European Journal of Medicinal Chemistry 46 (2011) 417–422

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Short communication

Synthesis and *in vitro* cytotoxic evaluation of some thiazolylbenzimidazole derivatives

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ARTICLE INFO

Article history:
Received 22 June 2010
Received in revised form
6 November 2010
Accepted 9 November 2010
Available online 27 November 2010

Keywords:
Thiazolyl benzimidazole
Anticancer
Synthesis
SMMC-7721
A549

ABSTRACT

A novel kind of thiazolylbenzimidazole derivatives were designed and synthesized and evaluated for their antitumor activity against SMMC-7721 and A549 cell lines. Most compounds showed good antitumor activities, and compound **11b** displayed remarkable *in vitro* anticancer activity comparable to taxol. The preliminary structure–activity relationship of these benzimidazole derivatives was discussed based on the experimental data obtained.

1. Introduction

Benzimidazole derivatives are recognized as a class of important heterocycles for their pharmacological properties in the past years [1]. Existing studies have proved their wide range of biological activity as antiviral [2–7], antibacterial [8,9], antifungal [10] and antitumor [11–13] agents. On the other hand, many substituted thiazole derivatives were also proven to exhibit anticancer and antimicrobial activities [14,15]. In addition, other studies on thiazole-containing antitumor agents indicate that substituents on the thiazole ring play an important role for their biological activity [16].

Despite many 2-(thiazol-4-yl)-1*H*-benzoimidazole compounds (compound **1**, Fig. 1) have been synthesized and studied for their biological activity, such as antifungal, antibacterial and anthelmintic activities [17–20] 2-(thiazole-2-yl)-1*H*-benzimidazol derivatives (compound **2**, Fig. 1) have been seldom reported in the literature. Recently, Garuti and co-workers reported thiazolylbenzimidazole-4,7-diones showed good antiproliferative activity against some tumor cell lines [21]. However, it is well known that quinones could precede reductive alkylation process [22], making quinone derivatives potentially toxic both to tumor cell lines and to normal counterparts.

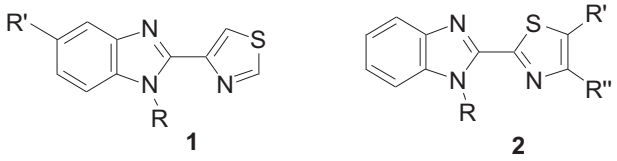

Fig. 1. The structures of two kinds of 2-thiazole-1H-benzimidazol.

In the light of the above reports and our continued interest in studying benzimidazole derivatives, we decided to synthesize a series of thiazolylbenzimidazole compounds and assess their antitumor activity. To increase the antitumor activity, efforts were exerted on the structure modification of the thiazole ring. Herein, we report the synthesis and antitumor activity of novel thiazolylbenzimidazole compounds related to the general structure **2**. And the preliminary structure–activity relationship was discussed (Fig. 1).

2. Chemistry

The synthesis of this kind of thiazolylbenzimidazoles started from 2-chloro-1*H*-benzoimidazole (**3**) (Scheme 1), which was

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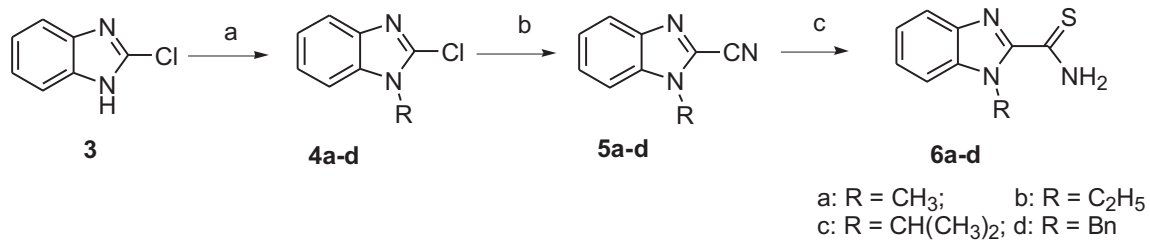
doi:10.1016/j.ejmech.2010.11.014



Scheme 1. Conditions and reagents: (a) organic iodides and bromides, NaH, DMF, 47–97%; (b) DMF, NaCN, 100 °C, 62–97%; (c) $(NH_4)_2S$, DMF, TEA, 50 °C, 47–90%.

prepared according the published method [23]. Reaction of **3** with organic iodide (methyl iodide) or bromides (ethyl bromide, 2-bromopropane and benzyl bromide) gave the corresponding *N*-alkyl benzimidazoles **4a**–**d**, which were subsequently treated with NaCN in DMF to afford the desired 2-cyano-benzimidazole intermediates **5a**–**d**. Using $(NH_4)_2S$ as sulfuration reagent [24], these cyano compounds were smoothly converted into thioformamides **6a**–**d**.

One subseries of the thiazolylbenzimidazoles was first synthesized from the key intermediate compounds **6a**–**d** [25]. Thus, reaction of compounds **6a**–**d** with three kinds of α-haloketones yielded the target compounds **7a**–**d**, **8a**–**c** and **9**, respectively (Scheme 2). This cyclization with ethyl 2-chloroacetoacetate proceeded smoothly and the yields were from moderate to good. However, reaction with ethyl bromopyruvate only resulted in poor to moderate yields. Reaction of **6a**–**d** with ethyl 4-chloroacetoacetate was also attempted, while only one thiazolylbenzimidazole compound **9** could be synthesized in a moderate yield.

