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# Synthesis and anticancer evaluation of bis(benzimidazoles), bis(benzoxazoles), and benzothiazoles

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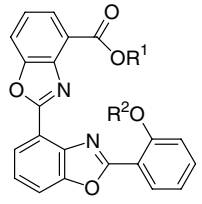
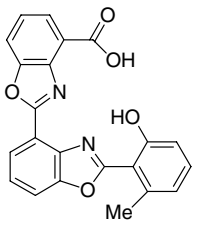
**Abstract**—Four classes of UK-1 analogues were synthesized and their cytotoxicity testing against human A-549, BFTC-905, RD, MES-SA, and HeLa carcinoma cell lines was determined. The results revealed that UK-1 and four of these analogues (**15**–**18**) are potent against the cancer cell lines. In particular, compound **16** is more potent than UK-1 against A-549 and HeLa cell lines, and compounds **15**, **17**, and **18** selectively exhibit potent cytotoxic activity against the BFTV-905 cells ($IC_{50}$ 9.6 μM), A-549 cells ($IC_{50}$ 6.6 μM), and MES-SA cells ($IC_{50}$ 9.2 μM), respectively.


## 1. Introduction

Taniguchi et al. isolated the bis(benzoxazole) natural product UK-1 from the mycelial cake of an actinomycete strain 517-02.[1–3] Subsequently, Tsuji et al. isolated AJI9561 from the mycelium extract of *Streptomyces* sp. AJ9561.[4] UK-1 exhibits significant cytotoxic activity against B16, HeLa, and P338 cells, but does not exhibit any growth-inhibiting activity against Gram-positive bacteria, yeasts or fungi at up to 100 μg/mL.[1] AJI9561 exhibited cytotoxic activity against both Jurkat and P388 cells with $IC_{50}$ values of 0.88 and 1.63 μM, respectively. However, the methyl derivative MUK-1 and alkaline hydrolysate demethyl derivative DMUK-1 both exhibit activity against Gram-positive and Gram-negative bacteria.[2,3] MUK-1 is also active against yeast and filamentous fungi.[2] Kerwin et al. investigated the metal ion coordination by UK-1 and found that UK-1 binds $Zn^{2+}$ and $Mg^{2+}$ to form stable complexes with an overall stoichiometry of 1:1.[5] $Fe^{3+}$ ions are bound slightly less strongly by UK-1, and $Ca^{2+}$ ions form weak complexes with this ligand. In the presence of $Mg^{2+}$, UK-1 binds to double-stranded DNA ten times more tightly than in the absence of $Mg^{2+}$. Like the antitumor agent quinobenzoxazines, which also bind $Mg^{2+}$ and bind to DNA in a magnesium ion-dependent fashion, UK-1 inhibits the activity of human topoisomerase II.



**UK-1**: $R^1$ = Me, $R^2$ = H
**MUK-1**: $R^1$ = Me, $R^2$ = Me
**DMUK-1**: $R^1$ = H, $R^2$ = H



**AJI9561**

Kerwin et al. subsequently reported the activity of UK-1 against a wide range of human cancer cell lines.[6] UK-1 exhibits a wide spectrum of potent anticancer activity against leukemia, lymphoma, and certain solid tumor-derived cell lines, with $IC_{50}$ values of as low as 20 nM, but is inactive against *Staphylococcus aureus*. A series of analogues of the bis(benzoxazole) natural product UK-1 in which the carbomethoxy-substituted benzoxazole ring of the natural product was modified were prepared, and their anticancer and antibacterial properties evaluated. An analogue of UK-1, in which the carbomethoxy-substituted benzoxazole ring was replaced by a carbomethoxy-substituted benzimidazole ring (compound **B**), was inactive against human cancer cell lines and the two strains of *S. aureus*. In contrast, a simplified analogue in which the carbomethoxy-substituted benzoxazole ring was replaced with a carbomethoxy group

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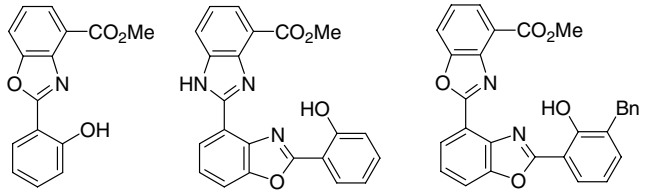
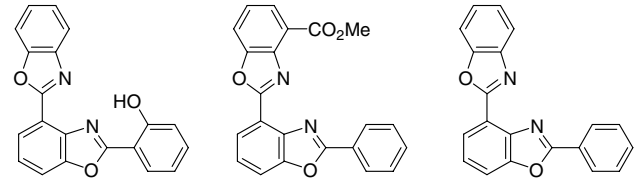
(compound **A**) was almost as active as UK-1 against the four cancer cell lines examined but exhibited no activity against *S. aureus*. The benzylated UK-1 analogue (compound **C**) is more active than UK-1 against HT-29 cells, about 10-fold less potent than UK-1 against MCF-7 and PC-3 cells, and 20-fold less active than UK-1 against HL60 cells. Metal ion binding investigations of these analogues indicate that they bind both $Zn^{2+}$ and $Ca^{2+}$ approximately as strongly as UK-1. The non-cytotoxic benzimidazole UK-1 analogue binds $Mg^{2+}$ ions 50 times more weakly than UK-1, while the simple benzoxazole analogue binds $Mg^{2+}$ ions almost as effectively as UK-1. These results are evidence of the involvement of $Mg^{2+}$ ion binding in the selective cytotoxicity of UK-1 and its provision of a minimal pharmacophore for the selective cytotoxic activity of the natural product.




**A** **B** **C**

Smith et al. synthesized three new analogues of UK-1 and evaluated their efficacies as topoisomerase II inhibitors.[7] Their results revealed that UK-1 and two of these analogues (compounds **D** and **E**) are catalytic inhibitors of topoisomerase II, whereas compound **F** shows no measurable activity, and identify a critical structural motif required for enzyme inhibition.




**D** **E** **F**

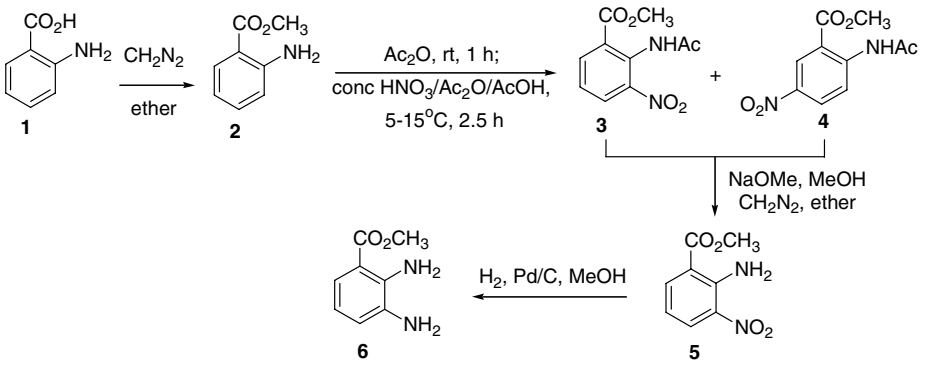
A number of structural analogues of this natural product were prepared to understand the structural basis of the selective cytotoxicity of UK-1. A comparison of the anticancer activity of these compounds with that of their structurally simplified analogues of UK-1 elucidates the natural product's selective activity against cancer cells.

## 2. Chemistry

A number of analogues of UK-1 in which the carbomethoxy-substituted benzoxazole ring was either modified or deleted were prepared. These UK-1 analogues were synthesized in a manner that was analogous to the previously reported total synthesis of the natural product[6–8] with some improvements. An analogue of UK-1, in which the carbomethoxy-substituted benzoxazole ring is replaced with a substituted benzimidazole ring, was prepared according to the Schemes 1–3. Compound **6** was obtained in five steps, starting with the esterification of 2-aminobenzoic acid (**1**) with diazomethane to yield methyl 2-aminobenzoate (**2**) in 94% yield (Scheme1). The nitration of compound **2** gave 2-acetylamino-3-nitro-benzoic acid methyl ester **3** with a 37% yield, and 2-acetylamino-5-nitro-benzoic acid methyl ester **4** with a 61% yield.[9] Following separation, the acetyl group of **3** was cleaved using sodium methoxide[10] and treated with diazomethane to esterify partially hydrolyzed ester **5** with a 87% yield. The nitro group of **5** was reduced by hydrogenation on Pd/C to yield 2,3-diaminobenzoic acid methyl ester **6** with a 96% yield.

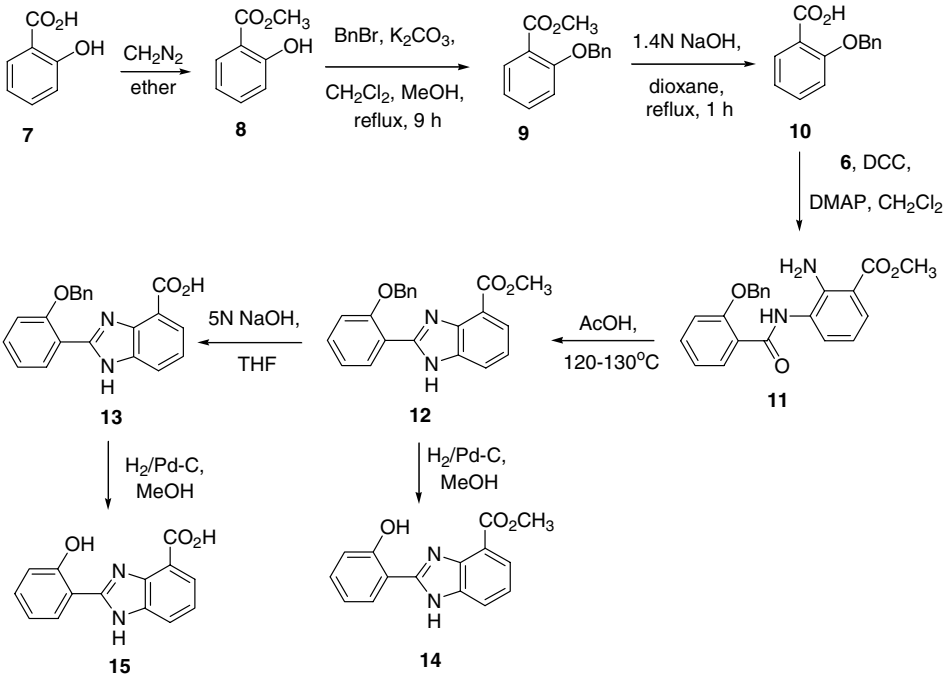
Compound **12** was prepared in five steps, starting with the esterification of salicylic acid **7** by diazomethane to generate methyl salicylate **8** in quantitative yield (Scheme 2). The hydroxyl group of **8** was protected with benzyl ether by treating with benzyl bromide and potassium carbonate to give **9** in 93% yield.[11] Compound **9** was transformed to the carboxylic acid **10** in quantitative yield by saponification reaction. Compound **10** was coupled with **6** by treating it with dicyclohexylcarbodiimide to furnish amide **11** with a 83% yield which was then converted to benzimidazole **12** in 87% yield via cyclization-dehydration by refluxing in acetic acid.[12] Compound **12** was saponified by 5 M NaOH to give **13** in 97% yield. The benzyl groups in **12** and **13** were cleaved by hydrogenation to generate the corresponding phenols **14** and **15** in 97% and 60% yields, respectively.

