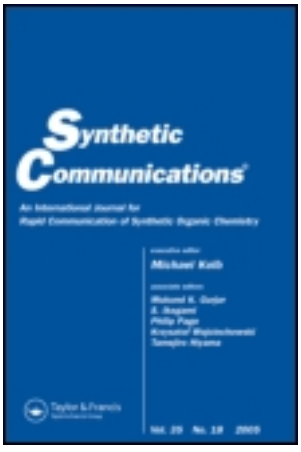

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Synthesis of 7-Keto-Gö6976 (ICP-103)

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Abstract: An efficient synthesis of 7-keto-Gö6976 (ICP-103) (**3**) is described that employs palladium(II) triflate in the final oxidative cyclization step.

Keywords: Indolo[2,3-*a*]carbazole, Gö6976, palladium(II) triflate

Indolo[2,3-*a*]carbazole and indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole alkaloids are an interesting class of natural products because of their high degree of biological activity, including antitumor, antifungal, and antimicrobial properties.[1] One member of this class, UCN-01 (**1**), generated considerable interest in our laboratory when it was found to be a potent inhibitor of DNA damage-induced S and G2 cell cycle checkpoints, which led to increased killing of tumor cells.[2] Although UCN-01 is well recognized as a protein kinase C inhibitor,[3] this checkpoint inhibition was attributed to its ability to inhibit Chk1.[4] In a clinical trial, UCN-01 was found to bind avidly to human serum proteins thereby compromising its potential therapeutic activity.[5] Therefore, we have initiated an Indolocarbazole Project (ICP) to identify

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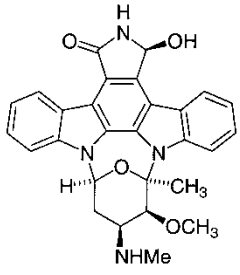
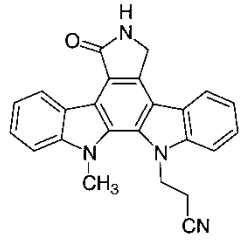
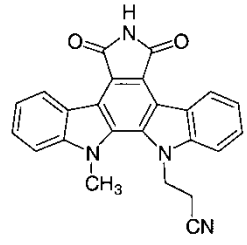
other potent and selective checkpoint inhibitors that would not be inactivated by human serum proteins. We initially synthesized and tested a K252a analogue, ICP-1, which overcame the problems of protein binding but had considerably reduced potency.[6] More recently, we established that Gö6976 (**2**) is a very potent checkpoint inhibitor even in the presence of human serum,[7] and **2** has subsequently been shown to potently inhibit Chk1.[8] As a consequence, we have begun structure/activity analysis of analogs of Gö6976. We now report an efficient synthesis of 7-keto-Gö6976 (**3**), which we name ICP-103.[9]



1 (UCN-01)



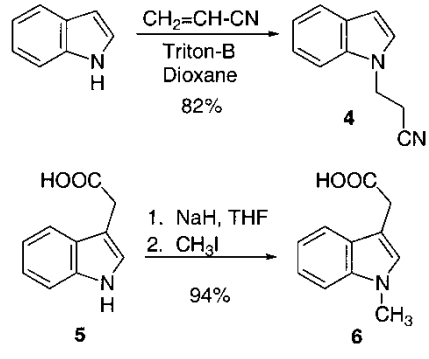
2 (Gö6976)



