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Structural Development of Biological Response Modifiers Based on Thalidomide

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Abstract—Thalidomide (*N*-α-phthalimidoglutarimide) is a teratogenic hypnotic/sedative agent which was used widely in the late 1950s and the early 1960s. In spite of its withdrawal from the market because of its severe teratogenicity, there has been a resurgence of interest in the drug in recent years due to its potential usefulness for the treatment of various diseases, including acquired immunodeficiency syndrome (AIDS) and various cancers. It has been revealed that thalidomide elicits pleiotropic effects and is a multi-target drug. Our structural development studies of thalidomide, focusing on tumor necrosis factor-α (TNF-α) production-regulating activity, anti-androgenic activity, puromycin-sensitive aminopeptidase-inhibiting activity, α-glucosidase-inhibiting activity, and inhibitory activities toward some other enzymes, are reviewed in relation to the pharmacological effects of thalidomide.

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Introduction

Increasing longevity is a general trend in advanced nations. This is at least partly due to the full-scale commercial appearance of antibiotics in the 1950s. The highest mortality rate in Japan before the 1950s had been due to infectious diseases such as tuberculosis, bronchitis and pneumonia. The death rates from these diseases dramatically dropped in the 1950s, and instead, the death rate from cancer has been rising. Currently, cancer is the number one cause of death in Japan, and one in three persons dies of cancer. In other words, the widespread use of antibiotics, drugs based on species-selective toxicity, has shifted the nature of our lethal diseases from infectious and acute to non-infectious and chronic. Biological response modifiers, which are not based on species-selective toxicity, might provide a means to meet this new challenge.

We have been engaged in development studies of two types of biological response modifiers, that is retinoids, which act directly on malignant cells at the gene expression level to make them behave normally,[1–3] and thalidomide-related molecules, which modulate internal processes of our body to restore a normal state.[3–6] In this paper, our researches on thalidomide-related molecules are reviewed.

History and Biological Activities of Thalidomide

Thalidomide (*N*-α-phthalimidoglutarimide: **1**; Fig. 1) was first synthesized in 1953 by the Swiss pharmaceutical company Ciba, who discontinued research because of the lack of apparent pharmacological effects of the compound. In spite of this, the same compound was synthesized in 1954 by the German company Chemie Grünenthal, who undertook its development and marketed the drug as an anticonvulsant for the treatment of epilepsy.[7,8] However, it was revealed that thalidomide is ineffective for this purpose. Instead, the drug was found to cause deep sleep with prompt action, without residual hangover, which led thalidomide to be remarketed as a hypnotic/sedative agent in 1956. Thalidomide soon became the most popular sleeping pill because of its lack of acute toxicity, and it was believed to be safe even for infants. By the end of the 1950s, 14 pharmaceutical companies were marketing the drug in more than 40 countries, except the United States, where the Food and Drug Administration (FDA) delayed the approval process.[9] Even nowadays, thalidomide is thought to be a unique sleeping drug, because it significantly increases the time spent in REM and stage 3–4 sleep, and decreases the time spent in stage 1 sleep, even though it has no effect on total sleep time.[10] Most hypnotic drugs currently available, such as barbiturates and benzodiazepine

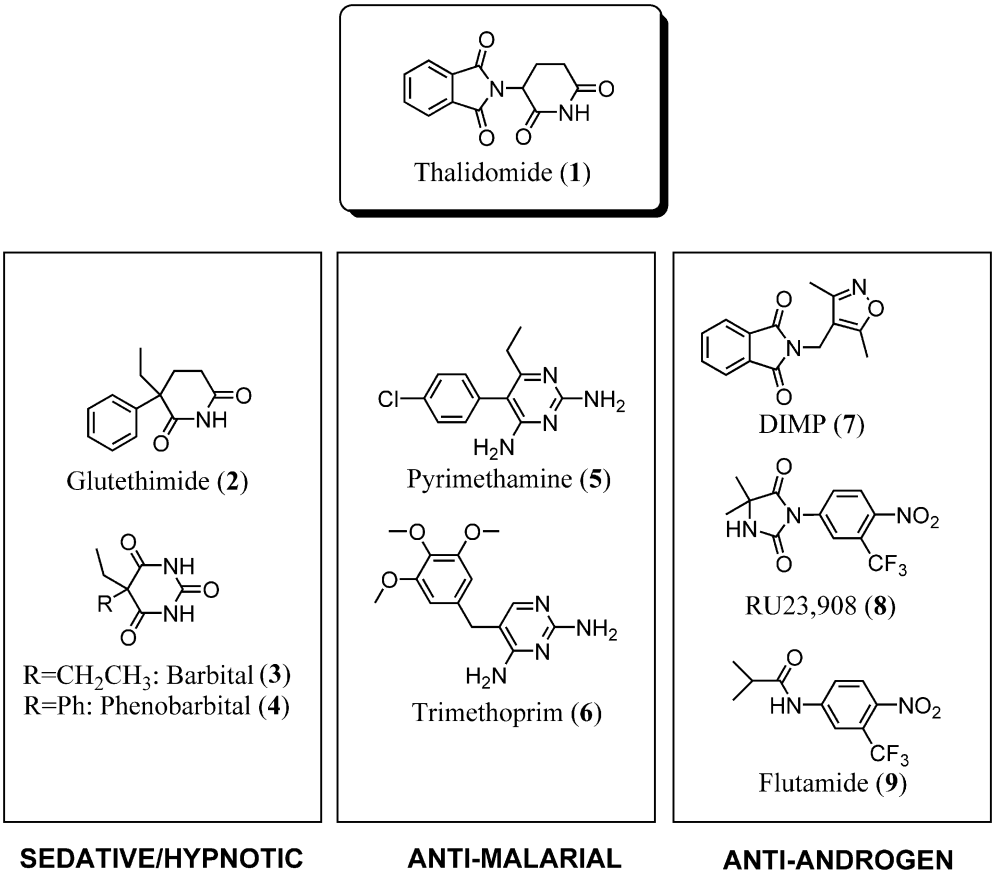


Figure 1. Structures of thalidomide (**1**) and some related medicaments.

derivatives, increase stage 2 sleep and reduce the amount of both REM and stage 3–4 sleep.[10]

Soon after that, escalating numbers of cases of birth defects, phocomelia, became apparent. Subsequently, Lenz and McBride almost simultaneously warned of the teratogenicity of thalidomide.[11–13] Thalidomide was subsequently withdrawn from the market, and the incidence of phocomelia declined abruptly. The scale of the disaster in the early 1960s seemed to condemn the drug to definitive proscription.

Surprisingly, even after the initial impact of the thalidomide disaster, the drug was still used in the therapy of many diseases by investigators.[14] In 1965, Sheskin reported that patients with leprosy, who received thalidomide as a sedative, experienced spectacular relief of symptoms.[15] Application research on thalidomide for the treatment of leprosy continued, and the drug was formally approved for this purpose by the FDA (USA) in 1998, under critical control. Sheskin's report led to new interest in the drug, and since then, many reports have appeared on its therapeutic usefulness in various diseases. The diseases for which thalidomide has potential therapeutic value include rheumatoid arthritis, photodermatitis, Behcet's syndrome, discoid lupus erythematosus, graft-versus-host diseases, malaria, tuberculosis, acquired immunodeficiency syndrome (AIDS), colon cancer, breast cancer, refractory multiple myeloma, prostate tumor, myelodysplastic syndromes, Crohn's disease, diabetes, glioblastoma, and so on.[4,16–27] Thalidomide is now supplied as THALOMID™ by the American pharmaceutical company Celgene, and further information can be obtained through the internet website, http://www.fda.gov/cder/news/thalinfo/default.htm, as well as from http://www.celgene.com/.

Although pharmacological applications of thalidomide have been widely investigated, the molecular basis of its actions has not been clarified yet. The beneficial pharmacological effects elicited by thalidomide include (1) anti-cachexia activity (cachexia is a major direct cause of cancer death), (2) anti-tumor-promoting activity, (3) anti-angiogenic activity, (4) anti-cell invasion (anti-metastasis) activity, (5) anti-viral activity, and (6) hypoglycemic effect. Thalidomide has been reported to regulate production of various cytokines, including tumor necrosis factor-α (TNF-α), interleukins (ILs) 2, 4, 5, 6, 10 and 12, and interferon-γ.[28] Regulation of these cytokines affects the function and population of T-cells, that is thalidomide potently activates $CD8^+$ T-cells and increases the Th2 cell population in the Th1/Th2 cell balance.[28] Though thalidomide affects production of various cytokines as mentioned above, the prevailing hypothesis is that all of the beneficial effects of thalidomide are elicited through regulation of TNF-α production.[29-31] This led us to start our studies on development of TNF-α production regulators based on thalidomide (vide infra). If the hypothesis were correct, potent and specific TNF-α production regulators should be obtainable by structural modification of thalidomide. If not, we should still be able to obtain compounds acting on other target phenomena/molecules which are relevant to the above six pharmacological effects (1–6) elicited by thalidomide. All of these six pharmacological effects are covered in this review.

Tumor Necrosis Factor-α (TNF-α) Production Regulators Derived from Thalidomide

TNF-α is a member of the TNF family of ligands and receptors, which is a large family of cell surface and secreted molecules mediating host defense and immune regulation. TNF-α, which is classified as an inflammatory/somnogenic cytokine, is produced mainly by macrophages and T cells (and also by other cell types including adipocytes and fibroblasts) in response to various stimuli. It is bioactive both as a transmembrane protein and as a homotrimeric secreted form, and shows a wide range of activities.[4] The activities elicited by TNF-α extend beyond the well-characterized pleiotropic pro-inflammatory properties to include diverse signals for cellular differentiation, proliferation and death. The growing understanding of the pathophysiological role of TNF-α in various diseases, including cancer, AIDS, diabetes, and rheumatoid arthritis, has led to the development of strategies to intercept the deleterious effects of TNF-α.[4,32] Because TNF-α is known to be one of the major factors which induce cachexia and angiogenesis, anti-cachexia and anti-angiogenic activity elicited by thalidomide might be attributed to TNF-α production-regulating activity. TNF-α was also reported to cause insulin-independency in Type II diabetes/obesity and enhancement of gene expression of human immunodeficiency virus (HIV) in AIDS.[33–35]

On the other hand, TNF-α itself has potential as an antitumor agent because it exhibits potent cytotoxicity selectively against various tumor cells.[4,36] Therefore, TNF-α can be regarded as possessing both favorable and unfavorable effects. These pleiotropic effects of TNF-α indicate that TNF-α production enhancers in some cases and production inhibitors in other cases would be useful as biological response modifiers in various circumstances. Moreover, tissue and/or cell type-specific TNF-α production regulators would be useful, because TNF-α is rapidly cleared from the circulation. In this chapter, the TNF-α production-regulating activity and the structural modification of thalidomide are reviewed.

Bi-directional TNF-α production-regulating activity of thalidomide

The regulating activity of thalidomide on TNF-α production has been shown to be bi-directional, depending on both cell types and stimulators.[4,37–39] The results obtained by the use of cultured human leukemia cell lines, HL-60 and THP-1, are shown in Fig. 2. Both of these cell lines begin to produce TNF-α when stimulated by various compounds, including tumor promoters, tetradecanoylphorbol 13-acetate (TPA, an activator of protein kinase C) and okadaic acid (OA, an inhibitor of protein phosphatase 2A).

Thalidomide acts as a TNF-α production enhancer on HL-60 cells when TPA is used as a stimulator (Fig. 2a).[4,37–40] On the other hand, it acts as an inhibitor on the same cell line, in the same concentration range, when OA is used as a stimulator (Fig. 2b). Thus, thalidomide is an inducer-dependent bi-directional TNF-α production regulator. It acts as a TNF-α production inhibitor in both TPA- (Fig. 2c) and OA-stimulated (Fig. 2d) THP-1 cells, indicating that thalidomide is also a cell type-dependent bi-directional TNF-α production regulator. These systems are excellent guide assays for structural development studies of thalidomide, and the HL-60/TPA and HL-60/OA systems can be regarded as assay systems which assess TNF-α production-enhancing and production-inhibiting activities, respectively.

Structural development studies

Thalidomide seems to possess pharmacophoric structures related to its known biological activities, which include sedative/hypnotic, anti-malarial and anti-androgenic (vide infra) activities (Fig. 1). A comparison of the structure with those of glutethimide (**2**), barbital (**3**), phenobarbital (**4**), pyrimethamine (**5**) and trimethoprim (**6**) (Fig. 1) led us to suppose that the glutarimide moiety of thalidomide (**1**) might be related to its sedative/hypnotic and anti-malarial effects. Similarly, comparison of the structure with those of DIMP (**7**), RU23,908 (**8**) and flutamide (**9**) (Fig. 1) led us to suppose that the *N*-substituted phthalimide structure might be related to the anti-androgenic activity. In addition, the glutamide structure of thalidomide has been reported to be critical for teratogenic activity. Because anti-androgenic activity is related to immuomodulatory activity through regulation of cytokine/growth factor production (vide infra), we decided to focus on the *N*-substituted phthalimide structure of thalidomide in our search for potent TNF-α production regulators.

