A NEW INDOLOPYRROLOCARBAZOLE ANTITUMOR SUBSTANCE, ED-110, A DERIVATIVE OF BE-13793C

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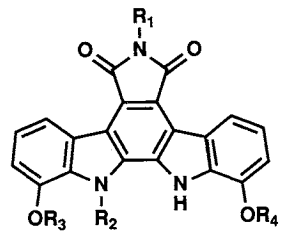
BE-13793C, 12,13-dihydro-1,11-dihydroxy-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7(6*H*)-dione (**1**) (Fig. 1), is an antitumor substance isolated from the culture broth of a strain of *Streptoverticillium*[1]. It showed antitumor activity against ip-implanted Ehrlich tumor cells in mice by ip-administration. We tried the derivatization of **1** and obtained ED-110. The structure of ED-110 is 12,13-dihydro-1,11-dihydroxy-12-*β*-D-glucopyranosyl-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7(6*H*)-dione (**6**) (Fig. 1). In this synthetic procedure, the benzyloxymethyl group was used as a protective group, as was used in the synthesis of rebeccamycin[2].

To a solution of **1** (180.5 mg, 0.505 mmol) in *N*,*N*-dimethylformamide (DMF, 37 ml) was added potassium carbonate (510 mg, 3.69 mmol) and benzyl bromide (170 μl, 1.43 mmol) at room temperature (r.t.). The mixture was stirred at r.t. for 2 hours and extracted with ethyl acetate (200 ml) after the addition of water (200 ml). The ethyl acetate layer was concentrated under reduced pressure and the residue was chromatographed on silica gel to obtain 1,11-dibenzyloxy-12,13-dihydro-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7(6*H*)-dione (**2**) (254 mg, yield 93.7%). Molecular formula $C_{34}H_{23}N_3O_4$; Rf 0.56 (Silica gel 60 F_{254}, Merck, ethyl acetate); FAB-MS *m/z* 538 $(M+H)^+$; ^1H NMR (300 MHz, DMSO-d_6) 5.36 (4H, s), 7.26 (4H, d, $J=4.8$ Hz), 7.3~7.5 (6H), 7.61 (4H, m), 8.54 (2H, m), 10.95 (1H, br s), 11.70 (2H, br s).

To a solution of **2** (143.8 mg, 0.267 mmol) in DMF (50 ml) was added sodium hydride (60% in oil, 128.8 mg, 3.22 mmol) and benzyloxymethyl chloride (36.5 μl, 0.262 mmol) at 0°C. The reaction mixture was stirred at 0°C for 1 hour and extracted with ethyl acetate after the addition of water. The ethyl acetate layer was concentrated and the residue was chromatographed on silica gel by using chloroform as the eluent to obtain 6-*N*-benzyloxymethyl-1,11-dibenzyloxy-12,13-dihydro-5*H*-indolo[2,3-*a*]-pyrrolo[3,4-*c*]carbazole-5,7(6*H*)-dione (**3**) (106.3 mg, yield 60.5%). Molecular formula $C_{42}H_{31}N_3O_5$; Rf 0.64 (Silica gel 60 F_{254}, toluene-methanol (50:1)); FAB-MS *m/z* 657 $(M)^+$, 658 $(M+H)^+$; ^1H NMR (300 MHz, $CDCl_3$) 4.67 (2H, s), 5.28 (4H, s), 5.30 (2H, s), 7.02 (2H, d, $J=7.8$ Hz), 7.10~7.30 (5H), 7.30~7.50 (8H), 7.60 (4H, m), 8.53 (2H, d, $J=7.5$ Hz), 9.00 (2H, s).

A mixture of **3** (5.4 mg, 8.21 μmol), benzene (5 ml), 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (51.6 mg, 0.125 mmol) and silver oxide (42.2 mg, 0.182 mmol) was refluxed for 2 hours. After the reaction, the remaining residue was filtered off and the obtained crude material was chromatographed on silica gel by using chloroform as the eluent to yield 6-*N*-benzyloxymethyl-1,11-dibenzyloxy-12,13-dihydro-12-*N*-(2,3,4,6-tetra-*O*-acetyl-*β*-D-glucopyranosyl)-5*H*-indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7(6*H*)-dione (**4**) (2.8 mg, yield 34.8%). Molecular formula $C_{56}H_{49}N_3O_{14}$; Rf 0.17 (Silica gel 60 F_{254}, *n*-hexane-ethyl acetate (2:1)); FAB-MS *m/z* 987 $(M)^+$, 988 $(M+H)^+$; ^1H NMR (300 MHz, $CDCl_3$) 1.09 (3H, s), 1.87 (3H, s), 2.02 (3H, s), 2.09 (3H, s), 3.69 (1H, m), 3.78 (1H, dd, $J=2.4$ and 12.6 Hz),

Fig. 1. The structures of **1**~**6**.



