Structural Development of Biological Response Modifiers Based on Retinoids and Thalidomide

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Abstract: The full-scale commercial appearance of antibiotics in the 1950's caused a shift of the nature of our lethal diseases from infectious/acute to non-infectious/chronic. In this situation, biological response modifiers (BRM's), which are not based on selective toxicity, are expected to be useful. There exist several types of BRM's, including retinoids which act directly on cells at the gene expression level, and thalidomide (and related molecules) which modulate internal circumstances of our body. We have been engaged in medicinal chemical/structural development studies based on these bio-active compounds. Retinoids include all-*trans*-retinoic acid (ATRA), a major active form of vitamin A (retinol), and its bio-isosters, which elicit their biological effects by binding to their nuclear receptors, RAR's. ATRA has been used in differentiation therapy [typically for the treatment of acute promyelocytic leukemia (APL)] and the treatment of dermatological diseases. Our structural development studies of retinoids, including computer-assisted molecular design has yielded class/subtype-selective agonists, synergists and antagonists of RAR's and their partner nuclear receptors, RXR's. Thalidomide elicits a wide range of pharmacological effects, including anti-cachexia, anti-angiogenic and anti-metastatic activities. We have found that thalidomide is a multi-target drug. Hypothetical target events/molecules of thalidomide include TNF-α production, nuclear androgen receptor, aminopeptidases, and α-glucosidase. Specific and potent compounds for each of these target phenomena/molecules have been prepared by appropriate modification of the thalidomide structure, and are expected to be superior lead compounds for novel immunomodulators, anti-angiogenic agents, and anti-tumor promoting agents.

Key words: Retinoid / Thalidomide / Structural development

INTRODUCTION

The full-scale commercial appearance of antibiotics in the 1950's has resulted in increased longevity in advanced nations. The highest mortality rates in Japan before the 1950's were due to infectious diseases such as tuberculosis. The death rates from these diseases dramatically dropped in the 1950's, 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 (BRM's), 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, *i.e.*, retinoids, which act directly on malignant cells at the gene expression level to make them behave normally [3,5,8], and thalidomide-related molecules, which modulate internal processes of our body to restore a normal state [4-6]. In this paper, our researches on structural development of BRM's are reviewed.

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RETINOIDS

Retinoid is a generic name for all-*trans*-retinoic acid (ATRA) and its bioisosters. ATRA is an active form of vitamin A (retinol), which is enzymatically oxidized to retinal, an important pigment in vision function, and further to ATRA, to which almost all of the biological activity of vitamin A in the maintenance of normal growth/life of mammals can be attributed. Retinoids, typically ATRA, have been used in differentiation-inducing therapy of tumors and the treatment of dermatological diseases. At the cellular level, their action mechanism is the regulation of cell differentiation, *i.e.*, not cytotoxicity, but alteration of the cell behavior. The most successful example of retinoid use is in the treatment of acute promyelocytic leukemia (APL). APL had been a lethal disease, but now, it can be cured by differentiation therapy using retinoids. Retinoids induce differentiation of leukemia cells, leading to complete remission (CR) to the patients without cell toxicity.

All retinoids elicit their effects by binding to their specific nuclear receptor, RAR (Fig. 1). RAR is a member of steroids/thyroid/vitamin D_3 nuclear receptor superfamily, and acts as a dimer with another nuclear receptor, retinoid X receptor (RXR). For both RAR and RXR, three subtypes, α, β and γ, exist. The clinical usefulness of ATRA, as well as its unfavorable chemical nature, has led many researchers to develop synthetic retinoids targeting RAR's. The major

