Cyclooxygenase Inhibitors Derived from Thalidomide

Mamiko SUIZU,[a] Yohei MUROYA,[b] Hiroki KAKUTA,[a] Hiroyuki KAGECHIKA,[b] Aya TANATANI,[a] Kazuo NAGASAWA,[a] and Yuichi HASHIMOTO*,[a]

[a] *Institute of Molecular & Cellular Biosciences, The University of Tokyo; 1–1–1 Yayoi, Bunkyo-ku, Tokyo 113–0032, Japan: and* [b] *Graduate School of Pharmaceutical Sciences, The University of Tokyo; 7–3–1 Hongo, Bunkyo-ku, Tokyo 113–0033, Japan.* Received April 25, 2003; accepted July 1, 2003

Several *N*-3,5-dimethylphenylphthalimide analogs possessing more potent cyclooxygenase-inhibiting activity than that of aspirin were prepared during structural development studies based on thalidomide. Substituent effects on the activity were investigated.

Key words thalidomide; cyclooxygenase; inhibitor; structure–activity relationship

Thalidomide (**1**) is a sedative/hypnotic drug, which was withdrawn from the market because of its severe teratogenicity.[1—3] In spite of this, research into thalidomide was not halted, and the drug has been established to be effective for the treatment of various diseases, including leprosy, myeloma, and AIDS.[2—4] We have demonstrated that thalidomide is a multi-target drug.[2,3,5—17] We have been engaged in structural development studies of thalidomide, and have obtained tumor necrosis factor (TNF)-α production regulators (including bi-directional ones, as well as pure inhibitors and enhancers),[2,3,5—7] androgen antagonists,[2,3,8,9] peptidase inhibitors,[3,10—13] glucosidase inhibitors,[15,16] and thymidine phosphorylase inhibitors.[16] We suspected that cyclooxygenase (COX) is another target molecule of thalidomide, because thalidomide is effective against colon and prostate cancers and possesses anti-angiogenic activity,[17,18] in which COX plays an important role.

COX is an enzyme which catalyzes the synthesis of prostaglandins from arachidonic acid, and is well-known as a target molecule of non-steroidal anti-inflammatory drugs (NSAIDs), including aspirin.[19—21] There are three isoforms of COX, of which COX-1 and -2 have been well investigated. COX-1 is constitutively expressed in most tissues, whereas COX-2 is inducible. Overexpression of COX-2 has been detected in various tumors and its role in carcinogenesis and angiogenesis has been well-documented.[21—23] Consequently, COX-2 has been suggested to be an important pharmacological target for the prevention and treatment of cancer.[21—23] Attempts have been made to apply COX-2 inhibitors, including celexocib and sulindac, for chemoprevention of various cancers, including colon and prostate cancers.[24,25] Recently, another subtype, COX-3, was shown to be a COX-1 variant, which is selectively inhibited by acetaminophen and other analgestic/antipyretic drugs.[26,27] Thus, inhibition of COX-3 is considered to represent the primary mechanism through which these drugs decrease pain and possibly fever.

Thalidomide suppresses lipopolysaccharide-induced expression of COX-2.[28,29] In addition, we have recently demonstrated that thalidomide directly inhibits COX-1/2, being comparable in potency to aspirin.[30] In this paper, we describe novel COX inhibitors derived from thalidomide, focusing on COX-1 and -2.

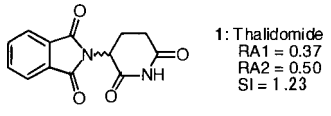


Fig. 1. Structure of Thalidomide and Its COX-Inhibiting Activity

RA1, RA2: Relative inhibitory activity (*versus* aspirin) for COX-1 and COX-2, respectively. SI: Selectivity index for COX-1 over COX-2. See text for details.

Results and Discussion

Substituted phthalimide (**2**—**28**) analogs, including deoxygenated derivatives, with *N*-3,5-dimethylphenyl substituents were selected based on our previous studies on COX-inhibitors derived from thalidomide, which suggested that the 3,5-dimethylphenyl substituent is superior for potent COX-inhibiting activity.[30]

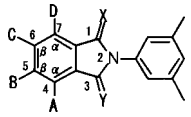
Compounds (**2**—**28**) were prepared by usual synthetic methods and the structures were confirmed by NMR and mass spectrometry, and by appropriate analytical values. Synthesis and chemical/physical data of several of the compounds listed in Table 1 have already been reported.[5—8,10—17,30,31] Inhibitory activity of the compounds toward COX-1 and COX-2 was assayed by the use of a Colorimetric COX (ovine) Inhibitor Screening Assay Kit (Cayman, No. 760111) according to the protocol recommended by the supplier.