Systematic investigation of *N*-alkylated phthalimide analogues revealed that phthalimides bearing a spherical alkyl group, such as an adamantyl group (**10**) (Fig. 3) and a carboranyl group, possess potent bi-directional TNF-α production-regulating activity.[41,42] *N*-Aryl-substituted and related phthalimide analogues have also been

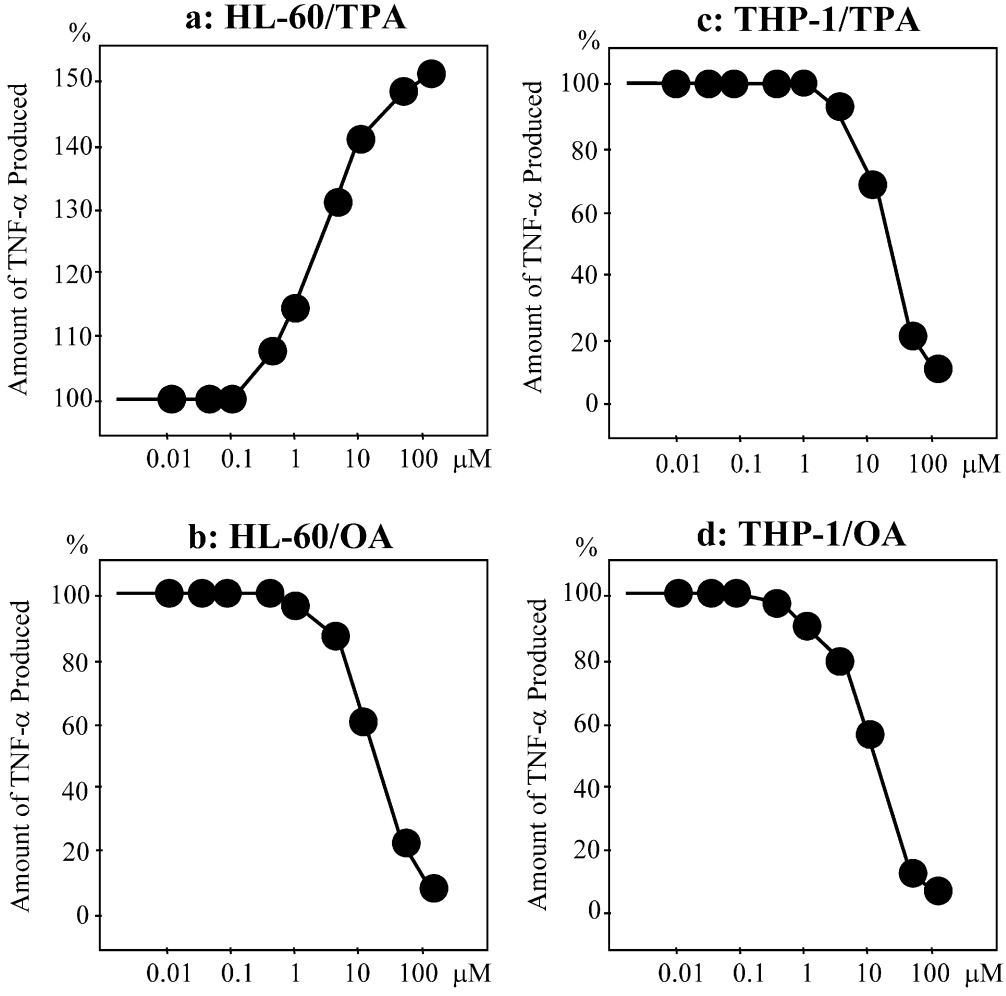


Figure 2. Bi-directional regulation of TNF-α production by thalidomide. Horizontal scale: concentration of added thalidomide. Vertical scale: amount of TNF-α (%) produced by HL-60 (panels a and b) and THP-1 (panels c and d) cells stimulated with 10 nM TPA (panels a and c) or 50 nM OA (panels b and d). The amount of TNF-a produced by HL-60 or THP-1 cells in the presence of the cell stimulator (TPA or OA) alone was defined as 100% in each panel.

investigated systematically.[4,43-49] In the series of substituted *N*-phenylphthalimide analogues, a spherical substituent resulted in a very potent TNF-α production-regulating activity, that is *N*-(2,6-diisopropylphenyl)phthalimide (PP-33: **11**, Fig. 3) showed much more potent activity than thalidomide (Fig. 4). The isopropyl groups of PP-33 (**11**) were the most effective substituents among the investigated alkyl groups, including methyl, ethyl, tertiary butyl, cyclohexyl and other longer normal alkyl groups, and the preferred site of substitution is *ortho* (Fig. 3).

Tetrafluorination of the phthalimide moiety of PP-33, that is FPP-33 (**12**), dramatically lowered the effective concentration to the nanomolar range (Fig. 4).[47,50] The effect seems to be specific to fluorination, because tetrachlorination or tetrabromination did not lower the effective concentration.[43,47] *N*-Phenethyl-4,5,6,7-tetrafluorophthalimide (**13**) is also a potent bi-directional TNF-α production-regulator.[46] Tetrafluorination of thalidomide, that is **14**, also enhanced the TNF-α production-regulating activity, but the efficacy was moderate.[50,51] Modification of the succinimide moiety, such as ring expansion, ring opening, decarbonylation, imination, and so on, generally lowered the activity, except for thiocarbonylation.[43,52]

These planar structure–activity relationships are similar in TNF-α production-enhancing and production-inhibiting systems. Partial separation of TNF-α production-enhancing activity from production-inhibiting activity has been achieved by introduction of a substituent into the phthaloyl moiety. Introduction of an electron-withdrawing nitro group (**15** and **16**, Table 1) caused an increase of the TNF-α production-enhancing activity and a decrease of the TNF-α production-inhibiting activity.[43] In contrast, introduction of an electron-donating group, such as an amino or a hydroxyl group (**17**–**20**, Table 1), resulted in a decrease of TNF-α production-enhancing activity.[43] In particular, introduction of a hydroxyl group at the 5-position (5HPP-33: **20**, Table 1) resulted in a decrease of the enhancing activity and an increase of TNF-α production-inhibiting activity.[43] Though the separation of TNF-α production-enhancing and inhibiting activities is not complete, the results suggest that the target molecules, possibly optically active macromolecules, of the TNF-α production-enhancing and production-inhibiting actions are different. This led us to try to separate the enhancing and inhibiting activities completely by changing the three-dimensional structure, that is by the introduction of optical activity.

Thalidomide (**1**) possesses an asymmetric carbon, and the drug has been used as a racemic mixture. A widely prevailing hypothesis is that only the *S*-isoform of thalidomide is teratogenic, and therefore, if *R*-thalidomide had been used, the thalidomide disaster could have been avoided. However, there has been only a single paper which supports this hypothesis experimentally, that is a report from Blaschke et al., who used rats, a species which is generally considered to be rather resistant to thalidomide teratogenicity. They found that only the *S*-isoform is teratogenic.[53] However, other researchers, including Fabro's group, who used more responsive New Zealand white rabbits, reported that they could not observe any difference in teratogenicity

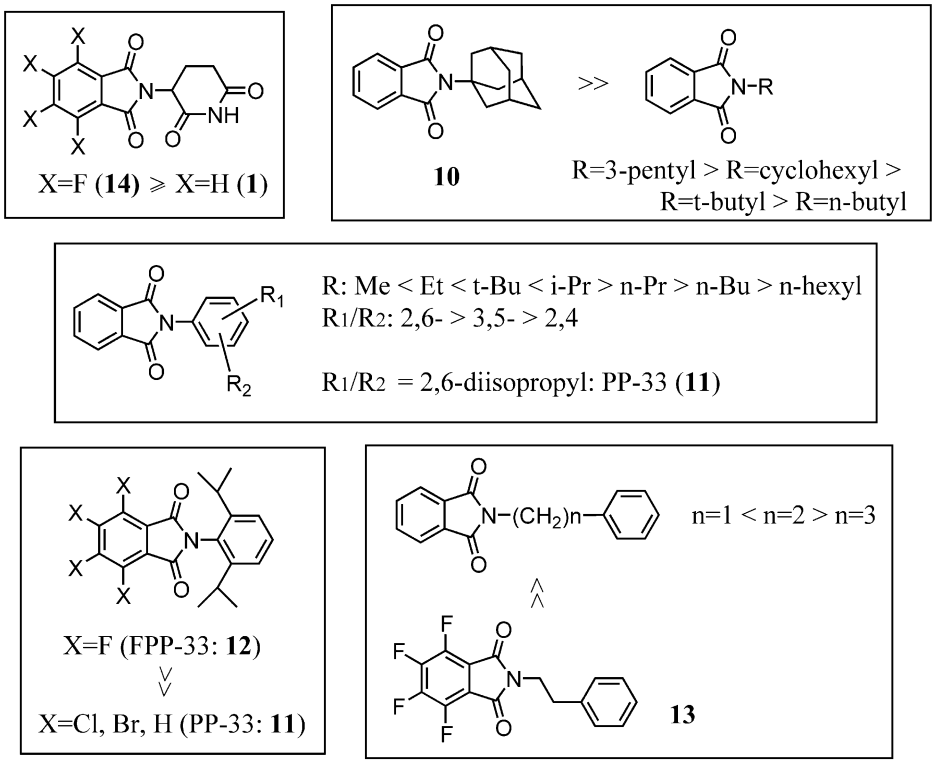


Figure 3. Structures of some typical bi-directional TNF-α production regulators and the structure–activity relationships.

between the *S*- and *R*-isoforms of thalidomide.[54] In addition, we and other researchers have shown that racemization of thalidomide under physiological conditions is fast, that is half-racemization takes place in less than 10 h.[55] As regards TNF-α production-regulating activity, pure *S*- and pure *R*-isoforms of thalidomide showed exactly the same activity, possibly owing to fast racemization.[55]

To clarify the difference of biological activity between *S*- and *R*-isoforms, we investigated the effects of non-racemizable analogues, that is *S*- (**21**) and *R*-methylthalidomides (**22**), on TNF-α production regulation (Table 2).[55] Synthetic methods to prepare optically pure methylthalidomide derivatives, including derivatives possessing a substituent(s) on the phthaloyl moiety, and an analytical method to confirm their optical purity were established.[51,55] Our results indicate that only *S*-methylthalidomide (**21**) possesses TNF-α production-enhancing activity, while the TNF-α production-inhibiting activity of *R*-methylthalidomide (**22**) is much more potent than that of the *S*-isoform (**21**).[38] The results indicated that complete separation of TNF-α production-enhancing and production-inhibiting activities might be achieved by the structural development of optically active derivatives. Subsequently, pure potent TNF-α production inhibitors without TNF-α production-enhancing activity [R-FPTP (**24**), R-FPTN (**26**), R-FPTH (**28**)] (Table 2) and a pure TNF-α production enhancer without TNF-α production-inhibiting activity [S-FP13P (**29**), Table 2] were prepared.[4,43,56]

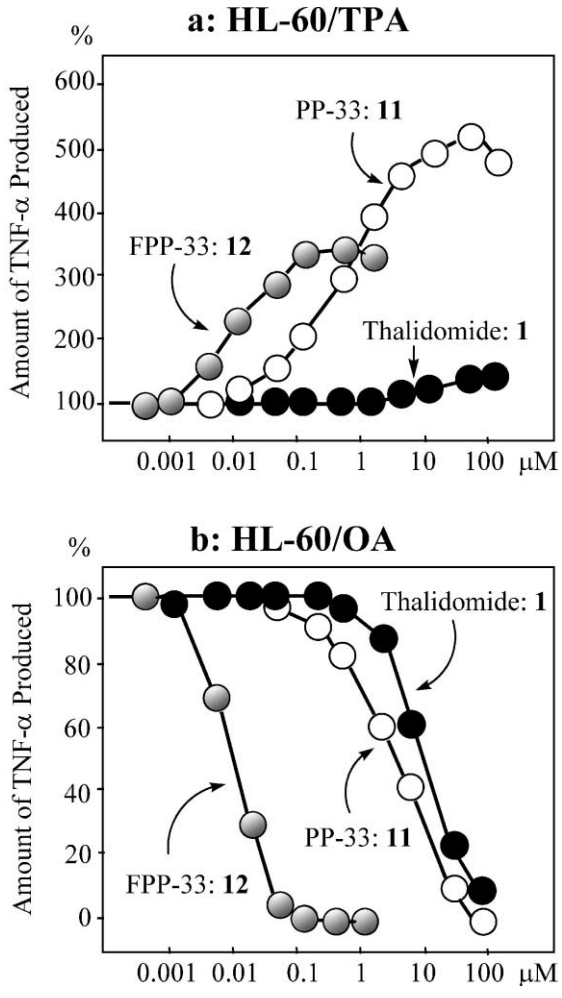
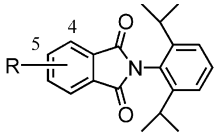


Figure 4. Dose–response curves of some bi-directional TNF-α production regulators. Horizontal scale: concentration of added compounds. Vertical scale: amount of TNF-α (%) produced by HL-60 cells stimulated with 10 nM TPA (panel a: assessment of enhancing effect) or 50 nM OA (panel b: assessment of inhibiting effect). The amount of TNF-a produced by HL-60 cells in the presence of the cell-stimulator (TPA or OA) alone was defined as 100% in each panel.