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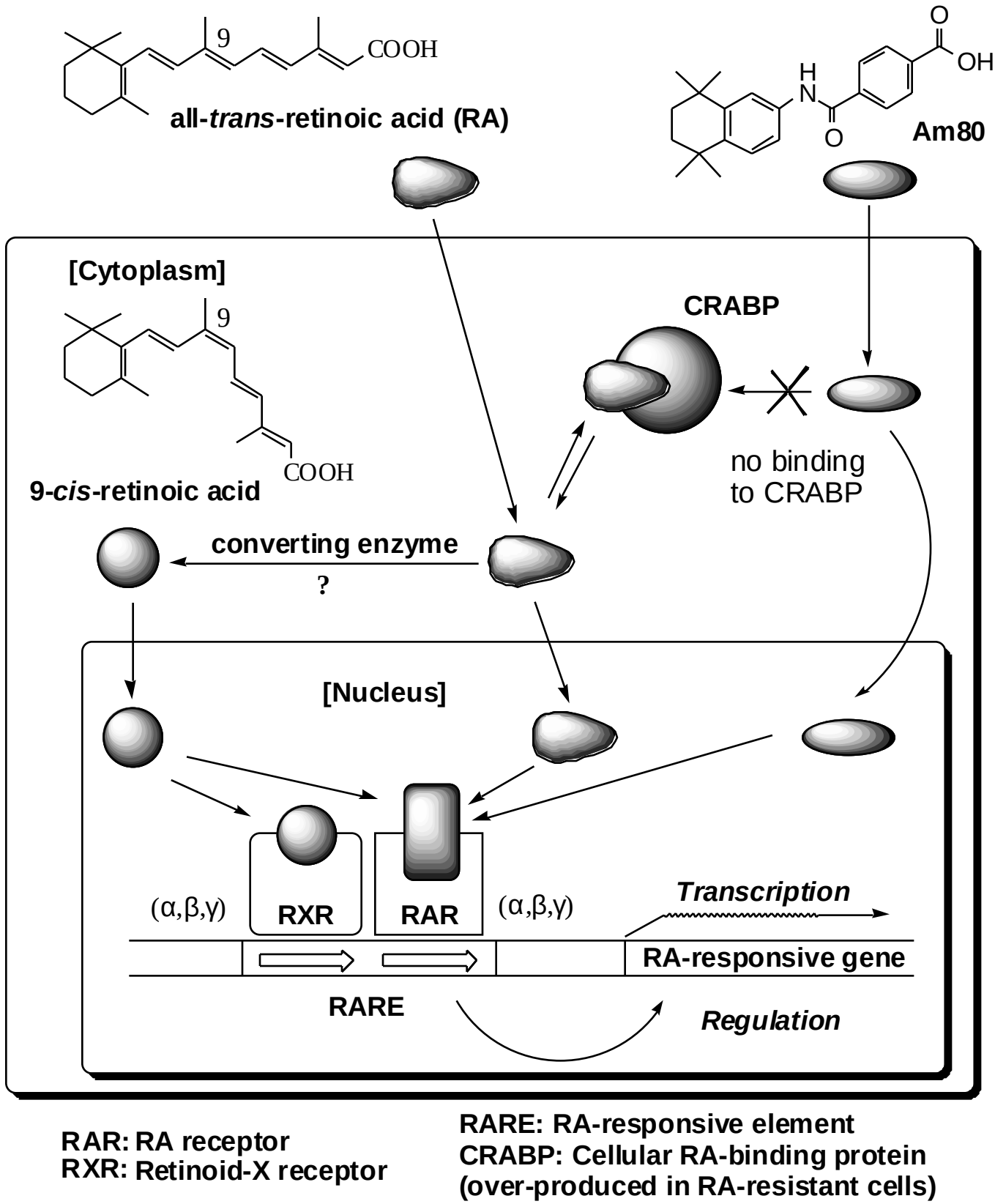

Fig. (1). Schematic illustration of the molecular mechanisms of retinoids.

disadvantages of ATRA include high lipophilicity and instability. In addition, appearance of ATRA-resistant cells during differentiation therapy is partly explained by over-production of cellular retinoic acid binding protein (CRABP), which is one of the fatty acid binding proteins.

To overcome these problems, we introduced heteroatoms into structural mimics of ATRA to increase polarity. Our typical retinoids (named retinobenzoic acids) are shown in Fig. 2 [3,5,8,9,25]. Am80 (Fig. 1 and 2) is an example of a potent synthetic retinoid [3,5,8,9]. Because Am80 does not

