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Review Article

Cyclopropapyrroloindole (CPI) bisalkylators

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Introduction

CC-1065, isolated from *Streptomyces zelensis* by researchers at Upjohn in 1978, is one of the most potent antitumor antibiotics (1, 2). This antibiotic was disclosed to be identical to rachelmycin, isolated from *Streptomyces* C-329 by investigators at Bristol-Myers in 1982 (3). Duocarmycin A and its structurally related congeners, duocarmycin B and C, were isolated from *Streptomyces* DO-88 and DO-89, respectively, by researchers at Kyowa Hakko in 1988 (4-8). Also in 1988, scientists at Meiji Seika reported the isolation of two new antitumor antibiotics, pyrindamycin A and B, from the culture broth of *Streptomyces* SF2582 (9, 10). These antibiotics were shown to be identical to duocarmycin C2 and C1, respectively (5). The newest natural product of this class is duocarmycin SA (SA means stable A), isolated from *Streptomyces* DO-113 by Kyowa Hakko in 1990 (11, 12). These antitumor antibiotics carry characteristic cyclopropapyrroloindole (CPI) moieties as the common pharmacophore. All of the CPI compounds thus far isolated have been revealed to be the minor groove binders of double-stranded B-DNA. Moreover, their CPI moieties have been recognized to be responsible for prominent cytotoxicity through their sequence-selective alkylations of adenine N-3 in the AT-rich region of double-stranded B-DNA (Fig. 1) (13-29).

Since CC-1065 exhibited unusual delayed lethality (30), the Upjohn group synthesized and evaluated various types of congeners in their search for less toxic analogs of the compound. Their research resulted in the development of U-73975 (adozelesin) (31-33) and the prodrug U-80244 (carzelesin) (34-38) as novel antitumor agents showing no delayed toxicity. Regarding duocarmycin A, synthetic efforts at Kyowa Hakko were devoted to the preparation of its congeners, culminating in the discovery of KW-2189 (39-47) as a semisynthetic prodrug. These two novel antitumor agents, carzelesin and KW-2189, are presently under clinical trials (Fig. 2). The synthesis, cytotoxicity and/or chemical stability of other CPI analogs structurally related to those described above have been reported (29, 48-52).

Recently, we reported the synthesis and antitumor activity of novel CPI derivatives which show prominent cytotoxicity and *in vivo* antitumor activity against P388 murine leukemia, S180 murine sarcoma and Colon 26 murine adenocarcinoma (53, 54). On the basis of these studies, the novel prodrug, 3-methoxycarbonyl-2-trifluoromethyl CPI (MCTFCPI; AT-3510) was discovered. This novel prodrug was found to exhibit better antitumor activity against human tumor xenografts than carzelesin, KW-2189 and the widely used anticancer agent, cisplatin (Fig. 3) (55, 56).

It is well known that some clinically useful anticancer agents such as cisplatin, mitomycin C, nitrosoureas and nitrogen mustards can form intra- or interstrand cross-links on DNA. The majority of these DNA cross-linkers covalently modify DNA by binding with its major groove, and are often guanine-selective (57). Among these known interstrand cross-linkers, cisplatin (which also forms an intrastrand cross-link on DNA), mitomycin C and nitrogen mustards cover a two base-pair region on DNA, while chloroethylnitrosourea only overlays one base-pair (57). The interstrand cross-linking ability of mitomycin C and nitrogen mustards is suggested to be responsible for their strong antitumor activity (58); however, the cross-linking efficacy of all the interstrand cross-linkers known to date is very low *in vitro* (57).

Recently, with the aim of developing novel anticancer agents and solving problems for recognition of DNA

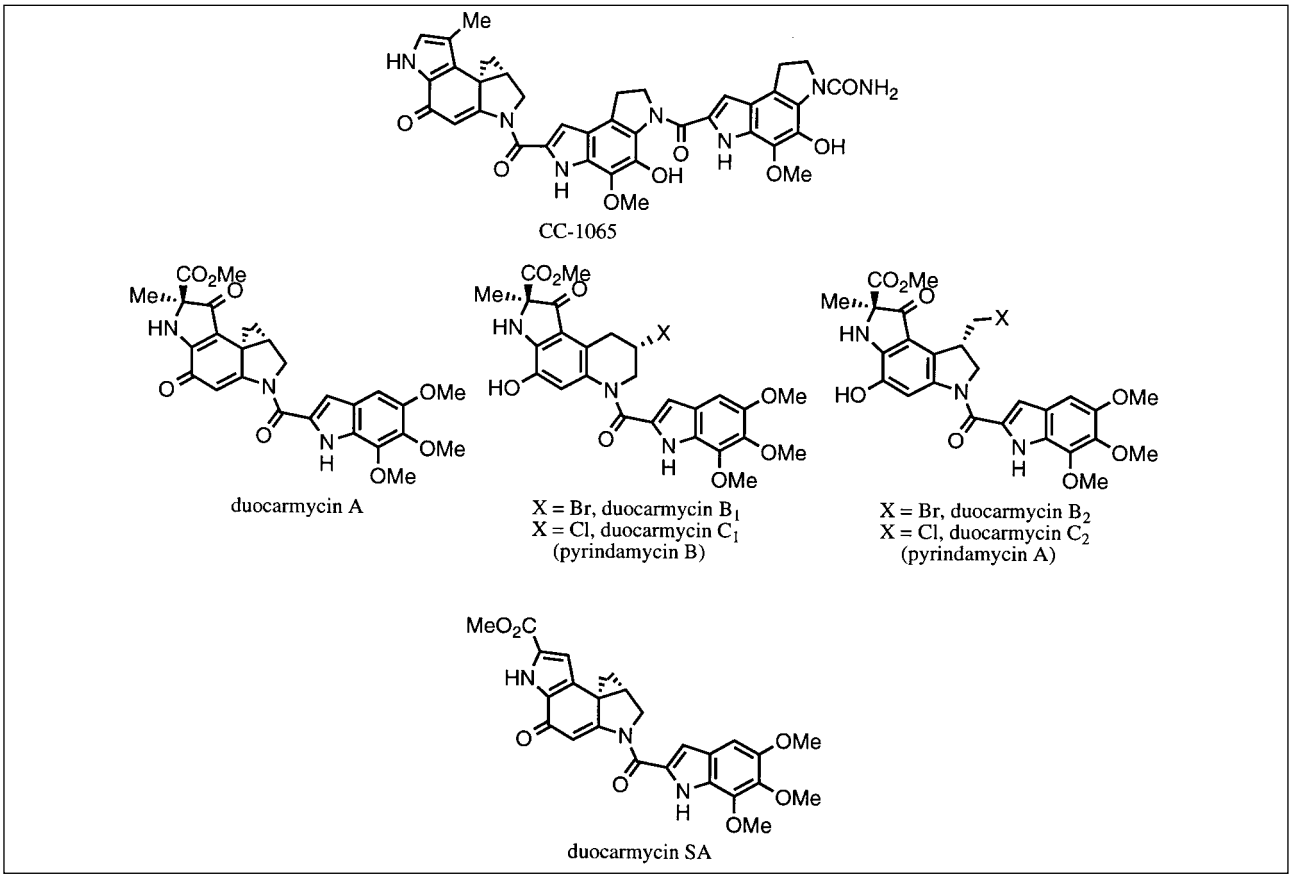


Fig. 1. Structures of natural CPI antitumor antibiotics.

sequences by interstrand cross-linkers, the CPI bisalkylators were synthesized (59-61). These compounds exhibited more potent cytotoxicity and *in vivo* antitumor activity than CC-1065 and adozelesin. They have been revealed to be interstrand cross-linkers based on their minor groove binding and sequence-selective alkylation of adenine N-3 in the AT-rich region of double-strand B-DNA. It is anticipated that the superior antitumor activity of the CPI bisalkylators to that of other interstrand cross-linkers thus far reported is due to their interstrand cross-linking

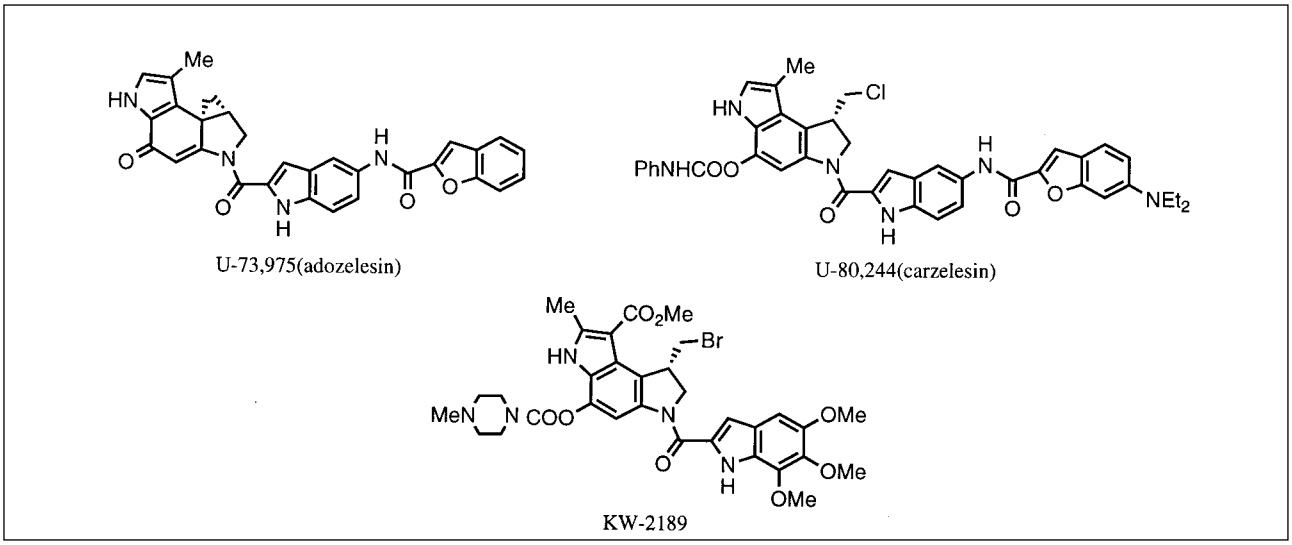


Fig. 2. Structures of CPI congeners.

