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Brief Article

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Discovery of Novel 2-Aryl-4-benzoyl-imidazole (ABI-III) Analogues Targeting Tubulin Polymerization As Antiproliferative Agents

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Supporting Information

ABSTRACT: Novel ABI-III compounds were designed and synthesized based on our previously reported ABI-I and ABI-II analogues. ABI-III compounds are highly potent against a panel of melanoma and prostate cancer cell lines, with the best compound having an average IC_{50} value of 3.8 nM. They are not substrate of Pgp and thus may effectively overcome Pgp-mediated multidrug resistance. ABI-III analogues maintain their mechanisms of action by inhibition of tubulin polymerization.

INTRODUCTION

Because of its critical role in mitosis and cell division, tubulin polymerization represents an excellent cancer drug target, with several clinical drugs developed (e.g., paclitaxel, docetaxe,l and vinblastine).[1,2] However, one major problem for current tubulin inhibitors is the development of drug resistance in cancer patients. P-glycoprotein (Pgp)-mediated multidrug resistance is one major reason for failure of treatment by paclitaxel and vinblastine.[3,4] Accordingly, the search for novel tubulin inhibitors that can overcome multidrug resistance has intensified in recent years; and as a result, a number of active compounds have been identified.[5−13] Our search has focused on the discovery and optimization of novel tubulin inhibitors with high potency and acceptable pharmacologic and pharmacokinetic properties.[9,10,12−16]

We previously reported the discovery of ABI-I/II (2-aryl-4-benzoyl-imidazole, Figure 1) analogues with potent antiproliferative activity against a panel of melanoma and prostate cancer cell lines.[12,13] We modified the A and B rings of these analogues by introducing different functional groups to explore their effects on the activity.[13] The A ring modification with a bulky 2-(3′,4′,5′-trimethoxyphneyl)-indole ring (Figure 1, **21b**) displacing a phenyl ring led to significantly decreased activity; B-ring modification by methylation or benzylation on the imidazole NH resulted in similar or decreased activity for N-methylated and N-benzylated compounds, respectively. Fused A/B ring (Figure 1, ABI-II) resulted in much lower activity for compounds with substitution on the position-6 of the indole ring, while compounds with substitution on position-5 showed moderate activity.

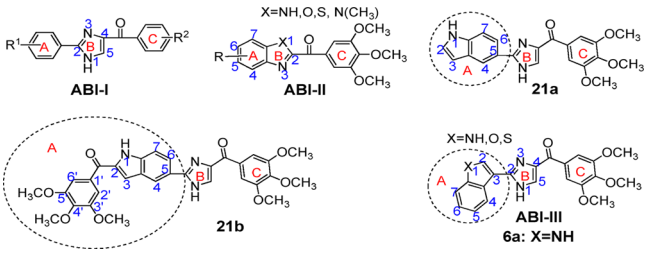

Figure 1. Structures of ABI-I/II/III.

Since indole is a very important building block for many biological active agents including tubulin inhibitors,[17−19] after analyzing the structure−activity relationships (SAR) of ABI-I and ABI-II analogues, we designed and attempted to synthesize compound **21a** (Figure 1) with an unsubstituted indole as the A ring. However, because of the lack of selectivity with the benzoylation reaction, we only obtained **21b** with an extra 3′,4′,5′-trimethoxyphenyl on the indole 2-position.[13] This compound showed only moderate activity, probably due to the extra bulkiness from the 3′,4′,5′-trimethoxyphenyl group in the indole 2-position, which may not fit into the binding pocket of tubulin. Our continued efforts resulted in the discovery of compound **6a** with a 3-indole as the A ring (Figure 1). We found that **6a** demonstrated significantly improved potency with an average IC_{50} value of 3.8 nM in five tested cancer cell lines. Encouraged by this discovery, we performed a focused SAR study on the indole ring (Figure 1, ABI-III) by (a) varying the X in the indole A-ring (X = NH, O, S)or (b) retaining the indole nitrogen but introducing a methyl or a bulkier phenylsulfonyl group on the indole nitrogen atom. In this article, we report the synthesis and biological testing for these highly potent compounds on both resistant and parental cancer cell lines. We confirmed that the mechanism of action of these novel compounds is through the inhibition of tubulin polymerization. In addition, we performed in vitro assays of Pgp function to further demonstrate that these new analogues are not a substrate of Pgp and thus can effectively overcome Pgp-mediated multidrug resistance that are common to several existing antimitotic drugs.

