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Structure–activity relationship study on benzoic acid part of diphenylamine-based retinoids

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ABSTRACT

Based on structure–activity relationship studies of the benzoic acid part of diphenylamine-based retinoids, the potent RXR agonist **4** was derivatized to obtain retinoid agonists, synergists, and an antagonist. Cinnamic acid derivatives **5** and phenylpropionic acid derivatives **6** showed retinoid agonistic and synergistic activities, respectively. The difference of the activities is considered to be due to differences in the flexibility of the carboxylic acid-containing substituent on the diphenylamine skeleton. Compound **7**, bearing a methyl group at the meta position to the carboxyl group, was an antagonist, dose-dependently inhibiting HL-60 cell differentiation induced by 3.3×10^{-10} M Am80.

Retinoids, which are natural and synthetic analogues of all-*trans*-retinoic acid (ATRA), play an important role in cell differentiation, proliferation and embryonic development in vertebrates,[1] and are used as therapeutic agents in the fields of dermatology and oncology.[2] Their biological activities are mediated by binding to and activating two types of specific nuclear receptors, retinoic acid receptors (RARs) and retinoid X receptors (RXRs), each of which has three subtypes, α, β, and γ.[3] The RARs and RXRs are ligand-inducible transcription factors, and their endogenous ligands are ATRA and 9-*cis*-retinoic acid (9CRA), respectively (Fig. 1).[4] Major retinoidal activities are elicited by RXR–RAR heterodimers, in which RXR is a silent partner.[5] Thus, the heterodimers can be activated by an RAR agonist such as Am80 (Fig. 1),[6] but not by an RXR agonist alone. Structurally, a hydrophobic moiety and a polar carboxyl group are necessary for binding to RARs and RXRs. The positional and spatial relations between these parts are significant for the activity. RAR ligands have a straight and planar skeleton, while RXR ligands have a bent and twisted structure. As established during the development of Am80, modification of the straight and planar ATRA structures with aromatic rings and the introduction of heteroatoms affords highly active and selective RAR ligands with remarkable chemical stability and good bioavailability.[7,8]

RXR agonists act as retinoid synergists, and dose-dependently increase the activity of low concentrations of retinoids.[9] Taking into account the bent structure of 9CRA, we replaced the benzanilide skeleton with a diphenylamine structure to obtain RXR agonists, such as DA124 (Fig. 1).[10] The diphenylamine skeleton has some advantages for drug discovery, because it is readily constructed by Pd-catalyzed coupling of aniline derivatives with aryl halides, and the linker nitrogen atom can also be readily modified with various substituents.[11] Indeed, the diphenylamine skeleton has been used as a central scaffold of selective nuclear receptor modulators for estrogen receptor (ER),[12] androgen receptor

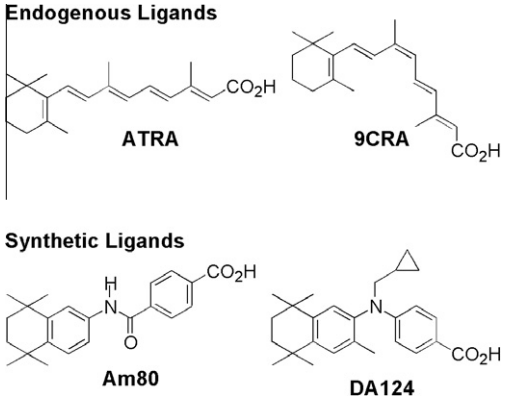

Figure 1. Structures of endogenous and synthetic RAR and RXR ligands.