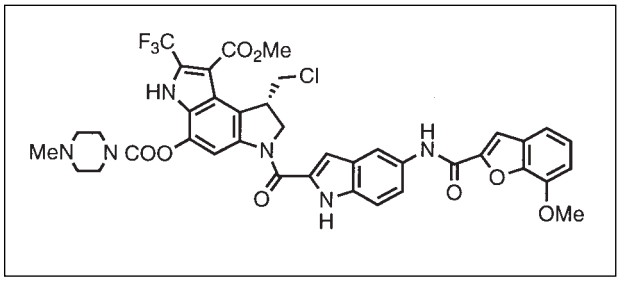


Fig. 3. Structure of AT-3510.

property. The following article summarizes the cytotoxicity and *in vivo* antitumor activity of the CPI bisalkylators, including those recently studied by our group.

Bisalkylators bearing flexible linkers

CPI bisalkylators were first reported by Upjohn in 1989 (59). The CPI bisalkylators **1** contain two CPI moieties of CC-1065 connected with a flexible methylene chain of variable length (Fig. 4). Cytotoxicity and interstrand cross-linking ability of these CPI bisalkylators were found to depend highly upon the methylene chain length (Table I). Among them, cytotoxicity and *in vivo* antitumor activity of **1b** against L1210 murine leukemia cells (IC_{50} = 0.0020 ng/ml, ILS% (μg/kg) = 100 (0.8)) were superior to those of CC-1065 (IC_{50} = 0.030 ng/ml, ILS% (μg/kg) = 68 (100)). It should be pointed out that cytotoxicity and interstrand cross-linking ability of the CPI bisalkylators **2** (IC_{50} = 0.20 ng/ml) and *ent*-**1g** (IC_{50} = 5.0 ng/ml) bearing the enantiomeric configurations at one and both end(s), respectively, are obviously weaker than those for **1g** (IC_{50} = 0.0050 ng/ml). The CPI bisalkylators **1** were shown to be interstrand cross-linkers by using alkaline agarose gel electrophoresis.

Table I: Cytotoxycity and relative cross-linking capacity of the bisalkylators bearing flexible linkers.

Compound	n	IC_{50} (ng/ml)[a]	Cross-linking score
1a	2	4.0	–
1b	3	0.002	+++
1c	4	0.02	++
1d	5	0.006	+++
1e	6	0.04	+
1f	7	0.2	–
1g	8	0.005	+
1h	9	9	–
1i	10	0.05	–
1j	11	2	–
1k	14	3	–
2	8	0.2	–
ent-**1g**	8	5	–
CC-1065	–	0.03	–

[a]Data from ref. 59.

Bisalkylators bearing rigid linkers (U-77779; bizelesin)

Based on the studies of CPI bisalkylators bearing flexible linkers, the cytotoxicity and *in vivo* antitumor activity of the seco-Cl CPI bisalkylators bearing rigid linkers against L1210 murine leukemia cells were reported (Fig. 5) (60, 61). All of these compounds exhibited strong cytotoxicity against L1210 murine leukemia cells (IC_{50} = 0.012-0.0010 ng/ml) (Table II). Among them, U-77779 (bizelesin) (Fig. 6), bearing a 1,3-bis(2-carbonyl-1*H*-indol-5-yl)urea group as the rigid linker, was shown to be the most potent (IC_{50} = 0.0010 ng/ml) (Fig. 5) (61-64). Bizelesin showed more potent *in vivo* antitumor activity against L1210 murine leukemia cells (cured 50% of the mice at 10 μg/kg) than adozelesin (ILS% (μg/kg) = 94 (100)) and other seco-Cl CPI bisalkylators (60). Bizelesin is presently reported to be in phase I/II clinical trials (65, 66). It has been shown that interstrand cross-linking by bizelesin occurs on adenine bases across 6 or 7 base-pairs apart in the AT-rich region of DNA (67-69). To

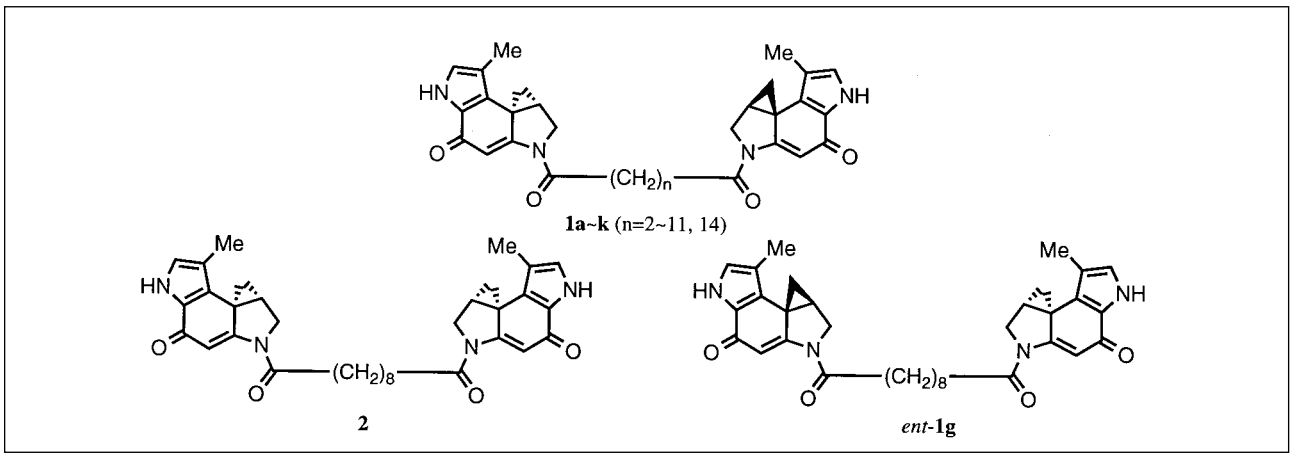


Fig. 4. Structures of CPI bisalkylators bearing flexible linkers.

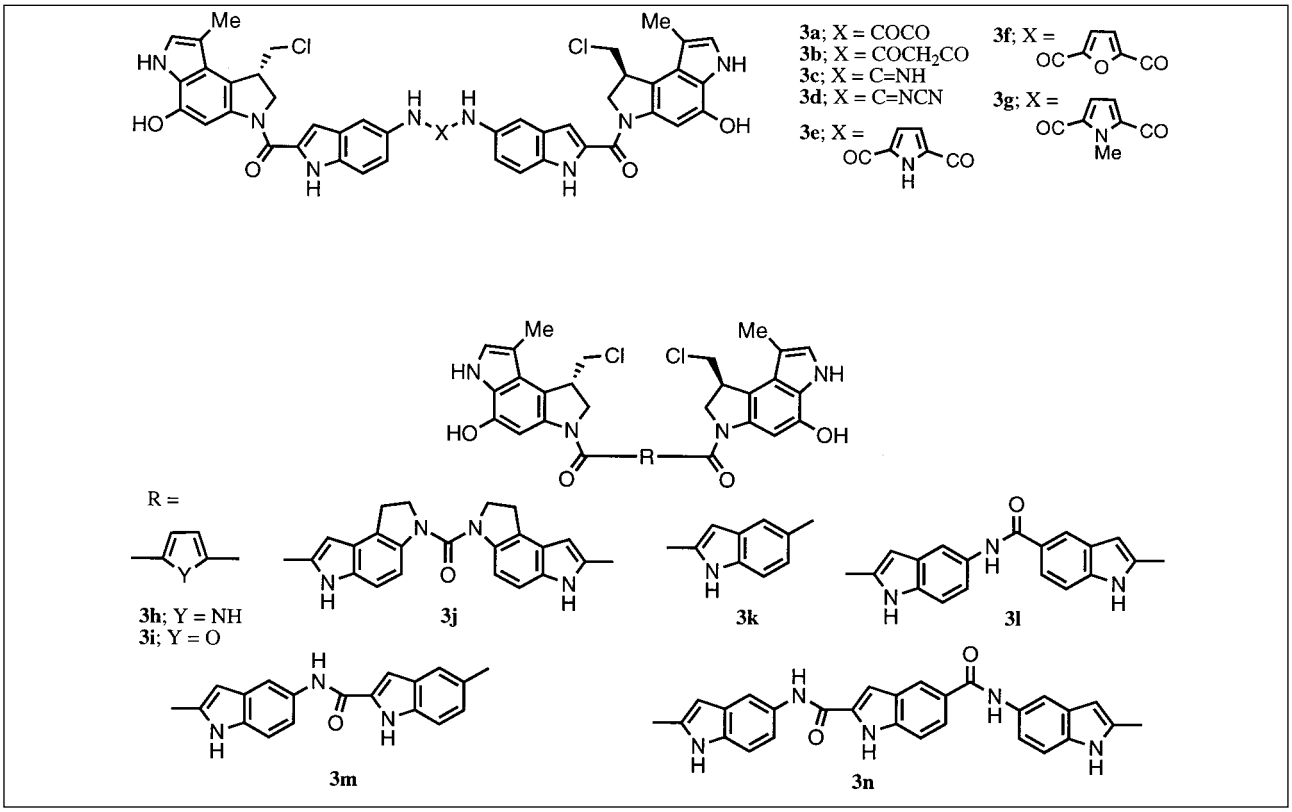


Fig. 5. Structures of CPI bisalkylators bearing rigid linkers.