Another subseries of the title compounds was prepared from **7d** (Scheme 3). Thus, **7d** was hydrolyzed to give the acid **10**, which was condensed with different primary or secondary amines to afford the amides **11a**–**g** in 42–94% yields.

3. Results and discussion

The cytotoxicity of the synthesized benzimidazoles, together with the reference drug taxol was evaluated in SMMC-7721 and A549 cell lines. The results are summarized in Table 1. The cytotoxicity of each compound was expressed as the concentration of compound required to kill 50% of the tumor cells.

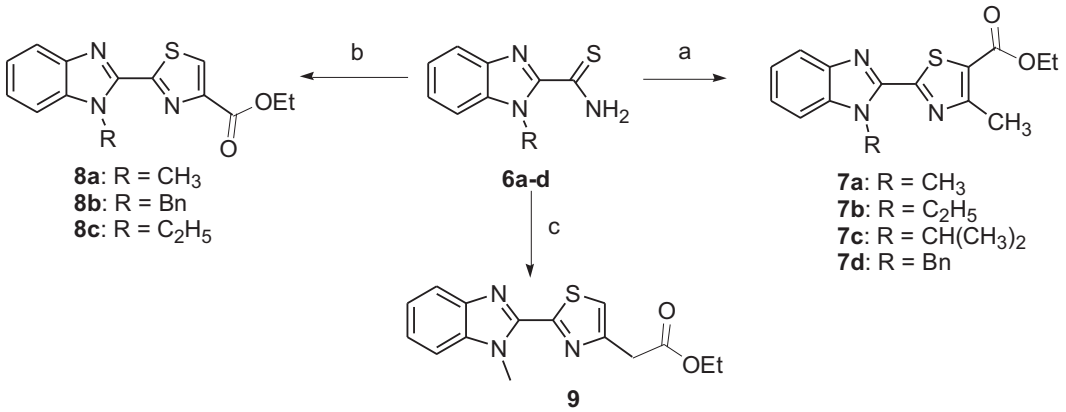
As shown in Table 1, most of these compounds exhibited good to moderate antitumor potency (IC_{50} from 1.14 to 33.31) against SMMC-7721 cell. The different kinds of substituents in *N*-1 position were first investigated. The *N*-CH_3 compound **7a** and *N*-C_2H_5 compound **7b** exhibited moderate activity (IC_{50} = 13.88 and 9.85, respectively), while replacement of the methyl or ethyl group with benzyl group resulted in a dramatically increased activity (**7d**, IC_{50} = 2.78), suggesting that the lipophilic character of substituents in *N*-1 position was an important determinant for the cytotoxicity. However, compound **7c** with an isopropyl group in *N*-1 position displayed much reduced antitumor potency (IC_{50} = 98.35).

To identify the effects of the thiazole ring on the biological profiles, this subseries of compounds **7**–**9** has different patterns of substitution on the thiazole's 4,5 positions. Compound **8a** and **8b** displayed moderate activity, while **9** showed slightly less potent cytotoxicity. However, both **8** and **9** were generally less potent antitumor activity than compounds **7** (with the exception of **7c**). This result suggested that both the electronic character and the position of substituents played important roles for the antitumor activity.

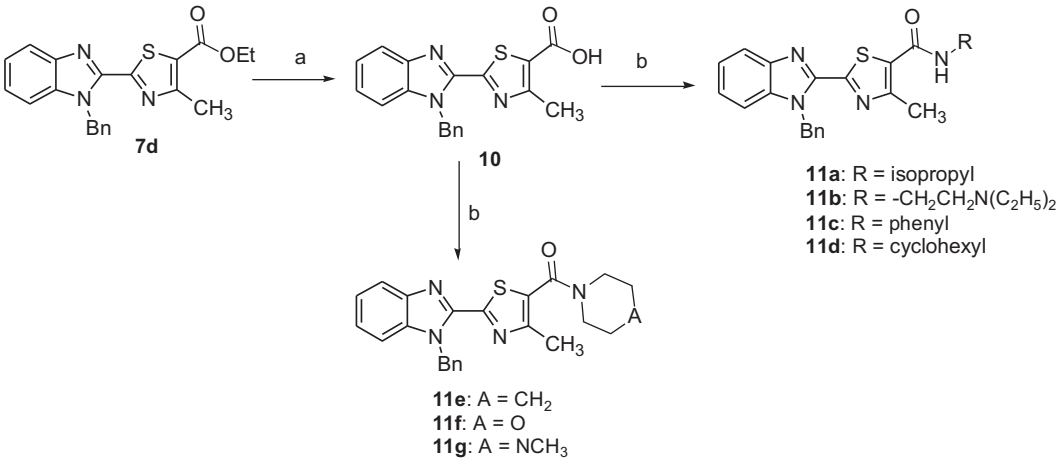
Although most of the above compounds exhibited antitumor activity against SMMC-7721 cell, none of them were active against A549 cell line. Nevertheless, **7d**, for its good antitumor activity, was selected as the benchmark compound for subsequent optimization.

