SYNTHESIS AND CYTOTOXICITY OF VARIOUS STRUCTUAL TYPES OF NOVEL CYCLOPROPAPYRROLOINDOLE(CPI) DERIVATIVES[1]

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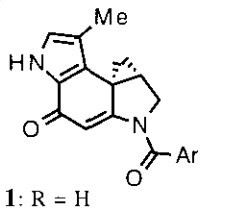
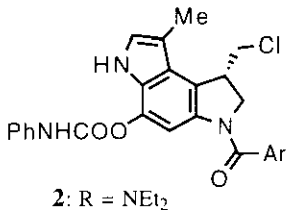
Abstract-The various structural types of novel cyclopropapyrroloindole(CPI) derivatives (**7~10**) were synthesized and their cytotoxicity was evaluated. Among these derivatives, 3-methoxycarbonylCPI(3-MCCPI) derivatives (**8a**,**b**) and cyclopropapyrrolocarbazole (CPC) derivative (**10a**) were found to exhibit comparable cytotoxicity to U-73,975 (adozelesin) (**1**).

The cyclopropapyrroloindole(CPI) derivatives, U-73,975 (adozelesin) (**1**),[2] U-80,244 (carzelesin) (**2**)[3], and KW-2189 (**3**),[4] are the promising antitumor agents, the two of which, **2** and **3**, are presently under

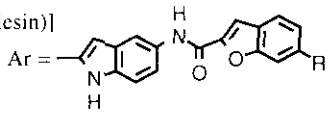
Figure 1

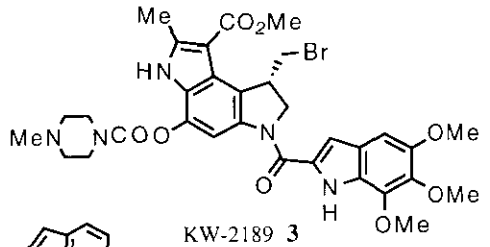


1: R = H
[U-73,975(adozelesin)]



2: R = NEt_2
[U-80,244(carzelesin)]

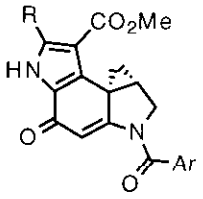




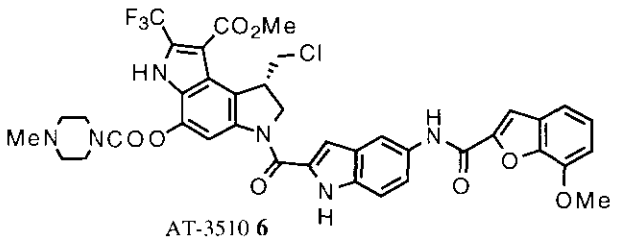
KW-2189 **3**

clinical trials (**Figure 1**). Recently, we reported the synthesis and antitumor activity of the novel CPI derivatives, bis(methoxycarbonyl)CPI (MC_2CPI) and 3-methoxycarbonyl-2-trifluoromethylCPI

