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Design and Synthesis of Indomethacin Analogues That Inhibit P-Glycoprotein and/or Multidrug Resistant Protein without Cox Inhibitory Activity

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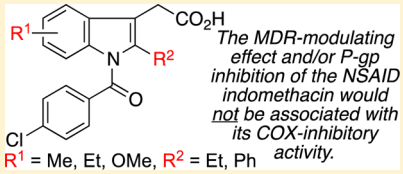
Supporting Information

ABSTRACT: We designed and synthesized conformationally restricted analogues and regioisomers of the nonsteroidal anti-inflammatory drug indomethacin. Evaluation of the inhibitory effects of these compounds on COX, P-glycoprotein, and multidrug resistance indicated that NSAIDS modulation of multidrug-resistant P-glycoprotein and multidrug-resistant protein-1 is not associated with COX-1 and COX-2 inhibitory activities.



INTRODUCTION

Doxorubicin is a chemotherapeutic drug widely used for the treatment of non-Hodgkin's lymphoma, multiple myeloma, acute leukemia, Kaposi's sarcoma, Ewing's sarcoma, Wilm's tumor, and cancers of the breast, adrenal cortex, endometrium, lung, ovary, and other sites. Its efficacy is often limited, however, by the development of multidrug resistance (MDR), which has been linked to the upregulation of P-glycoprotein (P-gp) and/or multidrug-resistant protein (MRP) in cancer cells. The nonselective cyclooxygenase (COX) inhibitor indomethacin and the COX-2-selective inhibitor 4-[5-(4-chlorophenyl)-3-(trifluoromethyl)-1*H*-pyrazol-1-yl]benzenesulfonamide (SC236) directly function as noncompetitive inhibitors of P-gp, and combined treatment using indomethacin or SC236 with doxorubicin may have significant potential clinical application, especially for circumventing P-gp-mediated MDR in cancer cells.[1] Further, indomethacin analogues potentiate the cytotoxicity of the chemotherapeutic agent doxorubicin and are therefore an attractive starting point for further development of multidrug-resistant protein-1 (MRP-1) inhibitors.[2] Although conventional nonsteroidal anti-inflammatory drugs (NSAIDs), i.e., COX-1 and COX-2-selective inhibitors, overcome MDR in some cancer cells,[1] it is not known whether COX-1 and/or COX-2 activity is directly related to the MDR properties of cancer cells.[3] Thus, we designed and synthesized conformationally restricted and regioisomeric analogues of indomethacin **1a–f** (Figure 1) to investigate the relationship between the COX inhibitory activity and the P-gp and MRP-1 modulating effects of MDR.

Compound conformation is one of the determinants of biological activity. Compounds sometimes have several different biological effects due to interactions with different target biomolecules, through which the bioactive conformation (conformation of the compound binding to its target biomolecule) can be changed depending on the target biomolecule.[4,5] Accordingly, conformational restriction of a lead compound having two or three different biological effects can be an efficient strategy for evaluating a compound that selectively exhibits one of the biological effects of the lead compound. Thus, we were interested in the conformational restriction of indomethacin.

The bioactive conformers of indomethacin in complexes with COX-1 and COX-2 are the s-trans[4] and s-cis forms,[5] respectively (Protein Data Bank entries: 4COX and 1PGG, respectively). Therefore, the binding conformations of indomethacin interacting directly with P-gp and/or MRP-1 might be the s-trans form or the s-cis form.[6]

We hypothesized that introduction of an alkyl substituent at the 2- or 7-position of indomethacin (**1a**) and its derivatives may restrict conformation to either the s-cis form or the s-trans form because of steric repulsion between the introduced substituent and the *N*-acyl side chain. When the substituent at the 7-position (R^1) is bulkier than that at the 2-position (R^2), the s-trans conformation is expected to be thermodynamically favored over the s-cis (Figure 1). Thus, we designed indomethacin analogues **1c–f**, substituted at the 2- or 7-

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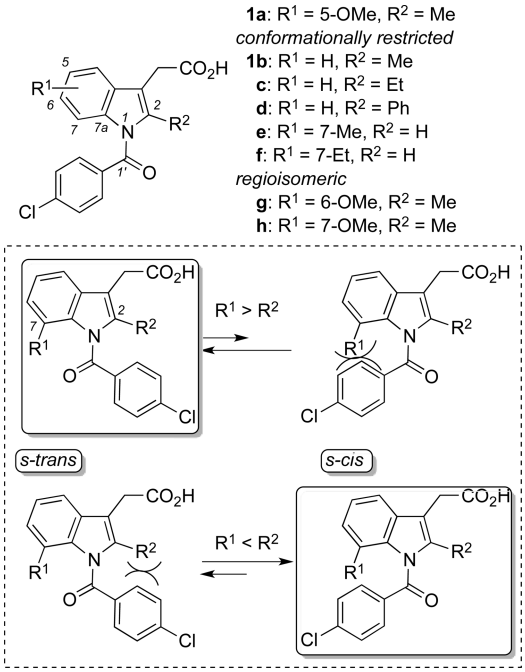

Figure 1. Indomethacin (**1a**) and designed indomethacin derivatives **1b–h**.

position,[7] and synthesized them using a one-pot isomerization/enamide-ene metathesis[8] as the key step. In this research project, we also planned to synthesize novel 6-methoxy and 7-methoxy regioisomers **1g** and **1h** of indomethacin, which, despite their pharmacologically interesting properties, have not been reported to date. All synthesized indomethacin derivatives were evaluated for their COX, P-gp, and MRP-1 inhibitory activities to clarify the relationship of these biological activities.

We identified the indomethacin analogues without COX inhibitory activity that enhanced doxorubicin cytotoxicity in MDR cells and showed that P-gp and MRP-1 inhibitory effects of indomethacin were not associated with COX-inhibitory activity. Here, we report the results of these studies in detail.

RESULTS AND DISCUSSION

Conformational Analysis by Calculations. Because of the importance of conformational restriction in this research project, we investigated the conformational stability of the designed indomethacin derivatives using theoretical calculations.

First, we investigated the stable conformation of **1a–h** by molecular mechanics calculation using the MacroModel software (Schrödinger, Portland, OR). The results indicated that **1a–c,g,h** favor the s-cis form (Figure 2) while **1d–f** favor the s-trans form (Figure 3). These two types of compounds exhibited significantly different energies, as indicated in the figures.

Next, the rotational barrier energy around the C7a–N1–C1′–O dihedral angle of model compounds **A–G**, in which the carboxymethyl group at the 3-position of the indomethacin analogues was replaced with a methyl group (Figure 4), was calculated based on density functional theory (DFT). The dihedral angle was rotated from 0° to 360° at 10° intervals, and the single point energies of the optimized conformers were calculated at B3LYP/6-31G* to obtain the energy profile. Dihedral drive indicated oscillation profiles for the range of 8

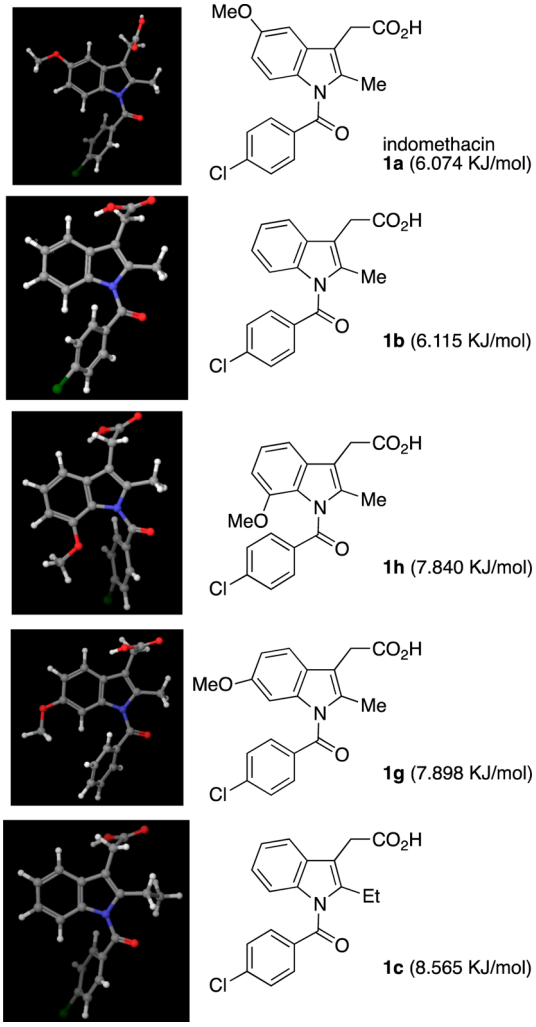

Figure 2. Compounds stable in the s-cis conformer by molecular field calculation. Numbers in parentheses indicate energy differences between the two comformers.

