Retinobenzoic Acids. 2. Structure-Activity Relationships of Chalcone-4-carboxylic Acids and Flavone-4'-carboxylic Acids

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The structure–activity relationships of (*E*)-chalcone-4-carboxylic acids, which are retinoidal benzoic acids represented by R-Ph-X-Ph-COOH (4, X = —COCH=CH—), are discussed on the basis of differentiation-inducing activity on human promyelocytic leukemia cells HL-60. The activity was increased by the substitution of a bulky alkyl group(s) (R), and among such compounds, (*E*)-4-[3-(3,5-di-*tert*-butylphenyl)-3-oxo-1-propenyl]benzoic acid (Ch55) and (*E*)-4-[3-oxo-3-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)-1-propenyl]benzoic acid (Ch80) are several times more active than retinoic acid. Though the stable conformer of chalcone derivatives is linear (s-cis form), the conformationally restricted analogue 4-(6,7,8,9-tetrahydro-6,6,9,9-tetramethyl-4*H*-4-oxonaphtho[2,3-*b*]pyran-2-yl)benzoic acid (Fv80) is more active than Ch80. While the effect of introduction of an oxygen atom varied, 4-[1-hydroxy-3-oxo-3-(5,6,7,8-tetrahydro-3-hydroxy-5,5,8,8-tetramethyl-2-naphthalenyl)-1-propenyl]benzoic acid (Re80), regarded as a derivative of Ch80 with two additional hydroxyl groups, has very strong activity.

Retinoic acid (RA, 1; Chart I) and its analogues (retinoids) are specific modulators of cell differentiation and proliferation of both normal and neoplastic cells.[1] The mechanism of retinoid function is not yet understood at the molecular level, though some hypotheses have recently been proposed.[2-4] In addition to their fundamental biological activities, retinoids are known to suppress of tumorigenesis, as well as having antipromoter activity.[5] So far, a number of compounds with retinoidal activities have been synthesized as candidate drugs for studies on chemoprevention in oncology and for the treatment of dermatological disease. Among them, etretinate (2)[6] and TTNPB (3)[7,8] have potent retinoidal activities, such as inhibition of papilloma formation. However, the retinoids synthesized up to now, including retinoic acid, have the disadvantage for clinical use because they exhibit high toxicities (hypervitaminosis A).[6] Structurally, most of them have hydrocarbon skeletons and are strongly hydrophobic, which may possibly cause their high and long-term toxicities. In our search for new active retinoids having different chemical properties or structures from those of retinoic acid and conventional synthetic retinoids, we found strong retinoidal activities in various benzoic acid derivatives, whose general structure is represented by 4 (Chart II).[9,10] The linking group X can be varied (—NHCO—,[11] —CONH—,[12] —SO_2NH—, —COCH=CH—,[13] —N=N—[14] and so on), and R represents a medium-sized alkyl group(s).[10] These compounds are collectively named "retinobenzoic acids". In the previous paper, we reported the structure–activity relationships of terephthalic monoanilides (X = -NHCO- in 4) and (arylcarboxamido)benzoic acids (X = -CONH- in 4), based on the ability to induce differentiation of HL-60 cells to mature granulocytes.[15] Among these synthetic amide compounds, Am80

Chart I

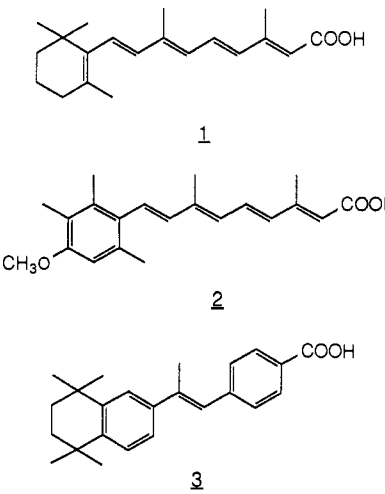

Chart II

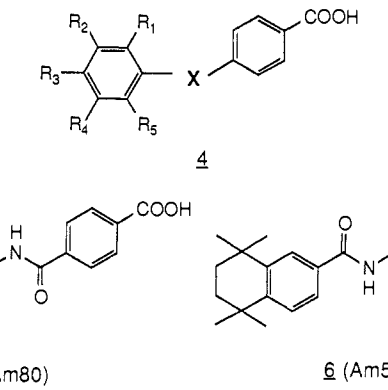

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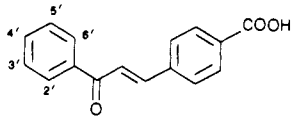
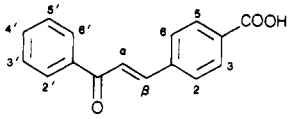
Table I. Differentiation-Inducing Activities of (*E*)-Chalcone-4-carboxylic Acids



name	substituents 2'	3'	4'	5'	6'	ED_{50},[a] M	rel act.[b]
RA						2.4×10^{-9}[c]	1
Ch00	H	H	H	H	H	inactive	
Ch20	H	Et	Et	H	H	1.7×10^{-7}	8.3×10^{-3}
Ch30	H	iPr	iPr	H	H	8.2×10^{-10}	1.8
Ch40	H	H	tBu	H	H	2.8×10^{-8}	3.9×10^{-2}
Ch50	tBu	H	H	tBu	H	4.5×10^{-8}	2.1×10^{-2}
Ch55	H	tBu	H	tBu	H	2.1×10^{-10}	6.4
Ch60	H	tBu	H	H	H	1.6×10^{-7}	8.1×10^{-3}
Ch80	H	$-(CH_3)_2CCH_2CH_2C(CH_3)_2-$		H	H	6.4×10^{-10}	2.8
Ch100	H	$-(CH_3)_2CCH_2CH_2C(CH_3)_2-$		H	CH_3	5.4×10^{-10}	2.1

[a] ED_{50} values of active compounds were calculated from the NBT reduction assay data. Experiments were repeated more than three times in most cases. The values shown are representative ones or means (when more than five repetitions were done). This is also the case in the other tables. [b] The ratio of ED_{50} (retinoic acid) to ED_{50} (a test compound) both values having been obtained in concurrent experiments. This is also the case in the other tables. [c] The deviation (σ_{n-1}) of retinoic acid is estimated to be 1.8×10^{-9} M ($n = 90$).