Although the IC_{50} values differed from experiment to experiment, the results were basically reproducible, and typical sets of data are presented in Fig. 2, and Table 1. In the assay system, the IC_{50} values of aspirin for COX-1 and COX-2 were determined to be 90—100 μM and 100—110 μM, suggesting that aspirin is a non-selective or slightly COX-1-selective inhibitor. The activities of compounds are presented as relative activities [RA values (RA1 and RA2, for COX-1 and COX-2 inhibitory activities, respectively)] defined as $IC_{50\,(aspirin)}/IC_{50\,(test\ compound)}$, and the selectivity index (SI values) was defined as $IC_{50\,(COX\text{-}1)}/IC_{50\,(COX\text{-}2)}$. For compounds with weak activity, IC_{30} (for RA values of less than 0.3) or IC_{10} (for RA values of less than 0.1) values were used instead of IC_{50} values. The assay was performed in duplicate, and repeated at least three times.

As shown in Table 1, phenylphthalimide analogs substituted at the 4-position (**2**—**6**) are inactive. However, substitution with the same series of functional groups at the 5-position (**7**—**10**) resulted in COX-inhibitory activity with comparable potency to that of thalidomide (Fig. 1), though the potency is lower than that of aspirin. Broadly speaking, removal of one carbonyl group of the phthalimide moiety, *i.e.*, isoin-

* To whom correspondence should be addressed. e-mail: hashimot@iam.u-tokyo.ac.jp

Table 1. COX-Inhibitory Activity of Compounds (**2**—**28**) Derived from 3,5-Dimethylphenylphthalimide



Compound	A	B	C	D	X	Y	RA1	RA2	SI
2	NO_2	H	H	H	O	O	<0.05	<0.05	—
3	NH_2	H	H	H	O	O	<0.05	<0.05	—
4	NHMe	H	H	H	O	O	<0.05	<0.05	—
5	NMe_2	H	H	H	O	O	<0.05	<0.05	—
6	NHAc	H	H	H	O	O	<0.05	<0.05	—
7	H	NO_2	H	H	O	O	0.3	0.9	2.7
8	H	NH_2	H	H	O	O	<0.05	0.3	(>5)
9	H	NMe_2	H	H	O	O	0.4	<0.05	(<0.01)
10	H	NHAc	H	H	O	O	0.2	1.2	5.4
11	H	H	H	H	O	H_2	<0.05	<0.05	—
12	H	H	H	NO_2	O	H_2	<0.05	<0.05	—
13	H	H	H	NH_2	O	H_2	1.9	1.5	0.7
14	H	H	H	NHMe	O	H_2	1.1	0.6	0.6
15	H	H	H	NMe_2	O	H_2	2.6	0.8	0.3
16	H	H	NH_2	H	O	H_2	25.0	2.8	0.1
17	H	H	NMe_2	H	O	H_2	12.1	5.0	0.4
18	H	NO_2	H	H	O	H_2	1.0	6.5	5.9
19	H	NH_2	H	H	O	H_2	1.2	<0.05	(<0.04)
20	H	NMe_2	H	H	O	H_2	0.6	0.5	0.7
21	NO_2	H	H	H	O	H_2	<0.05	<0.05	—
22	NH_2	H	H	H	O	H_2	0.9	<0.05	(<0.05)
23	NMe_2	H	H	H	O	H_2	0.5	0.5	0.9
24	NO_2	H	H	H	H_2	H_2	0.7	0.9	1.2
25	H	NO_2	H	H	H_2	H_2	33.6	71.4	2.4
26	H	NH_2	H	H	H_2	H_2	38.0	10.4	0.4
27	H	NMe_2	H	H	H_2	H_2	22.6	5.3	0.2
28	H	NHAc	H	H	H_2	H_2	18.4	5.5	0.3

RA1, RA2: relative inhibitory activity (*versus* aspirin) for COX-1 and COX-2, respectively. SI: selectivity index for COX-1 over COX-2. See text, for details.

