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SHORT STEP SYNTHESIS OF AN ANTIBIOTIC, 6-CYANO-5-METHOXY-12-METHYLINDOLO[2,3-*a*]CARBAZOLE[1]

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Abstract – For evaluating the effectiveness of organic synthesis, both intellectual property factor (IPF) and application potential factor (APF) are proposed. As a representative example, a highly effective synthetic method with IPF value of 53.8 and APF value of 100 has been established starting from indigo directing toward an antibiotic, 6-cyano-5-methoxy-12-methylindolo[2,3-*a*]carbazole, using only conventional reagents without using any protecting groups.

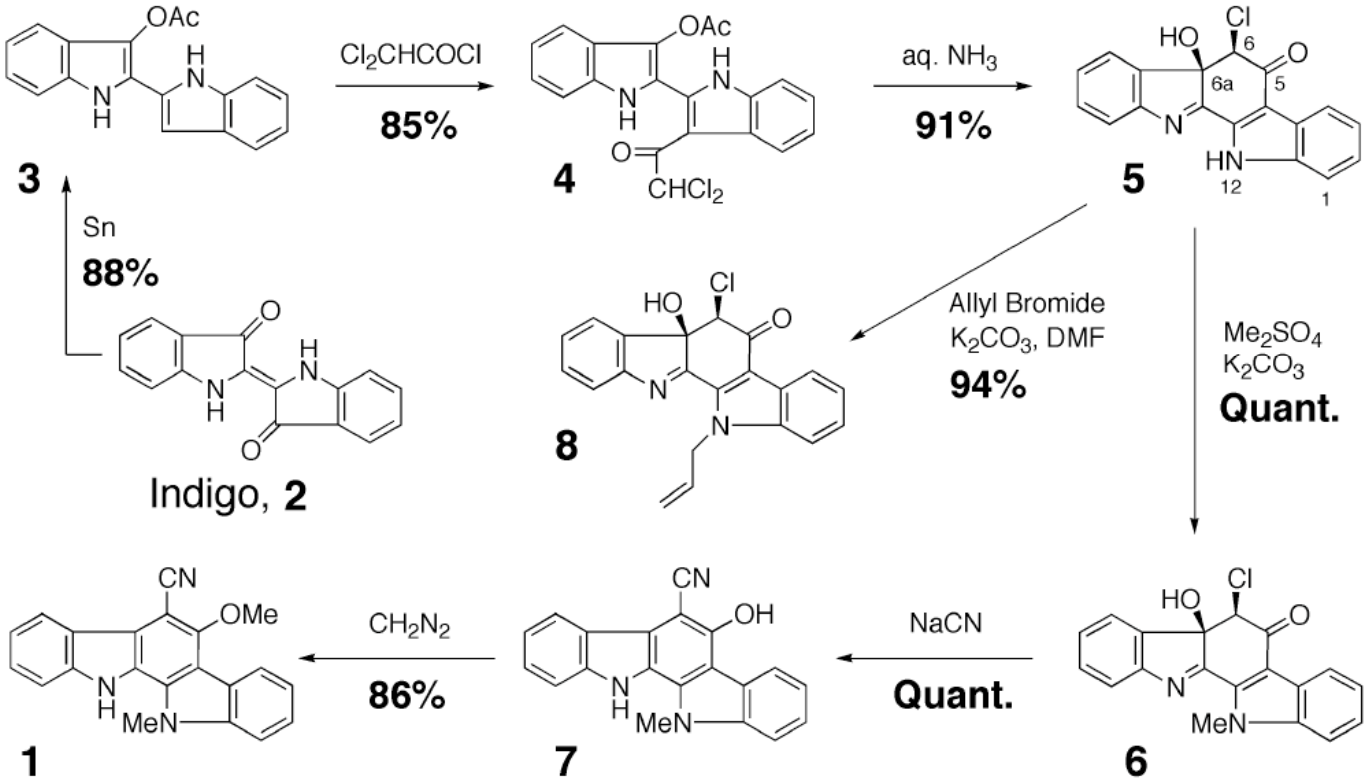
We have newly proposed two kinds of measures[2,3] for evaluating effectiveness of organic synthesis. The one is an intellectual property factor (IPF) and the other is an application potential factor (APF). The IPF is a total rate of intellectual property of every reaction step and every compound involved in the synthesis, including starting material, target compound, and all synthetic intermediates. It is obtained by the formula[2a] shown in the reference 2.[2] Similarly, APF is calculated according to the formula shown in the reference 3.[2b,3] As a typical example, we wish to report a synthesis of an antibiotic, 6-cyano-5-methoxy-12-methylindolo[2,3-*a*]carbazole (**1**), with an IPF value of 53.8 and APF value of 100.