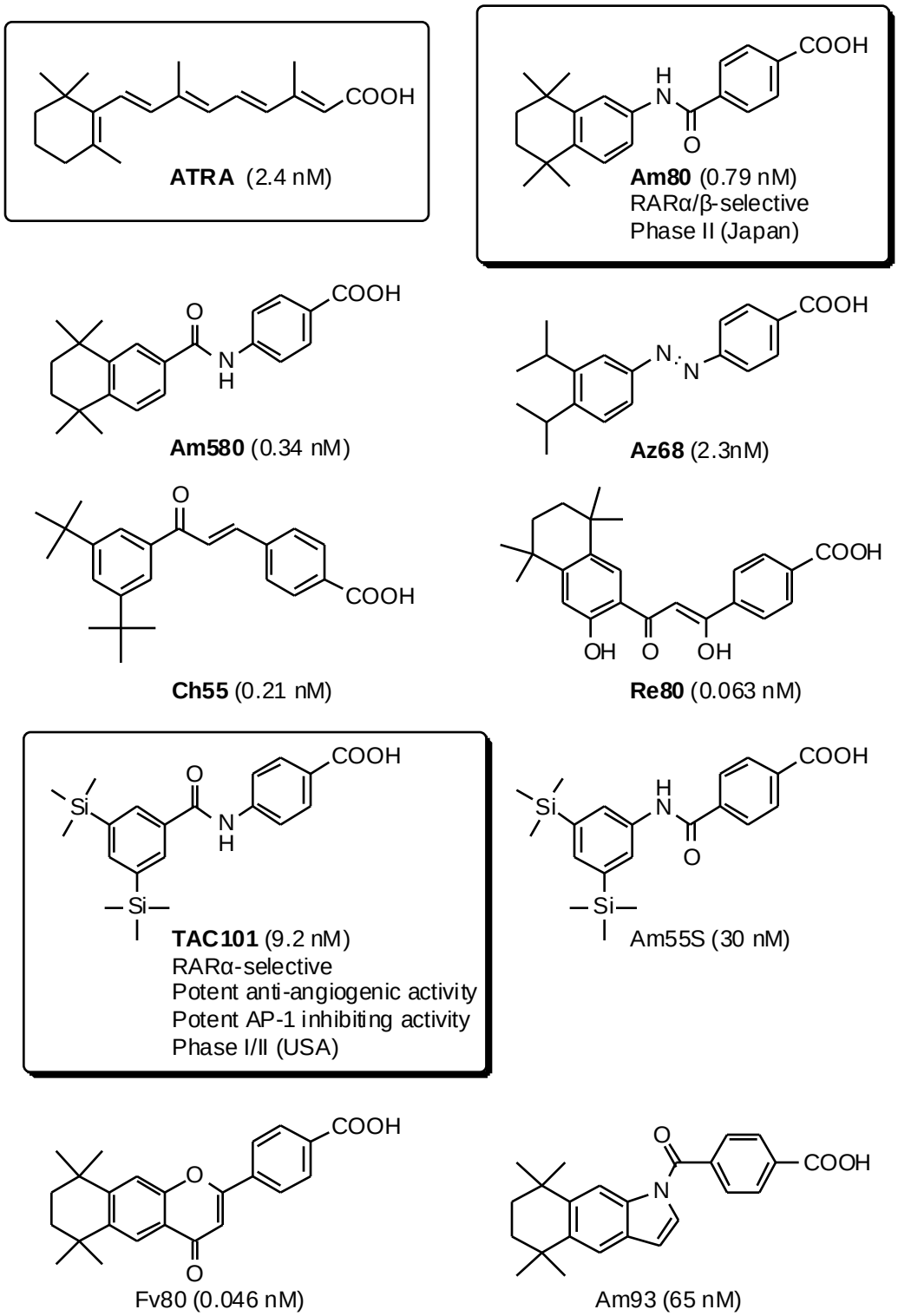

Fig. (2). ATRA and typical retinobenzoic acids. Values in parenthesis are the ED_{50} values of cell differentiation-inducing activity on human leukemia HL-60 cells.

bind CRABP [7], it is active toward CRABP-rich ATRA-resistant cells. In addition, Am80 has been established to be RARα/β-selective [3,5,7]. It has been assigned as an orphan drug in Japan, and is under phase II clinical trial for the treatment of APL. Another retinobenzoic acid which is under clinical study is TAC-101 (Fig. 2) [25]. TAC-101 is a potent RARα-selective retinoid, and possesses a unique structure with two trimethylsilyl groups forming a hydrophobic moiety which plays a critical role in the activation of RARα. In addition to its potent cell-differentiation-inducing activity, it also possesses anti-angiogenic activity and AP-1 inhibitory activity. TAC-101 effectively prolongs the survival of solid tumor-bearing mice (more efficiently than 5-fluorouracil or cisplatin), and is under phase I/II trial in the United States.