RESULTS AND DISCUSSION

Chemistry. The synthesis of compound **6a−c** is outlined in Scheme 1. Briefly, the indole-3-carboxaldehyde compound **1**

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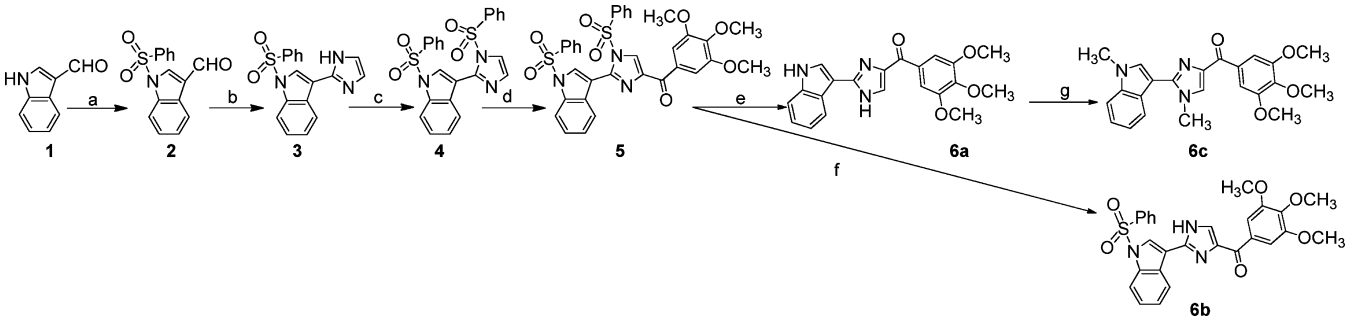
Scheme 1. Synthesis of 6a−c

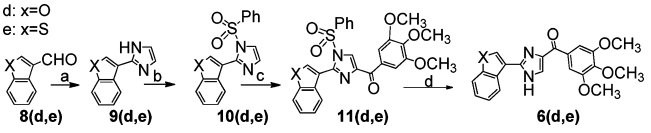


Reagents and conditions: (a) 1. KOH, ethanol; 2. $PhSO_2CI$, acetone, RT; (b) NH_4OH, glyoxal, ethanol, RT; (c) NaH, $PhSO_2CI$, THF, 0 °C to RT; (d) *t*-BuLi (1.7 M in pentane), 3,4,5-trimethoxybenzoyl chloride, THF, −78 °C; (e) NaOH, ethanol, H_2O, reflux; (f) TBAF, THF, RT; (g) NaH, CH_3I, THF.

was protected by the phenylsulfonyl group on the indole NH to afford N-protected indole-3-carboxaldehyde **2**. Compound **2** was reacted with glyoxal and ammonium hydroxide to generate the 2-aryl-1*H*-imidazole compound **3**. Protection of the imidazole NH on compound **3** with phenylsulfonyl provided the intermediate **4**, which was coupled with 3,4,5-trimethoxy benzoyl chloride in the presence of *tert*-butyl lithium to produce compound **5.** The benzoylation occurred in imidazole position-4 because of less steric hindrance in the two tautomers as confirmed by 2D NMR studies (Supporting Information S8−15). Removal of the protecting group from compound **5** by sodium hydroxide or by *tert*-butylammonium fluoride (TBAF) yielded compound **6a** and **6b**, respectively. Compound **6a** was further methylated by methyl iodide to produce the dimethylated compound **6c**.

Compounds **6d** and **6e** were synthesized following the route outlined in Scheme 2. The benzofuran (**8d**) or benzothiophene

Scheme 2. Synthesis of 6d and 6e



Reagents and conditions: (a) NH_4OH, glyoxal, ethanol, RT; (b) NaH, $PhSO_2CI$, THF, 0 °C to RT; (c) *t*-BuLi (1.7 M in pentane), 3,4,5-trimethoxybenzoyl chloride, THF, −78 °C; (d) TBAF, THF, RT.

compound (**8e**) was converted to compound **9**(**d**,**e**) in the presence of ammonium hydroxide and glyoxal to construct the imidazole scaffold. The imidazole ring of compound **9**(**d**,**e**) was protected by a phenylsulfonyl group to achieve N-protected compound **10**(**d**,**e**). Finally, compound **6**(**d**,**e**) was generated through a one-pot reaction by coupling **10**(**d**,**e**) with 3,4,5-trimethoxy benzoyl chloride followed by treating with *tert*-butylammonium fluoride to remove the protecting group.