Compound **13** was transformed to acyl chloride by treatment with thionyl chloride and then coupled with

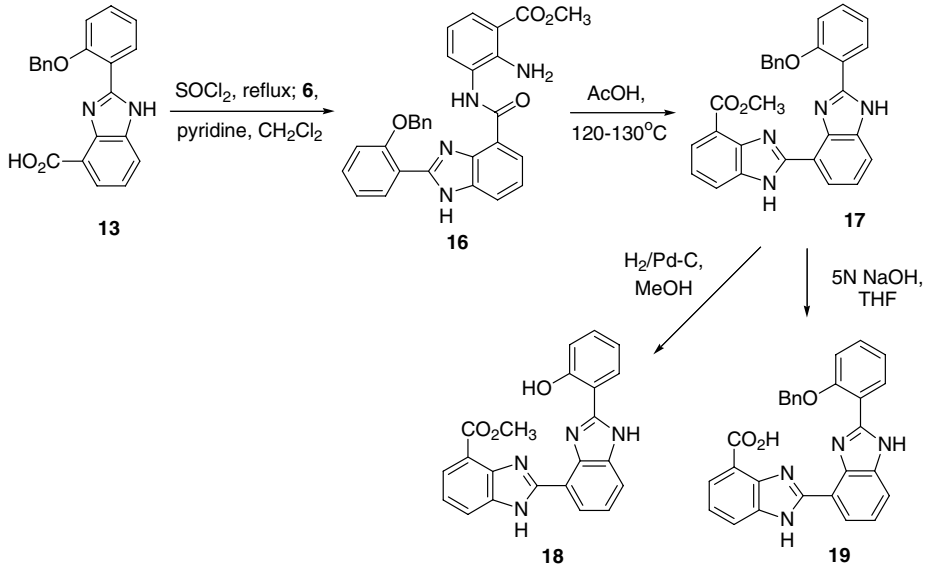



**1** **2** **3** **4** **5** **6**

**Scheme 1.** Synthesis of 2,3-diaminobenzoic acid methyl ester **6**.




**Scheme 2.** Synthesis of benzimidazole analogues **12**–**15**.

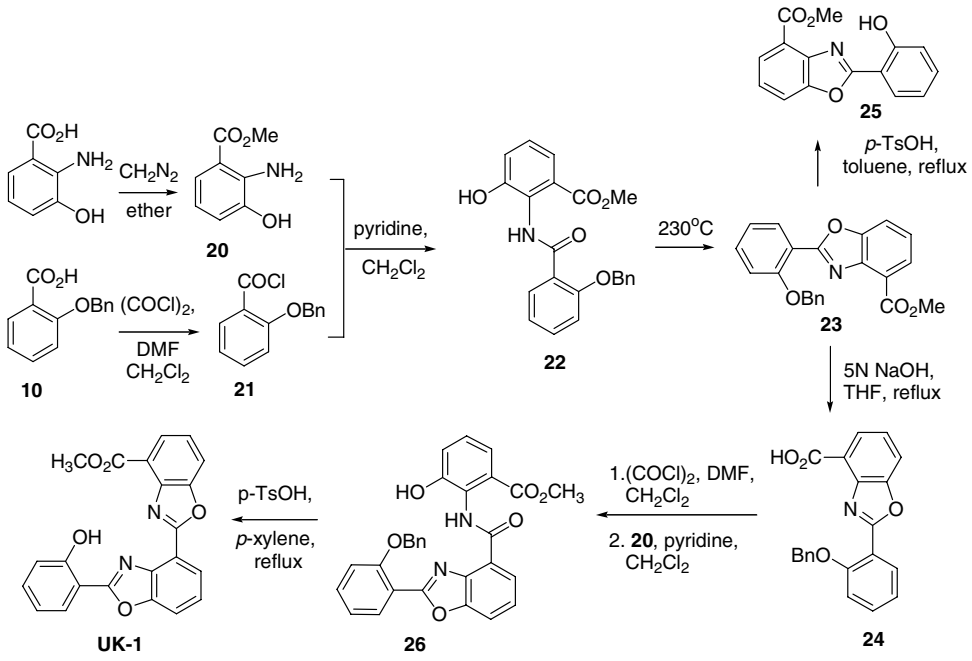



**Scheme 3.** Synthesis of bis(benzimidazole) analogues **17**–**19**.

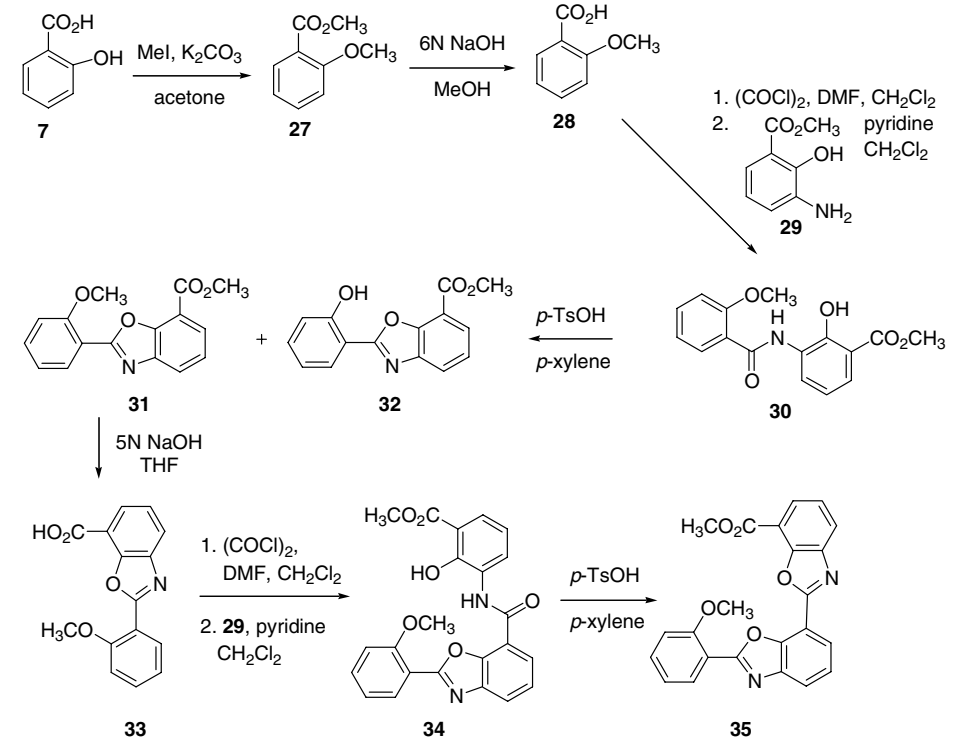
**6** to give **16** in 56% yield (Scheme 3). Compound **16** was refluxed in acetic acid to form the bis(benzimidazole) **17** in 85% yield, and this **17** was converted to **18** in quantitative yield by hydrogenation, and to **19** in 66% yield by saponification.

UK-1 is typically synthesized following the procedure of Kerwin et al. (Scheme 4).[5] Compound **20** was prepared from 2-amino-3-hydroxybenzoic acid by treating with diazomethane. Compound **10** was transformed to acyl chloride by treating with oxalyl chloride and *N*,*N*-dimethylformamide, and then reacted with **20** to give the amide **22** in 96% yield. Heating **22** to 230 °C produced a cyclization-dehydration product **23** in 86% yield, which was saponified to give **24** in 84% yield. Compound **24** was converted to acyl chloride by treating it with oxalyl chloride and *N*,*N*-dimethylformamide, and then reacting it with **20** to give **26** in 76% yield. Compound **23** was refluxed with *p*-toluenesulfonic acid in toluene to give compound **25** in 51% yield. Final cyclodehydration and debenzylation were performed by refluxing **26** with *p*-toluenesulfonic acid in *p*-xylene to give the **UK-1** in 43% yield.

One of the UK-1 analogues, **35**, was prepared from salicylic acid presented in Scheme 5. Salicylic acid

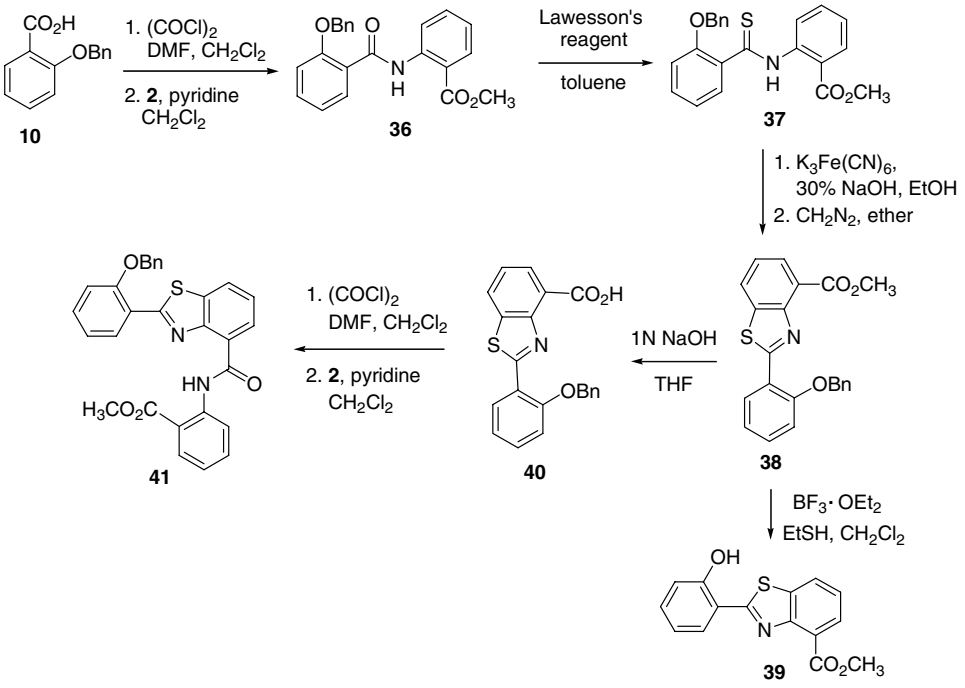



**Scheme 4.** Synthesis of **UK-1**.




**Scheme 5.** Synthesis of **31**–**35**.

was transformed to **27** by treating it with iodomethane and potassium carbonate.[13] It was then treated with sodium hydroxide to give **28** in 51% yield (in 2 steps). Compound **28** was converted to acyl chloride and coupled with **29** to produce amide **30** in 74% yield. Compound **30** was refluxed with *p*-toluenesulfonic acid in *p*-xylene to give **31** by cyclization-dehydration in 32% yield and a demethylation product **32** in 9% yield. Compound **31** was converted to **33** by saponification. Then, **33** was treated with oxalyl chloride and *N*,*N*-dimethylformamide to produce the corresponding acyl chloride, which was coupled with **29** to give the amide **34** in 61% yield (in two steps). Compound **34** was refluxed with *p*-toluenesulfonic acid in *p*-xylene to give **35** by cyclization-dehydration.




**Scheme 6.** Synthesis of **38**–**41**.

UK-1 analogues with benzothiazole moiety were prepared according to Scheme 6. Compound **10** was treated with oxalyl chloride and *N*,*N*-dimethylformamide to produce the corresponding acyl chloride, which was coupled with **2** to give the amide **36** in 81% yield. Then, **36** was converted to thioamide **37** in 51% yield by treatment with Lawesson's reagent.[14] Thioamide **37** was cyclized to benzothiazoles **38** by Jacobson synthesis with alkaline potassium ferricyanide. The crude products contained compounds **38** and **40**. Therefore, the crude products were treated with diazomethane and purified by flash column chromatography to give **38** in 21% yield. Compound **39** was prepared by the debenzylation of **38** using ethanethiol and boron trifluoride-diethyl etherate. Compound **38** was converted to acid **40** by saponification. Then, **40** was treated with oxalyl chloride and *N*,*N*-dimethylformamide and the corresponding acyl chloride was produced;[15] it was coupled with **2** to give the amide **41** in 51% yield (in two steps). Then, an attempt was made to convert **41** to thioamide by treating it with Lawesson's reagent and Jacobson synthesis with alkaline potassium ferricyanide was used to prepare the corresponding bis(benzothiazole), but the attempt was unsuccessful.

## 3. Biological assay

The cytotoxicity of UK-1 against P388, B16, and HeLa cell lines has been reported elsewhere.[1] A range of human cancer cell lines A-549 and HeLa cells were investigated to determine the spectrum of cell lines whose growth is inhibited by UK-1. The results, presented in Table 1, indicate that UK-1 exhibits potent anticancer activity against human lung and epithelial carcinoma cell lines. The cytotoxicities of the UK-1 analogues to cancer cells were examined in order to elucidate the structural basis for selective cytotoxicity of the natural product toward a unique cancer cell. Table 1 summarizes the results of cytotoxicity testing against human

**Table 1.** $IC_{50}$[a] (μM) values of tumor cell lines after 72 h continuous exposure to test compounds

| Compound | Tumor type/cell line | | | | |
|---|---|---|---|---|---|
| | A-549[b] | BFTC-905[c] | RD[d] | MES-SA[e] | HeLa[f] |
| **12** | 70.0 | 53.8 | >100 | 50.6 | 67.6 |
| **13** | 87.0 | 66.4 | >100 | 91.0 | 67.3 |
| **14** | 74.1 | 37.9 | 35.9 | 33.2 | 31.5 |
| **15** | 38.2 | 9.6 | 17.6 | 52.7 | 52.5 |
| **16** | 2.8 | 11.9 | 13.8 | <5 | 7.1 |
| **17** | 6.6 | 36.8 | 44.9 | 15.3 | nd |
| **18** | 22.2 | 29.9 | 26.5 | 9.2 | 14.5 |
| **19** | 46.9 | 21.4 | 28.0 | 33.2 | 29.3 |
| **23** | >100 | 89.2 | nd | 91.3 | 82.1 |
| **24** | >100 | 98.3 | >100 | 63.9 | 90.3 |
| **25** | >100 | 49.6 | 58.5 | >100 | 75.8 |
| **26** | 50.8 | 84.9 | 87.8 | 41.0 | 51.4 |
| **31** | >100 | >100 | >100 | >100 | 52.6 |
| **32** | >100 | 54.7 | >100 | 89.4 | 67.6 |
| **33** | >100 | >100 | >100 | >100 | >100 |
| **34** | nd[g] | nd | nd | nd | nd |
| **39** | >100 | 87.3 | 39.1 | 76.7 | 81.6 |
| **41** | nd | nd | nd | nd | nd |
| UK-1 | 5.1 | nd | nd | nd | 10.7 |

[a] $IC_{50}$ is the concentration that induces 50% growth inhibition compared with untreated control cells.
[b] human lung carcinoma cell line.
[c] human bladder carcinoma transitional cell line.
[d] human rhabdomyosarcoma cell line.
[e] human uterine sarcoma cell line.
[f] Human epithelial carcinoma cell line.
[g] nd, not determined.