dolone derivatives (**11**—**23**), generally enhanced the COX-inhibitory activity. Among the isoindolone derivatives, the 6-amino derivative (**16**) and the 5-nitro derivative (**18**) are the most potent COX-1 inhibitor (25.0 times more potent than aspirin) and COX-2 inhibitor (6.5 times more potent than aspirin), respectively. Further deoxygenation, *i.e.*, isoindolyl analogs (**24**—**28**), afforded much more potent COX-inhibitors than aspirin. Among this series of the compounds, the 5-amino (**26**) and 5-nitro (**25**) analogs are the most potent COX-1 (38.0 times more active than aspirin) and COX-2 (71.4 times more active than aspirin) inhibitors, respectively.

Though the structure-activity relationships remain to be fully established, some aspects can be discussed, as follows.

(1) Roughly speaking, compounds with a substituent of group "B" (**7**—**10**, **18**—**20**, **25**—**28**) or "C" (**16**—**17**) in Table 1 are more potent COX-inhibitors than the corresponding isomers with a substituent of group "A" (**2**—**6**, **21**—**24**) or "D" (**12**—**14**) in Table 1. This suggests that substitution at the β-positions (positions 5 and 6) is better than that at the α-positions (positions 4 and 7) for potent COX-inhibiting activity, regardless of the electronic nature of the substituent. Exceptions are the pairs of compounds **13**/**19** and **15**/**20**. In these pairs, 7-substituted compounds (**13**, **15**) possess more potent COX-inhibiting activity than the corresponding 5-substituted compounds (**19**, **20**, respectively).

(2) Among the compounds with potent/moderate COX-inhibiting activity, compounds with an electron-donating functional group [amino (**13**, **16**, **19**, **22**, **26**), methylamino (**14**), or dimethylamino (**9**, **15**, **17**, **20**, **23**, **27**) group] show COX-1 selectivity, with the sole exception of **8**, which is COX-2 selective. On the other hand, compounds with an electron-withdrawing nitro group (**7**, **18**, **24**, **25**) show COX-2 selectivity, though the selectivities of **24** and **25** are low. The effect of an acetylamino group on the COX-1/2 selectivity could not be interpreted. The dose–response curves for COX-1 and -2 inhibition of **16** and **25** are presented in Fig. 2.

Previously, we reported that the electronic nature of the substituents introduced into the phthalimide moiety of methylthalidomide dramatically changes the COX-1/2 selectivity of the compounds, depending on the position of the substituent introduced.[30] That finding, and the results presented in this paper, should be useful for the development of superior COX-inhibitors.

Experimental

General Melting points were determined by using a Yanagimoto hot-stage melting point apparatus and are uncorrected. Elemental analyses were carried out in the Microanalytical Laboratory, Faculty of Pharmaceutical Sciences, University of Tokyo, and were within ±0.3% of the theoretical values. NMR spectra were recorded on a JEOL JNM-GX400 (400 MHz) spectrometer. Chemical shifts are expressed in ppm relative to tetramethylsilane. Mass spectra were recorded on a JEOL JMS-DX303 spectrometer.

4-Substituted Phthalimides (General method) A mixture of 4-nitrophthalic anhydride (4.65 g, 24.1 mmol) and 3,5-xylidine (2.91 g, 24.0 mmol) in acetic acid (100 ml) was refluxed for 24 h. After cooling, the mixture was poured into ice water. The precipitates were collected, and recrystallized from CH_2Cl_2/EtOH to give **2** (4.09 g, 58%).

2: Yellow needles; mp 209—212 °C; ^{1}H-NMR ($CDCl_3$) 8.21 (1H, d, J=7.5 Hz), 8.15 (1H, d, J=8.0 Hz), 7.97 (1H, t, J=7.7 Hz), 7.07 (1H, s),