Table II. Effects of Polar Substituents on the Differentiation-Inducing Activity of Chalcone-4-carboxylic Acids



name	substituents 2'	3'	4'	5'	6'	β	3	ED_{50}, M	relative act.
RA								2.4×10^{-9}	1
Ch80	H	$-(CH_3)_2CCH_2CH_2C(CH_3)_2-$		H	H	H	H	6.4×10^{-10}	2.8
Ch70	H	$-(CH_3)_2CCH_2CH_2O-$		H	H	H	H	$>10^{-6}$[a]	$<10^{-4}$
Ch73	$-OCH_2CH_2C(CH_3)_2-$		H	H	H	H	H	6.9×10^{-7}	7.7×10^{-3}
Ch75	H	$-(CH_3)_2CCH_2CH_2S-$		H	H	H	H	$>10^{-6}$	$<10^{-4}$
Ch110	H	$-(CH_3)_2CCH_2CH_2C(CH_3)_2-$		H	OH	H	H	1.5×10^{-10}	1.4
Ch115	H	$-(CH_3)_2CCH_2CH_2C(CH_3)_2-$		H	OCH_3	H	H	3.9×10^{-9}	0.35
Ch120	H	$-(CH_3)_2CCH_2CH_2C(CH_3)_2-$		H	H	H	OH	1.7×10^{-9}	0.88
Ch220	H	CH_3O	CH_3O	H	H	H	H	inactive	
Re00	OH	H	H	H	H	OH	H	inactive	
Re40	OH	H	tBu	H	H	OH	H	5.6×10^{-7}	2.3×10^{-3}
Re60	OH	H	H	tBu	H	OH	H	$>10^{-6}$	$<10^{-4}$
Re80	OH	H	$-(CH_3)_2CCH_2CH_2C(CH_3)_2-$		H	OH	H	6.3×10^{-11}	7.6

[a] "$>10^{-6}$ M" means there was slight activity at 10^{-6} M.

(5) and Am580 (6) were found to be several times more active than retinoic acid in the assay. In this paper, the structure–activity relationships of (*E*)-chalcone-4-carboxylic acids (X = —COCH=CH— in 4) and the related compounds, flavone-4'-carboxylic acids, are discussed.

The retinoidal activities of these compounds in this study were also evaluated for the differentiation-inducing activities toward human promyelocytic leukemia cell line HL-60.[16] Retinoic acid induces these cells to differentiate into mature granulocytes[17] and this ability of retinoids correlates well with other retinoidal activities.[1,18] The morphological changes were examined after Wright-Giemsa staining, and the Nitroblue tetrazolium (NBT) reduction assay was employed as a functional marker of differentiation.[19] These two indexes of differentiation correlated well. Experiments were repeated more than three times in most cases, covering 5 orders of concentrations. The ED_{50} values of active compounds were calculated from the NBT reduction assay data. Relative activity was defined as the ratio of ED_{50} of retinoic acid to ED_{50} of a test compound, both values having been obtained in concurrent experiments. These two values shown in tables are representative ones or means when more than five repetitions were done.

Results

Differentiation-inducing activities of alkyl-substituted (*E*)-chalcone-4-carboxylic acids are shown in Table I. The simple (nonsubstituted) chalcone-4-carboxylic acid (Ch00) was completely inactive at concentrations below 10^{-6} M in this assay. However, alkyl group substitution resulted in significant activity, as in the case of the other retinobenzoic acids (4, X = amide, azo, etc.).[9,10] Thus, the 3',4'-diethyl derivative (Ch20) had differentiation-inducing activity, though it was weaker than that of retinoic acid by 2 orders of magnitude. A *tert*-butyl group, especially at the 4'-position (Ch40) rather than at the 3'-position (Ch60), increased activity more effectively. Compounds that have two bulky alkyl groups showed very strong ac-

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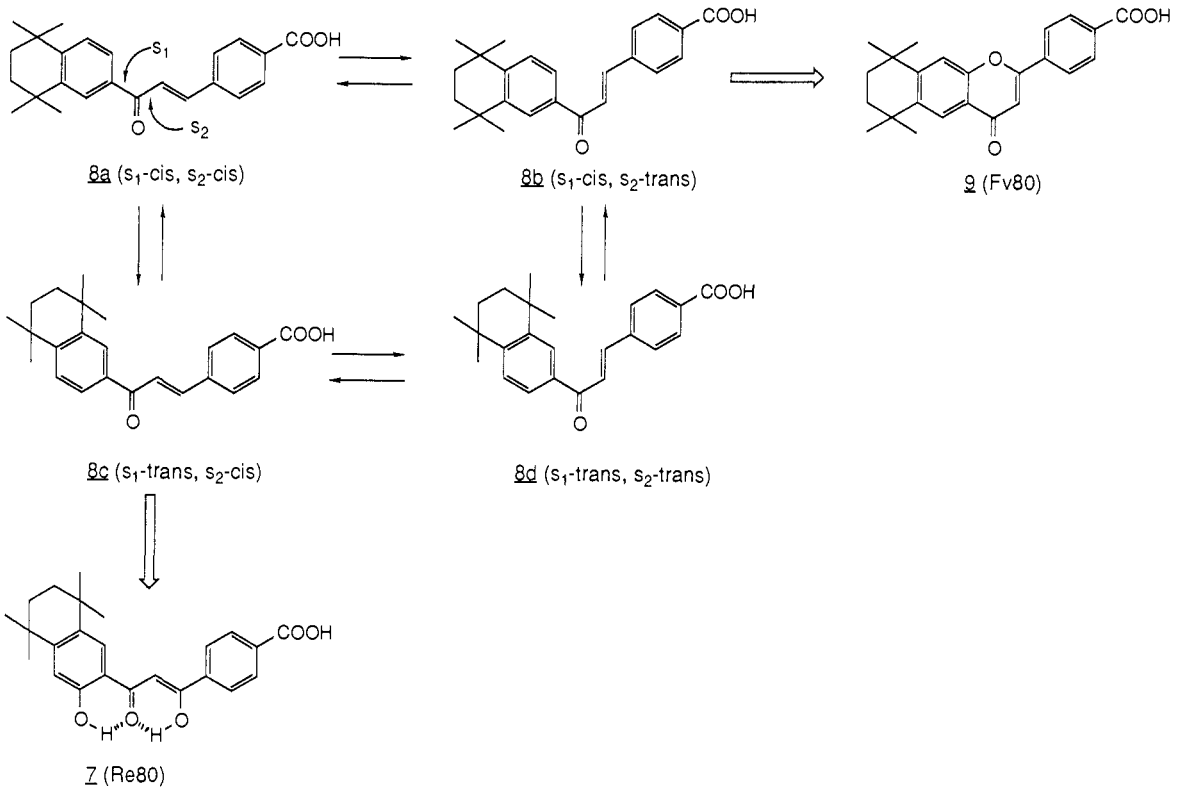