Dramatic change in antitumor activity was observed, when the ester group of **7d** was transformed into its derivatives. The acid **10** displayed reduced activity (IC_{50} = 15.30 μM) against SMMC-7721 cell, whereas exhibited low antitumor activity (IC_{50} = 68.05 μM) against A549 cell line. Condensation of acid **10** with different primary or secondary amines afforded the amides **11a**–**g** (Scheme 3). As shown in Table 1, the amides from primary amines (**11a**–**d**) were more toxic than those from secondary amines (**11e**–**g**). Compounds (**11a**–**c**) were not only active for SMMC-7721 cell, but also exhibited good cytotoxicity against A549 cell, suggesting the hydrophilic feature of the thiazole ring was closely related with the antitumor activity.

Compound **11b**, with a flexible and basic alkyl chain, exhibited the potent antitumor activity against both SMMC-7721 cell and A549 cell. Replacement of the flexible basic side chain with phenyl group resulted in a slight loss of cytotoxicity (IC_{50} = 3.70 and 3.96, respectively). However, replacement of 2-diethylamino-ethyl side chain with hydrophilic cyclohexyl ring (**11d**) resulted in a 12-fold



Scheme 2. Conditions and reagents: (a) ethyl 2-chloroacetoacetate, ethanol, reflux, 32–70%; (b) ethyl bromopyruvate, ethanol, reflux, 25–42%; (c) ethyl 4-chloroacetoacetate, ethanol, reflux, 32%.



Scheme 3. Conditions and reagents: (a) KOH, methanol, H_2O, reflux, 72%; (b) primary and secondary amines, CDI (*N*,*N*-carbonyldiimidazole), THF, 42–94%.

Table 1
The Inhibition Concentration 50% of Compounds **7**–**11**.

Compounds	SMMC-7721		A549	
	IC_{50} [μM]	95% confidence interval	IC_{50} [μM]	95% confidence interval
7a	13.88	6.94–27.74	>20	–
7b	9.85	2.27–43.37	>20	–
7c	> 20	26.80–361.1	>20	–
7d	2.78	0.79–4.45	>20	–
8a	16.48	4.35–61.78	>20	–
8b	13.00	3.44–48.84	>20	
8c	> 20	9.78–221.9	>20	–
9	> 20	5.21–86.28	>20	–
10	15.30	4.47–52.29	>20	26.07–177.4
11a	6.90	6.07–7.74	6.64	3.92–11.28
11b	1.14	0.67–1.95	2.43	0.65–9.35
11c	3.70	1.46–9.36	3.96	2.45–7.75
11d	12.42	3.63–42.44	5.23	2.90–9.39
11e	>20	9.88–54.76	>20	19.42–127.8
11f	>20	13.99–146.7	>20	19.56–501.6
11g	20.0	13.25–30.23	>20	15.64–49.98
Taxol	1.42	0.43–4.63	0.63	0.19–2.13

decrease of activity against SMMC-7721 and a 2-fold decrease of activity against A549 cell. This result suggested again the hydrophilic feature of the amide groups played a pivotal role for the antitumor potency.

4. Conclusions

A series of thiazolylbenzimidazole derivatives was synthesized and assessed for antitumor activity *in vitro* against SMMC-7721 and A549 cells. Compounds **11a**–**c** displayed the optimal profiles, with IC_{50} in μM range. The most promising compound **11b** with potent antitumor activity was an attractive candidate for further assessment *in vivo* as antitumor agent. These results also indicate that the thiazolylbenzimidazole compounds with no quinone moiety could also serve as potent antitumor agents, which might exhibit fewer side-effects than the quinone counterparts.

5. Experimental

5.1. Chemistry

^{1}H NMR spectra were recorded on a Bruker DRX-500 (500 MHz). Mass spectra (MS) were determined on a Finnigan MAT-95 mass spectrometer. Chemical shifts (δ) are reported in parts per million (ppm). Data are reported as follows: chemical shift, multiplicity (br s = broad singlet, d = doublet, dd = doublet of doublet, dt = doublet of triplet, m = multiple, s = singlet and t = triplet), coupling constants (Hz), integration.

5.2. General procedures for the synthesis of compounds ***4a*–*d***

2-Chloro-1H-benzoimidazole (3.04 g, 20 mmol) was dissolved in dry DMF (15 mL) at 0 °C, to the solution was added NaH (0.91 g, 22.7 mmol), and the mixture was stirred for 1 h at 0 °C, then halide (21.6 mmol) was added. The mixture was stirred overnight at room temperature and was poured into water (50 mL) and stirred for 1 h, filtrated, washed with water and dried to afford **4a**–**d**.

5.2.1. 1-Methyl-2-chloro-1H-benzoimidazole (***4a***)

4a(1.75 g, 53%), mp 113–114 °C. ^{1}H NMR ($CDCl_3$): δ 3.79 (s, 3H), 7.28–7.30 (m, 3H), 7.69 (d, J = 7.6 Hz, 1H). EI-MS: m/z 166 $[M]^+$.