Table II: Cytotoxicity and in vivo *antitumor activity of the bisalkylators bearing rigid linkers against L1210 murine leukemia cells.*

Compound	IC_{50} (ng/ml)[a]	ILS% (μg/kg)[a]
Bizelesin	0.001	50% cures (10)
3a	0.009	6 (6)
3b	0.006	94 (50)
3c	–	–
3d	–	–
3e	0.004	56 (1)
3f	0.012	78 (1 cure) (5)
3g	–	–
3h	0.004	56 (50)
3i	0.01	56 (600)
3j	0.002	143 (1/6 cure) (12)
3k	–	213 (1 cure) (6)
3l	–	181 (2)
3m	–	188 (13)
3n	–	188 (13)
Adozelesin	0.004	113 (100)

[a]Data from ref. 61.

elucidate the importance of the ureadiyl group involved in the linker of bizelesin in the cross-linking reaction, the cross-linking profiles were also studied on the structurally related guanidino and cyanoguanidino bisalkylators **3c** and **3d** (70). The cross-linking profile of **3d** was almost the same as that for bizelesin, while that of **3c** was quite different from that of bizelesin. The rate-determining steps of cross-linking reaction for bizelesin and **3c** were monoalkylation (first step) and bisalkylation (second step), respectively. As for **3e-3g**, their sequence selectivity was determined and their relative DNA reactivity was compared with that of bizelesin (71). The sequence selectivity of **3e** was different from that of bizelesin. The relative DNA reactivity of **3f** was weaker than that of bizelesin and **3e** and **3g**. The sequence selectivity and relative DNA reactivity of **3g** was similar to those of bizelesin. Two-dimensional NMR analysis was carried out for the bizelesin cross-linked 10-mer duplex adduct to determine mechanism of cross-linking reaction, conformation of adduct and hydrogen bonding of ureadiyl group to DNA (65).

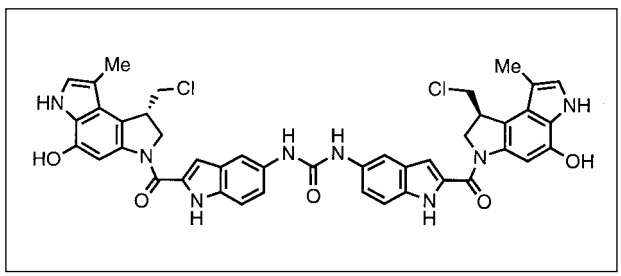


Fig. 6. Structure of U-77779 (bizelesin).

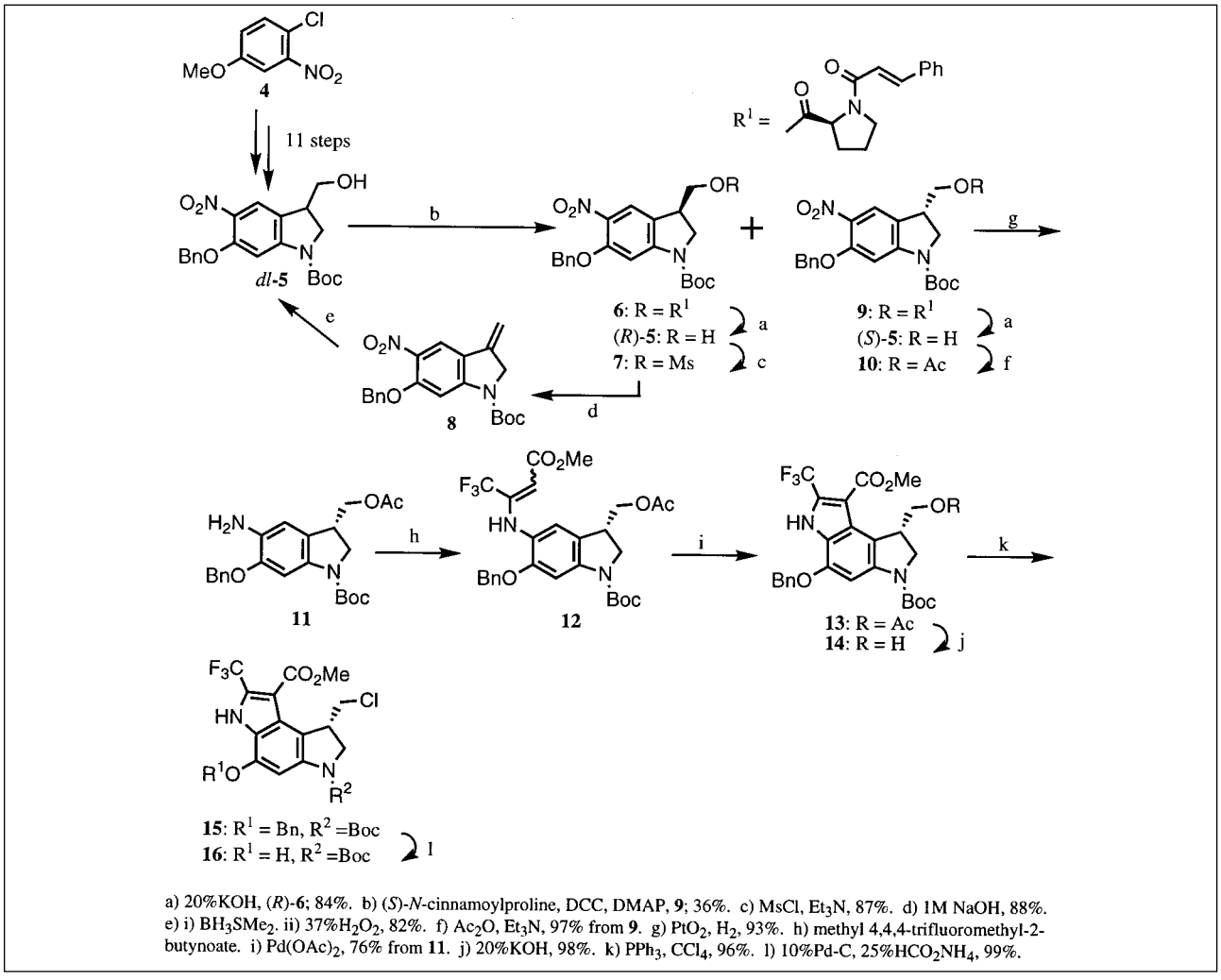


Fig. 7. Synthesis of the optically active MCTFCPI moiety.

MCTFCPI bisalkylators bearing rigid bis(indole)dicarbonyl linkers

As described in the introduction, we found that AT-3510 had excellent antitumor activity compared to that of carzelesin and KW-2189. In addition, it appeared that the *in vivo* antitumor activity of bizelesin bearing a 1,3-bis(2-carbonyl-1*H*-indol-5-yl)urea group as the rigid linker was more potent than that of adozelesin and other seco-CI CPI bisalkylators. Therefore, in the search for novel CPI bisalkylators having even better antitumor activity than bizelesin, we designed and synthesized the seco-CI CPI bisalkylators **19a-l**, bearing various lengths and types of rigid bis(indole)dicarbonyl linkers between the two seco-CI MCTFCPI moieties (Fig. 8).

Chemistry

According to earlier studies (54, 55), the optically active N-protected derivative **16** was prepared by the sequence involving optical resolution of *dl*-**5** (Fig. 7) (72). The nitroindoline derivative *dl*-**5** was produced from the commercially available nitrobenzene **4** in 11 steps (31, 73-76). Esterification of *dl*-**5** with (*S*)-N-cinnamoylproline gave a diastereomeric mixture of esters **6** and **9**. The less polar ester was separated by recrystallization, affording the pure diastereomer **9** in 36% yield. Alkaline hydrolysis of **9** furnished optically pure (*S*)-**5**. Subsequent acetylation of (*S*)-**5** followed by catalytic reduction of acetate **10** yielded the 5-aminoindoline **11**. Absolute configuration of optically pure **5** derived from **9** was determined to belong to (*S*)-series based on the successful total synthesis of CC-1065 and duocarmycin SA from **11** (77, 78). The undesired diastereomer **6** was recycled to *dl*-**5** usable for optical resolution by way of (*R*)-**5**, mesylate **7** and the achiral 3-methyleneindoline **8**. The explored synthetic route to optically pure (*S*)-**5** can be easily carried out in a kilogram scale. Michael addition of **11** with methyl 4,4,4-trifluoro-2-butynoate cleanly provided enamino ester **12** as a single product. Oxidative cyclization of **12**

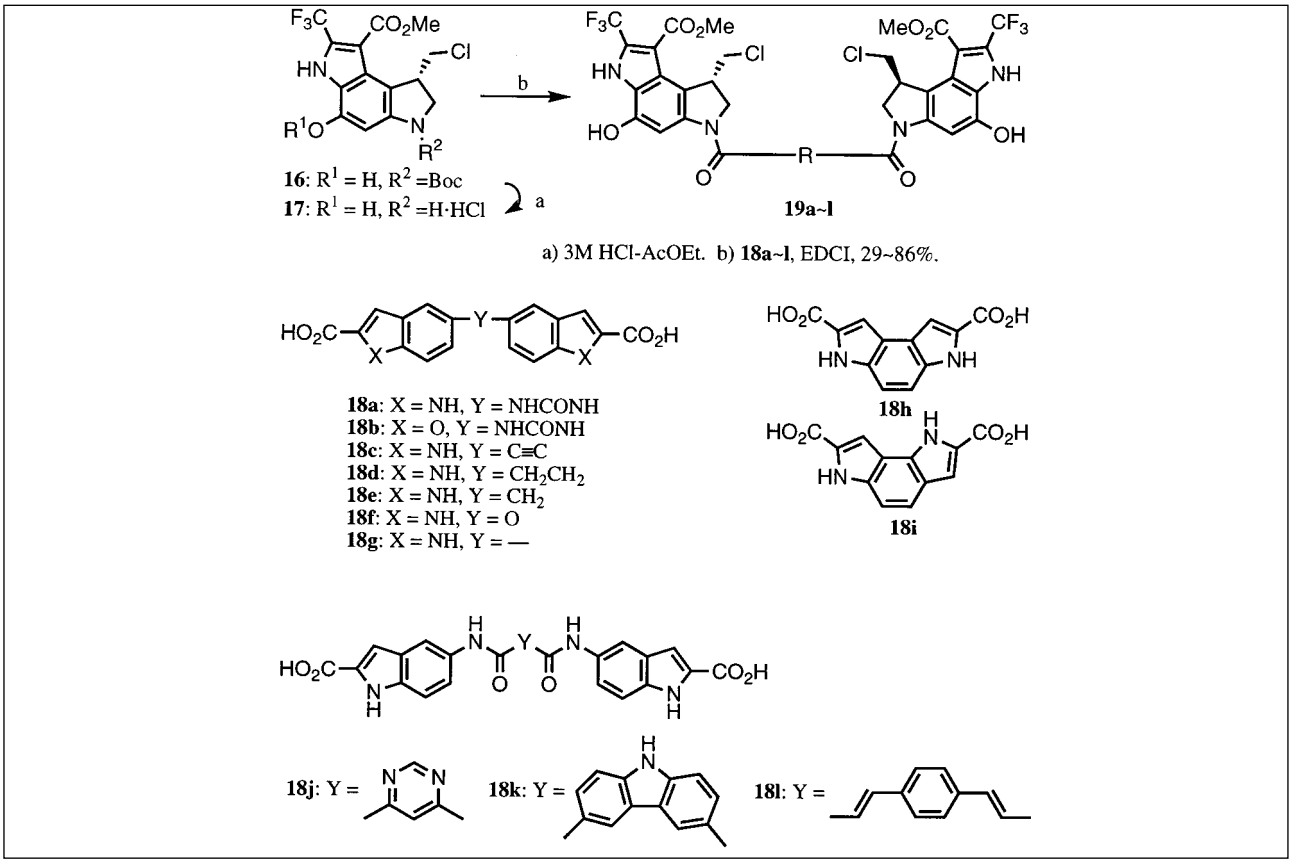


Fig. 8. Synthesis of MCTFCPI bisalkylators bearing rigid bis(indole)dicarbonyl linkers.