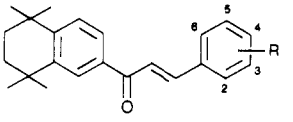
Figure 1.

tivities. Thus, dramatic increases in the activity were seen in the 3′,4′-diisopropyl derivative (Ch30) and 3′,5′-di-*tert*-butyl derivative (Ch55): their ED_{50}s are of the order of 10^{-10} M, and they are several times more active than retinoic acid. In the chalcone series, compounds with a tetramethyltetralin ring (Ch80 and Ch100), which corresponds to the alkyl group of Am80 or Am580, or to TTNPB, also had stronger activity than retinoic acid.

The effects of the substitution of a polar atom or group(s) are shown in Table II. Compound Ch220 having a 3′,4′-dimethoxy group, which sterically somewhat resembles the 3′,4′-diethyl group of Ch20, was inactive. The same tendency was seen in oxa or thia analogues of Ch80 (Ch70 and Ch75, respectively). This result indicates that hydrophobicity or low electrostatic properties of substituents are necessary for activity. The effect of introducing a hydroxyl group at other positions of Ch80 is worth discussing. The compound with a hydroxyl group at the position ortho (6′) to the ketone group (Ch110) did not show any decrease of the activity compared to Ch80 or Ch100, although the methoxy derivative (Ch115, methyl ether of Ch110) showed slightly weaker activity. Introduction of a hydroxyl group on the other benzene ring (ortho to the carboxylic acid, Ch120) also resulted in retention of the high activity.

Interestingly, very strong activity was observed when another hydroxyl group was introduced on the olefinic carbon in addition to the 3′-hydroxyl group; 4-[1-hydroxy-3-oxo-3-(5,6,7,8-tetrahydro-3-hydroxy-5,5,8,8-tetramethyl-2-naphthalenyl)-1-propenyl]benzoic acid (Re80) has an ED_{50} of 6.3×10^{-11} M. From ^1H and ^{13}C NMR studies (NOE enhancement, CH-COSY) of the methyl ester of Re80 (Re81), it was concluded that the 1,3-diketone moiety existed in the enol form in $CDCl_3$. The chemical shifts of the two hydroxyl groups were shifted to low field, suggesting the presence of hydrogen bonds between the hydroxyl groups and the carbonyl group. A large NOE enhancement (27%) between the olefinic proton and 6′-proton (Table II) indicated these protons are close to each other. Thus, Re80 also exists as the enol (7), as illustrated in Figure 1, and may be regarded as a conformationally fixed analogue of Ch80. In this Re series too (Table II), the unsubstituted compound (Re00) was inactive. Re40 having a *tert*-butyl group at the 4′-position had activity, but only $^1/_{10}$ of that of the corresponding chalcone-4-carboxylic acid (Ch40). Re60 having the 5′-*tert*-butyl group was nearly inactive, although the corresponding chalcone Ch60 had significant activity (about $^1/_5$ of that of Ch40). Nevertheless, Re80 is 7.6 times more active than retinoic acid and is more active than the corresponding chalcone Ch80. Re80, as well as Ch55, showed the strongest activity among the chalcone-4-carboxylic acids. The correspondence between the effects of the alkyl group(s) in the Ch and Re series is not complete. Possible structural correlations among these compounds are illustrated in Figure 1. The 5′-positions of the Ch series and 3′-positions of the Re series seem to correspond.

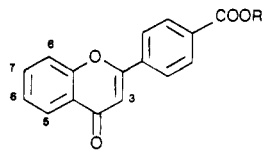
Table III. Effect of Modification of the Terminal Carboxyl Group on the Differentiation-Inducing Activity of Ch80



name	R	ED_{50}, M	relative act.
RA		2.4×10^{-9}	1
Ch80	4-COOH	6.4×10^{-10}	2.8
Ch81	4-$COOCH_3$	3.0×10^{-8}	4.3×10^{-2}
Ch82	4-COOnBu	2.7×10^{-8}	6.3×10^{-2}
Ch83	4-$CONH_2$	$>10^{-6}$	$<10^{-4}$
Ch84	4-COCl	3.3×10^{-9}	0.52
Ch85	4-CH_3	inactive	
Ch86	3-COOH	$>10^{-6}$	$<10^{-4}$

Table III shows the effects of the modification of the terminal carboxyl group. Esterification diminished the activity by 1 or 2 orders of magnitude (Ch81, Ch82) and derivatization to an amide caused disappearance of the

Table IV. Differentiation-Inducing Activities of Flavone-4′-carboxylic Acids



name	substituents 3	5	6	7	8	R	ED_{50}, M	rel act.
RA							2.4×10^{-9}	1
Fv00	H	H	H	H	H	H	inactive	
Fv40	H	H	H	tBu	H	H	4.8×10^{-7}	5.8×10^{-3}
Fv60	H	H	tBu	H	H	H	$>10^{-6}$	$<10^{-4}$
Fv70	H	H	$-OCH_2CH_2C(CH_3)_2-$		H	H	2.5×10^{-7}	1.7×10^{-2}
Fv80	H	H	$-(CH_3)_2CCH_2CH_2C(CH_3)_2-$		H	H	4.6×10^{-11}	27.4
Fv81	H	H	$-(CH_3)_2CCH_2CH_2C(CH_3)_2-$		H	CH_3	4.8×10^{-10}	8.5
Fv180	OH	H	$-(CH_3)_2CCH_2CH_2C(CH_3)_2-$		H	H	1.2×10^{-9}	1.25
Fv190	OCH_3	H	$-(CH_3)_2CCH_2CH_2C(CH_3)_2-$		H	H	$>10^{-6}$	$<10^{-4}$

activity (Ch83). The acid chloride (Ch84) had reduced activity, but it is uncertain whether it was active in the form of the acid chloride or after hydrolysis. The compound with a *m*-carboxyl group (Ch86) was nearly inactive. Other functional groups, such as methyl, methoxy, halogen (Cl, F), nitro, etc., instead of the carboxyl group of Ch40, Ch55, or Ch80, caused loss of activity (data not shown).