Antiproliferative Activities of ABI-III in Melanoma and Prostate Cancer Cells. The antiproliferative activity of these compounds was evaluated in two human metastatic melanoma cell lines (A375 and WM164) and three human prostate cancer cell lines (LNCaP, PC-3, and Du 145) using the methods described previously.[12,20] Colchicine was used as a positive control. The ability of these new analogues to inhibit the growth of cancer cell lines is summarized in Table 1. The 3-indole compound **6a** showed high activity (3.8 nM; unless specified, the IC_{50} value for each compound is the average of all five cancer cell lines), identifying this compound as the most potent compound in the ABI family (I/II/III) to date. The bulky phenylsulfonyl group in the 3-indole ring (**6b**) resulted in 50-fold lower activity (196.2 nM), suggesting the size of the substituent on the indole NH may play an important role in determining activity. Consistent with this hypothesis, the dimethylated 3-indole compound **6c** showed very good potency (39.8 nM), indicating that a smaller group on either the imidazole- or indole-NH was well tolerated. The benzofuran compound **6d** and benzothiophene compound **6e** showed relatively strong activity (41.2 nM, 35.0 nM for **6d** and **6e**, respectively), although it is about 10-fold less active than the 3-indole compound **6a**, implying that the replacement of the 3-indole NH with either an oxygen or a sulfur does not provide any beneficial effect on the activity. Resistance indices (RI) were calculated by dividing IC_{50} values on paclitaxel resistant cell lines PC-3/TxR by IC_{50} values on the matching sensitive parental cell line PC-3. The larger the RI value, the more resistant the drug.

Effects of ABI-III Compound on Paclitaxel Resistant Cell Line. Paclitaxel resistance is a major mechanism accounting for therapeutic failures for the clinical use of

Table 1. In Vitro Growth Inhibitory Effects of ABI-III Compounds on Melanoma and Prostate Cancer Cells

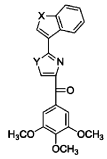
Structure	ID	X	Y	IC_{50}±SEM (nM)(n=4)					
				A375	WM164	LNCaP	PC-3	Du 145	Average
	**6a**	NH	NH	3.2±1.2	5.3±2.0	2.8±0.6	3.7±0.3	3.9±1.0	3.8
	6b	N-$PhSO_2$	NH	414.0±35.4	166.2±18.9	107.0±17.5	105.0±2.1	189.0±13.5	196.2
	6c	N-CH_3	N-CH_3	18.8±3.6	24.2±4.1	35.9±3.3	40.7±5.1	76.5±4.1	39.8
	6d	O	NH	53.4±12.5	73.6±15.2	14.1±6.2	33.3±2.1	45.0±9.6	41.2
	6e	S	NH	41.0±5.2	45.7±9.3	29.1±3.9	20.8±0.5	51.2±3.5	35.0
Colchicine				20.6±3.6	29.0±5.2	16.3±4.0	11.5±0.1	11.2±1.1	17.7

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Table 2. Antiproliferative Activity of ABI-III Analogues on Paclitaxel Resistant Cell Line

	IC_{50} ± SEM (nM)(n = 4)					
ID	**6a**	**6b**	**6c**	**6d**	**6e**	paclitaxel
PC-3	3.7 ± 0.3	33.3 ± 2.1	20.8 ± 0.5	40.7 ± 5.1	105.0 ± 2.1	0.4 ± 0.1
PC-3/TxR	3.7 ± 0.8	27.6 ± 9.8	22.4 ± 5.1	42.8 ± 11.9	98.6 ± 6.3	188.0 ± 22.0
RI*	1	0.9	1.1	1	0.9	437

paclitaxel and vinblastine.[21−23] Our previous ABI-I/II analogues were shown to effectively overcome paclitaxel resistance.[13] We hypothesized the ABI-III analogues modified based on ABI-I/II scaffold would retain the ability to circumvent paclitaxel resistance mechanisms. To support our hypothesis, we examined the antiproliferative activity of compounds **6a−e** in a paclitaxel resistance cell line: PC-3/TxR, with paclitaxel as positive control.[24] In the PC-3/TxR cell line, more than 200 genes are upregulated in addition to Pgp overexpression, which may represent additional paclitaxel drug resistance mechanisms.[24] As indicated in Table 2, compounds **6a−e** showed equal potency on both the parental (PC-3) and its paclitaxel resistant cell line (PC-3/TxR) with resistance indices ranging from 0.9 to 1.1. While paclitaxel showed pico-molar activity (0.4 nM) in the parental PC-3 cells, it demonstrated significantly lesser potency (188.0 nM) in the PC-3/TxR cell line with a resistance index of 437. These results clearly indicated that ABI-III analogues can overcome paclitaxel-resistance mechanisms and suggest that they may provide a therapeutic advantage over paclitaxel.