A-549, BFTC-905, RD, MES-SA, and HeLa carcinoma cell lines. UK-1 exhibits potent cytotoxic activity against the A-549 and HeLa cancer cells lines, but compound **16** is more potent than UK-1 against both A-549 cells ($IC_{50}$ 2.8 μM vs 5.1 μM for UK-1) and HeLa cells ($IC_{50}$ 7.1 μM vs 10.7 μM for UK-1). Compound **16** is the precursor of compound **18**, but compound **18** is less potent than **16** against A-549 cells ($IC_{50}$ 22.2 μM vs 2.8 μM for **16**), MES-SA ($IC_{50}$ 9.2 μM vs <5 μM for **16**), and HeLa cells ($IC_{50}$ 14.5 μM vs 7.1 μM for **16**). Only one benzimidazole ring was formed in compound **16**, and this skeleton relative to **18** may increase the flexibility to coordinate with $Mg^{2+}$ ion, and enhanced cytotoxicity against cancer cell lines. Compound **26** is the precursor of UK-1, but compound **26** is less potent than UK-1 against A-549 cells ($IC_{50}$ 50.8 μM vs 5.1 μM for UK-1) and HeLa cells ($IC_{50}$ 151.4 μM vs 10.7 μM for UK-1). Even though compound **18** has a bis(benzimidazole) skeleton and UK-1 has the corresponding bis(benzoxazole) skeleton, compound **18** is less potent than UK-1 against A-549 cells ($IC_{50}$ 22.2 μM vs 5.1 μM for UK-1) and HeLa cells ($IC_{50}$ 14.5 μM vs 10.7 μM for UK-1). Kerwin et al. reported the ability of UK-1 to form metal ion complexes that can bind to DNA[5,17] and inhibit DNA-processing enzymes,[5] which findings indicate that $Mg^{2+}$ ion binding by UK-1 may lead to biologically relevant complexes with a specific target in cancer cells. Therefore, the interaction between bis(benzoxazole) and the $Mg^{2+}$ ion may be stronger than that of bis(benzimidazole). Interestingly, compounds **15**, **17**, and **18** selectively exhibit potent cytotoxic activity against the BFTC-905 cells ($IC_{50}$ 9.6 μM), A-549 cells ($IC_{50}$ 6.6 μM), and MES-SA cells ($IC_{50}$ 9.2 μM), respectively.

Compounds **14**, **25**, **32**, and **39** have the same skeleton and differ only in their hetero atoms. Interestingly, the compound that has benzimidazole moiety exhibits stronger anticancer activity against cell lines than those that contain benzoxazole or benzothiazole. Compound **18** is less potent than UK-1 against cancer cell lines. Compound **18** contains bis(bezoimidazole) moiety, and UK-1 has bis(benzoxazole) moiety, perhaps because the steric energies[18] of chelating complexes of $Mg^{2+}$ with compounds **18** (11.71 kcal/mol) exceed that of UK-1 (9.29 kcal/mol). When ester **14** was hydrolyzed to form the corresponding acid **15**, the power of **15** to inhibit BFTC-905 was increased. The potency of compound **17** against A-549 is stronger than that of compound **18**, while the inhibitive potency against BFTC-905, RD, and MES-SA of compound **18** exceeds that of compound **17**. The hydroxyl group of compound **18** was converted to benzyl ether in **17**. Therefore, the binding sites in compounds **18** and **17** should differ from each other in the cell lines, because compound **17** is large sized and hydrophobic. Compound **16** is the most potent against cancer cell lines herein. The flexibility of **16** may increase its ability to chelate with $Mg^{2+}$ and to promote the interaction at the binding site. Unfortunately, compound **26** is the precursor of UK-1 that corresponds to the compound **16** as the precursor of compound **18**. It does not powerfully inhibit activity against cancer cell lines, even though it has good flexibility. Although compounds **16** and **26** have anilide functional groups, the positions of $-NH_2$ groups that form anilide differ, and the conformations of their complexes formed with $Mg^{2+}$ differ. This occurrence may imply that compound **26** is less potent than compound **16** and UK-1, even though it is the precursor of UK-1.

### 3.1. Conclusion

The natural product of bis(benzoxazole), UK-1, has potential for use against cancer cell lines. Structurally similar bis(benzimidazole) **17** and **18** were also found to exhibit potent anticancer activity. Interestingly, compound **16** is the synthetic precursor of compounds **17**-**19** and is more potent than UK-1 against human lung (A-549) and epithelial (HeLa) carcinoma cell lines. Therefore, further work with UK-1, compounds **15**–**18**, and their analogues, must be conducted to determine whether such targeting is involved in the selective cytotoxicity of UK-1 and compounds **15**–**18**, and whether the promising spectrum of the in vitro anticancer activity of UK-1 and **15**–**18** reported herein is also reflected in vivo.

## 4. Experimental

### 4.1. Chemistry

**4.1.1. General methods.** Melting points were recorded using a Yanagimoto Micro Melting Point Apparatus Model-S3 capillary melting point apparatus and are uncorrected. TLC analysis was carried out on silica gel 60 F254 precoated glass sheets and detected under UV light. $^1H$ and $^{13}C$ NMR spectra were recorded employing a Bruker AVANCE DPE-300 or VARIAN UNITYINOVA-500 spectrometer, and chemical shifts ($\delta$) are in ppm relative to $CHCl_3$ (7.26 ppm or 77 ppm), $CD_3OD$ ($\delta$ = 3.30 ppm or 49 ppm), and $(CD_3)_2SO$ (2.49 ppm or 39.7 ppm) as internal standard. Infrared spectra were recorded on a JASCO FT/IR 410 spectrometer. Mass spectra were recorded on a Micromass Platform II or Finnigan/Thermo Quest MAT 95XL spectrometer. In each case proton NMR showed the presence of indicated solvent(s). All chemicals and solvents were purchased from Aldrich Chemical Co., Fisher Scientific, or Lancaster.

**4.1.1.1. 2-Amino-benzoic acid methyl ester (2).** To a solution of 2-aminobenzoic acid (6.00 g, 44.0 mmol) in diethyl ether (100 mL) was added a solution of diazomethane in diethyl ether at 0 °C until ceased to generate nitrogen. After adding 1 mL of acetic acid, the reaction solution was concentrated and the resulting residue was purified by flash column chromatography using silica gel as the stationary phase and using ethyl acetate/hexane (1:49–1:19) as the mobile phase producing compound **2** (6.25 g, 41.0 mmol). Yield: 94%. $^1H$ NMR (300 MHz, $CDCl_3$, $\delta$): 7.87–7.83 (d, $J$ = 8.1 Hz, 1H), 7.29–7.23 (dd, $J$ = 8.4, 7.0 Hz, 1H), 6.67–6.61 (m, 2H), 5.71 (s, 2H), 3.86 (s, 3H). $^{13}C$ NMR (125.7 MHz, $CDCl_3$, $\delta$): 168.4, 149.6, 134.0, 131.1, 116.9, 116.7, 111.1, 51.5. IR (KBr): 3481, 3371, 1692, 1435, 1274, 752 $cm^{-1}$. MS $m/z$: 152 ($M^+$+1, 5), 151 ($M^+$, 65), 120

(31), 119 (100), 92 (50). HRMS-EI (*m*/*z*): $[M]^+$ calcd for $C_8H_9NO_2$, 151.0633; found, 151.0637.

**4.1.1.2. 2-Acetylamino-3-nitro-benzoic acid methyl ester (3) and 2-acetylamino-5-nitro-benzoic acid methyl ester (4).** A solution of compound **2** (6.30 g, 41.0 mmol) in acetic anhydride (47.0 mL) was stirred at room temperature for 1 h, then a solution of acetic anhydride (9.6 mL), acetic acid (8.4 mL), and concd nitric acid (13.7 mL) was added slowly at 5–15 °C. After stirring at 5–15 °C for 2.5 h, the reaction solution was poured into ice–water (50 mL) and the precipitate was filtered to give compound **4** (5.89 g, 25.0 mmol). The filtrate was diluted with water (50 mL) and stood overnight to give crystalline **3**. The mother liquid was extracted with dichloromethane, after concentration the residue was purified by flash column chromatography using silica gel as the stationary phase and using ethyl acetate/hexane (1:3, 1:1) as the mobile phase producing compound **3** (3.68 g, 15.0 mmol, combined with crystalline **3**).

**4.1.1.3. Compound 3.** Yield: 37%. mp: 120–122 °C. $^1$H NMR (300 MHz, $CDCl_3$, $\delta$): 10.34 (s, 1H), 8.22–8.19 (d, $J$ = 6.3 Hz, 1H), 8.11–8.07 (d, $J$ = 8.2 Hz, 1H), 7.36–7.31 (dd, $J$ = 8.1, 6.5 Hz, 1H), 3.96 (s, 3H), 2.23 (s, 3H). $^{13}$C NMR (125.7 MHz, $CDCl_3$, $\delta$): 168.6, 166.6, 144.2, 134.8, 132.1, 129.4, 123.8, 122.6, 53.0, 24.1. IR (KBr): 3307, 1683, 1527, 1438, 1365, 1267, 1120 cm$^{-1}$. MS *m*/*z*: 238 ($M^+$, 0.58), 196 (100), 192 (15), 165 (12), 164 (53), 90 (14). HRMS-EI (*m*/*z*): $[M]^+$ calcd for $C_{10}H_{10}N_2O_5$, 238.0590; found, 238.0591.

**4.1.1.4. Compound 4.** Yield: 61%. mp: 177–181 °C. $^1$H NMR (300 MHz, $CDCl_3$, $\delta$): 11.38 (s, 1H), 8.95–8.92 (m, 2H), 8.39–8.35 (d, $J$ = 9.4 Hz, 1H), 4.0 (s, 3H), 2.29 (s, 3H). $^{13}$C NMR (125.7 MHz, $CDCl_3$, $\delta$): 169.4, 167.2, 146.5, 141.6, 129.4, 126.8, 120.3, 114.4, 53.0, 25.5. IR (KBr): 3004, 1685, 1587, 1508, 1436, 1348, 902, 821 cm$^{-1}$. MS *m*/*z*: 238 ($M^+$, 18), 196 (100), 164 (45), 119 (12), 57 (28), 55 (15). HRMS-EI (*m*/*z*): $[M]^+$ calcd for $C_{10}H_{10}N_2O_5$, 238.0590; found, 238.0587.

**4.1.1.5. 2-Amino-3-nitro-benzoic acid methyl ester (5).** To a solution of compound **3** (0.50 g, 2.1 mmol) in anhydrous methanol (25 mL) was added a sodium methoxide solution (37.0 mL, 0.07 mmol, 1.92 M in methanol) at room temperature. After the reaction was completed, added acetic acid to neutralize the reaction solution. The solvent was removed, and the residue was extracted by ethyl acetate. The extract was dried by anhydrous magnesium sulfate and concentrated. The resulting residue was dissolved in a mixture of diethyl ether and methanol, and added diazomethane at 0 °C until ceased to generate nitrogen. After adding a few drops of acetic acid, the reaction solution was concentrated to produce product **5** (0.40 g, 2.0 mmol). Yield: 87%. mp: 97–100 °C. $^1$H NMR (300 MHz, $CD_3Cl_3$, $\delta$): 8.40–8.37 (d, $J$ = 8.4 Hz, 1H), 8.25–8.22 (d, $J$ = 7.7 Hz, 1H), 6.68–6.63 (dd, $J$ = 8.3, 7.8 Hz, 1H), 3.92 (s, 3H). $^{13}$C NMR (125.7 MHz, $CDCl_3$, $\delta$): 167.4, 147.2, 139.3, 133.1, 132.2, 114.3, 113.9, 52.2. IR (KBr): 3120, 3028, 953, 882, 847, 706 cm$^{-1}$. MS *m*/*z*: 197 ($M^+$+1, 9), 196 ($M^+$, 100), 165 (46), 119 (24), 118 (16), 90 (41). HRMS-EI (*m*/*z*): $[M]^+$ calcd for $C_8H_8N_2O_4$, 196.0484; found, 196.0489.

**4.1.1.6. 2,3-Diamino-benzoic acid methyl ester (6).** A mixture of compound **5** (1.00 g, 5.1 mmol) and Pd/C (0.10 g, 10% of Pd on charcoal) in methanol (30 mL) under hydrogen atmosphere (60 psi) was hydrogenated for 3 h. The catalyst was removed by filtration, the filtrate was concentrated, and the resulting residue was purified by flash column chromatography using silica gel as the stationary phase and using ethyl acetate/hexane (3:7) as the mobile phase producing compound **6** (0.81 g, 4.9 mmol). Yield: 96%. mp: 63–65 °C. $^1$H NMR (300 MHz, $CDCl_3$, $\delta$): 7.48–7.45 (d, $J$ = 8.1 Hz, 1H), 6.86–6.83 (d, $J$ = 7.5 Hz, 1H), 6.62–6.57 (dd, $J$ = 7.9, 7.7 Hz, 1H), 3.87 (s, 3H). $^{13}$C NMR (125.7 MHz, $CDCl_3$, $\delta$): 168.9, 141.1, 134.1, 122.6, 120.6, 116.6, 111.9, 51.5. IR (KBr): 3367, 1693, 1433, 1287, 1234, 733 cm$^{-1}$. MS *m*/*z*: 167 ($M^+$+1, 8), 166 ($M^+$, 100), 135 (19), 134 (74), 106 (97), 105 (28). HRMS-EI (*m*/*z*): $[M]^+$ calcd for $C_8H_{10}N_2O_2$, 166.0742; found, 166.0748.