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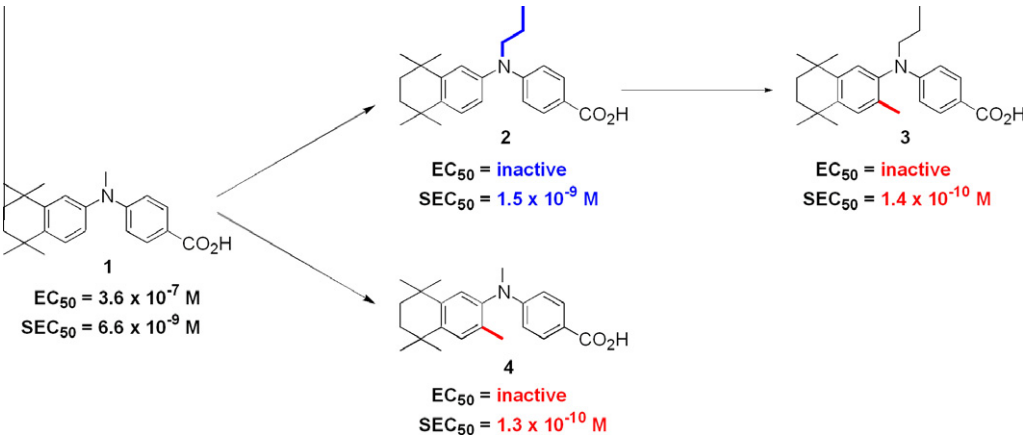


Figure 2. Enhancement of the potency and selectivity of retinoid synergistic activity by structural modifications of diphenylamine-based retinoids. EC_{50} and SEC_{50} (synergistic effective dose) mean half-maximal (50%) effective concentration of test compound alone and in the presence of the RAR agonist Am80 (3.3×10^{-10} M).[10]

(AR),[13] and thyroid hormone receptor (TR),[14] and is considered as a privileged structure for the development of specific nuclear receptor modulators. Several diphenylamine-based retinoids have been synthesized by using DA124 as a lead compound,[10,15–17] and the structure–activity relationships on the nitrogen atom and on the hydrophobic benzene ring of the diphenylamine skeleton have been well investigated (Fig. 2). Among the synthesized diphenylamine derivatives, compound **1** showed dual RAR and RXR agonistic activities, that is, compound **1** has both retinoid agonistic and synergistic activities.[9a] Introduction of a medium-sized *N*-alkyl substituent such as a *n*-propyl group into **1**, yielding **2**, remarkably diminished the retinoidal activity and enhanced the retinoid synergistic activity, which was further enhanced by introduction of a methyl group on the hydrophobic benzene ring, as observed in the RXR-selective compounds **3** and **4** (Fig. 2).[9a] On the other hand, there is little information on the structure–activity relationship for the benzoic acid part of diphenylamine-based retinoids, except in the case of aza analogs, such as pyridine- and pyrimidinecarboxylic acids. Therefore, we focused on structural modifications of the benzoic acid part of compound **1**, and designed cinnamic acid derivatives **5** and phenylpropionic acid derivatives **6** (Fig. 3). Compound **7** bearing a methyl group ortho to the linking amino group on the benzoic acid part was also designed with the aim of obtaining enhanced synergistic activity by twisting the conformation of the diphenylamine skeleton, by analogy with compounds **3** and **4**.

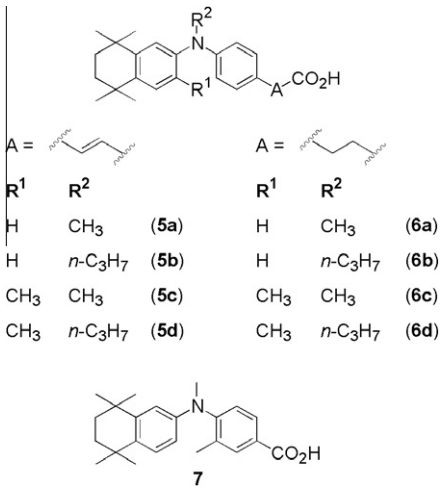
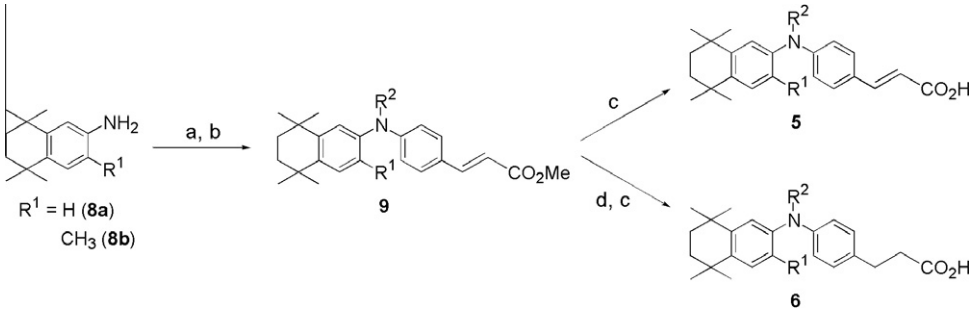


Figure 3. Target molecules for the structure–activity relationship study of the benzoic acid moiety.