5.2.2. 1-Ethyl-2-chloro-1H-benzoimidazole (***4b***)

4b (2.64 g, 73%), mp 52–53 °C. ^{1}H NMR ($CDCl_3$): δ 1.75 (t, J = 7.8 Hz, 3H), 4.96 (q, J = 7.8 Hz, 2H), 7.27–7.31 (m, 3H), 7.55 (d, J = 7.6 Hz, 1H). EI-MS: m/z 180 $[M]^+$.

5.2.3. 1-Isopropyl-2-chloro-1H-benzoimidazole (***4c***)

4c (1.84 g, 47%), mp 53–54 °C. ^{1}H NMR ($CDCl_3$): δ 1.65 (d, J = 7.1 Hz, 6H), 4.90–4.93 (m, 1H), 7.24–7.26 (m, 2H), 7.49 (dd, J = 6.0 Hz, J = 2.0 Hz, 1H), 7.69 (dd, J = 6.0 Hz, J = 2.0 Hz, 1H). EI-MS: m/z 194 $[M]^+$.

5.2.4. 1-Benzyl-2-chloro-1H-benzoimidazole (***4d***)

4d (4.72 g, 97%), mp 107–109 °C. ^{1}H NMR ($CDCl_3$): δ 5.39 (s, 2 H), 7.18 (d, J = 6.5 Hz, 2H), 7.23–7.34 (m, 6H), 7.72 (d, J = 7.0 Hz, 1H). EI-MS: m/z 242 $[M]^+$.

5.3. General procedures for the synthesis of compounds ***5a*–*d***

4 (8.7 mmol) was dissolved in dry DMF (15 mL), to the solution was added NaCN (9.2 mol), and the mixture was stirred for 2 h at 100 °C. The mixture was poured into water (50 mL), filtrated, washed with water and dried to afford **5**.

5.3.1. 1-Methyl-2-cyano-1H-benzoimidazole (***5a***)

5a (1.16 g, 75%), mp 179–180 °C. ^{1}H NMR ($CDCl_3$): δ 4.01 (s, 3H), 7.41–7.45(m, 2H), 7.48–7.50(m, 1H), 7.86 (d, J = 8.0 Hz, 1H). EI-MS: m/z 157 $[M]^+$.

*5.3.2. 1-Ethyl-2-cyano-1H-benzoimidazole (**5b**)*

5b (0.92 g, 62%), mp 86–88 °C. ^{1}H NMR ($CDCl_3$): δ 1.56 (t, J = 7.1 Hz, 3H), 4.45 (q, J = 7.1 Hz, 3H), 7.40–7.49 (m, 3H), 7.87 (d, J = 8.3 Hz, 1H). EI-MS: m/z 171 $[M]^+$.

*5.3.3. 1-Isopropyl-2-cyano-1H-benzoimidazole (**5c**)*

5c (1.35 g, 84%), mp 67–68 °C. ^{1}H NMR ($CDCl_3$): δ 1.76 (d, J = 7.0 Hz, 6H), 4.98–5.02 (m, 1H), 7.38–7.46 (m, 2H), 7.57 (d, J = 9.0 Hz, 1H), 7.86–7.88 (m, 1H). EI-MS: m/z 185 $[M]^+$.

*5.3.4. 1-Benzyl-2-cyano-1H-benzoimidazole (**5d**)*

5d (2.02 g, 97%), mp 139–141 °C. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 5.55 (s, 2H), 7.23–7.26 (m, 2H), 7.33–7.44 (m, 6H), 7.87 (d, J = 7.5 Hz, 1H). EI-MS: m/z 233 $[M]^+$.

*5.4. General procedures for the synthesis of compounds **6a–d***

To a solution of **5** (7.6 mmol) in dry DMF (15 mL) was added TEA (2.2 mL) and 20% $(NH_4)_2S$ (2.2 mL). The mixture was stirred for 2 h at 50 °C, and then poured into water (60 mL). The precipitation was filtrated, washed with water and dried to afford **6a–d**.

*5.4.1. 1-Methyl-1H-benzoimidazole-2-carbothioic acid amide (**6a**)*

6a (1.14 g, 82%), mp 156–158 °C. ^{1}H NMR ($CDCl_3$): δ 4.34 (s, 3H), 7.34–7.44 (m, 3H), 7.74 (br, 1H), 7.77 (d, J = 8.0 Hz, 1H); 9.15 (br, 1H). EI-MS: m/z 191 $[M]^+$.

*5.4.2. 1-Ethyl-1H-benzoimidazole-2-carbothioic acid amide (**6b**)*

6b (1.13 g, 73%), mp 156–157 °C. ^{1}H NMR ($CDCl_3$): δ 1.53 (t, J = 7.1 Hz, 3H), 5.05 (q, J = 7.1 Hz, 3H), 7.35–7.47 (m, 3H), 7.51 (br, 1H), 7.78 (d, J = 8.0 Hz, 1H); 9.03 (br, 1H). EI-MS: m/z 205 $[M]^+$.