was effected with $Pd(OAc)_2$ to afford the pyrroloindole derivative **13**. This was converted to the optically pure N-protected derivative **16** by way of alcohol **14** and chloride **15** by sequential transesterification, chlorination and removal of the benzyl group by transfer hydrogenolysis. The synthesis of compounds **19a-l** was completed by coupling two equivalents of the optically pure derivative **17** prepared from **16** by removal of the N-Boc group with one equivalent of various dicarboxylic acids **18a-l** (Fig. 8) (79).

Cytotoxicity

Compounds **19a-l** were subjected to a cytotoxicity assay against HeLaS3 human uterine cervix carcinoma cells. As shown in Table III, the cytotoxicity of **19a** (IC_{50} = 0.082 ng/ml), having the same linker bearing a ureadiyl group as bizelesin, was comparable to that of bizelesin (IC_{50} =0.060 ng/ml) (80). Bisbenzofuran analog **19b** (IC_{50} = 0.31 ng/ml) exhibited less cytotoxicity than **19a** and bizelesin. Analogs **19c** (IC_{50} = 0.35 ng/ml) and **19d** (IC_{50} = 0.21 ng/ml), which have an ethyne and an ethylene group between the two indole rings, also showed less cytotoxicity than **19a** and bizelesin. Much less cytotoxicity was observed for **19e** (IC_{50} = 33 ng/ml) and **19f** (IC_{50} = 22 ng/ml), which bear a methylene and an ether group between the two indole rings. Cytotoxicity of **19g** (IC_{50} = 0.0049 ng/ml), in which the two seco-CI MCTFCPI groups

Table III: Cytotoxicity against HeLaS3 human uterine cervix carcinoma cells and in vivo *antitumor activity of the MCTFCPI bisalkylators bearing rigid (indole)dicarbonyl linkers against Colon 26 murine adenocarcinoma cells.*

Compound	IC_{50} (ng/ml)[a]	TGI% (μg/kg)[b]	MTD[c]/TGI_{50}
19a	0.082	93 (7.81)	9.3
19b	0.031	92 (15.6)	9.1
19c	0.35	82 (31.3)	2.5
19d	0.21	87 (31.3)	7.3
19e	33	80 (4000)	3.3
19f	22	88 (4000)	8.8
19g	0.0049	84 (9.77)	3.3
19h	11	90 (2000)	12.2
19i	30	93 (4000)	6.5
19j	3.1	86 (62.5)	6.0
19k	32	90 (4000)	37.2
19l	1.6	84 (125)	3.6
Bizelesin	0.060	90 (15.6)	8.4

[a]Drug concentration required to inhibit the growth of HeLaS3 human uterine cervix carcinoma cells by 50%. [b]The percentage tumor growth inhibition as compared with the untreated group. [c]Maximum dose within 10% body weight loss.

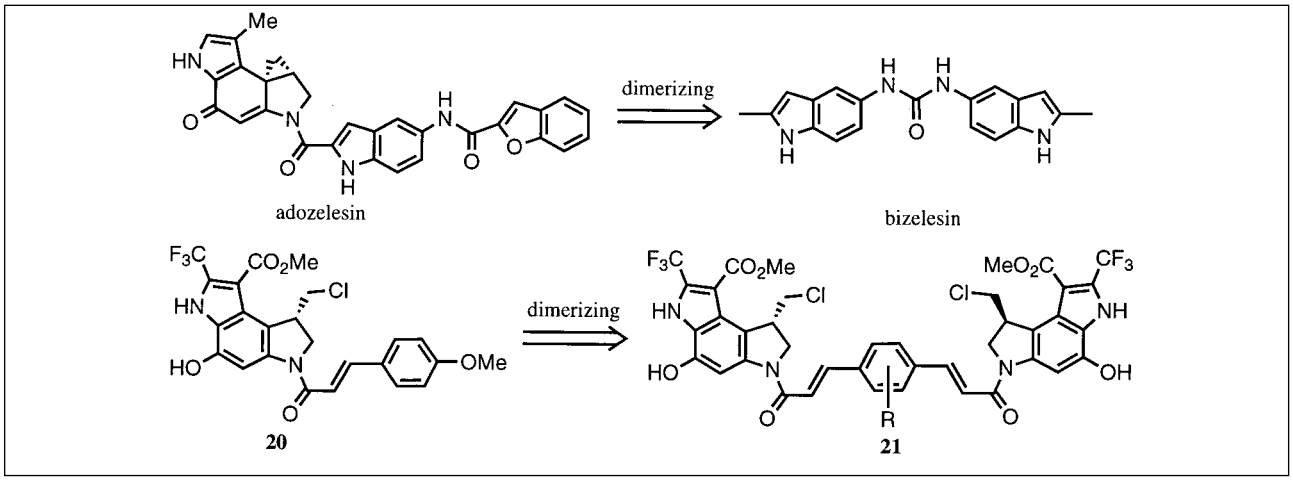


Fig. 9. Design concept for novel MCTFCPI bisalkylators bearing rigid 3,3'-aryldiacryloyl linkers.

are connected with a 5,5'-bis(2-carbonyl-1*H*-indole) group having a carbon-carbon single bond between the two indole rings, was obviously superior to that of bizelesin. In **19h** (IC_{50} = 11 ng/ml) and **19i** (IC_{50} = 30 ng/ml), attempts at shortening the linker of **19g** by superimposing its two benzene rings were unsuccessful for retaining potent cytotoxicity. On the other hand, **19j-l** (IC_{50} = 3.1, 32, 1.6 ng/ml), carrying longer linkers than bizelesin, were less cytotoxic than bizelesin, albeit their structures are anticipated to be similar to those of **3e-3g**.

In vivo *antitumor activity*

The *in vivo* antitumor activity of compounds **19a-l** against Colon 26 murine adenocarcinoma cells is summarized in Table III with therapeutic indices (T.I.= MTD/TGI_{50}). All of the compounds showed excellent *in vivo* antitumor activity (max.TGI% >80) within a dose range of 7.81-4000 μg/kg. *In vivo* antitumor activity and therapeutic indices of **19a** (max.TGI% = 93 (7.81 μg/kg), T.I. = 9.3) and **19b** (max.TGI% = 92 (15.6 μg/kg), T.I. = 9.1) were comparable to those of bizelesin (max.TGI% =90 (15.6 μg/kg), T.I.= 8.4). Although **19g** had the most potent cytotoxicity among **19a-l** and bizelesin, its therapeutic index (T.I. = 3.3) was narrower than that for bizelesin (T.I. = 8.4). Interestingly, **19k** (T.I. = 37.2) had the broadest therapeutic index.

MCTFCPI bisalkylators bearing rigid 3,3'-aryldiacryloyl linkers

Obviously, the design concept for bizelesin is simple dimerization of the structure of adozelesin or carzelesin. Therefore, the structure of MCTFCPI derivative **20**, bearing a *p*-methoxycinnamoyl group, was also dimerized as our next design for a novel linker, leading to the seco-Cl MCTFCPI bisalkylators **21**, bearing a 3,3'-aryldiacryloyl group as the linker (Fig. 9). These compounds were synthesized according to procedures similar to those shown in Figure 8 (Fig. 10) (81).

Cytotoxicity

Results of cytotoxicity testing of **21a-q** against HeLaS3 human uterine cervix carcinoma cells are shown in Table IV. The newly designed bisalkylator AT-760 (**21a**), bearing a 3,3'-(1,4-phenylene)diacryloyl group, exhibited more potent cytotoxicity than bizelesin and **19g**. The strong cytotoxicity observed for AT-760 disappeared for its positional isomers **21b** and **21c**. The cytotoxicity of **21d**, **21f** and **21g**, in which one or more phenylene group(s) are inserted into the linker of AT-760, was reduced dramatically. The bipyridyl analog **21e** had superior cytotoxicity as compared to the corresponding biphenyl derivative **21d**. The cytotoxicity of **21h-j**, in which the C-2 and C-3 positions of 1,4-phenylene group are substituted by dimethoxy, methylenedioxy or ethylenedioxy group(s), was comparable to that of AT-760. Interestingly, **21k**, carrying dimethoxy groups at the C-2 and C-5 positions of 1,4-phenylene group, was 3500 times less cytotoxic than AT-760. Different from the case of **21h**, the additional methoxy group at the C-5 position of the phenylene ring might have weakened cytotoxicity due to its steric hindrance to the interaction in a minor groove of duplex DNA. Although the naphthalene analogs **21m** and **21n** were 3- to 4-fold less cytotoxic than AT-760, the anthracene analogs **21o-q** were about 1000 times less cytotoxic than AT-760. These results indicate that the cytotoxicity of the bisalkylators depends not only upon the length but also the width of the linker.