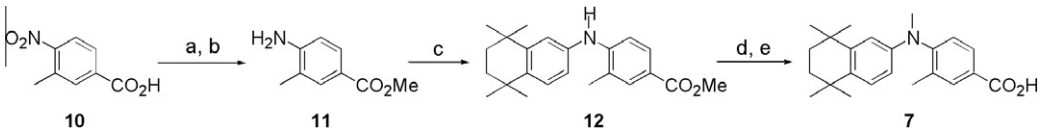
The cinnamic acid derivatives **5** and the phenylpropionic acid derivatives **6** were synthesized as illustrated in Scheme 1. 5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-2-naphthylamine (**8a**) and 5,6,7,8-tetrahydro-3,5,5,8,8-pentamethyl-2-naphthylamine (**8b**) were synthesized by the literature procedure,[6a] and then reacted with methyl 4-bromocinnamate under the conditions of the Buckwald amination reaction to afford the key intermediates with a diphenylamine skeleton.[13] They were treated with iodoalkane and NaH to obtain the N-alkylated diphenylamine derivatives **9**. The ester group of **9** was hydrolyzed with 20% potassium hydroxide to afford the corresponding carboxylic acids **5**. The unsaturated double bond of compounds **9** was reduced by catalytic hydrogenation with Pd/C, followed by hydrolysis, affording the phenylpropionic acid derivatives **6**.

Synthesis of compound **7** is summarized in Scheme 2. 3-Methyl-4-nitrobenzoic acid (**10**) was esterified with methanol, followed by reduction of the nitro group to afford an aniline derivative **11**. Compound **11** was reacted with 2-bromo-5,6,7,8-tetrahydro-5,5,8,8-tetramethylnaphthalene in the presence of Pd catalyst to afford the diphenylamine derivative **12**.[11] N-Methylation of **12**, followed by hydrolysis of the ester group, afforded the desired carboxylic acid **7**.

Biological activities of the newly prepared compounds were determined by assay of activity to induce differentiation of human acute promyelocytic leukemia cell line, HL-60.[16a] Differentiated cells were evaluated from the morphological changes, and also determined by nitro blue tetrazolium (NBT) reduction assay.[18] Figure 4 summarizes the retinoidal activity of compounds **5**–**7**. N-methylated derivatives **5a**, **5c**, **6a**, and **6c** showed moderate or weak differentiation-inducing activity, and have more potent retinoidal activity than the corresponding N-*n*-propylated derivatives **5b**, **5d, 6b**, and **6d**, as observed in the lower activity of **2** compared with **1**. That is, retinoidal activity can tolerate *N*-methyl group of the diphenylamine skeleton but can not N-*n*-propyl group. There is a remarkable difference of retinoidal activity between **5** and **6**; the activity of the cinnamic acid derivatives **5a** and **5c** was much greater than that of the corresponding phenylpropionic acid derivatives **6a** and **6c**. Thus, flexibility and planarity around the carboxylic acid group of the diphenylamine derivatives markedly influence the retinoidal activity. The activities of **5a** (EC_{50}: 2.2×10^{-8} M) and **5c** (EC_{50}: 3.8×10^{-8} M) are more active that the corresponding benzoic acid derivatives **1** and **4**, and the elongation in length between hydrophobic part and the polar carboxylic acid would increase the retinoid agonistic activity. Compound **7** bearing a methyl group on the phenyl ring exhibited no retinoidal activity.



