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Received November 15, 2001
Accepted November 26, 2001

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Cytotoxic activity of physalins and related compounds against HeLa cells

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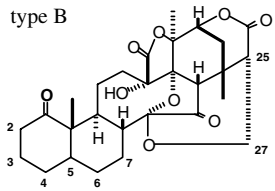
Physalins are 16,24-cyclo-13,14-secoergostane steroids which are classified to types B or A according to the presence or absence of a C(14)−O−C(27) acetalic linkage [1]. Some of the physalins including physalins B and H are known to demonstrate cytotoxic activity against tumor cells *in vitro* and *in vivo* [2, 3]. However, extensive study on the structure-cytotoxic activity relationship has not been reported. In this communication we will describe an extensive study on the cytotoxic activity against HeLa cells of naturally isolated physalins and their derivatives prepared by various chemical conversions.

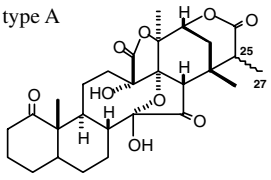
Cytotoxic activity of various physalins and their derivatives are summarized in the Table. Physalins B [4], C [5] and F [6] belong to most active physalins (entries 3, 58, 15) and some of halogen-containing derivatives are also potent (entries 30, 33, 42, 72). Conjugated 2-en-1-one moiety at A ring was shown to be essential for high activity by the lower activity of the 2,3-saturated derivatives (e.g., entries 47 vs. 3, 52 vs. 15) and the isomeric 3-en-1-one compounds (e.g., entries 11 vs. 3, 65 vs. 63). Type A physalins possessing a C(25)=C(27) double bond exhibited comparable activity to those of the corresponding type B physalins (entries 58 vs. 3, 62 vs. 8), which is not surprising considering the tautomerism under certain conditions [1], while the corresponding physalins possessing a C(27)-secondary methyl group exhibited lower cytotoxicity (entries 59 vs. 3, 63 vs. 8). In general introduction of a hydroxy group at C(25) [7] decreased the activity significantly (e.g., entries 3 vs. 4, 8 vs. 9) although in the cases of some inactive compounds their 25-hydroxy analogs were found to show activity (entries 2 and 18). Presence of a 7-hydroxy function also decreased the activity (e.g., entries 8 vs. 3, 62 vs. 58). As exemplified by physalins F and J [6] (entries 13 vs. 15) the 5,6-epoxy derivatives with β-configuration were more potent than the cor-

Table: Cytotoxic activity of physalins of types B and A and thier derivatives against HeLa cells

type B



type A



Entry	Type	AB ring (other than 1-oxo function)	C(25)−C(27)	IC_{50} (μg/ml)	References
1	B	Δ^2, Δ^4, Δ^6	CH−CH_2−O	>100	[4]
2	B	Δ^2, Δ^4, Δ^6	C(OH)−CH_2−O	10	
3	B	Δ^2, Δ^5	CH−CH_2−O	0.32	[4] (physalin B)
4	B	Δ^2, Δ^5	C(OH)−CH_2−O	13	[7]
5	B	Δ^2, Δ^4, 6α-OH	CH−CH_2−O	62	[9]
6	B	Δ^2, Δ^4, 6β-OH	CH−CH_2−O	37	[9]
7	B	Δ^2, Δ^4, 2-Cl, 6β-OH	CH−CH_2−O	30	
8	B	Δ^2, Δ^5, 7α-OH	CH−CH_2−O	1.2	[11] (physalin N)

Table (continued)