Figure 2



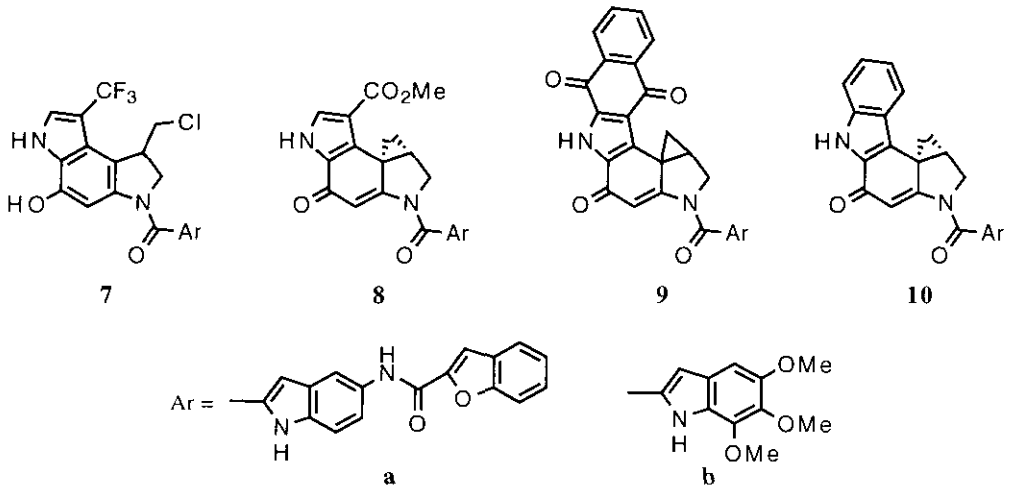
4: R = CO_2Me
5: R = CF_3



AT-3510 **6**

(MCTFCPI) derivatives (**4**) and (**5**), showing promising cytotoxicity (*in vitro*) and antitumor activity (*in vivo*) against murine leukemia and solid tumors (**Figure 2**).[5] Among these derivatives, **AT-3510** (**6**), the novel prodrug MCTFCPI derivative,[5c] was found to exhibit more excellent antitumor activity and less toxicity than all of the CPI derivatives so far reported.[2~5] Based on these studies, it appeared evident that structural characteristics of the indole ring involved in the CPI system give a significant influence on cytotoxicity and antitumor activity. Accordingly, in order to explore the novel CPI derivatives exhibiting more excellent antitumor activity, we next investigated the effect of substituent(s) present in the indole ring. Herein, we wish to report on the synthesis and cytotoxicity of various structural types of novel CPI derivatives, 3-trifluoromethylCPI(3-TFCPI) seco-chloride derivative (**7a**), 3-methoxycarbonylCPI(3-MCCPI) derivatives (**8a,b**), cyclopropapyrrolobenzocarbazole(CPBC) derivative (**9b**), and cyclopropapyrrolocarbazole(CPC) derivative (**10a**), which bear various substituents on the indole ring of the CPI system (**Figure 3**). Among these CPI derivatives, **7a** and **9b** were prepared in racemic forms while the synthesis of **8a,b** and **10a** was carried out in optically active compounds. This is because rough comparisons of cytotoxicity can be well done by its order of magnitude.[6]

Figure 3



7 **8** **9** **10**

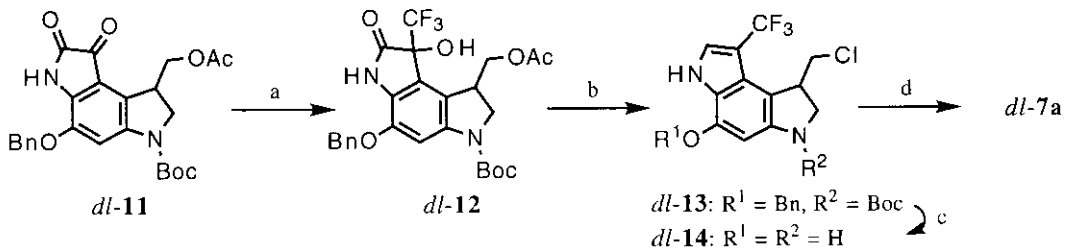
a **b**

Synthesis of the 3-TrifluoromethylCPI(3-TFCPI) Seco-chloride Derivative (*dl*-7a)

Synthesis of the 3-trifluoromethylCPI(3-TFCPI) seco-chloride derivative (*dl*-**7a**) corresponding to the trifluorinated analogue of the CPI system involved in **1** and **2** was first attempted. Thus, the introduction of a trifluoromethyl group was achieved by treating the isatin (*dl*-**11**)[7] with $TMSCF_3$[8] in the presence of tetra-*n*-butylammonium fluoride (TBAF), providing the trifluoromethylated product (*dl*-**12**) as a diastereomeric mixture (*ca.* 3:1 by ^{1}H-NMR). This was converted to chloride (*dl*-**13**) by reduction with borane-dimethyl sulfide complex followed by chlorination. Treatment of *dl*-**13** with boron tribromide underwent simultaneous deprotections of both the benzyl and Boc groups to give the indoline (*dl*-**14**). This was immediately coupled with 5-(benzofuran-2-ylcarbonyl)aminoindole-2-carboxylic acid (**15**)[9] in the presence of 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDCI), affording *dl*-**7a** (**Scheme 1**). Since spirocyclization of *dl*-**7a** to the 3-TFCPI system under basic conditions turned out to give a complex

mixture of the products probably due to the 3-trifluoromethyl group of indole ring, *dl*-**7a** was directly subjected to cytotoxicity assay as it stood.

Scheme 1

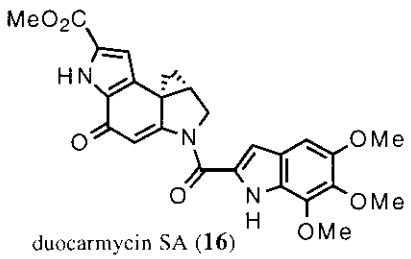


a) $TMSCF_3$, TBAF, THF, 0°C~10°C, 71%. b) i) BH_3, THF. ii) PPh_3, CCl_4, MeCN, rt, 18% (2 steps). c) BBr_3, CH_2Cl_2, -40°C~10°C. d) $ArCO_2H$ (**15**), EDCI, DMF, rt, 42% (2 steps).