ABI-III Compound 6a Is Not a Substrate of Pgp. The results from paclitaxel resistant cells not only confirmed our hypothesis that the ABI-III compounds can overcome paclitaxel resistance but also guided us to perform the Pgp-glo assay to examine the effect of compound **6a** on Pgp ATPase. In the Pgp-glo assay, ATP was incubated with recombinant human Pgp and then unmetabolized ATP in the presence of test compound was detected as a luciferase-generated luminescent signal. The decreases in luminescence after drug treatment reflect ATP consumption by Pgp. ATP consumption by Na_3VO_4 is attributed to minor non-Pgp ATPase activities in this system. The changes of luminescence of compound treated samples from the one of Na_3VO_4 treated samples were plotted to illustrate the stimulation or inhibition of Pgp ATPase activity by compound treatment (Figure 2). Verapamil, a well-known Pgp substrate, stimulated Pgp ATPase activity resulting in significantly decreased luminescence ($P < 0.05$). However, no significant difference in luminescence changes was observed between the vehicle control treated group and **6a** treated groups (up to 1 μM), suggesting that **6a** is neither a stimulator nor an inhibitor for Pgp ATPase. The result from Pgp-glo assay strongly indicates that 6a is not a substrate of Pgp and can at least partially explain the ability of ABI-III compounds on overcoming paclitaxel resistance.

Tubulin Polymerization Assay on ABI-III Compounds. We hypothesized that ABI-III compounds maintain their mechanism of action by inhibiting tubulin polymerization based on the structure similarity of ABI-III and ABI-I/II analogues. To confirm our hypothesis, we conducted in vitro tubulin polymerization assays on ABI-III analogues. Bovine brain tubulin (>97% pure) was incubated with compounds **6a−e**, at concentrations of 5 and 10 μM. Colchicine at 5 μM was used as a positive control. Compounds **6a−e** inhibited tubulin polymerization in a dose-dependent manner (Figure 3). Complete inhibition of tubulin polymerization was observed

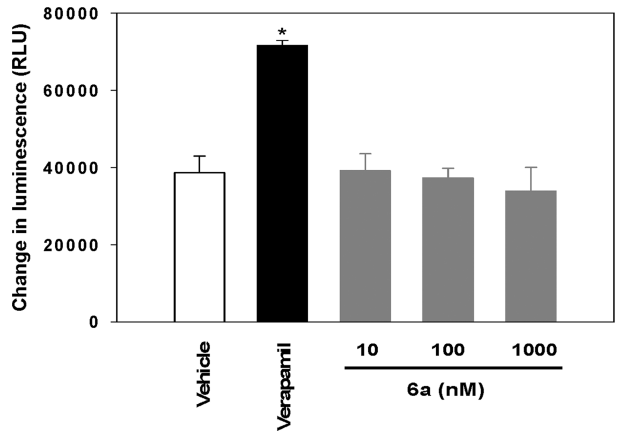

Figure 2. Effect of **6a** on Pgp ATPase activity. Change in luminescence compared to 100 μM Na_3VO_4 treated samples was plotted (mean ± SD, n = 3). Compound **6a** showed a similar effect as vehicle control on Pgp ATPase activity, indicating **6a** is not the substrate for Pgp. *, $p < 0.05$.

