Expedited Articles

Farnesyl Derivatives of Rigid Carboxylic Acids—Inhibitors of ras-Dependent Cell Growth

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Inhibitors of the enzyme that methylates ras proteins, the prenylated protein methyltransferase (PPMTase), are described. They are farnesyl derivatives of rigid carboxylic acids that recognize the farnesylcysteine recognition domain of the enzyme but do not serve as substrates. They also inhibit ras-dependent cell growth by a mechanism that is probably unrelated to inhibition of ras methylation, even though their potencies as PPMTase inhibitors and cell-growth inhibitors correlate well. The most potent inhibitor is *S-trans,trans*-farnesylthiosalicylic acid (FTS) (**2**). FTS (**2**) selectively inhibits the growth of human Ha-ras-transformed Rat1 cells *in vitro* (EC_{50} = 7.5 μM).

Introduction

ras proteins play a key role in tyrosine kinase growth factor receptor signaling.[1] These proteins bind GTP and propagate the growth factors' signal to the mitogen-activated protein (MAP) kinase cascade.[2] They are associated with the plasma membrane, where activation of c-raf1 occurs through direct ras–raf interaction.[3] Recent studies have shown that the ras-GTP complex recruits raf to the plasma membrane, where an additional component probably activates raf.[3] Termination of growth factor signaling involves hydrolysis of the ras-bound GTP to GDP. Oncogenic ras proteins do not hydrolyze GTP and are therefore in a permanently active state.[2] This contributes to the uncontrolled growth of tumor cells that express activated ras proteins.[4] Mutated ras proteins are frequently found in human cancers.[4] In some types of tumors, such as colon and pancreatic carcinomas, the incidence of activated ras is higher than 50%.[4] Therefore, pharmacological methods to curtail ras activity may be of use for the treatment of certain types of human cancers.

A pharmacological approach aimed at inhibiting ras oncoprotein activity has recently been described.[5] It was demonstrated that specific cell-active inhibitors of the CAAX farnesyltransferase (C = cysteine, A = aliphatic acid, and X = any amino acid) inhibit ras-dependent cell growth and reverse the transformed phenotype of cells expressing activated ras. These studies were prompted by earlier experiments indicating that farnesylation of ras oncoproteins is an absolute requirement for their membrane anchorage and transforming activity.[6] Because ras protein farnesylation is followed by proteolytic removal of their AAX and subsequent carboxyl methylation of the farnesylcysteine,[6,7] inhibitors of the protease or the methyltransferase would be expected to affect ras activity. However, point mutation analysis of the processing and activity of ras oncoproteins indicated that farnesylation alone is sufficient to confer membrane anchorage and activity.[6d] It was also reported that *N*-acetyl-*trans,trans*-farnesyl-L-cysteine (AFC), a substrate for the prenylated protein methyltransferase (PPMTase),[8,9] can inhibit ras methylation in ras-transformed NIH 3T3 cells but does not inhibit their growth.[8] Despite these considerations, there were several reasons to develop PPMTase inhibitors for studies on ras proteins and as potential blockers of ras functions. Firstly, PPMTase inhibitors may help in elucidating the yet unknown role of ras carboxyl methylation. Secondly, because the PPMTase is the last enzyme in the cascade of ras processing,[7b] it is plausible that its substrate recognition site would share some similarities with analogous sites that associate with the carboxy-terminal farnesylcysteine of ras. Accordingly, PPMTase inhibitors may recognize and interact with a membrane farnesylcysteine recognition domain that is important for ras functions. We report here on new farnesyl derivatives of rigid carboxylic acids, which are inhibitors of the PPMTase in a cell-free system while at the same time inhibiting ras-dependent cell growth by a mechanism unrelated to inhibition of methylation. The most potent such inhibitor obtained to date is *S-trans,trans*-farnesylthiosalicylic acid (FTS) (**2**), which affects tumor cell growth *in vitro*.

