7, 148.0, 149.8, 187.6 ppm; **14e**: ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.89 (3H, s, OCH_3), 3.91 (3H, s, OCH_3), 3.95 (3H, s, NCH_3), 6.79 (1H, d, J = 2.0 Hz, $CH_3OC_6H_3$), 6.85 (1H, d, J = 16.1 Hz, C*H*=CHAr), 6.96 (1H, d, J = 8.0 Hz, $CH_3OC_6H_3$), 6.98 (1H, dd, J = 8.8 and 2.4 Hz, $CH_3OC_6H_3$), 7.12 (1H, s, benzimidazole-C_6H_3), 7.16 (1H, d, J = 8.3 Hz, benzimidazole-C_6H_3), 7.69 (1H,

d, J = 9.0 Hz, benzimidazole-C_6H_3), 7.74 (1H, d, J = 15.2 Hz, benzimidazole-CH=C*H*), 7.77 (1H, d, J = 16.1 Hz, CH=C*H*Ar), 7.91 (1H, d, J = 15.0 Hz, benzimidazole-C*H*=CH)ppm; ^{13}C NMR (100 MHz, DMSO-d_6) δ 29.9, 55.5, 55.7, 93.3, 111.9, 113.3, 115.7, 120.0, 123.0, 123.7, 126.2, 127.0, 127.1, 129.8, 137.3, 137.4, 144.1, 148.0, 149.8, 157.0, 187.6 ppm; **14f**: ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.91 (3H, d, J = 6.3 Hz, NCH_3), 3.96 (3H, s, OCH_3), 6.85 (1H, dd, J = 16.3 and 2.50 Hz, C*H*=CHAr), 6.97 (1H, d, J = 8.0 Hz, $CH_3OC_6H_3$), 7.12 (1H, s, $CH_3OC_6H_3$), 7.17 (1H, dd, J = 8.0 and 2.0 Hz, $CH_3OC_6H_3$), 7.29 (1H, dd, J = 8.8 and 1.8 Hz, benzimidazole-C_6H_3), 7.35 (1H, dd, J = 39.3 and 1.5 Hz, benzimidazole-C_6H_3), 7.71 (1H, t, J = 4.3 Hz, benzimidazole-CH=C*H*), 7.77 (1H, d, J = 16.0 Hz, benzimidazole-C_6H_3), 7.78 (1H, dd, J = 16.0 and 2.00 Hz, CH=C*H*Ar), 7.97 (1H, dd, J = 15.0 and 2.0 Hz, benzimidazole-C*H*=CH)ppm; ^{13}C NMR (100 MHz, DMSO-d_6) δ 30.2, 56.0, 109.7, 109.9, 110.6, 115.0, 119.7, 120.9, 124.0, 124.3, 124.5, 124.7, 125.6, 127.0, 130.6, 130.9, 145.2, 147.0, 148.8, 187.9 ppm; **14g**: ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.97 (3H, s, OCH_3), 3.99 (3H, s, NCH_3), 6.88 (1H, d, J = 16.0 Hz, C*H*=CHAr), 6.98 (1H, d, J = 8.2 Hz, $CH_3OC_6H_3$), 7.14 (1H, d, J = 1.6 Hz, $CH_3OC_6H_3$), 7.20 (1H, dd, J = 8.3 and 1.7 Hz, $CH_3OC_6H_3$), 7.47 (1H, d, J = 9.0 Hz, benzimidazole-C_6H_3), 7.74 (1H, d, J = 15.0 Hz, benzimidazole-CH=C*H*), 7.81 (1H, d, J = 16.0 Hz, CH=C*H*Ar), 8.05 (1H, d, J = 15.0 Hz, benzimidazole-C*H*=CH), 8.28 (1H, dd, J = 8.9 and 2.1 Hz, benzimidazole-C_6H_3), 8.74 (1H, d, J = 1.9 Hz, benzimidazole-C_6H_3) ppm; ^{13}C NMR (100 MHz, DMSO-d_6) δ 30.5, 56.0, 106.7, 109.7, 109.8, 115.0, 116.7, 119.4, 124.1, 124.4, 124.9, 126.9, 132.4, 134.4, 140.1, 142.2, 145.7, 146.9, 148.9, 187.6.ppm; **14h**: ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.85 (3H, s, OCH_3), 3.92 (3H, s, NCH_3), 6.87 (1H, d, J = 8.0 Hz, $CH_3OC_6H_3$), 6.90 (1H, d, J = 9.2 Hz, benzimidazole-C_6H_2), 