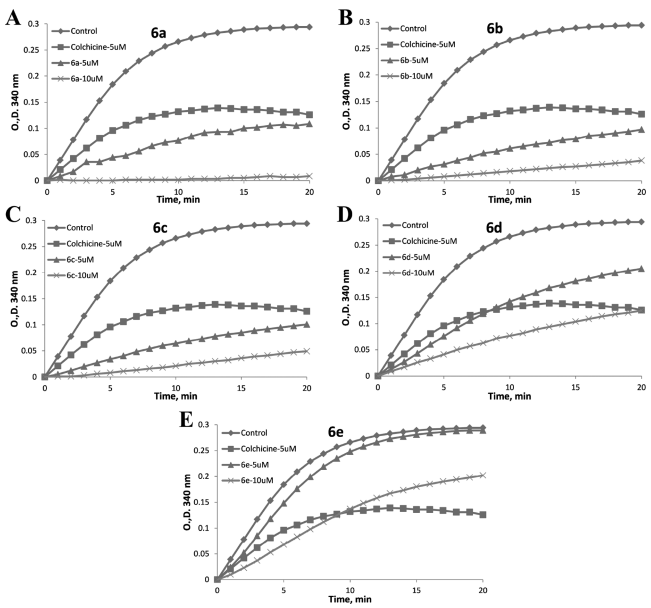

Figure 3. Effect of ABI-III compounds on tubulin polymerization in vitro. Tubulin (0.4 mg/assay) was exposed to 5, 10 μM ABI-III compounds (vehicle control, 5% DMSO): **6a** (A), **6b** (B), **6c** (C), **6d** (D), **6e** (E), or colchicine (positive control, at 5 μM). Absorbance at 340 nm was monitored at 37 °C every minute for 20 min.

after 20 min of treatment with **6a** (Figure 3A) at 10 μM, while 70% inhibition was achieved by **6a** at 5 μM. Compound **6b** (Figure 3B) inhibited tubulin polymerization by 67% and 85% at 5 and 10 μM, respectively. Compound **6c** (Figure 3C) inhibited 65% and 80% of tubulin polymerization at 5 and 10 μM, respectively, while 30% and 60% inhibition of tubulin polymerization was observed for compound **6d** (Figure 3D) at

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5 and 10 μM, respectively. Compound **6e** (Figure 3E) at 10 μM inhibited tubulin polymerization to an extent of 30% but had no effect on tubulin polymerization 5 μM (same as control DMSO). These results clearly indicate that the ABI-III compounds are strong inhibitors of tubulin polymerization and the inhibitory effects are roughly proportional to the relative antiproliferative potency for **6a** (IC_{50} = 3.8 nM), **6b** (IC_{50} = 196.2 nM), **6c** (IC_{50} = 39.8 nM), **6d** (IC_{50} = 41.2 nM), and **6e** (IC_{50} = 35.0 nM).

Molecular Modeling Studies on 6a. Tubulin polymerization assay suggests that ABI-III compounds exert their effects by inhibiting tubulin polymerization. To better understand how ABI-III analogues interact with tubulin, we investigated the theoretical binding mode of **6a** at colchicine binding site in tubulin dimer using Schrodinger 2011 molecular modeling suite (Schrodinger, Inc., New York, NY). We tried to dock compound **6a** and **21b** into two different tubulin crystal structures (PDB ID code: 1SA0 or 3HKD). However, compound **21b** did not fit the binding pockets in either crystal structures due to the extra bulkiness provided by the 3′,4′,5′-trimethoxyphenyl on the indole 2-position, which may explain the low activity of this compound (2.9 μM).[13] Compound **6a** demonstrated excellent binding in these models with glide scores of −7.42 and −10.50 for 1SA0 and 3HKD, respectively. This result is consistent with our previous observation that TN16-tubulin complex (3HKD) fits ABI scaffold better than the colchicine-tubulin complex (1SA0).[13]

The overview of the binding site of **6a** and TN-16 in 3HKD is shown in Figure 4A in which the mesh indicates residue

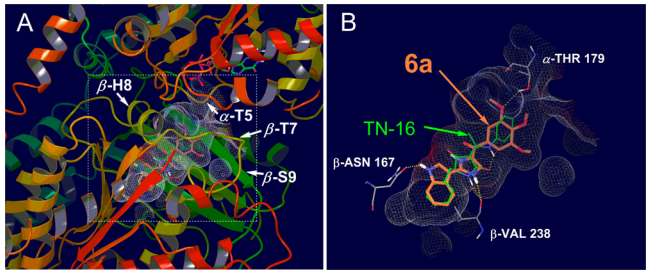

Figure 4. Binding modes of **6a** in the colchicine binding site of tubulin.