The indole alkaloid (**1**) was isolated as a cytotoxic and antiviral compound from blue-green alga *Nostoc sphaericum* (strain EX-5-1) by Moore and co-workers.[4] In our project to achieve effective synthesis of **1**, we have established five synthetic routes.[5] Among them, the following became a satisfied synthetic route[5a] for **1** with respect to the IPF and APF values, the overall yield, the length of synthetic process, and the originality rate (OR).[6]

3-Acetoxy-2,2'-biindolyl[7] (**3**) was prepared in one step from indigo (**2**) in 88% yield according to our findings.[5c,d] Then **3** was reacted with dichloroacetyl chloride in refluxing ethyl acetate to give 3-acetoxy-3'-dichloroacetyl-2,2'-biindolyl (**4**) in 85% yield. Subsequently we discovered novel cyclization[1b,5a] of **4**

to *cis*-6-chloro-6a-hydroxy-5-oxo-5,6,6a,12-tetrahydroindolo[2,3-*a*]carbazole[5b] (**5**) in 91% yield by treatment with aqueous 1.3% ammonia in MeOH-DMF at room temperature. Methylation of **5** with dimethyl sulfate in the presence of K_2CO_3 produced 12-methyl compound (**6**) in a quantitative yield. A reductive cyanation[5b] of **6** was newly found to produce **7** in a quantitative yield by the reaction with NaCN in DMF-H_2O. Finally methylation of **7** with diazomethane afforded **1** in 86% yield. Dimethyl sulfate could also be applied for the final step.

Scheme 1



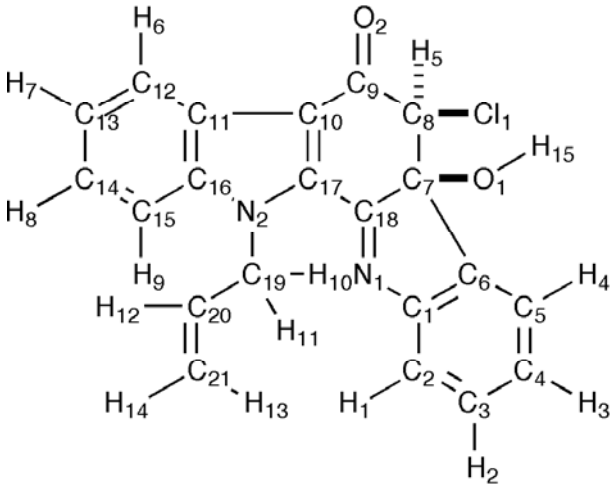
The *cis*-positioning of the 6-chlorine atom and the 6a-hydroxy group in the compound (**5**) was previously deduced based on its chemical behavior.[5b] In order to determine the stereochemistry, **5** was converted to the corresponding 12-allyl compound (**8**) in 94% yield by the treatment with allyl bromide and K_2CO_3. The compound (**8**) was suitable prisms for X-Ray single crystallographic analysis. The results shown in Figure 1 unequivocally prove the expected *cis*-configuration.

Thus, the antibiotic (**1**) is now available from indigo (**2**) in six steps with an overall yield of 59% using only conventional reagents without using any protecting groups.[8]

In the above synthesis, starting material is a widely used dye.[9] The target compound is an antibiotic.[4] The compound (**3**) was found to exhibit potent biological activity against telomerase.[10] In addition, we have discovered that **4**, **5**, **6**, and **7** are potent leads[11] for the development of inhibitor of blood platelet aggregation. Since every compound has either a biological activity or a useful functionality, the APF value of this synthesis is 100 (100 x 7/7). The IPF value of the synthesis is 53.8 (100 x 7/13) because **4**, **5**,