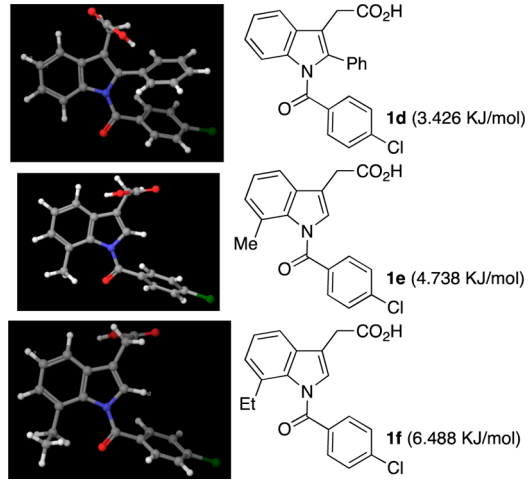

Figure 3. Compounds stable in the s-trans conformer by molecular field calculation. Numbers in parentheses indicate energy differences between the two comformers.

kJ/mol. In **D–F**, the s-trans form was relatively more stable than the s-cis form while in **A–C** and **G**, the s-cis form was more stable than the s-trans form (Figure 4). We did not

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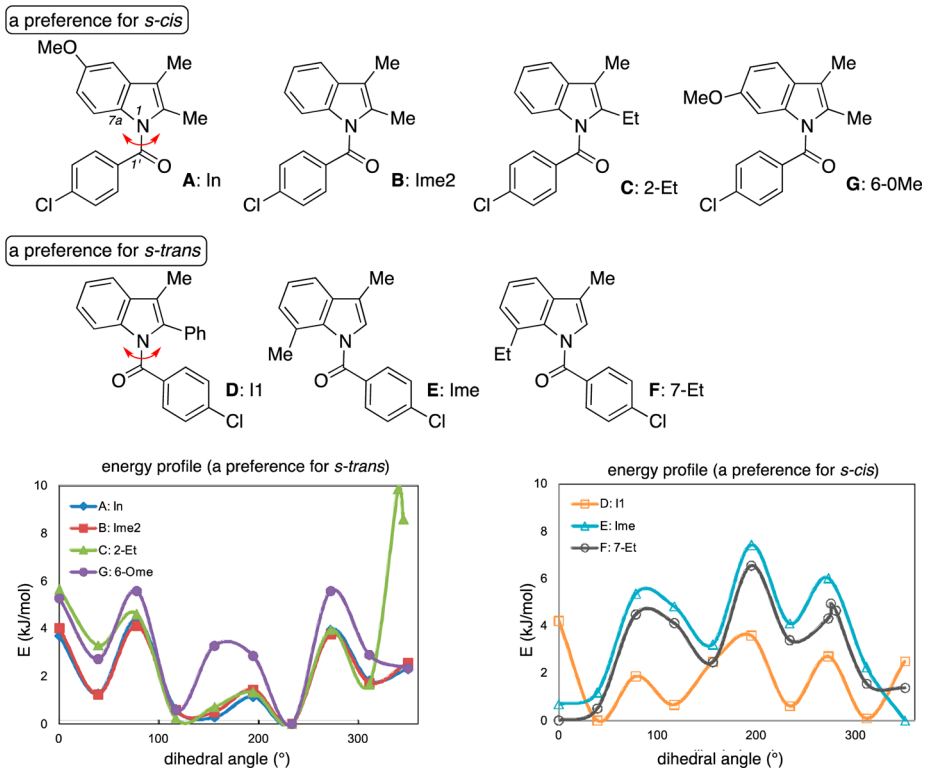
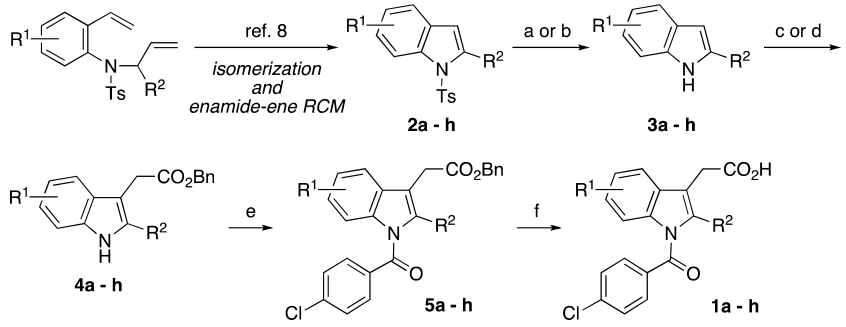


Figure 4. DFT calculation.

Scheme 1. Synthesis of Indomethacin (1a) and Its Derivatives (1b–h)[a]



[a]Reagents and conditions: (a) KOH, EtOH, THF, reflux, 77–98%. (b) Mg, MeOH, THF, 50 °C to rt, quant. (c) ICH_2CO_2Bn, *n*BuLi, $ZnCl_2$, THF, 0 °C to rt, 28–81%. (d) (i) Me_2NH, HCHO, AcOH. (ii) MeI, toluene. (iii) KCN, MeCN:H_2O = 1:3, reflux. (iv) Concentrated HCl, dioxane, reflux. (v) BnBr, $CsCO_3$, DMF, 0 °C rt, 51–85% (five steps). (e) 4-ClC_6H_4COCl, *t*BuOK, THF, −78 °C, 71–95%. (f) 10% Pd/C, H_2, AcOEt, rt or −15 °C, 80–95%.

process the same calculation for compound **H**, because of the result by molecular mechanics calculation (Figure 2).

Hence, both molecular mechanistic calculations and DFT calculations revealed almost the same results, indicating that introduction of a substituent at the 2- or 7-position on the indole ring effectively restricted the conformation of the indomethacin derivatives, as expected.

Chemistry. Most of the known indomethacin analogues prepared by traditional Fisher-indole synthesis are modified at the carboxylic acid moiety or at the 5-position of the indole ring.[9] The target compounds (**1b–h**) designed here, however, were not systematically prepared using traditional methods. The synthetic route used to construct the target compounds (**1b–h**) is depicted in Scheme 1. Substituted indoles (**3b–h**), key synthetic compounds, were obtained from the corresponding *N*-allyl-*N*-toluenesulfonyl-2-vinylaniline derivatives by a one-pot isomerization/enamide-ene metathesis and subsequent removal of *p*-toluenesulfonyl group on the nitrogen. Introduction of a substituent at the 3-position on the indole ring was accomplished by conventional 3-alkylation or Mannich reaction to yield **4a–h**. 4-Chlorobenzoylation of **4a–h** and subsequent hydrogenation led to **1a–h**, which have not been prepared previously (except for **1a** and **1b**,[10] which are known compounds), probably due to the limitation of the previous synthetic method described above.

Conformational Analysis by NMR. NOE experiments for **1a,c,e–g** were performed to investigate the conformation of the compounds in solution. Irradiation of the aromatic methine proton at the ortho-position of the benzoyl carbonyl of **1a** led to an NOE enhancement with the methine proton at the 7-position of the indole ring of 1.7% and with the methyl proton at the 2-position of 0.54% (Figure 5). These results suggest that

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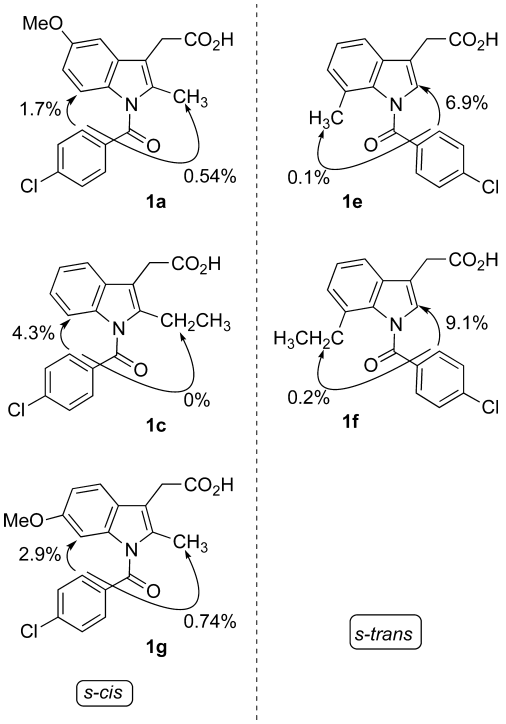

Figure 5. NOE correlations of **1a**,**c**,**e**−**g**.

the aromatic methine proton at the ortho-position of the benzoyl group is close to the methine proton at the 7-position of the indole ring. Thus, **1a** would be restricted in the s-cis form to some extent, as hypothesized. Furthermore, as shown in Figure 5, the NOE data suggested that **1c** and **1g** would be significantly restricted in the s-cis form and **1e** and **1f** in the s-trans form. The NOE data suggested that an ethyl group more effectively restricted the conformation than a methyl group (**1a** vs **1c**, **1e** vs **1f**). These results support our hypothesis that the introduction of substituents at the 2- or 7-position of indomethacin can restrict its conformation in the s-trans or the s-cis form because of steric repulsion of the substituent at the 2- or 7-position to the *N*-acyl side chain.

Conformational Analysis by the X-ray Method. X-ray analysis showed that a crystal of **1a** comprised units of the s-cis and s-trans forms at a 2:1 ratio, which is consistent with a previously reported polymorphism of indomethacin.[9b,11] On the other hand, compound **1d** was crystallized only in the s-trans form and was identical with the stable conformer obtained by the theoretical analysis described above (Figure 6).[7]

The structure of solid **1g** was determined using the X-ray powder diffraction method (Figure 7). This method allows for structural determination using very small crystals unsuitable for standard X-ray crystal graphic analysis. A crystal of **1g** comprised the s-cis and s-trans forms at a 1:1 ratio. These results, consistent with the NOE data, suggest that 2-phenyl derivative **1d** was highly restricted in the s-trans form, while conformational restriction to the s-cis form due to the 2-methyl group was not as pronounced in the case of **1a** and **1g**.

Biological Activities. *Cyclooxygenase Inhibitory Activity.* Table 1 shows the COX-1 and COX-2 inhibitory activities of indomethacin **1a** and its derivatives **1b**−**h** with their IC_{50} values. Indomethacin (**1a**) and **1b** showed potent COX-1 inhibitory activity and weak COX-2 inhibitory activity, while **1a** is known as a nonselective COX inhibitor. Compound **1g** inhibited COX-2 selectively, and the COX-2 selectivity was 4 times higher than that of COX-1. On the other hand, compounds **1c**−**f**,**h** were almost inactive to both COX-1 and COX-2. Thus, introduction of a substituent at the 7-position of indole abolished its COX-inhibitory activities, and a substituent bulkier than the methyl group at the 2- or 6-position of indole significantly decreased its COX-inhibitory activities.

