

## Novel Retinoidal Tropolone Derivatives. Bioisosteric Relationship of Tropolone Ring with Benzoic Acid Moiety in Retinoid Structure

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Several tropolone derivatives (4–7) were designed as novel retinoids on the assumption that the tropolone ring may mimic the benzoic acid moiety in retinoid structures, such as Am80 (2). Among the synthesized compounds, 5-[2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthyl)ethynyl]tropolone (7a) showed moderate potency as a differentiation-inducer of HL-60 cells. The activities of the tropolones were greatly enhanced in the presence of HX630, an RXR agonist (retinoid synergist).

**Key words** retinoid; tropolone; bioisoster; cell differentiation

Retinoids, retinoic acid (all-*trans*, 1, Fig. 1) and its analogues modulate various biological functions, such as cell differentiation, proliferation, and embryonic development in vertebrates.<sup>1,2)</sup> The biological activities of retinoids are mediated by two types of nuclear receptors, retinoic acid receptors (RARs) and retinoid X receptors (RXRs).<sup>3)</sup> Retinoids bind to the RAR site of RXR-RAR heterodimers and the liganded heterodimers regulate the expression of specific genes. RXR-specific ligands cannot activate RXR-RAR heterodimers, but can enhance the potency of RAR ligands.<sup>4)</sup> Since retinoids have significant preventive or therapeutic potential in the fields of dermatology and oncology, a number of synthetic retinoids have been synthesized.<sup>1,5)</sup> Most potent retinoids, such as Am80 (2), have a benzoic acid or other aromatic carboxylic acid moiety instead of the unstable polyenecarboxylic acid of retinoic acid (1). As shown in the generic structure (3) of retinoidal benzoic acids, their structures consist of a hydrophobic part, terminal carboxyl group and the linking group (X) between them. The structure at the hydrophobic region or the linking group (X) can be varied with retention of high activity, while the terminal carboxylic acid generally can not be replaced by other functional groups (bioisosters), such as an aminosulfonyl, amidino or tetrazolyl group, without a significant decrease or loss of activity.<sup>6)</sup> One exception is the successful replacement of the carboxylic acid of RAR and RXR ligands with a thiazolidinedione moiety.<sup>7)</sup> Here, we describe the design, synthesis, and biological activities of tropolone derivatives as novel retinoids without a carboxyl group.

Tropolone (8), 2-hydroxy-2,4,6-cycloheptatrien-1-one, an isomer of benzoic acid, is a seven-membered, non-benzenoid aromatic molecule possessing three double bonds conjugated with a carbonyl group.<sup>8,9)</sup> Since tropolone is regarded as a vinylog of a carboxylic acid,<sup>10)</sup> we considered that the tropolone ring might be bioisosteric with benzoic acid (Fig.

1). Based on this hypothesis, several tropolone derivatives (4–7, Fig. 2) were synthesized. The synthetic routes are illustrated in Chart 1. Nitrosation (90%) of 8, followed by reduction afforded 5-aminotropolone (9, 95%).<sup>8)</sup> The reaction of non-protected 9 with 5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthoyl chloride (10, 2 eq) gave the diacylated compound 11 (83%), which was hydrolyzed under basic conditions to afford 4. Since the hydroxyl group of 9 is more reactive than the amino group, the reaction of 9 with mesyl chloride (1 eq) gave the *O*-mesylated compound 12 (52%), which was reacted with the isocyanate 14 (15%), followed by hydrolysis to afford 5 (quant). The azo compound 6 was synthesized by reaction of 8 with the diazonium salt, prepared from the amine 15 (23%). In order to synthesize compound 7 having an acetylene group, 9 was converted to 5-iodo-2-methoxytropolone (17, 31%). 6-Bromo-1,2,3,4-tetrahydro-1,1,4,4-tetramethylnaphthalene (18) was coupled with (trimethylsilyl)acetylene by means of the Sonogashira reaction (79%). After removal of the TMS group (94%), 20 was coupled with 17 by means of the Sonogashira reaction to give 7b (81%), which was hydrolyzed to afford 7a (quant). The methoxyl group of 7b was also converted to hydrazine (7c, 90%),<sup>14)</sup> and then a hydrogen atom (7d, 7%).