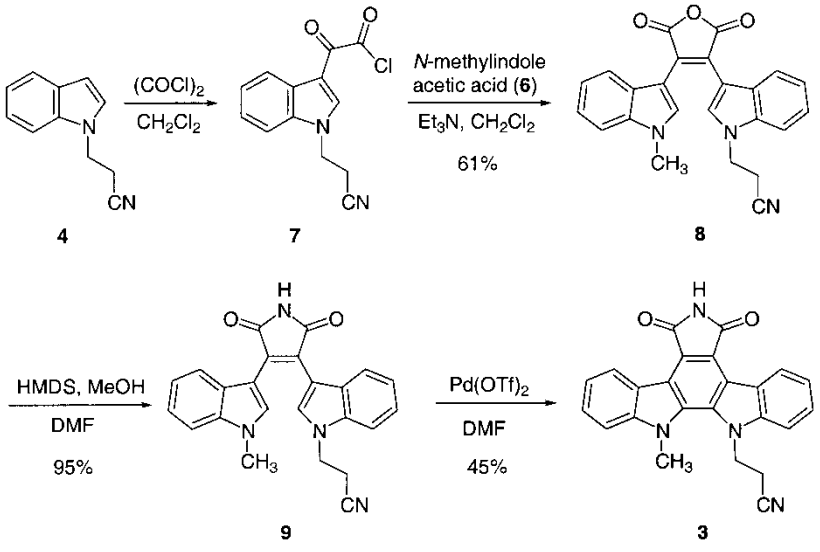
3 (ICP-103)

The requisite *N*-methylindole and *N*-cyanoethylindole fragments were synthesized as shown in Scheme 1. Indole-1-propionitrile (**4**) was prepared in 82% yield from the reaction of indole with acrylonitrile in presence of Triton-B.[10] Alkylation of indole-3-acetic acid (**5**) afforded 1-methylindole-3-acetic acid (**6**)[11] in excellent yield (Scheme 1).

Indole-1-propionitrile (**4**) was treated with oxalyl chloride to give [1-(2-cyanoethyl)indolyl]-3-glyoxylyl chloride (**7**).[12] The crude reaction mixture was allowed to react with 1-methylindole-3-acetic acid (**6**) in presence of triethylamine. Anhydride **8** was obtained in 61% yield over the two steps



Scheme 1.



Scheme 2.

(Scheme 2), and was converted to imide **9** under mild amination conditions. Due to the potentially labile nitrile functionality, ammonia was generated in situ from 1,1,1,3,3,3-hexamethyldisilazane (HMDS) and methanol and these conditions were used to convert anhydride **8** to imide **9** at room temperature.[13]

The final cyclization step to afford the target molecule **3** was initially attempted by using 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (DDQ) in presence of catalytic *p*-toluenesulfonic acid.[14] This particular oxidative cyclization can be difficult in the indolocarbazole system and, therefore, we delayed this reaction to the final step of our synthesis. Indeed, poor yields were obtained using DDQ as the oxidant. Different solvent systems (such as benzene,[15] chlorobenzene[16]) and temperatures did not improve the yield, and inseparable mixtures were obtained. The use of $PdCl_2$ did not increase the yield of this cyclization reaction.[17] Finally, we found $Pd(OTf)_2$ to be a superior reagent for this cyclization, especially with bulky substituents on *N*-12 and *N*-13 of the bisindolemaleimide.[18] Thus, using $Pd(OTf)_2$ in DMF, we were able to improve the yield of this oxidative cyclization to 45%. Overall, ICP-103 (**3**) was obtained in 26% yield from **4**.

In work to be reported separately, we find that ICP-103 (**3**) is almost as potent as Gö6976 (**2**) at abrogating DNA damage-induced cell cycle arrest. Using an assay previously described,[7] Gö6976 abrogated S and G2 arrest at 10 nM, whereas ICP-103 was effective at 30 nM.

EXPERIMENTAL

Melting points were determined with a Mel-Temp Laboratory Device apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on either a Varian XL-300 or 500 Fourier-transform NMR spectrometer. Both low- and high-resolution mass spectra were carried out at the Mass Spectrometry Laboratory, School of Chemical Sciences, University of Illinois at Urbana Champaign. Elemental analyses were done by Atlantic Microlab Inc. THF, methylene chloride, and triethylamine were distilled before use.