In vivo *antitumor activity*

The *in vivo* antitumor activity of **21a-g** against Colon 26 murine adenocarcinoma cells is summarized in

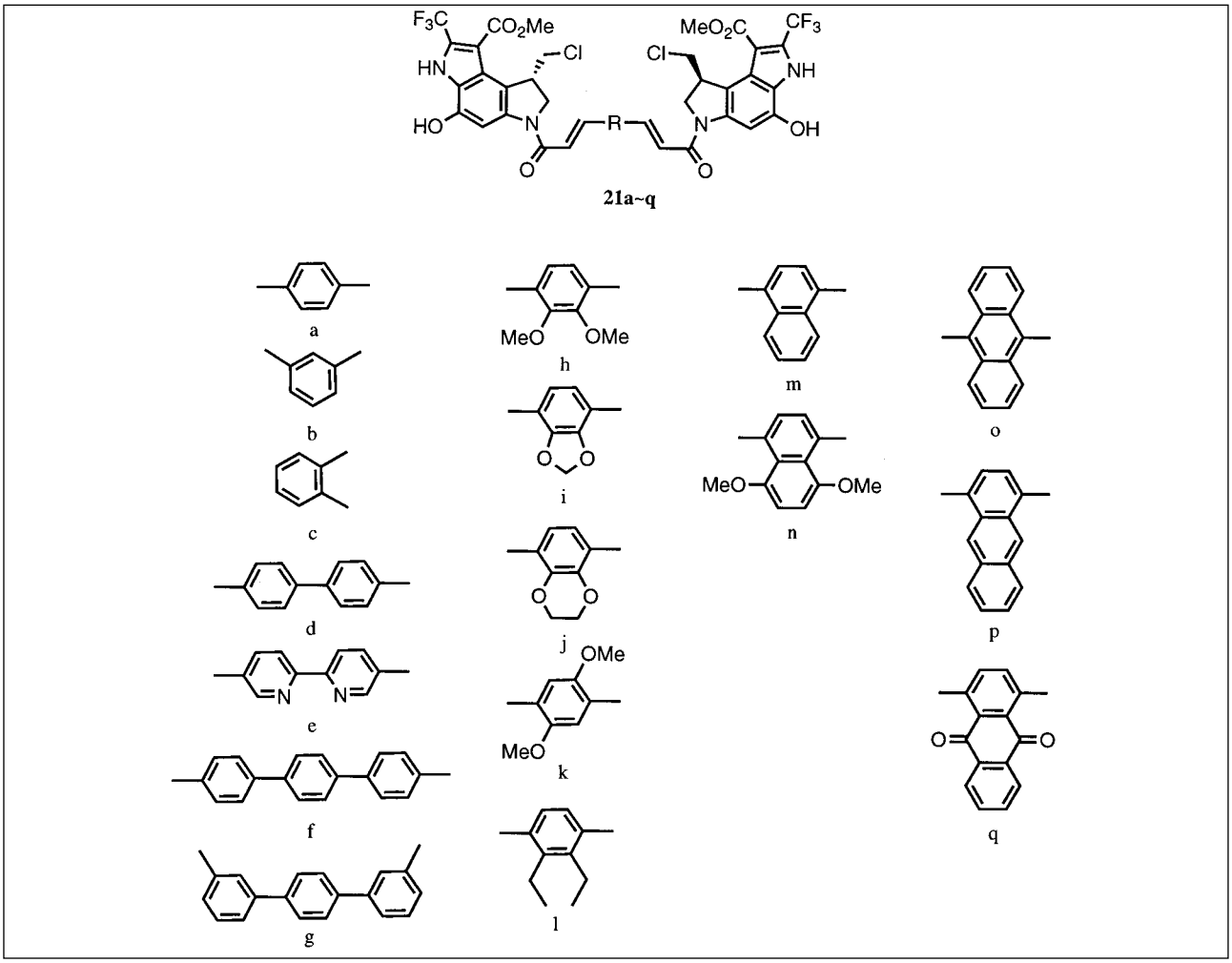


Fig. 10. Structures of MCTFCPI bisalkylators bearing rigid 3,3'-aryldiacryloyl linkers.

Table IV with therapeutic indices (T.I. = MTD/TGI_{50}). Among them, AT-760 (max.TGI% = 89 (1.95 μg/kg), T.I. = 30.7) and **21i** (max.TGI% = 90 (1.95 μg/kg), T.I. = 13.3) were found to have the most potent antitumor activity with a broader therapeutic index than bizelesin.

CPI bisalkylators bearing a 3,3'-(1,4-phenylene)diacryloyl linker

As described above, we found that AT-760 (**21a**) had better cytotoxicity and antitumor activity, with a broad therapeutic index, than bizelesin. Therefore, we then focused our efforts on the synthesis of **24a-e**, in which the two CPI moieties are connected with a 3,3'-(1,4-phenylene)diacryloyl group as the linker. According to the same synthetic procedures as for AT-760, **24a-e** were synthesized by coupling two equivalents of the optically pure seco-Cl CPI derivatives (*S*)-**23a-e** with one equivalent of 3,3'-(1,4-phenylene)diacrylic acid (Fig. 11) (82). Various structural types of (*S*)-**23a-e** were derived from the

Table IV: Cytotoxicity against HeLaS3 human uterine cervix carcinoma cells and in vivo *antitumor activity of the MCTFCPI bisalkylators bearing 3,3'-aryldiacryloyl linkers against Colon 26 murine adenocarcinoma cells.*

Compound	IC_{50} (ng/ml)[a]	TGI% (μg/kg)[b]	MTD[c]/TGI_{50}
21a (AT-760)	0.00274	89 (1-95)	30.7
21b	54.6	91 (4000)	nt
21c	87.6	70 (4000)	nt
21d	0.141	83 (15.6)	3.7
21e	0.0198	88 (3.91)	3.6
21f	48.3	84 (4000)	nt
21g	>100	nt	nt
21h	0.00158	89 (0.977)	4.6
21i	0.00595	90 (1.95)	13.3
21j	0.00276	91 (0.488)	9.0
21k	9.71	81 (2000)	>6.5
21l	0.00318	nt	nt
21m	0.011	85 (0.977)	7.4
21n	0.00934	nt	nt
21o	1.51	75 (4000)	nt
21p	3.25	82 (15.6)	3.5
21q	0.523	84 (15.6)	6.6
Bizelesin	0.060	90 (15.6)	8.4

[a,b,c]See legend of Table III; nt _ not tested.

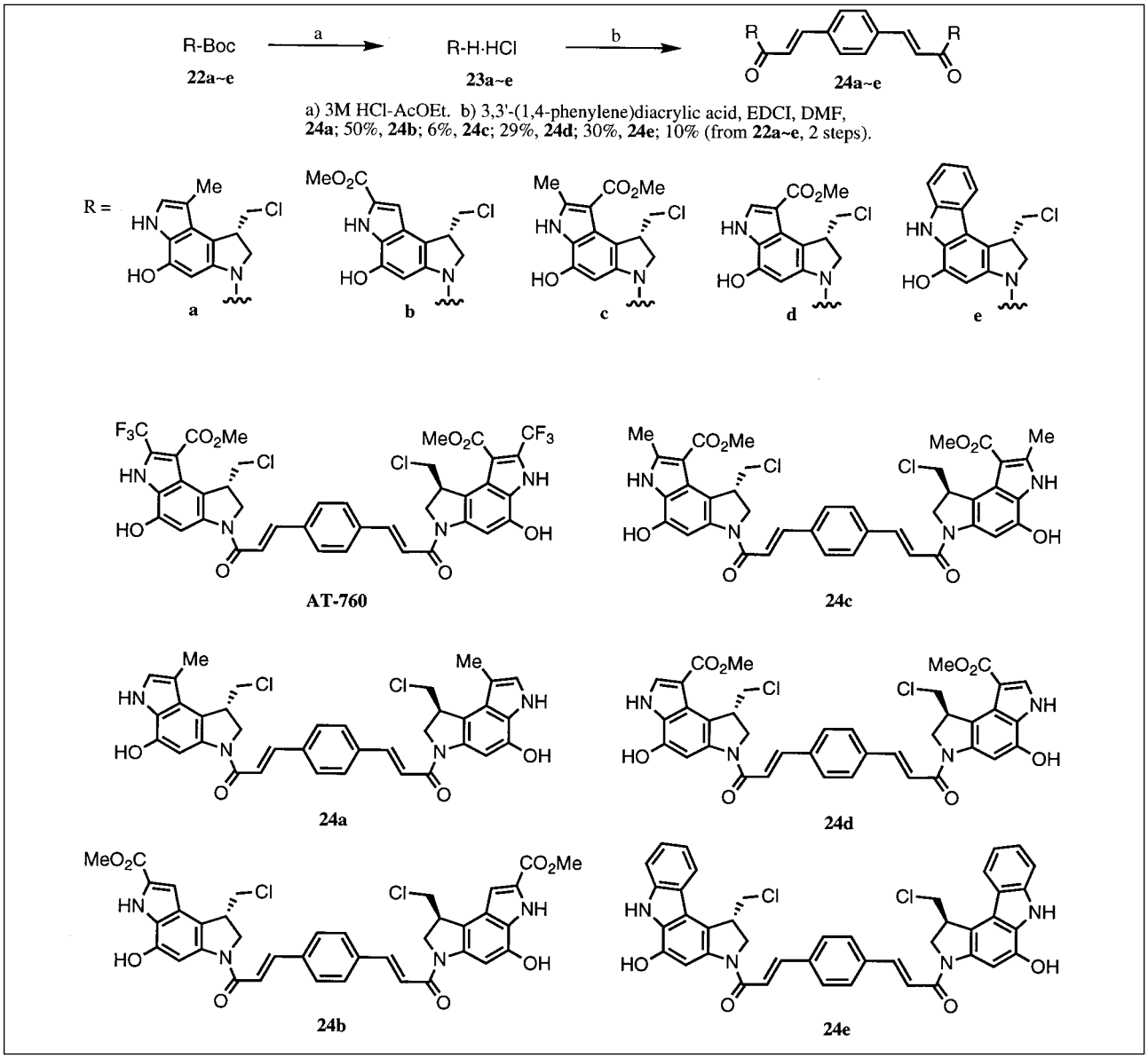


Fig. 11. Synthesis and structures of CPI bisalkylators bearing a 3,3'-(1,4-phenylene)diacryloyl group as the linker.

optically pure N-protected indolines (*S*)-**22a-e** by deprotection (83).