Based on the above results, that is (1) the inducer- and cell type-dependent bi-directional TNF-α production-regulating activity of thalidomide, and (2) the enantio-dependence of the bi-directional regulating activity and success in their complete separation, we hypothesized that there might be two target molecules for thalidomide and related TNF-α production regulators (Fig. 5).[4,38] One is an enhancing factor which plays a role in enhancement of TPA-induced TNF-α production. The enhancing factor is hypothesized to bind *S*-isoforms of compounds preferentially. The other is an inhibiting factor which plays a role in inhibition of OA-induced TNF-α production, and it binds *R*-isoforms preferentially. The inhibiting factor is hypothesized also to act on the TPA-induced TNF-α production system. However, if the enhancing and inhibiting factors both exist, the former is hypothesized to be dominant. The assumption that HL-60 cells possess both factors, and THP-1 cells lack the enhancing factor (or possess inactivated enhancing factor), can then interpret all the experimental results (Fig. 5).[4,38] By the use of a photoaffinity labeling technique, candidate proteins for the enhancing and inhibiting factors have been detected on SDS–PAGE.[44] Structural and functional characterization of these proteins is in progress. These target proteins might be transcription factors or related factors, because mechanistic studies of our TNF-α production regulators suggested that they act at the gene expression level.[57]

Table 1. Effects of substitutents at the phthaloyl moiety of PP33 (**11**) on TNF-α production-regulating activity

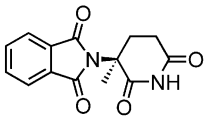


Compound	R	Amount of TNF-α	
		HL-60/TPA	HL60/OA
PP-33 (**11**)	H	602	21
4NPP-33 (**15**)	4-NO_2	792	54
5NPP-33 (**16**)	5-NO_2	683	54
4APP-33 (**17**)	4-NH_2	248	21
5APP-33 (**18**)	5-NH_2	242	28
4HPP-33 (**19**)	4-OH	239	64
5HPP-33 (**20**)	5-OH	153	7

HL-60 cells were treated with 10 nM TPA or 50 nM OA in the presence of 30 μM test compound.
The amount of TNF-α produced in the absence of test compound was defined as 100%.

Table 2. Enantio-dependence of bidirectional TNF-α production regulation and complete separation of the bidirectional activities

S-isoforms



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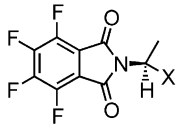

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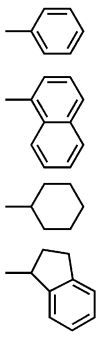
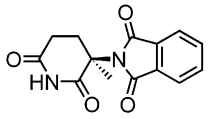


23 **25** **27** **29**

R-isoforms



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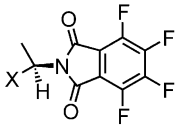

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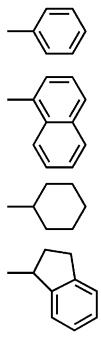


24 **26** **28** **30**

Compound	Amount of TNF-α (%)		Compound	Amount of TNF-α (%)	
	HL-60/TPA	HL60/OA		HL-60/TPA	HL60/OA
21	402	66	**22**	100	18
S-FPTP (**23**)	143	70	R-FPTP (**24**)	97	2
S-FPTN (**25**)	101	101	R-FPTN (**26**)	100	2
S-FPTH (**27**)	124	65	R-FPTH (**28**)	98	10
S-FP13P (**29**)	392	98	R-FP13P (**30**)	233	43

HL-60 cells were treated with 10 nM TPA or 50 nM OA in the presence of test compound. Methylthalidomides (**21** and **22**) were added at the concentration of 100 mM, and other compounds (**23**–**30**) were added at the concentration of 0.3 mM. The amount of TNF-α produced in the absence of test compound was defined as 100%.

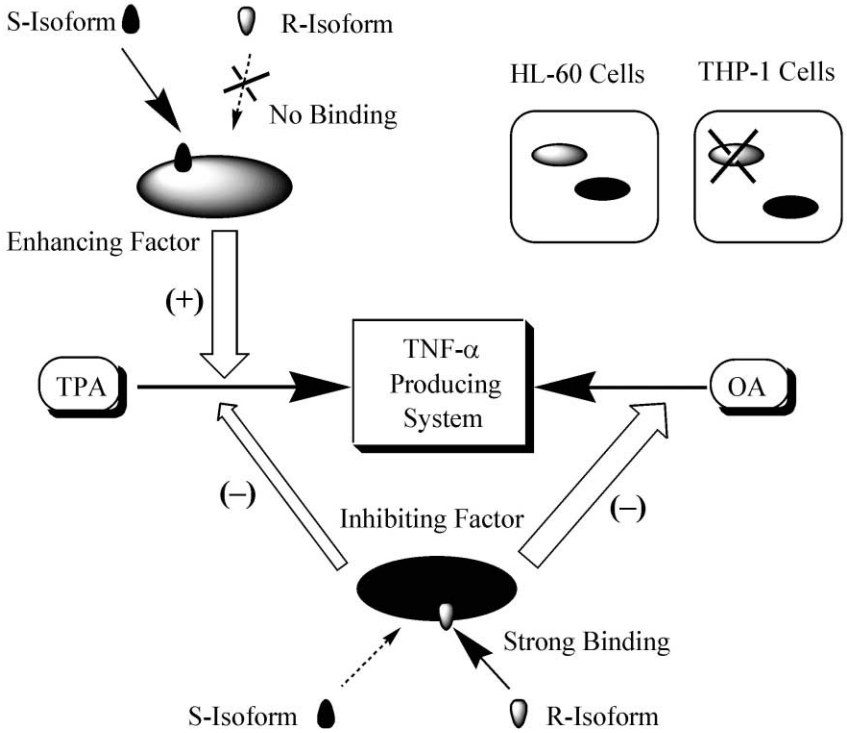


Figure 5. A possible explanation for cell type-/inducer-specific bi-directional regulation of TNF-α production and its enantio-dependence. (+) enhancement; (−) inhibition.

Anti-angiogenic activity

As described above, we obtained specific and potent TNF-α production regulators which are more potent than thalidomide, including bi-directional ones [PP-33 (**11**) and FPP-33 (**12**)], pure inhibitors [R-FPTP (**24**), R-FPTN (**26**) and R-FPTH (**28**), Table 2] and a pure enhancer [S-FP13P (**29**)] (Table 2). Because TNF-α is known to be a cachexia-inducing factor, its production inhibitors are expected to possess anti-cachexia activity. In fact, some of our bi-directional TNF-α production-regulators and inhibitors prolonged the life span of mice with cachexia induced by lipopolysaccharide injection (preliminary unpublished results). The anti-angiogenic activity of thalidomide has been well documented.[25,58] On-going clinical phase II/III studies of thalidomide as an anti-angiogenic agent prompted us to assess the anti-angiogenic activity of our compounds. Angiogenesis is a critical step for solid tumor tissue growth/enlargement and metastasis. Therefore, anti-angiogenic agents are candidate anti-tumor agents.

Anti-angiogenic activity of our compounds was evaluated in a murine model of angiogenesis induced by human basic fibroblast growth factor.[59] Some of our TNF-α production-regulators, especially R-FPTP (**24**), showed more potent anti-angiogenic activity than thalidomide at a much lower dose than thalidomide (Fig. 6).[59] Clinical application studies of these potent anti-angiogenic compounds are in progress.

Though TNF-α is reported to be an angiogenesis-inducing factor, we could not find any clear relationship between the anti-angiogenic activity and the TNF-α production-regulating activity of our compounds (Fig. 6). This suggests that the pharmacological effects, including the anti-angiogenic effect, elicited by thalidomide may not be due to TNF-α production-regulating activity alone. Moreover, in other biological assays, including evaluation of hypoglycemic effect and anti-rheumatoid effect, in which thalidomide is effective, our compounds were less effective than thalidomide. This led us to consider structural modifications of thalidomide based on different target molecules/phenomena (other than TNF-α), which are considered to be related to the above-mentioned six pharmacological effects elicited by thalidomide.

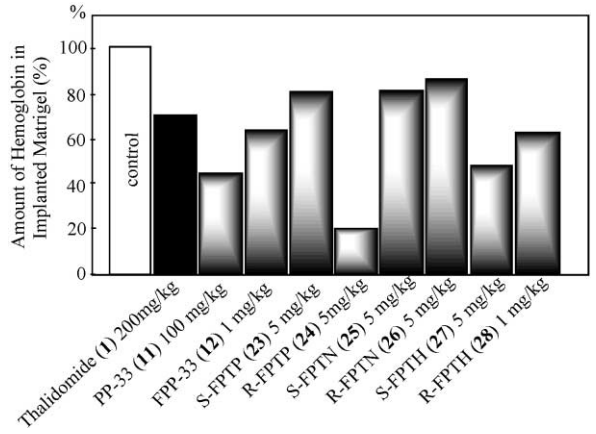


Figure 6. Anti-angiogenic activity of thalidomide (**1**) and TNF-α production regulators (**11**, **12**, **23**–**28**).

For anti-angiogenic activity, we considered thymidine phosphorylase (TP)/platelet-derived endothelial cell growth factor (PD-ECGF) as a putative target molecule. TP is an enzyme which catalyzes the reversible phosphorolytic degradation of thymidine to thymine and 2-deoxyribose-1-phosphate, and is considered to be essential in the thymidine salvage pathway. TP was shown to be identical to PD-ECGF, which is an important factor involved in angiogenesis, and is expressed in abnormally high levels in some human cancers.[60,61] TP/PD-ECGF also recognizes other pyrimidine nucleoside derivatives with anti-tumor/anti-virus activities, including 5-fluoro-2′-deoxyuridine and 5-trifluoromethyl-2′-deoxyuridine. The forward reaction is regarded as a deactivation of anti-tumor/anti-viral agents. Therefore, inhibitors of TP/PD-ECGF may elicit beneficial biological effects by acting both as anti-angiogenic agents (such as thalidomide), and as subsidiary agents enhancing the bioavailability of deoxynucleoside-type anti-tumor/anti-viral agents. Only a few inhibitors of TP/PD-ECGF have yet been reported, and all of them are pyrimidine or purine derivatives, including 5-nitrouracil (5-NU: **31**, Fig. 7).

Our structural development study targeting TP/PD-ECGF-inhibiting activity yielded several homophthalimide analogues (Fig. 7). Among them, **32** showed the most potent inhibitory activity with an IC_{50} value lower than that of 5-NU (**31**).[6,62] Lineweaver–Burk plot analysis indicated that **32** inhibits TP/PD-ECGF in a mixed-type mode, while 5-NU (**31**) acts as an competitive inhibitor.[62] Other compounds, **33** and **34**, possess almost the same level of inhibitory activity as 5-NU (**31**).[62] These compounds are considred to be lead compounds for the development of novel type(s) of TP/PD-ECGF inhibitors.

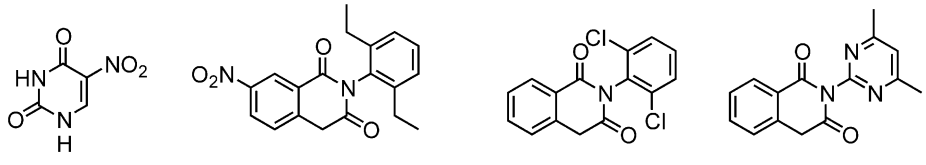


Figure 7. Structures of a classical TP/PD-ECGF inhibitor, 5-NU (**31**), and inhibitors derived from thalidomide (**32**–**34**).