Synthesis of the 3-MethoxycarbonylCPI(3-MCCPI) Derivatives (8a,b)

Taking into account of the antitumor activity of **3~6** so far reported,[5] we next designed the 3-methoxycarbonylCPI(3-MCCPI) derivatives (**8a**,**b**) which are regioisomeric to duocarmycin SA (**16**)

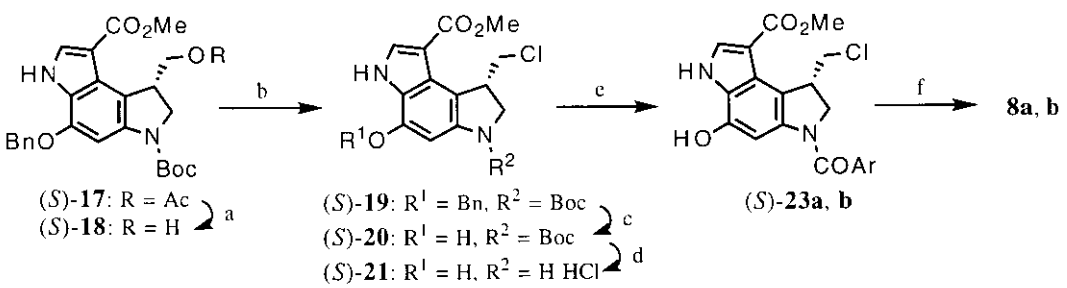
Figure 4



duocarmycin SA (**16**)

(**Figure 4**).[10] Thus, the acetyl group of the pyrroloindole [(*S*)-**17**][11] was removed by methanolysis under basic conditions, giving rise to alcohol [(*S*)-**18**]. Conversion of (*S*)-**18** to chloride [(*S*)-**19**] followed by removal of the benzyl group by transfer hydrogenolysis provided the phenol [(*S*)-**20**]. Deprotection of (*S*)-

Scheme 2



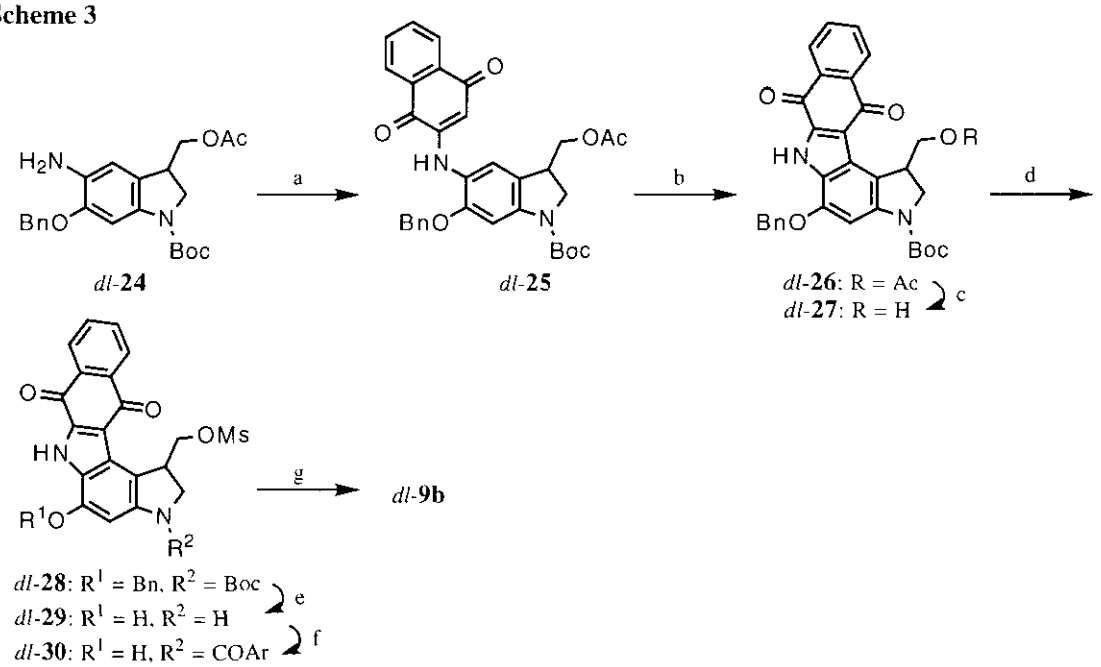
a) 20% KOH, MeOH, rt, 89%. b) PPh_3, CCl_4, MeCN, rt, 90%. c) 10%Pd-C, 25% HCO_2NH_4, THF, 0°C, 98%. d) 3M HCl-AcOEt. e) $ArCO_2H$ (**15** or **22**), EDCI, DMF, rt, (*S*)-**23a** ; 79% (2 steps), (*S*)-**23b** ; 71% (2 steps). f) DBU, MeCN, rt, **8a** ; 92%, **8b** ; 65%.