Figure 1

Numbering of Atoms



ORTEP Drawing of **8**

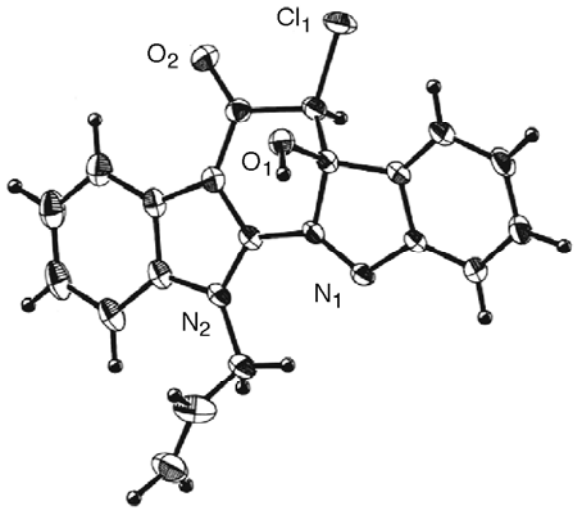


Table 1. Positional Parameters and *B* (eq) for **8**

atom	*x*	*y*	*z*	*B* (eq)	atom	*x*	*y*	*z*	*B* (eq)
Cl (1)	0.18862 (8)	0.1252 (1)	0.49317 (8)	4.82 (4)	C (17)	–0.1321 (2)	0.3634 (4)	0.3160 (3)	3.2 (1)
O (1)	0.0647 (2)	0.2953 (3)	0.2852 (2)	3.6 (1)	C (18)	–0.0329 (2)	0.4430 (4)	0.3554 (2)	2.9 (1)
O (2)	–0.0274 (2)	0.0050 (3)	0.3690 (2)	4.4 (1)	C (19)	–0.2706 (3)	0.5631 (5)	0.2565 (4)	5.1 (2)
N (1)	–0.0140 (2)	0.5742 (3)	0.3852 (2)	3.0 (1)	C (20)	–0.3415 (4)	0.5989 (6)	0.1440 (4)	6.0 (2)
N (2)	–0.2360 (2)	0.4135 (3)	0.2730 (2)	3.6 (1)	C (21)	–0.4351 (4)	0.6580 (6)	0.1040 (4)	6.2 (2)
C (1)	0.1002 (2)	0.5950 (3)	0.4274 (2)	2.9 (1)	H (1)	0.114 (3)	0.807 (4)	0.458 (3)	4.42 (2)
C (2)	0.1539 (3)	0.7224 (4)	0.4608 (3)	3.5 (1)	H (2)	0.310 (3)	0.807 (5)	0.523 (3)	4.71 (2)
C (3)	0.2652 (3)	0.7217 (4)	0.4969 (3)	4.1 (1)	H (3)	0.396 (4)	0.601 (5)	0.511 (4)	6.57 (3)
C (4)	0.3170 (3)	0.5971 (5)	0.4971 (3)	4.2 (2)	H (4)	0.303 (3)	0.387 (4)	0.465 (3)	3.31 (1)
C (5)	0.2620 (3)	0.4698 (4)	0.4623 (3)	3.8 (1)	H (5)	0.063 (3)	0.255 (4)	0.504 (3)	3.19 (1)
C (6)	0.1527 (3)	0.4685 (4)	0.4285 (2)	3.1 (1)	H (6)	–0.254 (3)	–0.051 (5)	0.281 (3)	5.31 (2)
C (7)	0.0680 (3)	0.3562 (3)	0.3768 (2)	3.0 (1)	H (7)	–0.439 (4)	–0.056 (6)	0.209 (4)	6.15 (2)
C (8)	0.0678 (3)	0.2268 (4)	0.4421 (3)	3.2 (1)	H (8)	–0.534 (4)	0.158 (5)	0.164 (4)	6.48 (3)
C (9)	–0.0327 (3)	0.1345 (4)	0.3728 (3)	3.2 (1)	H (9)	–0.463 (3)	0.380 (4)	0.188 (3)	4.13 (1)
C (10)	–0.1314 (3)	0.2161 (4)	0.3194 (3)	3.3 (1)	H (10)	–0.322 (5)	0.577 (6)	0.295 (4)	8.45 (4)
C (11)	–0.2417 (3)	0.1709 (4)	0.2754 (3)	3.5 (1)	H (11)	–0.208 (4)	0.620 (5)	0.293 (4)	6.31 (3)
C (12)	–0.2930 (4)	0.0384 (5)	0.2582 (3)	4.5 (2)	H (12)	–0.328 (5)	0.571 (6)	0.092 (5)	9.34 (5)
C (13)	–0.4032 (4)	0.0361 (6)	0.2164 (4)	5.4 (2)	H (13)	–0.457 (5)	0.689 (6)	0.151 (5)	9.13 (5)
C (14)	–0.4631 (4)	0.1612 (6)	0.1890 (4)	5.4 (2)	H (14)	–0.486 (5)	0.680 (6)	0.020 (5)	9.52 (4)
C (15)	–0.4166 (3)	0.2935 (5)	0.2040 (3)	4.6 (2)	H (15)	0.055 (3)	0.363 (4)	0.244 (4)	4.79 (2)
C (16)	–0.3038 (3)	0.2968 (4)	0.2477 (3)	3.7 (1)					