Androgen Antagonists Derived from Thalidomide

A preliminary study indicated that our TNF-α production-regulators show moderate anti-tumor promoting activity. This is reasonable, because TNF-α is reported to be one of the endogenous tumor promoters.[63] To develop more potent anti-tumor-promoting agents, we focused on another endogenous tumor promoter, that is fibroblast growth factor 10 (FGF-10). FGF-10 is reported to act as a tumor promoter especially in prostate cancer, and its production is induced by steroid hormone, androgen (Fig. 8). Considering the effectiveness of thalidomide in the treatment of prostate cancer and its structural similarity to a classical androgen antagonist, DIMP (**7**, Fig. 1), we expected that superior non-steroidal androgen antagonists might be prepared by structural development of thalidomide. Moreover, recent results indicated that FGF-10-knock-out mice lack limbs (Fig. 9).[64] This defect is morphologically similar to the defects induced by thalidomide. This and the known teratogenicity of androgens led us suspect a relationship between thalidomide and androgens.

Androgens, typically testosterone (**35**) and its active metabolite, 5α-dihydrotestosterone [DHT (**36**)], elicit their biological activity by binding and activating a specific receptor, nuclear androgen receptor (AR), which is a member of the steroid/retinoid/thyroid/vitamin D_3 nuclear receptor superfamily and is a ligand-dependent specific transcription factor. Our aim is to create androgen antagonists which antagonize the biological response induced by endogenous or exogenous androgens, by competitively inhibiting their binding to AR (Fig. 8).

First, the TNF-α production regulators derived from thalidomide (vide supra) were screened for anti-androgenic activity. Evaluation of the activity was performed by two well-established assay methods, that is CAT (chloramphenicol acetyltransferase) assay which measures the inhibitory activity of test compounds on androgen-induced activation of AR, and a growth inhibition assay of the androgen-dependent clonal cell line SC-3, derived from Shionogi carcinoma 115.[65] As expected, several of our compounds showed much more potent anti-androgenic activity than flutamide (**9**, Fig. 1), which is widely used for the treatment of prostate cancer, and DIMP (**7**, Fig. 1) (Table 3).[4,65] For example, S-FPTN (**25**) showed complete inhibition in CAT assay, and S-FPTP (**23**) and R-FPTH (**28**) showed almost complete inhibition in SC-3 assay. Based on the struc-

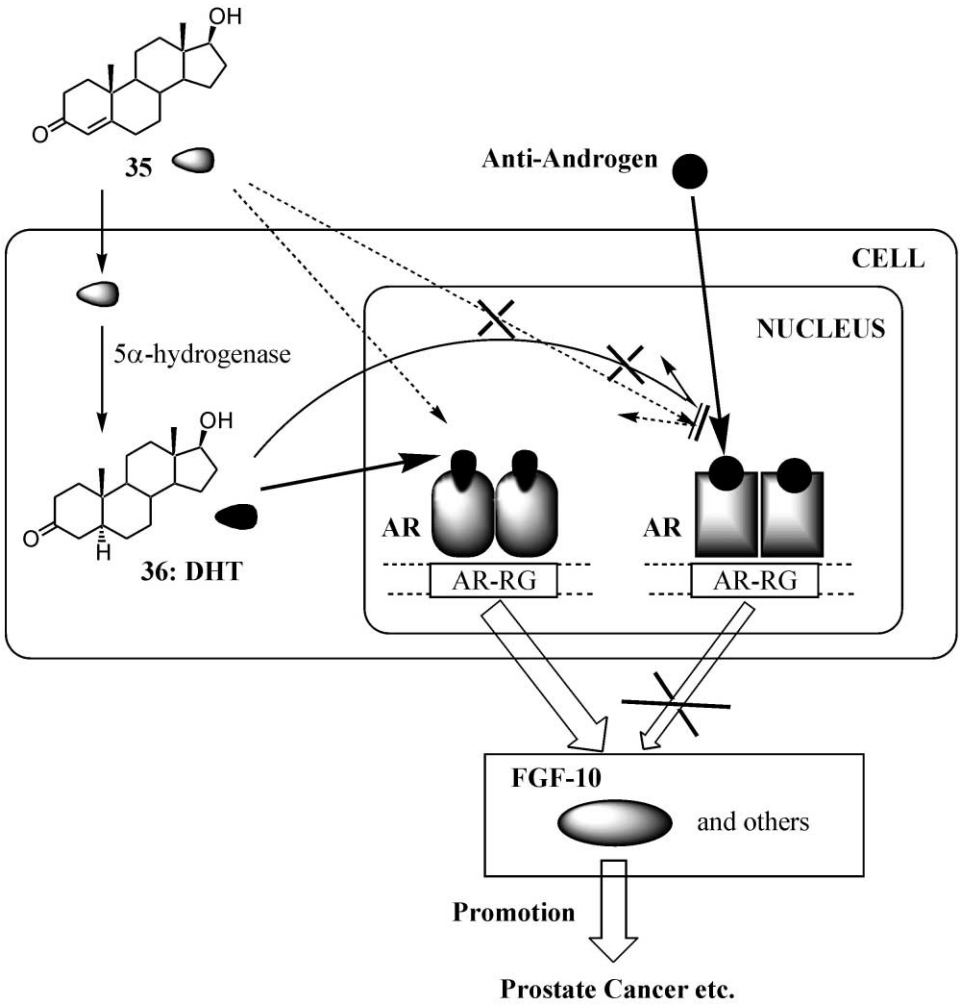


Figure 8. Schematic illustration of mechanisms of androgenic action and anti-androgenic action. AR, nuclear androgen receptor (acts as a head-to-head homodimer); AR-RG, androgen-responsive gene.

tural similarity between DIMP (**7**) and thalidomide (**1**), some analogues of DIMP (**37**–**40**, Fig. 10) were also prepared. Among them, the aza-analogue (**39**) was the most potent in our assay system (Table 3). The ring-opened analogue (**41**, Fig. 10) also showed potent anti-androgenic activity (Table 3).

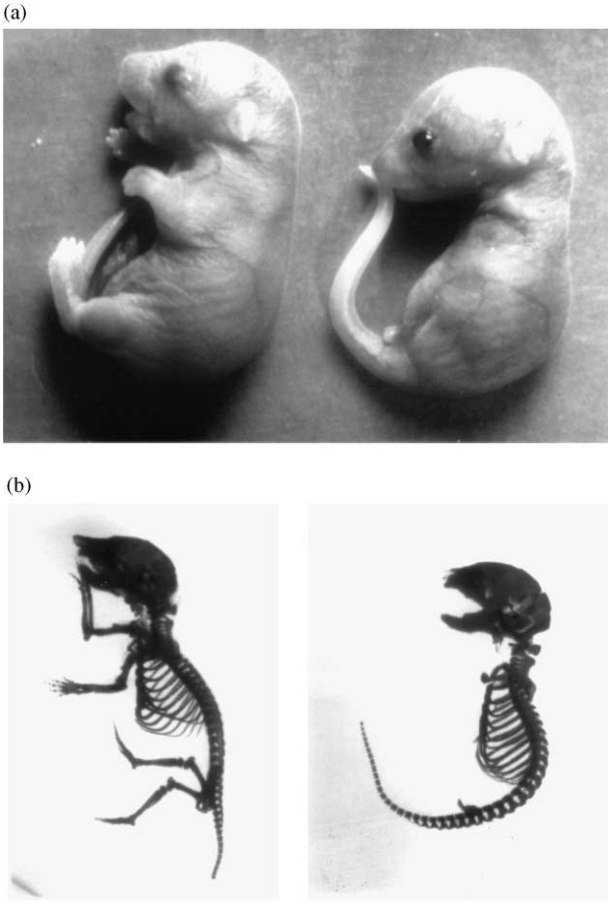


Figure 9. Morphology of FGF-10 knock-out mouse:[64] (a) phtotographs of newborn mice; (b) skeletal malformation stained with alizarin red and alcian blue. Left: control. Right: a FGF-10 knock-out mouse. Photographs supplied by Prof. S. Kato (IMCB, Univ. Tokyo).

Table 3. Anti-androgenic activity of non-steroidal compounds

Compound	Concentration (μM)	Inhibition	
		CAT assay	SC-3 growth
None	—	0	0
DIMP (**7**)	30	75	56
Flutamide (**9**)	30	23	36
S-FPTP (**23**)	0.3	92	97
R-FPTP (**24**)	0.3	95	84
S-FPTN (**25**)	0.3	100	86
R-FPTN (**26**)	0.3	91	91
S-FPTH (**27**)	0.3	85	95
R-FPTH (**28**)	0.3	89	98
37	0.3	90	41
38	0.3	60	47
39	0.3	67	85
40	0.3	87	76
41	0.3	59	68
42	10	49	56
43	10	31	60
44	10	53	82

Structures **37**–**44** are shown in Figure 10.

AR is a member of the steroid/retinoid/thyroid/vitamin D_3 nuclear receptor superfamily, and we have reported on the structural inter-relationship of the ligands for these nuclear receptors (ligand superfamily concept).[1–3,66] Our studies suggested that compounds with two aromatic rings connected with an amide, azo, or azoxy linkage are candidate nuclear receptor ligands.[1,2,67–71] In fact, the potent anti-androgenic ring-opened amide-type compound **41** mentioned above falls into this structural category.[65,72] We therefore prepared azo/azoxy-benzene analogues systematically, and evaluated their anti-androgenic activity by SC-3 assay and AR-binding assay using recombinant human AR.[71] Some azo/azoxy-benzene analogues (**42**–**44**, Fig. 10) were shown to possess moderate anti-androgenic activity (Table 3).[71]

Our previous reports on the ligand superfamily concept suggest the existence of a general antagonistic substituent group for nuclear receptor ligands.[1–3,66] Such a general antagonistic group, if it exists, is expected to hinder conformational change of the receptor to the active form without reducing the binding affinity of the ligand to which it is attached. A dialkylaminophenyl group might be such a general antagonistic group, as found in the well-known estrogen antagonist, tamoxifen, and other steroid antagonists. This consideration and the results of computational superposition studies of our anti-androgens, **39** and **41**, led us to design oxazolone-type compounds (Fig. 11).[73] In addition, a computer-assisted docking search of a database of commercially available compounds suggested that the oxazolone **51** would fit in the ligand-binding pocket of AR (Fig. 12).[73] Oxazolone-type compounds were systematically prepared and their anti-androgenic activity was evaluated by means of SC-3 cell growth inhibition and AR-binding assays (vide supra). Oxazolone-type compounds **45**–**67** showed moderate to potent anti-androgenic activity (Fig. 11). Evaluation of AR-binding affinity showed that the chlorophenyloxazolone derivative **59** is the most potent among the prepared compounds, that is it binds AR with an affinity 220-fold higher than that of flutaminde (**9**). The benzyloxazolone derivative **53** also possesses very potent activity. Evaluation of these novel, non-steroidal, potent androgen antagonists in an in vivo system is in progress.

Peptidase Inhibitors Derived from Thalidomide

Dipeptidyl peptidase IV

Dipeptidyl peptidase IV (DPP-IV) is a membrane-associated serine protease which is widely distributed in mammalian tissues and body fluids, and is identical with the T-cell activation marker CD26 in the human immune system.[74,75] DPP-IV preferentially liberates Xaa-proline or Xaa-alanine dipeptides from the N-terminal of some polypeptides. It appears to be involved in various pathophysiological effects, including tumor cell adhesion and the entry of human immunodeficiency virus

(HIV) into CD4$^+$ T cells.[76] Therefore, DPP-IV inhibitors are expected to be immunomodulators and to have potential pharmacological/clinical applications. The usefulness of thalidomide in the treatment of AIDS,[34] the effect of thalidomide on T-cell activation,[28] and its anti-metastatic activity suggested that it might be a useful lead compound for the development of novel DPP-IV inhibitors.