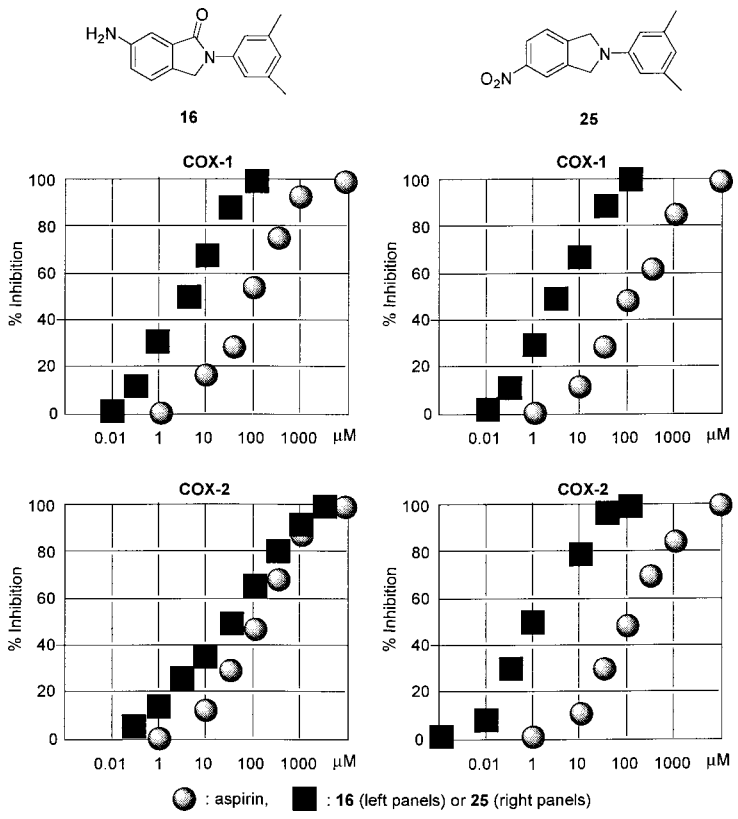


Fig. 2. The Dose–Response Curves for Inhibition of COX-1 (Upper Panels) and COX-2 (Lower Panels) by Compounds **16** (Left Panels) and **25** (Right Panels) and the Comparison with Aspirin

7.01 (2H, s), 2.38 (6H, s); *Anal.* Calcd for $C_{16}H_{12}N_2O_4$: C, 64.86; H, 4.08; N, 9.46. Found: C, 64.86; H, 4.33; N, 9.45.

A mixture of **2** (1.21 g, 4.07 mmmol) and 10% Pd–C (118 mg) in AcOEt (140 ml) was stirred under H_2 for 3 h. After filtration, the filtrate was evaporated, and the residue was purified by flash column chromatography (AcOEt : hexane 1 : 2) to give **3** (1.03 g, 95%).

3: Yellow needles (CH_2Cl_2/hexane); mp 194—197 °C; ^{1}H-NMR ($CDCl_3$) 7.47 (1H, t, J=7.7 Hz), 7.24 (1H, d, J=7.0 Hz), 7.02 (1H, s), 7.00 (2H, s), 6.90 (1H, t, J=8.2 Hz), 5.30 (2H, br s), 2.37 (6H, s); *Anal.* Calcd for $C_{16}H_{14}N_2O_2$: C, 72.16; H, 5.30; N, 10.52. Found: C, 71.96; H, 5.33; N, 10.26.

Paraformaldehyde (75 mg, 2.5 mmol) and $NaBH_3CN$ (81 mg, 1.3 mmol) were added to a solution of **3** (66 mg, 0.25 mmol) in acetic acid (6 ml), and the mixture was stirred for 4 h. The mixture was poured into 2 N NaOH, and extracted with AcOEt. The organic layer was washed with brine, dried over Na_2SO_4, and evaporated. The residue was purified by flash column chromatography (AcOEt : hexane 1 : 3) to give **4** (36 mg, 52%) and **5** (31 mg, 42%).

4: Yellow plates (CH_2Cl_2/hexane); mp 179—181 °C; ^{1}H-NMR ($CDCl_3$) 7.55 (1H, dd, J=8.5, 7.2 Hz), 7.16 (1H, d, J=7.2 Hz), 7.01 (1H, s), 7.00 (2H, s), 6.90 (1H, d, J=8.6 Hz), 6.36 (1H, br s), 3.00 (3H, s) 2.36 (6H, s); *Anal.* Calcd for $C_{17}H_{16}N_2O_2$: C, 72.84; H, 5.75; N, 9.99. Found: C, 72.72; H, 6.01; N, 9.84.