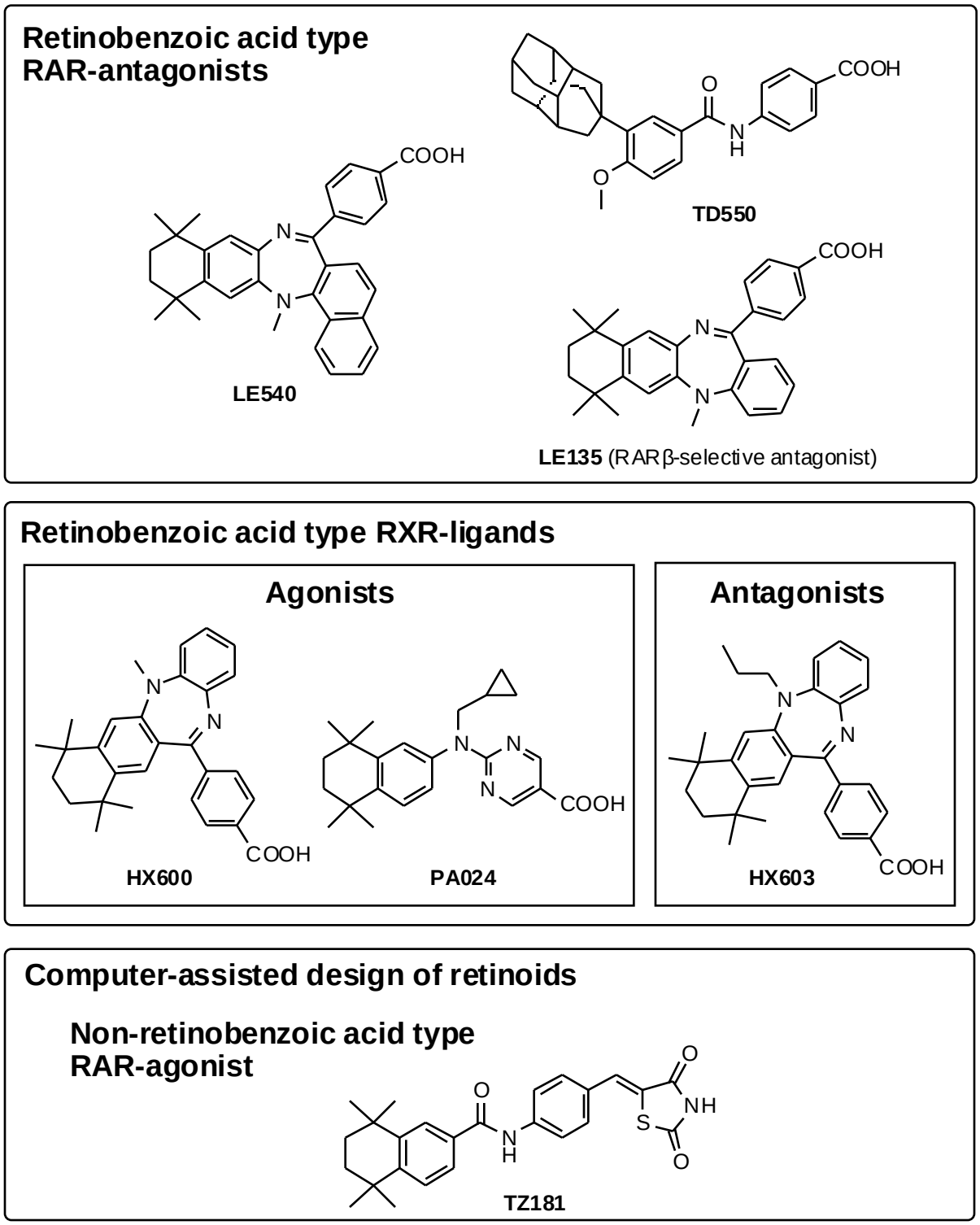

Fig. (3). Typical RAR and RXR ligands.