The most potent reversible specific DPP-IV inhibitors are boronic acid derivatives of peptides, including ProboroPro (PBP: **68**, Fig. 13), which are considered to be transition-state analogues. We have systematically prepared *N*-phenylphthalimide derivatives using DPP-IV inhibition as a guide. Several potent inhibitors with IC_{50} values lower than that of PBP were obtained (**69**–**74**, Fig. 13).[5,77] Among these phthalimide analogues, only **72** is a specific inhibitor which is completely inac-

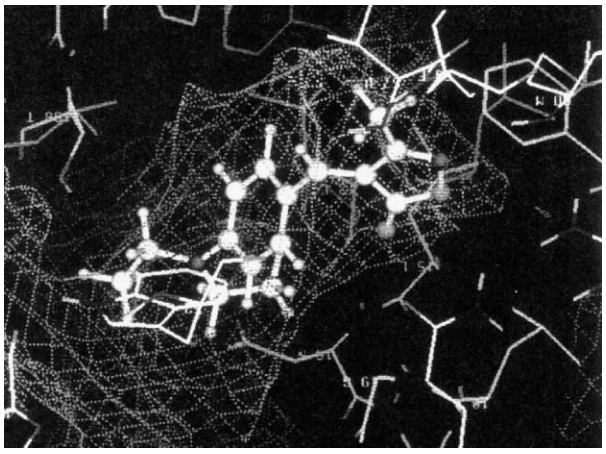


Figure 12. Computer-assisted docking model of oxazolone (**51**) with AR. The docking study was performed by Itai et al. at IMMD (Tokyo).[73]

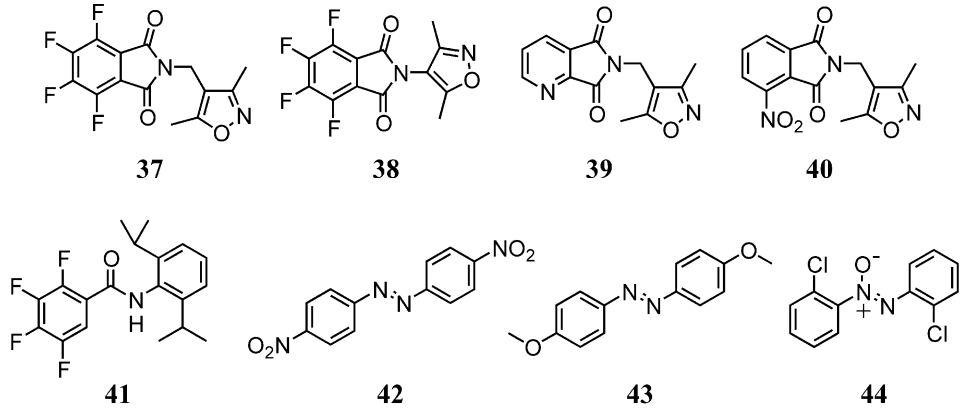


Figure 10. Structures of some novel non-steroidal anti-androgens.

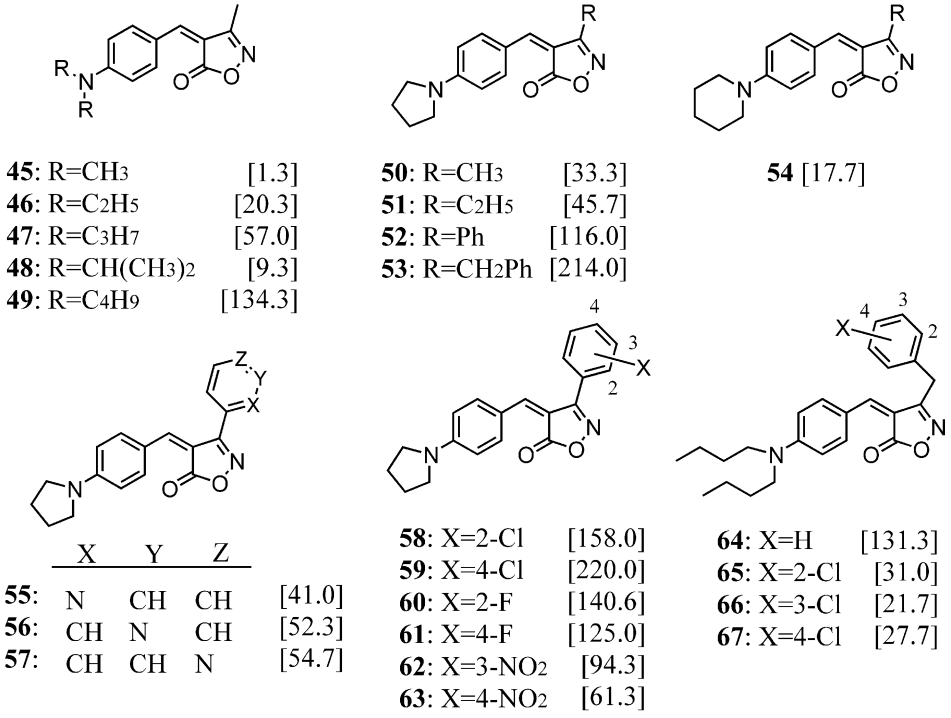
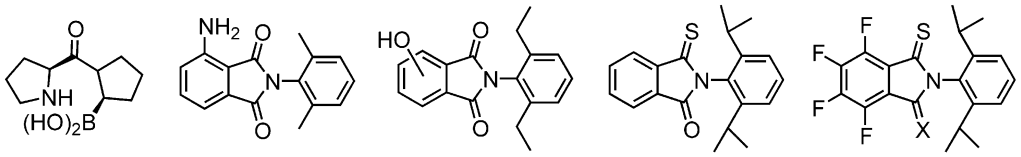


Figure 11. Structures of potent oxazolone-type anti-androgens. Values in parentheses are the relative affinity of the compound to AR compared with that of flutamide (**9**). Affinity of flutamide (**9**) toward AR was defined as [1.0].

tive toward another aminopeptidase, aminopeptidase N (APN), while the other phthalimides also inhibit APN.[5,77,78] These compounds should be superior lead compounds for the development of novel, non-peptide, small-molecular DPP-IV-specific inhibitors and DPP-IV/APN dual inhibitors.

Puromycin-sensitive aminopeptidase

During our structural development studies of thalidomide, we noticed that some of our compounds affect cell morphology. The change of the cell shape is similar to that induced by classical aminopeptidase inhibitors, bestatin (**75**) and actinonin (**76**) (Figs 14 and 15). Bestatin is known to be inactive toward DPP-IV, but possesses a wide range of inhibitory activities toward various aminopeptidases, including neutral alanine-aminopeptidases, APN and puromycin-sensitive aminopeptidase (PSA), which have been reported to play a critical role in tumor cell invasion and metastasis, though the detailed physiological role of the latter enzyme has not been clarified yet.[5,6,79] Bestatin (**75**) has been used clinically as an anti-tumor agent. Although some of our phthalimides (**69**–**71**, **73** and **74**, Fig. 13) showed moderate aminopeptidase-inhibiting activity, they are not specific. Our preliminary structural development studies aimed at a specific aminopeptidase inhibitor led us to homophthalimide analogues, as was the



68 [88.2] **69** [60.1] **70**: 4-OH [43.3] **71**: 5-OH [43.2] **72** [57.8] **73**: X= O [30.4] **74**: X= S [36.7]

Figure 13. Structures of potent DPP-IV inhibitors. Values in parentheses are the IC_{50} values (μM).

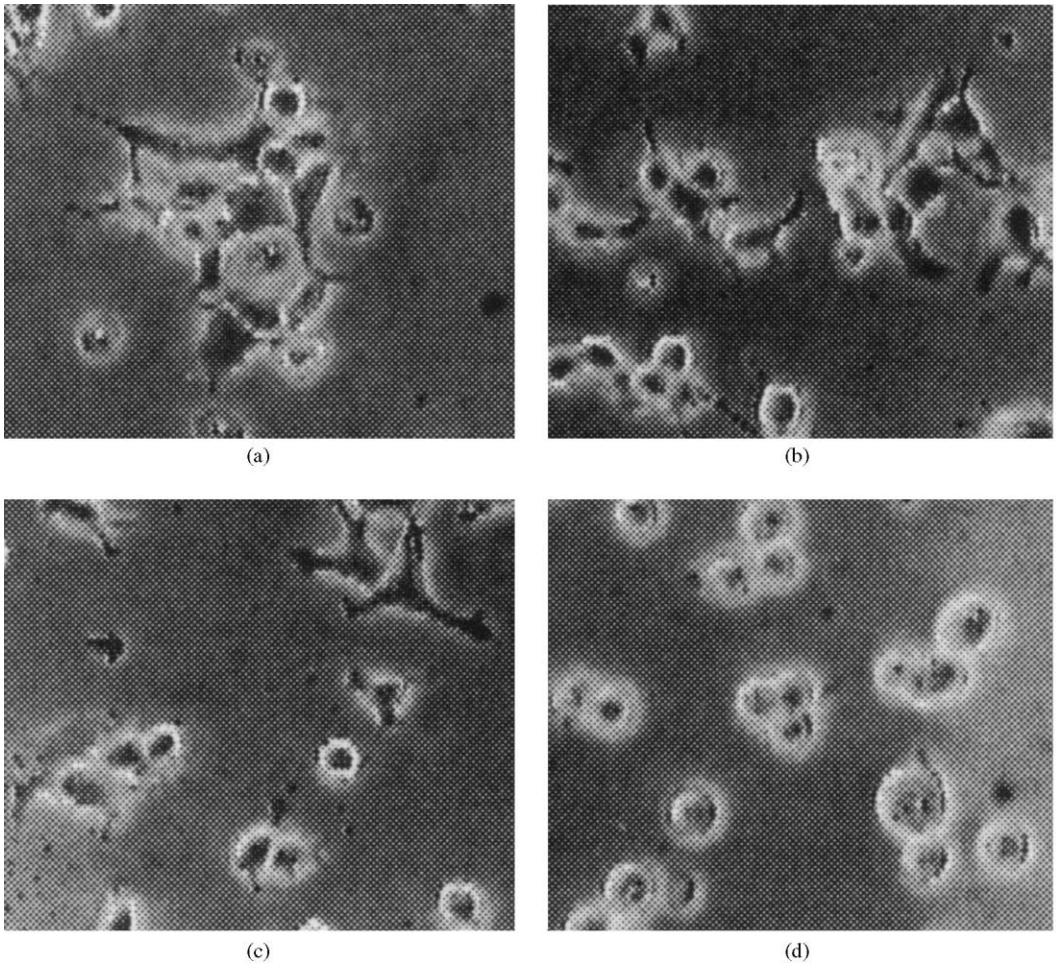


Figure 14. Effects of bestatin (**75**) and PIQ-22 (**78**) on cell morphology.[81] B16F10/L5 cells (a) were treated with 100 μM bestatin (b), 10 μM (c) and 100 μM (d) PIQ-22.

case for TP/PD-ECGF inhibitors (**32**–**34**, Fig. 7) (vide supra).[5,78-81] Among them, PIQ-22 (**78**, Fig. 15) is the most potent specific aminopeptidase inhibitor with an IC_{50} value much lower than that of bestatin (**75**) or actinonin (**76**). A structure–activity relationship study indicated that ethyl substitutents at the *ortho*-positions are optimum for the activity.[5,78,79] Though the decarbonylated analogue (**81**) retained moderate activity, the *N*-(2,6-diethylphenyl)homophthalimide structure seems to be strictly recognized. Analysis of the effect of introduction of an electron-donating or -withdrawing substituent (**78** and **79**) on the aminopeptidase-inhibiting activity suggested the possible importance of keto/enol tautomerism of the homophthalimide compounds.[79] Though an enol tautomer should be less stable than a keto form, the former might be the active form in aminopeptidase inhibition.

In accordance with its potent aminopeptidase-inhibiting activity, PIQ-22 (**77**) showed much more potent tumor cell invasion-inhibiting activity than bestatin (**75**) or actinonin (**76**), as evaluated in the human plasma fibronectin-coated Chemotaxicell chamber test using mouse melanoma B16F10/L5 cells (Fig. 16).[81] This potent inhibition by PIQ-22 (**78**) of tumor cell invasion seems to be elicited through inhibition of cell extension by aminopeptidase inhibition.[81] Isolation and characterization of the target aminopeptidase of PIQ-22 revealed

