Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 7, No. 11, pp. 1483-1488, 1997

0960-894X/97 $17.00 + 0.00

PII: S0960-894X(97)00249-7

POTENT NOVEL NONSTEROIDAL ANDROGEN ANTAGONISTS WITH A PHTHALIMIDE SKELETON

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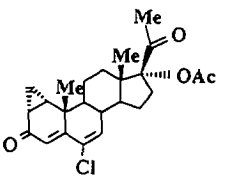
Abstract: Anti-androgenic activity of various phthalimide analogs was evaluated based on inhibition of androgen-induced activation of nuclear androgen receptor (CAT assay) and on growth inhibition of the androgen-dependent clonal cell line SC-3. Some compounds showed very potent androgen-antagonistic activity. © 1997 Elsevier Science Ltd.

Androgen antagonists are compounds which antagonize the biological responses induced by endogenous or exogenous androgens, by inhibiting competitively their binding to the nuclear androgen receptor. Because the growth of several kinds of tumors, especially prostate tumors, is stimulated by androgen, androgen antagonists are expected to be effective for treatment of these androgen-dependent tumors.[1,2]

There are two structural types of androgen antagonists, *i.e.*, steroidal and nonsteroidal types. A typical steroidal androgen antagonist is cyproteron acetate (**1**), the first such drug to have been used in therapeutics;[3] however, **1** also interacts with progestin and glucocorticoid receptors.[4] Typical nonsteroidal androgen antagonists include flutamide (**2**)[5] and Anandron (**3**),[6] both of which are pure androgen antagonists used in the treatment of prostate cancer.[7] The common feature of these two pure androgen antagonists is their very weak relative binding affinity for nuclear androgen receptor, 50 to 100 times less than that of the natural androgen testosterone.[4]

During our studies on tumor necrosis factor-alpha (TNF-α) production-inhibitors, we noticed that some TNF-α production inhibitors are similar in structure and/or structure-activity relationships to nonsteroidal androgen antagonists.[1,8-11] Because TNF-α is a deleterious factor in tumorigenesis,[12-15] androgen antagonists possessing TNF-α production-inhibiting activity might be superior clinical tools for cancer chemotherapy. In this paper, we

describe evaluation of the androgen-antagonistic activity of twelve TNF-α production-inhibitors (**4**–**15**), some of which proved to exhibit much more potent androgen-antagonistic activity than flutamide (**2**).



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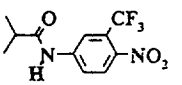

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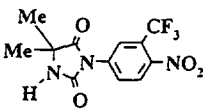

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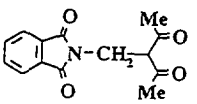

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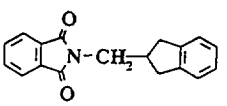

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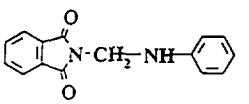

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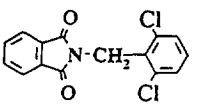

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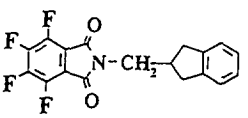

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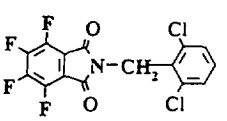

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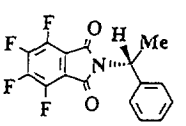

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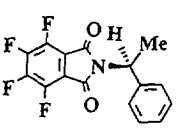

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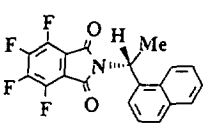

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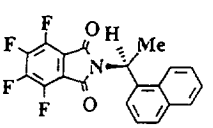

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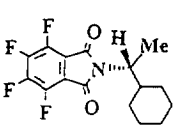

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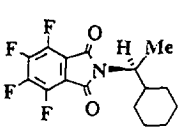

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Compounds **4**–**15** were prepared by condensation of phthalic anhydride or its tetrafluoro derivative with an appropriate amine in good yields. The structures and purity were supported by elemental analysis (including halogen), ^{1}H-NMR and mass specroscopy (*details and physicochemical data of the compounds will be published elsewhere*).

For evaluation of the androgen-antagonistic activity of the compounds, two well-established methods were applied, *i.e.*, (i) CAT (chloramphenicol acetyltransferase) assay which measures the inhibitory activity of test compounds on androgen-induced activation of

nuclear androgen receptor,[16] and (ii) growth inhibition assay of the androgen-dependent clonal cell line SC-3, derived from Shionogi carcinoma 115.[17-20] Both assays were performed basically by the reported methods.[16,17] The results are shown in Table 1.

Table 1. Androgen-Antagonistic Activities of Compounds **2** and **4**–**15**.