Chemistry

Recent studies of PPMTase have demonstrated that neither peptide derivatives nor farnesylated amino acids are required for substrate activity.[7–11] *S-trans,trans*-Farnesylthiopropionic acid (FTP) is an excellent substrate for the enzyme.[9,10] Moreover, it was demonstrated that shortening the distance between the thiofarnesyl and the carboxyl moieties by one methylene group, as in farnesylthioacetic acid (FTA),[9] resulted in a potent inhibition of the PPMTase.[9–11] By employing subtle modifications of the FTP structure that lead to

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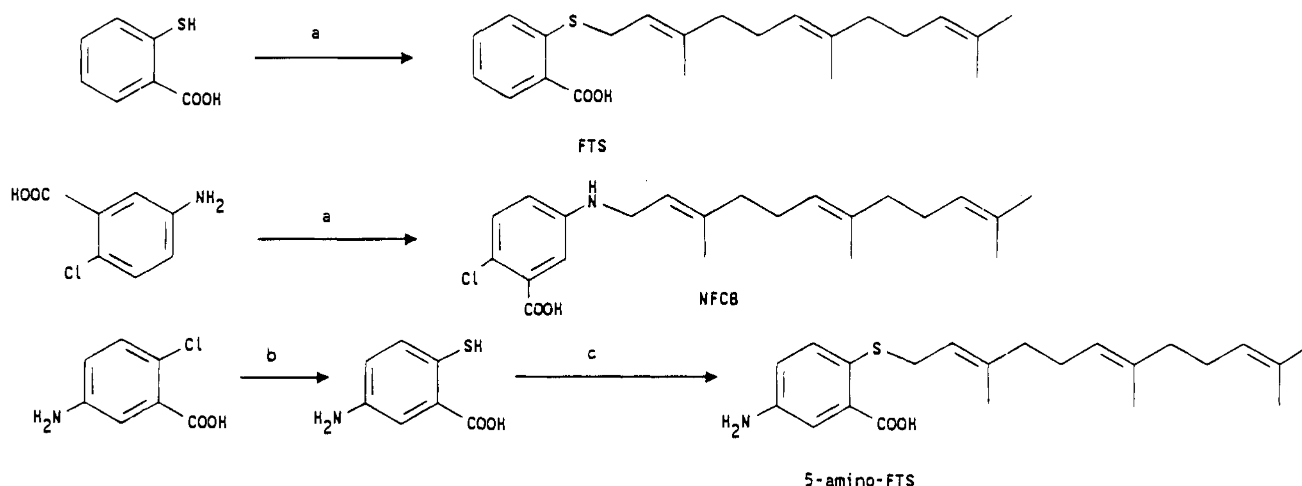
Scheme 1[a]



[a] (a) Farnesyl bromide, guanidino carbonate in acetone; (b) NaHS in water, reflux; (c) DMF, water, $KHCO_3$, then farnesyl bromide.

better recognition of the PPMTase but lack the substrate's properties, we prepared farnesyl derivatives of rigid carboxylic acids and examined their effects on enzyme activity and cell growth. Farnesylthiosalicylic acid (**2**) appears to serve as a prototype compound because its thiofarnesyl and carboxylic acid moieties are located at a distance of two carbon atoms (as in the enzyme substrates AFC and FTP) but are forced into a cis-configuration by the aromatic ring. Another related rigid carboxylic acid derivative, 5-amino-FTS (**6**), in which an amino group (present in the natural enzyme substrates) is substituted for hydrogen, was also prepared and tested. To better mimic the original position of the nitrogen atom in AFC, an additional derivative was prepared, namely farnesyl-2-mercaptonicotinic acid (FTN) (**8**), although in this compound the nitrogen has a tertiary planar sp^2 symmetry, while AFC has an sp^3 amine with more degrees of freedom. We also prepared and tested a farnesylamino derivative in which the sulfide linkage is replaced by an amine one, namely 2-chloro-5-(farnesylamino)benzoic acid (NFCB) (**4**).

The three pathways used for the preparation of these new farnesyl derivatives are summarized in Scheme 1. The products were purified by chromatography, and their structures were assigned on the basis of ^{1}H-NMR and mass spectrometry by analogy with related structures. The syntheses and analytical data of all the compounds are described in the Experimental Section.