P-Glycoprotein Inhibitory Activity. Table 1 also shows the P-gp inhibitory activities of indomethacin (**1a**) and its derivatives **1b**−**h** with their IC_{50} values. All of these indomethacin derivatives as well as verapamil, a well-known P-gp inhibitor, had potent and similar P-gp inhibitory activities. These results suggest that the P-gp inhibitory activity of the NSAID indomethacin is not related to its COX-inhibitory activity. This finding is crucial, as several previous studies have

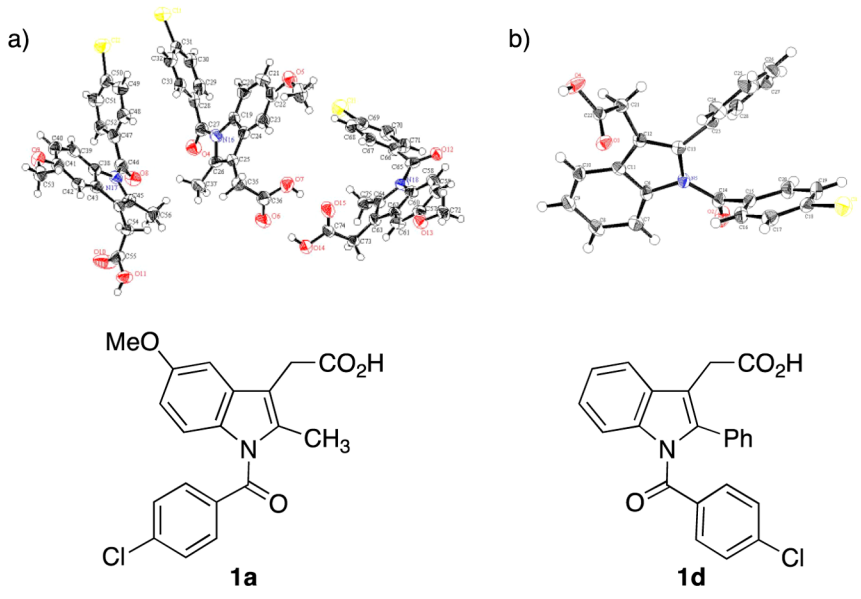

Figure 6. ORTEP drawing of (a) **1a** and (b) **1d**.

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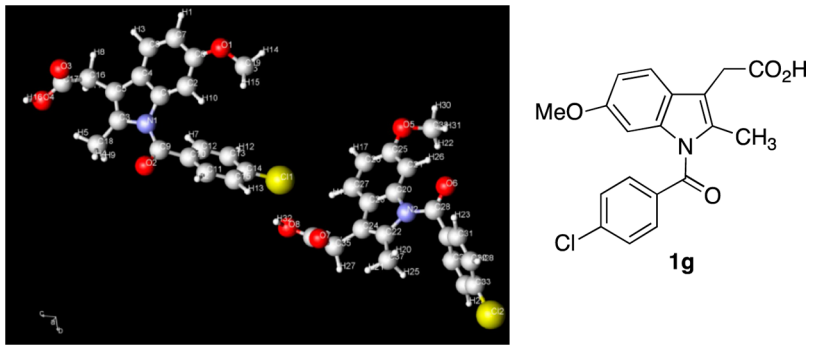

Figure 7. 3D structure of **1g** obtained by the X-ray powder diffraction method.

Table 1. Structure–Activity Relationship Studies of COX and P-gp Inhibition by 1a–h (n = 3)

	IC_{50} (μM)		
compound	COX-1	COX-2	P-gp
1a (synthesized)	0.01	18	28 ± 3.1
1b	0.01[a]	39[a]	20 ± 2.7
1c	>400	>400	20 ± 5.1
1d	>400	>400	18 ± 3.0
1e	>400	>400	15 ± 3.6
1f	>400	>400	17 ± 2.4
1g	>200	49	9.8 ± 0.97
1h	>400	>400	15 ± 2.2
1a (purchased)	0.12	20	32 ± 16
verapamil	–	–	18 ± 3.6

[a]These data are consisent with previously reported findings.[12]

posited a link between COX-2 expression and P-gp expression.[13–15]

Effect on MDR. MDR is a limiting factor in antitumor chemotherapy. MDR caused by MDR-associated MRP-1 is modulated by indomethacin (**1a**).[16] To date, few studies have examined how MRP-1 induces MDR.[17] The development of potent agents to inhibit MRP-1-induced MDR with minimal side effects is an important aim of medical and pharmacological research. Thus, using the previously reported procedure,[2] **1a–h** were evaluated in a cell biological cytotoxicity assay using the MRP-1-expressing human glioblastoma cell line T98G as a model system of MDR tumors at a concentration of 50 μM without or with 0.3, 1, or 3 μM doxorubicin (Figure 8).

As shown in Figure 8, doxorubicin showed moderate cytotoxicity in this cell line due to the MRP-1 expression, e.g., about 40% inhibition of the cell growth at 1 μM. Consistent with previously reports,[2] indomethacin (**1a**) effectively enhanced the cytotoxicity of doxorubicin by approximately 2-fold. In this system, 7-methoxyindole derivative **1h**, as well as 2-substituted indole derivatives **1c** and **1d**, without COX-1 and -2 inhibitory activity (IC_{50} >400 μM), enhanced the cytotoxicity of doxorubicin similar to **1a**. 7-Methylindole derivative **1e** and 7-ethylindole derivative **1f** exhibited only weak, nonsignificant inhibitory activities on COX-1, COX-2, and MDR. Previous studies indicated that the COX inhibitory effect of NSAIDs might be related to the MDR of cancer cells.[1,2,18] The present results in Table 1 and Figure 8, however, established that the MDR-modulating effect of the NSAID indomethacin is not associated with its COX-inhibitory activity.

CONCLUSION

We designed and synthesized a series of conformationally restricted and regioisomeric indomethacin analogues and suggested, that their conformation was restricted to the *s-cis*

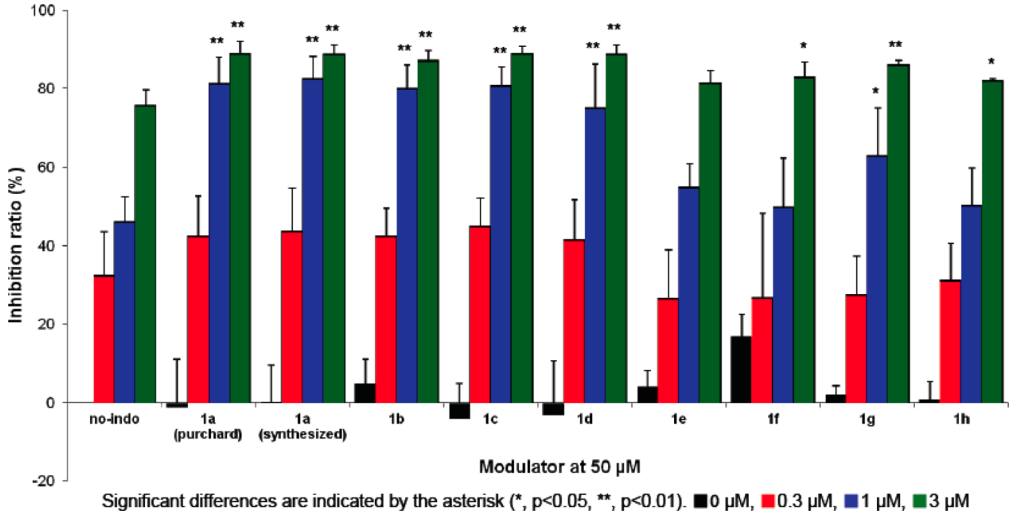

Figure 8. Effects of indole compounds **1a–h** on the cytotoxicity of doxorubicin. T98G cells were pretreated with an indomethacin analogue (50 μM) for 2 h and then cotreated with doxorubicin (0, 0.3, 1, or 3 μM) for 4 days. Survival was measured by crystal violet staining as described in Materials and Methods. Data shown are the mean percentage ± SD for a minimum of three experiments.

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or *s-trans* form at least to some extent due to steric effects of an added substituent at the 2- or 7-position on the indole ring. Evaluation of the COX inhibitory, P-gp inhibitory, and MDR modulating effects of the compounds indicated that the MDR-modulating effects of NSAIDs due to P-gp and/or MRP-1 inhibition are not associated with their COX-1 and COX-2 inhibitory activities.

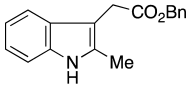
EXPERIMENTAL SECTION

MacroModel. The following protocol was used to generate various conformations of each compound: Execute an exhaustive conformational search using the molecular modeling program MacroModel (version 8.5).[19] For calculations of all compounds, the latest development of the current best general-purpose force-field for medicinal chemistry, MMFF94s,[20] was used in combination with the GB/SA solvation model.[21] GB/SA treats the solvent as an analytical dielectric continuum that starts near the van der Waals surface of the solute and extends to infinity. The model includes both generalized Born-based (GB) solvent polarization terms and surface area-based (SA)[22] solvent displacement terms. All nonbonded cutoffs were set to infinity for all calculations. Energy minimizations were performed with the Truncated Newton−Raphson conjugate gradient[23] method, which involves the use of second derivatives; the derivative convergence criterion was set to 0.05 kJ/Å-mol. The conformational search was performed using the Monte Carlo[24] method for random variation of all of the rotatable bonds combined with the so-called low mode conformational search[25] algorithm. For each calculation 10 000 Monte Carlo steps were performed.

DFT. All ab initio and DFT calculations were performed using the GAUSSIAN 03 W. The C50−C10−C1−H1dihedral angle of the compounds was rotated from 0° to 360° at 10° intervals, and the conformations were optimized at B3LYP/6-31G*. Finally, single point energies were calculated at RB3LYP/6-31G*.