*5.4.3. 1-Isopropyl-1H-benzoimidazole-2-carbothioic acid amide (**6c**)*

6c (0.79 g, 47%), mp 163–164 °C. ^{1}H NMR ($CDCl_3$): δ 1.65 (d, J = 7.0 Hz, 6H), 6.50–6.53 (m, 1H), 7.29–7.33 (m, 2H), 7.64–7.66 (m, 1H), 7.73–7.75 (m, 1H), 8.01 (br, 1H), 9.35 (br, 1H). EI-MS: m/z 219 $[M]^+$.

*5.4.4. Benzyl-1H-benzoimidazole-2-carbothioic acid amide (**6d**)*

6d (1.83 g, 90%), mp 162–164 °C. ^{1}H NMR ($CDCl_3$): δ 6.36 (s, 2H), 7.14 (d, J = 7.1 Hz, 2H), 7.24–7.36 (m, 6H), 7.61 (br, 1H), 8.01 (d, J = 6.1 Hz, 1H), 9.08 (br, 1H). EI-MS: m/z 267 $[M]^+$.

*5.5. General procedures for the synthesis of compounds **7a–d***

6 (1 mmol) and ethyl 2-chloroacetoacetate (1.2 mmol) were added into EtOH (10 mL). The reaction mixture was heated at reflux for 8 h, and then poured into water (30 mL), extracted with $CHCl_3$. The combined organic layer was washed with saturated brine, dried over anhydrous Na_2SO_4, and evaporated under reduced pressure. The residue was subjected to silica column chromatography (EtOAc/hexane) to give **7a–d** as white solids.

*5.5.1. 4-Methyl-2-(1-methyl-1H-benzoimidazol-2-yl)-thiazole-5-carboxylic acid ethyl ester (**7a**)*

7a (0.16 g, 57%), mp 105–107 °C. ^{1}H NMR ($CDCl_3$): δ 1.33 (t, J = 7.1 Hz, 3H), 2.81 (s, 3H), 4.27 (s, 3H), 4.33 (q, J = 7.1 Hz, 2H), 7.32 (t, J = 8.0 Hz, 1H), 7.41 (t, J = 8.0 Hz, 1H), 7.69 (d, J = 8.0 Hz, 1H), 7.74 (d, J = 8.0 Hz, 1H). ^{13}C NMR ($CDCl_3$): δ 162.25, 161.38, 160.98, 145.08, 142.87, 137.24, 133.92, 124.58, 123.51, 120.59, 110.22, 61.60, 32.35, 17.79, 14.42. EI-MS: m/z 301 $[M]^+$. HRMS (EI): Cal. for: 301.0885, found: 301.0879.

*5.5.2. 4-Methyl-2-(1-ethyl-1H-benzoimidazol-2-yl)-thiazole-5-carboxylic acid ethyl ester (**7b**)*

7b (0.10 g, 32%), mp 111–114 °C. ^{1}H NMR ($CDCl_3$): δ 1.35 (t, J = 7.1 Hz, 3H), δ: 1.53 (t, J = 7.1 Hz, 3H), 2.84 (s, 3H), 4.40 (q, J = 7.1 Hz, 2H), 4.92 (q, J = 7.1 Hz, 2H), 7.34–7.42 (m, 2H), 7.48 (d, J = 7.9 Hz, 1H), 7.86 (d, J = 8.1 Hz, 1H). ^{13}C NMR ($CDCl_3$): δ 162.30, 161.86, 160.88, 145.01, 144.12, 135.22, 132.40, 124.10, 129.95, 121.23, 111.87, 61.53, 45.83, 22.66, 17.67, 14.42. EI-MS: m/z 315 $[M]^+$. HRMS (EI): Cal. for: 315.1041, found: 315.1039.

*5.5.3. 4-Methyl-2-(1-isopropyl-1H-benzoimidazol-2-yl)-thiazole-5-carboxylic acid ethyl ester (**7c**)*

7c (0.14 g, 42%), mp 149–152 °C. ^{1}H NMR ($CDCl_3$): δ 1.38 (t, J = 7.2 Hz, 3H), δ: 1.71 (d, J = 7.0 Hz, 6H), 2.80 (s, 3H), 4.36 (q, J = 7.2 Hz, 2H), 6.40–7.45 (m, 1H), 7.28–7.32 (m, 2H), 7.66–7.68 (m, 1H), 7.82–7.84 (m, 1H). ^{13}C NMR ($CDCl_3$): δ 162.29, 161.85, 160.88, 144.53, 143.97, 135.06, 132.49, 124.01, 123.17, 121.03, 113.31, 61.59, 48.92, 21.51, 17.87, 14.45. EI-MS: m/z 329 $[M]^+$. HRMS (EI): Cal. for: 329.1198, found: 329.1196.

*5.5.4. 4-Methyl-2-(1-benzyl-1H-benzoimidazol-2-yl)-thiazole-5-carboxylic acid ethyl ester (**7d**)*

7d (0.26 g, 70%), mp 179–181 °C. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 1.38 (t, J = 7.2 Hz, 3H), δ: 2.76 (s, 3H), 4.36 (q, J = 7.2 Hz, 2H), 6.16 (s, 2H), 7.20–7.38 (m, 8H), 7.83–7.86 (m, 1H); ^{13}C NMR ($CDCl_3$): δ 163.13, 158.12, 157.54, 148.50, 144.46, 143.05, 140.57, 136.83, 129.90, 128.20, 127.25, 124.82, 123.70, 120.64, 111.06, 61.60, 39.64, 32.35, 14.43. EI-MS: 377 $[M]^+$, 348, 332, 300, 286, 272, 258, 234, 219, 207, 188, 175, 169, 152, 144, 129, 116, 102, 91, 85, 77, 71, 65, 51, 45. HRMS: Cal. for: 377.1198, found: 377.1199.