5: Yellow needles (CH_2Cl_2/hexane); mp 127—128 °C; ^{1}H-NMR ($CDCl_3$) 7.55 (1H, dd, J=8.4, 7.0 Hz), 7.37 (1H, d, J=7.0 Hz), 7.13 (1H, d, J=8.4 Hz), 7.01 (1H, s), 7.00 (2H, s), 3.13 (6H, s), 2.36 (6H, s); *Anal.* Calcd for $C_{18}H_{18}N_2O_2$: C, 73.45; H, 6.16; N, 9.52. Found: C, 73.16; H, 6.26; N, 9.32.

A solution of **3** (67 mg, 0.25 mmol) and pyridine (0.5 ml) in acetic anhydride (4 ml) was heated at 70 °C for 16 h. After cooling, the reaction mixture was poured into water, and extracted with AcOEt. The organic layer was washed with brine, dried over Na_2SO_4, and evaporated. The residue was purified by flash column chromatography (AcOEt : hexane 1 : 3) to give **6** (72 mg, 93%).

6: Colorless needles (CH_2Cl_2/hexane); mp 155—157 °C; ^{1}H-NMR (DMSO-d_6) 9.74 (1H, s), 8.50 (1H, d, J=8.2 Hz), 7.83 (1H, d, J=8.2 Hz), 7.62 (1H, d, J=7.3 Hz), 7.08 (1H, s), 7.03 (2H, s), 2.49 (6H, s) 2.19 (3H, s); *Anal.* Calcd for $C_{18}H_{16}N_2O_3$: C, 70.12; H, 5.23; N, 9.09. Found: C, 70.05; H, 5.43; N, 9.01.

5-Substituted phthalimides **7**—**10** were prepared according to the general method.

7: Yellow prisms; mp 222—224 °C; ^{1}H-NMR ($CDCl_3$) 8.76 (1H, d, J=2.0 Hz), 8.66 (1H, dd, J=8.1, 2.0 Hz), 8.14 (1H, t, J=8.0 Hz), 7.09 (1H, s), 7.01 (2H, s), 2.39 (6H, s); *Anal.* Calcd for $C_{16}H_{12}N_2O_4$: C, 64.86; H, 4.08; N, 9.46. Found: C, 64.88; H, 4.22; N, 9.45.

8: Yellow needles (CH_2Cl_2/hexane); mp 162 °C; ^{1}H-NMR ($CDCl_3$) 7.69 (1H, d, J=8.1 Hz), 7.11 (1H, d, J=2.0 Hz), 7.01 (1H, s), 6.99 (2H, s), 6.87 (1H, dd, J=8.1, 2.2 Hz), 4.37 (2H, br s), 2.36 (6H, s); *Anal.* Calcd for $C_{16}H_{14}N_2O_2$: C, 72.16; H, 5.30; N, 10.52. Found: C, 72.05; H, 5.42; N, 10.44.

9: Yellow needles (ether); mp 135—137 °C; ^{1}H-NMR ($CDCl_3$) 7.72 (1H, d, J=8.4 Hz), 7.13 (1H, d, J=8.4 Hz), 6.99 (3H, s), 6.83 (1H, dd, J=8.5, 2.4 Hz), 3.13 (6H, s), 2.35 (6H, s); *Anal.* Calcd for $C_{18}H_{18}N_2O_2$: C, 73.45; H, 6.16; N, 9.52. Found: C, 73.19; H, 6.30; N, 9.32.

10: Colorless flakes (CH_2Cl_2/benzene); mp >300 °C; ^{1}H-NMR (DMSO-d_6) 10.59 (1H, s), 8.25 (1H, s), 7.88 (2H, s), 7.06 (1H, s), 7.01 (2H, s), 2.31 (6H, s) 2.31 (3H, s); *Anal.* Calcd for $C_{18}H_{16}N_2O_3$: C, 70.12; H, 5.23; N, 9.09. Found: C, 69.86; H, 5.37; N, 9.00.

Isoindolinone 11 A mixture of *o*-phthalaldehyde (268 mg, 2.00 mmol), 3,5-xylidine (239 mg, 1.97 mmol) and a drop of acetic acid in THF (5 ml) was refluxed for 5 h. After evaporation, the residue was purified by flash column chromatography (CH_2Cl_2) to give **11** (176 mg, 38%).

11: Pale colored needles (ether/hexane); mp 136—139 °C; ^{1}H-NMR ($CDCl_3$) 7.92 (1H, d, J=8.3 Hz), 7.59 (1H, t, J=6.8 Hz), 7.52 (4H, m), 6.84 (1H, s), 4.85 (2H, s), 2.37 (6H, s); *Anal.* Calcd for $C_{16}H_{15}NO$: C, 80.98; H, 6.37; N, 5.90. Found: C, 80.81; H, 6.49; N, 5.76.

