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

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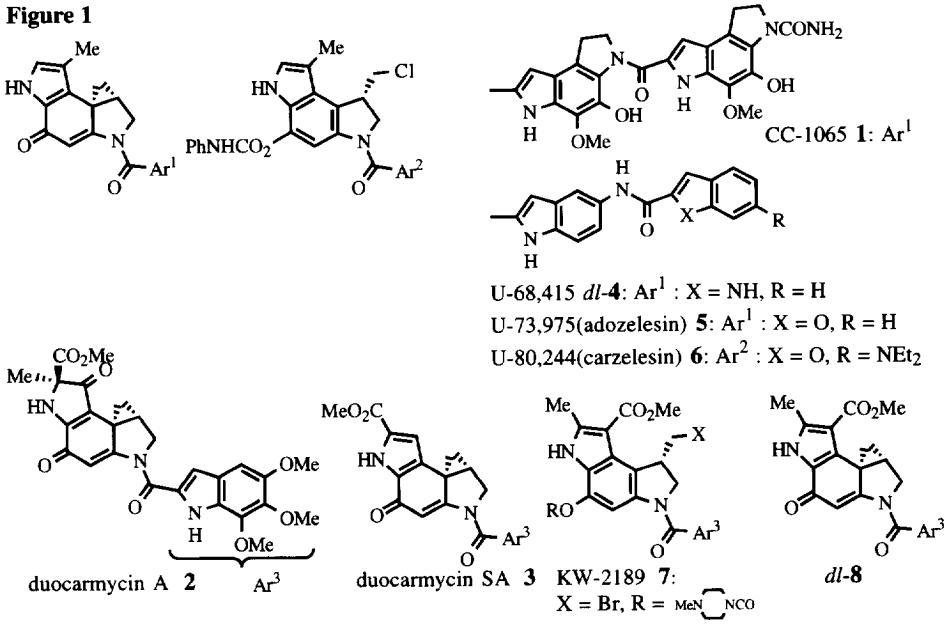
Abstract: The title synthesis was achieved by employing oxidative cyclization of the enaminoester as a key step. Some of these novel 3-methoxycarbonyl-2-trifluoromethylcyclopropapyrroloindole (MCTFCPI) derivatives, *dl*-**10c,d**, (+)-**10d**, and (*S*)-**21b** were found to exhibit antitumor activity against murine leukemia and murine solid tumors more prominent than that of the known CPI derivatives *dl*-**4** and the clinical trial candidates **5** and **7**.

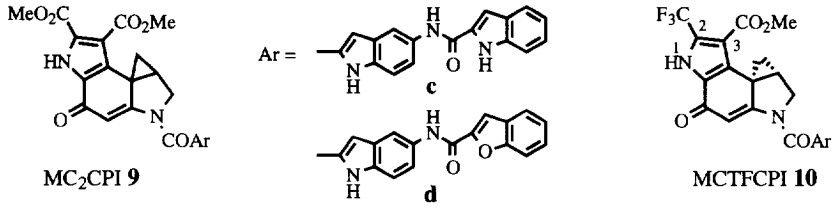
CC-1065 (**1**),[1] duocarmycin A (**2**),[2] and duocarmycin SA (**3**)[3] carrying a cyclopropapyrroloindole(CPI) moiety as the common pharmacophore are potent antitumor antibiotics isolated from *Streptomyces sp.* The CPI system has been recognized to be responsible for their prominent cytotoxicity through sequence selective alkylation of double strand DNA.[4] Since unusual delayed lethality was observed for **1**,[5] various types of congeners have been synthesized and evaluated to explore less toxic analogues of **1**, resulting in the development of U-68,415 (*dl*-**4**),[4c] U-73,975 (adozelesin) (**5**),[6] and U-80,244 (carzelesin) (**6**)[7] as novel antitumor agents showing no delayed toxicity. As for **2**, synthetic efforts have been devoted to the preparation of its congeners,

Figure 1



culminating in the exploration of KW-2189 (**7**)[8] as a semi-synthetic antitumor agent. These novel antitumor agents (**5~7**) are presently under clinical trials (**Figure 1**).