**4.1.1.7. 2-Hydroxy-benzoic acid methyl ester (8).** To a solution of compound **7** (7.80 g, 57.0 mmol) in diethyl ether and methanol was added a solution of diazomethane in diethyl ether at 0 °C until ceased to generate nitrogen. After adding a few drops of acetic acid, the reaction solution was concentrated to produce product **8** (8.60 g, 57.0 mmol). Yield: 100%. $^1$H NMR (300 MHz, $CDCl_3$, $\delta$): 10.75 (s, 1H), 7.85–7.82 (d, $J$ = 8.0 Hz, 1H), 7.46–7.42 (dd, $J$ = 8.7, 8.4 Hz, 1H), 6.99–6.96 (d, $J$ = 8.4 Hz, 1H), 6.90–6.85 (dd, $J$ = 8.2, 8.1 Hz, 1H), 3.95 (s, 3H). $^{13}$C NMR (125.7 MHz, $CDCl_3$, $\delta$): 170.5, 161.5, 135.6, 129.8, 119.1, 117.5, 112.3, 52.2. IR (KBr): 3186, 1678, 1305, 1090, 845, 757 cm$^{-1}$. MS *m*/*z*: 152 ($M^+$, 49), 121 (27), 120 (100), 92 (53), 65 (16). HRMS-EI (*m*/*z*): $[M]^+$ calcd for $C_8H_8O_3$, 152.0473; found, 152.0479.

**4.1.1.8. 2-Benzyloxy-benzoic acid methyl ester (9).** To a solution of compound **8** (10.00 g, 28.0 mmol) in anhydrous dichloromethane (99.0 mL) and anhydrous methanol (99.0 mL) were added potassium carbonate (20.00 g, 145.0 mmol) and benzyl bromide (15.70 mL, 132.0 mmol), and refluxed for 40 h under nitrogen atmosphere. After filtration, the filtrate was concentrated, and the residue was purified by flash column chromatography using silica gel as the stationary phase and using ethyl acetate/hexane (1:49) as the mobile phase producing compound **9** (14.79 g, 61.0 mmol). Yield: 93%. $^1$H NMR (300 MHz, $CDCl_3$, $\delta$): 7.84–7.80 (d, $J$ = 7.6 Hz, 1H), 7.51–7.31 (m, 6H), 7.02–6.99 (m, 2H), 5.19 (s, 2H), 3.91 (s, 3H). $^{13}$C NMR (125.7 MHz, $CDCl_3$, $\delta$): 166.7, 158.0, 136.6, 133.3, 131.3, 128.4, 127.6, 126.6, 120.6, 120.4, 113.7, 70.4, 51.8. IR (KBr): 2949, 1727, 1599, 1450, 1249, 754 cm$^{-1}$. MS *m*/*z*: 242 ($M^+$, 3), 210 (4), 92 (9), 91 (100), 65 (8). HRMS-EI (*m*/*z*): $[M]^+$ calcd for $C_{15}H_{14}O_3$, 242.0943; found, 242.0951.

**4.1.1.9. 2-Benzyloxy-benzoic acid (10).** To a solution of compound **9** (5.20 g, 21.0 mmol) in 1,4-dioxane (50 mL) was added 1.4 M sodium hydroxide solution (22 mL) and refluxed for 2 h. After concentration, the

remained concentrated solution was acidified to pH 1 by adding 1 N hydrochloric acid. The acidic solution was extracted with ethyl acetate, and the extract was dried over anhydrous magnesium sulfate, filtered, and concentrated to produce compound **10** (5.00 g, 22.0 mmol). Yield: 100%. mp: 73–75 °C. $^1$H NMR (300 MHz, $CDCl_3$, $\delta$): 10.8 (s, 1H), 8.23–8.2 (d, $J$ = 7.8 Hz, 1H), 7.57–7.56 (m, 1H), 7.45–7.42 (m, 5H), 7.18–7.12 (m, 2H), 5.26 (s, 2H). $^{13}$C NMR (125.7 MHz, $CDCl_3$, $\delta$): 165.3, 157.3, 135.0, 134.2, 133.8, 129.16, 129.12, 127.8, 122.4, 117.9, 113.0, 72.1. IR (KBr): 3048, 2952, 1727, 1600, 1450, 1251, 1083, 755 cm$^{-1}$. MS *m*/*z*: 228 ($M^+$, 1), 92 (11), 91 (100), 69 (9), 57 (15). HRMS-EI (*m*/*z*): $[M]^+$ calcd for $C_{14}H_{12}O_3$, 228.0786; found, 228.0795.

**4.1.1.10. 2-Amino-3-(2-benzyloxy-benzoylamino)-benzoic acid methyl ester (11).** To a solution of compound **10** (1.40 g, 6.0 mmol) and compound **6** (0.50 g, 3.0 mmol) in anhydrous dichloromethane (15 mL) were added dicyclohexylcarbodiimide (1.23 g, 6.0 mmol) and 4-(dimethylamino)pyridine (12 mg) under argon atmosphere, and stirred at room temperature for 7 h. The reaction mixture was filtered through celite, and the filtrate was concentrated. The residue was purified by flash column chromatography using silica gel as the stationary phase and using ethyl acetate/hexane (1:19, 1:9, 3:17) as the mobile phase producing compound **11** (0.94 g, 25.0 mmol). Yield: 83%. mp: 115–117 °C. $^1$H NMR (300 MHz, $CDCl_3$, $\delta$): 9.16 (s, 1H), 8.28–8.26 (d, $J$ = 7.8 Hz, 1H), 7.76–7.73 (d, $J$ = 8.1 Hz, 1H), 7.54–7.44 (m, 7H), 7.18–7.13 (m, 2H), 6.67–6.62 (dd, $J$ = 7.9, 7.9 Hz, 1H), 5.56 (s, 2H), 5.26 (s, 2H), 3.85 (s, 3H). $^{13}$C NMR (75.4 MHz, $CDCl_3$, $\delta$): 168.4, 164.0, 156.6, 145.0, 135.1, 133.3, 132.6, 130.1, 129.1, 128.8, 128.6, 124.6, 121.9, 121.8, 115.8, 112.7, 112.3, 71.7, 51.6. IR (KBr): 3282, 2935, 1631, 1446, 1243, 1091, 746 cm$^{-1}$. MS *m*/*z*: 377 ($M^+$+1, 5), 376 ($M^+$, 24), 211 (20), 165 (16), 121 (12). HRMS-EI (*m*/*z*): $[M]^+$ calcd for $C_{22}H_{20}N_2O_4$, 376.1423; found, 376.1419.

**4.1.1.11. 2-(2-Benzyloxy-phenyl)-1*H*-benzoimidazole-4-carboxylic acid methyl ester (12).** A solution of compound **11** (0.48 g, 1.3 mmol) in acetic acid (6 mL) was refluxed at 120–130 °C for 3 h. After cooled to room temperature, the reaction solution was poured into ice water and neutralized by sodium bicarbonate. The aqueous solution was extracted with dichloromethane, and the extract was washed with brine and dried over anhydrous magnesium sulfate. The dry solution was concentrated and dried under vacuum to give compound **12** (0.40 g, 1.1 mmol). Yield: 87%. mp: 103–105 °C. $^1$H NMR (300 MHz, $CDCl_3$, $\delta$): 11.45 (s, 1H), 8.62–8.59 (d, $J$ = 6.4 Hz, 1H), 8.03–8 (d, $J$ = 7.7 Hz, 1H), 7.9–7.87 (d, $J$ = 1 Hz, 1H), 7.56–7.53 (m, 2H), 7.46–7.42 (m, 4H), 7.34–7.29 (m, 1H), 7.21–7.16 (m, 2H), 5.36 (s, 2H), 3.66 (s, 3H). $^{13}$C NMR (75.4 MHz, $CDCl_3$, $\delta$): 166.3, 156.4, 150.9, 143.8, 135.7, 134.2, 131.6, 130.5, 128.9, 128.5, 128.0, 124.5, 124.3, 121.9, 121.7, 118.0, 113.4, 113.0, 71.3, 45.1. IR (KBr): 3437, 1719, 1598, 1461, 1286, 994, 746 cm$^{-1}$. MS *m*/*z*: 359 ($M^+$+1, 7), 358 ($M^+$, 33), 252 (14), 207 (11), 91 (100). HRMS-EI (*m*/*z*): $[M]^+$ calcd for $C_{22}H_{18}N_2O_3$, 358.1317; found, 358.1324.

**4.1.1.12. 2-(2-Benzyloxy-phenyl)-1*H*-benzoimidazole-4-carboxylic acid (13).** To a solution of compound **12** (0.37 g, 1.0 mmol) in tetrahydrofuran (7.3 mL) was added 5 M sodium hydroxide solution (4.5 mL), and refluxed for 12 h. After cooled to room temperature, the reaction solution was acidified by concd hydrochloric acid to give a white precipitate. The precipitate was filtered and washed with water, and dried under vacuum to produce compound **13** (0.34 g, 1.0 mmol). Yield: 97%. mp: 180–182 °C. $^1$H NMR (300 MHz, $CD_3OD$, $\delta$): 8.22–8.19 (d, $J$ = 7.6 Hz, 1H), 8.07–8.05 (d, $J$ = 8 Hz, 2H), 7.79–7.07 (m, 2H), 7.51–7.48 (m, 3H), 7.36–7.29 (m, 4H), 5.46 (s, 2H). $^{13}$C NMR (125.7 MHz, $CDCl_3$, $\delta$): 167.1, 158.9, 150.0, 137.0, 136.8, 133.5, 131.8, 131.5, 129.8, 129.5, 129.2, 128.9, 127.3, 123.1, 119.8, 118.4, 115.7, 112.5, 72.8. IR (KBr): 3351, 2607, 1697, 1128, 848, 622 cm$^{-1}$. MS *m*/*z*: 345 ($M^+$+1, 8), 344 ($M^+$, 39), 298 (8), 238 (8), 236 (9), 91 (100). HRMS-EI (*m*/*z*): $[M]^+$ calcd for $C_{21}H_{16}N_2O_3$, 344.1161; found, 344.1159.

**4.1.1.13. 2-(2-Hydroxy-phenyl)-1*H*-benzoimidazole-4-carboxylic acid methyl ester (14).** A mixture of compound **12** (0.21 g, 0.6 mmol) and Pd/C (0.02 g, 10% of Pd on charcoal) in ethyl acetate (10 mL) under hydrogen atmosphere (60 psi) was hydrogenated for 2 h. The catalyst was removed by filtration, and the filtrate was concentrated to give compound **14** (0.15 g, 0.6 mmol). Yield: 97%. mp: 205–207 °C. $^1$H NMR (300 MHz, $CD_3Cl_3$, $\delta$): 10.72 (s, 1H), 7.96–7.91 (dd, $J$ = 8.2, 7.8 Hz, 2H), 7.70–7.67 (d, $J$ = 7.8 Hz, 1H), 7.43–7.32 (m, 2H), 7.15–7.12 (d, $J$ = 8.1 Hz, 1H), 7.02–6.97 (dd, $J$ = 7.8, 7.3 Hz, 1H), 4.04 (s, 3H). $^{13}$C NMR (75.4 MHz, $CDCl_3$, $\delta$): 167, 159.1, 152.2, 142.4, 133.4, 125.0, 123.9, 122.3, 119.2, 118.1, 113.2, 111.7, 52.3. IR (KBr): 3394, 1702, 1463, 1284, 750 cm$^{-1}$. MS *m*/*z*: 269 ($M^+$+1, 8), 268 ($M^+$, 49), 237 (17), 236 (100), 208 (18). HRMS-EI (*m*/*z*): $[M]^+$ calcd for $C_{15}H_{12}N_2O_3$, 268.0848; found, 268.0843.

**4.1.1.14. 2-(2-Hydroxy-phenyl)-1*H*-benzoimidazole-4-carboxylic acid (15).** A mixture of compound **13** (0.20 g, 0.6 mmol) and Pd/C (0.02 g, 10% of Pd on charcoal) in methanol (15 mL) under hydrogen atmosphere (60 psi) was hydrogenated for 2 h. The catalyst was removed by filtration, and the filtrate was concentrated, and the resulting residue was purified by flash column chromatography using silica gel as the stationary phase and using methanol/ethyl acetate (1:4) as the mobile phase producing compound **15** (0.09 g, 0.4 mmol). Yield: 60%. mp: 310 °C (decomposed). $^1$H NMR (300 MHz, DMSO-$d_6$, $\delta$): 8.26 (b, 1H), 7.76–7.74 (b, 2H), 7.34–7.28 (b, 1H), 7.24–7.16 (b, 2H), 6.91 (b, 1H). $^{13}$C NMR (125.7 MHz, DMSO, $\delta$): 169.1, 157.6, 151.5, 141.2, 134.0, 131.6, 127.7, 124.2, 121.5, 120.3, 119.0, 117.1, 113.5. IR (KBr): 3396, 1585, 1488, 1384, 1292, 1257, 748 cm$^{-1}$. MS *m*/*z*: 255 ($M^+$+1, 6), 254 ($M^+$, 43), 237 (15), 236 (100), 208 (20). HRMS-EI (*m*/*z*): $[M]^+$ calcd for $C_{14}H_{10}N_2O_3$, 254.0691; found, 254.0684.