7-Nitroisoindolinone 12 A solution of methyl 2-methyl-6-nitrobenzoate (196 mg, 1.00 mmol) and *N*-bromosuccinimide (396 mg, 2.22 mmol) in CCl_4 (10 ml) was refluxed for 7 h under an Ar atmosphere. The mixture was filtered, and the filtrate was evaporated. The residue was purified by flash column chromatography (AcOEt : hexane 1 : 5) to give methyl 2-(bromomethyl)-6-nitrobenzoate (197 mg, 72%). A solution of methyl 2-(bromomethyl)-6-nitrobenzoate (2.50 g, 9.10 mmol), 3,5-xylidine (1.84 g, 15.2 mmol) and pyridine (1 ml) in ethanol (100 ml) was refluxed for 2 d. After cooling, the mixture was poured into water. The precipitates was col-

lected, washed with water and ethanol, and recrystallized from acetone to give **12** (1.93 g, 75%).

12: Pale yellow needles; mp 209—212 °C; ^{1}H-NMR (DMSO-d_6) 7.92 (2H, m), 7.86 (1H, m), 7.48 (2H, m), 6.86 (1H, s), 5.08 (2H, s), 2.30 (6H, s); *Anal.* Calcd for $C_{16}H_{14}N_2O_3$: C, 68.07; H, 5.00; N, 9.92. Found: C, 67.90; H, 5.14; N, 9.64.

12 was converted to **13**—**15** according to the general method.

13: Colorless needles (CH_2Cl_2/benzene); mp 158—159 °C; ^{1}H-NMR ($CDCl_3$) 7.42 (2H, s), 7.29 (1H, d, J=7.7 Hz), 6.79 (1H, s), 6.72 (1H, d, J=7.4 Hz), 6.57 (1H, d, J=8.1 Hz), 5.32 (2H, br s), 4.74 (2H, s), 2.34 (6H, s); *Anal.* Calcd for $C_{16}H_{16}N_2O$: C, 76.16; H, 6.39; N, 11.10. Found: C, 76.19; H, 6.54; N, 11.03.

14: Colorless needles (CH_2Cl_2/benzene); mp 197—199 °C; ^{1}H-NMR ($CDCl_3$) 7.41 (2H, s), 7.37 (1H, t, J=7.8 Hz), 6.78 (1H, s), 6.77 (1H, br s), 6.65 (1H, d, J=7.3 Hz), 6.52 (1H, d, J=8.1 Hz), 4.72 (2H, s), 2.92 (3H, d, J=5.1 Hz), 2.30 (6H, s); *Anal.* Calcd for $C_{17}H_{18}N_2O$: C, 76.66; H, 6.81; N, 10.52. Found: C, 76.68; H, 6.66; N, 10.33.

15: Pale yellow prisms (CH_2Cl_2/benzene); mp 140—141 °C; ^{1}H-NMR ($CDCl_3$) 7.46 (2H, s), 7.41 (1H, t, J=7.8 Hz), 6.94 (1H, d, J=7.4 Hz), 6.88 (1H, d, J=8.3 Hz), 6.80 (1H, s), 4.75 (2H, s), 3.04 (6H, s), 2.35 (6H, s); *Anal.* Calcd for $C_{18}H_{20}N_2O$: C, 77.11; H, 7.19; N, 9.99. Found: C, 76.96; H, 7.25; N, 9.90.

6- (16) and 5-Aminoisoindolinone (19) A mixture of **7** (1.199 g, 4.05 mmol) and Sn (9.21 g, 57.07 mmol) in ethanol (50 ml) and hydrochloric acid (30 ml) was heated at 75 °C for 8 h. After cooling, the mixture was poured into 2 N NaOH, and extracted with AcOEt. The organic layer was washed with brine, dried over Na_2SO_4, and evaporated. The residue was purified by flash column chromatography (CH_2Cl_2 : AcOEt 4 : 1) to give **16** (312 mg, 31%) and **19** (392 mg, 38%).