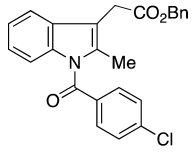
Synthesis. ^{1}H NMR spectra were recorded in $CDCl_3$ at 25 °C unless otherwise noted, at 400 or 500 MHz, with TMS as an internal standard. ^{13}C NMR spectra were recorded in $CDCl_3$ at 25 °C unless otherwise noted, at 400 or 500 MHz. Flash column chromatography was performed with silica gel 60 N (spherical, neutral, 40−50 μm, Kanto Chemical Co., Inc.). **3b**,**e**,**f** are commercially available. **1a**,**g** and **2c**,**d** were prepared according to the reported procedures.[8] Elemental analysis was performed to confirm ≥95% sample purity (within ±0.4% of the calculated value).

*2-[1-(4-Chlorobenzoyl)-2-methylindol-3-yl]acetic Acid (**1b**).* To a solution of **3b** (0.59 mmol, 77 mg) in tetrahydrofuran (2.0 mL) was added dropwise a solution of *n*-BuLi in hexane (1.59 M, 0.97 mmol, 0.62 mL) at 0 °C. The solution was warmed to rt and stirred for 30 min. To the mixture was added a solution of $ZnCl_2$ (1.0M, 0.6 mmol, 0.6 mL), and the whole was continuously stirred for 30 min. To the mixture was cannulated a solution of iodoacetic acid benzyl ester(1.10 mmol, 303 mg) in tetrahydrofuran (1.0 mL), and the whole was stirred for further 17 h. To the mixture was added sat. aq NH_4Cl. The solvent was partially removed by evaporation, and organic compounds were extracted with AcOEt. Organic layers were washed with brine and dried over Na_2SO_4. The solvents were filtered and evaporated under reduced pressure. The obtained residue was subjected to column chromatography (neutral silica gel, hexane to hexane/AcOEt = 9:1) to give **4b** (0.48 mmol, 134 mg, 88%) as yellow needles.



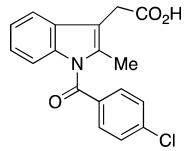
Mp 84−84 °C (EtOH); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.84 (br, 1 H, N*H*), 7.51 (d, 1 H, *J* = 7.4 Hz, indole-aromatic), 7.35−7.29 (m, 5 H, CH_2*Ph*), 7.25 (d, 1 H, *J* = 7.4 Hz, indole-aromatic), 7.12 (dd, 1 H, *J* = 7.4, 7.4 Hz, indole-aromatic), 7.08 (dd, 1 H, *J* = 7.4, 7.4 Hz, indole-aromatic), 5.11 (s, 2 H, C*H*$_2$Ph), 3.73 (s, 2 H, Ar-C*H*$_2$CO), 2.38 (s, 3 H, Ar-C*H*$_3$); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 171.85, 135.99, 135.03, 132.69, 128.44, 128.40, 128.05, 121.19, 119.50, 118.06, 110.21, 104.35, 66.42, 30.37, 11.66; LRMS (EI) *m*/*z* 279.15 (M^+); HRMS (EI) calcd for $C_{18}H_{17}NO_2$ (M^+) 279.1259, found 279.1259. Anal. Calcd for $C_{18}H_{17}NO_2 \cdot 0.1H_2O$: C, 76.90; H, 6.17; N, 4.98. Found: C, 76.70; H, 6.14; N, 5.01.

To a solution of **4b** (0.22 mmol, 61 mg) in tetrahydrofuran (2.0 mL) was added dropwise a solution of *t*BuOK in tetrahydrofuran (1.0 M, 0.26 mmol, 264 μL) at −78 °C, and the mixture was stirred for 1 h. To the mixture was cannulated a solution of *p*-chlorobenzoyl chloride (0.33 mmol, 42 μL) in tetrahydrofuran (1.0 mL), and the whole was stirred for 3 h. To the mixture was added sat. aq NH_4Cl. The solvent was partially removed by evaporation, and organic compounds were extracted with AcOEt. Organic layers were washed with brine and dried over Na_2SO_4. The solvents were filtered and evaporated under reduced pressure. The obtained residue was subjected to column chromatography (neutral silica gel, hexane/AcOEt = 20:1) to give **5b** (0.21 mmol, 87 mg, 95%) as a yellow oil.



^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.65 (d, 2 H, *J* = 8.6 Hz), 7.49 (d, 1 H, *J* = 7.4 Hz), 7.45 (d, 2 H, *J* = 8.6 Hz), 7.35−7.29 (m, 5 H), 7.17 (dd, 1 H, *J* = 7.4 8.0 Hz), 7.06 (dd, 1 H, *J* = 7.4, 8.0 Hz), 6.97 (d, 1 H, *J* = 8.0 Hz), 5.13 (s, 2 H), 3.74 (s, 2 H), 2.38 (s, 3 H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.66, 168.44, 139.37, 136.07, 135.67, 135.16, 133.67, 131.22, 129.53, 129.09, 128.48, 128.21, 128.08, 123.19, 122.70, 118.37, 113.96, 112.35, 66.70, 30.24, 13.16. LRMS (EI) *m*/*z* 417.16 (M^+); HRMS (EI) calcd for $C_{25}H_{20}ClNO_3$ 417.1132 (M^+), found 417.1133. Anal. Calcd for $C_{25}H_{20}ClNO_3 \cdot 0.2H_2O$: C, 71.24; H, 4.88; N, 3.32. Found: C, 71.32; H, 5.08; N, 3.25.

To a solution of **5b** (72 μmol, 33 mg) in AcOEt (2.0 mL) was added 10% Pd on carbon (15 mg) under H_2 atmosphere (1 atm), and the mixture was stirred for 1 h. After Celite545 pad filtration, the crude was subjected to column chromatography (acidic silica gel, $CHCl_3$/MeOH =97:3) to give **1b** (55 μmol, 18 mg, 76%) as a colorless solid.



^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.68 (d, 2 H, *J* = 8.6 Hz), 7.50 (d, 1 H, *J* = 7.4 Hz), 7.47 (d, 2 H, *J* = 8.6 Hz), 7.19 (dd, 1 H, *J* = 7.4, 7.4 Hz), 7.06 (dd, 1 H, *J* = 7.4, 8.0 Hz), 6.94 (d, 1 H, *J* = 8.0 Hz), 3.73 (s, 2 H), 2.41 (s, 3 H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 178.32, 169.05, 140.41, 137.33, 135.78, 134.66, 130.35, 132.21, 130.75, 124.00, 122.89, 119.34, 114.78, 113.98, 31.09. 13.25; LRMS (EI) *m*/*z* 327.08 (M^+)

*2-[1-(4-Chlorobenzoyl)-2-ethylindol-3-yl]acetic Acid (**1c**).* To a solution of **2c** (0.64 mmol, 192 mg) in MeOH (5.0 mL) was added KOH (19.2 mmol, 1.08 g), and the mixture was refluxed for 4 h. To the mixture was added 1 N HCl, and the organic compounds were extracted with AcOEt. Organic layers were washed with brine and dried over Na_2SO_4. The solvents were filtered and evaporated under reduced pressure. The obtained residue was subjected to column chromatography (NH_2 silica gel, hexane/AcOEt = 6:1) to give **3c** (0.63 mmol, 91 mg, 98%) as a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (br, 1 H), 7.52 (d, 1 H, *J* = 7.2 Hz), 7.25 (d, 1 H, *J* = 7.7 Hz), 7.13−7.04 (m, 2 H), 6.23 (s, 1 H), 2.75 (q, *J* = 7.7 Hz, 2 H), 1.32 (t, *J* = 7.7 Hz, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 141.41, 135.73, 128.67, 120.81, 119.66, 119.46, 110.31, 98.44, 21.21, 13.14; LRMS (EI) *m*/*z* 145 (M^+).

To a solution of **3c**[28] (0.73 mmol, 106 mg) in tetrahydrofuran (5.0 mL) was added dropwise a solution of *n*-BuLi in tetrahydrofuran (1.59 M, 0.95 mmol, 0.73 mL) at 0 °C. The solution was warmed to rt and stirred for 50 min. To the mixture was added a solution of $ZnCl_2$ (1.0M, 0.73 mmol, 0.73 mL), and the whole was continuously stirred

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for 50 min. To the mixture was cannulated a solution of iodoacetic acid benzyl ester (2.19 mmol, 605 mg) in tetrahydrofuran (3.0 mL) and the whole was stirred for further 18 h. To the mixture was added sat. aq NH_4Cl. The solvent was partially removed by evaporation, and the organic compounds were extracted with AcOEt. Organic layers were washed with brine and dried over Na_2SO_4. The solvents were filtered and evaporated under reduced pressure. The obtained residue was subjected to column chromatography (NH_2 silica gel, hexane to hexane/AcOEt = 30:1) to give **4c** (0.22 mmol, 64 mg, 30%) as a yellow oil.

^{1}H NMR (500 MHz, $CDCl_3$) d 7.91 (br, 1 H), 7.53 (d, 1 H, J = 8.0 Hz), 7.33−7.26 (m, 5 H), 7.20 (d, 1 H, J = 8.0 Hz), 7.12−7.06 (m, 2 H), 5.08 (s, 2 H), 3.73 (s, 2 H), 2.70 (q, 2 H, J = 7.4 Hz), 1.20 (t, 3 H, J = 7.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.89, 138.43, 135.90, 134.98, 128.38, 128.02, 121.14, 119.41, 118.19, 110.36, 103.30, 66.39, 30.30, 19.29, 13.89; LRMS (EI) m/z 293.16 (M^+); HRMS (EI) calcd for $C_{19}H_{19}NO_2$ (M^+); 293.1416, found 293.1404.