***In Vitro* Inhibition of PPMTase.** Each of the new derivatives was first examined for its ability to serve as a substrate for PPMTase *in vitro* in a specific and sensitive assay, which uses synaptosomal membranes of young rat cerebellum. These membranes are highly enriched in PPMTase.[10,11] [*Methyl*-^{3}H]-*S*-adenosyl-L-methionine ([*methyl*-^{3}H]AdoMet) serves as a methyl donor, and exogenously added derivatives serve as methyl acceptors. Chloroform–methanol extraction was followed by base hydrolysis of the [^{3}H]methyl esters formed and vapor-phase diffusion of [^{3}H]methanol into a scintillation coctail, which was used to estimate formation of carboxymethyl esters. This assay can detect about 3 pmol of [^{3}H]methyl esters (approximately 3 times higher than the background level). As shown in Table 1, the enzyme substrates AFC and FTP[8–10] were methylated by the cerebellar PPMTase but the known enzyme inhibitor FTA[9,10] was not. Similarly,

Table 1. Farnesyl Derivatives of Rigid Carboxylic Acids That Do Not Serve as Substrates for PPMTase

compound	[^{3}H]methyl esters formed (pmol)
	0.85 ± 0.12
AFC (substrate)[a]	52.5 ± 6.7
FTP (substrate)[a]	26.3 ± 1.8
FTA (inhibitor)[a]	0.64 ± 0.08
FTS (**2**) (inhibitor)[a]	0.76 ± 0.10
NFCB (**4**) (inhibitor)[a]	0.37 ± 0.09
5-amino-FTS (**6**) (inhibitor)[a]	0.89 ± 0.12
FTN (**8**) (inhibitor)[a]	0.73 ± 0.08

[a] The compounds (150 μM final concentration) were tested for their ability to serve as methyl acceptors, with rat brain cerebellar synaptosomes used as a source for PPMTase (100 μg of protein) and 25 μM [*methyl*-^{3}H]AdoMet (300 000 cpm/mmol) as a methyl donor. The assay protocol was as described elsewhere.[10,11,13] Data represent the amount of [^{3}H]methyl esters formed in 15 min ($\pm$SEM), as determined in quadruplicate.

none of the farnesylated carboxylic acid derivatives yielded any significant signal above the background level in the PPMTase assay (Table 1), indicating that they did not serve as substrates for the enzyme. Evidence that the new derivatives do recognize PPMTase *in vitro* is presented in Figure 1, which demonstrates that they inhibit the enzymatic methylation of AFC in a dose-dependent manner. The calculated K_i values for the various farnesyl derivatives (summarized in Table 2) indicate that their rank order of potency is FTS (**2**) > NFCB (**4**) ≥ FTA > FTN (**8**) > 5-amino-FTS (**6**).

The general picture emerging from these results is that the rigid structure of the farnesylated carboxylic acid derivatives tested here confers an appropriate structural element that is recognized by rat cerebellum PPMTase. This structural element is apparently sufficient to allow binding to the enzyme but is not sufficient for the enzymatic methylation reaction to occur. It thus appears to be worth exploiting and further modifying the aromatic carboxylic structure for the design of new inhibitors of PPMTase. While FTS (**2**) was found to be the most potent of the PPMTase inhibitors prepared and tested here, our results show that other farnesylated aromatic carboxylic acids may also have an inhibitory potency. The similarity between the K_i values of the farnesylamino derivatives (NFCB) (**4**) and FTA suggests that the sulfide linkage is not necessary for potent inhibition. In contrast, modifica-