Entry	Type	AB ring (other than 1-oxo function)	C(25)–C(27)	IC_{50} (μg/ml)	References
9	B	Δ^2, Δ^5, 7α-OH	C(OH)–CH_2–O	31	
10	B	Δ^2, Δ^6, 5α-OH	CH–CH_2–O	>100	[8]
11	B	Δ^3, Δ^5	CH–CH_2–O	11	[4]
12	B	Δ^3, Δ^5, 7α-OH	C(OH)–CH_2–O	>100	
13	B	Δ^2, 5α,6α-epoxy	CH–CH_2–O	9.4	[6] (physalin J)
14	B	Δ^2, 5α,6α-epoxy	C(OH)–CH_2–O	88	
15	B	Δ^2, 5β,6β-epoxy	CH–CH_2–O	0.35	[6] (physalin F)
16	B	Δ^2, 5β,6β-epoxy	C(OH)–CH_2–O	1.9	
17	B	Δ^2, 5α-OH, 6β-OH	CH–CH_2–O	>100	[17] (physalin D)
18	B	Δ^2, 5α-OH, 6β-OH	C(OH)–CH_2–O	89	
19	B	Δ^2, 5α-OH, 6β-OAc	CH–CH_2–O	>100	[11]
20	B	Δ^2, 5α-OMe, 6β-OH	CH–CH_2–O	11	[17] (physalin I)
21	B	Δ^2, 5α-OMe, 6β-OH	C(OH)–CH_2–O	68	
22	B	Δ^2, 5α-OEt, 6β-OH	CH–CH_2–O	41	[12]
23	B	Δ^2, 5α-OEt, 6β-OH	C(OH)–CH_2–O	>100	
24	B	Δ^2, 5α-OH, 6β-Cl	CH–CH_2–O	28	[3]
25	B	Δ^2, 5α-F, 6β-OH	CH–CH_2–O	84	
26	B	Δ^2, 5α-F, 6β-OH	C(OH)–CH_2–O	>100	
27	B	Δ^2, 5α-Cl, 6β-OH	CH–CH_2–O	1.4	[3] (physalin H)
28	B	Δ^2, 5α-Cl, 6β-OH	C(OH)–CH_2–O	2.6	
29	B	Δ^2, 5α-Cl, 6β-OAc	CH–CH_2–O	9.4	
30	B	Δ^2, 5α-Br, 6β-OH	CH–CH_2–O	0.71	[3]
31	B	Δ^2, 5α-Br, 6β-OH	C(OH)–CH_2–O	2.0	
32	B	Δ^2, 5α-Br, 6β-Br	CH–CH_2–O	3.9	
33	B	Δ^2, 5α-I, 6β-OH	CH–CH_2–O	0.58	
34	B	Δ^2, 5α-I, 6β-OH	C(OH)–CH_2–O	1.8	
35	B	Δ^2, 5α-OH, 6-oxo	CH–CH_2–O	>100	
36	B	Δ^2, 5α-OH, 6-oxo	C(OH)–CH_2–O	>100	
37	B	Δ^2, 5α-Cl, 6-oxo	CH–CH_2–O	68	
38	B	Δ^2, 4α,5α-epoxy, 6α-OH	CH–CH_2–O	1.2	[9]
39	B	Δ^2, 4α,5α-epoxy, 6β-OH	CH–CH_2–O	2.9	[9]
40	B	Δ^2, 4β,5β-epoxy, 6α-OH	CH–CH_2–O	80	[9]
41	B	Δ^2, 4β,5β-epoxy, 6β-OH	CH–CH_2–O	>100	[9]
42	B	Δ^2, 2-Cl, 5α-Cl, 6β-OH	CH–CH_2–O	0.73	
43	B	Δ^3, 2α,5α-epidioxy, 6α-OH	CH–CH_2–O	>100	[9]
44	B	Δ^3, 2α,5α-epidioxy, 6β-OH	CH–CH_2–O	>100	[9] (physalin K)
45	B	Δ^3, 2β,5β-epidioxy, 6α-OH	CH–CH_2–O	>100	[9]
46	B	Δ^3, 2β,5β-epidioxy, 6β-OH	CH–CH_2–O	>100	[9] (physalin Q)
47	B	Δ^5	CH–CH_2–O	18	[4]
48	B	Δ^5	C(OH)–CH_2–O	62	
49	B	Δ^5, 7α-OH	C(OH)–CH_2–O	>100	
50	B	saturated and unsubstituted	CH–CH_2–O	>100	[4]
51	B	5α,6α-epoxy	CH–CH_2–O	>100	
52	B	5β,6β-epoxy	CH–CH_2–O	34	
53	B	5α-OH, 6β-OH	CH–CH_2–O	>100	[13] (physalin T)
54	B	5α-OH, 6β-OH	C(OH)–CH_2–O	>100	
55	B	5α-Cl, 6β-OH	CH–CH_2–O	73	
56	B	2β,3β-epoxy, 5β,6β-epoxy	C(OH)–CH_2–O	>100	
57	B	2β-Cl, 3α-Cl, 5α-Cl, 6β-OH	CH–CH_2–O	13	
58	A	Δ^2, Δ^5	C=CH_2	0.32	[1, 5] (physalin C)
59	A	Δ^2, Δ^5	CH–CH_3 (*S*)	5.4	[2]
60	A	Δ^2, Δ^5	C(OH)–CH_3 (*S*)	6.7	
61	A	Δ^3, Δ^5	CH–CH_3 (*S*)	28	[14] (physalin M)
62	A	Δ^2, Δ^5, 7α-OH	C=CH_2	3.4	[4] (physalin A)
63	A	Δ^2, Δ^5, 7α-OH	CH–CH_3 (*S*)	28	[11] (physalin O)
64	A	Δ^2, Δ^5, 7α-OH	C(OH)–CH_3 (*S*)	68	
65	A	Δ^3, Δ^5, 7α-OH	CH–CH_3 (*S*)	>100	[15] (physalin L)
66	A	Δ^5	CH–CH_3 (*S*)	>100	[5]
67	A	Δ^5	C(OH)–CH_3 (*S*)	>100	
68	A	Δ^5, 7α-OH	CH–CH_3 (*R*)	>100	[4]
69	A	Δ^5, 7α-OH	CH–CH_3 (*S*)	>100	[4]
70	A	Δ^5, 7α-OH	C(OH)–CH_3 (*S*)	>100	
71	A	Δ^2, 5α,6α-epoxy, 7α-OH	C=CH_2	>100	
72	A	Δ^2, 2–Cl, 5α-Cl, 6β-OH	C=CH_2	0.55	
73	A	saturated and unsubstituted	CH–CH_3 (*S*)	>100	[4]