The retinoidal activity of the synthesized compounds was examined in terms of the ability to induce differentiation of human promyelocytic leukemia cells HL-60.<sup>11)</sup> Differentiated cells were determined by means of nitro blue tetrazolium (NBT) reduction assay.<sup>12)</sup> Tropolone derivatives, except 5, in-

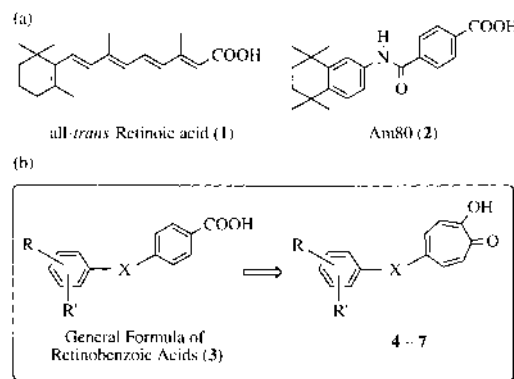


Fig. 1. (a) Structures of Retinoids. (b) Design of Tropolone Derivatives as Novel Retinoid Candidates

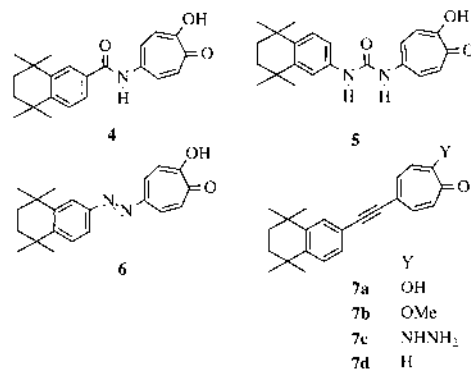
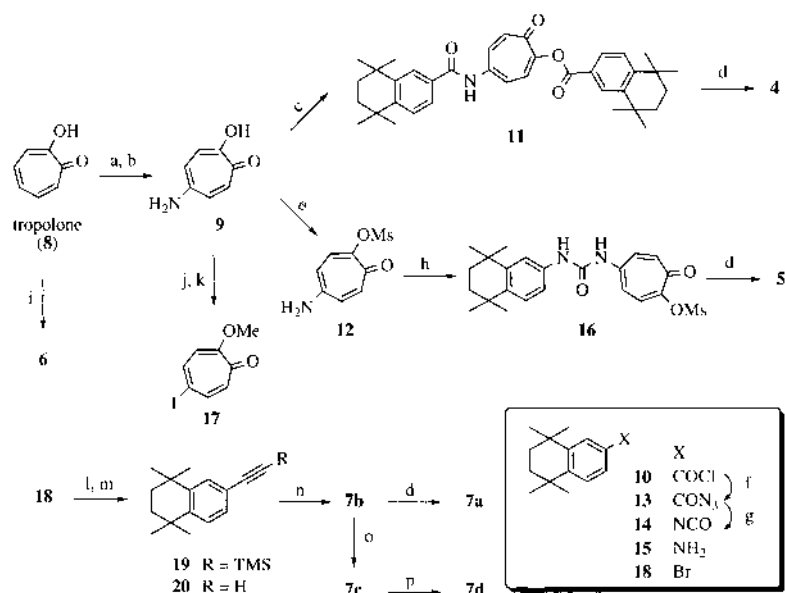


Fig. 2. Structures of Tropolone Derivatives

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(a)  $\text{NaNO}_2$ ; (b)  $\text{PbO}_2 / \text{H}_2$ ; (c) **10** / DMAP / pyridine; (d) NaOH; (e)  $\text{MsCl} / \text{Et}_3\text{N}$ ; (f)  $\text{NaN}_3$ ; (g)  $\Delta$ ; (h) **14** / DMAP /  $\Delta$ ; (i) **15** /  $\text{H}_2\text{SO}_4$  /  $\text{NaNO}_2$  / NaOH; (j)  $\text{NaNO}_2$  /  $\text{H}_2\text{SO}_4$ ; KI; (k)  $\text{Ti}_2\text{O}$  / 2,6-lutidine; MeOH /  $\text{NMe}_3$ ; (l)  $\text{TMS-C}\equiv\text{CH} / \text{Pd}(\text{PPh}_3)_2\text{Cl}_2 / \text{CuI} / \text{NEt}_3$ ; (m)  $\text{K}_2\text{CO}_3$ ; (n) **17** /  $\text{Pd}(\text{PPh}_3)_2\text{Cl}_2 / \text{CuI} / \text{NEt}_3$ ; (o)  $\text{NH}_2\text{NH}_2$ ; (p)  $\text{CuSO}_4$