Further studies on structural development of retinobenzoic acids led to RAR-antagonists (*ex.* TD550, LE540 and LE135) [1,2], RXR-agonists (*ex.* HX600) and RXR-antagonists (*ex.* HX603) [24] (Fig. 3). RAR-antagonists were designed on the basis of the ligand superfamily concept, which stresses the importance of the ligand-induced conformational change of RAR accompanying the folding of helix 12 [2]. Therefore, all of the RAR-antagonists thus designed are based on a retinobenzoic acid structure with steric hindrance, *i.e.*, structures which bind to the ligand-fitting pocket of RAR, but sterically hinder the folding of helix 12. Among them, LE135 is RARβ-selective antagonist [1,12,13]. RXR-agonists and antagonists were designed on the basis of a similar strategy to that used for RAR-agonists and antagonists, respectively, and the RXR-agonists act as retinoid synergists which enhance retinoidal activity elicited by low-dose RAR-agonists [24].

Structurally different types of RAR-agonists and antagonists *i.e*., RAR-agonists of non-benzoic acid structure and RAR-antagonists without a bulky hydrophobic group(s), respectively, could be designed by computer-assisted molecular design. Computer-assisted retrieval from a chemical database on the basis of docking study with a three-dimensional RAR structure followed by optimization of the structure resulted in RAR-agonists without a carboxylic acid moiety (*e.g.,* TZ181) (Fig. 3) and RAR-antagonists without a bulky hydrophobic group(s) (*unpublished results*). The computer-assisted molecular design methodology used can be applied not only to compound databases, but also to create a virtual combinatorial library in the computer.

THALIDOMIDE

Thalidomide is a hypnotic/sedative drug which was once withdrawn from the market because of its severe teratogenicity [4-6]. However, thalidomide has been established to be useful for the treatment of leprosy, and the drug was formally approved for this purpose by the FDA (USA) in 1998 under critical control. Many reports have appeared on its therapeutic usefulness in various diseases, including various cancers, rheumatoid arthritis, graft-*versus*-host diseases, acquired immunodeficiency syndrome (AIDS), colon cancer, and others [4-6]. 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 (*A*) anti-cachexia activity (cachexia is a major direct cause of cancer death), (*B*) anti-tumor-promoting activity, (*C*) anti-angiogenic activity, (*D*) anti-cell invasion (anti-metastasis) activity, (*E*) anti-viral activity, and (*F*) hypoglycemic effect (Fig. 4). Though thalidomide affects production of various cytokines, the prevailing hypothesis is that all of the beneficial effects of thalidomide are elicited through regulation of tumor necrosis factor-α (TNF-α) production.