6.97 (1H, s, benzimidazole-C_6H_3), 7.24 (1H, d, J = 16.0 Hz, C*H*=CHAr), 7.28 (1H, dd, J = 8.2 and 1.80 Hz, $CH_3OC_6H_3$), 7.45 (1H, d, J = 2.0 Hz, $CH_3OC_6H_3$), 7.53 (1H, d, J = 8.8 Hz, benzimidazole-C_6H_3), 7.45 (1H, s, benzimidazole-C_6H_3), 7.74 (1H, d, J = 14.8 Hz, benzimidazole-CH=C*H*), 7.75 (1H, d, J = 14.8 Hz, CH=C*H*Ar), 7.81 (1H, d, J = 16.0 Hz, benzimidazole-C*H*=CH) 9.80 (2H, s, OH)ppm; ^{13}C NMR (100 MHz, DMSO-d_6) δ 30.2, 56.2, 95.6, 112.4, 114.0, 116.2, 120.5, 123.5, 124.1, 126.7, 127.6, 130.0, 137.2, 138.0, 144.5, 148.1, 148.4, 150.2, 155.4, 188.0 ppm.; **15a**: ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.84 (3H, s, OCH_3), 3.99 (3H, s, NCH_3), 7.01 (1H, d, J = 8.8 Hz, $CH_3OC_6H_3$), 7.22 (1H, d, J = 16.0 Hz, C*H*=CHAr), 7.27–7.34 (2H, m, $CH_3OC_6H_3$), 7.27–7.34 (2H, m, benzimidazole-C_6H_4), 7.64 (1H, d, J = 7.6 Hz, benzimidazole-C_6H_4), 7.70 (1H, d, J = 7.2 Hz, benzimidazole-C_6H_4), 7.71 (1H, d, J = 16.0 Hz, benzimidazole-CH=C*H*), 7.72 (1H, d, J = 15.6 Hz, CH=C*H*Ar), 7.83 (1H, d, J = 15.2 Hz, benzimidazole-C*H*=CH), 9.24 (1H, s, OH)ppm; ^{13}C NMR (100 MHz, DMSO-d_6) δ 29.7, 55.7, 110.8, 112.0, 114.6, 119.3, 122.2, 122.8, 123.1, 123.3, 127.2, 127.5, 131.4, 136.4, 142.7, 144.1, 146.7, 148.7, 150.5, 187.7 ppm; **15b**: ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.51 (3H, s, CH_3), 3.92 (3H, s, OCH_3), 3.95 (3H, s, NCH_3), 5.31 (1H, s, OH), 6.86 (1H, d, J = 16.1 Hz, C*H*=CHAr), 6.89 (1H, d, J = 8.3 Hz, $CH_3OC_6H_3$), 7.14 (1H, dd, J = 8.4 and 1.9 Hz, $CH_3OC_6H_3$), 7.18 (1H, d, J = 8.0 Hz, benzimidazole-C_6H_3), 7.24 (1H, d, J = 1.9 Hz, $CH_3OC_6H_3$), 7.28 (1H, d, J = 8.4 Hz, benzimidazole-C_6H_3), 7.61 (1H, s, benzimidazole-C_6H_3), 7.76 (1H, d, J = 15.1 Hz, benzimidazole-CH=C*H*), 7.78 (1H, d, J = 16.2 Hz, CH=C*H*Ar), 7.98 (1H, d, J = 15.1 Hz, benzimidazole-C*H*=CH)ppm; ^{13}C NMR (100 MHz, DMSO-d_6) δ 21.2, 29.9, 55.6, 110.3, 111.9, 114.5, 118.9, 122.2, 123.1, 125.0, 127.3, 127.5, 131.0, 132.0, 134.7, 143.1, 144.0, 146.7, 148.5, 150.4, 187.7 ppm; **15c**: ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.73 (3H, s, CH_3), 3.92 (3H, s, OCH_3), 3.94 (3H, s, NCH_3), 6.88 (1H, d, J = 15.9 Hz, C*H*=CHAr), 6.89 (1H, d, J = 8.2 Hz, $CH_3OC_6H_3$), 7.12–7.28 (2H, m, $CH_3C_6H_3$), 7.12–7.28 (3H, m, benzimidazole-C_6H_3), 7.77 (1H, d, J = 15.4 Hz, benzimidazole-CH=C*H*), 7.77 (1H, d, J = 15.4 Hz, CH=C*H*Ar), 7.96 (1H, d, J = 15.0 Hz, benzimidazole-C*H*=CH)ppm; ^{13}C NMR (100 MHz, DMSO-d_6) δ 16.9, 30.5, 