16: Colorless plates (AcOEt); mp 197—200 °C; ^{1}H-NMR ($CDCl_3$) 7.46 (2H, s), 7.26 (1H, d, J=8.1 Hz), 7.17 (1H, d, J=2.2 Hz), 6.89 (1H, dd, J=8.1, 2.2 Hz), 6.82 (1H, s), 4.73 (2H, s), 3.86 (2H, br s), 2.36 (6H, s); *Anal.* Calcd for $C_{16}H_{16}N_2O$: C, 76.16; H, 6.39; N, 11.10. Found: C, 75.90; H, 6.50; N, 10.99.

19: Colorless needles (AcOEt); mp 236—238 °C; ^{1}H-NMR ($CDCl_3$) 7.68 (1H, d, J=8.1 Hz), 7.46 (2H, s), 6.79 (1H, s), 6.73 (1H, dd, J=8.2, 2.0 Hz), 6.70 (1H, s), 4.71 (2H, s), 4.06 (2H, br s), 2.35 (6H, s); *Anal.* Calcd for $C_{16}H_{16}N_2O$: C, 76.16; H, 6.39; N, 11.10. Found: C, 75.87; H, 6.42; N, 10.94.

16 and **19** were alkylated according to the general method to afford **17** and **20**, respectively.

17: Colorless needles (CH_2Cl_2/hexane); mp 193 °C; ^{1}H-NMR ($CDCl_3$) 7.49 (2H, s), 7.33 (1H, d, J=8.3 Hz), 7.20 (1H, d, J=2.4 Hz), 6.97 (1H, dd, J=8.3, 2.4 Hz), 6.82 (1H, s), 4.75 (2H, s), 3.03 (6H, s), 2.36 (6H, s); *Anal.* Calcd for $C_{18}H_{20}N_2O$: C, 77.11; H, 7.19; N, 9.99. Found: C, 77.05; H, 7.34; N, 9.95.

20: Colorless prisms (CH_2Cl_2/hexane); mp 211—213 °C; ^{1}H-NMR ($CDCl_3$) 7.72 (1H, d, J=8.6 Hz), 7.46 (2H, s), 6.76 (2H, m), 6.66 (1H, s), 4.72 (2H, s), 3.06 (6H, s), 2.34 (6H, s); *Anal.* Calcd for $C_{18}H_{20}N_2O$: C, 77.11; H, 7.19; N, 9.99. Found: C, 77.13; H, 7.32; N, 9.99.

5-Nitroisoindolinone 18 and 5-nitroisoindoline 25 Borane in THF (1 M, 30 ml) was added to a solution of **7** (2.98 g, 10.0 mmol) in 50 ml of THF, and the mixture was refluxed for 16 h. After cooling, the mixture was poured into 1 N HCl, stirred for 1 h, and extracted with AcOEt. The organic solution was washed with brine, and dried over $MgSO_4$. After evaporation, the crude product was purified by flash column chromatography (AcOEt/hexane 1 : 10) to give **18** (0.17 g, 6%) and **25** (1.71 g, 67%).

18: Yellow needles (CH_2Cl_2/hexane); mp 205—207 °C; ^{1}H-NMR (DMSO-d_6) 8.49 (1H, d, J=8.3 Hz), 8.20 (1H, d, J=7.5 Hz), 7.85 (1H, t, J=7.8 Hz), 7.56 (2H, s), 6.86 (1H, s), 5.39 (2H, s), 2.08 (6H, s); *Anal.* Calcd for $C_{16}H_{14}N_2O_3$: C, 68.07; H, 5.00; N, 9.92. Found: C, 67.80; H, 5.07; N, 9.79.

25: Brown solid (CH_2Cl_2/hexane); mp 171—173 °C; ^{1}H-NMR ($CDCl_3$) 8.20 (2H, m), 7.48 (1H, d, J=8.8 Hz), 6.51 (1H, s), 6.37 (2H, s), 4.74 (4H, s), 2.34 (6H, s); *Anal.* Calcd for $C_{16}H_{16}N_2O_2 \cdot 1/6H_2O$: C, 70.83; H, 6.07; N, 10.32. Found: C, 70.93; H, 6.05; N, 10.23.

25 was converted to **26** and **28** according to the general method.