To a solution of **4c** (0.13 mmol, 38 mg) in tetrahydrofuran (1.0 mL) was added dropwise a solution of *t*BuOK in tetrahydrofuran (1.0 M, 0.17 mmol, 170 μL) at −40 °C, and the mixture was stirred for 1 h. To the mixture was cannulated a solution of *p*-chlorobenzoyl chloride (0.21 mmol, 27 μL) in tetrahydrofuran (0.1 mL), and the whole was stirred for 2 h. To the mixture was added sat. aq NH_4Cl. The solvent was partially removed by evaporation, and organic compounds were extracted with AcOEt. Organic layers were washed with brine and dried over Na_2SO_4. The solvents were filtered and evaporated under reduced pressure. The obtained residue was subjected to column chromatography (neutral silica gel, hexane/AcOEt = 19:1) to give **5c** (90 μmol, 39 mg, 69%) as a yellow oil.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.68 (d, 2 H, J = 8.3 Hz), 7.51 (d, 1 H, J = 7.4 Hz), 7.46 (d, 2 H, J = 8.3 Hz), 7.35−7.29 (m, 5 H), 7.15 (dd, 1 H, J = 7.1, 7.4 Hz), 7.00 (dd, 1 H, J = 7.1, 8.6 Hz), 6.72 (d, 1 H, J = 8.6 Hz), 5.14 (s, 2 H), 3.77 (s, 2 H), 2.95 (q, 2 H, J = 7.4 Hz), 1.14 (t, 3 H, J = 7.4 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.77, 168.51, 141.62, 139.65, 136.24, 135.70, 133.48, 131.39, 129.49, 129.19, 128.50, 128.23, 128.15, 123.02, 122.49, 118.69, 113.78, 111.61, 66.75, 30.24, 19.13, 14.43; LRMS (EI) 431.15 (M^+); HRMS (EI) calcd for $C_{26}H_{22}ClNO_3$ (M^+); 431.1288, found 431.1275

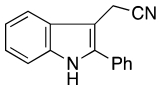
To a solution of **5c** (0.10 mmol, 44 mg) in AcOEt (1.0 mL) was added 10% Pd on carbon (15 mg) under H_2 atmosphere (1 atm), and the mixture was stirred for 4 h. After Celite545 pad filtration, the crude was subjected to column chromatography (acidic silica gel, hexane/AcOEt = 5:1) to give **1c** (90 μmol, 30 mg, 90%) as yellow needles.

Mp 112−113 °C (50% aq EtOH); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.70 (d, 1 H, J = 8.6 Hz), 7.52 (d, 1 H, J = 8.0 Hz), 7.47 (d, 1 H, J = 8.6 Hz), 7.17 (dd, 1 H, J = 7.4, 8.0 Hz), 7.01 (dd, 1 H, J = 7.4, 7.4 Hz), 6.70 (d, 1 H, J = 7.4 Hz), 3.76 (s, 2 H), 2.97 (q, 2 H, J = 7.4 Hz), 1.18 (t, 3 H, J = 7.4 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 176.90, 168.53, 141.88, 139.74, 136.21, 133.39, 131.43, 129.35, 123.13, 122.56, 118.56, 113.86, 110.97, 29.89, 19.13, 14.42; LRMS (EI) m/z 341.12 (M^+); HRMS (EI) calcd for $C_{19}H_{16}ClNO_3$ (M^+); 341.0819, found 341.0819.

*2-[1-(4-Chlorobenzoyl)-2-phenylindole-3-yl]acetic Acid (**1d**).* To a solution of **2d** (0.15 mmol, 53 mg) in MeOH (1.5 mL) was added KOH (1.53 mmol, 86 mg), and the mixture was refluxed for 4 h. To the mixture was added 1 N HCl, and the organic compounds were extracted with AcOEt. Organic layers were washed with brine and dried over Na_2SO_4. The solvents were filtered and evaporated under reduced pressure. The obtained residue was subjected to column chromatography (NH_2 silica gel, hexane/AcOEt = 6:1) to give **3d**[29] (0.12 mmol, 23 mg, 78%) as colorless needles.

Mp 89 °C (CH_2Cl_2, ref 85−86 °C); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.33 (br, 1 H), 7.68−7.65 (m, 2 H), 7.63 (d, 1H, J = 8.0 Hz), 7.46−7.42 (m, 2 H), 7.40 (d, 1 H, J = 8.0 Hz), 7.33 (t, 1 H, J = 7.7 Hz), 7.20 (ddd, 1 H, J = 0.9, 1.4, 8.0 Hz), 7.12 (ddd, 1 H, J = 0.9, 1.4, 8.0 Hz), 6.83 (s, 1 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 137.83, 136.73, 132.28, 129.19, 128.98, 127.68, 125.10, 122.31, 120.63, 120.24, 110.89, 99.92; LRMS (EI) m/z 193 (M^+).

To a solution mixture of 50% Me_2NH (3.39 mmol, 305 μL), acetic acid (0.7 mL), and 36−38% formic acid (3.13 mmol, 254 μL) was added **3c** (2.61 mmol, 504 mg) at 0 °C, and the mixture was stirred for 3 h. The pH of the solution was changed to 12 with 20% KOH at 0 °C, and organic compounds were extracted with Et_2O. Organic layers were washed with water and brine and dried over Na_2SO_4. After filtration, solvents were evaporated under reduced pressure. To a solution of the obtained residue in toluene (30 mL) was added iodomethane (5.22 mmol, 325 μL), and the mixture was stirred for 12 h. After evaporation, to a solution of the obtained solid in water (21 mL) and MeCN (7 mL) was added KCN (3.13 mmol, 204 mg), and the mixture was refluxed for 5 h. After cooling, organic compounds were extracted with AcOEt. Organic layers were washed with water and brine and dried over Na_2SO_4. The solvents were filtered and evaporated under reduced pressure. The obtained residue was subjected to column chromatography (neutral silica gel, hexane/AcOEt = 3:1) to give 2-phenyl-1*H*-indol-3-ylacetonitrile[30] (2.24 mmol, 521 mg, three steps 86%) as colorless plates.

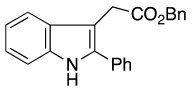


Mp 130 °C (CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.26 (br, 1 H, NH), 7.70 (d, 1 H, J = 7.7 Hz, aromatic), 7.53−7.51 (m, 3 H, aromatic), 7.46−7.40 (m, 2 H, aromatic), 7.29−7.19 (m, 3 H, aromatic), 3.88 (s, 2 H, CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 136.07, 135.41, 131.07, 128.89, 128.26, 127.87, 127.39, 122.71, 120.21, 118.37, 117.98, 111.17, 100.17, 13.43; LRMS (EI) m/z 232.15 (M^+).

To a solution of 2-phenyl-1*H*-indol-3-ylacetonitrile (851 mg, 3.36 mmol) in dioxane (18 mL) was added 12 N HCl (10 mL), and the mixture was refluxed for 2 h. After cooling, organic compounds were extracted with AcOEt. Organic layers were washed with water and brine and dried over Na_2SO_4. The solvents were filtered and evaporated under reduced pressure. The obtained residue was subjected to column chromatography (hexane/AcOEt = 2:1) to give **4d**[31] (3.57 mmol, 898 mg, 98%) as a colorless needle.

Mp 174−175 °C (CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.15 (br, 1 H), 7.66 (d, 1 H, J = 8.2 Hz), 7.62 (d, 2 H, J = 8.6 Hz), 7.49 (dd, 2 H, J = 7.2, 7.2 Hz), 7.42−7.38 (m, 2 H), 7.26−7.22 (m, 1 H), 7.17 (dd, 1 H, J = 7.2. 7.2 Hz), 3.87 (s, 2 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 177.64, 136.43, 135.66, 132.15, 129.01, 128.82, 128.24, 128.20, 122.73, 120.23, 119.21, 110.92, 104.83, 30.69; LRMS (EI) m/z 251.15 (M^+).

To a solution of **4d** (4.06 mmol, 1.02 g) in *N*,*N*-dimethylformamide (30 mL) were added $CsCO_3$ (2.23 mmol, 728 mg) and BnBr (4.47 mmol, 530 μL) at 0 °C, and the mixture was stirred at rt for 5 h. To the mixture was added sat. aq NH_4Cl. Organic compounds were extracted with AcOEt. Organic layers were washed with brine and dried over Na_2SO_4. The solvents were filtered and evaporated under reduced pressure. The obtained residue was subjected to column chromatography (silica gel, hexane to hexane/AcOEt = 10:1) to give benzyl 2-phenyl-3-indoleacetate (3.66 mmol, 918 mg, 90%) as yellow statues.



Mp 110 °C (AcOEt); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.15 (br, 1 H), 7.65 (d, 1 H, J = 8.0 Hz), 7.58 (dd, 2 H, J = 7.4, 7.4 Hz), 7.39 (t, 2 H, J = 7.4 Hz), 7.35−7.28 (m, 7 H), 7.18 (dd, 1 H, J = 6.9, 7.4 Hz), 7.12 (dd, 1 H, J = 6.9, 8.0 Hz), 5.13 (s, 2 H), 3.87 (s, 2 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 172.11, 136.10, 135.70, 135.60, 132.12, 128.76, 128.69, 128.35, 128.07, 128.01, 127.79, 122.32, 119.80, 119.05, 110.90, 105.02, 66.52, 30.96; LRMS (EI) m/z 341.22 (M^+); Anal. Calcd for $C_{23}H_{19}NO_2$: C, 80.92; H, 5.61; N, 4.10. Found: C, 81.13; H, 5.65; N, 4.09.

To a solution of benzyl 2-phenyl-3-indoleacetate (3.26 mmol, 1.11 g) in tetrahydrofuran (20 mL) was added dropwise a solution of *t*BuOK in tetrahydrofuran (1.0 M, 3.91 mmol, 170 μL) at −40 °C, and the mixture was stirred for 1 h. To the mixture was cannulated a solution of *p*-chlorobenzoyl chloride (4.89 mmol, 627 μL) in

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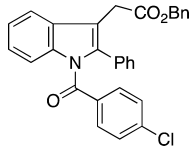
tetrahydrofuran (5 mL), and the whole was stirred for 3 h. To the mixture was added sat. aq NH_4Cl. The solvent was partially removed by evaporation, and organic compounds were extracted with AcOEt. Organic layers were washed with brine and dried over Na_2SO_4. The solvents were filtered and evaporated under reduced pressure. The obtained residue was subjected to column chromatography (neutral silica gel, hexane/AcOEt = 19:1) to give **5d** (2.76 mmol, 1.32 g, 85%) as a colorless statue.