*5.6. General procedures for the synthesis of compounds **8a–c***

6 (1 mmol) and ethyl bromopyruvate (1.2 mmol) were added into EtOH (10 mL). The reaction mixture was heated at reflux for 8 h, and then poured into water (30 mL), extracted 3 times with $CHCl_3$. The combined organic layer was washed with saturated brine, dried over anhydrous Na_2SO_4, and evaporated under reduced pressure. The residue was subjected to silica column chromatography (EtOAc/hexane) to give **8** as white solids.

*5.6.1. 2-(1-Methyl-1H-benzoimidazol-2-yl)-thiazole-4-carboxylic acid ethyl ester (**8a**)*

8a (0.12 g, 42%), mp 197–201(dec)°C. ^{1}H NMR ($CDCl_3$): δ 1.36 (t, J = 7.0 Hz, 3H), 4.32 (s, 3H), 4.39 (q, J = 7.0 Hz, 2H), 7.34 (t, J = 8.0 Hz, 1H), 7.41 (t, J = 8.0 Hz, 1H), 7.71 (d, J = 8.0 Hz, 1H), 7.76 (d, J = 8.0 Hz, 1H), 8.73 (s, 1H). ^{13}C NMR ($CDCl_3$): δ 160.32, 151.88, 149.37, 139,97, 133.79, 132.59, 132.32, 127.90, 127.77, 117.13, 111.81, 62.35, 34.07, 29.87, 14.50. ESI-MS: 288 $[M+1]^+$. HRMS: Cal. for: 287.0728, found: 287.0723.

*5.6.2. 2-(1-Benzyl-1H-benzoimidazol-2-yl)-thiazole-4-carboxylic acid ethyl ester (**8b**)*

8b (0.13 g, 35%), mp 163–166 °C. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 1.43 (t, J = 7.2 Hz, 3H), 4.44 (q, J = 7.2 Hz, 2H), 6.17 (s, 2H), 7.23–7.27 (m, 3H), 7.32–7.36 (m, 4H), 7.46–7.48 (m, 1H), 7.86–7.88 (m, 1H), 8.26 (s, 1H); ^{13}C NMR ($CDCl_3$): δ 160.96, 158.41, 157.65, 148.58, 143.43, 140.58, 136.13, 130.20, 128.88, 127.69, 125.51, 124.59, 119.87, 111.38, 61.75, 49.04, 14.49. EI-MS: 363 $[M]^+$, 334, 318, 258, 205, 161, 154, 144, 129, 116, 102, 90, 83 77, 71, 65, 51, 45. HRMS: Cal. for: 363.1041, found: 363.1036.

*5.6.3. 2-(1-Ethyl-1H-benzoimidazol-2-yl)-thiazole-4-carboxylic acid ethyl ester (**8c**)*

8c (80 mg, 25%), mp 181–184 °C. ^{1}H NMR ($CDCl_3$): δ 1.36 (t, J = 7.0 Hz, 3H), δ: 1.55 (t, J = 7.1 Hz, 3H), 4.38 (q, J = 7.1 Hz, 2H), 4.45

(q, J = 7.0 Hz, 2H), 7.36 (t, J = 8.0 Hz, 1H), 7.43 (t, J = 8.0 Hz, 1H), 7.72 (d, J = 8.0 Hz, 1H), 7.78 (d, J = 8.0 Hz, 1H), 8.75 (s, 1H). ^{13}C NMR ($CDCl_3$): δ 160.49, 152.80, 149.32, 140,24, 134.06, 132.53, 132.25, 127.96, 127.71, 116.93, 111.82, 65.27, 61.18, 40.29, 37.57, 15.87. EI-MS: m/z 287 $[M]^+$. HRMS (EI): Cal. for: 287.0728, found: 287.0827.

5.7. [2-(1-Methyl-1H-benzoimidazol-2-yl)-thiazol-4-yl]-acetic acid ethyl ester (**9**)

A mixture of **6a** (1 mmol) and ethyl 4-chloroacetoacetate (1.2 mmol) in EtOH (10 mL) was heated at reflux for 8 h. Then the mixture was poured into water (30 mL), extracted with $CHCl_3$. The combined organic layer was washed with saturated brine, dried over anhydrous Na_2SO_4, and evaporated to dryness under reduced pressure. The residue was subjected to silica column chromatography (EtOAc/hexane) to give **9** as a white solid (0.10 g, 3%), mp 95–97 °C. ^{1}H NMR ($CDCl_3$): δ 1.30 (t, J = 7.2 Hz, 3H), 3.93 (s, 2H), 4.24 (q, J = 7.2 Hz, 2H), 4.30 (s, 3H), 7.30–7.38 (m, 3H), 7.43 (d, J = 7.9 Hz, 1H), 7.82 (d, J = 7.8 Hz, 1H), 8.73 (s, 1H). ^{13}C NMR ($CDCl_3$): δ 159.21, 150.06, 144.71, 142.80, 135.99, 124.14, 123.73, 123.25, 120.32, 118.94, 110.19, 66.49, 61.02, 37.94, 15.19. EI-MS: m/z 301 $[M]^+$. HRMS (EI): Cal. for: 301.0885, found: 301.0883.