**4.1.1.15. 2-Amino-3-{[2-(2-benzyloxy-phenyl)-1*H*-benzoimidazole-4-carbonyl]-amino}-benzoic acid methyl ester (16).** A solution of compound **13** (0.34 g, 1.0 mmol) in

thionyl chloride (2 mL) was refluxed for 1 h, and the thionyl chloride was removed by distillation. The remained residue was dissolved in anhydrous dichloromethane, to this solution was added a solution of compound **6** (0.17 g, 1.0 mmol), triethylamine (1.0 mL) in anhydrous dichloromethane (5 mL) by canula. The resulting solution was stirred at room temperature overnight and quenched by adding water. The aqueous solution was extracted with dichloromethane, and the extract was dried over anhydrous magnesium sulfate. The organic solution was concentrated, and the residue was purified by flash column chromatography using silica gel as the stationary phase and using methanol/ethyl acetate (1:1, 3:1) as the mobile phase producing compound **16** (0.27 g, 0.6 mmol). Yield: 56%. mp: 178–181 °C. $^1$H NMR (300 MHz, $CD_3OD$, $\delta$): 11.68 (b, 1H), 10.84 (b, 1H), 8.63–8.60 (d, $J$ = 9.0 Hz, 1H), 8.21–8.18 (d, $J$ = 7.3 Hz, 1H), 8.07–8.05 (d, $J$ = 7.4 Hz, 1H), 7.82–7.79 (d, $J$ = 8.0 Hz, 1H), 7.52–7.14 (m, 10H), 6.81–6.76 (dd, $J$ = 7.4, 7.3 Hz, 1H), 6.32 (b, 2H), 5.32 (s, 2H), 3.91 (s, 3H). $^{13}$C NMR (75.4 MHz, $CDCl_3$, $\delta$): 168.8, 164.3, 156.4, 150.2, 144.4, 140.4, 135.7, 133.6, 132.1, 130.4, 129.24, 129.21, 129.0, 127.9, 127.8, 125.6, 124.3, 122.7, 122.4, 122.3, 117.0, 116.0, 114.6, 113.0, 111.9, 71.5, 51.6. IR (KBr): 3392, 1704, 1673, 1292, 1249, 742 cm$^{-1}$. MS $m/z$: 493 ($M^+$+1, 18), 492 ($M^+$, 54), 475 (44), 474 (42), 327 (57). HRMS-EI ($m/z$): $[M]^+$ calcd for $C_{29}H_{24}N_4O_4$, 492.1798; found, 492.1792.

**4.1.1.16. 2′-(2-Benzyloxy-phenyl)-1*H*,1′*H*-[2,4′]bibenzoimidazolyl-4-carboxylic acid methyl ester (17).** A solution of compound **16** (0.17 g, 0.4 mmol) in acetic acid (3 mL) was refluxed at 120–130 °C for 2 h. After cooled to room temperature, the reaction solution was poured into ice water and neutralized by sodium bicarbonate. The aqueous solution was extracted with dichloromethane, and the extract was washed with brine and dried over anhydrous magnesium sulfate. The dry solution was concentrated and dried under vacuum to give compound **17** (0.14 g, 0.3 mmol). Yield: 85%. mp: 202–206 °C. $^1$H NMR (300 MHz, $CDCl_3$, $\delta$): 13.66 (s, 1H), 10.74 (s, 1H), 9.01–8.98 (d, $J$ = 7.8 Hz, 1H), 8.40–8.37 (m, 1H), 8.07–8.04 (d, $J$ = 8.0 Hz, 1H), 7.99–7.96 (d, $J$ = 7.7 Hz, 1H), 7.54–7.49 (m, 6H), 7.37–7.31 (m, 4H), 7.22–7.19 (d, $J$ = 8.8 Hz, 1H), 5.36 (s, 2H), 4.18 (s, 3H). $^{13}$C NMR (75.4 MHz, $CDCl_3$, $\delta$): 166.6, 156.2, 152.4, 150.2, 144.6, 140.5, 135.8, 134.3, 133.8, 131.7, 130.4, 129.1, 128.9, 127.8, 124.7, 124.2, 122.9, 121.8, 121.4, 120.8, 118.5, 117.3, 113.6, 112.6, 112.5, 71.2, 52.0. IR (KBr): 3413, 1720, 1432, 1280, 744 cm$^{-1}$. MS $m/z$: 475 ($M^+$+1, 33), 474 ($M^+$, 100), 473 (20), 352 (25), 351 (33). HRMS-EI ($m/z$): $[M]^+$ calcd for $C_{29}H_{22}N_4O_3$, 474.1692; found, 474.1684.

**4.1.1.17. 2′-(2-Hydroxy-phenyl)-1*H*,1′*H*-[2,4′]bibenzoimidazolyl-4-carboxylic acid methyl ester (18).** A mixture of compound **17** (0.05 g, 0.1 mmol) and Pd/C (2.5 mg, 10% of Pd on charcoal) in methanol (25 mL) under hydrogen atmosphere (1 atm) was hydrogenated overnight. The catalyst was removed by filtration, and the filtrate was concentrated to give compound **18** (0.03 g, 0.07 mmol). Yield: 66%. mp: 283–286 °C. $^1$H NMR (300 MHz, $CDCl_3$, $\delta$): 12.0 (s, 1H), 10.88 (s, 1H), 8.12–8.09 (d, $J$ = 8.1 Hz, 1H), 7.97–7.95 (d, $J$ = 7.9 Hz, 1H), 7.89–7.86 (m, 2H), 7.77–7.74 (d, $J$ = 7.8 Hz, 1H), 7.46–7.37 (m, 3H), 7.17–7.15 (d, $J$ = 7.5 Hz, 1H), 7.09–7.04 (dd, $J$ = 7.8, 7.4 Hz, 1H), 4.06 (s, 3H). $^{13}$C NMR (125.7 MHz, DMSO-$d_6$, $\delta$): 165.9, 156.2, 152.0, 151.6, 144.3, 140.1, 135.8, 133.8, 131.9, 129.1, 124.2, 124.1, 122.4, 121.7, 120.1, 119.6, 116.8, 115.0, 113.3, 52.3. IR (KBr): 3486, 3378, 3189, 3068, 2977, 2817, 1675, 1608, 1583, 784 cm$^{-1}$. MS $m/z$: 385 ($M^+$+1, 25), 384 (100), 353 (24), 352 (93), 162 (13). HRMS-EI ($m/z$): $[M]^+$ calcd for $C_{22}H_{16}N_4O_3$, 384.1222; found, 384.1219.

**4.1.1.18. 2′-(2-Benzyloxy-phenyl)-1*H*,1′*H*-[2,4′]bibenzoimidazolyl-4-carboxylic acid (19).** To a solution of compound **17** (0.05 g, 0.1 mmol) in tetrahydrofuran (2 mL) was added 5 M sodium hydroxide solution (3 mL), and refluxed for 48 h. After cooled to room temperature, the reaction solution was acidified by concentrated hydrochloric acid to give a white precipitate. The precipitate was filtered and washed with water, and dried under vacuum to produce compound **19** (0.08 g, 0.2 mmol). Yield: 100%. mp: 256 °C (decomposed). $^1$H NMR (300 MHz, $CD_3OD$, $\delta$): 8.55–8.53 (d, $J$ = 7.5 Hz, 1H), 8.02–7.99 (d, $J$ = 7.5 Hz, 2H), 7.88–7.85 (d, $J$ = 9.0 Hz, 1H), 7.78–7.76 (d, $J$ = 6.0 Hz, 6H), 7.56–7.28 (m, 8H), 7.16–7.13 (d, $J$ = 8.4 Hz, 1H), 7.06–7.02 (dd, $J$ = 7.5, 6.0 Hz, 1H), 5.33 (s, 2H). $^{13}$C NMR (125.7 MHz, $d$-DMSO, $\delta$): 166.6, 155.9, 151.1, 149.8, 140.2, 136.9, 135.4, 132.4, 131.7, 130.3, 128.5, 127.7, 127.1, 126.2, 124.3, 123.0, 121.6, 120.7, 116.9, 115.8, 114.1, 69.1. IR (KBr): 3390, 3200, 3095, 3050, 1625, 1575, 1550, 1095, 750 cm$^{-1}$. MS $m/z$: 460 ($M^+$, 1.78), 418 (45), 253 (14), 252 (100), 91 (26). HRMS-EI ($m/z$): $[M]^+$ calcd for $C_{28}H_{20}N_4O_3$, 460.1535; found, 460.1541.

**4.1.1.19. 2-Amino-3-hydroxy-benzoic acid methyl ester (20).** To a solution of 2-amino-3-hydroxybenzoic acid (0.15 g, 0.9 mmol) in diethyl ether (10.0 mL) and methanol (2.0 mL) was added a solution of diazomethane in diethyl ether at 0 °C until ceased to generate nitrogen. After adding 1 mL of acetic acid, the reaction solution was concentrated and the resulting residue was purified by flash column chromatography using silica gel as the stationary phase and using ethyl acetate/hexane (1:9) as the mobile phase producing compound **20** (0.15 g, 0.8 mmol). Yield: 92%. mp: 94–97 °C. $^1$H NMR (300 MHz, $CDCl_3$, $\delta$): 7.50–7.47 (d, $J$ = 8.7 Hz, 1H), 6.83–6.81 (d, $J$ = 6.6 Hz, 1H), 6.52–6.47 (dd, $J$ = 8.0, 7.9 Hz, 1H), 3.87 (s, 3H). $^{13}$C NMR (125.7 MHz, $CDCl_3$, $\delta$): 168.7, 143.1, 140.5, 123.1, 117.9, 115.0, 111.3, 51.5. IR (KBr): 3409, 1704, 1439, 1293, 743 cm$^{-1}$. MS $m/z$: 167 ($M^+$, 100), 136 (26), 135 (79), 108 (12), 107 (85). HRMS-EI ($m/z$): $[M]^+$ calcd for $C_8H_9NO_3$, 167.0582; found, 167.0589.

**4.1.1.20. 2-(2-Benzyloxy-benzoylamino)-3-hydroxy-benzoic acid methyl ester (22).** A solution of compound **10** (0.28 g, 1.2 mmol) in anhydrous dichloromethane (10 mL) was added oxalyl chloride (0.22 mL, 2.5 mmol), and stirred for 10 min at room temperature; following added 5 drops of *N*,*N*-dimethylformamide and stirred for 2 h. Then the solvent was removed and dried under vacuum for 3 h. The residue was dissolved in anhydrous

dichloromethane (10 mL). To this solution was added a solution of compound **20** (0.15 g, 0.8 mmol) and pyridine (2 mL) in anhydrous dichloromethane (10 mL) via canula, and stirred overnight. The reaction was quenched by adding water and extracted with dichloromethane. The organic phase was dried over anhydrous magnesium sulfate. After filtration and concentration, the resulting residue was purified by flash column chromatography using silica gel as the stationary phase and using ethyl acetate/hexane (1:19, 1:9) as the mobile phase producing compound **22** (0.30 g, 0.8 mmol). Yield: 96%. mp: 101–102 °C. $^1$H NMR (300 MHz, $CDCl_3$, $\delta$): 12.03 (s, 1H), 9.39 (s, 1H), 8.26–8.23 (d, $J$ = 7.9 Hz, 1H), 7.60–7.57 (d, $J$ = 7.7 Hz, 1H), 7.47–7.40 (m, 3H), 7.36-7.28 (m, 4H), 7.23–7.18 (m, 1H), 7.11–7.05 (dd, $J$ = 7.9, 7.9 Hz, 1H), 7.04–7.01 (d, $J$ = 8.3 Hz, 1H), 5.47 (s, 2H), 3.78 (s, 3H). $^{13}$C NMR (125.7 MHz, $CDCl_3$, $\delta$): 167.5, 165.5, 157.0, 150.7, 136.2, 133.9, 132.7, 128.6, 128.2, 128.0, 126.9, 126.0, 125.7, 122.9, 121.5, 121.3, 120.9, 113.3, 70.8, 52.2. IR (KBr): 1710, 1627, 1455, 1288, 989, 751 cm$^{-1}$. MS *m*/*z*: 377 ($M^+$, 22), 211 (74), 166 (22), 121 (17), 91 (100). HRMS-EI (*m*/*z*): $[M]^+$ calcd for $C_{22}H_{19}NO_5$, 377.1263; found, 377.1257.