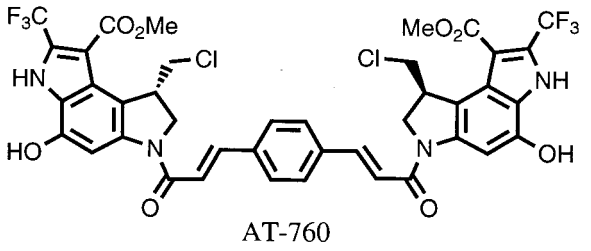
Cytotoxicity

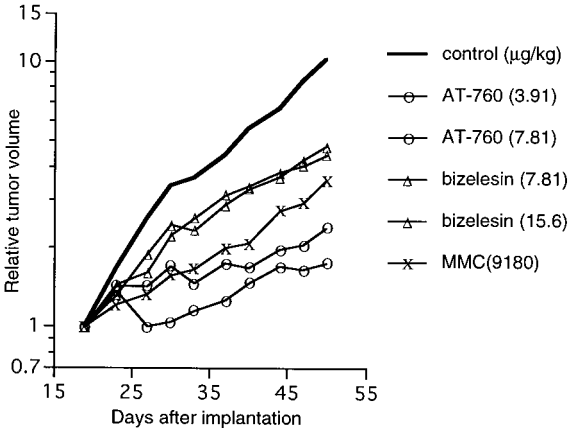
Results of cytotoxicity assays of **24a-e** against HeLaS3 human uterine cervix carcinoma cells are shown in Table V. The cytotoxicity of **24a** (IC_{50} = 0.0013 ng/ml) and **24b** (IC_{50} = 0.023 ng/ml), bearing the seco-Cl CPI moieties of CC-1065 and duocarmycin SA, was superior to that of bizelesin. Among **24a-e**, **24c** (IC_{50} = 0.00046 ng/ml) and **24d** (IC_{50} = 0.00035 ng/ml), bearing the seco-Cl CPI moiety of KW-2189 and that regioisomeric to the seco-Cl CPI moiety of duocarmycin SA, respectively, exhibited the most potent cytotoxicity (84).

Table V: Cytotoxicity against HeLaS3 human uterine cervix carcinoma cells and in vivo *antitumor activity of the CPI bisalkylators bearing 3,3'-aryldiacryloyl group as the linker against Colon 26 murine adenocarcinoma cells.*

Compound	IC_{50} (ng/ml)[a]	TGI% (μg/kg)[b]	MTD[c]/TGI_{50}
21a (AT-760)	0.0027	89 (1.95)	30.7
24a	0.0013	92 (15.6	5.5
24b	0.023	82 (31.3)	–
24c	0.00046	87 (31.3)	8.8
24d	0.00035	80 (4000)	10.3
24e	0.072	88 (4000)	8.2
Bizelesin	0.060	90 (15.6)	8.4

[a,b,c]See legend of Table III.





Compound	Dose (mg/kg)[a]	TGI (%)[b]
AT-760	3.91	77
	7.81	83
Bizelesin	7.81	53
	15.6	56
MMC	9180	65

TGI: Tumor growth inhibition. [a]Tumor fragments of human colon CX-1 were implanted s.c. on day 0. Drugs were administered i.v. on day 19. [b]The percentage tumor growth inhibition as compared with the untreated group.

Fig. 12. Antitumor activity of bisalkylators in athymic mice implanted with human colon CX-1 tumor xenografts.

The cytotoxicity observed for **24e** (IC_{50} = 0.072 ng/ml), bearing the cyclopropapyrrolocarbazole (CPC) moiety, was comparable to that of bizelesin.

In vivo *antitumor activity*

The *in vivo* antitumor activity of **24a-e** against Colon 26 murine adenocarcinoma cells is summarized in Table V with therapeutic indices (T.I. = MTD/TGI_{50}). Among AT-760 and **24a-e,** AT-760 showed the most potent *in vivo* antitumor activity with the broadest therapeutic index. Interestingly, **24b**, carrying the seco-Cl CPI moiety of duocarmycin SA, had weak *in vivo* antitumor activity. Since AT-760 appeared to be the most promising compound among the various bisalkylators thus far synthesized, its *in vivo* antitumor activity against human colon CX-1 tumor xenografts was compared with that of bizelesin. As shown in Figure 12, AT-760 had antitumor activity superior to that of bizelesin and mitomycin C. Further investigations on the pharmacological profile of AT-760 are in progress.

Miscellaneous bisalkylators

The cytotoxicity and DNA alkylation properties of 9a-chloromethyl-1,2,9,9a-tetrahydrocyclopropa[c]benz[e] indol-4-one (C2BI) bisalkylators **25a-d** were reported by Boger *et al.* (Fig. 13) (85, 86). Compounds **25a**-d showed poor cytotoxicity and DNA alkylation compared to the CBI and CPI derivatives **26a-d** and **27a-d**, and CC-1065 (Table VI). This was considered to be the consequence of a significant steric deceleration of the alkylation reaction attributable to the additional 9a-chloromethyl substituent.

Conclusions

Isolation of CC-1065 from *Streptomyces zelensis* opened a new area in the development of novel anticancer agents. CC-1065, which showed prominent antitumor activity attributed to its minor groove binding capability and sequence-selective adenine N-3 alkylation ability, had been expected to be a novel anticancer agent. The CPI moiety of CC-1065, which undergoes sequence-selective adenine N-3 alkylation in the AT-rich region of double-stranded B-DNA, has been recognized as the pharmacophore responsible for its cytotoxicity. Since CC-1065 exhibited unusual delayed lethality, synthetic efforts led to the development of the clinical trial candidate carzelesin (U-80244), which exhibits no delayed lethality. Isolation of duocarmycin A from *Streptomyces* DO-88 led to the discovery of the clinical trial candidate KW-2189. Our studies on the CPI derivatives, commencing with the total synthesis of duocarmycin A, culminated in the discovery of AT-3510, bearing the novel MCTFCPI moiety. AT-3510 was found to exhibit more potent antitumor activity than carzelesin and KW-2189.

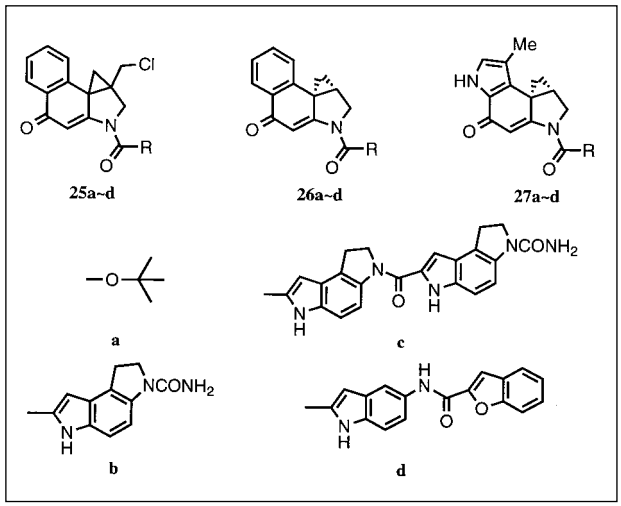


Fig. 13. Structures of C2BI bisalkylators and CBI and CPI derivatives.

Table VI: Cytotoxicity of the C2BI bisalkylators and the CBI and CPI derivatives against L1210 murine leukemia cells.

Compound	IC_{50} (ng/ml)[a]	Compound	IC_{50} (ng/ml)[a]	Compound	IC_{50} (ng/ml)[a]
25a	12000	**26a**	80	**27a**	300
25b	6300	**26b**	0.005	**27b**	0.04
25c	6	**26c**	0.005	**27c**	0.02
25d	900	**26d**	0.01	**27d**	0.04
CC-1065	0.02				

[a]Data from ref. 86.

The CPI bisalkylators, second-generation derivatives bearing two CPI moieties in the same molecule and showing more potent antitumor activity than the monoalkylators, were the first example of bisalkylators undergoing interstrand cross-linkings on adenines in the AT-rich region of DNA. Among the CPI bisalkylators, bizelesin was selected as the most promising compound and subjected to clinical testing. Various investigations on the CPI bisalkylators, including bizelesin, led to the understanding of DNA recognition by a small molecular ligand, and indicated the possibility of developing a potent antitumor agent by dimerizing CPI monoalkylators. Research on the novel CPI derivatives and bisalkylators led to the development of the MCTFCPI bisalkylator AT-760, bearing a 3,3'-(1,4-phenylene)diacryloyl group as the linker. Results of structure-activity studies with linkers disclosed that the cytotoxicity of the bisalkylators depends not only on the length but also the width of the linker. It appears that the MCTFCPI moiety introduced into AT-760 is the most potent pharmacophore against Colon 26 murine adenocarcinoma cells among those for the bisalkylators carrying a 3,3'(1,4-phenylene)diacryloyl group as the linker. Moreover, the antitumor activity of AT-760 against Colon 26 murine adenocarcinoma cells and human colon CX-1 tumor xenografts is superior to that of bizelesin, possibly because of the cross-linking property of AT-760 carrying a shorter molecular size than bizelesin.

Considering the results from our studies, continuing research in this area should reveal further subtle relationships between molecular structures, interstrand cross-linking properties and antitumor activities of the CPI bisalkylators. Therefore, the area of CPI bisalkylators still offers an intriguing challenge to both medicinal chemists and pharmacologists for discovering novel antitumor agents.

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References

1. Hanka, L.J., Dietz, A., Gerpheide, S.A., Kuentzel, S.L., Martin, D.G. *CC-1065 (NSC-298223), a new antitumor antibiotic. Production, in vitro biological activity, microbiological assays, and taxonomy of the producing organism.* J Antibiot 1978, 31: 1211-7.

2. Martin, D.G., Biles, C., Gerpheide, S.A. et al. *CC-1065 (NSC 298223), a potent new antitumor agent. Improved production and isolation, characterization, and antitumor activity.* J Antibiot 1981, 34: 1119-25.