20 under acidic conditions gave the indoline [(*S*)-**21**] as its hydrochloride. This was immediately coupled with **15**[9] and 5,6,7-trimethoxyindole-2-carboxylic acid (**22**)[7] in the presence of EDCI to afford the seco-chlorides (*S*)-**23a** and (*S*)-**23b**, respectively. Finally, treatments of (*S*)-**23a,b** with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) effected spirocyclization, furnishing **8a**, $[\alpha]_D^{26}$ = +170° (c = 0.20, THF) and **8b**, $[\alpha]_D^{27}$ = +205° (c = 0.20, THF) (**Scheme 2**).

Synthesis of the Cyclopropapyrrolobenzocarbazole(CPBC) Derivative (*dl*-9b)

In the CPI derivatives (**3~6** and **16**), one or two carbonyl group(s) are attached at the C_2- or C_3-position, or both the C_2- and C_3-positions of CPI system in a form of methoxycarbonyl group. Therefore, we further designed the cyclopropapyrrolobenzocarbazole(CPBC) derivative (*dl*-**9b**) carrying two carbonyl groups in a form of quinone system. Thus, according to the reported method,[12] the treatment of the 5-aminoindoline (*dl*-**24**)[7] with 1,4-naphthoquinone gave rise to the aminoquinone (*dl*-**25**). Oxidative cyclization of *dl*-**25** was effected with $Pd(OAc)_2$ in acetic acid, affording the pyrrolobenzocarbazole (*dl*-**26**) as a sole product. The acetyl group of *dl*-**26** was removed by methanolysis under basic conditions, and the resulting alcohol (*dl*-**27**) was converted to the mesylate (*dl*-**28**). Treatment of *dl*-**28** with boron tribromide underwent simultaneous deprotection of both the benzyl and Boc groups giving the indoline (*dl*-**29**). This was immediately coupled with **22**[7] in the presence of EDCI to give the seco-mesylate (*dl*-**30**). Finally, treatment of *dl*-**30** with DBU effected spirocyclization, affording *dl*-**9b** (**Scheme 3**).