Chart 1

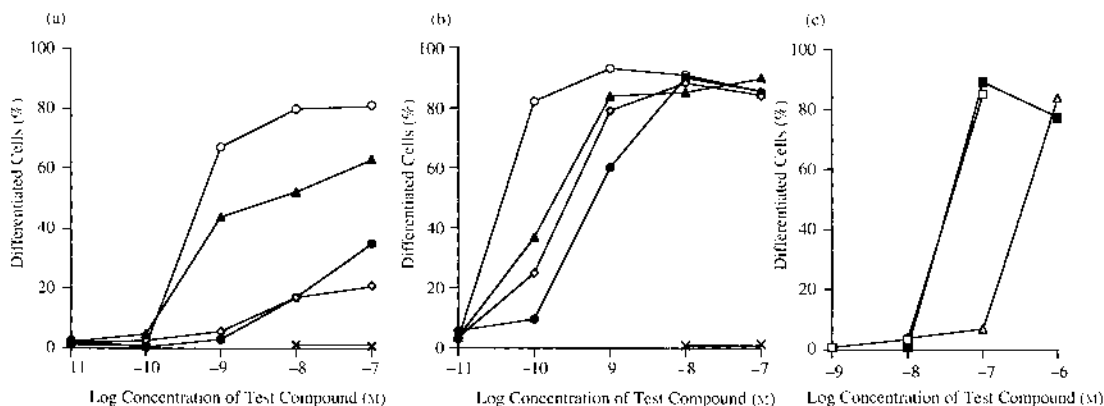


Fig. 3. Differentiation-Inducing Activity of Tropolone Derivatives (a) Alone or (b, c) in the Presence of a Retinoid Synergist, HX630 ( $1 \times 10^{-7} \text{ M}$ ), in HL-60 Cells

The vertical scale is the percentage of differentiated cells evaluated from NBT reduction assay, and the horizontal scale is the molar concentration of test compounds. Added compound: (○) Am80, (●) **4**, (×) **5**, (◇) **6**, (▲) **7a**, (△) **7b**, (■) **7c**, or (□) **7d**.

duced HL-60 cell differentiation into mature granulocytes in a similar manner as **2**, as shown in Fig 3a. However, their potency is weaker than that of **2**, and the maximum responses at high concentration ( $1 \times 10^{-7} \text{ M}$ ) are smaller than that of **2**. Even the acetylenic compound **7a**, the most active among the tropolone derivatives, induced at most 60% of the cells to differentiate. However, the differentiation-inducing activities of tropolone derivatives were strongly enhanced in the presence of an RXR agonist, HX630 (Figs. 3b, 4). HX630 does not exhibit retinoidal activity alone, but enhances the activities of various RAR agonists in HL-60 assay.<sup>13</sup> The  $\text{EC}_{50}$  values of **2** are  $5.5 \times 10^{-10} \text{ M}$  and  $3.6 \times 10^{-11} \text{ M}$  in the absence and presence of  $1 \times 10^{-7} \text{ M}$  HX630, respectively. Thus, HX630 enhances the activity of **2** by one order of magnitude. The ac-

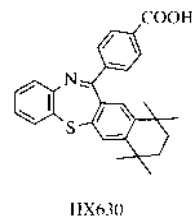


Fig. 4. Structure of HX630

tivity of **7a** was also enhanced by HX630, and the  $\text{EC}_{50}$  value of **7a** in the presence of  $1 \times 10^{-7} \text{ M}$  HX630 is  $1.3 \times 10^{-10} \text{ M}$ , lower than that of **2** alone, but only about 1/4 as potent as the combination of **2** and HX630. Further, it is significant that

the maximum response to **7a** reached a similar value (90%) to that of **2**. More remarkable increases in the activity were observed for compounds **4** and **6**. These compounds are poor differentiation-inducers alone, but highly active in combination with HX630. Their  $EC_{50}$  values ( $4.4 \times 10^{-10}$  M for **4** and  $2.0 \times 10^{-10}$  M for **6**) in the presence of  $1 \times 10^{-7}$  M HX630 are similar to that of **7a** under the same conditions. Only compound **5** was inactive in this experiment, which suggests that the ureido bond may be too long a linking group between the hydrophobic aromatic and tropolone rings. These results indicate that the tropolones **4**, **6** and **7a** are potent retinoids (RAR agonists) only in the presence of RXR agonist.

Compounds **7b—d** are nearly inactive alone (data not shown), and exhibited differentiation-inducing activity only at high concentrations even in the presence of HX630 (Fig. 3c). The hydroxyl group on the tropone ring seems to be important for potent activity.

In conclusion, we found that the tropolone ring can act as a bioisoster of the benzoic acid moiety in retinoid structures. Some tropolone derivatives elicited potent retinoidal activity in combination with the RXR agonist HX630, although they showed poor activity alone in HL-60 assay. At present, the reason for the dramatic change in potency is unknown, but it is presumably related to some specific affinity to RARs, RXRs, or their heterodimers, including related cofactors. We are now investigating the action mechanisms of the tropolone derivatives using these receptors and cofactors. Since retinoidal tropolones may exhibit different pharmacological behaviors from retinoidal benzoic acids, as well as unique bi-

ological activity, they may provide further scope for employing retinoids in clinical applications.

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