3. Nettleton, D.E., Bush, J.A., Bradner, W.T. *Fermentation process for making rachelmycin.* US 4301248.

4. Ichimura, M., Muroi, K., Asano, K. et al. *DC89-A1, a new antitumor antibiotic from Streptomyces.* J Antibiot 1988, 41: 1285-8.

5. Takahashi, I., Takahashi, K., Ichimura, M. et al. *Duocarmycin A, a new antitumor antibiotic from Streptomyces.* J Antibiot 1988, 41: 1915-7.

6. Ogawa, T., Ichimura, M., Katsumata, S., Morimoto, M., Takahashi, K. *New antitumor antibiotics, duocarmycin B1 and B2.* J Antibiot 1989, 42: 1299-301.

7. Yasuzawa, T., Iida, T., Muroi, K., Ichimura, M., Takahashi, K., Sano, H. *Structures of duocarmycins, novel antitumor antibiotics produced by Streptomyces sp.* Chem Pharm Bull 1988, 36: 3728-31.

8. Yasuzawa, T., Muroi, K., Ichimura, M. et al. *Duocarmycins, potent antitumor antibiotics produced by Streptomyces sp. Structures and chemistry.* Chem Pharm Bull 1995, 43: 378-91.

9. Ohba, K., Watabe, H., Sasaki, T. et al. *Pyrindamycin A and B, new antitumor antibiotics.* J Antibiot 1989, 41: 1515-9.

10. Ishii, S., Nagasawa, M., Kariya, Y., Yamamoto, H., Inoue, S. *Antitumor activity of pyrindamycin A and B.* J Antibiot 1989, 42: 1713-7.

11. Ichimura, M., Ogawa, T., Takahashi, K. et al. *Duocarmycin SA, a new antitumor antibiotic from Streptomyces sp.* J Antibiot 1990, 43: 1037-8.

12. Yasuzawa, T., Saitoh, Y., Ichimura, M., Takahashi, I., Sano, H. *Structure of duocarmycin SA, a potent antitumor antibiotic.* J Antibiot 1991, 44: 445-7.

13. Li, L.H., Swenson, S.L., Schpok, S.L.F., Kuentzel, S.L., Dayton, B.D., Krueger, W.C. *CC-1065 (NSC 298223), a novel agent that interacts strongly with double-stranded DNA.* Cancer Res 1982, 42: 999-1004.

14. Swenson, S.L., Li, L.H., Hurley, L.H. et al. *Mechanism of interaction of CC-1065 (NSC 298223) with DNA.* Cancer Res 1982, 42: 2821-8.

15. Hurley, L.H., Reynolds, V.L., Swenson, D.H., Petzold, G.L., Scahill, T.A. *Reaction of the antitumor antibiotic CC-1065 with DNA: Adduct with DNA sequence specificity.* Science 1984, 226: 843-6.

16. Reynolds, V.L., Molineux, C.J., Caplan, D.J., Swenson, D.H., Hurley, L.H. *DNA sequence selectivity of the antibiotic CC-1065.* Biochemistry 1985, 24: 6228-37.

17. Needham-VanDevanter, D.R., Hurley, L.H., Reynolds, V.L., Theriault, N.Y., Krueger, W.C., Wierenga, W. *Characterization of*

an adduct between CC-1065 and a defined oligonucleotide duplex. Nucleic Acids Res 1984, 12: 6159-64.

18. Hurley, L.H., Lee, C.-S., McGovren, J.P. et al. *Molecular basis for sequence-specific DNA alkylation by CC-1065.* Biochemistry 1988, 27: 3886-92.

19. Boger, D.L., Coleman, R.S., Invergo, B.J. et al. *Synthesis and evaluation of aborted and extended CC-1065 functional analogues: (+)- and (–)-CPI-CDPI3. Preparation of key partial structures and definition of an additional functional role of the CC-1065 central and right-hand subunits.* J Am Chem Soc 1990, 112: 4623-32.

20. Hurley, L.H., Warpehoski, M.A., Lee, C.-S. et al. *Sequence specificity of DNA alkylation by the unnatural enantiomer of CC-1065 and its synthetic analogues.* J Am Chem Soc 1990, 112: 4633-49.

21. Boger, D.L., Ishizaki, T., Zarrinmayeh, H., Kitos, P.A., Suntomwat, O. *Synthesis and preliminary evaluation of agents incorporating the pharmacophore of the duocarmycin/pyrindamycin alkylation subunit: Identification of the CC-1065/duocarmycin common pharmacophore.* J Org Chem 1990, 55: 4499-502.

22. Sugiyama, H., Hosoda, M., Saito, I., Asai, A., Saito, H. *Covalent alkylation of DNA with duocarmycin A. Identification of a basic site structure.* Tetrahedron Lett 1990, 31: 7197.

23. Chin, H.L., Dinshaw, J.P. *Site-specific covalent duocarmycin A intramolecular DNA triplex complex.* J Am Chem Soc 1992, 114: 10658.

24. Boger, D.L., Yun, W., Terashima, S. et al. *DNA alkylation properties of the duocarmycins: (+)-Duocarmycin A, epi-(+)-duocarmycin A, ent-(–)-duocarmycin A and epi,ent-(-)-duocarmycin A.* Bioorg Med Chem Lett 1992, 2: 759-65.

25. Sugiyama, H., Ohmori, K., Chan, K.L. et al. *A novel guanine N3 alkylation by antitumor antibiotic duocarmycin A.* Tetrahedron Lett 1993, 34: 2179-82.

26. Yamamoto, K., Sugiyama, H., Kawanishi, S. *Concerted DNA recognition and novel site-specific alkylation by duocarmycin A with distamycin A.* Biochemistry 1993, 32: 1059-66.

27. Boger, D.L., Johnson, D.S., Yun, W. *(+)- and ent-(–)-Duocarmycin SA and (+)- and ent-(–)-N-Boc-DSA DNA alkylation properties. Alkylation site models that accommodate the offset AT-rich adenine N3 alkylation selectivity of the enantiomeric agents.* J Am Chem Soc 1994, 116: 1635-56.

28. Boger, D.L., Munk, S.A., Zarrinmayeh, H., Ishizaki, T., Haught, J., Bina, M. *An alternative and convenient strategy for generation of substantial quantities of singly 5'-^{32}P-end-labeled double-stranded DNA for binding studies: Development of a protocol for examination of functional features of (+)-CC-1065 and the duocarmycins that contribute to their sequence-selective DNA alkylation properties.* Tetrahedron 1991, 47: 2661-82.

29. Boger, D.L., Johnson, D.S. *CC-1065 and the duocarmycins: Understanding their biological function through mechanistic studies.* Angew Chem Int Ed Engl 1996, 35: 1438-74.

30. McGovren, J.P., Clarke, G.L., Pratt, E.A., DeKoning, T.F. *Preliminary toxicity studies with the DNA-binding antibiotic, CC-1065.* J Antibiot 1984, 37: 63-70.

31. Warpehoski, M.A., Gebhard, I., Kelly, R.C. et al. *Stereoelectronic factors influencing the biological activity and DNA interaction of synthetic antitumor agents modeled on CC-1065.* J Med Chem 1988, 31: 590-603.

32. Weiland, K.L., Dooley, T.P. *In vitro and in vivo DNA bonding by the CC-1065 analogue U-73975.* Biochemistry 1991, 30: 7559-65.

33. Bhuyan, B.K., Smith, K.S., Adams, E.G., Petzold, G.L., McGovren, J.P. *Lethality, DNA alkylation, and cell cycle effects of adozelesin (U-73975) on rodent and human cells.* Cancer Res 1992, 52: 5687-92.

34. Wierenga, W. *Sequence-selective DNA-interactive antitumor agents.* Drugs Fut 1991, 16: 741-50.

35. Li, L.H., DeKoning, T.F., Kelly, R.C. et al. *Cytotoxicity and antitumor activity of carzelesin, a prodrug cyclopropapyrroloindole analogue.* Cancer Res 1992, 52: 4904-13.

36. De Vries, J.D., De Graaff-Teulen, J.A., Henrar, R.E.C., Vanden Bosch, J., Bult, A., Beijnen, J.H. *Pharmaceutical development of a parenteral formulation of the novel antitumor agent carzelesin (U-80244).* Invest New Drugs 1994, 12: 303-14.

37. Houghton, P.J., Cheshire, P.J., Hallman, J.D. II, Houghton, J.A. *Therapeutic efficacy of the cyclopropapyrroloindole, carzelesin, against xenografts derived from adult and childhood solid tumors.* Cancer Chemother Pharmacol 1995, 36: 45-52.

38. Wolff, I., Bench, K., Beijnen, J.H. et al. *Phase I clinical and pharmacokinetic study of carzelesin (U-80244) given daily for five consecutive days.* Clin Cancer Res 1996, 2: 1717-23.

39. Asai, A., Nagamura, S., Saito, H. *A novel property of duocarmycin and its analogues for covalent reaction with DNA.* J Am Chem Soc 1994, 116: 4171-7.

40. Kobayashi, E., Okamoto, A., Asada, M. et al. *Characteristics of antitumor activity of KW-2189, a novel water-soluble derivative of duocarmycin, against murine and human tumors.* Cancer Res 1994, 54: 2404-10.

41. Ogasawara, H., Nishio, K., Takeda, Y. et al. N. *A novel antitumor antibiotic, KW-2189 is activated by carboxyl esterase and induces DNA strand breaks in human small cell lung cancer cells.* Jpn J Cancer Res 1994, 85: 418-25.

42. Nagamura, S., Kanda, Y., Kobayashi, E., Gomi, K., Saito, H. *Synthesis and antitumor activity of duocarmycin derivatives.* Chem Pharm Bull 1995, 43: 1530-5.

43. Nagamura, S., Asai, A., Kanda, Y., Kobayashi, E., Gomi, K., Saito, H. *Synthesis and antitumor activity of duocarmycin derivatives: Modification of segment A of duocarmycin B2.* Chem Pharm Bull 1996, 44: 1723-30.

44. Nagamura, S., Kanda, Y., Asai, A., Kobayashi, E., Gomi, K., Saito, H. *Wagner-Meerwein rearrangement of duocarmycins.* Chem Pharm Bull 1996, 44: 933-9.