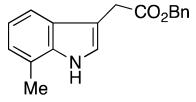


Mp 130 °C (AcOEt); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.78 (d, 1 H, J = 7.4 Hz), 7.64 (d, 1 H, J = 6.9 Hz), 7.41 (d, 2 H, J = 8.6 Hz), 7.35−7.28 (m, 7 H), 7.24−7.22 (m, 2 H), 7.17−7.15 (m, 3 H), 7.12 (d, 2 H, J = 8.6 Hz), 5.18 (s, 2 H), 3.72 (s, 2 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 171.07, 168.74, 138.67, 138.02, 136.96, 135.66, 133.55, 131.39, 131.31, 129.94, 129.27, 128.53, 128.33, 128.31, 128.19, 128.02, 124.86, 123.35, 119.33, 115.12, 113.56, 66.85, 30.95; LRMS (EI) m/z 479.20 (M^+); Anal. Calcd for $C_{30}H_{22}ClNO_3$: C, 75.07; H, 4.62; N, 2.92. Found: C, 74.61; H, 4.73; N, 2.92.

To a solution of **5d** (1.13 mmol, 540 mg) in AcOEt (10 mL) was added 10% Pd on carbon (170 mg) under H_2 atmosphere (1 atm), and the mixture was stirred for 4 h. After Celite545 pad filtration, the crude was subjected to column chromatography (acidic silica gel, hexane/AcOEt = 3:1) to give **1d** (1.07 mmol, 417 mg, 95%) as a colorless plate.

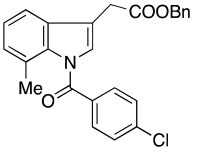
Mp 195 °C (AcOEt); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.79−7.77 (m, 1 H), 7.67−7.68 (m, 1 H), 7.46 (d, 2 H, J = 8.6 Hz), 7.35−7.33 (m, 2 H), 7.27−7.26 (m, 2 H), 7.24−7.20 (m, 3 H), 7.17 (d, 2 H, J = 8.6 Hz), 3.72 (s, 2 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 177.33, 168.75, 138.81, 138.26, 136.96, 131.54, 134.36, 131.26, 129.94, 128.88, 128.40, 128.32, 128.18, 124.99, 123.44, 119.28, 114.24, 112.92, 30.71; LRMS (EI) m/z 389.12 (M^+); HRMS (EI) calcd for $C_{23}H_{16}ClNO_3$ (M^+); 389.0819, found 389.0811.

*2-[1-(4-Chlorobenzoyl)-2,7-dimethylindole-3-yl]acetic Acid (**1e**).* To a solution of **3e** (10 mmol, 1.31 g) in tetrahydrofuran (60 mL) was added dropwise a solution of *n*-BuLi in tetrahydrofuran (1.57 M, 12 mmol, 7.64 mL) at 0 °C. The solution was warmed to rt and stirred for 30 min. To the mixture was added a solution of $ZnCl_2$ (1.0 M in Et_2O, 10 mmol, 10 mL), and the whole was continuously stirred for 30 min. To the mixture was cannulated a solution of iodoacetic acid benzyl (15 mmol, 4.14 g) in tetrahydrofuran (5.0 mL), and the whole was stirred for further 15 h. To the mixture was added sat. aq NH_4Cl. The solvent was partially removed by evaporation, and organic compounds were extracted with AcOEt. Organic layers were washed with brine and dried over Na_2SO_4. The solvents were filtered and evaporated under reduced pressure. The obtained residue was subjected to column chromatography (neutral silica gel, hexane:AcOEt = 10:1) to give **4e** (3.57 mmol, 997 mg, 36%) as a yellow oil.



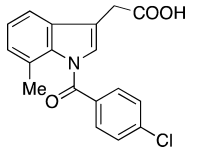
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02 (br, 1 H), 7.45 (d, 1 H, J = 7.2 Hz), 7.33−7.25 (m, 5 H), 7.17 (s, 1 H), 7.07−6.99 (m, 2 H), 5.15 (s, 2 H), 3.83 (2 H), 2.47 (s, 3 H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 172.02, 135.82, 135.55, 128.45, 128.17, 128.12, 126.61, 122.88, 122.56, 120.34, 119.75, 116.45, 108.46, 66.52, 31.38, 16.41; LRMS (EI) m/z 279.15 (M^+); HRMS (EI) calcd for $C_{18}H_{17}NO_2$ (M^+) 279.1259, found 279.1259 (M^+).

To a solution of **4e** (1.15 mmol, 320 mg) in tetrahydrofuran (10 mL) was added dropwise a solution of *t*BuOK in tetrahydrofuran (1.0 M, 1.50 mmol, 1.5 mL) at −78 °C, and the mixture was stirred for 1 h. To the mixture was cannulated a solution of *p*-chlorobenzoyl chloride (2.29 mmol, 294 μL) in tetrahydrofuran (1 mL), and the whole was stirred for 3 h. To the mixture was added sat. aq NH_4Cl. The solvent was partially removed by evaporation, and organic compounds were extracted with AcOEt. Organic layers were washed with brine and dried over Na_2SO_4. The solvents were filtered and evaporated under reduced pressure. The obtained residue was subjected to column chromatography (neutral silica gel, hexane:AcOEt = 20:1) to give **5e** (0.96 mmol, 402 g, 84%) as a yellow oil.



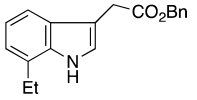
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.85 (d, J = 8.6 Hz, 2 H), 7.50 (d, J = 8.3 Hz, 2 H), 7.40 (d, 1 H, J = 7.2 Hz), 7.33−7.25 (m, 7 H), 7.21−7.19 (m, 2 H), 5.14 (s, 2 H), 3.74 (s, 2 H), 2.46 (s, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.53, 166.17, 139.41, 135.78, 135.47, 132.10, 131.65, 131.49, 129.02, 128.46, 128.26, 128.22, 127.91, 127.08, 126.11, 124.02, 116.64, 113.70, 66.75, 30.87, 21.51; LRMS (EI) m/z 417.16 (M^+); HRMS (EI) calcd for $C_{25}H_{20}ClNO_3$ (M^+) 417.1132, found 417.11

To a solution of **5e** (67 μmol, 28 mg) in AcOEt (1 mL) was added 10% Pd on carbon (7 mg) at −15 °C under H_2 atmosphere (1 atm), and the mixture was stirred for 2 h. After Celite545 pad filtration, the crude was subjected to column chromatography (acidic silica gel, $CHCl_3$:MeOH = 97:3) to give **1e** (54 μmol, 18 mg, 81%) as a yellow needle.



Mp 102−103 °C (from 50% aq EtOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.87 (d, 2 H, J = 8.6 Hz), 7.52 (d, J = 8.6 Hz, 2 H), 7.42 (d, J = 8.0 Hz, 1 H), 7.29 (dd, J = 7.5, 8.0 Hz, 1 H), 7.21 (d, J = 7.5 Hz, 1 H), 7.19 (s, 1 H), 3.73 (s, 2 H), 2.46 (s, 3 H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 176.42, 166.29, 139.63, 132.10, 131.75, 131.51, 131.39, 129.15, 128.85, 128.06, 127.33, 126.25, 124.17, 116.68, 113.22, 29.67, 21.55; LRMS (EI) m/z 327.11 (M^+); HRMS (EI) calcd for $C_{18}H_{14}ClNO_3$ (M^+) 327.0662, found 327.0656.

*1-(4-Chlorobenzoyl)-7-ethyl-2-methylindole-3-yl acetic Acid (**1f**).* To a solution of **3f** (10 mmol, 1.37 g) in tetrahydrofuran (60 mL) was added dropwise a solution of *n*-BuLi in tetrahydrofuran (1.57 M, 12 mmol, 7.64 mL) at 0 °C. The solution was warmed to rt and stirred for 30 min. To the mixture was added a solution of $ZnCl_2$ (1.0 M in Et_2O, 10 mmol, 10 mL), and the whole was continuously stirred for 30 min. To the mixture was cannulated a solution of iodoacetic acid benzyl ester (15 mmol, 4.14 g) in tetrahydrofuran (5.0 mL), and the whole was stirred for further 15 h. To the mixture was added sat. aq NH_4Cl. The solvent was partially removed by evaporation, and organic compounds were extracted with AcOEt. Organic layers were washed with brine and dried over Na_2SO_4. The solvents were filtered and evaporated under reduced pressure. The obtained residue was subjected to column chromatography (neutral silica gel, hexane:AcOEt = 10:1) to give **4f** (2.78 mmol, 815 mg, 28%) as a yellow oil.



^{1}H NMR (400 MHz, $CDCl_3$) δ 8.04 (br, 1 H), 7.45 (d, 1 H, J = 7.7 Hz), 7.33−7.24 (m, 5 H), 7.16 (s, 1 H), 7.08−7.03 (m, 2 H), 5.15 (s, 2 H), 3.83 (s, 2 H), 2.84 (q, 2 H, J = 7.7 Hz), 1.34 (t, 3 H, J = 7.7 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.08, 135.78, 134.79, 128.41, 128.11, 128.09, 126.78, 126.55, 122.85, 120.47, 119.77, 116.40, 108.23, 66.48, 31.33, 23.70, 13.69; LRMS (EI) m/z 293.17 (M^+); HRMS (EI) calcd for $C_{19}H_{19}NO_2$ (M^+) 293.1416, found 293.1408.