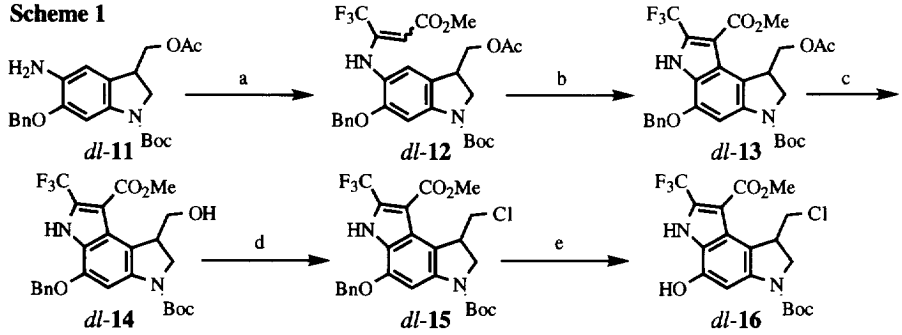
As described in the preceding paper,[9] we have succeeded in design and synthesis of the bis(methoxycarbonyl)CPI (MC_2CPI) system **9** bearing two methoxycarbonyl groups at the vicinal positions of the pyrrole ring. Among these MC_2CPI derivatives, **9c,d** were found to exhibit promising cytotoxicity (*in vitro*) and antitumor activity (*in vivo*). These results let us develop a novel CPI system which can exhibit even more prominent antitumor activity than **9c,d** and the other known CPI derivatives depicted in **Figure 1**. Taking into account structural characteristics of the CPI systems so far reported, we designed a novel CPI system, the 3-methoxycarbonyl-2-trifluoromethylCPI (MCTFCPI) system, which carries methoxycarbonyl and trifluoromethyl



groups at the vicinal positions of the pyrrole ring. It is well known that various fluorinated drugs often show unique pharmacological properties. Therefore, the antitumor activity of the novel MCTFCPI derivatives **10** was expected to be quite promising. Herein, we wish to report on the synthesis and antitumor activity of **10** prepared by employing oxidative cyclization of the enamino ester as a key step in a similar manner to that for **9**.

According to the results disclosed in preceding paper,[9] the synthesis of *dl*-**10** was first attempted. Thus, Michael addition of the 5-aminoindoline *dl*-**11** with methyl 4,4,4-trifluoro-2-butynoate[10] in methanol cleanly provided enamino ester *dl*-**12** as a single product.[11] Oxidative cyclization of *dl*-**12** was effected with $Pd(OAc)_2$

Scheme 1

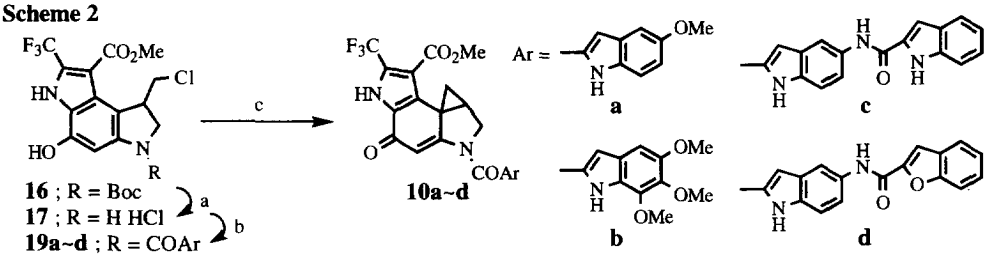


a) methyl 4,4,4-trifluoro-2-butynoate, MeOH, 0°C~r.t., 1h, 97%. b) $Pd(OAc)_2$, DMA, 70°C, 3.5h, 58%. c) K_2CO_3, MeOH, 0°C~r.t., 7h, 99%. d) PPh_3, CCl_4, MeCN, r.t., 5h, 99%. e) 10%Pd-C, 25%HCO_2NH_4, THF, 0°C, 1h, 99%.

in *N,N*-dimethylacetamide (DMA) to afford the novel pyrroloindole system *dl*-**13**. The acetyl group of *dl*-**13** was removed by methanolysis under basic conditions, giving rise to alcohol *dl*-**14**. Conversion of *dl*-**14** to chloride *dl*-**15** followed by removal of the benzyl group by transfer hydrogenolysis provided the phenol *dl*-**16** without competitive reduction of the chloride part (**Scheme 1**).

The remaining task to complete the synthesis of *dl*-**10** is the coupling with various indole-2-carboxylic acids (Ar-CO_2H, **18a~d**[9]) and subsequent spirocyclization to the novel MCTFCPI system. Towards this end, deprotection of *dl*-**16** under acidic conditions gave the indoline *dl*-**17** as its hydrochloride. This was immediately coupled with **18a~d** in the presence of 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDCI) to afford four sorts of the seco-chlorides *dl*-**19a~d**. Finally, treatment of *dl*-**19a~d** with 1,8-diazabicyclo-[5.4.0]undec-7-ene (DBU) effected spirocyclization, affording *dl*-**10a~d** in excellent yields (**Scheme 2**).

