In vitro effect of organotin-substituted steroids in human tumor cell lines

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Abstract

Four steroidal organotin compounds have been prepared and compared *in vitro* with a parent steroid and two model compounds in a series of human tumor cell lines. The organotin steroids (compounds **1** and **2**) showed promising *in vitro* activity. Another compound with important characteristics in terms of bond angles and stereochemistry (compound **3**) was a highly effective antitumor agent. This compound may serve as a model for further investigation on the structure–activity relationship in antitumor organotin compounds.

Introduction

Platinum compounds such as cis-platin and carboplatin are well known antitumor agents. Derivatives of other metals also display antitumor activity. We are designing organotin compounds intended to be drugs which are more active and/or less toxic than platinum analogs and/or non-cross-resistant with other cytotoxic agents. A variety of diorganotin compounds of the type $RR'(R''COO)_2Sn$ and $\{[RR'(R''COO)Sn]_2O\}_2$ [1], showing promising *in vitro* antitumor activity, was prepared [1–5]. The organic groups, R and R', bound to the tin atoms, can be alkyl or aryl groups, while R''COO denotes a carboxylate group.

Because steroidal compounds are known to possess antitumor activity and are in use in endocrine cancer therapy our strategy was to develop novel organotin compounds containing a steroid ligand. Organotin steroids were hardly studied. Only a few compounds in which the steroid moiety is linked to the tin by Sn–O bonds, were tested [6–8]. It appeared interesting to prepare organotin steroids, in which the steroid moiety is linked to the tin atom by Sn–C bonds rather than by Sn–O bonds. The organotin steroids containing Sn–C bonds were expected to be less sensitive to hydrolysis.

A second goal in our strategy is to identify structural elements in the organotin steroids, which are essential for the antitumor activity. To this purpose we synthesized two model compounds (**3** and **6**) possessing important characteristics in terms of bond angles and stereochemistry.

The steroidal tin compounds **1**, **2**, **4** and **5** and the model compounds associated therewith, **3** and **6**, are presented in Fig. 1, together with the parent ethynyl compounds **7–9**.

Materials and methods

Compounds **1**, **2** and **3** were synthesized from the triphenyltin analogs, **4**, **5** and **6**, respectively (see Fig. 1), by the cleavage of two phenyl–tin bonds by iodine, as described earlier [9]. A 101.6 mg amount of iodine (0.4 mmol) dissolved in 10 ml of chloroform was added to 0.2 mmol of compounds **4**, **5** or **6** dissolved in 2 ml of chloroform. The color of iodine disappeared immediately upon addition of the first equivalent, but much more slowly upon addition of the second one. The solvent was evaporated.

Compounds **4–6** were synthesized by addition of triphenyltin hydride onto the triple bonds of the parent ethynyl compounds **7–9**, as described earlier [9]. 5 mmol

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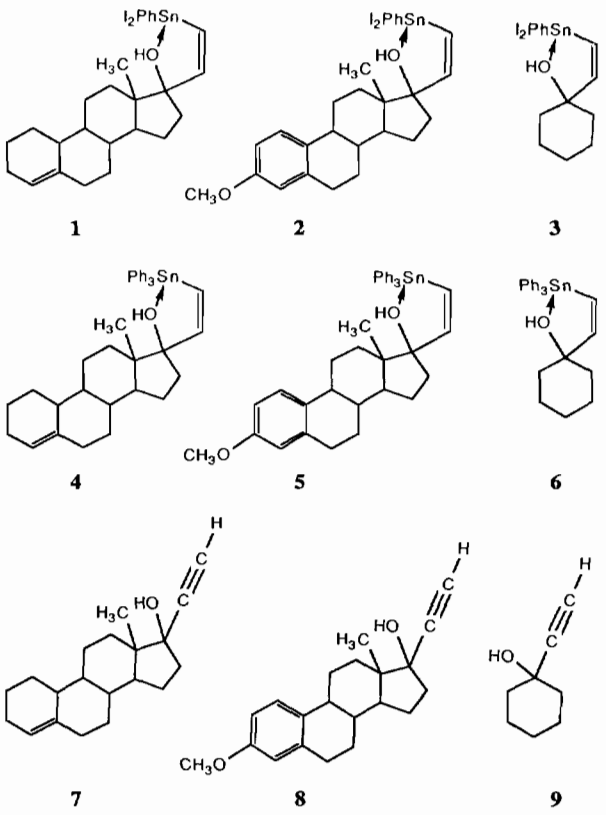

Fig. 1. Structures of compounds **1–9**.

of compounds **7**, **8** or **9** and 1.76 g Ph_3SnH (5 mmol) were dissolved in 30 ml of dry ether in a two-necked flask and 10 mg of dibenzoyl peroxide was added. After 20 h at room temperature, ether was evaporated and the viscous material obtained was crystallized from ethanol. A small amount of hexaphenylditin, only slightly soluble in ethanol, was separated as a side product.

