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SYNTHESIS AND ANTITUMOR ACTIVITY OF NOVEL CYCLOPROPAPYRROLOINDOLE (CPI) DERIVATIVES BEARING BIS(METHOXYCARBONYL) GROUPS

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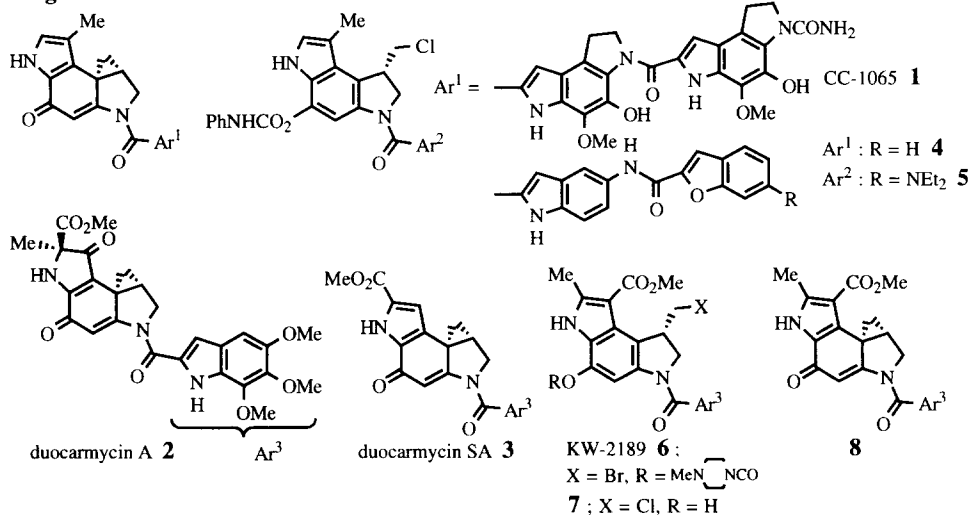
Abstract: The title synthesis was achieved by employing oxidative cyclization of the enaminodiester prepared by Michael addition of the 5-aminoindoline with dimethyl acetylenedicarboxylate, as a key step. Some of these novel bis(methoxycarbonyl)cyclopropapyrroloindole (MC_2CPI) derivatives **9c**, **d** and their seco-chlorides **18c**, **d** were found to exhibit prominent cytotoxicity and antitumor activity against P388 murine leukemia.

CC-1065 (**1**),[1] duocarmycin A (**2**),[2] and duocarmycin SA (**3**)[3] isolated from *Streptomyces sp.* are potent antitumor antibiotics carrying a characteristic cyclopropapyrroloindole(CPI) moiety as the common pharmacophore. The CPI system has been recognized to be responsible for their prominent cytotoxicity through sequence selective alkylation of double strand DNA.[4] Since **1** showed unusual delayed lethality,[5] various types of congeners have been synthesized and evaluated to explore less toxic analogues of **1**, resulting in the development of U-73,975 (adozelesin) (**4**)[6] and U-80,244 (carzelesin) (**5**)[7] as novel antitumor agents showing

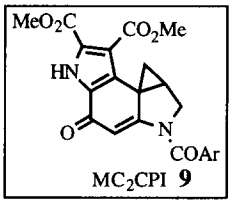
Figure 1



no delayed toxicity. As for **2**, synthetic efforts have been devoted to the preparation of its congeners (for example, **6~8**), culminating in the exploration of KW-2189 (**6**)[8] as a semi-synthetic antitumor agent. These novel antitumor agents (**4~6**) are presently under clinical trials (**Figure 1**).

These CPI derivatives **1~4**, **8** and their seco-chlorides **5~7** bear a methyl group (see, **1**, **4**, and **5**), an oxo group and a quaternary carbon carrying methyl and methoxycarbonyl groups (see, **2**), a methoxycarbonyl group

(see, **3**), or a methyl and a methoxycarbonyl group (see, **6~8**) on the pyrrole ring, respectively. Taking into account these structural characteristics, we designed a novel CPI system, the bis(methoxycarbonyl)CPI (MC_2CPI) system, which carries two methoxycarbonyl groups at the vicinal positions of the pyrrole ring. This novel system can be regarded as the addition of the CPI systems of **3** and **8**. The CPI derivative **8** has been reported as an active form of **6**.[8]