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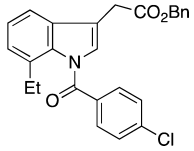
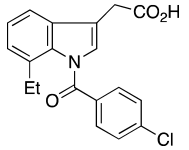
To a solution of **4f** (0.52 mmol, 153 mg) in tetrahydrofuran (5 mL) was added dropwise a solution of *t*BuOK in tetrahydrofuran (1.0 M, 0.78 mmol, 780 μL) at −78 °C, and the mixture was stirred for 1 h. To the mixture was cannulated a solution of *p*-chlorobenzoyl chloride (1.56 mmol, 200 μL) in tetrahydrofuran (1 mL), and the whole was stirred for 3 h. To the mixture was added sat. aq NH_4Cl. The solvent was partially removed by evaporation, and organic compounds were extracted with AcOEt. Organic layers were washed with brine and dried over Na_2SO_4. The solvents were filtered and evaporated under reduced pressure. The obtained residue was subjected to column chromatography (neutral silica gel, hexane:AcOEt = 20:1) to give **5f** (0.37 mmol, 159 mg, 71%) as a yellow oil.



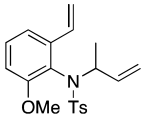
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.85 (d, *J* = 8.6 Hz, 2 H), 7.50 (d, *J* = 8.3 Hz, 2 H), 7.41−7.25 (m, 8 H), 7.17 (s, 1 H), 5.14 (s, 2 H), 3.74 (s, 2 H), 2.92 (q, 2 H, *J* = 7.4 Hz), 1.16 (t, 3 H, *J* = 7.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.57, 166.41, 139.47, 135.49, 134.91, 132.19, 132.09, 131.69, 131.48, 129.83, 129.09, 128.48, 128.22, 127.16, 125.99, 124.19, 116.77, 113.70, 66.77, 30.87, 27.41, 13.61; LRMS (EI) *m*/*z* 431.18 (M^+); HRMS (EI) calcd for $C_{26}H_{22}ClNO_3$ (M^+) 431.1288, found 431.1271.

To a solution of **5f** (76 μmol, 33 mg) in AcOEt (1 mL) was added 10% Pd on carbon (10 mg) at −15 °C under H_2 atmosphere (1 atm), and the mixture was stirred for 2 h. After Celite545 pad filtration, the crude was subjected to column chromatography (acidic silica gel, $CHCl_3$:MeOH = 97:3) to give **1f** (61 μmol, 21 mg, 80%) as yellow needles.



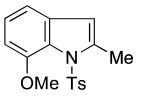
Mp 112−113 °C (from 50% aq EtOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.87 (d, 2 H, *J* = 8.0 Hz), 7.52 (d, *J* = 8.0 Hz, 2 H), 7.42 (d, *J* = 8.0 Hz, 1 H), 7.32 (dd, *J* = 7.5, 8.0 Hz, 1 H), 7.27 (d, *J* = 7.5 Hz, 1 H), 7.17 (s, 1 H), 3.72 (s, 2 H), 2.92 (q, 2 H, *J* = 7.5 Hz), 1.16 (t, 3 H, *J* = 7.5 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 176.95, 166.50, 139.65, 134.99, 132.27, 132.02, 131.76, 129.18, 128.85, 127.40, 126.09, 124.31, 116.79, 113.13, 30.73, 27.30, 13.60; LRMS (EI) *m*/*z* 341.11 (M^+); HRMS (EI) calcd for $C_{19}H_{16}ClNO_3$ (M^+) 341.0819, found 341.0811.

*1-(4-Chlorobenzoyl)-7-methoxy-2-methylindole-3-yl acetic Acid (**1h**).* To a solution of 2-ethenyl-6-methoxy-*N*-*p*-toluenesulfonylaniline (2.81 mmol, 851 mg)[32] in THF (25 mL) were added triphenylphosphine (5.61 mmol, 2.94 g) and 3-buten-2-ol (5.61 mmol, 486 μL). To the mixture was added dropwise diisopropyl azodicarboxylate (5.61 mmol, 1.1 mL) at 0 °C, and the mixure was stirred at rt for 2 h. The solvents were removed, and the obtained residue was subjected to column chromatography (neutral SiO_2, hexane:AcOEt = 25:1) to give *N*-(but-1-en-3-yl)-6-ethenyl-2-methoxy-*N*-*p*-toluenesulfonylaniline (2.84 mmol, 1.02 g, quant. as a rotamer of 6:4) as a colorless oil.



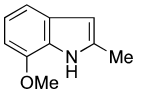
^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.69 (d, 1.2 H, *J* = 8.6 Hz), 7.66 (d, 0.8 H, *J* = 8.6 Hz), 7.29−7.18 (m, 4.4 H), 7.07 (dd, 0.6 H, *J* = 10.9, 17.8 Hz), 6.77−6.73 (m, 0.6 H), 6.70 (dd, 0.4 H, *J* = 1.7, 7.5 Hz), 5.86−5.70 (m, 2 H), 5.34 (dd, 0.4 H, *J* = 1.1, 10.9 Hz), 5.26 (dd, 0.4 H, *J* = 1.1, 10.9 Hz), 5.11 (dd, 0.6 H, *J* = 1.1, 17.2 Hz), 4.99−4.96 (m, 1 H), 4.86 (d, 0.4 H, *J* = 1.1, 10.3 Hz), 4.72−4.69 (m, 0.4 H), 4.66−4.61 (m, 0.4 H), 3.47 (s, 1.8 H), 3.33 (s, 1.2 H), 2.42 (s, 1.8 H), 2.40 (s, 1.2 H), 1.17 (d, 1.2 H, *J* = 6.9 Hz), 1.08 (d, 1.8 H, *J* = 6.9 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 158.02, 157.69, 142.43, 142.32, 141.54, 141.19, 139.07, 139.01, 138.99, 134.19, 134.13, 129.26, 128.66, 128.50, 128.04, 127.93, 124.01, 123.84, 117.39, 117.34, 115.67, 115.60, 115.49, 114.78, 110.21, 110.17, 59.84, 59.46, 54.57, 54.22, 21.36, 21.34, 19.59, 18.90; LRMS (EI) *m*/*z* 357 (M^+); Anal. Calcd for C 67.20, H 6.49, N 3.92; found C 67.21, H 6.40, N 3.94.

In a glovebox, to a solution of *N*-(but-1-en-3-yl)-6-ethenyl-2-methoxy-*N*-*p*-toluenesulfonylaniline (0.18 mmol, 66 mg) in toluene (4.4 mL) was added carbonylchlorohydridotris(triphenylphosphine)-ruthenium(II) (18 μmol, 17 mg), and the whole was refluxed for 4 h. To the mixture was added second generation Grubbs catalyst (18 μmol, 15 mg), and the solution was refluxed for 3 h. The solvents were removed under reduced pressure, and the obtained residue was subjected to column chromatography (neutral SiO_2, hexane:AcOEt = 30:1) to give **2h** (0.16 mmol, 52 mg, two steps 92%) as colorless statues.



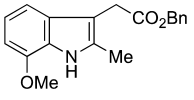
Mp 108.0−109.5 °C (from MeOH); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.70 (d, 2 H, *J* = 8.6 Hz), 7.26 (d, 2 H, *J* = 8.6 Hz), 7.08 (dd, 1 H, *J* = 7.4, 8.0 Hz), 7.02 (d, 1 H, *J* = 7.4 Hz), 6.63 (d, 1 H, *J* = 8.0 Hz), 6.36 (s, 1 H), 3.61 (s, 3 H), 2.73 (s, 3 H), 2.39 (s, 3 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 147.36, 143.46, 140.58, 138.95, 132.15, 129.20, 126.53, 126.32, 124.17, 112.77, 109.40, 107.22, 55.52, 21.54, 17.14; LRMS (EI) *m*/*z* 315 (M^+); Anal. Calcd for $C_{17}H_{17}NO_3S$: C, 64.74; H, 5.43; N, 4.44. Found: C, 64.91; H, 5.40; N, 4.43.

To a solution of **2h** (2.00 mmol, 53 mg) in MeOH (20 mL) was added KOH (100 mmol, 5.6 g), and the mixture was refluxed for 4 h. To the mixture was added 1 N HCl, and organic compounds were extracted with AcOEt. Organic layers were washed with brine and dried over Na_2SO_4. The solvents were filtered and evaporated under reduced pressure. The obtained residue was subjected to column chromatography (NH_2 silica gel, hexane:AcOEt = 15:1) to give **3h**[33] as a colorless statue (1.54 mmol, 249 mg, 77%).



Mp 79−80 °C (from AcOEt, ref 83−85 °C); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.08 (br, 1 H), 7.12 (d, 1 H, *J* = 8.2 Hz), 6.98 (dd, 1 H, *J* = 7.7, 8.2 Hz), 6.58 (d, 1 H, *J* = 7.7 Hz), 6.19 (s, 1 H), 3.94 (s, 3 H), 2.43 (s, 3 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 145.28, 134.56, 130.23, 126.10, 119.72, 112.49, 101.02, 100.49, 55.05, 13.25; LRMS (EI) *m*/*z* 161 (M^+).

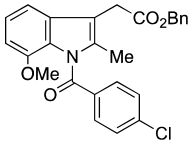
To a solution of **3h** (0.226 mmol, 69 mg) in tetrahydrofuran (1.5 mL) was added dropwise a solution of *n*-BuLi in *n*-hexane (1.63 M, 0.51 mmol, 310 μL) at 0 °C. The solution was warmed to rt and stirred for 30 min. To the mixture was added a solution of $ZnCl_2$ (1.0 M in Et_2O, 0.51 mmol, 510 μL), and the whole was continuously stirred for 30 min. To the mixture was cannulated a solution of iodoacetic acid benzyl ester (0.51 mmol, 142 mg) in tetrahydrofuran (0.5 mL), and the whole was stirred for further 15 h. To the mixture was added sat. aq NH_4Cl. The solvent was partially removed by evaporation, and organic compounds were extracted with AcOEt. Organic layers were washed with brine and dried over Na_2SO_4. The solvents were filtered and evaporated under reduced pressure. The obtained residue was subjected to column chromatography (neutral silica gel, hexane:AcOEt = 10:1) to give **4h** (0.15 mmol, 45 mg, 34%) as a yellow oil.



dx.doi.org/10.1021/jm301084z | J. Med. Chem. 2012, 55, 8152−8163

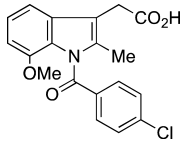
^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.06 (br, 1 H), 7.32−7.29 (m, 5 H), 7.13 (d, 1 H, J = 8.0 Hz), 7.00 (dd, 1 H, J = 7.4, 8.0 Hz), 6.99 (d, 1 H, J = 7.4 Hz), 5.10 (s, 2 H), 3.93 (s, 3 H), 3.72 (s, 2 H), 2.39 (s, 3 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 171.81, 145.43, 136.04, 132.23, 129.72, 128.44, 128.04, 125.22, 119.91, 111.06, 104.85, 101.50, 66.39, 55.28, 30.58, 11.71; LRMS (EI) m/z 309.16 (M^+); HRMS (EI) calcd for $C_{19}H_{19}NO_3$ (M^+) 309.1365, found 309.1368.