6, and **7** are our coumpounds, and the first,[5c,d] the third,[5b] and the fifth synthetic processes[1b,5a] are our own reactions. The originality rate[6] of the synthesis is 57% (100 x 4/7)

The results of biological evaluations of **4**, **5**, **6**, and **7** will be reported in due course.[11]

EXPERIMENTAL

IR spectra were determined with a HORIBA FT-720 spectrophotometer and ^{1}H-NMR spectra with a JEOL GSX-500 spectrometer with tetramethylsilane as an internal standard. MS spectra were recorded on a JEOL SX-102A spectrometer. Column chromatography was performed on silica gel (SiO_2, 100–200 mesh, from Kanto Chemical Co. Inc.) throughout the present study.

3-Acetoxy-3'-dichloroacetyl-2,2'-biindolyl (4) from 3-Acetoxy-2,2'-biindolyl (3)[5c,d,7] — Dichloroacetyl chloride (3.35 mL, 34.8 mmol) was added to a solution of **3**[5c,d,7] (1.01 g, 3.48 mmol) in AcOEt (120 mL) and the mixture was refluxed for 3 h with stirring. After addition of H_2O, the whole was extracted with AcOEt. The extract was washed with brine, dried over Na_2SO_4, and evaporated under reduced pressure to leave a residue, which was column-chromatographed on SiO_2 with AcOEt–hexane (1:2, v/v) to give **4** (1.19 g, 85%). **4**: mp 227—229 °C (decomp, pale yellow fine needles, recrystallized from AcOEt). IR (KBr): 3340, 1745, 1637, 742 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 2.24 (3H, s), 6.71 (1H, s), 7.16 (1H, t, *J*=7.3 Hz), 7.29—7.36 (3H, m), 7.50 (1H, d, *J*=7.3 Hz), 7.52 (1H, d, *J*=8.5 Hz), 7.54 (1H, d, *J*=8.5 Hz), 8.12 (1H, d, *J*=7.3 Hz), 11.93 (1H, br s, disappeared on addition of D_2O), 12.67 (1H, br s, disappeared on addition of D_2O). MS *m/z*: 404, 402, 400 (M^+). *Anal.* Calcd for $C_{20}H_{14}N_2O_3Cl_2$: C, 59.87; H, 3.52; N, 6.98. Found: C, 59.60; H, 3.35; N, 6.68.

***cis*-6-Chloro-6a-hydroxy-5-oxo-5,6,6a,12-tetrahydroindolo[2,3-*a*]carbazole (5) from 4** — 28% Aqueous NH_3 (2.0 mL) was added to a solution of **4** (2.44 g, 6.0 mmol) in MeOH (20 mL) and DMF (20 mL), and the mixture was stirred at rt for 1 h. After evaporation of the solvent under reduced pressure, the residue was column-chromatographed on SiO_2 with AcOEt–hexane (1:2, v/v) to give **5** (1.44 g, 91%). **5**: mp 236—238 °C (decomp, yellow powder, recrystallized from AcOEt). IR (KBr): 3335, 1680, 1593, 1473, 758, 738 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 5.32 (1H, s), 6.80 (1H, s, disappeared on addition of D_2O), 7.34 (1H, dt, *J*=0.7, 7.7 Hz), 7.37 (1H, dt, *J*=0.7, 7.7 Hz), 7.43 (1H, dt, *J*=1.2, 7.7 Hz), 7.53 (1H, dt, *J*=1.2, 7.7 Hz), 7.60 (1H, dt, *J*=8.1, 1.2 Hz), 7.72 (1H, d, *J*=7.7 Hz), 7.82 (1H, ddd, *J*=0.5, 1.2, 7.7 Hz), 8.11 (1H, dt, *J*=8.1, 1.2 Hz), 13.12 (1H, br s, disappeared on addition of D_2O). MS *m/z*: 324, 322 (M^+). *Anal.* Calcd for $C_{18}H_{11}N_2O_2Cl\cdot 1/4H_2O$: C, 66.06; H, 3.54; N, 8.56. Found: C, 66.16; H, 3.37; N, 8.53.