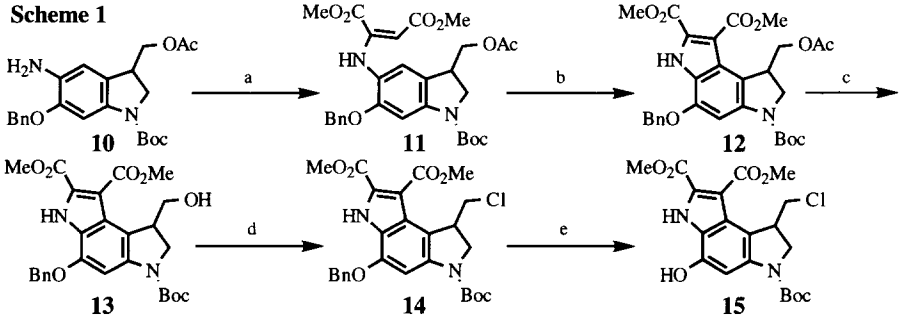


MC_2CPI **9**

In the previous syntheses of **1**,[9] **2**,[10] **4**,[9] and **5**,[11] the 5-aminoindoline **10**[10] had been employed as the common intermediate to construct their CPI and seco-halide systems. With these notable facts in mind, a novel synthetic scheme to the MC_2CPI system was designed which similarly commences with **10**. After some preliminary experiments,[12] we have succeeded in constructing the MC_2CPI system by the oxidative cyclization of enaminodiester **11** derived from **10**.[13] Herein, we wish to report on the synthesis and antitumor activity of the novel MC_2CPI derivatives **9** prepared by employing the oxidative cyclization of **11** as a key step.[14] These synthesized compounds, **9** and its seco-chlorides **18**, were found to exhibit equal or little weaker antitumor activity than *dl*-**7** and *dl*-**8**.[16]

Thus, Michael addition of **10** with dimethyl acetylenedicarboxylate in methanol cleanly provided **11**. Treatment of **11** with $Pd(OAc)_2$ in *N,N*-dimethylacetamide (DMA) effected the oxidative cyclization, affording the novel MC_2CPI system **12**. Some oxidative cyclization reactions using $Pd(OAc)_2$ have been reported to proceed well in AcOH or MeCN.[13] However, in our case, DMA was found to be more promising as a reaction solvent than AcOH, MeCN, and *N,N*-dimethylformamide (DMF). The acetyl group of **12** was removed by methanolysis under basic conditions, giving rise to primary alcohol **13**. Conversion of **13** to chloride **14** followed by the removal of the benzyl group by transfer hydrogenolysis afforded the phenol **15** without competitive reduction of the chloride part (**Scheme 1**).

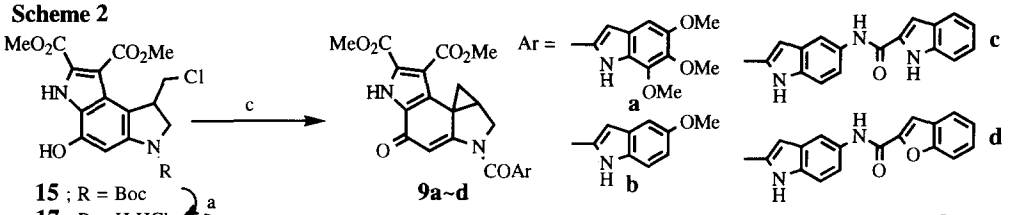
Scheme 1



a) dimethyl acetylenedicarboxylate, MeOH, 0°C~r.t., 1h, 95%. b) $Pd(OAc)_2$ (2eq), DMA, 70°C, 3.5h, 49%. c) K_2CO_3, MeOH, 0°C~r.t., 5h, 95%. d) PPh_3, CCl_4, CH_2Cl_2, r.t., overnight, 91%. e) 10%Pd-C, 25%HCO_2NH_4, THF, 0°C, 1.5h, 99%.

The remaining task to complete the projected synthesis of **9** is the couplings with various indole-2-carboxylic acids (Ar-CO_2H, **16a~d**[17]) and subsequent spirocyclizations to the MC_2CPI system. Towards this end, **15** was deprotected under acidic conditions, affording the indoline **17** as its hydrochloride. This was immediately coupled with **16a~d**[17] in the presence of 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDCI) to give four sorts of the seco-chlorides **18a~d**. Finally, spirocyclizations of **18a~d** were effected by treating with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) to provide **9a~d** in excellent yields (**Scheme 2**).

Scheme 2



a) 3MHCl-AcOEt b) **16a~d**, EDCI, DMF, r.t., 3h, **a** ; 75%, **b** ; 71%, **c** ; 76%, **d** ; 67%.
c) DBU, MeCN, r.t., 2.5h, **a** ; 87%, **b** ; 62%, **c** ; 71%, **d** ; 72%.