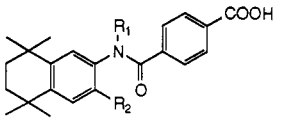
Flavones can be regarded as chalcone compounds whose C_3 unit is structurally restricted by the formation of a pyran ring. Therefore, it is interesting to compare the activities of flavone-4′-carboxylic acids with those of chalcone-4-carboxylic acids. Thus, some flavone-4′-carboxylic acids were synthesized and their activities were examined (shown in Table IV). Their structure–activity relationships are very similar to those of chalcone-4-carboxylic acids, in particular those of the Re series compounds. Nonsubstituted flavone-4′-carboxylic acid (Fv00) was completely inactive. The effect of a *tert*-butyl group was the same as that in the Re series. That is, Fv40, which has a *tert*-butyl group para (7) to the ketone, had differentiation-inducing activity, but Fv60 with the meta (6) *tert*-butyl group was nearly inactive. Furthermore, Fv80, the compound with the same alkyl group as Am80, Am580, and Ch80, was 27 times more active than retinoic acid. In this case also, the substitution of a polar atom reduced activity. Fv70, an oxa analogue of Fv80, was 4 orders of magnitude less active. A hydroxyl group at the 3-position of the flavone skeleton reduced the activity by 2 orders of magnitude (Fv180), and a methoxy group at this position caused further reduction of activity (Fv190). The esterification of the terminal carboxyl group diminished activity by 1 order of magnitude (Fv81), as in the case of the other retinobenzoic acids.

Discussion

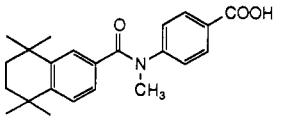
Previously, we reported the structure–activity relationships of aromatic amides with retinoidal activities, including Am80 (**5**) and Am580 (**6**).[15] In these amide series, (i) a bulky alkyl group, such as an isopropyl or *tert*-butyl group at the meta position to the linking group X and (ii) the carboxyl group at the para position of the other benzene ring are necessary for the activity. In addition, (iii) the conformation of the amide group is a very important factor. With respect to these features, the structure–activity relationships of chalcone- and flavone-carboxylic acids will be compared with those of the amide compounds.

First, the effects of alkyl substitutions in the chalcone-4-carboxylic acids are, as a whole, closely similar to those in the aromatic amides; that is, a bulky alkyl group(s) such as an isopropyl or *tert*-butyl group is necessary for activity. But there are subtle differences, as follows: (i) A *tert*-butyl group is more effective at the para (4′-) position than at the meta (3′-) position in chalcone-4-carboxylic acids, whereas the opposite is the case with the aromatic amides. This is more marked in the Re series or flavone-4′-carboxylic acids, where compounds having a *tert*-butyl group at the meta position to the ketone are nearly inactive. (ii) The reduction of activity by the ortho substituent of the chalcone-4-carboxylic acids is less than that of the aromatic amides. Among amide compounds, ortho substitution resulted in a large reduction of the activity, probably because of the large change of conformation of the amide bond. However, 2′,5′-di-*tert*-butylchalcone-4-carboxylic acid (Ch50), having two *tert*-butyl groups at the ortho and meta positions, is more active than Ch60, which has only the meta-*tert*-butyl group. The derivative of Ch80 substituted with one methyl group at the position ortho to the ketone, Ch100, had the same activity as Ch80, while the corresponding analogue of Am80 (Am100) was far less active than Am80 (Chart III).[15] This difference should result from the different degree of conformational change on the α,β-unsaturated ketone and the amide groups caused by the ortho substituent. The chalcone skeleton links two benzene rings by three atoms, being different from the other retinobenzoic acids such as amides (X = —NHCO—) and azo compounds (X = —N=N—), where the two benzene rings are linked by two atoms. So, the chalcone skeleton will be more flexible, even when an ortho substituent is present. (iii) Among all the chalcone-4-carboxylic acids, Ch55 possesses the highest activity (6.4 times more active than retinoic acid). It is 3 times more active than Ch80. Am80 and Am580 have the strongest activities of all the amide derivatives. Am55 and Am555,

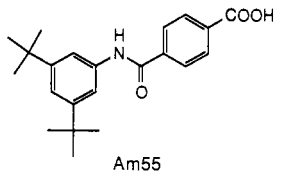
Chart III



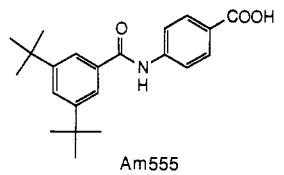
Am90 R_1=CH_3, R_2=H
Am100 R_1=H, R_2=CH_3



Am590



Am55



Am555

which have two *tert*-butyl groups at the meta positions, like Ch55, are $^1/_{30}$–$^1/_{100}$ as active as Am80 and Am580, respectively.[15] It is interesting that the *m*-alkyl group substitution of chalcone-4-carboxylic acids is less effective in increasing activity than in the case of aromatic amides. (iv) Chalcone-4-carboxylic acids have two kinds of polar substituent effects. Oxa or thia analogues of the alkyl groups substituted on the chalcone skeletons show strongly diminished activities (Ch70, Ch75, and Ch220). The presence of an electronegative atom at the region favorable for alkyl substitution seems to decrease the activity. On the other hand, the substitution of a hydroxyl group at other positions did not reduce the activity. In fact, Re80 (having two hydroxyl groups) is more active than Ch80.

Second, the importance of the terminal carboxyl group in chalcone derivatives is the same as in the amide derivatives. Thus, the shift of the *p*-carboxyl group to the meta position caused the disappearance of the activity. Modification to carboxyl derivatives such as an ester, acid chloride, or amide diminished activity. However, these ester or amide compounds should be active when their groups are hydrolyzed to the carboxylic acid, and so would be expected to be useful as prodrugs in vivo.