Compound	CAT activity (%)[a]	Number of SC-3 cells (%)[b]	Amount of TNF-α produced (%)[c]
none	100	100	100
2	75	66	103 (30 μM)[d]
4	71	49	72 (30 μM)
5	68	56	50 (30 μM)
6	96	91	73 (30 μM)
7	85	69	39 (30 μM)
8	62	61	43 (300 nM)
9	64	45	76 (300 nM)
10	5	16	2 (300 nM)
11	8	3	70 (300 nM)
12	9	9	2 (300 nM)
13	0	14	101 (300 nM)
14	11	2	10 (300 nM)
15	15	5	65 (300 nM)

a. The CAT activity in the cell extract prepared from cells incubated with testosterone alone was defined as 100%.

b. The number of cells incubated with testosterone alone was defined as 100%.

c. The amount of TNF-α produced by HL-60 cells in the presence of okadaic acid (50 nM) alone was defined as 100%.

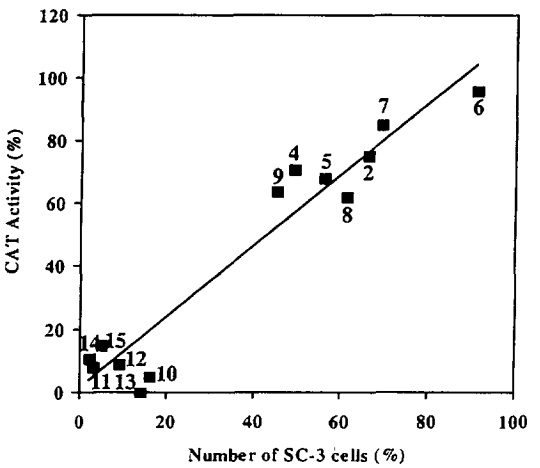
d. Concentration of the added compound.

Briefly, CAT assay was performed by using HeLa cells cotransfected with a CAT reporter plasmid bearing the response element (binding site) for nuclear androgen receptor and expression vectors for the nuclear androgen receptor and β-galactosidase. The cells were treated or not treated with 10 nM testosterone in the presence or absence of 1 μM test compound. Cell extracts were prepared by freeze-thawing and assayed for CAT activity after normalization for β-galactosidase activity as described.[21-23]

For SC-3 cell growth-inhibition assay, the cells were incubated under usual conditions with 10 nM testosterone in the presence or absence of 1 μM test compound for three days. The number of cells was counted under a microscope. In both CAT and SC-3 cell growth assay systems, no cell-killing toxicity or apparent cell damage was caused by the test compounds in the range of concentrations used.

In addition, TNF-α production-inhibiting activity of the compounds on HL-60 cells stimulated with okadaic acid was also assayed as described previously.[8-11,24] Briefly, exponentially growing HL-60 cells were incubated with okadaic acid (50 nM) in the presence or absence of test compounds (30 μM or 300 nM) for 16 h. The amount of TNF-α secreted in the medium was measured with an ELISA TNF-α assay kit. These results are also shown in Table 1.

Fig. 1. Correlation of CAT and SC-3 Assay Results



Vertical scale: CAT activity (%) evaluated in the CAT assay as shown in Table 1.
Horizontal scale: Number of SC-3 cells (%) counted in growth inhibition assay as shown in Table 1.
The correlation curve (r=0.961) was drawn by the method of least squares.

As shown in the table, tetrafluorophthalimide derivatives (**10** - **15**) showed much more potent androgen-antagonistic activity than flutamide (**2**) in both CAT and SC-3 growth inhibition assay systems. Other compounds except **6**, *i.e.*, compounds **4**, **5** and **7**-**9**, showed moderate androgen-antagonistic activities which are comparable to those of **2** in these two assay systems. The inhibitory activity on androgen-induced activation of nuclear androgen receptor evaluated in CAT assay was well correlated to the androgen-antagonistic activity evaluated by growth inhibition assay on androgen-dependent SC-3 cells. The correlation

coefficient (*r*-factor) was 0.961 (Fig. 1).

The good correlation shown in Fig. 1 strongly indicates that the androgen-antagonistic activity of these compounds (**4**, **5**, **7**–**15**) is elicited by binding to and inactivating the nuclear androgen receptor, as is the case for flutamide (**2**).

On the other hand, the androgen-antagonistic activity seems not to correlate with the TNF-α production-inhibiting activity (Table 1). For example, (i) the classical nonsteroidal androgen antagonist flutamide (**2**) does not show TNF-α production-inhibiting activity, (ii) the strong androgen antagonist **13** shows no TNF-α production-inhibiting activity, and (iii) clear *enantio*-dependence is observed in TNF-α production-inhibiting activity [(R)-isomers (**10**, **12**, **14**) shows much more potent inhibiting activity than the corresponding (S)-isomers (**11**, **13**, **15**)], while no such *enantio*-dependence was observed in androgen antagonistic activities.

In conclusion, novel nonsteroidal androgen antagonists were found, with some of them, **10**–**15**, being much more potent than flutamide (**2**). In particular, compounds **10**, **12** and **14** exhibit very potent androgen-antagonistic activity as well as TNF-α production-inhibiting activity (they are pure TNF-α production inhibitors[10]). These compounds should be superior lead compounds for the development of novel bioresponse modifiers for clinical treatment of androgen-dependent tumors.

Acknowledgement: This work was partially supported by a Grant-in Aid for Scientific. Research from the Minisitry of Education, Science and Culture, Japan.

References and Notes

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(*Received in Japan* 25 *April* 1997; *accepted* 6 *May* 1997)