Indole-1-propionitrile (4)

To a magnetically stirred solution of indole (2.93 g, 25 mmol) in dioxane (25 mL) at 0°C was added acrylonitrile (2.5 mL, 37.5 mmol) followed by the dropwise addition of Triton-B (0.8 mL). The resulting mixture was slowly allowed to warm to room temperature (rt) and stirred for 20 h. Then 5% acetic acid was added dropwise to neutralize the base catalyst. The solution was washed with brine and dried over sodium sulfate. The solvent was removed in vacuo and the residue was purified by column chromatography (6:4 hexanes:ethyl acetate) to give 3.49 g (82%) of **4** as a colorless oil that solidified on prolonged drying to furnish a white solid; m.p. 44–46°C (Lit.[19] 47–48°C); ^{1}H ($CDCl_3$) δ 7.73 (d, 1H, $J = 7.8$ Hz), 7.37–7.18 (m, 4H), 6.62 (dd, 1H, $J = 3.2$ Hz, 0.5 Hz), 4.44 (t, 2H, $J = 6.7$ Hz), 2.80 (t, 2H, $J = 6.8$ Hz); ^{13}C ($CDCl_3$) δ 135.5, 129.1, 127.6, 122.4, 121.6, 120.3, 117.4, 108.8, 103.0, 42.2, 19.2.

1-Methylindole-3-acetic Acid (6)

To a magnetically stirred suspension of NaH (6 g, 150 mmol, 60% mineral oil dispersion) in THF (125 mL) at 0°C was added a solution of indole-3-acetic acid (**5**) (5.25 g, 30 mmol) in THF (50 mL). After stirring the reaction mixture at the same temperature for 30 min, a solution of methyl iodide (14.2 g, 100 mmol) in THF (50 mL) was added and stirred for 16 h at rt. Excess hydride was carefully destroyed by slow addition of MeOH followed by water. The mixture was extracted with Et_2O. The aqueous layer was acidified with 6N HCl and extracted with CH_2Cl_2. The combined organic extracts were dried over sodium sulfate and concentrated to about 40–50 mL. Petroleum ether (b.p. 38.5–55°C) was then added slowly until a cream-colored solid precipitated. The solid was recrystallized from ethanol to give 5.33 g (94%) of **6**; m.p. 127–128°C (Lit.[20] 127–128.5°C); ^{1}H ($CDCl_3$) δ 7.64–7.62 (m, 1H), 7.34–7.26 (m, 2H), 7.19–7.15 (m, 1H), 7.07 (s, 1H), 3.83 (s, 2H), 3.78 (s, 3H); ^{13}C ($CDCl_3$) δ 178.8, 137.0, 128.1, 127.7, 122.0, 119.5, 119.1, 109.5, 106.2, 32.9, 31.2.

3-(1-Cyanoethyl-3-indolyl)-4-(1-methyl-3-indolyl)maleic Anhydride (8)

To a magnetically stirred solution of indole-1-propionitrile (**4**) (0.85 g, 5 mmol) in dichloromethane (50 mL) at 0°C was added dropwise oxalyl chloride (0.7 g, 5.5 mmol). The mixture was stirred at 0–5°C for 30 min. The solvent was removed in vacuo and the organic residue was redissolved in dichloromethane (50 mL). This solution was then added dropwise to a stirred mixture of 1-methylindole-3-acetic acid (**6**) (0.95 g, 5 mmol) and triethylamine (1.0 g, 10 mmol) in dichloromethane (25 mL). After 8 h of stirring at rt, the solvent was evaporated in vacuo. The residue was purified by column chromatography (99:1 dichloromethane:methanol) to give 1.2 g (61%) of **8** as a red solid; m.p. 203–205°C; ^{1}H (acetone-d_6) δ 7.94 (d, 2H, $J = 13.2$ Hz), 7.62 (d, 1H, $J = 8.4$ Hz), 7.43 (d, 1H, $J = 8.4$ Hz), 7.15–7.04 (m, 3H), 6.82–6.70 (m, 3H), 4.71 (t, 2H, $J = 6.6$ Hz), 3.95 (s, 3H), 3.08 (t, 2H, $J = 6.6$ Hz); LRMS (EI): m/z 395 (M^+), 365, 340, 323, 259, 205, 183.1, 171, 155, 143, 131 (100%), 103, 77; HRMS (EI): Calcd. for $C_{24}H_{17}N_3O_3$: 395.1270. Found: 395.1268; Anal. Calcd. for $C_{24}H_{17}N_3O_3$: C, 72.90; H, 4.33; N, 10.63. Found: C, 72.77; H, 4.25; N, 10.62.