Third, the conformation of chalcone-4-carboxylic acids affects activity. In the case of the aromatic amides, only the compounds with trans amide groups showed potent activities. Compounds with cis amide groups (e.g., *N*-methyl derivatives of Am80 or Am580, Am90 and Am590, respectively) were inactive in the assay.[15] It is conceivable that (*E*)-chalcone-4-carboxylic acids adopt minimum-energy conformations, such as s-trans or s-cis isomer of the Ar—CO or CO—C(=C) single bonds. Therefore, Ch80 can exist in or equilibrate among four conformations, approximately as illustrated in Figure 1. Generally, a simple (*E*)-chalcone exists predominantly in the s-cis [CO—C(=C)] form in solution and in the crystal.[20,21] Indeed, the present retinoidal chalcone derivatives also exist in the s-cis form about the CO—C(=C) bond as judged from NMR and IR studies. The observation of NOE enhancement from the Cα proton to the proton at the ortho position (2′) of CH55 indicated that, in solution ($CHCl_3$), Ch55 exists in the s-cis form. From X-ray crystallography, both Ch80 and Ch55 exist in the s-cis form (that is, in the elongated form) in the crystal. Therefore, the conformation of the biologically active form is supposed to be s-cis form about the CO—C(=C) bond, though this is not conclusive. One reasonable method for the elucidation of this problem is studying conformationally restricted analogues of these flexible compounds, such as flavone-4′-carboxylic acids, which are conformationally restricted in the s-trans form (**8b** in Figure 1) of the chalcone-4-carboxylic acids, the minor or unfavorable conformer of chalcones. The fact that Fv80 has strong activity, stronger than that of Ch80, superficially indicates that the s-trans conformer is possibly the active form of chalcone-4-carboxylic acids. On the other hand, Re80 appears to exist in enolate form in solution. Since its conformation is restricted by two hydrogen bonds (**8c** in Figure 1), it is necessarily in the s-cis form about the CO—C(=C) bond. Re80 is as active as or more active than Ch80, indicating that the s-cis form may also be the active conformer of Ch80. There is a possibility that Re80 is active after conversion to Fv80 by cyclization in the cells although this path, chemically, requires an acid treatment. Consequently, at present, it is uncertain which conformation (or both) of chalcone-4-carboxylic acid is the active form: further conformational studies are required. Interestingly, the most active compound among the synthetic retinobenzoic acids, Fv80, has the structure with the most restricted conformation, and therefore should provide a clue to the elucidation of this problem. Substitution (OH or OCH_3) at the 3-position of Fv80 caused a major reduction of the activity. Considering that the linking group X in generic formula 4 can be varied regardless of the electronic properties, this reduction should not be due to the electronic effect of the substituent, but to the steric effect. The three-dimensional conformational analysis of retinobenzoic acids is in progress.

Since the chalcone- and flavonecarboxylic acids induce the differentiation of HL-60 cells, other retinoidal activities of the compounds, in particular, of Ch55, were examined.[4] Ch55 induced mouse teratocarcinoma cells F9 to differentiate morphologically to endoderm-like cells and functionally as indicated by plasminogen activator induction.[22] Ch55 inhibited the proliferation of mouse melanoma S91,[22] the proliferation and keratinization of rat bladder cancer cell line BES20B,[23] and the keratinization of hamster tracheal cells.[22] In these assays, Ch55 was always more active than retinoic acid or aromatic amides Am80 and Am580. Some biochemical activities related to the mechanism of action of retinoids were also examined with Ch55. Ch55 inhibited the induction of ornithine decarboxylase (ODC) by the tumor promoters TPA[22] and teleocidin.[24] The enhanced expression of *c-myc* gene in HL-60 cells was suppressed by Ch55 prior to the morphological and functional differentiation.[25] Ch55 also suppressed the protooncogene *c-mos*.[26] The binding of epidermal growth factor (EGF) to the cellular receptors in NRK cells is enhanced by retinoids, probably via an increase of EGF receptors.[27] Ch55 also enhanced the binding of EGF more strongly than did retinoic acid. Thus, Ch55 acts as a modulator of cell differentiation and proliferation or of gene expression, and shows the strongest activities among other retinoids (including retinoic acid, Am80, and Am580) in most cases. Moreover, Ch55 promoted the growth of vitamin A deficient rats, and therefore it (and possibly related compounds generalized by the formula 4) can reasonably be classified as a retinoid.[28]

Most significant, the chalcone-4-carboxylic acids Ch55 and Ch80, although they possess strong activities, essentially did not bind to the cellular retinoic acid binding protein (CRABP).[22,24,29] This result indicates that CRABP is not the true or crucial specific receptor related to the retinoidal action. The modulation of cell differentiation and proliferation by retinoids is so specific that there should be a specific receptor(s), whose binding abilities to various retinoids are correlated to their activities. Ch55 or the other retinobenzoic acids discussed above should be useful tools for the identification of the retinoid receptor(s). This idea, in fact, has led to the identification

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Table V. Chemical and Physical Properties of Chalcone- and Flavonecarboxylic Acids