Scheme 3

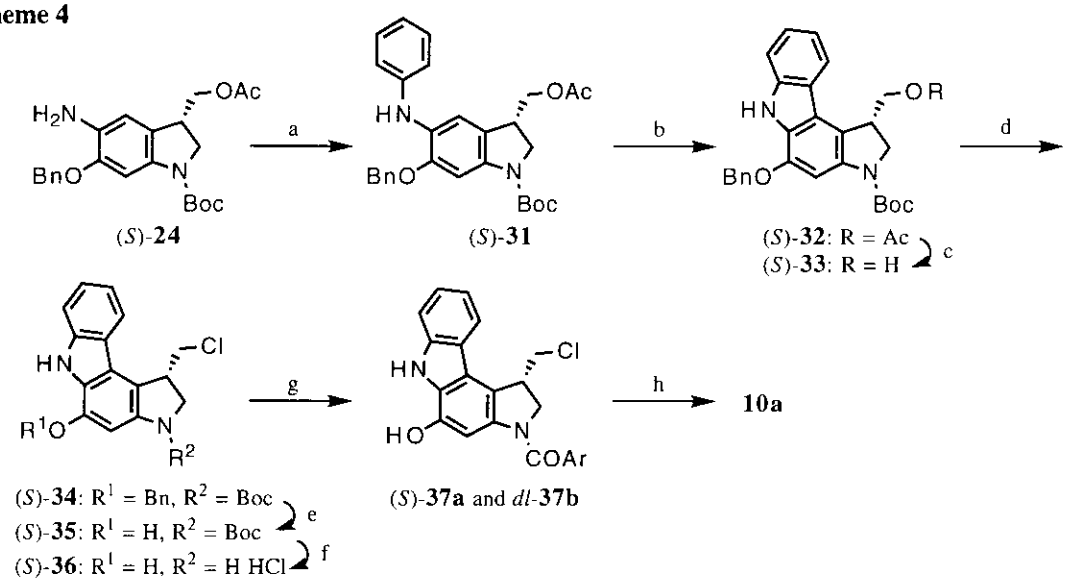


a) 1,4-naphthoquinone, AcOH, H_2O, rt, 73%. b) $Pd(OAc)_2$, AcOH, 100°C, 29%. c) 20% KOH, MeOH, rt. d) MsCl, Et_3N, THF, 0°C, 86% (2 steps). e) BBr_3, CH_2Cl_2, -40°C~0°C. f) $ArCO_2H$ (**22**), EDCI, DMF, rt, 47% (2 steps). g) DBU, MeCN, rt, 78%.

Synthesis of the Cyclopropapyrrolocarbazole(CPC) Derivative (10a)

With *dl*-**7a**, **8a**,**b**, and *dl*-**9b** in hand, the synthesis of the cyclopropapyrrolocarbazole(CPC) derivative carrying the CPI system fused with a benzene ring at its C_2- and C_3-positions was next attempted. According to the reported method,[13] phenylation of (*S*)-**24**[6] with triphenyl bismuthine in the presence of $Cu(OAc)_2$ provided the aniline [(*S*)-**31**]. Oxidative cyclization of (*S*)-**31** was effected with $Pd(OAc)_2$ to afford the pyrrolocarbazole [(*S*)-**32**] as a sole product. The acetyl group of (*S*)-**32** was removed by methanolysis under basic conditions, giving rise to alcohol [(*S*)-**33**]. Conversion of (*S*)-**33** to chloride

Scheme 4



a) triphenyl bismuthine, $Cu(OAc)_2$, CH_2Cl_2, rt, 93%. b) $Pd(OAc)_2$, dichloroacetic acid, MeCN, 80°C, 25%. c) 20% KOH, MeOH, 50°C, 96%. d) PPh_3, CCl_4, CH_2Cl_2, rt, 72%. e) 10%Pd-C, 25% HCO_2NH_4, THF, 0°C, 99%. f) 3M HCl-AcOEt. g) $ArCO_2H$ (**15** or **22**), EDCI, DMF, rt, **37a** ; 82% (2 steps), *dl*-**37b** ; 47% (2 steps). h) DBU, MeCN, rt, 71%.

[(*S*)-**34**] followed by removal of the benzyl group by transfer hydrogenolysis provided the phenol [(*S*)-**35**]. Further deprotection of (*S*)-**35** under acidic conditions afforded the indoline [(*S*)-**36**] as its hydrochloride. This was immediately coupled with **15**[7] in the presence EDCI to give the seco-mesylate [(*S*)-**37a**]. Finally, treatment of (*S*)-**37a** with DBU effected spirocyclization, furnishing (*S*)-**10a**, $[\alpha]_D^{24}$ = +216° (c = 0.20, THF). By employing the similar procedure, *dl*-**37b** was synthesized from *dl*-**24**[7] (**Scheme 3**).

Cytotoxicity Assay of Various Structural Types of Novel CPI Derivatives

With the novel CPI derivatives (**8a**,**b**, *dl*-**9b**, and **10a**) in hand, they were subjected to cytotoxicity assay (*in vitro*) against HeLaS3 human uterine cervix carcinoma along with the seco-chlorides (*dl*-**7a**) and (*dl*-**37b**). From the results shown in **Table 1**, cytotoxicity of **8a**,**b**, *dl*-**37b** and **10a** was found to be comparable to that of **1**.[14] It is well known that seco-halides exhibit cytotoxicity after they are first converted to the corresponding CPI derivatives,[15] and that 5-(benzofuran-2-ylcarbonyl)aminoindole-2-carbonyl group shows a similar effect to 5,6,7-trimethoxyindole-2-carbonyl group in the cytotoxicity

Table 1. Cytotoxicity Against HeLaS3 Human Uterine Cervix Carcinoma

	7a	8a	8b	9b	*dl*-37b	10a	1
IC_{50} (ng/ml)[a)]	0.28	0.048	0.052	1.4	0.019	0.035	0.036

a) Drug concentration required to inhibit the growth of HeLaS3 cells by 50%.

assay.[5a,b] Taking into account of these facts, it appeared evident that the novel 3-MCCPI and CPC systems might be useful as promising scaffolds to explore novel anticancer agents. Further investigations on the 3-MCCPI and CPC systems are in progress.

ACKNOWLEDGEMENTS

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REFERENCES AND NOTES

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