**4.1.1.21. 2-(2-Benzyloxy-phenyl)-benzoxazole-4-carboxylic acid methyl ester (23).** Compound **22** (0.11 g, 0.3 mmol) was heated up to 230 °C under vacuum (ca. 40 torr) for 5 h, and purified by flash column chromatography using silica gel as the stationary phase and using ethyl acetate/hexane (1:49, 1:19) as the mobile phase producing compound **23** (0.09 g, 0.3 mmol). Yield: 86%. mp: 104–105 °C. $^1$H NMR (300 MHz, $CDCl_3$, $\delta$): 8.28–8.26 (d, $J$ = 7.9 Hz, 1H), 8.06–8.03 (d, $J$ = 7.8 Hz, 1H), 7.77–7.75 (d, $J$ = 7.6 Hz, 1H), 7.67–7.64 (d, $J$ = 7.2 Hz, 2H), 7.54–7.25 (m, 5H), 7.15–7.10 (m, 2H), 5.31 (s, 2H), 4.01 (s, 3H). $^{13}$C NMR (125.7 MHz, $CDCl_3$, $\delta$): 165.9, 163.6, 157.7, 151.2, 141.3, 136.6, 133.2, 131.9, 128.4, 127.7, 126.87, 126.83, 124.1, 121.9, 121.0, 116.2, 114.7, 113.6, 70.6, 52.4. IR (KBr): 1703, 1601, 1450, 1240, 752 cm$^{-1}$. MS *m*/*z*: 360 ($M^+$+1, 19), 359 ($M^+$, 80), 342 (15), 327 (16), 91 (100). HRMS-EI (*m*/*z*): $[M]^+$ calcd for $C_{22}H_{17}NO_4$, 359.1158; found, 359.1165.

**4.1.1.22. 2-(2-Benzyloxy-phenyl)-benzoxazole-4-carboxylic acid (24).** To a solution of compound **23** (0.09 g, 0.3 mmol) in tetrahydrofuran (2 mL) was added 5 N sodium hydroxide solution (1 mL), and refluxed for 2 h. After cooled to room temperature, the reaction solution was acidified by concd hydrochloric acid and extracted with ethyl acetate. The extract was dried over anhydrous magnesium sulfate. After filtration and concentration, the residue was dried under vacuum to give product **24** (0.07 g, 0.2 mmol). Yield: 84%. mp: 99–102 °C. $^1$H NMR (300 MHz, $CDCl_3$, $\delta$): 11.75 (s, 1H), 8.25–8.22 (d, $J$ = 7.9 Hz, 1H), 8.15–8.12 (d, $J$ = 7.8 Hz, 1H), 7.80–7.77 (d, $J$ = 8.1 Hz, 1H), 7.61–7.34 (m, 7H), 7.21–7.14 (m, 2H), 5.34 (s, 2H). $^{13}$C NMR (125.7 MHz, $CDCl_3$, $\delta$): 165.1, 163.0, 158.1, 149.8, 141.0, 136.0, 134.3, 131.6, 128.8, 128.2, 127.2, 126.9, 126.2, 125.3, 121.1, 119.9, 115.2, 114.4, 113.6, 70.8. IR (KBr): 1745, 1604, 1430, 1251, 1020, 746 cm$^{-1}$. MS *m*/*z*: 346 ($M^+$+1, 13), 345 ($M^+$, 59), 328 (9), 237 (10), 91 (100). HRMS-EI (*m*/*z*): $[M]^+$ calcd for $C_{21}H_{15}NO_4$, 345.1001; found, 345.0995.

**4.1.1.23. 2-(2-Hydroxy-phenyl)-benzoxazole-4-carboxylic acid methyl ester (25).** A solution of compound **23** (0.05 g, 0.1 mmol) and *p*-toluenesulfonic acid (0.06 g, 0.3 mmol) in anhydrous toluene (2 mL) was refluxed for 1.5 h, and the reaction was quenched by adding saturated sodium bicarbonate solution. The aqueous phase was extracted with ethyl acetate, and the extract was dried over anhydrous magnesium sulfate. After filtration and concentration, the residue was purified by flash column chromatography using silica gel as the stationary phase and using ethyl acetate/hexane (1:49, 1:19) as the mobile phase producing compound **25** (0.02 g, 0.07 mmol). Yield: 51%. mp: 131–135 °C. $^1$H NMR (300 MHz, $CDCl_3$, $\delta$): 11.9 (s, 1H), 8.09–8.02 (m, 2H), 7.82–7.79 (d, $J$ = 8.0 Hz, 1H), 7.51–7.43 (m, 2H), 7.16–7.13 (d, $J$ = 7.9 Hz, 1H), 7.05–7.0 (dd, $J$ = 7.9, 7.6 Hz, 1H), 4.06 (s, 3H). $^{13}$C NMR (125.7 MHz, $CDCl_3$, $\delta$): 165.5, 164.3, 159.3, 149.7, 139.4, 134.2, 127.4, 127.2, 124.7, 121.2, 119.2, 117.6, 114.9, 109.9, 52.4. IR (KBr): 1717, 1633, 1548, 1301, 747 cm$^{-1}$. MS *m*/*z*: 269 ($M^+$, 62), 238 (23), 237 (100), 211 (36), 209 (15), 133 (13). HRMS-EI (*m*/*z*): $[M]^+$ calcd for $C_{15}H_{11}NO_4$, 269.0688; found, 269.0684.

**4.1.1.24. 2-{[2-(2-Benzyloxy-phenyl)-benzoxazole-4-carbonyl]-amino}-3-hydroxy-benzoic acid methyl ester (26).** A solution of compound **24** (0.07 g, 0.2 mmol) in anhydrous dichloromethane (10 mL) was added oxalyl chloride (0.15 mL, 1.7 mmol), and stirred for 10 minutes at room temperature; following added 5 drops of *N*,*N*-dimethylformamide, and stirred for 2 h. Then the solvent was removed and dried under vacuum for 3 h. The residue was dissolved in anhydrous dichloromethane (15 mL). To this solution was added a solution of compound **20** (0.04 g, 0.2 mmol) and pyridine (0.5 mL) in anhydrous dichloromethane (6 mL) via canula, and stirred overnight. The reaction was quenched by adding water and extracted with dichloromethane. The organic phase was dried over anhydrous magnesium sulfate. After filtration and concentration, the resulting residue was purified by flash column chromatography using silica gel as the stationary phase and using ethyl acetate/hexane (1:9) as the mobile phase producing compound **26** (0.08 g, 0.2 mmol). Yield: 76%. mp: 155–158 °C. $^1$H NMR (300 MHz, $CDCl_3$, $\delta$): 12.57 (s, 1H), 8.80 (s, 1H), 8.49–8.46 (d, $J$ = 7.7 Hz, 1H), 8.30–8.27 (d, $J$ = 7.8 Hz, 1H), 7.80–7.77 (d, $J$ = 8.1 Hz, 1H), 7.64–7.61 (d, $J$ = 7.7 Hz, 1H), 7.53–7.50 (m, 4H), 7.37–7.27 (m, 4H), 7.23–7.21 (m, 1H), 7.19–7.10 (m, 1H), 5.24 (s, 2H), 3.77 (s, 3H). $^{13}$C NMR (125.7 MHz, $CDCl_3$, $\delta$): 167.1, 164.4, 163.4, 157.9, 151.2, 150.8, 139.9, 136.2, 133.6, 132.3, 128.3, 127.8, 127.6, 126.7, 126.5, 126.3, 125.6, 124.7, 123.6, 123.0, 122.5, 121.2, 115.7, 114.7, 113.7, 70.7, 52.2. IR (KBr): 3208, 1713, 1527, 1458, 1291, 750 cm$^{-1}$. MS *m*/*z*: 494 ($M^+$, 39), 328 (100), 238 (48), 237 (31), 91 (25). HRMS-EI (*m*/*z*): $[M]^+$ calcd for $C_{29}H_{22}N_2O_6$, 494.1478; found, 494.1482.

**4.1.1.25. 2′-(2-Hydroxy-phenyl)-[2,4′]bibenzoxazolyl-4-carboxylic acid methyl ester (UK-1).** A solution of compound **26** (0.06 g, 0.1 mmol) and *p*-toluenesulfonic acid (0.06 g, 0.3 mmol) in anhydrous *p*-xylene (2 mL) was refluxed for 2 h, and the reaction was quenched by adding saturated sodium bicarbonate solution. The aqueous phase was extracted with ethyl acetate, and the extract was dried over anhydrous magnesium sulfate. After filtration and concentration, the residue was purified by flash column chromatography using silica gel as the stationary phase and using ethyl acetate/hexane (1:19, 1:9) as the mobile phase producing **UK-1** (0.02 g, 0.1 mmol). Yield: 43%. mp: 209–210 °C. $^1$H NMR (300 MHz, $CDCl_3$, $\delta$): 12.06 (s, 1H), 8.40–8.37 (d, $J$ = 7.7 Hz, 1H), 8.13–8.11 (d, $J$ = 7.3 Hz, 1H), 8.10–8.07 (d, $J$ = 7.9 Hz, 1H), 7.90–7.88 (d, $J$ = 8.0 Hz, 1H), 7.83–7.80 (d, $J$ = 8.2 Hz, 1H), 7.58–7.46 (m, 3H), 7.21–7.18 (d, $J$ = 8.3 Hz, 1H), 7.05–7.0 (t, $J$ = 7.7, 7.2 Hz, 1H), 4.13 (s, 3H). $^{13}$C NMR (125.7 MHz, $CDCl_3$, $\delta$): 166.1, 164.5, 161.8, 159.5, 151.2, 149.8, 141.4, 138.6, 134.2, 127.4, 127.1, 125.28, 125.25, 124.8, 122.3, 119.5, 117.7, 117.5, 115.0, 113.8, 109.9, 52.6. IR (KBr): 2923, 1724, 1546, 1427, 1259, 744 cm$^{-1}$. MS *m*/*z*: 387 ($M^+$+1, 24), 386 ($M^+$, 100), 355 (26), 354 (83), 328 (28). HRMS-EI (*m*/*z*): $[M]^+$ calcd for $C_{22}H_{14}N_2O_5$, 386.0903; found, 386.0904.

**4.1.1.26. 2-Methoxy-benzoic acid methyl ester (27).** To a solution of salicylic acid **7** (2.20 g, 16.0 mmol) in anhydrous acetone (30 mL) were added potassium carbonate (5.53 g, 40.0 mmol) and iodomethane (2.18 mL, 35.0 mmol), and refluxed overnight. After cooled to room temperature, the reaction mixture was filtered through Celite, and the filtrate was concentrated. The residue was purified by flash column chromatography using silica gel as the stationary phase and using ethyl acetate/hexane (1:19, 1:9) as the mobile phase producing compound **27** (2.40 g, 14.0 mmol). Yield: 91%. $^1$H NMR (300 MHz, $CDCl_3$, $\delta$): 7.81–7.78 (d, $J$ = 7.9 Hz, 1H), 7.50–7.44 (m, 1H), 7.0–6.95 (m, 2H), 3.91 (s, 3H), 3.89 (s, 3H). $^{13}$C NMR (125.7 MHz, $CDCl_3$, $\delta$): 166.6, 159.0, 133.5, 131.6, 120.0, 119.9, 111.9, 55.9, 51.9. IR (KBr): 1728, 1492, 1254, 1085, 756 cm$^{-1}$. MS *m*/*z*: 166 ($M^+$, 38), 135 (100), 133 (33), 105 (14), 92 (16), 77 (41). HRMS-EI (*m*/*z*): $[M]^+$ calcd for $C_9H_{10}O_3$, 166.0630; found, 166.0632.

**4.1.1.27. 2-Methoxy-benzoic acid (28).** To a solution of compound **27** (2.40 g, 14.0 mmol) in methanol (15 mL) was added 6 M sodium hydroxide solution (10 mL), and refluxed for 3 h. After concentration, the remained concentrated solution was acidified by adding 6 N hydrochloric acid to give a precipitate. The precipitate was filtered and dried under vacuum to produce compound **28** (1.71 g, 11.0 mmol). Yield: 56%. mp: 108–111 °C. $^1$H NMR (300 MHz, $CDCl_3$, $\delta$): 10.97 (s, 1H), 8.21–8.17 (d, $J$ = 7.8 Hz, 1H), 7.61–7.55 (dd, $J$ = 8.6, 7.2 Hz, 1H), 7.17–7.12 (dd, $J$ = 7.4, 7.1 Hz, 1H), 7.08–7.05 (d, $J$ = 8.4 Hz, 1H), 4.08 (s, 3H). $^{13}$C NMR (125.7 MHz, $CDCl_3$, $\delta$): 165.3, 158.0, 135.0, 133.8, 122.2, 117.5, 111.5, 56.6. IR (KBr): 1692, 1492, 1255, 1087, 1019, 761 cm$^{-1}$. MS *m*/*z*: 152 ($M^+$, 100), 135 (29), 123 (51), 105 (78), 79 (19), 77 (33). HRMS-EI (*m*/*z*): $[M]^+$ calcd for $C_8H_8O_3$, 152.0473; found, 152.0471.