Compound	R_1	R_2	R_3	R_4
1	H	H	H	H
2	H	H	Bzl	Bzl
3	CH_2OBzl	H	Bzl	Bzl
4	CH_2OBzl	$(Ac)_4Glc^a$	Bzl	Bzl
5	CH_2OH	$(Ac)_4Glc^a$	Bzl	Bzl
6	H	Glc^b	H	H

[a] 2,3,4,6-Tetra-*O*-acetyl-*β*-D-glucopyranosyl.
[b] *β*-D-Glucopyranosyl.

4.07 (1H, dd, J=8.4 and 12.6 Hz), 4.80 (2H, s), 5.05~5.30 (3H), 5.34 (2H, s), 5.36 (2H, s), 5.37 (2H, s), 7.05~7.65 (20H), 8.80 (1H, d, J=8.1 Hz), 8.99 (1H, d, J=8.0 Hz), 10.44 (1H, br s).

To a solution of **4** (411 mg, 416 μmol) in ethyl acetate - ethanol (1 : 6) (105 ml) was added a catalytic amount of palladium-black and hydrogenation was performed at r.t. for 3 hours. The reaction mixture was filtered by a glass filter and palladium-black was washed successively with methanol and tetrahydrofuran. The filtrate and washings were combined and concentrated to obtain 12,13-dihydro-1,11-dihydroxy-6-*N*-hydroxymethyl-12-*N*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-5*H*-indolo-[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7(6*H*)-dione (**5**). Rf 0.3 (Silica gel 60 F_{254}, chloroform - methanol (19 : 1)). Without further purification, to a solution of **5** in methanol (50 ml) was added concd ammonia water (40 ml) and stirred at r.t. for 1 hour. The reaction mixture was concentrated. The residue was chromatographed on silica gel by using ethyl acetate - methanol (7 : 1) as the eluent to yield ED-110 (**6**) (134 mg, 258 μmol, yield 62%). Molecular formula $C_{26}H_{21}N_3O_9$; Rf 0.68 (Silica gel 60 F_{254}, chloroform - methanol (10 : 1)); FAB-MS m/z 520 $(M+H)^+$; ^{1}H NMR (300 MHz, DMSO-d_6) 3.48 (1H, m), 3.64 (2H, m), 3.74 (1H, m), 4.02 (2H, m), 4.88 (1H, br d, J=5.3 Hz), 5.19 (1H, br d, J=5.3 Hz), 5.35 (1H, br t, J=5.0 Hz), 5.41 (1H, br d, J=5.6 Hz), 6.99 (1H, d, J=8.0 Hz), 7.03 (1H, d, J=8.0 Hz), 7.05 (1H, d, J=9.4 Hz), 7.17 (2H, t, J=8.0 Hz), 8.52 (1H, d, J=8.0 Hz), 8.70 (1H, d, J=8.0 Hz), 9.91 (1H, br s), 10.31 (1H, br s), 10.91 (1H, br s), 11.04 (1H, br s).

Cell growth inhibition activities (IC_{50}) of ED-110 against human stomach (MKN-45), colon (LS-180) and lung (PC-13) tumor cell lines were examined. The media used for the culture of MKN-45 and LS-180 were 10% fetal bovine serum (FBS) containing DULBECCO's modified EAGLE's medium (DMEM). 10% FBS containing RPMI-1640 medium was used for the culture of PC-13. Each cell line was cultured in 96-well microplates (3×10^3 cells/well) with or without the test sample under 5% CO_2 at 37°C for 72 hours. After fixing with 50% trichloroacetic acid, cells were stained by 0.4% sulforhodamine B and the dye was extracted from the stained cells with 10 mM Tris(hydroxymethyl)-aminomethane solution. Absorbance of the extract was read at 540 nm. IC_{50} values of ED-110 for MKN-45, LS-180 and PC-13 were 0.28, 1.65 and 1.70 μg/ml, respectively. In acute toxicity test of ED-110 in CDF_1 female mice, no death was found on 5th day, when 400 mg/kg was intraperitoneally administered. Biochemical experiments revealed that ED-110 stabilized the cleavable complex of topoisomerase I and DNA[3]. In a preliminary *in vivo* experiment (CDF_1 female mice, ip-ip, Q1D, day 1~10), ED-110 showed strong antitumor activities against P388 murine leukemia with an ILS value of 66% at a dose of 30 mg/kg/day. Details of *in vivo* experiments will be presented elsewhere.

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