5.8. 2-(1-Benzyl-1H-benzoimidazol-2-yl)-4-methyl-thiazole-5-carboxylic acid (**10**)

KOH (1.6 g) was dissolved in H_2O/MeOH (40 mL/8 mL), then **7d** (3.0 g, 8.6 mmol) was added. The reaction mixture was heated at reflux for 2.5 h, cooled and adjusted to pH = 3–4 with 2 N HCl. The resultant precipitation was filtrated, washed with water and dried to give **10** as a yellow solid (2.0 g, 72%), mp 225–228 °C (dec). ^{1}H NMR (DMSO-d_6): δ 2.62 (s, 3H), 6.17 (s, 2H), 7.20–7.29 (m, 7H), 7.58 (d, J = 7.0 Hz, 1H), 7.72 (d, J = 6.8 Hz, 1H). ^{13}C NMR (DMSO-d_6): δ 164.74, 155.86, 152.84, 144.84, 142.33, 137.35, 136.16, 128.56, 127.41, 127.07, 124.03, 123.80, 122.92, 119.53, 111.33, 66.76, 47.54, 16.58. EI-MS: m/z 349 $[M]^+$. HRMS (EI): Cal. for: 349.0883, found: 349.0885.

5.9. General procedures for the synthesis of compounds **11a–g**

A mixture of **10** (100 mg, 0.29 mmol) and CDI (100 mg, 0.62 mmol) in dry THF (10 mL) was stirred at room temperature for 0.5 h. Then amine (0.58 mmol) was added and the resultant solution was stirred at room temperature overnight. After the reaction was completed, the mixture was poured into water. The precipitation was filtrated, washed with water, dried and recrystallized from ethanol to give **11a–g** as white solids.

5.9.1. 2-(1-Benzyl-1H-benzoimidazol-2-yl)-4-methyl-thiazole-5-carboxylic acid isopropylamide (**11a**)

11a (50 mg, 42%), mp 238–241 °C. ^{1}H NMR ($CDCl_3$): δ 1.26 (d, J = 6.6 Hz, 6H), 2.71 (s, 3H), 4.21–4.25 (m, 1H), 5.59–5.61 (m, 1H), 6.14 (s, 2H), 7.12–7.24 (m, 2H), 7.25–7.28 (m, 3H), 7.30–7.32 (m, 2H), 7.36–7.38 (m, 1H), 7.81–7.84 (m, 1H). ^{13}C NMR (DMSO-d_6): δ 159.72, 157.31, 154.40, 143.84, 142.21, 137.08, 136.28, 128.73, 128.66, 127.49, 127.06, 124.36, 123.28, 119.73, 111.52, 47.57, 41.44, 22.17, 16.83. ESI-MS: m/z 391 $[M + 1]^+$. HRMS (EI): Cal. for: 390.1514, found: 390.1512.

5.9.2. 2-(1-Benzyl-1H-benzoimidazol-2-yl)-4-methyl-thiazole-5-carboxylic acid (2-diethylamino-ethyl)-amide (**11b**)

11b (0.10 g, 78%), mp 136–138 °C. ^{1}H NMR ($CDCl_3$): δ 1.05 (t, J = 7.1 Hz, 6H), 2.59 (q, J = 7.1 Hz, 4H), 2.65 (t, J = 5.4 Hz, 2H), 2.73 (s, 3H), 3.47–3.49 (m, 2H), 6.14 (s, 2H), 6.84 (s, 1H), 7.19–7.31 (m, 7H), 7.33–7.35 (m, 1H), 7.82–7.84 (m, 1H). ^{13}C NMR (DMSO-d_6): δ 160.45, 157.47, 154.58, 143.78, 142.18, 137.07, 136.28, 128.65, 127.45, 127.07, 124.45, 123.32, 119.73, 111.54, 51.17, 47.59, 46.64, 17.03, 16.87, 11.94. EI-MS: m/z 447 $[M]^+$. HRMS (EI): Cal. for: 447.2093, found: 447.2096.

5.9.3. 2-(1-Benzyl-1H-benzoimidazol-2-yl)-4-methyl-thiazole-5-carboxylic acid phenylamide (**11c**)

11c (80 mg, 66%), mp 212–214 °C. ^{1}H NMR (DMSO-d_6): δ 2.74 (s, 3 H), 6.19 (s, 2 H), 7.16–7.20 (m, 6 H), 7.29–7.37 (m, 5H), 7.52 (d, J = 7.0 Hz, 1H), 7.65 (d, J = 6.8 Hz, 1H), 7.70 (d, J = 7.0 Hz, 1H), 7.89 (s, 1H). ^{13}C NMR (DMSO-d_6): δ 160.55, 157.42, 152.48, 139.66, 138.16, 136.92, 130.74, 128.71, 128.63, 127.54, 127.03, 126.97, 124.87, 123.61, 121.74, 119.95, 118.33, 118.14, 47.76, 17.64. EI-MS: m/z 424 $[M]^+$. HRMS (EI): Cal. for: 424.1358, found: 424.1363.