name	mp, °C	crystal form	recrystn solvent	formula
Ch00	209–211	pale yellow prisms	AcOEt-*n*-hexane	$C_{16}H_{12}O_3 \cdot {}^1/_8H_2O$
Ch20	178.5–180	pale yellow plates	AcOEt-*n*-hexane	$C_{20}H_{20}O_3$
Ch30	197.5–199	pale yellow needles	benzene-*n*-hexane	$C_{22}H_{24}O_3$
Ch40	245–246	pale yellow prisms	AcOEt-*n*-hexane	$C_{20}H_{20}O_3$
Ch50	215–216	pale yellow prisms	benzene-*n*-hexane	$C_{24}H_{28}O_3$
Ch55	202–203.5	pale yellow prisms	benzene-*n*-hexane	$C_{24}H_{28}O_3$
Ch60	199–200	pale yellow prisms	CH_3OH	$C_{20}H_{20}O_3$
Ch70	228–228.5	pale yellow flakes	AcOEt-*n*-hexane	$C_{21}H_{20}O_4$
Ch73	250–252	pale yellow prisms	AcOEt-*n*-hexane	$C_{21}H_{20}O_4$
Ch75	217–219	pale yellow prisms	AcOEt-*n*-hexane	$C_{21}H_{20}SO_3 \cdot {}^1/_4H_2O$
Ch80	203–204	pale yellow prisms	AcOEt-*n*-hexane	$C_{24}H_{26}O_3$
Ch81	93.5–94	pale yellow plates	CH_3OH	$C_{25}H_{28}O_3$
Ch82	128–129.5	pale yellow prisms	CH_3OH	$C_{28}H_{34}O_3$
Ch83	208.5–209	pale yellow needles	CH_2Cl_2-*n*-hexane	$C_{24}H_{27}NO_2 \cdot {}^1/_6H_2O$
Ch84	144–145	pale yellow needles	CH_2Cl_2-*n*-hexane	$C_{24}H_{25}O_2Cl$
Ch85	114.5–116	colorless prisms	CH_3OH	$C_{24}H_{28}O$
Ch86	167.5–168.5	yellow prisms	AcOEt-*n*-hexane	$C_{24}H_{26}O_3 \cdot {}^1/_8H_2O$
Ch100	227.5–228	pale yellow prisms	AcOEt-*n*-hexane	$C_{25}H_{28}O_3$
Ch110	267–269	orange prisms	AcOEt-EtOH	$C_{24}H_{26}O_4$
Ch115	233–234	pale yellow prisms	AcOEt-*n*-hexane	$C_{25}H_{28}O_4$
Ch120	261.5–263	pale yellow prisms	AcOEt-*n*-hexane	$C_{24}H_{26}O_4$
Ch220	208.5–209.5	pale yellow needles	AcOEt-*n*-hexane	$C_{18}H_{18}O_5$
Re00	262 dec	pale yellow needles	AcOEt-*n*-hexane	$C_{16}H_{12}O_5$
Re40	245–247	pale yellow needles	AcOEt-*n*-hexane	$C_{20}H_{20}O_5$
Re60	228–229	pale yellow plates	AcOEt-*n*-hexane	$C_{20}H_{20}O_5$
Re80	232–233	pale yellow needles	AcOEt-EtOH	$C_{24}H_{26}O_5$
Fv00	302–303	pale yellow prisms	CH_3OH	$C_{16}H_{10}O_4$
Fv40	284.5–285.5	pale yellow flakes	CH_3OH	$C_{20}H_{18}O_4$
Fv60	291–292	colorless prisms	CH_3OH	$C_{20}H_{18}O_4$
Fv70	>300	colorless prisms	CH_3OH	$C_{21}H_{18}O_5$
Fv80	>300	pale colored prisms	CH_3OH	$C_{24}H_{24}O_4$
Fv81	175–176	colorless flakes	CH_2Cl_2-*n*-hexane	$C_{25}H_{26}O_4$
Fv180	298–300	yellow prisms	AcOEt	$C_{24}H_{24}O_5$
Fv190	300	pale yellow needles	AcOEt-*n*-hexane	$C_{25}H_{26}O_5$

of a retinoid-specific binding protein (RSBP), which binds specifically and strongly to Ch55 and Am80 as well as retinoic acid.[2] This, together with the identification of the c-DNA for retinoic acid receptor,[3,4] may open up a new era in the biochemistry of retinoid-binding proteins.

Conclusion

Chalcone-4-carboxylic acids and flavone-4′-carboxylic acids are potent inducers of differentiation of HL-60 cells. Some of them are more active than retinoic acid. From biological activities and the structure–activity relationships, these compounds should be classified as retinoids. Ch55, Re80, and Fv80, which have quite different chemical structures from conventional retinoids, show high retinoidal activities. Not only should they be powerful tools for the elucidation of the mechanisms of retinoidal actions but also may be candidates for clinical use in dermatology and oncology.

Experimental Section

Cells and Culture. The human promyelocytic leukemia cells, HL-60, were provided by Prof. F. Takaku (Faculty of Medicine, University of Tokyo) and have been maintained in continuous suspension culture. The cells were cultured in plastic flasks in RPMI1640 medium supplemented with 5% fetal calf serum (FCS) and antibiotics (penicillin G and streptomycin), at 37 °C in a humidified atmosphere of 5% CO_2 in air.

Test compounds were dissolved in ethanol at 0.2 mM and added to the cells (seeded at about 8×10^4 cells/mL), while the final ethanol concentration was kept below 0.5%. Control cells were given only the same volume of ethanol. Retinoic acid, a positive control, was always assayed at the same time. The cells were incubated for 4 days and stained with Wright-Giemsa. Differential counts were then performed under a light microscope on a minimum of 200 cells. Nitroblue tetrazolium (NBT) reduction was assayed as described.[19] Cells were incubated for 20 min at 37 °C in RPMI1640 medium (5% FCS) and an equal volume of phosphate-buffered saline (PBS) containing NBT (0.2%) and 12-*O*-tetradecanoylphorbol 13-acetate (TPA; 200 ng/mL). The percentage of cells containing blue-black formazan was determined on a minimum of 200 cells. The results of these two evaluations were always in good agreement.

The assays of test compounds were performed at least three times. ED_{50} values of active compounds were calculated from the NBT reduction assay data. Relative activities were calculated as the ratio of ED_{50} of retinoic acid to ED_{50} of a test compound obtained in concurrent experiments.

Chemistry. Melting points were determined by using a Yanagimoto hot-stage melting point apparatus and are uncorrected. Elemental analyses were carried out in the Microanalytical Laboratory, University of Tokyo, and were within ±0.3% of the theoretical values. NMR spectra were recorded on JEOL FX 100 MHz and JEOL GX 400 MHz NMR spectrometers. Chemical shifts are expressed in ppm relative to tetramethylsilane.

(*E*)-4-[3-(3,5-Di-*tert*-butylphenyl)-3-oxo-1-propenyl]benzoic Acid (Ch55). 3,5-Di-*tert*-butylacetophenone[15] (170 mg, 0.73 mmol) and terephthalaldehydic acid methyl ester (120 mg, 0.73 mmol) were dissolved in 5 mL of CH_3OH, and 3 mL of 1 N NaOH was added. The mixture was stirred overnight and then poured into 1 N HCl and extracted with AcOEt. The organic layer was washed with H_2O and brine and dried over $MgSO_4$. After evaporation, the crude product was purified by recrystallization to give Ch55. Ch55: pale yellow prisms (from benzene–*n*-hexane), mp 202–203.5 °C; ^1H NMR (100 MHz, $CDCl_3$) δ 1.41 (s, 18 H), 7.64 (d, 1 H, J = 16 Hz), 7.69 (d, 1 H, J = 2 Hz), 7.70 (d, 2 H, J = 8 Hz), 7.74 (d, 1 H, J = 16 Hz), 7.83 (d, 2 H, J = 2 Hz), 8.10 (d, 2 H, J = 8 Hz). Anal. ($C_{24}H_{28}O_3$) C, H, N.

Other chalconecarboxylic acids were also prepared according to this method, and their chemical and physical properties are listed in Table V.