56.2, 108.7, 112.4, 115.2, 122.6, 123.3, 123.6, 123.9, 127.8, 128.0, 129.5, 131.9, 136.6, 142.7, 144.3, 147.2, 148.3, 150.9, 188.1 ppm; **15d**: ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.33 (3H, s, CH_3), 2.37 (3H, s, CH_3), 3.84 (3H, s, OCH_3), 3.93 (3H, s, NCH_3), 7.01 (1H, d, J = 8.8 Hz, $CH_3OC_6H_3$), 7.20 (1H, d, J = 16.0 Hz, C*H*=CHAr), 7.21–7.26 (2H, m, $CH_3OC_6H_3$), 7.40 (1H, s, benzimidazole-C_6H_2), 7.45 (1H, s, benzimidazole-C_6H_2), 7.65 (1H, d, J = 15.2 Hz, benzimidazole-CH=C*H*), 7.68 (1H, d, J = 15.8 Hz, CH=C*H*Ar), 7.78 (1H, d, J = 15.6 Hz, benzimidazole-C*H*=CH), 9.21 (1H, s, OH)ppm; ^{13}C NMR (100 MHz, DMSO-d_6) δ 20.0, 20.2, 29.8, 55.7, 110.6, 112.0, 114.6, 119.2, 122.3, 123.2, 127.4, 127.5, 130.4, 131.6, 132.6, 135.1, 141.5, 143.8, 146.7, 147.7, 150.4, 187.6 ppm; **15e**: ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.89 (3H, s, OCH_3), 3.91 (3H, s, OCH_3), 3.95 (3H, s, NCH_3), 6.79 (1H, d, J = 2.4 Hz, $CH_3OC_6H_3$), 6.86 (1H, d, J = 16.1 Hz, C*H*=CHAr), 6.89 (1H, d, J = 8.3 Hz, $CH_3OC_6H_3$), 6.98 (1H, dd, J = 8.9 and 2.3 Hz, $CH_3OC_6H_3$), 7.14 (1H, dd, J = 8.3 and 2.0 Hz, benzimidazole-C_6H_3), 7.23 (1H, d, J = 2.0 Hz, benzimidazole-C_6H_3), 7.70 (1H, d, J = 8.8 Hz, benzimidazole-C_6H_3), 7.74 (1H, d, J = 16.3 Hz, benzimidazole-CH=C*H*), 7.77 (1H, d, J = 15.6 Hz, CH=C*H*Ar), 7.91 (1H, d, J = 15.7 Hz, benzimidazole-C*H*=CH)ppm; ^{13}C NMR (100 MHz, DMSO-d_6) δ 29.9, 55.5, 55.6, 93.3, 111.9, 113.3, 114.5, 120.0, 122.1, 123.2, 127.2, 127.3, 130.0, 137.2, 137.3, 143.8, 146.7, 147.7, 150.4, 157.0, 187.6 ppm; **15f**: ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.91 (3H, d, J = 6.4 Hz, NCH_3), 3.93 (3H, s, OCH_3), 6.84 (1H, d, J = 16.3 Hz, C*H*=CHAr), 6.89 (1H, d, J = 8.4 Hz, $CH_3OC_6H_3$), 7.14 (1H, d, J = 8.3 Hz, $CH_3OC_6H_3$), 7.24 (1H, d, J = 1.8 Hz, $CH_3OC_6H_3$), 7.30 (1H, dd, J = 8.5 and 1.8 Hz, benzimidazole-C_6H_3), 7.35 (1H, dd, J = 22.7 and 1.8 Hz, benzimidazole-C_6H_3), 7.71 (1H, d, J = 15.0 Hz, benzimidazole-CH=C*H*), 7.74 (1H, d, J = 11.6 Hz, benzimidazole-C_6H_3), 7.77 (1H, d, J = 16.3 Hz, CH=C*H*Ar), 8.00 (1H, dd, J = 15.1 and 2.8 Hz, benzimidazole-C*H*=CH)ppm; ^{13}C NMR (100 MHz, DMSO-d_6) δ 30.1, 55.7, 78.2, 111.9, 112.3, 114.6, 122.3, 123.1, 126.8, 127.3, 127.5, 132.2, 135.3, 137.1, 143.4, 144.3, 146.7, 150.2, 150.3, 187.6 ppm; **15g**: ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.84 (3H, s, OCH_3), 4.06 (3H, s, NCH_3), 7.02 (1H, d, J = 8.2 Hz, $CH_3OC_6H_3$), 7.24 (1H, d, J = 16.0 Hz, C*H*=CHAr), 7.27 (1H, s, $CH_3OC_6H_3$), 7.29 (1H, dd, J = 8.3 and 1.7 Hz, $CH_3OC_6H_3$), 7.74 (1H, d, J = 15.0 Hz, benzimidazole-CH=C*H*), 7.81 (1H, d, J = 16.0 Hz, CH=C*H*Ar), 7.82 (1H, d, J = 9.0 Hz, benzimidazole-C_6H_3), 7.89 (1H, d, J = 15.0 Hz, benzimidazole-C*H*=CH), 8.23 (1H, dd, J = 9.0 and 2.1 Hz, benzimidazole-C_6H_3), 8.57 (1H, d, J = 1.9 Hz, benzimidazole-C_6H_3), 9.25 (1H, s, OH)ppm; ^{13}C NMR (100 MHz, DMSO-d_6) δ 30.3, 56.2, 106.7, 109.8, 109.8, 115.2, 116.6, 119.6, 124.0, 124.6, 124.9, 126.9, 132.2, 134.7, 140.3, 142.7, 145.5, 146.9, 148.9, 187.8 ppm; **15h**: ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.83 (3H, s, OCH_3), 3.87 (3H, s, NCH_3), 6.79 (1H, d, J = 8.0 Hz, benzimidazole-C_6H_3), 6.86 (1H, s, benzimidazole-C_6H_3), 7.00 (1H, d, J = 8.0 Hz, benzimidazole-C_6H_3), 7.21 (1H, d, J = 12.0 Hz, C*H*=CHAr), 7.26 (2H, s, $CH_3OC_6H_3$), 7.48 (1H, d, J = 12.0 Hz, $CH_3OC_6H_3$), 7.60 (1H, d, J = 12.0 Hz, benzimidazole-CH=C*H*), 7.67 (1H, d, J = 12.0 Hz, CH=C*H*Ar), 7.77 (1H, d, J = 12.0 Hz, benzimidazole-C*H*=CH) 9.23 (1H, s, OH), 9.57 (1H, s, OH)ppm; ^{13}C NMR (100 MHz, DMSO-d_6) δ 30.2, 56.1, 95.5, 112.4, 114.0, 115.0, 120.5, 122.6, 123.7, 127.9, 128.0, 130.1, 137.2, 138.0, 144.1, 147.1, 147.7, 150.8, 155.4, 188.1 ppm.

21. All experiments were carried out 24 h after cells were seeded. Chemical compounds were dissolved in DMSO and diluted with DMEM medium to final concentrations of 1, 5, 10, 20, and 40 μM. The tumor cells were incubated with chemical compounds for 24 or 48 h before the MTT assay. A 3-(4,5-dimethylthiazol-yl-2)-2,5-diphenyl tetrazolium bromide (MTT) reduction was determined to examine the effect of chemical compounds on cell viability. Briefly, following the treatment of cells with different concentrations of chemical compound (final concentration, 1, 5, 10, 20, and 40 μM) for 24 or 48 h, cells were incubated with MTT solution (0.5 mg/ml) for 3 h at 37 °C. The violet precipitate (formazan) in MTT-treated cells was dissolved with 100 μL of DMSO and then analyzed at a wavelength of 595 nm with a microplate reader (Beckman Coulter, Brea, CA). Each assay was performed in triplicate. IC_{50} was calculated by nonlinear regression analysis from a sigmoid dose–response curve using the GraphPad Prism software ver 3.0 (Graph-Pad Software, CA, U.S.A.) when R^2 >0.95.
22. MCF-7: Breast adenocarcinoma; SH-SY5Y: Human neuroblastoma; Hep-G2: Human hepatocellular carcinoma; H460: Lung carcinoma.