responding α-epoxy compounds. In the case of diastereomeric 4,5-epoxy-6-hydroxy derivatives their activity was in the order: αα > αβ > βα > ββ (entries 38–41), which was similar to the stereochemistry-antitumor activity relationship of the withanolide derivatives [8]. All the 2,5-epidioxy compounds including physalins K and Q [9] were inactive (entries 43–46). Physalin H [3] and other 5α-halo-6β-hydroxy compounds exhibited cytotoxicity and their potency was in the order: iodohydrin > bromohydrin > chlorohydrin > fluorohydrin (entries 33, 30, 27, 25). Taking into account the strong antitumor activity of prostanoids chlorinated at the α-position of the cyclic enone system [10], 2-chloro derivatives of physalins were prepared which showed slightly higher activity than the parent compounds (entries 7 vs. 6, 42 vs. 27).

Experimental

1. Materials

Physalins were isolated from epigeal parts of *Physalis alkekengi* var. *francheti* as already reported [4, 5, 9, 11, 14, 15]. Physalin C was prepared by the isomerization of physalin B [1]. Derivatives of physalins were prepared as already reported or as described below.

25-Hydroxy derivatives: These are prepared from the corresponding physalins or derivatives using activated charcoal-mediated hydroxylation [7].

5α-Halo-6β-hydroxy derivatives: The fluoro-, bromo- and iodocompounds were prepared from physalin F in the similar manner to the reported preparation of the chlorohydrin (physalin H) [3].

2-Chloro derivatives: Cl_2 gas was introduced to the solution of physalin H (282 mg) in $CHCl_3$ (20 ml) for 24 h. After evaporating volatiles the residue was dissolved in DMF (5 ml), to which KOH (60 mg) was added at 0 °C and was stirred at 0 °C for 2 h. The reaction mixture was poured into aqueous 10% NH_4Cl solution and was extracted with AcOEt. After removing the intermediate 2β,3α-dichloro-2,3-dihydrophysalin H, by silica gel column chromatography ($CHCl_3$–MeOH), reversed phase chromatography (H_2O–MeCN) afforded 2-chlorophysalin H (38 mg, 13%) and 2,5α-dichloro-6β-hydroxy-5,6-dihydrophysalin C (10 mg, 3%). KOH treatment of 2β,3α-dichloro-2,3-dihydrophysalin H in DMF at room temperature for 7.5 h afforded 2-chloro-6α-hydroxy-4,5-didehydro-5,6-dihydrophysalin B, in 28% yield.

2. Methods

Cytotoxic assay: Test samples were dissolved initially in DMSO at a concentration of 10 mg/ml and further diluted with cell culture medium (Eagle's MEM) to the desired concentration. HeLa cells in complete medium were added to 96-well plate at a final concentration of 5×10^3 cells/well in a total volume of 200 µl. The plate was incubated for 72 h at 37 °C in a 5% CO_2-in-air atmoshpere. At the end of the incubation, cell growth was determined by XTT-assay [16] and IC_{50} values were defined as the sample concentrations resulting in a 50% decrease in cell growth as compared to the control culture in the absence of inhibitor.

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Received August 16, 2001
Accepted September 15, 2001

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