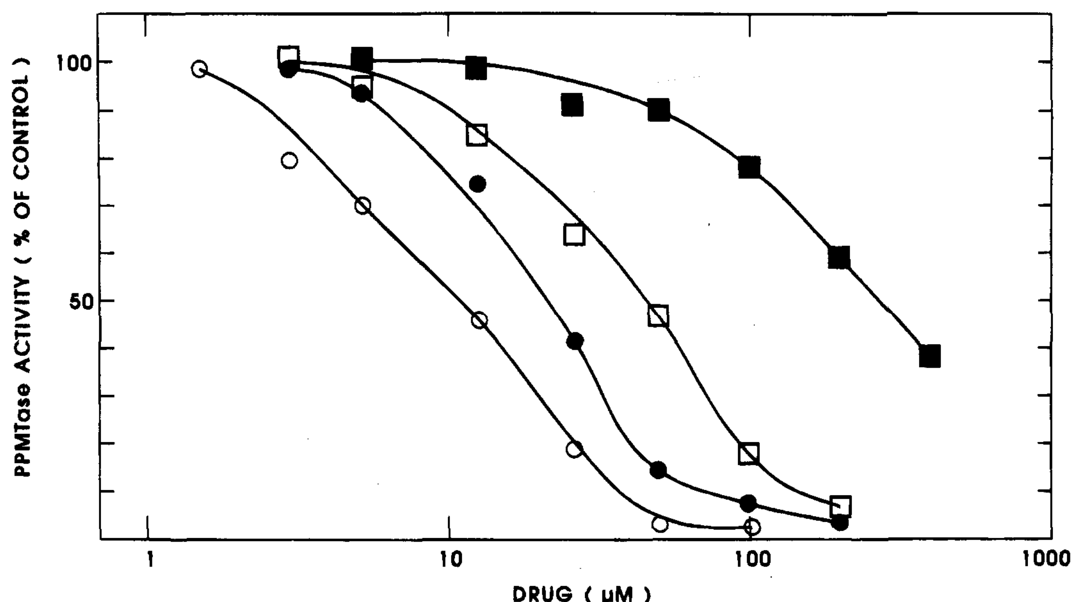


Figure 1. Dose-dependent inhibition of PPMTase by various farnesyl derivatives. Rat cerebellum synaptosomal membranes (100 μg of protein) were used as a source for PPMTase.[10,11] Enzyme assays were performed in duplicate as described,[10,11] using 50 μM AFC as substrate and 25 μM [*methyl*-^{3}H]AdoMet. Reactions were allowed to proceed at 37 °C for 15 min in the absence and presence of the indicated drug concentrations. Data are presented in terms of PPMTase activity in the drug's presence as a percentage of PPMTase activity in its absence. Incubation curves were generated with FTS (○), NFCB (●), FTN (□), and 5-amino-FTS (■).

Table 2. Inhibition Constants of Carboxylic Acid Farnesyl Derivatives

compound	inhibition of PPMTase in cell-free systems[a] K_i (μM)	inhibition of growth of ras-transformed cells[b] EC_{50} (μM)	cytotoxic end point[c] (μM)
FTS (**2**)	2.8 ± 1.3	7.5 ± 3.7	200
NFCB (**4**)	8.0 ± 2.5	30.8 ± 6.1	200
FTA	8.1 ± 3.7	37.2 ± 4.9	200
FTN (**8**)	14.9 ± 2.6	125.7 ± 20.5	200
5-amino-FTS (**6**)	72.8 ± 10.3	(not active at 200)	25

[a] K_i values were calculated from the IC_{50} values determined in inhibition curves as shown in Figure 1, using the equation $K_i = IC_{50}/(1 + S/K_M)$, where S = 50 μM AFC and K_M = 25 μM. Values represent means (±SD) of three separate determinations. [b] EC_{50} values (means of three to five determinations ± SD) represent the effective concentration that yields 50% inhibition of growth of human Ha-ras-transformed Rat1 (EJ)[15] cells, as determined from the dose–response curves for each of the compounds. The experimental protocol is described in ref 13. [c] The highest nontoxic concentration for the EJ cells, determined by MTT staining by a protocol described in ref 13.

tion of the FTS structure by the addition of a nitrogen atom in two different positions, namely as in FTN (**8**), and in the meta position of the carboxylic acid, as in 5-amino-FTS (**6**), resulted in reduced affinity in both cases (Table 2). In this regard it is interesting to note that modifications in the analogous positions of non-aromatic *S*-farnesyl derivatives also affect their interactions with PPMTase. Farnesylcysteine, unlike AFC, does not serve as a substrate for the enzyme,[8] while FTP—which lacks the amine group—is a PPMTase substrate.[9,10] Also, farnesylcysteine derivatives in which the α-amino group has been modified by substituents other than the acetyl group (as in AFC), e.g., isobutyryl, can serve as PPMTase substrates provided that these groups are not bulky as they are, for example, in benzoyl or pivolyl.[12]