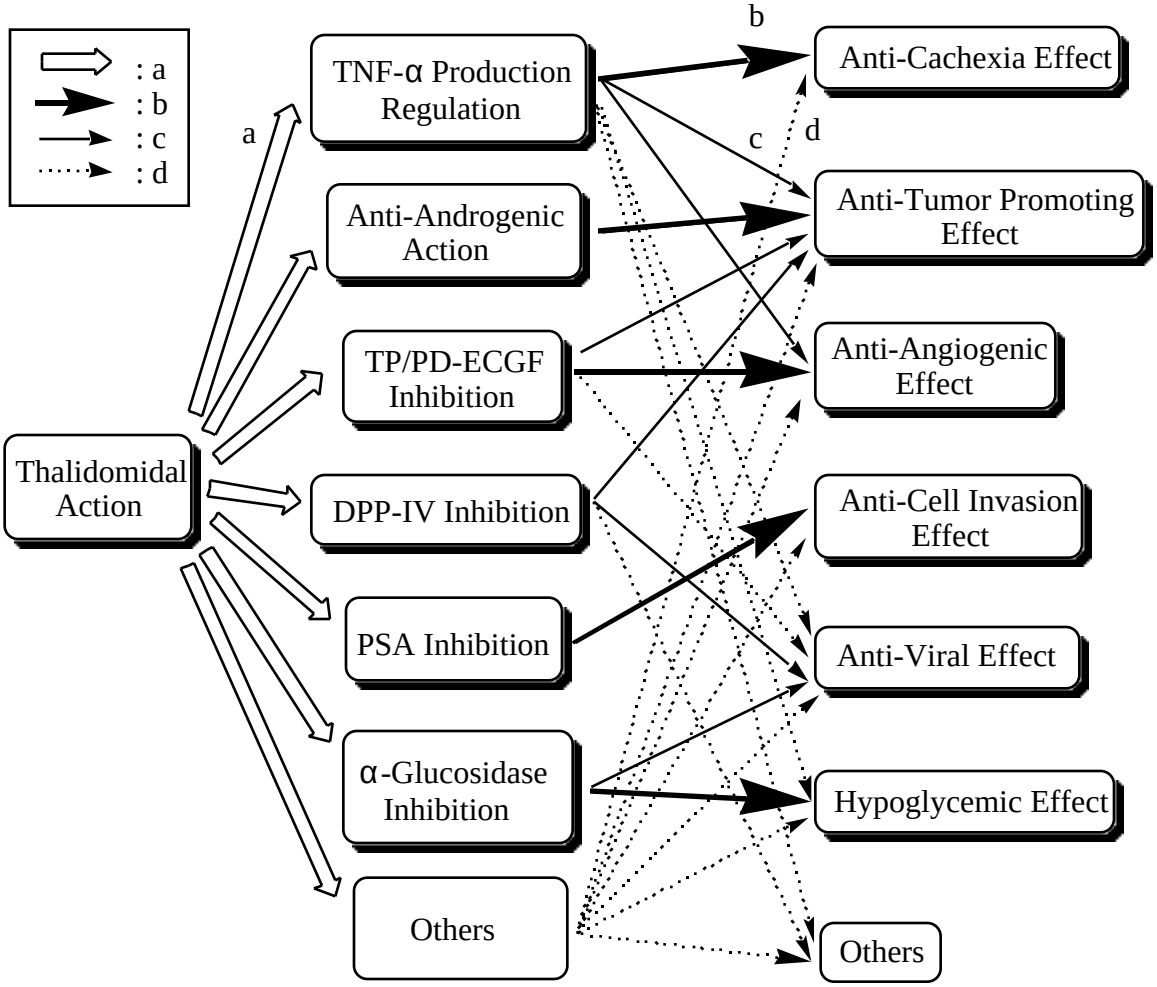

Fig. (4). Pharmacological effects elicited by thalidomide and their possible target phenomena/molecules.

a: Established or possible target.

b: Major contribution.

c: Partial contribution.

d: Minor or unknown contribution.

Our studies on the TNF-α production-regulating activity of thalidomide revealed that the effect elicited by the drug is bidirectional, depending on both cell types and cell stimulators [4-6,15,16,18]. Studies on structural development of thalidomide resulted in very potent bidirectional TNF-α production regulators (*e.g.,* PP-33 and FPP-33) and complete separation of the bidirectionality [pure inhibitors (*e.g.,* R-FPTP and R-FPTN) and pure enhancers (*e.g.,* S-FP13P) (Fig. 5) [4-6,14,18,20]. Some of our bi-directional TNF-α production-regulators and inhibitors prolonged the life span of mice with cachexia induced by lipopolysaccharide injection. 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. Some of our TNF-α production-regulators, especially R-FPTP, showed more potent anti-angiogenic activity than thalidomide at a much lower dose than thalidomide [6]. Clinical application studies of these potent anti-angiogenic compounds are in progress. However, the structure-activity relationship studies indicated that the pharmacological effects of thalidomide cannot be attributed to its TNF-α production regulating activity alone [4-6]. 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 (*A*-*F*) elicited by thalidomide.

For anti-angiogenic activity, we considered thymidine phosphorylase (TP)/platelet-derived endothelial cell growth factor (PD-ECGF) as a putative target molecule (Fig. 4). Our structural development study targeting TP/PD-ECGF-inhibiting activity yielded several homophthalimide analogs, including NPIQ (Fig. 5) which showed more potent inhibitory activity than the classical inhibitor, 5-nitrouracil [6,10]. NPIQ and related inhibitors are considered to be lead compounds for the development of novel type(s) of TP/PD-ECGF inhibitors.

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. To develop more potent anti-tumor-promoting agents, we focused on another endogenous tumor promoter, *i.e*., 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. Considering the effectiveness of thalidomide in the treatment of prostate cancer and its structural similarity to a classical androgen antagonist, DIMP, we expected that superior non-steroidal androgen antagonists might be prepared by structural development of thalidomide [6,17]. Androgens, typically testosterone and its active metabolite, 5α-dihydrotestosterone (DHT), 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. 4). Structural development studies of thalidomide based on anti-androgenic activity resulted in several compounds showing much more potent anti-androgenic activity than flutamide, which is widely used for the treatment of prostate cancer (*e.g.,* S-FPTN and R-FPTH) (Fig. 5) [4-6,17]. Further structural development assisted by computer (docking study using the three-dimensional structure of AR) resulted in oxazolone-type compounds, including OX59 (Fig. 5) [6]. Evaluation of AR-binding affinity showed that OX59 binds AR with an affinity 220-fold higher than that of flutamide. Evaluation of these novel, non-steroidal, potent androgen antagonists *in vivo* is in progress.