surface within 4 Å from TN-16. This binding pocket is located on the interface between the α- and β-subunits of the tubulin dimer and extended inside to the nucleoside-binding domain of the β-subunit.[25,26] Figure 4B illustrated the close view of the potential binding pose. Generally, **6a** (orange tube model) overlapped very well with TN-16 (green wire model). The 3-indole group of **6a** penetrates deeply into the bottom of the pocket. The interaction was strongly stabilized by two hydrogen bonds: the first one provided by the indole NH and ASN167 in β-S5; the second formed between imidazole NH and VAL238 in β-H7, while the native ligand, TN-16, has only one hydrogen bond with VAL238 in β-H7 in this modeling study. The 3,4,5-trimethoxybenzoyl group (C ring) of **6a** extends toward the α/β interface, similar to the mode of the active ABI-II compound.[13] The C ring moiety contributes to the binding interaction by forming the third hydrogen bond between one of its methoxyl groups and the hydroxyl group of THR197 in α-T5, which further enhances the interaction between **6a** and the tubulin dimer. The three observed hydrogen bonds along with the possible hydrophobic interaction provided by the indole A ring and phenyl C ring, can at least partially explain the high potency of **6a**.

CONCLUSIONS

Novel ABI-III analogues were synthesized, and a focused SAR study was performed. The ABI-III compounds were highly potent against melanoma and prostate cancers in vitro. The 3-indole compound **6a** was identified as the most potent ABI analogue. The ABI-IIIs were not substrates of Pgp, and demonstrated equal potency on both a paclitaxel resistant cancer cell line and its matching parental cell line. Thus, they can effectively overcome Pgp-mediated multidrug resistance and paclitaxel resistance. The mechanism of the action studies suggests that the ABI-III compounds exert their effect by inhibiting tubulin polymerization. Molecular modeling provides insights into the binding of ABI-III analogues in tubulin. In summary, novel ABI-III analogues represent a potent, new class of tubulin inhibitors that hold great potential for development into more effective therapeutics for treatment of paclitaxel resistant cancers.

EXPERIMENTAL PROCEDURES

All reagents for the synthesis were purchased from commercial sources and were used without further purification. Moisture-sensitive reactions were carried out under an argon atmosphere. NMR spectra were obtained on an Agilent Inova-500 MHz spectrometer (Agilent Technologies Inc., Santa Clara, CA). Mass spectral data was collected on a Bruker ESQUIRE-LC/MS system (Bruker Daltonics, Billerica, MA) equipped with an ESI source. The purity of the final compounds was analyzed by an Agilent 1100 HPLC system (Santa Clara, CA). HPLC conditions: 90% methanol at flow rate of 1.0 mL/min using a Luna-PFP 5 μM column (250 × 4.6 mm) purchased from Phenomenex (Torrance, CA) at ambient temperature. UV detection was set at 280 nm. Purities of the compounds were established by careful integration of areas for all peaks detected and ≥95%.

2-(1*H*-Indol-3-yl)-1*H*-imidazol-4-yl)(3,4,5-trimethoxyphenyl) Methanone (6a). To a solution of (1-(phenylsulfonyl)-2-(1-(phenylsulfonyl)-1*H*-indol-3-yl)-1*H*-imidazol-4-yl)(3,4,5-trimethoxyphenyl)-methanone 5 (0.66 g, 1 mmol) in ethanol (40 mL) and water (4 mL) was added sodium hydroxide (0.4 g, 10 mmol) and stirred overnight under refluxing conditions in darkness. The reaction mixture was diluted by 50 mL of water and extracted by ethyl acetate (200 mL). The organic layer was dried over magnesium sulfate and concentrated. The residue was purified by flash column chromatography (hexane/ethyl acetate 1:1) to give a yellow solid. Yield: 60%. Mp 210−212 °C. ^{1}H NMR (CD_3OD, 500 MHz) δ 8.31 (d, J = 6.5 Hz, 1 H), 7.99 (s, 1 H), 7.90 (s, 1 H), 7.48−7.52 (m, 3 H), 7.24−7.28 (m, 2 H), 4.00 (s, 6 H), 3.93 (s, 3 H). MS (ESI) calcd for $C_{21}H_{19}N_3O_4$, 377.1; found, 400.1 $[M + Na]^+$. HPLC: t_R 4.33 min, purity >99%.

The synthesis and chromatographic data for **6b−d** and their intermediates are detailed in the Supporting Information.

ASSOCIATED CONTENT

Supporting Information

Synthesis of **6b−e**; NMR and LC-MS. Procedures for cell culture, cytotoxicity assay, Pgp ATPase assay, in vitro microtubule polymerization assay, and molecular modeling. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

ABI, 2-aryl-4-benzoyl-imidazoles; MDR, multidrug resistance; Pgp, P-glycoprotein; SAR, structure−activity relationships; TMS, tetramethylsilane; TBAF, tert-butylammonium fluoride; RT, room temperature

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