***cis*-6-Chloro-6a-hydroxy-5-oxo-12-methyl-5,6,6a,12-tetrahydroindolo[2,3-*a*]carbazole (6) from 5** — K_2CO_3 (233.4 mg, 1.70 mmol) and Me_2SO_4 (0.34 mL, 3.41 mmol) were added to a solution of **5**(110.1 mg, 0.34 mmol) in DMF (8 mL), and the mixture was stirred at rt for 3 h. After addition of H_2O under ice water bath, the whole was extracted with AcOEt. The extract was washed with brine, dried over Na_2SO_4,

and evaporated under reduced pressure to leave a residue, which was column-chromatographed on SiO_2 with AcOEt–hexane (1:1, v/v) to give **6** (116.5 mg, 100%). **6**: mp 223—225 ˚C (decomp, yellow prisms, recrystallized from AcOEt). IR (KBr): 3385, 1645, 1575, 758 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 4.26 (3H, s), 5.32 (1H, s), 6.82 (1H, s, disappeared on addition of D_2O), 7.39 (1H, td, *J*=7.3, 1.2 Hz), 7.41 (1H, td, *J*=7.3, 1.2 Hz), 7.52 (1H, td, *J*=7.8, 1.5 Hz), 7.55 (1H, td, *J*=7.8, 1.5 Hz), 7.74—7.80 (2H, m), 7.83 (1H, dd, *J*=7.3, 1.2 Hz), 8.17 (1H, dd, *J*=7.8, 1.5 Hz). MS *m/z*: 338, 336 (M^+). *Anal.* Calcd for $C_{19}H_{13}N_2O_2Cl\cdot 1/2H_2O$: C, 66.00; H, 4.08; N, 8.10. Found: C, 66.27; H, 3.85; N, 7.90.

6-Cyano-5-hydroxy-12-methylindolo[2,3-*a*]carbazole (7) from 6 — NaCN (761.5 mg, 14.8 mmol) was added to a solution of **6** (165.7 mg, 0.49 mmol) in DMF (10 mL) and H_2O (5 mL), and the mixture was stirred at 70 ˚C for 15 min. After addition of H_2O, the whole was extracted with AcOEt. The extract was washed with brine, dried over Na_2SO_4, and evaporated under reduced pressure to leave a residue, which was column-chromatographed on SiO_2 with AcOEt–hexane (1:1, v/v) to give **7** (152.7 mg, 100%). **7**: mp >300 ˚C (pale gray powder, recrystallized from AcOEt–hexane). IR (KBr): 3200, 2220, 1638, 1578, 1327, 740 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 4.36 (3H, s), 7.27 (1H, t, *J*=7.6 Hz), 7.32 (1H, t, *J*=7.6 Hz), 7.47 (1H, t, *J*=8.0 Hz), 7.51 (1H, t, *J*=8.0 Hz), 7.71 (1H, d, *J*=8.0 Hz), 7.74 (1H, d, *J*=8.0 Hz), 8.37 (1H, d, *J*=7.6 Hz), 8.44 (1H, d, *J*=7.6 Hz), 10.71 (1H, br s, disappeared on addition of D_2O), 11.74 (1H, s, disappeared on addition of D_2O). MS *m/z*: 311 (M^+). *Anal.* Calcd for $C_{20}H_{13}N_3O\cdot 1/8H_2O$: C, 76.60; H, 4.26; N, 13.40. Found: C, 76.81; H, 4.23; N, 13.31.