Methyl (*E*)-4-[3-Oxo-3-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)-1-propenyl]benzoate (Ch81). Ch80 (200 mg, 0.55 mmol) was dissolved in 10 mL of dry benzene and 3 mL of $SOCl_2$ and refluxed for several hours. The solvent was removed to give the crude acid chloride of Ch80 (Ch84). The crude Ch84 was dissolved in 10 mL of dry benzene and 1 mL of pyridine and then 2 mL of CH_3OH was added at 0 °C. After the mixture was stirred for 1 h, the solvent was removed under vacuum and

the residue was extracted with AcOEt. The organic layer was washed successively with H_2O, 1 N $NaHCO_3$, H_2O, and brine and dried over $MgSO_4$. The crude product was chromatographed on silica gel to give Ch81. Ch84: pale yellow needles (from CH_2Cl_2–*n*-hexane), mp 144–145 °C; ^1H NMR (100 MHz, $CDCl_3$) δ 1.33 (s, 6 H), 1.37 (s, 6 H), 1.74 (s, 4 H), 7.45 (d, 1 H, J = 8 Hz), 7.64 (d, 1 H, J = 15 Hz), 7.75 (d, 2 H, J = 8.5 Hz), 7.76 (d, 1 H, J = 15 Hz), 7.77 (dd, 1 H, J = 2.5, 8 Hz), 7.99 (d, 1 H, J = 2.5 Hz), 8.16 (d, 2 H, J = 8.5 Hz). Anal. ($C_{24}H_{25}O_2Cl$) C, H, N. Ch81: pale yellow plates (from CH_3OH), mp 93.5–94 °C; ^1H NMR (400 MHz, $CDCl_3$) 1.32 (s, 6 H), 1.35 (s, 6 H), 1.73 (s, 4 H), 3.95 (s, 3 H), 7.44 (d, 1 H, J = 8.5 Hz), 7.58 (d, 1 H, J = 15.5 Hz), 7.70 (d, 2 H, J = 8.5 Hz), 7.77 (dd, 1 H, J = 8.5 Hz), 7.79 (d, 1 H, J = 15.5 Hz), 8.00 (d, 1 H, J = 2 Hz), 8.09 (d, 2 H, J = 8.5 Hz). Anal. ($C_{25}H_{28}O_3$) C, H, N.

Other derivatives of Ch80 were prepared according to this method, and their chemical and physical properties are listed in Table V.

Terephthalic Acid 3-Acetyl-5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthyl Methyl Ester. *m*-Chloroperbenzoic acid (79% purity, 45 g, 0.18 mol) was added to a solution of 5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthyl methyl ketone (36.8 g, 0.16 mol) in 200 mL of $CHCl_3$, and the mixture was refluxed for 4 h. After filtration and the removal of the solvent, the residue was purified by silica gel column chromatography to give 6-acetoxy-1,2,3,4-tetrahydro-1,1,4,4-tetramethylnaphthalene (34.5 g, 87.6%). $AlCl_3$ (37 g, 0.28 mol) was added to the acetate (62 g, 0.25 mol) in a 500-mL flask equipped with a mechanical stirrer and heated at 130-140 °C for 30 min. Then 300 mL of ice was added to the dark mixture and the whole was extracted with CH_2Cl_2. The organic layer was washed with H_2O and brine and dried over $MgSO_4$. The solvent was removed and the residue was chromatographed on silica gel to give 5,6,7,8-tetrahydro-3-hydroxy-5,5,8,8-tetramethyl-2-naphthyl methyl ketone (48.2 g, 77.7%). This phenolic compound (21.4 g, 0.087 mol) and terephthalic acid monomethyl ester chloride (36 g, 0.18 mol) were dissolved in 200 mL of pyridine and the solution was stirred overnight. The solvent was removed under vacuum and the residue was diluted with AcOEt and H_2O. After filtration, the organic layer was washed successively with 2 N HCl (twice), H_2O, 1 N $NaHCO_3$, H_2O, and brine and dried over $MgSO_4$. The solvent was removed and the crude product was purified by silica gel column chromatography and recrystallization from CH_3OH to give terephthalic acid 3-acetyl-5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthyl methyl ester (22.9 g, 64.5%). Terephthalic acid 3-acetyl-5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthyl methyl ester: colorless prisms (from CH_3OH), mp 166–167.5 °C; ^1H NMR (60 MHz, $CDCl_3$) δ 1.35 (s, 12 H), 1.69 (s, 4 H), 2.51 (s, 3 H), 3.94 (s, 3 H), 7.13 (s, 1 H), 7.84 (s, 1 H), 8.22 (br s, 4 H). Anal. ($C_{25}H_{28}O_5$) C, H, N.

4-[1-Hydroxy-3-oxo-3-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)-1-propenyl]benzoic Acid (Re80). To a solution of terephthalic acid 3-acetyl-5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthyl methyl ester (10.84 g, 0.027 mol) in 150 mL of pyridine was added KOH (85% purity, 3.82 g, 0.057 mol), and the mixture was stirred overnight. The yellow solution was poured into 1.5 L of 20% AcOH and extracted with AcOEt. The organic layer was washed with H_2O several times and dried over $MgSO_4$. After evaporation, the crude mixture was purified by silica gel column chromatography to give methyl 4-[1-hydroxy-3-oxo-3-(5,6,7,8-tetrahydro-3-hydroxy-5,5,8,8-tetramethyl-2-naphthalenyl)-1-propenyl]benzoate (Re81, 7.9 g, 72.9%), which was hydrolyzed to Re80 by the usual method (2 N NaOH in ethanol). Re81: pale yellow needles (from CH_2Cl_2–*n*-hexane), mp 161–162 °C; ^1H NMR (100 MHz, $CDCl_3$) δ 1.31 (s, 6 H), 1.35 (s, 6 H), 1.72 (s, 4 H), 3.97 (s, 3 H), 6.83 (s, 1 H), 6.96 (s, 1 H), 7.68 (s, 1 H), 8.00 (d, 2 H, J = 8 Hz), 8.10 (d, 2 H, J = 8 Hz), 11.62 (s, 1 H), 15.51 (s, 1 H). Anal. ($C_{25}H_{28}O_5$) C, H, N. Re80: pale yellow needles (from AcOEt–EtOH), mp 232–233 °C. Anal. ($C_{24}H_{26}O_5$) C, H, N.