Effects of PPMTase Inhibitors on Cell Growth. In related studies we have shown that the most potent available PPMTase inhibitor, FTS (**2**), selectively effects ras-dependent cell growth *in vitro*.[13] Apparently FTS (**2**) inhibits the mitogenic effect of epidermal and basic fibroblast growth factors in Rat1 fibroblasts that are not otherwise affected by FTS and inhibits the growth of human Ha-ras-transformed Rat1 fibroblasts or human endometrial carcinoma HEC1A cells that express an activated *K* ras.[13] The other PPMTase inhibitors studied here, except 5-amino-FTS (**6**), could also inhibit growth of Ha-ras-transformed Rat1 cells without cytotoxicity. Control and inhibitor-treated cultures were stained with MTT or trypan blue to detect respectively live and dead cells. Except 5-amino-FTS, with which dead cells were detected already at 25 μM, in all other cases cytotoxic effects were apparent only at relatively high concentrations (≥200 μM). Inhibition of the ras-transformed Rat1 cells growth was dose-dependent. A typical example of the effect of NFCB (**4**) on the ras-transformed cells grown in the presence of the inhibitor is shown in Figure 2. These cells usually grow in multilayer clumps typical of malignant transformation (Figure 2a). An apparent reversal of the transformed morphology was observed following treatment for 7 days with 5 μM NFCB (**4**) (Figure 2b), and the inhibitory effect was increased with the dose (Figure 2c,d). Similar effects were observed with FTS (**2**), FTA, and FTN (**8**). Dose–response curves were generated for each of the compounds using the experimental paradigm described in Figure 2 except that the number of cells was determined by direct counting. From these curves, EC_{50} values (effective concentration causing 50% inhibition of cell growth) were estimated (Table 2). The rank order of potency for the compounds was FTS (**2**) > NFCB (**4**) ≥ FTA > FTN (**8**). The compound 5-amino-FTS (**6**) was