**4.1.1.28. 2-Hydroxy-3-(2-methoxy-benzoylamino)-benzoic acid methyl ester (30).** A solution of compound **28** (0.15 g, 1.0 mmol) in anhydrous dichloromethane (15 mL) was added oxalyl chloride (0.26 mL, 3.0 mmol), and stirred for 10 minutes at room temperature; following added 5 drops of *N*,*N*-dimethylformamide, and stirred for 2 h. Then the solvent was removed and dried under vacuum for 3 h. The residue was dissolved in anhydrous dichloromethane (7 mL). To this solution was added a solution of compound **29** (0.15 g, 0.9 mmol) and pyridine (1 mL) in anhydrous dichloromethane (10 mL) via canula, and stirred overnight. The reaction was quenched by adding water and extracted with dichloromethane. The organic phase was dried over anhydrous magnesium sulfate. After filtration and concentration, the resulting residue was purified by flash column chromatography using silica gel as the stationary phase and using ethyl acetate/hexane (3:17, 3:7) as the mobile phase producing compound **30** (0.20 g, 0.7 mmol). Yield: 74%. mp: 166–168 °C. $^1$H NMR (300 MHz, $CDCl_3$, $\delta$): 11.35 (s, 1H), 10.77 (s, 1H), 8.86–8.83 (d, $J$ = 8.0 Hz, 1H), 8.32–8.29 (d, $J$ = 7.9 Hz, 1H), 7.58–7.54 (d, $J$ = 8.3 Hz, 1H), 7.53–7.47 (m, 1H), 7.16–7.10 (dd, $J$ = 7.5, 7.5 Hz, 1H), 7.06–7.03 (d, $J$ = 8.3 Hz, 1H), 6.96–6.91 (dd, $J$ = 8.3, 8.1 Hz, 1H), 4.09 (s, 3H), 3.98 (s, 3H). $^{13}$C NMR (125.7 MHz, $CDCl_3$, $\delta$): 171.0, 163.2, 157.4, 150.9, 133.2, 132.3, 128.3, 125.5, 123.4, 121.7, 121.4, 119.2, 111.5, 56.1, 52.4. IR (KBr): 1665, 1536, 1344, 1286, 756 cm$^{-1}$. MS *m*/*z*: 302 ($M^+$+1, 5), 301 ($M^+$, 31), 269 (6), 136 (8), 135 (100). HRMS-EI (*m*/*z*): $[M]^+$ calcd for $C_{16}H_{15}NO_5$, 301.0950; found, 301.0946.

**4.1.1.29. 2-(2-Methoxy-phenyl)-benzoxazole-7-carboxylic acid methyl ester (31) and 2-(2-hydroxy-phenyl)-benzoxazole-7-carboxylic acid methyl ester (32).** A solution of compound **30** (0.24 g, 0.8 mmol) and *p*-toluenesulfonic acid (0.38 g, 2.0 mmol) in anhydrous *p*-xylene (9.7 mL) was refluxed for 4 days, and the reaction was quenched by adding saturated sodium bicarbonate solution. The aqueous phase was extracted with ethyl acetate, and the extract was washed with brine and dried over anhydrous magnesium sulfate. After filtration and concentration, the residue was dried under vacuum to produce compound **31** (0.11 g, 0.07 mmol) and compound **32** (20 mg, 0.07 mmol).

**4.1.1.30. Compound 31.** Yield: 32%. mp: 88–90 °C. $^1$H NMR (300 MHz, $CDCl_3$, $\delta$): 8.26–8.23 (d, $J$ = 7.7 Hz, 1H), 8.05–7.99 (m, 2H), 7.57–7.52 (m, 1H), 7.44–7.39 (dd, $J$ = 7.9, 7.9 Hz, 1H), 7.19–7.10 (m, 1H), 4.07 (s, 3H), 4.05 (s, 3H). $^{13}$C NMR (125.7 MHz, $CDCl_3$, $\delta$): 164.7, 162.3, 158.7, 149.0, 143.3, 133.3, 131.4, 127.0, 124.9, 124.1, 120.8, 115.3, 114.6, 112.1, 56.2, 52.3. IR (KBr): 1716, 1621, 1428, 1301, 1037, 740 cm$^{-1}$. MS *m*/*z*: 283 ($M^+$, 70), 252 (21), 251 (37), 224 (17), 223 (100), 167 (16). HRMS-EI (*m*/*z*): $[M]^+$ calcd for $C_{16}H_{13}NO_4$, 283.0845; found, 283.0852.

**4.1.1.31. Compound 32.** Yield: 9%. mp: 137–139 °C. $^1$H NMR (300 MHz, $CDCl_3$, $\delta$): 11.28 (s, 1H), 8.16–8.13 (d, $J$ = 7.8 Hz, 1H), 8.04–8.01 (d, $J$ = 7.8 Hz, 1H), 7.94–7.91 (d, $J$ = 7.9 Hz, 1H), 7.50–7.43 (m, 2H), 7.15–7.12 (d, $J$ = 8.4 Hz, 1H), 7.07–7.02 (dd, $J$ = 7.9, 7.6 Hz, 1H), 4.07 (s, 3H). $^{13}$C NMR (125.7 MHz, $CDCl_3$, $\delta$): 164.4, 163.7, 158.7, 148.1, 141.4, 134.0, 127.5, 127.3, 124.7, 123.8, 119.7, 117.4, 115.0, 110.0, 52.5. IR (KBr): 1728, 1631, 1290, 1060, 754 cm$^{-1}$. MS $m/z$: 270 ($M^+$+1, 12), 269 ($M^+$, 73), 238 (24), 237 (100), 209 (9). HRMS-EI ($m/z$): $[M]^+$ calcd for $C_{15}H_{11}NO_4$, 269.0688; found, 269.0680.

**4.1.1.32. 2-(2-Methoxy-phenyl)-benzoxazole-7-carboxylic acid (33).** To a solution of compound **31** (52 mg, 0.18 mmol) in tetrahydrofuran (3 mL) was added 5 N sodium hydroxide solution (0.73 mL), and refluxed for 2 days. After cooled to room temperature, the reaction solution was acidified by concd hydrochloric acid to give a white precipitate. The precipitate was filtered and washed with water, and dried under vacuum to produce compound **33** (45 mg, 0.17 mmol). Yield: 91%. mp: 275 °C (decomposed). $^1$H NMR (300 MHz, $CD_3OD$, $\delta$): 8.32–8.28 (dd, $J$ = 7.8, 7.8 Hz, 1H), 7.86–7.83 (d, $J$ = 7.7 Hz, 1H), 7.79–7.76 (d, $J$ = 7.9 Hz, 1H), 7.60–7.54 (dd, $J$ = 7.8, 7.7 Hz,1H), 7.40–7.35 (dd, $J$ = 7.8, 7.8 Hz, 1H), 7.24–7.21 (d, $J$ = 7.9 Hz, 1H), 7.16–7.10 (m, 1H), 4.0 (s, 3H). $^{13}$C NMR (125.7 MHz, $CDCl_3$, $\delta$): 170.7, 161.9, 158.7, 148.4, 142.2, 133.1, 131.0, 126.5, 123.7, 123.2, 120.6, 120.3, 115.1, 111.9, 54.9. IR (KBr): 3432, 1581, 1400, 1124, 763, 617 cm$^{-1}$. MS $m/z$: 269 ($M^+$, 4) 175 (100), 154 (50), 137 (25), 136 (34), 57 (22). HRMS-FAB ($m/z$): $[M + H]^+$ calcd for $C_{15}H_{11}NO_4$, 270.0766; found, 270.0775.

**4.1.1.33. 2-Hydroxy-3-{[2-(2-methoxy-phenyl)-benzoxazole-7-carbonyl]-amino}-benzoic acid methyl ester (34).** A solution of compound **33** (23 mg, 0.09 mmol) in anhydrous dichloromethane (7 mL) was added oxalyl chloride (22 μL, 0.26 mmol), and stirred for 10 min at room temperature; following added 5 drops of *N*,*N*-dimethylformamide, and stirred for 2 h. Then the solvent was removed and dried under vacuum for 3 h. The residue was dissolved in anhydrous dichloromethane (10 mL). To this solution was added a solution of compound **29** (18 mg, 0.1 mmol) and pyridine (0.1 mL) in anhydrous dichloromethane (5 mL) via canula, and stirred overnight. The reaction was quenched by adding water and extracted with dichloromethane. The organic phase was dried over anhydrous magnesium sulfate. After filtration and concentration, the resulting residue was purified by flash column chromatography using silica gel as the stationary phase and using ethyl acetate/hexane (1:4, 3:7) as the mobile phase producing compound **34** (30 mg, 0.07 mmol). Yield: 67%. mp: 235–238 °C. $^1$H NMR (300 MHz, $CDCl_3$, $\delta$): 11.61 (s, 1H), 9.89 (s, 1H), 8.90–8.87 (d, $J$ = 7.8 Hz, 1H), 8.43–8.40 (d, $J$ = 7.9 Hz, 1H), 8.22–8.20 (d, $J$ = 7.8 Hz, 1H), 8.03–8.01 (d, $J$ = 7.8 Hz, 1H), 7.66–7.51 (m, 3H), 7.22–7.12 (m, 2H), 7.03–6.98 (dd, $J$ = 7.8, 7.8 Hz, 1H), 4.05 (s, 3H), 4.01 (s, 3H). $^{13}$C NMR (125.7 MHz, $CDCl_3$, $\delta$): 171.0, 161.1, 158.7, 150.8, 146.6, 142.6, 133.5, 131.4, 127.7, 126.7, 125.7, 124.8, 124.3, 124.0, 120.7, 119.4, 117.4, 114.8, 112.0, 111.7, 56.1, 52.5. IR (KBr): 1670, 1548, 1349, 1253, 765 cm$^{-1}$. MS $m/z$: 419 ($M^+$+1, 13), 418 ($M^+$, 49), 386 (11), 253 (16), 252 (100). HRMS-EI ($m/z$): $[M]^+$ calcd for $C_{23}H_{18}N_2O_6$, 418.1165; found, 418.1167.

**4.1.1.34. 2′-(2-Methoxy-phenyl)-[2,7′]bibenzoxazolyl-7-carboxylic acid methyl ester (35).** A solution of compound **34** (0.14 g, 0.33 mmol) and *p*-toluenesulfonic acid (0.16 g, 0.84 mmol) in anhydrous *p*-xylene (7.5 mL) was refluxed for 32.5 h, and the reaction was quenched by adding saturated sodium bicarbonate solution. The aqueous phase was extracted with ethyl acetate, and the extract was washed with brine and dried over anhydrous magnesium sulfate. After filtration and concentration, the resulting residue was purified by flash column chromatography using silica gel as the stationary phase and using ethyl acetate/hexane (1:9, 3:7, 1:1) as the mobile phase producing compound **35** (40 mg, 0.1 mmol). Yield: 30%. mp: 164–166 °C. $^1$H NMR (300 MHz, $CDCl_3$, $\delta$): 8.51–8.49 (d, $J$ = 6.1 Hz, 1H), 8.32–8.29 (d, $J$ = 8.1 Hz, 1H), 8.09–8.06 (m, 3H), 7.61–7.46 (m, 3H), 7.22–7.13 (m, 2H), 4.12 (s, 3H), 4.09 (s, 3H). $^{13}$C NMR (75 MHz, $CDCl_3$, $\delta$): 164.6, 162.4, 160.8, 158.8, 150.8, 149.6, 147.7, 143.8, 143.4, 133.2, 131.4, 127.5, 125.0, 124.9, 124.6, 123.8, 120.7, 115.5, 115.0, 112.1, 111.0, 56.3, 52.4. IR (KBr): 3397 (br), 3079, 3020, 2916, 2852, 1731, 1617, 1542, 1291, 1431, 746 cm$^{-1}$. MS $m/z$: 401 ($M^+$+1, 26), 154 (35), 81 (39), 69 (60), 57 (74), 55 (100). HRMS-FAB ($m/z$): $[M + H]^+$ calcd for $C_{23}H_{16}N_2O_5$, 401.1137; found, 401.1142.

**4.1.1.35. 2-(2-Benzyloxy-benzoylamino)-benzoic acid methyl ester (36).** A solution of compound **10** (2.00 g, 8.8 mmol) in anhydrous dichloromethane (60 mL) was added oxalyl chloride (3.8 mL, 43.6 mmol), and stirred for 10 min at room temperature; following added *N*,*N*-dimethylformamide (0.1 mL), and stirred for 2 h. Then the solvent was removed and dried under vacuum for 3 h. The residue was dissolved in anhydrous dichloromethane (50 mL). To this solution was added a solution of compound **2** (1.32 g, 8.8 mmol) and pyridine (3 mL) in anhydrous dichloromethane (40 mL) via canula, and stirred overnight. The reaction was quenched by adding water and extracted with dichloromethane. The organic phase was dried over anhydrous magnesium sulfate. After filtration and concentration, the resulting residue was purified by flash column chromatography using silica gel as the stationary phase and using ethyl acetate/hexane (1:49) as the mobile phase producing compound **36** (2.55 g, 7.0 mmol). Yield: 81%. mp: 116-119 °C. $^1$H NMR (300 MHz, $CDCl_3$, $\delta$) : 12.7 (s, 1H), 8.95–8.92 (d, $J$ = 8.6 Hz, 1H), 8.13–8.11 (d, $J$ = 7.8 Hz, 1H), 8.02–7.99 (d, $J$ = 8 Hz, 1H), 7.45–7.42 (dd, $J$ = 7.3, 6.4 Hz, 1H), 7.45–7.28 (m, 6H), 7.13–7.03 (m, 2H), 7.0–6.97 (d, $J$ = 8.3 Hz, 1H), 5.43 (s, 2H), 3.73 (s, 3H). $^{13}$C NMR (125.7 MHz, $CDCl_3$, $\delta$): 167.6, 164.7, 156.3, 141.2, 136.7, 134.1, 132.8, 132.1, 130.7, 128.5, 127.7, 126.8, 123.7, 122.5, 121.8, 121.2, 116.5, 113.3, 70.4, 51.9. IR (KBr): 1710, 1586, 1450, 1265, 757 cm$^{-1}$. MS $m/z$: 361 ($M^+$, 5), 241 (16), 240 (19), 151 (29), 92 (14), 91 (100). HRMS-EI ($m/z$): $[M]^+$ calcd for $C_{22}H_{19}NO_4$, 361.1314; found, 361.1308.