26: Brown prisms (CH_2Cl_2/hexane); mp 140.5—142.5 °C; ^{1}H-NMR ($CDCl_3$) 7.10 (1H, d, J=7.9 Hz), 6.66 (1H, s), 6.63 (1H, t, J=8.0 Hz), 6.40 (1H, s), 6.29 (2H, s), 4.53 (2H, s), 4.52 (2H, s), 3.68 (2H, br s), 2.31 (6H, s); *Anal.* Calcd for $C_{16}H_{18}N_2$: C, 80.63; H, 7.61; N, 11.75. Found: C, 80.51; H, 7.70; N, 11.62.

28: Brown prisms (CH_2Cl_2/hexane); mp 256—259 °C; ^{1}H-NMR (DMSO-d_6) 9.96 (1H, s), 7.68 (1H, s), 7.42 (1H, dd, J=8.2, 1.5 Hz), 7.28 (1H, d, J=8.1 Hz), 6.31 (1H, s), 6.27 (2H, s), 4.51 (2H, s), 4.49 (2H, s), 2.22 (6H, s), 2.04 (3H, s); *Anal.* Calcd for $C_{18}H_{20}N_2O \cdot 1/6H_2O$: C, 76.30; H, 7.23; N, 9.89. Found: C, 76.36; H, 7.29; N, 9.81.

4-Nitroisoindolinone **21** was prepared according to the synthetic procedure for **12**, and was converted to **22** and **23** according to the general method.

21: Pale yellow needles (acetone); mp 258—260 °C; ^{1}H-NMR ($CDCl_3$) 8.45 (1H, d, J=8.0 Hz), 8.27 (1H, d, J=7.5 Hz), 7.75 (1H, t, J=7.8 Hz), 7.51 (2H, s), 6.90 (1H, s), 5.31 (2H, s), 2.40 (6H, s); *Anal.* Calcd for $C_{16}H_{14}N_2O_3$: C, 68.07; H, 5.00; N, 9.92. Found: C, 68.14; H, 5.18; N, 9.80.

22: Colorless needles (CH_2Cl_2/hexane); mp 198—200 °C; ^{1}H-NMR ($CDCl_3$) 7.47 (2H, s), 7.35 (1H, d, J=7.0 Hz), 7.29 (1H, t, J=7.5 Hz), 6.85 (1H, d, J=7.9 Hz), 6.81 (1H, s), 4.64 (2H, s), 3.77 (2H, br s), 2.35 (6H, s); *Anal.* Calcd for $C_{16}H_{16}N_2O$: C, 76.16; H, 6.39; N, 11.10. Found: C, 76.11; H, 6.62; N, 11.06.

23: Colorless solid (methanol); mp 181—185 °C; ^{1}H-NMR ($CDCl_3$) 7.49 (2H, s), 7.48 (1H, d, J=7.9 Hz), 7.39 (1H, t, J=7.9 Hz), 7.03 (1H, d, J=7.9 Hz), 6.84 (1H, s), 4.89 (2H, s), 2.96 (6H, s), 2.37 (6H, s); *Anal.* Calcd for $C_{18}H_{20}N_2O$: C, 77.11; H, 7.19; N, 9.99. Found: C, 77.16; H, 7.29; N, 9.98.

4-Nitroisoindoline **24** and 4-(dimethylamino)isoindoline **27** were prepared according to the synthetic procedure for **25**.

24: Brown solid (CH_2Cl_2/hexane); mp 229—231 °C; ^{1}H-NMR ($CDCl_3$) 8.18 (1H, d, J=8.2 Hz), 7.65 (1H, d, J=7.2 Hz), 7.50 (1H, t, J=7.8 Hz), 6.48 (1H, s), 6.39 (2H, s), 5.09 (2H, m), 4.72 (2H, m), 2.35 (6H, s); *Anal.* Calcd for $C_{16}H_{16}N_2O_2 \cdot 1/6H_2O$: C, 70.83; H, 6.07; N, 10.32. Found: C, 70.65; H, 5.99; N, 10.08.

27: Pale brown flakes (CH_2Cl_2/hexane); mp 115—116 °C; ^{1}H-NMR (DMSO-d_6) 7.17 (1H, d, J=8.6 Hz), 6.73 (1H, s), 6.69 (1H, d, J=8.4 Hz), 6.30 (1H, s), 6.25 (2H, s), 4.48 (2H, s), 4.44 (2H, s), 2.89 (6H, s), 2.22 (6H, s); *Anal.* Calcd for $C_{18}H_{22}N_2$: C, 81.16; H, 8.32; N, 10.52. Found: C, 81.22; H, 8.33; N, 10.46.

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