4-(6,7,8,9-Tetrahydro-6,6,9,9-tetramethyl-4*H*-4-oxonaphtho[2,3-*b*]pyran-2-yl)benzoic Acid (Fv80). Concentrated H_2SO_4 (0.2 mL) was added to a solution of Re81 (80 mg, 0.2 mmol) in 5 mL of AcOH and refluxed for 30 min. The solution was poured into H_2O and extracted with AcOEt. The organic layer was washed with H_2O until the aqueous layer showed pH = ca. 7 and dried over $MgSO_4$. After the removal of the solvent, the crude product was purified by silica gel column chromatography to give methyl 4-(6,7,8,9-tetrahydro-6,6,9,9-tetramethyl-4*H*-4-oxonaphtho[2,3-*b*]pyran-2-yl)benzoate (Fv81, 32 mg, 41.8%), which was hydrolyzed with equimolar KOH in EtOH at room temperature to give Fv80. Fv81: colorless flakes (from CH_2Cl_2–*n*-hexane), mp 175–176 °C; ^1H NMR (100 MHz, $CDCl_3$) δ 1.37 (s, 12 H), 1.76 (s, 4 H), 3.96 (s, 3 H), 6.81 (s, 1 H), 7.49 (s, 1 H), 7.98 (d, 2 H, J = 8 Hz), 8.15 (s, 1 H), 8.17 (d, 2 H, J = 8 Hz). Anal. ($C_{25}H_{26}O_4$) C, H, N. Fv80: colorless prisms (from CH_3OH), mp >300 °C; ^1H NMR (100 MHz, $CDCl_3$–DMSO-d_6) δ 1.37 (s, 12 H), 1.77 (s, 4 H), 2.8–3.7 (br s, OH), 6.86 (s, 1 H), 7.57 (s, 1 H), 8.05 (d, 2 H, J = 8 Hz), 8.06 (s, 1 H), 8.16 (d, 2 H, J = 8 Hz). Anal. ($C_{24}H_{24}O_4$) C, H, N.

Other flavone-4′-carboxylic acids were prepared according to this method and their chemical and physical properties are listed in Table V.

4-(6,7,8,9-Tetrahydro-3-hydroxy-6,6,9,9-tetramethyl-4*H*-4-oxonaphtho[2,3-*b*]pyran-2-yl)benzoic Acid (Fv180). 5,6,7,8-Tetrahydro-3-hydroxy-5,5,8,8-tetramethyl-2-naphthyl methyl ketone (1 g, 4.07 mmol; see the section on Fv80) and terephthalaldehydic acid methyl ester (670 mg, 4.08 mmol) were dissolved in 20 mL of EtOH and 10 mL of 2 N NaOH, and the mixture was stirred overnight. The mixture was poured into 1 N HCl, and the precipitates were collected and then recrystallized to give (*E*)-4-[3-oxo-3-(5,6,7,8-tetrahydro-3-hydroxy-5,5,8,8-tetramethyl-2-naphthalenyl)-1-propenyl]benzoic acid (Ch110). To a suspension of Ch110 (215 mg, 0.57 mmol) in CH_3OH, 4 mL of 2 N NaOH was added dropwise at 0 °C. The suspension turned into a clear, dark red solution, to which 0.8 mL of 30% H_2O_2 was added slowly at 0 °C. The reaction mixture was kept in a refrigerator overnight and then poured into 1 N HCl and extracted with AcOEt. The organic layer was washed with H_2O and brine and dried over $MgSO_4$. The solution was concentrated and the residue was recrystallized to give Fv180. Ch110: orange prisms (from AcOEt–EtOH), mp 267–269 °C; ^1H NMR (100 MHz, $CDCl_3$–DMSO-d_6) δ 1.30 (s, 6 H), 1.36 (s, 6 H), 1.69 (s, 4 H), 6.91 (s, 1 H), 7.68 (d, 1 H, J = 16 Hz), 7.72 (d, 2 H, J = 8 Hz), 7.81 (s, 1 H), 7.86 (d, 1 H, J = 16 Hz), 8.08 (d, 2 H, J = 8 Hz), 12.25 (s, 1 H). Anal. ($C_{24}H_{26}O_4$) C, H, N. Fv180: yellow prisms (from AcOEt), mp 298–300 °C; ^1H NMR (100 MHz, $CDCl_3$–DMSO-d_6) δ 1.39 (s, 12 H), 1.77 (s, 4 H), 7.51 (s, 1 H), 8.13 (s, 1 H), 8.14 (d, 2 H, J = 8 Hz), 8.34 (d, 2 H, J = 8 Hz), 7.6–8.0 (br s, OH).

4-(6,7,8,9-Tetrahydro-3-methoxy-6,6,9,9-tetramethyl-4*H*-4-oxonaphtho[2,3-*b*]pyran-2-yl)benzoic Acid (Fv190). The ethyl ester of Fv180 (100 mg, 0.24 mmol) in 2 mL of DMF was added to a suspension of 60% NaH (15 mg, 0.375 mmol; washed twice with *n*-hexane) in 4 mL of DMF. After the mixture was stirred for 20 min at room temperature, a clear, dark orange solution was obtained, to which 2 mL of CH_3I was added. The mixture was heated at 90 °C for 1–2 min, another 0.5 mL of CH_3I was added, and heating was continued for 1 min. The solvent was removed under vacuum and the residue was chromatographed on silica gel to give methyl 4-(6,7,8,9-tetrahydro-3-methoxy-6,6,9,9-tetramethyl-4*H*-4-oxonaphtho[2,3-*b*]pyran-2-yl)benzoate (Fv191, 88 mg, 85.2%). A 2 N NaOH solution (2 mL) was added to a solution of Fv191 (88 mg, 0.20 mmol) in 8 mL of CH_3OH, and the mixture was stirred for several hours, poured into 1 N HCl, and extracted with AcOEt. The organic layer was washed with H_2O and brine and dried over $MgSO_4$. After evaporation, the crude product was purified by recrystallization to give Fv190 (81 mg, 98.4%). Fv190: pale yellow needles (from AcOEt–*n*-hexane), mp 300 °C; ^1H NMR (100 MHz, $CDCl_3$) δ 1.38 (s, 12 H), 1.76 (s, 4 H), 2.6–3.6 (br s, OH), 3.91 (s, 3 H), 7.45 (s, 1 H), 8.18 (s, 1 H), 8.21 (s, 4 H). Anal. ($C_{25}H_{26}O_5$) C, H, N.

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