In vitro activity against the human tumor cell lines MCF-7 (mammary tumor) and WiDr (colon carcinoma) was determined using an automated *in vitro* technique described previously [10]. The organotin compound is dissolved in DMSO and this solution is diluted with medium. The final mixture contains less than 0.25% DMSO,which does not affect the test results. Incubation was allowed for 120 h. The test results are given in ID_{50} values (ng/ml).

The *in vitro* colony inhibition in a six cell line panel was likewise described earlier [11]. Cells from human tumor xenografts (HTX) serially transplanted in nude mice were plated in 24 well plates. Incubation occurred continuously for at least one week with several drug concentrations. Effects are expressed as percentage *T/C* (test/control) ratios of the number of colonies generated. Activity is considered to be statistically significant at $T/C < 30\%$. The organotin compounds are dissolved in DMSO and further diluted with medium. The final concentration of at most 0.1% of DMSO does not affect the test results.

Results and discussion

The ID_{50} *in vitro* values (ng/ml) of compounds **1–7** against the MCF-7 and WiDr human tumor cell lines, are presented in Table 1. The cell lines were selected from a wider panel of human tumor cell lines on the basis of their predictive value for organotin compounds [1–5].

When compared with the reference drugs cis-platin (**10**) and etoposide (**11**) the steroidal organotin compounds **1** and **2** exhibit higher antitumor activities in the MCF-7 as well as in the WiDr cell line. The model compound **3** displayed a higher *in vitro* activity than cis-platin in the MCF-7 cell line only. The reference compound **7**, the ethynylsteroid used to prepare **1**, is almost inactive. Compounds **4** and **5** are even more inactive, while compound **6** was more active than the reference drugs cis-platin and etoposide. Because of their relative water insolubility no further studies were undertaken on compounds **4–6**.

On the basis of their promising *in vitro* activity, compounds **1–3** were further evaluated in an established panel of six human tumor cell lines: colorectal and gastric carcinoma, lung adeno-carcinoma (large cell), breast cancer, melanoma and ovarian carcinoma. Each compound was tested in the concentration range 0.001–100 μg/ml. For all compounds a clear dose–response relationship was found. Colony formation was inhibited at dose levels between 0.1 and 100 μg/ml. The results at the concentration of 1 μg/ml are presented in Table 2. Compound **1** was active in 3/6 cell lines, compound **2** in 5/6 and compound **3** in 6/6.

The activity of compounds **1–3** detected in the initial two tumor cell line screen is confirmed in the six human tumor cell lines. However, compound **3**, which was the least active one in the former screen, appears now to be the most active one in the latter screen. Since the tumor cell lines in the latter panel are different from those in the initial screen, a slight variation in test outcome may occur. Another phenomenon, already mentioned above, which may affect the results to some extent also, is the water solubility of the compounds. Compound **3** appears to have a much higher solubility than compounds **1** and **2**.

Overall the model compound **3** shows *in vitro* antitumor activity in most cell lines of a magnitude comparable to or higher than that of compounds **1** and **2**. This suggests the presence in model compound **3** of structural elements essentially responsible for *in vitro* antitumor activity. These might be related either to

TABLE 1. *In vitro* ID_{50} values (ng/ml) of compounds **1–7** and two reference compounds against two human tumor cell lines

Compounds	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**10**[c]	**11**[d]
MCF-7[a]	113	49	321	5943	2846	416	1661	850	187
WiDr[b]	165	140	1195	6931	2361	345	2607	624	624

[a]Mammary carcinoma. [b]Colon carcinoma. [c]Cis-platin. [d]Etoposide.

TABLE 2. *In vitro* effect of compounds **1–3** in human tumor cell lines at a drug concentration of 1 μg/ml

Tumor[a]	Control colony	Test/control (%)[b]		
		1	**2**	**3**
CXF	106	31 +	16 ++	5+++
GXF	82	79 −	42 +	9+++
LXFL	135	3+++	1+++	1+++
MAXF	22	76 −	16 ++	21 ++
MEXF	137	18 ++	11 ++	13 ++
OVXF	125	2+++	4+++	13 ++
Active (++, +++)/total		3/6	5/6	6/6
(%)		50	83	100

[a]CXF: colorectal; GXF: gastric; LXFL: lung large cell; MAXF: breast; MEXF: melanoma xenograft; OVXF: ovarian cancer xenograft. [b]−: ($T/C \geqslant 50$), +: ($30 \leqslant T/C < 50$), ++: ($10 \leqslant T/C < 30$), +++: ($T/C < 10$).

the different ring size or to the strength of the coordinative HO→Sn interaction.

Conclusions

The idea of including steroidal structures into antitumor organotin compounds has proven to be fruitful. Two such compounds, **1** and **2**, proved to be active. Compound **3**, being the most active, suggestably serves as a suitable model for achieving a tin–ligand configuration optimal with regards to its antitumor activity. The results obtained merit further studies on the structure–activity relationship of antitumor organotin compounds of this type.

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