6-Cyano-5-methoxy-12-methylindolo[2,3-*a*]carbazole (1) from 7 — Excess CH_2N_2 in Et_2O was added to a suspension of **7** (30.0 mg, 0.096 mmol) in MeOH (4 mL) and the mixture was stirred at rt for 50 min. After evaporation of the solvent under reduced pressure, the residue was column-chromatographed on SiO_2 with $CHCl_3$ to give **1** (26.7 mg, 86%). All spectral data of **1** are identical with those of natural product.[4]

***cis*-12-Allyl-6-chloro-6a-hydroxy-5-oxo-5,6,6a,12-tetrahydroindolo[2,3-*a*]carbazole (8) from 5** — K_2CO_3 (754.1 mg, 5.44 mmol) and allyl bromide (2.70 mL, 31.1 mmol) were added to a solution of **5** (501.4 mg, 1.56 mmol) in anhydrous DMF (10 mL), and the mixture was stirred at rt for 30 min. Since the work-up using H_2O was found to decompose **8**, the residue, obtained after evaporation of the solvent under reduced pressure, was directly column-chromatographed on SiO_2 with AcOEt–hexane (1:2, v/v) to give **8** (540.3 mg, 96%). **8**: mp 202—203 ˚C (decomp, yellow prisms, recrystallized from AcOEt). IR (KBr): 1668, 1582, 1478, 750 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 5.16 (1H, br dd, *J*=17.1, 1.5 Hz), 5.22 (1H, br dd, *J*=10.3, 1.5 Hz), 5.35 (1H, s), 5.36 (1H, dd, *J*=16.3, 5.4 Hz), 5.53 (1H, dd, *J*=16.3, 5.4 Hz), 6.07—6.16 (1H, m), 6.84 (1H, s, disappeared on addition of D_2O), 7.39 (1H, t, *J*=8.1 Hz), 7.40 (1H, t, *J*=8.1 Hz), 7.50 (1H, t, *J*=7.5 Hz), 7.55 (1H, t, *J*=7.5 Hz), 7.75 (1H, d, *J*=8.1 Hz), 7.77 (1H, d, *J*=7.5 Hz),

7.84 (1H, d, *J*=7.5 Hz), 8.19 (1H, d, *J*=8.1 Hz). MS *m/z*: 364, 36 (M^+). *Anal.* Calcd for $C_{21}H_{15}N_2O_2Cl$: C, 69.52; H, 4.17; N, 7.72. Found: C, 69.49; H, 4.17; N, 7.41.

X-Ray Crystallographic Analysis of 8 — A single crystal (0.50 x 0.30 x 0.50 mm) of **8** was obtained by recrystallization from AcOEt. All measurements were made on a Rigaku AFC5R diffractometer with graphite monochromated Cu-$K\alpha$ radiation (λ=1.54178 Å). Crystal data: $C_{21}H_{15}N_2O_2Cl$, *M*=362.81, monoclinic, space group $P2_1/c$ (#14), *a*=14.141 (2) Å, *b*=9.364 (1) Å, *c*=14.471 (1) Å, β=119.152 (7)°, *V*=1673.6 (3) $Å^3$, *Z*=4, D_{calc}=1.440 g/cm^3, *F*(000)=752, and μ(Cu$K\alpha$)=21.81 cm^{-1}. The structure was solved by direct methods using MITHRIL.[12] The non-hydrogen atoms were refined anisotropically. The final cycle of full-matrix least-squares refinement was based on 2222 observed reflections ($I>3.00\sigma(I)$, $2\theta<120.2°$) and 295 variable parameters. The final refinement converged with *R*=0.066 and R_w=0.089.

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2. a) Definition of an intellectual property factor (IPF) in synthesis: IPF is the total rate of one's own intellectual property of every compound involved in the synthesis, including starting material, target compound, and all synthetic intermediates. It is calculated by the following formula.

$$\text{IPF} = 100 \times \frac{\left(\begin{array}{c}\text{The Number of Compound}\\ \text{Having Intellectual Property}\end{array}\right) + \left(\begin{array}{c}\text{The Number of Synthetic Process}\\ \text{Having Intellectual Property}\end{array}\right)}{2 \times (\text{The Number of Synthetic Process}) + 1}$$

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3. Definition of an application potential factor (APF) in synthesis: APF is the total potential of application of every compound involved in the synthesis, including starting material, target

compound, and all synthetic intermediates. It is calculated by the following formula.

$$APF = 100 \times \frac{\text{The Number of Compound Having either Biological Activity or Other Useful Functionality}}{\text{The Number of Synthetic Process} + 1}$$

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