Concerning anti-cell invasion/adhesion activity, we focused on aminopeptidase inhibitory activity. Our structural development studies of thalidomide resulted in specific dipeptidyl peptidase IV (DPP-IV) inhibitors (*e.g.,* PPS-33) (Fig. 5) and specific puromycin-sensitive aminopeptidase (PSA) inhibitors (*e.g.,* PIQ-22 and PAQ-22) (Fig. 5) [6,10,11,19,21,22]. DPP-IV appears to be involved in various pathophysiological effects, including tumor cell adhesion and the entry of human immunodeficiency virus (HIV) into $CD4^+$ T cells, and therefore DPP-IV inhibitors are expected to be immunomodulators and to have potential pharmacological/clinical applications. Though the physiological role of PSA has not been clarified in detail yet, specific and potent inhibitors, PIQ-22 and PAQ-22 (Fig. 5), which are more potent than bestatin or actinonin, showed much more potent tumor cell invasion-inhibiting activity than bestatin or actinonin [6,10,11]. This suggests that PSA could be a novel target molecule for the development of anti-metastatic agents. PIQ-22 and PAQ-22 are completely inactive toward other aminopeptidases, including aminopeptidase N (APN), which has almost the same substrate selectivity as PSA, and leucine aminopeptidase (LAP), against which bestatin and actinonin are potently active. Lineweaver-Burk plot analysis indicates that PIQ-22 and PAQ-22 are non-competitive inhibitors of PSA, while puromycin and bestatin are competitive inhibitors [6,10,11]. This mode of action might explain the high specificity of PIQ-22 and PAQ-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 and PAQ-22 are non-competitive inhibitors, it is supposed that PIQ-22 and PAQ-22 bind at a specific site of PSA other than its substrate-binding site. 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.

Of the six pharmacological effects of thalidomide shown in Fig. 4, *i.e*., (*A*) anti-cachexia effect, (*B*) anti-tumor promotion effect, (*C*) anti-angiogenic effect, (*D*) anti-cell invasion effect, (*E*) anti-viral effect, and (*F*) hypoglycemic effect, only the anti-cachexia effect (*A*) can definitely be interpreted in terms of TNF-α production-regulating activity. The anti-tumor promotion effect (*B*) 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 (*C*) 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 (*F*). The anti-viral effect, especially against immunodeficiency virus (HIV), might be partly explained by TNF-α production-regulating activity. The anti-cell invasion effect (*D*) can be interpreted in terms of PSA-inhibiting activity. As for the remaining effects, (*E*) and in part (*F*), we suspected that α-glucosidase-inhibiting activity might be important. 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. A well-established classical α-glucosidase inhibitor is 1-deoxynojirimycin (dNM). Some derivatives of dNM have been shown to be effective against AIDS and B- and C-types of viral hepatitis. Our structural development studies based on α-glucosidase-inhibiting activity resulted in potent non-competitive inhibitors (*e.g.*, CP0P) and potent competitive inhibitors (*e.g.*, CP4P) (Fig. 5) [6,23]. Comparison of the IC_{50} values indicates that CP0P and CP4P are about 13 and 16 times more potent than dNM, respectively.