Scheme 2



a) 3MHCl-AcOEt. b) **18a~d**, EDCI, DMF, r.t., overnight, **a** ; 55%, **b** ; 82%, **c** ; 46%, **d** ; 56%.
c) DBU, MeCN, r.t., 2.5-4h, **a** ; 82%, **b** ; 89%, **c** ; 96%, **d** ; 85%.

With the novel MCTFCPI derivatives *dl*-**10a~d** in hand, they were subjected to cytotoxicity assay (*in vitro*) against P388 murine leukemia and antitumor activity assays (*in vivo*) against P388 murine leukemia and S180 murine sarcoma. These results are shown in **Table 1**. Interestingly, it was found that *dl*-**10b** whose structure corresponds to the trifluorinated parent CPI derivative *dl*-**8** of KW-2189 (**7**), shows comparable cytotoxicity and

Table 1. Cytotoxicity (*in vitro*) Against P388 Murine Leukemia and Antitumor Activity (*in vivo*) Against P388 Murine Leukemia and S180 Murine Sarcoma

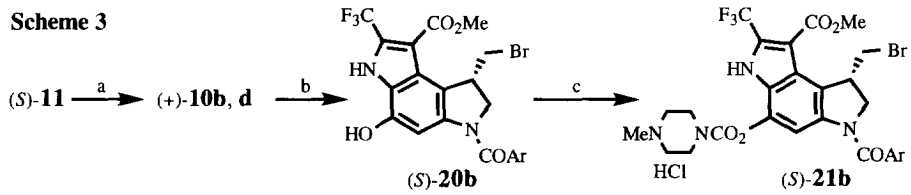
compound	IC_{50}(ng/ml)[a]	P388 ILS(%)[b] (dose, mg/kg)[c]	S180 TGI(%)[e] (dose, mg/kg)[f]	compound	IC_{50}(ng/ml)[a]	P388 ILS(%)[b] (dose, mg/kg)[c]	S180 TGI(%)[e] (dose, mg/kg)[f]
dl-**10a**	0.23	69(0.125)	54(0.25)	*dl*-**9c**	0.31	102(0.125)	44(0.5)
dl-**10b**	0.24	94(0.125)	92(0.5)	*dl*-**9d**	0.66	79(0.125)	26(0.5)
dl-**10c**	0.86	2/2[d](0.25)	84(0.25)	*dl*-**4**	0.028	210(0.125)	86(0.125)
dl-**10d**	0.53	2/2(0.25)	83(0.25)	*dl*-**8**	0.34	80(0.125)	81(0.5)

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. d) Cured mice (>60 days survivors). e) The percentage tumor growth inhibition as compared with the untreated group. f) S180 ($4.5x10^6$~$5x10^6$/mouse) cells were inoculated s.c. into male ICR mice on day 0. Drugs were administered i.v. on day 1

antitumor activity against P388 murine leukemia to those of *dl*-**8**.[11] Antitumor activity of *dl*-**10c,d** against P388 murine leukemia was obviously superior to that of *dl*-**9c,d**. It was also uncovered that *dl*-**10c** carrying the same 5-(indole-2-ylcarbonyl)aminoindole-2-carbonyl group as for *dl*-**4**, exhibits less cytotoxicity (ca.30 times) and more prominent antitumor activity against P388 murine leukemia than *dl*-**4**.[12] While the MC_2CPI derivatives, *dl*-**9c,d** were inactive against S180 murine sarcoma, antitumor activity of *dl*-**10b~d** against S180 murine sarcoma was comparable to that of *dl*-**4** and *dl*-**8**. As described above, it appeared evident that the MCTFCPI derivatives *dl*-**10b~d** exhibit promising antitumor activity against murine leukemia and solid tumor.