Scheme 1. Syntheses of the cinnamic acids **5** and phenylpropionic acids **6**. Reagents and conditions: (a) $Pd_2(dba)_3$, *rac*-BINAP, NaO^{t}Bu, methyl 4-bromocinnamate, toluene; (b) NaH, iodoalkane, DMF; (c) 20% KOH aq, EtOH; (d) Pd/C, H_2, MeOH.



Scheme 2. Synthesis of **7**. Reagents and conditions: (a) c.H_2SO_4, MeOH; (b) Pd/C, H_2, MeOH; (c) $Pd_2(dba)_3$, *rac*-BINAP, NaO^{t}Bu, 2-bromo-5,6,7,8-tetrahydro-5,5,8,8-tetramethylnaphthalene, toluene; (d) NaH, iodomethane, DMF; (e) 20% KOH aq, MeOH.

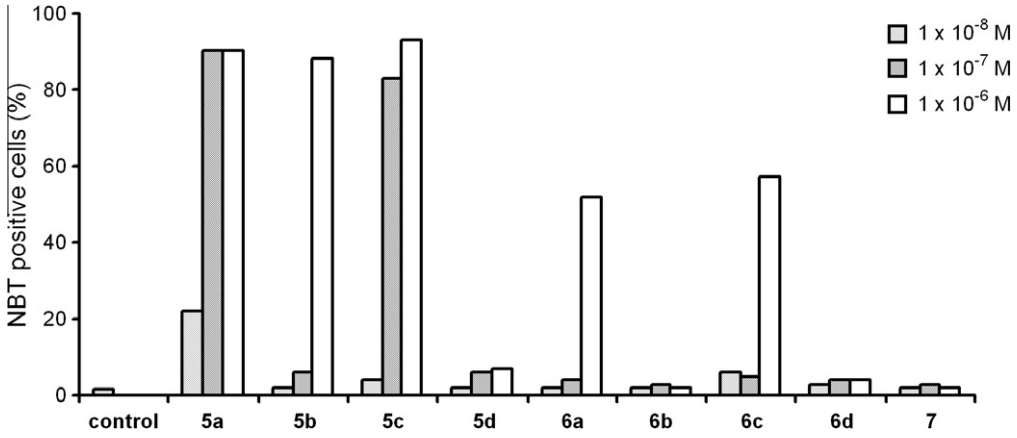


Figure 4. Retinoidal activities of diphenylamine derivatives **5**–**7** in HL-60 cell assay.

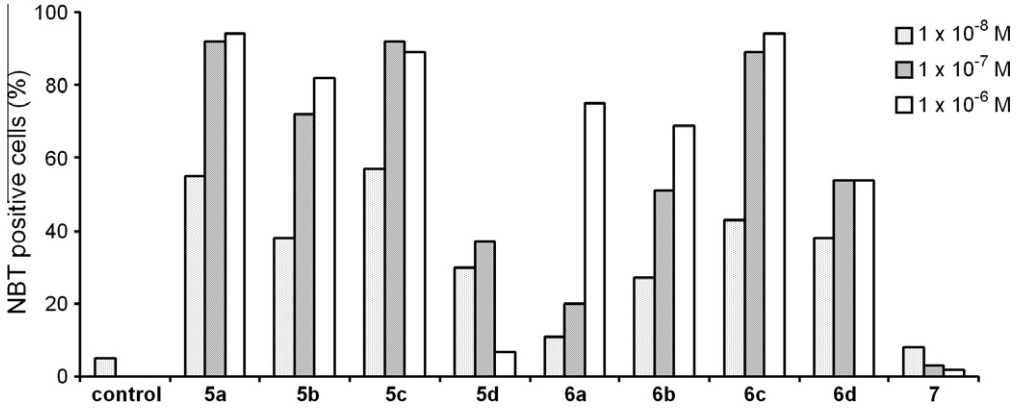


Figure 5. Retinoid synergistic activity of compounds **5**–**7** in the presence of 1×10^{-10} M Am80 in HL-60 cell differentiation assay. 1×10^{-10} M Am80 alone (control) induced differentiation of less than 10% cells.