With novel MC_2CPI derivatives **9a~d** and their seco-chlorides **18a~d** in hand, they were subjected to cytotoxicity (*in vitro*) and antitumor activity (*in vivo*) assays against P388 murine leukemia. As shown in **Table 1**, it appeared evident that **9a~d** and **18a~d** show strong cytotoxicity (*in vitro*) and antitumor activity (*in vivo*). However, the activities of **9a** and **18a** were found to be little weaker than those of *dl*-**8** and *dl*-**7**[16] carrying the same acyl moiety [**a**] as that for **9a** and **18a**. Interestingly, **9c**, **d** and **18c**, **d** which bear a 5-(indole-2-ylcarbonyl)aminoindole-2-ylcarbonyl or a 5-(benzofuran-2-ylcarbonyl)-aminoindole-2-ylcarbonyl group, exhibited more prominent antitumor activity than **9a**, **b** and **18a**, **b** carrying a 5,6,7-trimethoxyindole-2-ylcarbonyl or a 5-methoxyindole-2-ylcarbonyl group. The activities of **9c**, **d** and **18c**, **d** were comparable to those of *dl*-**8** and *dl*-**7**.[16] It is also worth noting that, although **18a~d** show stronger cytotoxicity than **9a~d**, they require the dose levels higher than those for **9a~d** (ca. 5 times) to observe the antitumor activity which is equal to that for **9a~d**.

Table 1. Cytotoxicity (*in vitro*) and Antitumor Activity (*in vivo*) Against P388 Murine Leukemia

compound	IC_{50}(ng/ml)[a]	ILS(%)[b] (dose, mg/kg)[c]	compound	IC_{50}(ng/ml)[a]	ILS(%)[b] (dose, mg/kg)[c]
9a	0.74	43(0.063)	**18a**	0.31	73(1.0)
9b	0.63	49(0.125)	**18b**	0.27	43(1.0)
9c	0.31	102(0.125)	**18c**	0.24	90(0.5)
9d	0.66	79(0.125)	**18d**	0.32	115(0.625)
dl-**8**	0.34	80(0.125)	*dl*-**7**	0.22	74(0.25)

a) Drug concentration required to inhibit the growth of P388 cells by 50%. b) The percentage increase in life span as compared with the untreated group. c) P388 cells were inoclated i.p. on day 0. Drugs were administered i.p. on day 1.

As described above, we have succeeded in the synthesis of **9a~d** by featuring the oxidative cyclization of **11** derived from **10**. Among the novel MC_2CPI derivatives **9a~d** and their seco-chlorides **18a~d**, **9c**, **d** and **18c**, **d** were found to exhibit promising antitumor activity simiraly to *dl*-**8** and *dl*-**7**. Exploration of the characteristics of their antitumor activity is currently in progress in our laboratories. Synthesis of other novel CPI systems which might show more prominent antitumor activity are also being examined by employing similar oxidative cyclization and will be reported in due course along with preliminary results of their antitumor activity.

Acknowledgment

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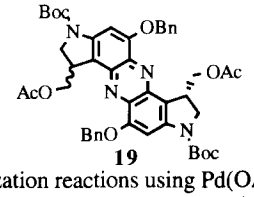
12. At the outset, the preparation of **12** was examined by employing the intramolecular Heck reaction. However, all the attempts to brominate the C_4-position of **10** met with failure. For example, bromination of **10** with *N*-bromosuccinimide gave the dimeric compound **19** as the sole product. As the second method to construct **12**, the intramolecular radical cyclization of bromoenaminodiester **20** was attempted. Although the radical reaction seemed to be promising, it could not be reproduced in a large scale reaction.



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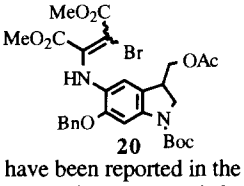

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13. Similar oxidative cyclization reactions using $Pd(OAc)_2$ have been reported in the following references. a) Chen, L.-C.; Yang, S.-C. *Heterocycles*, **1990**, *31*, 911. b) Bittner, S.; Krief, P.; Massil, T. *Synthesis*, **1991**, 215. c) Yogo, M.; Ito, C.; Furukawa, H. *Chem. Pharm. Bull.*, **1991**, *39*, 328.
14. These MC_2CPI derivatives, **9** and **18**, were synthesized in *dl* forms since we observed in our previous studies on duocarmycins that the unnatural enantiomers exhibit weaker cytotoxicity (100 times) than the natural enantiomers.[15]
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16. The known CPI derivatives, *dl*-**7** and *dl*-**8**, were synthesized in our laboratories and used as the standard compounds. The synthesis of *dl*-**7** and *dl*-**8** will be reported separately. It was impossible to use **3** as the standard compound since the synthesis of **3** had not been completed.
17. For the synthesis of **16a~d**, see, ref. 4e and 11 (for **16a**) and ref. 4c (for **16c**, **d**). The acid (**16b**) is commercially available.

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