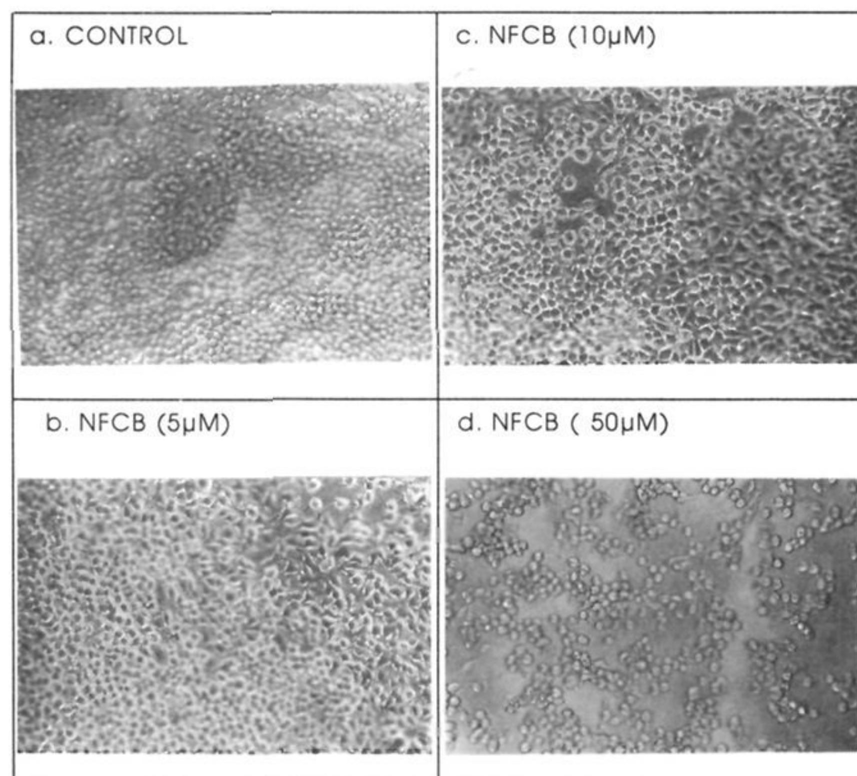


Figure 2. Inhibition of growth and reversal of the transformed phenotype of ras-transformed Rat1 cells by NFCB. Cells were grown in 24-well plates in DMEM/10% FCS in the presence of 0.1% DMSO (a, control) or at the indicated concentrations of NFCB in 0.1% DMSO (b–d). The experimental protocol was as described elsewhere.[13] Photomicrographs (magnification × 100) are of 7-day cultures. The original photograph has been reduced to 48% of its original size for publication purposes.

not active at all. A good correlation was observed between the *in vitro* K_i values for inhibition of rat cerebellum PPMTase in the cell-free system and the *in vitro* estimated EC_{50} values in intact cells (Table 2). These results seem to suggest that the farnesyl derivatives may inhibit ras-dependent cell growth by a mechanism related directly to the inhibition of ras methylation. Other experiments, however, which were performed with the most potent PPMTase inhibitor, FTS (**2**), do not support such a suggestion.[13] Apparently, FTS (**2**) is not an effective methylation inhibitor in intact Ha-ras-transformed Rat1 cells. It can partially inhibit methylation of ras and other proteins but at concentrations significantly higher (50–100 μM) than those required to inhibit cell growth (0.1–10 μM).[13] Therefore, it is unlikely that FTS (**2**) or the less potent PPMTase inhibitors affect cell growth by inhibiting protein methylation. It is also unlikely that these compounds affect the farnesylation or the proteolytic removal of the AAX carboxy-terminus of ras because these processing steps precede the methylation step.[6,7] Our *in vitro* studies confirmed that FTS (**2**) does not inhibit farnesylation of ras.[13] In view of these considerations, we propose that FTS (**2**) and related compounds selectively affect ras-dependent cell growth by interacting with a farnesylcysteine recognition domain distinct from the one present in the active site of the PPMTase. The presence of such a farnesylcysteine recognition domain for ras is already predicted from the observation that blocking of ras farnesylation prevents its membranes anchoring and transforming activity.[6] We know from other studies that FTS does not interfere with v-raf transformation (whose normal cellular form, c-raf1, is recruited to the membrane by ras)[3] but does affect ErbB2 transformation,[13] which acts upstream of ras.[14] It is therefore plausible that the selective effect of FTS, and probably of the related compounds, is due to interference with a membrane farnesylcysteine recognition domain that is important for ras–raf communication.[3] The good correlation between the K_i values of the compounds for inhibiting PPMTase and their EC_{50} values for affecting ras-dependent cell growth suggests similarities between farnesylcysteine recognition domains of the PPMTase and a ras-binding site. The experimental data suggest that FTS and NFCB bind to the latter site with a higher affinity than that with which they bind to PPMTase. Notably, the cytotoxic indexes of FTS (**2**) and NFCB (**4**) are at least 1 order of magnitude higher than their EC_{50} values for cell-growth inhibition (Table 2). Under the conditions used here, the doubling time of the Ha-ras-transformed Rat1 cells was 25 h, similar to that of the v-raf-transformed NIH 3T3 cells (24 h). In the presence of 5 μM NFCB or FTS, doubling times of the ras-transformed, but not of the v-raf-transformed, cells were increased to 36 and 30 h, respectively. These altered doubling times are similar to the one of the untransformed Rat1 cells which were grown under the same conditions (32 h). Together these observations are consistent with the notion that farnesylated rigid carboxylic acid derivatives may be effective nontoxic cytostatic drugs and that the *in vitro* PPMTase inhibition model may be successfully used for the design of additional potent inhibitors of ras-dependent cell growth.