To a solution of **4h** (0.15 mmol, 47 mg) in tetrahydrofuran (2 mL) was added dropwise a solution of *t*BuOK in tetrahydrofuran (1.0 M, 0.18 mmol, 180 μL) at −78 °C, and the mixture was stirred for 1 h. To the mixture was cannulated a solution of *p*-chlorobenzoyl chloride (0.30 mmol, 38 μL) in tetrahydrofuran (1 mL), and the whole was stirred for 3 h. To the mixture was added sat. aq NH_4Cl. The solvent was partially removed by evaporation, and organic compounds were extracted with AcOEt. Organic layers were washed with brine and dried over Na_2SO_4. The solvents were filtered and evaporated under reduced pressure. The obtained residue was subjected to column chromatography (neutral silica gel, hexane:AcOEt = 20:1) to give **5h** (0.13 mmol, 57 mg, 85%) as a yellow oil.



^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.49 (d, J = 8.6 Hz, 2 H), 7.35−7.26 (m, 7 H), 7.17−7.09 (m, 2 H), 6.55 (d, J = 7.7 Hz, 1 H), 5.14 (s, 2 H), 3.76 (s, 2 H), 3.34 (s, 3 H), 2.40 (s, 3 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 171.40, 169.76, 146.08, 139.08, 135.82, 135.61, 134.97, 130.83, 130.32, 128.56, 128.49, 128.17, 128.02, 125.73, 123.00, 111.24, 109.57, 104.94, 66.62, 54.74, 30.50, 11.67; LRMS (EI) m/z 447.23 (M^+)

To a solution of **5h** (25 μmol, 11 mg) in AcOEt (1 mL) was added 10% Pd on carbon (4 mg) at −15 °C under H_2 atmosphere (1 atm), and the mixture was stirred for 2 h. After Celite545 pad filtration, the crude was subjected to column chromatography (acidic silica gel, $CHCl_3$:MeOH = 97:3) to give **1h** (23 μmol, 8 mg, 95%) as yellow needles.



Mp 82−83 °C (from 50% aq EtOH); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.51 (d, 2 H, J = 8.6 Hz), 7.35 (d, J = 8.6 Hz, 2 H), 7.17 (d, J = 7.4 Hz, 1 H), 7.14 (dd, J = 6.9, 7.4 Hz, 1 H), 6.55 (d, J = 6.9 Hz, 1 H), 3.75 (s, 2 H), 3.34 (s, 3 H), 2.43 (s, 3 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 177.10, 169.73, 146.12, 139.15, 135.78, 131.53, 130.69, 130.37, 128.85, 128.58, 123.08, 111.09, 108.96, 105.03, 54.76, 30.15, 11.61; LRMS (EI) m/z 357.12 (M^+); HRMS (EI): calcd for $C_{19}H_{16}ClNO_4$ (M^+) 357.0768; found 357.0763.

X-ray Powder Diffraction. X-ray powder diffraction experiments were performed using SPring-8 on the BL14B2 Beamline. A powder sample of **1g** was introduced into a glass capillary (0.3 mm internal diameter). The wavelength of the incident X-rays was 1.000 Å. Powder data were collected at 300 K, and the exposure time was 20 min. Two-dimensional powder data were recorded on an image plate, and the two-dimensional data were converted to one-dimensional powder data in the range of 2.2−60.0°.

All the processes in structure determination were performed using a Rigaku PDXL structure analysis package. To obtain the diffracted peaks, the X-ray powder pattern of **1g** was decomposed by the Pawley method,[26] and the diffracted peaks were indexed with the N-TREOR[27] program. The molecular geometry of **1g** was generated based on the molecular structure of indomethacin (**1a**) and described by Z-matrix coordinates. The initial structure was determined using direct-space methods with a parallel tempering algorithm. After the direct-space method process, the coordinates of all atoms of the molecules were converted to fractional coordinates and the structural parameters were refined by the Rietveld method with restraints on bond lengths and angles. Planar restraints were also applied to a phenyl ring, an indole ring, and a carboxyl group.

In Vitro Cyclooxygenase Assay. The in vitro biological activity of the compounds was assessed with a colorimetric COX (ovine) inhibitory screening assay kit (Cayman Chemical, catalog no. 760111) according to the supplier's protocol. Each experiment was performed at least four times, and the mean value was calculated. The results are shown as mean ± SEM.

In Vitro P-Glycoprotein Assay. To evaluate the inhibitory activities of the test compounds on P-gp in vitro, we used the Pgp-Glo assay system (Promega, Madison, WI). The assay was performed according to the manufacturer's instructions. Briefly, various concentrations of test compounds (10-fold dilution series, range from 0.781 to 200 μM) were incubated with 5 mM MgATP and 25 mg recombinant human Pgp membranes at 37 °C for 40 min. Luminescence was initiated by the ATP detection buffer. After incubation, the luminescence signal was developed at room temperature for 20 min on 384 multiwell plates (Corning, NY), and the signal was measured on Varioskan Flash multimode reader (Thermo Fisher Scientific, Waltham, MA). The sample concentration and luminescence were plotted, and the IC_{50} values were calculated using the nonlinear least-squares method.

MDR. *Reverse Transcription PCR.* The expression of the MRP gene was controlled by reverse-transcription PCR (RT-PCR) of the MRP transcript. RNA was purified from T89G cells using standard methods (RNeasy Mini Kit, Qiagen, Hilden, Germany). For PCR, the purified RNA was reverse-transcribed into cDNA using Omniscript Reverse Transcriptase (Qiagen). The following primers were used for PCR of the MRP cDNA:14 MRP sense, 5′-CGTGTACTCCAACGCTGAC-3′; MRP antisense, 5′-CTGGACCGCTGACGCCCGTGAC-3′. The template was denatured for 5 min at 95 °C, and PCR was carried out for 35 cycles of 45 s at 95 °C, 45 s at 55 °C, and 60 s at 72 °C. The MRP product was expected to be 326 bp long. For a positive control, β-actin was directly amplified by OneStep RT-PCR (Qiagen) from RNA. The following primers were used: β-actin sense 5′-GCGGGATCCTCGACAACGGCTCCGGCAT-3′; β-actin antisense 5′-GCGGTCGACGGATCTTCATGAGGTAGTCAG-3′. The RT-PCR program was as follows: 30 min at 50 °C, 15 min at 95 °C, and 35 cycles at 30 s at 94 °C, 30 s at 60 °C, and 60 s at 72 °C. The resulting β-actin fragment was expected to be 628 bp long. The PCR result was controlled by electrophoresis in 2% agarose gels using a 100-bp ladder marker (Fermentas, Burlington, Ontario). Gels were stained by ethidium bromide.

Western Blot. The T98G cells were analyzed for the presence of the MRP-1 protein. Subconfluent cells were harvested and resuspended in PBS including a mix of protease inhibitors (Complete from Roche, Basel, Switzerland). Cells were lysed on ice by ultrasound sonication. The total lysate was centrifuged for 10 min at 13 000 rpm. The resulting supernatant and the total lysate were adjusted to have equal protein concentrations as determined using the Bradford protein concentration assay. Total protein (35 mg) of each sample was separated by SDS−PAGE on a 7.5% gel and blotted on a PVDF membrane (Amersham Biosciences, Buckinghamshire, UK). The membrane was blocked with blocking buffer (5% powdered milk, 0.1% Tween20 in PBS) for 2 h. The membrane was incubated with anti-MRP-1 antibody (QCRL-1 clone from Santa Cruz Biotechnology, Santa Cruz, CA) at 1:100 dilution in blocking buffer overnight. The membrane was then incubated with HRP-coupled antimouse-antibody (Amersham Biosciences) at 1:20 000 dilution in blocking buffer for 1 h at room temperature. The membrane was developed using the ECL Plus Western blot detection system (Amersham Biosciences).

Cell Line and Cytotoxicity Assay. The human glioblastoma cell line T98G was purchased from American Type Culture Collection (Rockville, MD). Cells were cultured under standard conditions. Cytotoxicity was evaluated by measuring the metabolic activity of the cells using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.[18] Briefly, the T98G cells were seeded at 10^4

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cells/well in 96-well plates, adhered for 24 h, and exposed to 10 μM of the drugs for 4 d. A 100-μL aliquot of the cultured medium was removed, and the cells were treated with 20 μL of PBS containing 5 mg/mL MTT (Sigma). After incubation at 37 °C in a humidified air atmosphere (7.5% CO_2) for 2 h, 150 μL of 2-propanol containing 0.04 mol/L HCl was added to each well to dissolve the formazan crystals produced by the reduction of MTT in viable cells. After incubation for 45 min and trituation, the absorbance of each well was measured at 620 nm. The results are expressed as percentages relative to control cells. The experiments were performed in triplicate and repeated at least three times.

■ ASSOCIATED CONTENT

Supporting Information

^{1}H and ^{13}C NMR charts and X-ray analysis. 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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