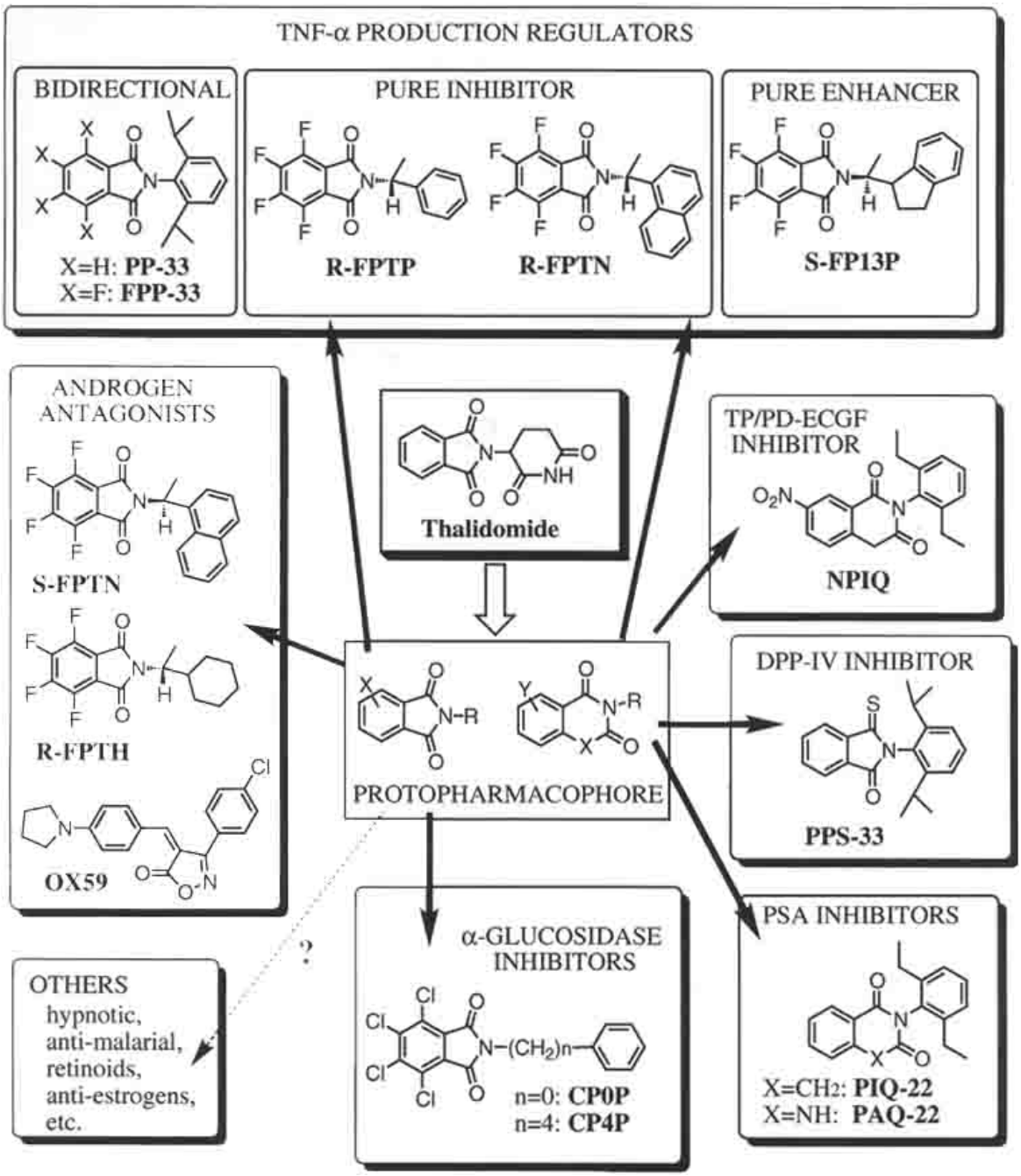

Fig. (5). Typical biological response modifiers and enzyme inhibitors derived from thalidomide.

DISCUSSION

We have been engaged in structural development studies of BRM's based on retinoids and thalidomide. Concerning retinoids, we have created various types of synthetic retinoids, including RAR-agonistic retinobenzoic acids (Fig. 1 and 2), their antagonists, and RXR-ligands (Fig. 3). Some of our retinoids are now under clinical phase I/II studies. Computer-assisted molecular design resulted in novel retinoids with completely different skeletons from retiobenzoic acids (Fig. 3). This expansion of the structural variation of retinoids should ultimately afford superior differentiation therapy medicaments.

Concerning thalidomide research, 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. 4). 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 intrinsically possesses pharmacophores with a wide range of activities in 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 bidirectional ones, pure inhibitors and pure enhancers, TP/PD-ECGF inhibitors, androgen antagonists, DPP-IV inhibitors, PSA inhibitors, and α-glucosidase inhibitors (Fig. 5). 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. 4. Inhibition of phosphodiesterases, cyclo-oxygenase 2, μ-calpain, and NO synthase, and a transcription factor NF-κB, are candidate actions, as well as induction of cell differentiation [6]. Thalidomide itself has relatively low potency, or is inactive, towards some of the target molecules listed in Fig. 4. 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.

Finally, we should emphasize 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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