Next, the retinoid synergistic activity of the synthesized compounds was evaluated in the presence of 1×10^{-10} M Am80. At this concentration, Am80 induced differentiation of 5–10% of the cells (Fig. 5).[12a] Compounds **5** showed potent differentiation-inducing activity, but this appeared to be mostly due to their own intrinsic activity (compare Fig. 4 with Fig. 5). Phenylpropionic acid derivatives **6** showed little or no activity alone, but they remarkably enhanced the retinoidal activity of 1×10^{-10} M Am80, although the

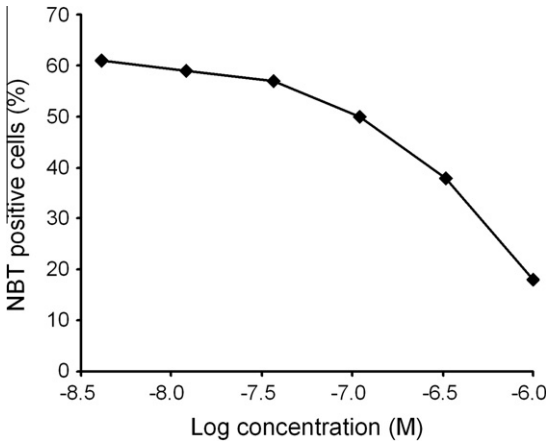


Figure 6. Dose-dependent inhibition by compound **7** of HL-60 cell differentiation induced by 3.3×10^{-10} M Am80. 3.3×10^{-10} M Am80 alone (control) induced differentiation of about 60% cells.

synergitic potency is lower than benzoic derivatives **2**–**4**. Interestingly, compound **7** was again inactive as a retinoid synergist in the presence of RAR ligand.

Compound **7** with a methyl group on the benzoic acid part did not show either retinoid agonistic or synergistic activity. So, we next examined the retinoid antagonistic activity of compound **7**. Compound **7** inhibited the cell differentiation induced by 3.3×10^{-10} M Am80 which induced differentiation of about 60% of cells (Fig. 6). The antagonistic activity of compound **7** is unexpected, since our previous studies (Fig. 2) showed that introduction of a methyl group on the hydrophobic tetrahydrotetramethylnaphthalene ring of the diphenylamine-based retinoids markedly increased the synergistic activity (RXR agonistic activity),[16a,c] probably because the two benzene rings of the twisted diphenylamine structure take a molecular shape similar to that of 9CRA, in contrast to planar diphenylamine structure. Some diphenylamine-type compounds that possess a long or bulky substituent on the linking nitrogen atom or hydrophobic aromatic ring (ortho to the amino group) exhibit retinoid antagonistic activity.[15b] Presumably such a substituent inhibits the proper folding of helix-12 of the receptor that is necessary for receptor activation. In the case of compound **7**, there is no such large substituent that can inhibit the folding of helix-12, but the methyl group on the benzene ring results in a bent diphenylamine structure, as in the case of the isomeric compound **4**, which shows potent synergistic activity. The opposite activities of compounds **4** and **7** presumably results from differences in the nature of the bending, that is, the two compounds have different spatial orientations and directions of the hydrophobic aromatic moiety and the carboxyl group. The bent structure of **7** may not inhibit the folding of helix-12, but may induce a different folding structure from the proper activated form, resulting in receptor inactivation. Detailed mechanistic studies of the binding of antagonist **7** to RARs and RXRs are in progress.

In conclusion, structural modifications of the benzoic acid ring of diphenylamine-based retinoids generated retinoid agonists, synergists, and an antagonist. Introduction of an olefinic bond between the diphenylamine skeleton and the carboxyl group afforded cinnamic acid derivatives **5** with retinoid agonistic activity, while reduction of the olefinic bond of **5** gave propionic acid derivatives **6** with potent retinoid synergistic activity. The differences in planarity and flexibility of the substituent containing the carboxylic acid moiety seem to be responsible for the difference in activity between **5** and **6**. Unexpectedly, introduction of a methyl group onto the benzoic acid moiety generated a retinoid antagonist **7**. Overall, the present and previous results indicate that diphenylamine-based retinoids change their mode of action in a substituent-dependent manner. The structural information obtained here and the active compounds that we have obtained should be useful to develop novel modulators of retinoid actions with potential clinical applications.

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