Conclusions

We have described the preparation of several farnesylated rigid carboxylic acid derivatives that serve as pure inhibitors of the prenylated protein methyltransferase. While these compounds are potent inhibitors of the enzyme in a cell-free system, even the most potent derivative, FTS (K_i = 2.6 μM), is a poor methylation inhibitor in intact cells. Yet, FTS and several related analogs inhibit ras-dependent cell growth with potencies that correlate well with their inhibition constants for PPMTase. The structure–activity relationships described here, together with the demonstrated selectivity of FTS (**2**) toward ras-dependent cell growth in *in vitro* and its low cytotoxicity, may provide new strategies for the design of cancer treatment. They also provide important information for the design of additional nontoxic cytostatic drugs and a tool for studies of farnesylcysteine recognition domains that bind ras in the plasma membrane.

Experimental Section

N-Acetyl-L-cysteine, *S*-adenosyl-L-methionine, and mercaptoacetic acid were purchased from Sigma. *trans,trans*-Farnesyl bromide thiosalicylic acid, 5-amino-2-chlorobenzoic acid, and 2-mercaptonicotinic acid were purchased from Aldrich. [*Methyl*-^{3}H]-*S*-adenosyl-L-methionine (75 Ci/mmol) was from RCI. All other chemicals were of AR grade and purchased from Merck, Aldrich, or Sigma. Tissue culture supplies (media, sera, and antibiotics) were from Beit-Haemek, Israel. Tissue culture plates were from Corning, U.K. Silica gel for column chromatography (Merck art. no. 7733) and thin layer chromatography (TLC; Merck art. no. 5575) was from Merck, Germany. *N*-Acetyl-*S*-*trans,trans*-farnesyl-L-cysteine (AFC) and *S*-*trans,trans*-farnesylthioacetic acid (FTA) were prepared from *trans,trans*-farnesyl bromide, *N*-acetyl-L-cysteine, and mercaptoacetic acid as described in detail previously[9] with only minor modifications. Synthesis of the new farnesyl derivatives is described below. All analogs were purified on silica gel columns and analyzed by proton nuclear magnetic resonance (^{1}H-NMR), mass spectroscopy, and TLC visualized by iodine

vapor. The NMR data were determined with a Bruker AMX 360-WB NMR spectrometer, using deuterated chloroform ($CDCl_3$) as solvent and tetramethylsilane (TMS) as internal standard. Abbreviations used are singlet (s), broad singlet (bs), and multiplet (m). Mass spectra were determined using a DuPont 21-49113 spectrometer. High-resolution mass spectra (HRMS) were determined using a Varian MAT Model 711 spectrometer in The Mass Spectrometry Center, The Technion, Haifa, Israel. The ^{1}H-NMR and mass spectra data obtained for AFC and FTA were identical with those obtained by Tan et al. (1991).[9] Elementary analyses (Anal.) were determined using a Perkin Elmer CHN analyzer in the Microanalysis Laboratory, The Hebrew University, Jerusalem.

Synthesis of Farnesylthiosalicylic Acid (FTS) (2). Thiosalicylic acid (1) (0.9 g, 6 mmol), guanidine carbonate (1.3 g, 7 mmol), and *trans,trans*-farnesyl bromide (**9**) (1.7 g, 6 mmol) were mixed overnight in 75 mL of acetone at room temperature. After the acetone had evaporated, chloroform was added together with a few drops of 2 N HCl. The mixture was washed with water, and the organic phase was separated and dried on magnesium sulfate and then evaporated. A yellowish oil was obtained. The product, FTS (**2**), was purified on silica gel with mixtures of chloroform and ethyl acetate (5:1–1:5) and then ethyl acetate as eluants (85% yield). FTS (**2**): IUPAC name, (3,7,11-trimethyldodeca-2,6,10-trienyl)-2-thiobenzoic acid; appearance, pale yellow solid; mp 63 °C; TLC (silica gel, chloroform/ethyl acetate, 1:1) $R_f = 0.67$; MS *m/e* 358 (M^+), 222, 204, 152, 136, 121, 107, 93, 81, 69, 68; HRMS *m/e* 358 ($C_{22}H_{30}O_2S$); ^{1}H-NMR ($CDCl_3$, TMS) δ 1.55 (3H, s), 1.57 (3H, s), 1.6 (3H, s), 1.67 (3H, s), 1.97 (m, 4H), 2.02 (m, 4H), 3.45 (bs, 2H), 5.1 (m, 2H), 5.25 (m, 1H), 7.0 (m, 1H), 7.2 (m, 3H), 7.9 (m, 1H). Anal. ($C_{22}H_{30}O_2S$) C, H, N.