Aiming to definitely explore the fact that the MCTFCPI system is superior to the known CPI systems in light of antitumor activity, we next examined the synthesis of the optically active MCTFCPI derivatives (+)-**10d** and (*S*)-**21b** which bear the same acyl moieties as for the clinical trial candidates **5** and **7**,[12] respectively, to compare them with **5** and **7**. Based on the previous studies,[13] it became evident that the CPI derivative having the natural configuration at the cyclopropane ring possesses stronger cytotoxicity than that having the unnatural configuration. Accordingly, the synthesis of (+)-**10d** and (*S*)-**21b** having the natural configurations was attempted. Thus, starting with (*S*)-**11** which had been prepared by optical resolution and subsequent manipulations,[14] the synthesis of (+)-**10b,d** was accomplished following the same reaction sequence as developed for *dl*-**10b,d**. Further treatment of (+)-**10b** with a HBr solution provided the seco-bromide (*S*)-**20b**. The phenolic hydroxyl group was masked with a *N*-methylpiperazinylcarbamoyl group which had been introduced as the prodrug moiety of **7**,[15] giving rise to (*S*)-**21b** (**Scheme 3**).

Scheme 3



a) see **Scheme 1** and **2**. b) 1MHBr in MeCN, r.t., 1.5h. c) i) $ClCO_2PhNO_2$, Et_3N, 0°C, 1h. ii) N-methylpiperazine, 0°C~r.t., overnight, 49% from (+)-**10b**. iii) 3MHCl-AcOEt, 92%.

With completion of the synthesis of the optically active MCTFCPI derivatives (+)-**10d** and (*S*)-**21b**, they were subjected to cytotoxicity assay (*in vitro*) against HeLaS3 human uterine cervix carcinoma and antitumor activity assay (*in vivo*) against Colon 26 murine adenocarcinoma. From the results shown in **Table 2**, it appeared that the cytotoxicity of (+)-**10d** is 10 times weaker than that of **5**, and that the prodrugs (*S*)-**21b** and **7** exhibit comparable weak cytotoxicity. On the other hand, the antitumor activity of (+)-**10d** and (*S*)-**21b** against Colon 26 murine adenocarcinoma was found to be comparable to that of **5** and **7**, respectively. Furthermore, therapeutic indices (MTD/TGI_{50}) of (+)-**10d** and (*S*)-**21b** were obviously superior to those of **5** and **7**. Accordingly, it appeared evident that the MCTFCPI derivatives (+)-**10d** and (*S*)-**21b** definitely show less toxicity than the clinical trial candidates **5** and **7**, respectively (**Table 3**).

Table 2. Cytotoxicity (*in vitro*) Against HeLaS3 Human Uterine Cervix Carcinoma
a) Drug concentration required to inhibit the growth of HeLaS3 cells by 50%.

	(+)-**10d**	**5**	(*S*)-**21b**	**7**
IC_{50}[a] (ng/ml)	0.365	0.0364	18.8	18.1

Table 3. Antitumor Activity (*in vivo*) Against Colon 26 Murine Adenocarcinoma[a]

	Dose (mg/kg)	TGI[b] (%)	TGI_{50} (mg/kg)	MTD[c]/ TGI_{50}	Body weight change (%)[d]
Control	Vehicle	0			2
(+)-**10d**	0.177	85	0.0410	**4.3**	-3
	0.125	87			-4
	0.0625	68			-3
	0.0313	39			0
5	0.0884	79	0.0444	2.0	-9
	0.0625	59			-4
	0.0313	38			-4
	0.0156	33			2
(*S*)-**21b**	0.500	93	0.0429	**11.7**	-8
	0.250	90			-5
	0.125	76			-1
	0.0625	58			-2
7	0.707	90	0.209	3.4	-9
	0.500	85			-1
	0.250	62			-2
	0.125	26			1

a) Colon 26 (10^6/mouse) cells were inoculated s.c. into male CDF1 mice on day 0. Drugs were administered i.v. on day 7. b) The percentage tumor growth inhibition as compared with the untreated group. c) Maximum dose within 10% body weight loss. d) Day 7 to day 12.

As described above, we have succeeded in synthesizing of the novel *dl*- and optically active MCTFCPI derivatives bearing methoxycarbony and trifluoromethyl groups on the pyrrole ring by featuring oxidative cyclization of the enamino ester derived from *dl*- and (*S*)-**11**. It should be noted that some of the MCTFCPI derivatives *dl*-**10c**,**d**, (+)-**10c**, and (*S*)-**21b** exhibit less cytotoxicity and more excellent antitumor activity against murine leukemia and solid tumors than the known CPI derivatives *dl*-**4** and the clinical trial candidates **5** and **7**. It is of interest to confirm a significant influence of the trifluoromethyl group of MCTFCPI derivatives on cytotoxicity and antitumor activity. Investigations along this line and research for other MCTFCPI derivatives which bear novel acyl moieties and might exhibit more prominent antitumor activity, are in progress and will be reported shortly.

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