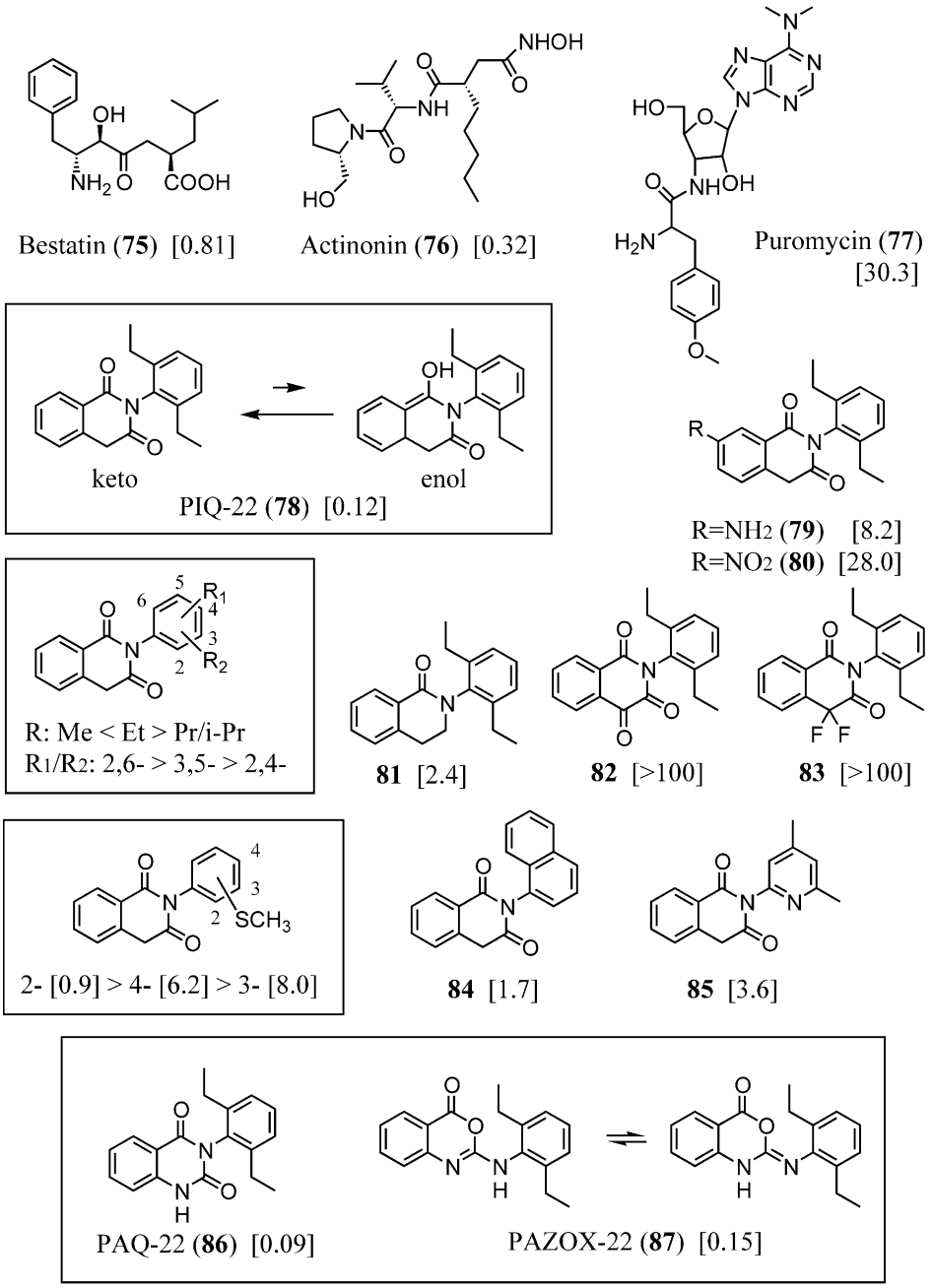


Figure 15. Structures of classical aminopeptidase inhibitors (**75**–**77**) and novel homophthalimimde type inhibitors and related compounds (**78**–**87**). Values on parentheses are the IC_{50} values (μg/mL).

that PIQ-22 is a potent and specific inhibitor of PSA.[79] This suggests that PSA could be a novel target molecule for the development of anti-metastatic agents. PIQ-22 is completely inactive toward other aminopeptidases, including APN, which has almost the same substrate selectivity with PSA, and leucine aminopeptidase (LAP), against which bestatin and actinonin are potently active.[79] Lineweaver–Burk plot analysis indicates that PIQ-22 (**78**) is a non-competitive inhibitor of PSA, while puromycin (**77**) and bestatin are competitive inhibitors.[79] This mode of action might explain the high specificity of PIQ-22 for PSA. Generally, aminopeptidase family members possess similar substrate selectivity, with similar structures of the substrate-binding pocket. Therefore, competitive inhibitors generally cross-inhibit aminopeptidases, as bestatin does. Because PIQ-22 is a non-competitive inhibitor, it is supposed that PIQ-22 binds at a specific site of PSA other than its substrate-binding site.

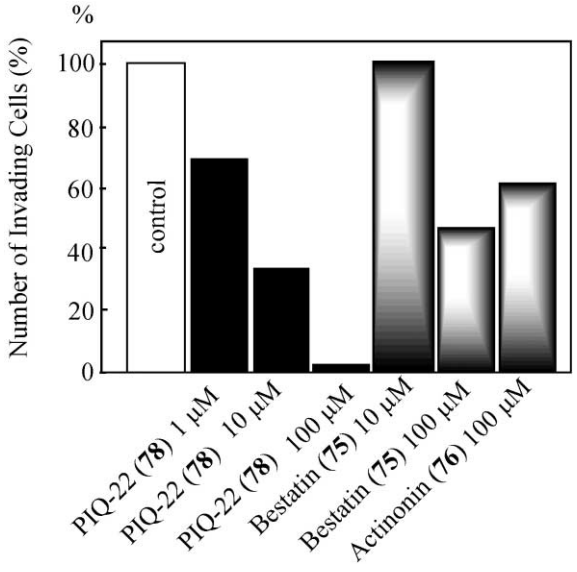
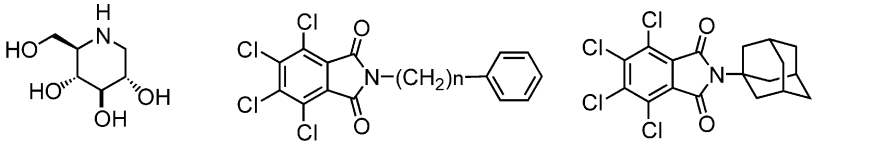


Figure 16. Effects of PIQ-22 (**78**), bestatin (**75**) and actinonin (**76**) on the invasion of B16F10/L5 cells into matrigel.

Though PIQ-22 (**78**) is a superior, potent, specific PSA inhibitor which potently inhibits tumor cell invasion, the compound possesses disadvantageous characteristics for clinical applications. The major problem is its easy oxidation at the benzylic site to give an inactive triketo derivative (**82**).[6,79] To avoid this oxidation, the difluoro analogue (**83**) was designed and synthesized. However, the difluoro analogue **83** was also inactive, suggesting the possible importance of the benzylic proton(s).[6,80] Next, PAQ-22 (**86**) and PAZOX-22 (**87**) were designed and prepared (Fig. 15).[80] As expected, PAQ-22 and PAZOX-22 showed potent and specific PSA-inhibiting activity, comparable to that of PIQ-22. In addition, these two compounds overcame another problem of PIQ-22 for clinical application, that is solubility. These PSA-specific, potent, non-peptide, small-molecular inhibitors should be useful as probes to investigate in detail the physiological function of PSA and as lead compounds to develop superior anti-metastatic agents.

α-Glucosidase Inhibitors Derived from Thalidomide

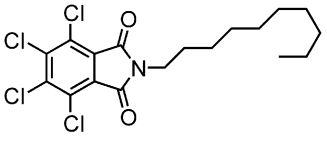
Of the six pharmacological effects of thalidomide mentioned in the introduction part of this article, that is (1) anti-cachexia effect, (2) anti-tumor promotion effect, (3) anti-angiogenic effect, (4) anti-cell invasion effect, (5) anti-viral effect, and (6) hypoglycemic effect, only the anti-cachexia effect (1) can definitly be interpreted in terms of TNF-α production-regulating activity. The anti-tumor promotion effect (2) can also be partly interpreted in terms of the same activity, but is more likely to be mainly due to anti-androgenic activity, especially in the case of prostate cancer. Anti-angiogenic effect (3) can be interpreted partly in terms of TNF-α production-regulating activity and partly TP/PD-ECGF-inhibiting activity. The latter activity might also play a role in the anti-viral effect (6). The anti-viral effect, especially against immunodeficiency virus (HIV), might be partly explained by TNF-α production-regulating activity (vide supra).[34] The anti-cell invasion effect (4) can be interpreted in terms of PSA-inhibiting activity. As for the remaining effects, (5) and in part (6), we suspected that α-glucosidase-inhibiting activity might be important.



dNM (**88**) [34.6]

89: n=0 [2.6]
90: n=1 [10.9]
91: n=2 [6.0]
92: n=3 [4.5]
93: n=4 [2.0]
94: n=5 [3.5]
95: n=6 [7.0]
96: n=10 [6.8]

97 [2.9]



98 [0.7]

Figure 17. Structures of 1-deoxynojirimycin (**88**) and novel tetrachlorophthalimide type α-glucosidase inhibitors (**89**–**98**). Values in parentheses are the IC_{50} values (μM).

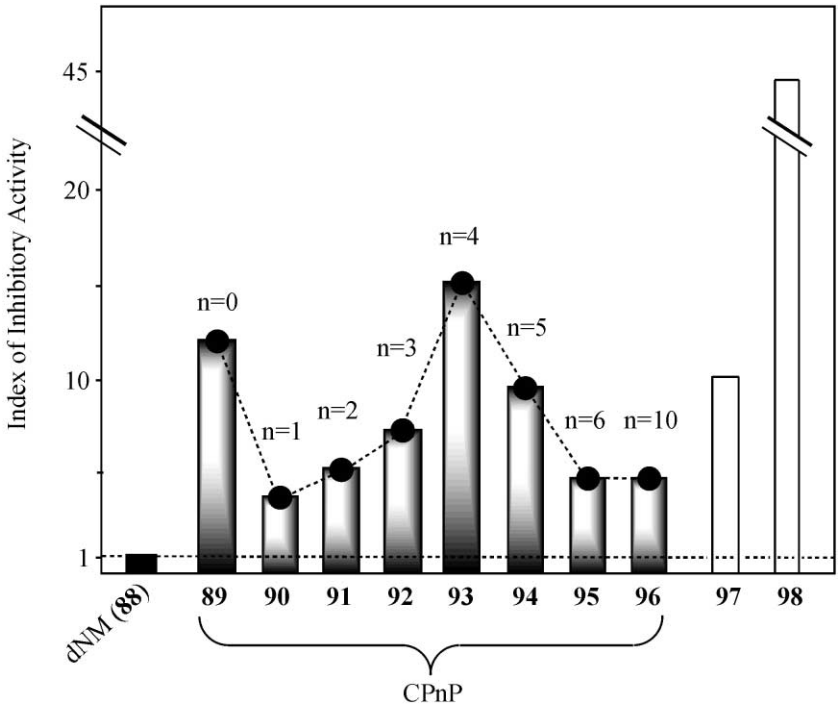


Figure 18. α-Glucosidase-inhibiting activity of dNM (**88**) and tetrachlorophthalimide-type compounds (**89**–**98**). Vertical scale: index (ratio of IC_{50} values) of α-glucosidase-inhibiting activity. The inhibitory activity of dNM was defined as 1.0.

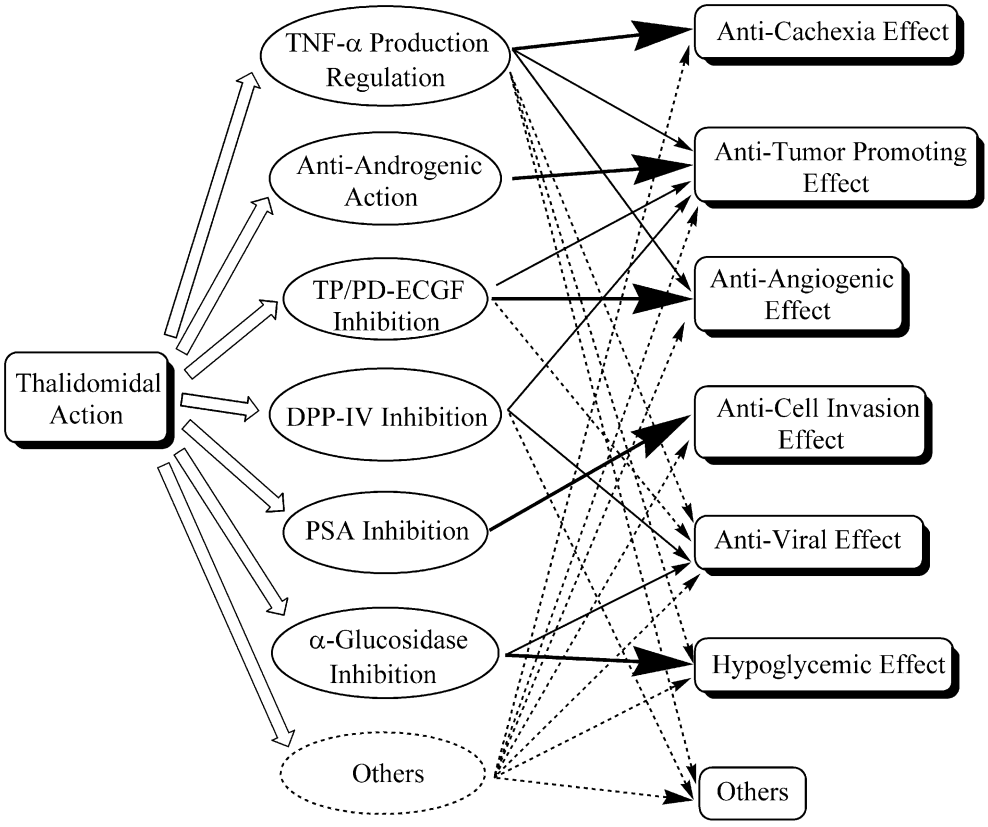


Figure 19. Pharmacological effects elicited by thalidomide and their possible target phenomena/molecules. ⇨: established or possible target; ➔: major contribution, ⟶: partial contribution; ⇢: minor or unknown contribution.