2-(1-Cyanoethyl-3-indolyl)-3-(1-methyl-3-indolyl)maleimide (9)

A magnetically stirred solution of anhydride **8** (0.4 g, 1 mmol) in DMF (5 mL) was added a 1,1,1,3,3,3-hexamethyldisilazane (1.61 g, 10 mmol) followed by methanol (0.16 g, 5 mmol). The completely closed solution was stirred at rt for 18 h. Then the mixture was poured into water and extracted thrice with ethyl acetate. The combined organic extracts were washed with water. The separated organic layer was dried over magnesium sulfate and the solvent was removed in vacuo to give 0.37 g (95%) of **9** as a red solid: m.p. 249–251°C; ^{1}H (DMSO-d_6) 10.96 (brs, 1H), 7.86 (d, 2H, $J = 17.6$ Hz), 7.57 (d, 1H, $J = 8.1$ Hz), 7.40 (d, 1H, $J = 8.1$ Hz), 7.07–6.99 (m, 2H), 6.88 (d, 1H, $J = 8.1$ Hz), 6.72–6.58 (m, 3H), 4.57 (t, 2H, $J = 6.4$ Hz), 3.37 (s, 3H), 3.00 (t, 2H, $J = 6.4$ Hz); LRMS (EI): m/z 394 (M^+, 100%), 354, 339, 311, 283, 268, 177, 142, 121; HRMS (EI): Calcd. for $C_{24}H_{18}N_4O_2$: 394.1430. Found: 394.1429; Anal. Calcd. for $C_{24}H_{18}N_4O_2$: C, 73.08; H, 4.60; N, 14.20. Found: C, 72.99; H, 4.67; N, 14.16.

ICP-103 (3)

A mixture of maleimide **9** (39.4 mg, 0.1 mmol) and $Pd(OTf)_2$ (166 mg, 0.5 mmol) in DMF (15 mL) was heated at 90°C for 2 h. The reaction mixture was then cooled, diluted with ethyl acetate and washed with 0.5N

HCl. The organic phase was dried over sodium sulfate and filtered through Hyflo. The solvent was removed in vacuo and the residue was purified by column chromatography (97:3 dichloromethane:methanol) to give 17.7 mg (45%) of **3** as a fluorescent yellow solid; m.p. 291–293°C; ^{1}H (DMSO-d_6) δ11.17 (brs, 1H), 9.17–9.10 (m, 2H), 7.99–7.96 (m, 1H), 7.83–7.81 (m, 1H), 7.71–7.67 (m, 2H), 7.49–7.44 (m, 2H), 5.16–5.12 (t, 2H, $J = 6.5$ Hz), 4.23 (s, 3H), 2.83–2.79 (t, 2H, $J = 6.6$ Hz); LRMS (EI): m/z 392 (M^+), 352, 339, 53 (100%); HRMS (EI): Calcd. for $C_{24}H_{16}N_4O_2$: 392.1273. Found: 392.1274. Anal. Calcd. for $C_{24}H_{16}N_4O_2.1/3H_2O$: C, 72.35; H, 4.22; N 14.06. Found: C, 72.49; H, 4.13; N, 14.21.

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