**4.1.1.36. 2-(2-Benzyloxy-thiobenzoylamino)-benzoic acid methyl ester (37).** To a solution of compound **36** (0.36 g, 1.0 mmol) in anhydrous toluene (4 mL) was added the Lawesson's reagent (0.40 g, 1.0 mmol) in glove box. The resulting solution was refluxed for 7.5 h under argon atmosphere, and the reaction was quenched by adding water. The aqueous solution was extracted with ethyl acetate, and the extract was dried over anhydrous magnesium sulfate. After filtration and concentration, the residue was purified by flash column chromatography using silica gel as the stationary phase and using ethyl acetate/hexane (1:49) as the mobile phase producing compound **37** (0.19 g, 0.5 mmol). Yield: 51%. mp: 74–76 °C. $^1$H NMR (300 MHz, $CDCl_3$, $\delta$): 12.71 (b, 1H), 9.59–9.56 (d, $J$ = 8.2 Hz, 1H), 8.04–8.01 (d, $J$ = 7.6 Hz, 1H), 7.96–7.94 (d, $J$ = 7.2 Hz, 1H), 7.65–7.60 (dd, $J$ = 7.6, 6.8 Hz, 1H), 7.37–7.32 (m, 3H), 7.23–7.21 (m, 4H), 7.06–6.98 (m, 2H), 5.20 (s, 2H), 3.79 (s, 3H). $^{13}$C NMR (125.7 MHz, $CDCl_3$, $\delta$): 134.0, 133.7, 133.6, 132.3, 131.5, 130.9, 128.6, 128.4, 127.7, 126.6, 124.9, 121.9, 121.3, 118.4, 113.3, 70.6, 52.3. IR (KBr): 1703, 1588, 1449, 1349, 1267, 759 cm$^{-1}$. MS *m/z*: 377 ($M^+$, 0.61), 345 (25), 344 (100), 318 (20), 312 (27). HRMS-EI (*m/z*): $[M]^+$ calcd for $C_{22}H_{19}NO_3S$, 377.1086; found, 377.1091.

**4.1.1.37. 2-(2-Benzyloxy-phenyl)-benzothiazole-4-carboxylic acid methyl ester (38).** To a solution of potassium ferricyanide(III) (2.87 g, 8.7 mmol) in water (2 mL) at 80–90 °C was added a solution of compound **37** (0.82 g, 2.2 mmol) in ethanol (1 mL), 30 % sodium hydroxide solution (1.68 mL), and water (0.72 mL) at a rate of 5 mL per minute. After stirred for 1 h, the reaction solution was cooled to room temperature and poured into water. The resulting solution was extracted with ethyl acetate, and the extract was dried over anhydrous magnesium sulfate. After filtration and concentration, the residue was dissolved in a mixture of diethyl ether and methanol, and added diazomethane at 0 °C until ceased to generate nitrogen. After adding a few drops of acetic acid, the reaction solution was concentrated, and the residue was purified by flash column chromatography using silica gel as the stationary phase and using hexane and then ethyl acetate/hexane (1:49) as the mobile phase producing compound **38** (0.17 g, 0.5 mmol). Yield: 21%. mp: 112–116 °C. $^1$H NMR (300 MHz, $CDCl_3$, $\delta$): 8.73–8.70 (d, $J$ = 7.9 Hz, 1H), 8.11–8.04 (m, 2H), 7.54–7.52 (d, $J$ = 7.6 Hz, 2H), 7.45–7.32 (m, 5H), 7.17–7.09 (m, 2H), 5.36 (s, 2H), 4.07 (s, 3H). $^{13}$C NMR (125.7 MHz, $CDCl_3$, $\delta$): 166.9, 164.8, 156.4, 149.9, 137.8, 135.9, 132.2, 130.3, 128.68, 128.66, 128.3, 127.8, 125.7, 124.3, 123.7, 122.3, 121.4, 112.7, 71.0, 52.4. IR (KBr): 1696, 1452, 1292, 1241, 1113, 751 cm$^{-1}$. MS *m/z*: 376 ($M^+$+1, 14), 375 ($M^+$, 54), 358 (20), 269 (12), 211 (11). HRMS-EI (*m/z*): $[M]^+$ calcd for $C_{22}H_{17}NO_3S$, 375.0929; found, 375.0925.

**4.1.1.38. 2-(2-Hydroxy-phenyl)-benzothiazole-4-carboxylic acid methyl ester (39).** To a solution of compound **38** (91.0 mg, 0.2 mmol) in anhydrous dichloromethane (2 mL) were added ethanethiol (1.5 mL) and boron trifluoride diethyl etherate (1.0 mL), and stirred at room temperature for 5 days. The reaction was quenched by adding saturated sodium bicarbonate solution and extracted with diethyl ether. The extract was dried over anhydrous magnesium sulfate. After filtration and concentration, the residue was purified by flash column chromatography using silica gel as the stationary phase and using ethyl acetate/hexane (1:49, 1:19) as the mobile phase producing compound **39** (56.0 mg, 0.2 mmol). Yield: 81%. mp: 163–165 °C. $^1$H NMR (300 MHz, $CDCl_3$, $\delta$): 8.24–8.21 (d, $J$ = 7.7 Hz, 1H), 8.12–8.09 (d, $J$ = 8.0 Hz, 1 H), 7.71–7.68 (d, $J$ = 7.9 Hz, 1H), 7.52–7.47 (dd, $J$ = 7.9, 7.8 Hz, 1H), 7.45–7.40 (dd, $J$ = 8.0, 7.9 Hz, 1H), 7.18–7.16 (d, $J$ = 8.4 Hz, 1H), 7.00-6.94 (t, $J$ = 8.0, 6.4 Hz, 1H), 4.09 (s, 3H). $^{13}$C NMR (125.7 MHz, $CDCl_3$, $\delta$): 170.7, 165.9, 158.7, 149.4, 134.1, 133.5, 129.7, 128.4, 126.0, 125.0, 123.1, 119.4, 118.2, 116.3, 52.5. IR (KBr): 1717, 1487, 1258, 1114, 741 cm$^{-1}$. MS *m/z*: 286 ($M^+$+1, 9), 285 ($M^+$, 59), 254 (18), 253 (100), 225 (10). HRMS-EI (*m/z*): $[M]^+$ calcd for $C_{15}H_{11}NO_3S$, 285.0460; found, 285.0466.

**4.1.1.39. 2-(2-Benzyloxy-phenyl)-benzothiazole-4-carboxylic acid (40).** To a solution of compound **38** (0.21 g, 0.6 mmol) in tetrahydrofuran (10.5 mL) was added 1 N sodium hydroxide solution (16.1 mL), and refluxed overnight. After cooled to room temperature, the reaction solution was acidified by concd hydrochloric acid to give a white precipitate. The precipitate was filtered and washed with water, and dried under vacuum to produce compound **40** (0.19 g, 0.5 mmol). Yield: 95%. mp: 223–225 °C. $^1$H NMR (300 MHz, $CDCl_3$, $\delta$)g 8.47–8.43 (d, $J$ = 7.9 Hz, 1H), 8.38–8.35 (d, $J$ = 8.0 Hz, 1H), 8.09–8.06 (d, $J$ = 8.0 Hz, 1H), 7.55–7.50 (m, 4H), 7.47–7.41 (m, 3H), 7.22–7.16 (m, 2H), 5.39 (s, 2H). $^{13}$C NMR (125.7 MHz, $CDCl_3$, $\delta$): 166.0, 156.7, 149.6, 135.8, 135.4, 133.4, 129.6, 129.5, 128.7, 128.6, 128.0, 126.3, 125.1, 121.7, 121.6, 120.5, 113.0, 71.2. IR (KBr): 1731, 1454, 1248, 994, 747 cm$^{-1}$. MS *m/z*: 362 ($M^+$+1, 12), 361 ($M^+$, 53), 317 (15), 253 (16), 211 (10). HRMS-EI (*m/z*): $[M]^+$ calcd for $C_{21}H_{15}NO_3S$, 361.0773; found, 361.0780.

**4.1.1.40. 2-{[2-(2-Benzyloxy-phenyl)-benzothiazole-4-carbonyl]-amino}-benzoic acid methyl ester (41).** A solution of compound **40** (0.10 g, 0.3 mmol) in anhydrous dichloromethane (4 mL) was added oxalyl chloride (1.0 mL, 11.5 mmol), and stirred for 10 minutes at room temperature; following added 5 drops of *N*,*N*-dimethylformamide, and stirred for 2 h. Then the solvent was removed and dried under vacuum for 3 h. The residue was dissolved in anhydrous dichloromethane (2 mL). To this solution was added a solution of compound **2** (0.05 g, 0.3 mmol) and pyridine (0.5 mL) in anhydrous dichloromethane (3 mL) via canula, and stirred overnight. The reaction was quenched by adding water and extracted with dichloromethane. The organic phase was dried over anhydrous magnesium sulfate. After filtration and concentration, the resulting residue was purified by flash column chromatography using silica gel as the stationary phase and using ethyl acetate/hexane (1:49) as the mobile phase producing compound **41** (0.08 g, 0.2 mmol). Yield: 54%. mp: 233–235 °C. $^1$H NMR (300 MHz, $CDCl_3$, $\delta$): 12.9 (s, 1H), 8.73–8.70 (d, $J$ = 7.6 Hz, 1H), 8.65–8.62 (d, $J$ = 7.6 Hz, 1H), 8.47–8.45 (d, $J$ = 7.6 Hz, 1H), 8.12–8.09 (d, $J$ = 8.0 Hz, 1H), 8.06–8.03 (d, $J$ = 7.9 Hz, 1H),

7.57–7.42 (m, 8H), 7.24–7.18 (dd, $J = 7.6$, 7.6 Hz, 1H), 7.14–7.05 (m, 2H), 5.38 (s, 2H), 3.74 (s, 3H). $^{13}C$ NMR (125.7 MHz, $CDCl_3$, $\delta$): 167.2, 164.4, 164.1, 156.6, 148.8, 140.1, 136.9, 135.8, 133.6, 132.3, 131.0, 130.5, 128.8, 128.7, 128.4, 127.9, 126.7, 125.0, 124.5, 124.1, 123.4, 121.9, 121.1, 119.2, 112.7, 71.1, 52.1. IR (KBr): 1718, 1662, 1535, 1449, 1244, 752 $cm^{-1}$. MS *m/z*: 495 ($M^+$+1, 33), 494 ($M^+$, 100), 344 (30), 254 (37), 253 (94). HRMS-EI (*m/z*): $[M]^+$ calcd for $C_{29}H_{22}N_2O_4S$, 494.1300; found, 494.1304.

### 4.2. Biological evaluation

**4.2.1. Cell culture.** The human bladder papillary transitional cell carcinoma cell line BFTC-905, the human lung carcinoma cell line A-549, the human uterus sarcoma cell line MES-SA, and the human embryonal rhabdomyosarcoma cell line RD were obtained from the Bioresources Collection & Research Center (BCRC, Taiwan). All cancer cell lines were maintained in the BCRC recommended medium supplemented with 10% fetal bovine serum and penicillin–streptomycin. After 24 h incubation at 37 °C in humidified 5% carbon dioxide to allow cell attachment, the cells were treated with various concentrations of UK-1 derivatives and incubated for 72 h under the same conditions. Even though the UK-1 derivatives were dissolved in DMSO, in the treatment stage the final DMSO concentration never exceeded 0.5%.

**4.2.2. Cytotoxicity assay.** Cell viability was determined by measuring the metabolism of a tetrazolium substrate, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT).[16] Briefly, the cells ($1 \times 10^4$ cells per well) were incubated in 96-well plates in which each cell contained 200 μl of the growth medium at 37 °C. Following overnight incubation, the growth medium was removed from cultures and replaced with 200 μl of the test article solution. The MTT test was conducted using six concentrations, 100 μM, 75 μM, 50 μM, 25 μM, 5 μM, and 0 μM, in four cell lines. Tests at each concentration were conducted in triplicate. Test article solutions were incubated with target cells for approximately 72 h at 37 ± 1 °C with 5 ± 1% $CO_2$. Following exposure, the test solutions were removed and replaced with 500 μg/ml MTT solution. After around 6 h of incubation, the MTT solution was removed. Sodium dodecyl sulfate (SDS) solution (10%) was added into cell cultures and the optical density (OD) values were read using an ELISA reader (SUNRISE, TECAN, Switzerland) at a wavelength of 570 nm. The mean and standard deviation of each group were calculated. The OD reading of every group was first substrate with blank control (background). The relative survival rate was the OD reading of the treated group divided by that of the negative control group, multiplied by 100. The percentage of toxicity was calculated as (1 − survival rate) × 100%. Therefore, the negative control was set to 100% survival or 0% toxicity.

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