α-Glucosidase is an enzyme which catalyzes the final step in the digestion of carbohydrate. Inhibitors of this enzyme may retard the uptake of dietary carbohydrates and suppress post-prandial hyperglycemia, and could be useful in the treatment of diabetes, obesity, and certain forms of hyperlipoproteinemia. They also have potential as anti-viral agents controlling viral infectivity through interference with the normal biosynthesis of N-linked oligosaccharides by glycosidation of viral coat/envelope glycoproteins, and are being investigated for the treatment of both cancer and AIDS.[82,83] A well-established classical α-glucosidase inhibitor is 1-deoxynojirimycin (dNM: **88**, Fig. 17). Some derivatives of dNM have been shown to be effective against AIDS and B- and C-types of viral hepatitis.[84]

We therefore screened our compounds for α-glucosidase-inhibiting activity. Several compounds already mentioned in this article showed moderate activity, but the potent activity was exhibited by tetrachlorophenylphthalimide (CP0P: **89**, Fig. 17), whose IC_{50} value is one order lower than that of dNM (**88**).[85] Tetrachlorination at the phthaloyl moiety seems to be critical for this potent activity, because the corresponding non-halogenated analogue, *N*-phenylphthalimide (**90**), and the tetrafluorinated analogue (**91**), are inactive or very weak inhibitors.[78] On the basis of these screening results, we developed the structure of CP0P (**89**), using α-glucosidase-inhibiting activity as a guide.[6,85–87]

In the structure–activity relationship study of dNM (**88**), the importance of the three-dimensional position of a hydrophobic group has been emphasized. This led us to prepare analogues of CP0P (**89**) by inserting methylene units between the phthalimide and the phenyl moieties (**90**–**96**, Fig. 17).[85,86] All of these compounds

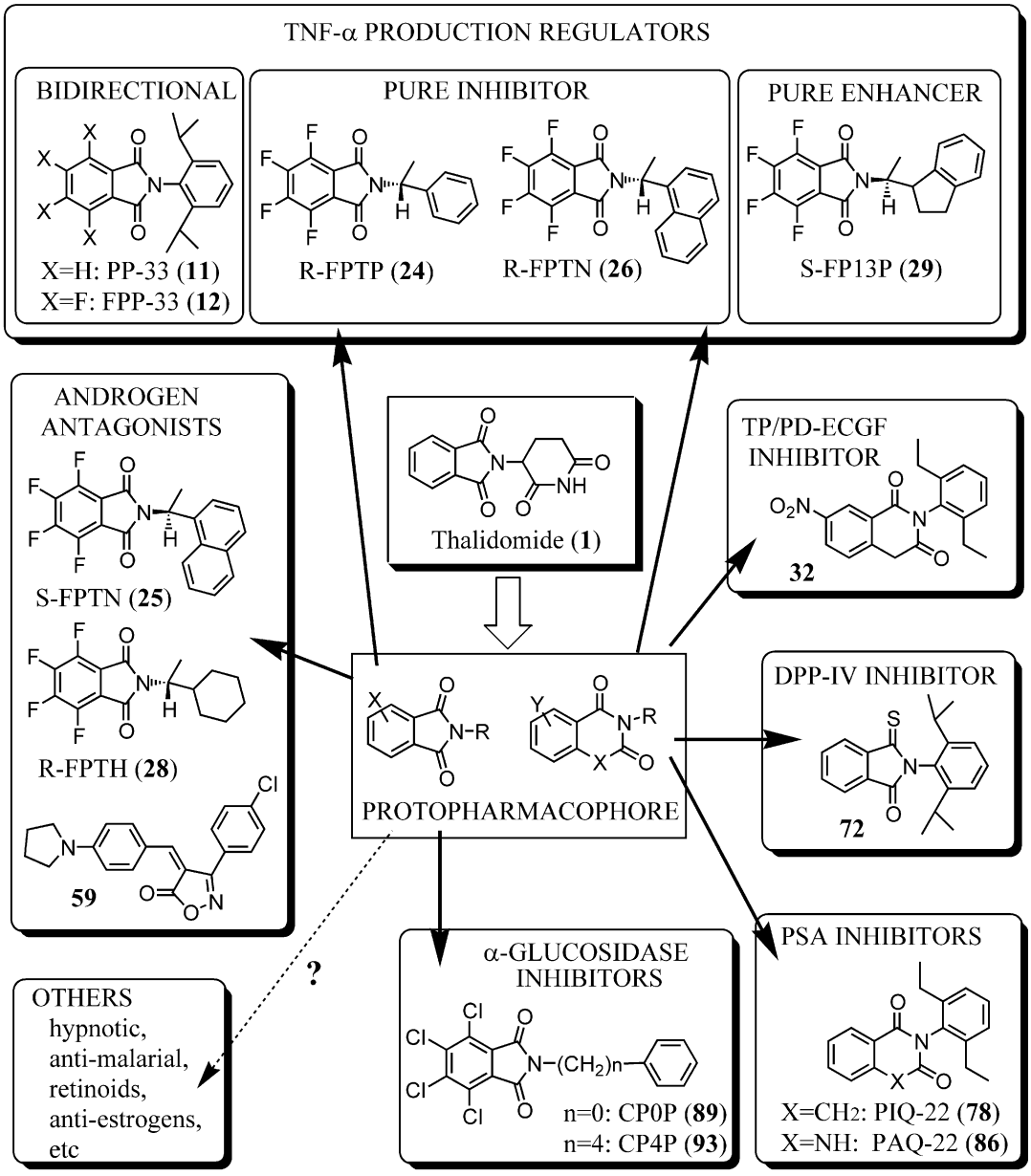


Figure 20. Typical biological response modifiers and enzyme inhibitors derived from thalidomide.

were shown to be potent α-glucosidase inhibitors (Fig. 18). Comparison of the IC_{50} values indicates that CP0P (**89**) is about 13 times more potent than dNM (**88**). Insertion of one methylene unit, that is CP1P (**90**), resulted in a decrease of the activity, but further elongation of the methylene spacer increased the activity with the maximum at four methylene units, CP4P (**93**). Further elongation caused a decrease of the activity again. This biphasic curve (Fig. 18) implies that the mechanisms of α-glucosidase inhibition by CP0P (**90**) and CP4P (**93**) are different. In fact, Lineweaver–Burk plot analysis indicated that CP0P is a non-competitive inhibitor, while CP4P and dNM are competitive inhibitors. Identification of the binding site by photoaffinity labeling followed by location of the labeled site(s) by the endoproteinase combination technique[88–90] is under way.

N-Adamantyl (**97**) and *N*-carboranyl derivatives are also non-competitive potent inhibitors.[86,87] Among competitive inhibitors, the *N*-decyl analogue (**98**) which corresponds to CP4P (**93**) possesses more potent activity than CP4P (Figs 17 and 18).[86]

Discussion and Concluding Remarks

Our studies have indicated that the effectiveness and potential of thalidomide for the treatment of various diseases can not be attributed solely to its TNF-α production-regulating activity. Thalidomide should be recognized as a multi-target drug, acting on AR, TP/PD-ECGF, DPP-IV, PSA, and α-glucosidase, at least (Fig. 19). As mentioned in this article, specific and potent compounds for each of these target molecules/phenomena could be prepared by appropriate modification of the thalidomide structure. This means that thalidomide (**1**) intrinsically possesses pharmacophores with a wide range of activities within its small molecular skeleton.

In our studies, we extracted the phthalimide and homophthalimide structures of thalidomide and by the usage of these skeletons, were able to obtain specific and potent TNF-α production regulators including bi-directional ones, pure inhibitors and pure enhancers, TP/PD-ECGF inhibitors, androgen antagonists, DPP-IV inhibitors, PSA inhibitors, and α-glucosidase inhibitors (Fig. 20). We believe that the same strategy will allow the development of hypnotic, anti-malarial, and other agents. Creation of anti-estrogens based on thalidomide structure was also partially successful. There may also be further biological effects of thalidomide other than those listed in Fig. 19. Inhibition of phosphodiesterases, cyclo-oxygenase 2, μ-calpain, and NO synthase, and a transcription factor NF-κB, are candidate actions,[6,91,92] as well as induction of cell differentiation.[93]

Thalidomide itself has relatively low potency, or is inactive, towards some of the target molecules listed in Fig. 19. There are at least two possible interpretation of this. One is that the overall effects of thalidomide on the target molecules are additive, and thereby appear as clinically useful effects. The other interpretation involves metabolism of thalidomide. Thalidomide is both chemically and metabolically labile, and various metabolites are known to be produced in vivo. Therefore, one or more metabolites might possess very potent activity on some or a specific target molecule among those listed above. In fact, teratogenicity of thalidomide has been reported to be attributed to a metabolite rather than to thalidomide itself. Also, some thalidomide metabolites are known to possess potent cell differentiation-inducing activity, which thalidomide itself does not possess.[93]

Finally, we should emphasize that this article is focused on our strategy for the structural development of thalidomide. Firstly, we identified six pharmacological and biological effects of thalidomide. We then formed a hypothesis as to the molecular target or target phenomenon which might be relevant to each pharmacological/biological effect. It is important to note that it does not matter whether thalidomide itself really binds to the hypothetical molecular target. The aim is simply to reproduce the relevant pharmacological/biological effect specifically by using newly prepared compounds. The third step is the creation of potent and specific compounds. Compounds thus prepared, of course, might merely mimic thalidomide's pharmacological/biological effects, but have no relation to thalidomide at the molecular mechanistic level. Nevertheless, we believe that, by preparing compounds that mimic the pharmacological/biological effects elicited by thalidomide (even if the molecular mechanism is different from that of thalidomide), and using combinations of the prepared compounds, we will be able to reproduce or reconstruct the spectrum of pharmacological/biological effects of thalidomide.

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References and Notes

1. Hashimoto, Y. *Cell Struct. Funct.* **1991**, *16*, 113.
2. Hashimoto, Y.; Shudo, K. *Cell Biol. Rev.* **1991**, *25*, 209.
3. Hashimoto, Y. *Recent Res. Develop. Org. Chem.* **2000**, *4*, 87.
4. Hashimoto, Y. *Curr. Med. Chem.* **1998**, *5*, 163.