45. Nagamura, S., Kobayashi, E., Gomi, K., Saito, H. *Synthesis and antitumor activity of duocarmycin derivatives: A-ring pyrrole analogues of duocarmycin B2.* Bioorg Med Chem 1996, 4: 1379-91.

46. Nagamura, S., Kobayashi, E., Gomi, K., Saito, H. *Studies on the active metabolite (DU-86) of KW-2189, a novel derivative of duocarmycin.* Bioorg Med Chem Lett 1996, 6: 2147-50.

47. Nagamura, S., Asai, A., Amishiro, N., Kobayashi, E., Gomi, K., Saito, H. *Synthesis and antitumor activity of duocarmycin derivatives: A-ring pyrrole compounds bearing cinnamoyl groups.* J Med Chem 1997, 40: 972-9.

48. Boger, D.L., Boyce, C.W., Garbaccio, R.M., Goldberg, J.A. *CC-1065 and the duocarmycins: Synthetic studies.* Chem Rev 1997, 97: 787-828.

49. Tercel, M., Denny, W.A., Wilson, W.R. *Nitrogen and sulfur analogs of the seco-CI alkylating agent. Synthesis and cytotoxicity.* Bioorg Med Chem Lett 1996, 6: 2735-40.

50. Atwell, G.J., Wilson, W.R., Denny, W.A. *Synthesis and cytotoxicity of amino analogs of the potent DNA alkylating agent seco-CBI-TMI.* Bioorg Med Chem Lett 1997, 7: 1493-6.

51. Patel, V.F., Andis, S.L., Enkema, J.K. et al. *Total synthesis of seco (+)- and ent-(–)-oxaduocarmycin SA: Construction of the (chloromethyl)indoline alkylating subunit by a novel intramolecular aryl radical cyclization onto a vinyl chloride.* J Org Chem 1997, 62: 8868-74.

52. Lukhtanov, E.A., Kutyavin, I.V., Gorn, V.V. et al. *Sequence and structure dependence of the hybridization-triggered reaction of oligonucleotides bearing conjugated cyclopropapyrroloindole.* J Am Chem Soc 1997, 119: 6214-25.

53. Fukuda, Y., Oomori, Y., Kusama, Y., Terashima, S. *Synthesis and antitumor activity of novel cyclopropapyrroloindole (CPI) derivatives bearing bis(methoxycarbonyl) groups.* Bioorg Med Chem Lett 1997, 7: 749-52.

54. Fukuda, Y., Furuta, H., Shiga, F. et al. *Synthesis and antitumor activity of novel cyclopropapyrroloindole (CPI) derivatives bearing methoxycarbonyl and trifluoromethyl groups.* Bioorg Med Chem Lett 1997, 7: 1683-8.

55. Terashima, S., Fukuda, Y., Oomori, Y. *Trifluoromethylpyrroloindolecarboxilic acid ester and process for production thereof.* US 5629430.

56. Fukuda, Y., Furuta, H., Kusama, Y., Ebisu, H., Oomori, Y., Terashima, S. In preparation.

57. Ding, Z.-M., Hurley, L.H. *DNA interstrand cross-linking, DNA sequence specificity, and induced conformational changes produced by a dimeric analog of (+)-CC-1065.* Anti-Cancer Drug Des 1991, 6: 427-52.

58. Sun, D., Hurley, L.H. *Analysis of the monoalkylation and cross-linking sequence specificity of bizelesin, a bifunctional alkylation agent related to (+)-CC-1065.* J Am Chem Soc 1993, 115: 5925-33.

59. Mitchell, M.A., Johnson, P.D., Williams, M.G., Aristoff, P.A. *Interstrand DNA cross-linking with dimers of the spirocyclopropyl alkylating moiety of CC-1065.* J Am Chem Soc 1989, 111: 6428-9.

60. Mitchell, M.A., Kelly, R.C., Wicnienski, N.A. et al. *Synthesis and DNA cross-linking by a rigid CPI dimer.* J Am Chem Soc 1991, 113: 8994-5.

61. Kelly, R.C., Aristoff, P.A. *Novel CC-1065 analogs having two CPI subunits.* EP 0359454.

62. Lee, C.-S., Gibson, N.W. *DNA damage and differential cytotoxicity produced in human carcinoma cells by CC-1065 analogs, U-73,975 and U-77,779.* Cancer Res 1991, 51: 6586-91.

63. Butryn, R.K., Smith, K.S., Adams, E.G. et al. *V79 Chinese hamster lung cells resistant to the bisalkylator bizelesin are multidrug-resistant.* Cancer Chemother Pharmacol 1994, 34: 44-50.

64. Walker, D.L., Reid, J.M., Ames, M.M. *Preclinical pharmacology of bizelesin, a potent bifunctional analog of the DNA-binding antibiotic CC-1065.* Cancer Chemother Pharmacol 1994, 34: 317-22.

65. Seaman, F.C., Hurley, L.H. *Manipulative interplay of the interstrand cross-linker bizelesin with d(TAA7A)$_2$ to achieve sequence recognition of DNA.* J Am Chem Soc 1996, 118: 10052-64.

66. *Interim report on phase I testing of bizelesin, a bifunctional alkylating agent.* Prous Science Daily Essentials June 26, 1998.

67. Seaman, F.C., Hurley, L.H. *Analysis of the monoalkylation and cross-linking sequence specificity of bizelesin, a bifunctional alkylation agent related to (+)-CC-1065.* J Am Chem Soc 1993, 115: 5925-33.

68. Lee, C.-S., Gibson, N.W. *DNA interstrand cross-links induced by the cyclopropylpyrroloindole antitumor agent bizelesin are reversible upon exposure to alkali.* Biochemistry 1993, 32: 9108-14.

69. Thompson, A.S., Sun, D., Hurley, L.H. *Monoalkylation and cross-linking of DNA by cyclopropapyrroloindoles entraps bent and straight forms of A-tracts.* J Am Chem Soc 1995, 117: 2371-2.

70. Lee, S.-J., Seaman, F.C., Sun, D., Xiong, H., Kelly, R.C., Hurley, L.H. *Replacement of the bizelesin ureadiyl linkage by a guanidium moiety retards translocation from monoalkylation to cross-linking sites on DNA.* J Am Chem Soc 1997, 119: 3434-42.

71. Park, H.-J., Kelly, R.C., Hurley, L.H. *The chemical evolution of DNA-DNA interstrand cross-linkers that recognize defined mixed AT and GC.* J Am Chem Soc 1996, 118: 10041-51.

72. Nakatani, K., Fukuda, Y., Terashima, S. *Synthesis and cytotoxicity of natural (+)-duocarmycin A and its three possible stereoisomers.* Pure Appl Chem 1994, 66: 2255-8.

73. Fukuda, Y., Nakatani, K., Ito, Y., Terashima, S. *First total synthesis of dl-duocarmycin A.* Tetrahedron Lett 1990, 31: 6699-702.

74. Fukuda, Y., Ito, Y., Nakatani, K., Terashima, S. *Synthetic studies on duocarmycin. 1. Total synthesis of dl-duocarmycin A and its 2-epimer.* Tetrahedron 1994, 50: 2793-808.

75. Fukuda, Y., Nakatani, K., Terashima, S. *Synthesis and cytotoxicity of enantiomeric pairs of duocarmycin A and its 2-epimer.* Bioorg Med Chem Lett 1992, 2: 755-8.

76. Fukuda, Y., Nakatani, K., Terashima, S. *Synthetic studies on duocarmycin. 2. Synthesis and cytotoxicity of natural (+)-duocarmycin A and its three possible stereoisomers.* Tetrahedron 1994, 50: 2809-20.

77. Fukuda, Y., Furuta, H., Shiga, F., Asahina, Y., Terashima, S. *Novel synthesis of optically active CC-1065, U-73,975 (adozelesin), U-80,244 (carzelesin), U-77,779 (bizelesin), KW-2189, and DU-86.* Heterocycles 1997, 45: 2303-8.

78. Fukuda, Y., Terashima, S. *A novel synthesis of (+)-duocarmycin SA.* Tetrahedron Lett 1997, 38: 7207-8.

79. Fukuda, Y., Furuta, H., Kusama, Y., Ebisu, H., Oomori, Y., Terashima, S. *The novel cyclopropapyrroloindole (CPI) bisalkylators bearing methoxycarbonyl and trifluoromethyl groups.* Bioorg Med Chem Lett 1998, 8: 1387-90.

80. Bizelesin used as the standard compound was synthesized in our laboratories. See ref. 75.

81. Fukuda, Y., Seto, S., Furuta, H., Ebisu, H., Oomori, Y., Terashima, S. In preparation.

82. Fukuda, Y., Seto, S., Furuta, H., Ebisu, H., Oomori, Y., Terashima, S. *The novel cyclopropapyrroloindole (CPI) bisalkylators bearing 3,3'-(1,4-phenylene)diacryloyl group as a linker.* Bioorg Med Chem Lett 1998, 8: 2003-4.

83. For the synthesis of compounds **22a-e**, see refs. 77, 78 and 84.

84. Fukuda, Y., Furuta, H., Kusama, Y., Ebisu, H., Oomori, Y., Terashima, S. *Synthesis and cytotoxicity of various structural types of novel cyclopropapyrroloindole (CPI) derivatives.* Heterocycles 1998, 49: 53-8.

85. Boger, D.L., Palanki, M.S.S. *Functional analogs of CC-1065 and the duocarmycins incorporating the 9a(chloromethyl)-1,2,9,9a-tetrahydrocyclopropa[clbenz[e]indol-4-one (C2BI) alkylation subunit: Synthesis and preliminary DNA alkylation studies.* J Am Chem Soc 1992, 114: 9318-27.

86. Boger, D.L., Johnson, D.S., Palanki, M.S.S. *Evaluation of functional analogs of CC-1065 and the duocarmycins incorporating the cross-linking 9a-chloromethyl-1,2,9,9a-tetrahydrocyclopropa[clbenz[e]indol-4-one (C2BI) alkylation subunit.* Bioorg Med Chem 1993, 1: 27-38.