5.9.4. 2-(1-Benzyl-1H-benzoimidazol-2-yl)-4-methyl-thiazole-5-carboxylic acid cyclohexylamide (**11d**)

11d (0.12 g, 94%), mp 228–230 °C. ^{1}H NMR ($CDCl_3$): δ 1.07–1.09 (m, 4H), 1.66–1.72 (m, 6H), 2.70 (s, 3H), 3.45–3.48 (m, 1H), 5.65 (s, 1H), 6.13 (s, 2H), 7.19–7.24 (m, 3H), 7.29–7.35 (m, 5H), 7.80–7.82 (m, 1H). ^{13}C NMR ($CDCl_3$): δ 160.79, 157.99, 156.86, 144.56, 143.09, 136.84, 136.72, 128.98, 127.98, 127.61, 127.37, 124.79, 123.75, 120.66, 111.11, 48.70, 26.46, 24.67, 16.57. EI-MS: m/z 430 $[M]^+$. HRMS (EI): Cal. for: 430.1827, found: 430.1826.

5.9.5. [2-(1-Benzyl-1H-benzoimidazol-2-yl)-4-methyl-thiazol-5-yl]-piperidin-1-yl-methanone (**11e**)

11e (90 mg, 76%), mp 180–181 °C. ^{1}H NMR ($CDCl_3$): δ 1.68–1.70 (m, 6H), 2.48 (s, 3H), 3.57–3.59 (m, 4H), 6.14 (s, 2H), 7.21–7.31 (m, 7H), 7.35 (d, J = 8.1 Hz, 1H), 7.84 (d, J = 8.1 Hz, 1H). ^{13}C NMR ($CDCl_3$): δ 162.20, 158.47, 153.27, 144.81, 143.08, 136.90, 136.78, 128.91, 127.86, 127.28, 127.04, 124.57, 123.56, 120.60, 111.01, 48.70, 26.46, 24.67, 16.57. EI-MS: m/z 416 $[M]^+$. HRMS (EI): Cal. for: 416.1671, found: 416.1673.

5.9.6. [2-(1-Benzyl-1H-benzoimidazol-2-yl)-4-methyl-thiazol-5-yl]-morpholin-4-yl-methanone (**11f**)

11f (0.10 g, 83%), mp 126–130 °C. ^{1}H NMR ($CDCl_3$): δ 2.50 (s, 3H), 3.67–3.73 (m, 8H), 6.13 (s, 2H), 7.20–7.24 (m, 1H), 7.29–7.37 (m, 5H), 7.82–7.84 (m, 1H). ^{13}C NMR (DMSO-d_6): δ 162.31, 158.79, 153.75, 144.61, 143.00, 136.80, 136.74, 128.86, 127.85, 127.21, 126.31, 124.62, 123.58, 120.56, 110.99, 55.14, 48.65, 46.09, 17.14, 16.64. EI-MS: m/z 418 $[M]^+$. HRMS (EI): Cal. for: 418.1463, found: 418.1464.

5.9.7. [2-(1-Benzyl-1H-benzoimidazol-2-yl)-4-methyl-thiazol-5-yl]-(4-methyl-piperazin-1-yl)-methanone (**11g**)

11g (70 mg, 57%), mp 188–191 °C. ^{1}H NMR ($CDCl_3$): δ 2.33 (s, 3H), 2.42–2.46 (m, 4H), 2.49 (s, 3H), 3.67–3.69 (m, 4H), 6.13 (s, 2H), 7.20–7.33 (m, 7H), 7.35 (d, J = 7.4 Hz, 1H), 7.84 (d, J = 7.4 Hz, 1H). ESI-MS: m/z 432 $[M + 1]^+$. HRMS (EI): Cal. for: 432.1867, found: 432.1866.

5.10. Antitumor activity assay

Compounds **7–11** were studied for their *in vitro* anticancer activities against HCT-116 cells by MTT-based assay. Cells were maintained as a suspension in RPMI-1640 medium supplemented with 10% fetal calf serum at 37 °C in a humidified atmosphere containing 5% CO_2. Then the cells were planted onto standard 96-well plates at the concentration 104 cells per well and allowed to proliferate for 24 h under the above conditions. The compounds were added in six threefold dilutions (10 μg/mL to 0.04 μg/mL) with taxol co-assayed as a positive control. After 72 h exposure period, then 15 μL of 5 mg/mL MTT were added to each well and the plates were incubated for 4 h at 37 °C. The medium was then aspirated

and the formazan product was solubilized by 100 μL DMSO. The absorbance was measured at 570 nm using an ELISA Reader. Each assay was carried out at least three times, and the results of the experiment were summarized in the Table 1.

Acknowledgements

We gratefully acknowledge the financial support for Shanghai Municipal Natural Science Foundation (10ZR1409600). And we also thank Lab of Organic Functional Molecules, the Sino-French Institute of ECNU for supports.

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