5. Shimazawa, R.; Takayama, H.; Fujimoto, Y.; Komoda, M.; Dodo, K.; Yamasaki, R.; Shirai, R.; Koiso, Y.; Miyata, K.; Kato, F.; Kato, M.; Miyachi, H.; Hashimoto, Y. *J. Enzyme Inhibit*. **1999**, *14*, 259.
6. Kakuta, H.; Takahashi, H.; Sou, S.; Kita, T.; Nagasawa, K.; Hashimoto, Y. *Recent Res. Develop. Med. Chem*. **2001**, in press.
7. Rendall, T. *J. Am. Med. Assoc* **1990**, *263*, 1474.
8. Ochonisky, S.; Revuz, J. *Eur. J. Dermatol*. **1994**, *4*, 9.
9. Kelsey, F. O. *Teratology* **1988**, *38*, 221.
10. Kanbayashi, T.; Shimizu, T.; Takahashi, Y.; Kitajima, T.; Takahashi, K.; Saito, Y.; Hishikawa, Y. *Sleep* **1999**, *22*, 113.
11. Lenz, W. *Lancet*. **1962**, *1*, 271.
12. McBride, W. G. *Lancet*. **1961**, *2*, 1358.
13. Lenz, W. *Teratology* **1988**, *38*, 203.
14. Rendall, T. *J. Am. Med. Assoc*. **1990**, *263*, 1467.
15. Sheskin, J. *Clin. Pharmacol. Ther*. **1965**, *6*, 303.
16. Raje, N.; Anderson, K. *New Eng. J. Med*. **1999**, *341*, 1606.
17. Moraes, M.; Russo, G. *Am. J. Med. Sci*. **2001**, *321*, 321.
18. Singhal, S.; Mehta, J. *Biodrugs* **2001**, *15*, 163.
19. Allen, B. R. *Brit. J. Dermatol*. **2001**, *144*, 227.
20. Adlard, J. W. *Anti-Cancer Drugs* **2000**, *11*, 787.
21. Thomas, D. A.; Kantarjian, H. M. *Curr. Opin. Oncol*. **2000**, *12*, 564.
22. Peuckmann, V.; Fisch, M.; Bruera, E. *Drugs* **2000**, *60*, 273.
23. Calabrese, L.; Fleischer, A. B. *Am. J. Med*. **2000**, *108*, 487.
24. Singhal, S.; Mehta, J. *Biodrugs* **2001**, *15*, 163.
25. Short, S. C.; Taish, D.; Dowe, A.; Hines, F.; Gore, M.; Brada, M. *J. Neuro-Oncol*. **2001**, *51*, 41.
26. Davies, F. E.; Raje, N.; Hideshima, T.; Lentzsch, S.; Young, G.; Tai, Y. T.; Lin, B.; Podar, K.; Gupta, D.; Chauhan, D.; Treon, S. P.; Richardson, P. G.; Schlossman, R. L.; Morgan, G. J.; Muller, G. W.; Stirling, D. I.; Anderson, K. C. *Blood* **2001**, *98*, 210.
27. Moraes, M.; Russo, G. *Am. J. Med. Sci*. **2001**, *321*, 321.
28. Hashimoto, Y. *Clin. Immunol*. **1999**, *31*, 577.
29. Gershon, D. *Nat. Med*. **1995**, *1*, 1230.
30. Kaplan, G. *Immunology* **1994**, *191*, 564.
31. Black, R. A.; Birde, T. A.; Mohler, K. M. *Ann. Rep. Med. Chem*. **1997**, *32*, 241.
32. Pfizenmaier, K.; Wajant, H.; Grell, M. *Cytokine Growth Factor Rev*. **1996**, *7*, 272.
33. Stephens, J. M.; Lee, J.; Pilch, P. F. *J. Biol. Chem*. **1997**, *272*, 971.
34. Makonkawkeyoon, S.; Limson-Pombre, R. N. R.; Moreira, A. L.; Schauf, V.; Kaplan, G. *Proc. Natl. Acad. Sci. U.S.A*. **1993**, *90*, 5974.
35. Uysal, K.; Wiesbrock, S. M.; Marino, M. W.; Hotamisligil, G. S. *Nature* **1997**, *389*, 610.
36. Natoli, G.; Costanzo, A.; Ianni, A.; Templeton, D. J.; Woodgett, J. R.; Balsano, C.; Levrero, M. *Science* **1997**, *275*, 200.
37. Miyachi, H.; Azuma, A.; Hioki, E.; Iwasaki, S.; Kobayashi, Y.; Hashimoto, Y. *Biochem. Biophys. Res. Comm*. **1996**, *224*, 426.
38. Miyachi, H.; Azuma, A.; Hioki, E.; Iwasaki, S.; Kobayashi, Y.; Hashimoto, Y. *Biochem. Biophys. Res. Comm*. **1996**, *226*, 439.
39. Hashimoto, Y. *Clin. Immunol*. **1997**, *29*, 1403.
40. Nishimura, K.; Hashimoto, Y.; Iwasaki, S. *Biochem. Biophys. Res. Comm*. **1994**, *199*, 455.
41. Shibata, Y.; Shichita, M.; Sasaki, K.; Nishimura, K.; Hashimoto, Y.; Iwasaki, S. *Chem. Pharm. Bull*. **1995**, *43*, 177.
42. Tsuji, M.; Koiso, Y.; Takahashi, H.; Hashimoto, Y.; Endo, Y. *Biol. Pharm. Bull*. **2000**, *23*, 513.
43. Miyachi, H.; Azuma, A.; Ogasawara, A.; Uchimura, E.; Watanabe, N.; Kobayashi, Y.; Kato, F.; Kato, M.; Hashimoto, Y. *J. Med. Chem*. **1997**, *40*, 2858.
44. Miyachi, H.; Ogasawara, A.; Azuma, A.; Hashimoto, Y. *Bioorg. Med. Chem*. **1997**, *5*, 2095.
45. Shibata, Y.; Sasaki, K.; Hashimoto, Y.; Iwasaki, S. *Chem. Pharm. Bull*. **1996**, *44*, 156.
46. Sasaki, K.; Shibata, Y.; Hashimoto, Y.; Iwasaki, S. *Biol. Pharm. Bull*. **1995**, *18*, 1228.
47. Shibata, Y.; Sasaki, K.; Hashimoto, Y.; Iwasaki, S. *Biochem. Biophys. Res. Comm*. **1994**, *205*, 1992.
48. Shibata, H.; Sasaki, K.; Nishimura, K.; Hashimoto, Y.; Iwasaki, S. *Biol. Pharm. Bull*. **1994**, *17*, 1532.
49. Sasaki, K.; Shibata, Y.; Nishiumra, K.; Hashimoto, Y.; Iwasaki, S. *Biol. Pharm. Bull*. **1994**, *17*, 1313.
50. Niwayama, S.; Loh, C.; Turk, B. E.; Liu, J. O.; Miyachi, H.; Hashimoto, Y. *Bioorg. Med. Chem. Lett*. **1998**, *8*, 1071.
51. Miyachi, H.; Koiso, Y.; Shirai, R.; Niwayama, S.; Liu, J. O.; Hashimoto, Y. *Chem. Pharm. Bull*. **1998**, *46*, 1165.
52. Miyachi, H.; Azuma, A.; Hioki, E.; Kobayashi, Y.; Iwasaki, S.; Hashimoto, Y. *Chem. Pharm. Bull*. **1996**, *44*, 1980.
53. Blaschke, G.; Kraft, H. P.; Fickentscher, K.; Koehler, F. *Arzneimittelforschung* **1979**, *29*, 1640.
54. Fabro, S.; Smith, R. L.; Williams, R. T. *Nature* **1967**, *215*, 296.
55. Nishimura, K.; Hashimoto, Y.; Iwasaki, S. *Chem. Pharm. Bull*. **1994**, *42*, 1157.
56. Miyachi, H.; Azuma, A.; Hioki, E.; Iwasaki, S.; Hashimoto, Y. *Bioorg. Med. Chem. Lett*. **1996**, *6*, 2293.
57. Azuma, A.; Miyachi, H.; Shibata, Y.; Hashimoto, Y.; Iwasaki, S. *Biol. Pharm. Bull*. **1996**, *19*, 1001.
58. D'Amato, R. J.; Loufhnan, M. S.; Flynn, E.; Folkman, J. *Proc. Natl. Acad. Sci. U.S.A*. **1994**, *91*, 4082.
59. Shimazawa, R.; Miyachi, H.; Takayama, H.; Kuroda, K.; Kato, F.; Kato, M.; Hashimoto, Y. *Biol. Pharm. Bull*. **1999**, *22*, 224.
60. Moghaddam, A.; Zang, H. T.; Fan, T. P.; Hu, D. E.; Lees, V. C.; Turley, H.; Fox, S. B.; Gatter, K. C.; Harris, A. L.; Bicknell, R. *Proc. Natl. Acad. Sci. U.S.A*. **1995**, *92*, 998.
61. Miyadera, K.; Simizawa, T.; Haraguchi, M.; Yoshida, H.; Konstanty, W.; Yamada, Y.; Akiyama, S. *Cancer Res*. **1995**, *55*, 1687.
62. Kita, T.; Takahashi, H.; Hashimoto, Y. *Biol. Pharm. Bull*. **2001**, *24*, 860.
63. Komori, A.; Suganuma, M.; Okabe, S.; Zou, X.; Tius, M. A.; Fujiki, H. *Cancer Res*. **1993**, *53*, 3462.
64. Sekine, K.; Ohuchi, H.; Fujiwara, M.; Yamasaki, M.; Yoshizawa, T.; Sato, T.; Yagishita, N.; Matsui, D.; Koga, Y.; Itoh, N.; Kato, S. *Nat. Genet*. **1999**, *21*, 138.
65. Miyachi, H.; Azuma, A.; Kitamoto, T.; Hayashi, K.; Kato, K.; Koga, M.; Sato, B.; Hashimoto, Y. *Bioorg. Med. Chem. Lett*. **1997**, *7*, 1483.
66. Eyrolles, L.; Kawachi, E.; Matsushima, Y.; Nakajima, O.; Kagechika, H.; Hashimoto, Y.; Shudo, K. *Med. Chem. Res*. **1992**, *2*, 361.
67. Kagechika, H.; Kawachi, E.; Hashimoto, Y.; Himi, T.; Shudo, K. *J. Med. Chem*. **1988**, *31*, 2182.
68. Kagechika, H.; Himi, T.; Namikawa, K.; Kawachi, E.; Hashimoto, Y.; Shudo, K. *J. Med. Chem*. **1989**, *32*, 1098.
69. Kagechika, H.; Kawachi, E.; Hashimoto, Y.; Shudo, K. *Chem. Pharm. Bull*. **1985**, *33*, 5597.
70. Kagechika, H.; Kawachi, E.; Hashimoto, Y.; Shudo, K. *Chem. Pharm. Bull*. **1986**, *34*, 2275.
71. Takahashi, H.; Ishioka, T.; Koiso, Y.; Sodeoka, M.; Hashimoto, Y. *Biol. Pharm. Bull*. **2000**, *23*, 1387.
72. Ishioka, T., Koiso, Y., Kubo, A; Muto, S.; Itai, A., Nagasawa, K., Hashimoto, Y. In preparation.
73. Kagechika, H.; Kawachi, E.; Hashimoto, Y.; Shudo, K. *Chem. Pharm. Bull*. **1984**, *32*, 4209.
74. Yaron, A.; Naider, F. *Crit. Rev. Biochem. Mol. Biol*. **1993**, *28*, 31.
75. Marguet, D.; David, F.; Vivier, I.; Bernard, A. M.; Pierres, M. *Adv. Neuroimmunol*. **1993**, *3*, 209.

76. Subramanyam, M.; Gutheil, W.; Bachovchin, W. W.; Huber, B. T. *J. Immunol.* **1993**, *150*, 2544.
77. Shimazawa, R.; Takayama, H.; Kato, F.; Kato, M.; Hashimoto, Y. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 559.
78. Miyachi, H.; Kato, M.; Kato, F.; Hashimoto, Y. *J. Med. Chem.* **1998**, *41*, 263.
79. Komoda, M.; Kakuta, H.; Takahashi, H.; Fujimoto, Y.; Kadoya, S.; Kato, F.; Hashimoto, Y. *Bioorg. Med. Chem.* **2001**, *9*, 121.
80. Kakuta, H.; Takahashi, H.; Nagasawa, K.; Hashimoto, Y. *Heterocycles* **2001**, *55*, 1433.
81. Kagechika, H.; Komoda, M.; Fujimoto, Y.; Koiso, Y.; Takayama, H.; Kadoya, S.; Miyata, K.; Kato, F.; Kato, M.; Hashimoto, Y. *Biol. Pharm. Bull.* **1999**, *22*, 1010.
82. Gruters, R. A.; Neefjes, J. J.; Tersmette, M.; deGoede, R. E. Y.; Tulp, A.; Huisman, H. G.; Miedema, F.; Ploegh, H. L. *Nature* **1987**, *330*, 72.
83. Karpas, A.; Fleet, G. W. J.; Dwek, R. A.; Petursson, S.; Namgoong, S. K.; Ramsden, N. G.; Jacob, G. S.; Rademacher, T. W. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 9229.
84. Zitzmann, N.; Mehta, A. S.; Carouee, S.; Tutters, T. D.; Platt, F. M.; McCauley, J.; Blumberg, B. S.; Dwek, R. A.; Block, T. M. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 11878.
85. Sou, S.; Mayumi, S.; Takahashi, H.; Yamasaki, R.; Kadoya, S.; Sodeoka, M.; Hashimoto, Y. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1083.
86. Takahashi, H.; Sou, S.; Yamasaki, R.; Sodeoka, M.; Hashimoto, Y. *Chem. Pharm. Bull.* **2000**, *48*, 1494.
87. Sou, S.; Takahashi, H.; Yamasaki, R.; Kakuta, H.; Endo, Y.; Hashimoto, Y. *Chem. Pharm. Bull.* **2001**, *49*, 791.
88. Sasaki, T.; Hashimoto, Y. *Methods in Mol. Biol.* **1998**, *89*, 293.
89. Sasaki, T.; Shimazawa, R.; Sawada, T.; Iijima, T.; Fukasawa, H.; Shudo, K.; Hashimoto, Y.; Iwasaki, S. *Biochem. Biophys. Res. Comm.* **1995**, *207*, 444.
90. Sawada, T.; Kobayashi, H.; Hashimoto, Y.; Iwasaki, S. *Biochem. Pharmacol.* **1993**, *45*, 1387.
91. Rowhand, T. L.; McHugh, S. M.; Deighton, J.; Ewan, P. W.; Dearman, R. J.; Kimber, I. *Int. Immunopharmacol.* **2001**, *1*, 49.
92. Anon/Celgene, Drug News Perspect **2000**, *13*, 146.
93. Hatfill, S. J.; Fester, E. D.; de Beer, D. P.; Bohm, L. *Leukemia Res.* **1991**, *15*, 129.

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