Synthesis of 2-Chloro-5-(farnesylamino)benzoic Acid (NFCB) (4). 5-Amino-2-chlorobenzoic acid (1.58 g, 5.8 mmol) was dissolved in 75 mL of dry acetone. Guanidine carbonate (1.3 g, 7 mmol) and *trans,trans*-farnesyl bromide (1.3 g, 4.6 mmol) were then added, and the reaction mixture was mixed for a few hours at room temperature. An additional portion of 1.3 g of guanidine carbonate was then added for overnight mixing at room temperature. The reaction mixture was filtered and the solid washed with acetone. The filtrates were combined and evaporated. The product, NFCB, was purified on a silica gel column with mixtures of chloroform and ethyl acetate (4:1–1:9) as eluants (60% yield). It was characterized by TLC, mass spectrometry, and NMR. NFCB (**4**): IUPAC name, 5′-[(3,7,11-trimethyldodeca-2,6,10-trienyl)amino]-2′-chlorobenzoic acid; appearance, white solid; TLC (silica gel, ethyl acetate) $R_f = 0.6$; MS *m/e* 375/377 (M^+), 342, 306, 237, 204, 171; HRMS *m/e* 377/375 (M^+) ($C_{22}H_{30}NO_2Cl$); ^{1}H-NMR ($CDCl_3$, TMS) δ 1.55 (s, 3H), 1.57 (s, 3H), 1.60 (s, 3H), 1.67 (s, 3H), 1.97 (m, 4H), 2.0 (m, 4H), 3.8 (d, 2H), 5.1 (m, 4H), 6.6 (m, 1H), 7.05 (d, 1H), 7.1 (m, 1H).

Synthesis of 5-Amino-2-farnesylthiosalicylic Acid (5-amino-FTS) (6). 5-Amino-2-chlorobenzoic acid (1.5 g, 5.5 mmol) was dissolved in water, and NaHS (1.2 g, 0.022 mol) was added. The mixture was refluxed for 2 h and the water then evaporated to yield 2.1 g of a solid gray material (5-amino-2-mercaptobenzoic acid). A portion of the product (3.6 g, 3.5 mmol) was dissolved in a minimal volume of dimethylformamide (DMF), and $KHCO_3$ (0.4 g, 4 mmol) was added. Following continuous mixing for 1 h, *trans,trans*-farnesyl bromide (1 g, 3.5 mmol) was added and the reaction allowed to proceed overnight at room temperature under constant mixing. The DMF and water were then removed by distillation, and the resulting solid was dissolved in methanol and filtered to remove insoluble material. The soluble portion was purified on a silica gel column with mixtures of methanol and chloroform (1:9–9:1) and then methanol as eluants. The expected product was further purified on a preparative silica gel plate developed with methanol/2% ammonia ($R_f = 0.4$) (1% yield). 5-amino-FTS (**6**): IUPAC name, 5′-amino-2′-(3,7,11-trimethyldodeca-2,6,10-trienyl)thiobenzoic acid; appearance, pale yellowish oil; MS *m/e* 290 [373(K^+) $(CH_3)_2CCHCH_2CH_2$], 256, 237 (*S*-farnesyl), 235, 204, 171, 135 (molecular peak not visible); TLC (silica gel, chloroform/methanol, 9:1) $R_f = 0.5$; ^{1}H-NMR (DMSO-d_6) δ 1.13 (s, 6H), 1.60 (s, 3H), 1.65 (s, 3H), 1.9–2.1 (m, 8H), 3.8 (d, 2H), 5–5.2 (2 multiplets, 3H), 6.4 (dd, 1H), 6.65 (bs, 1H), 6.95 (d, 1H).

Synthesis of Farnesyl-2-mercaptonicotinic Acid (FTN) (8). 2-Mercaptonicotinic acid (0.6 g, 6 mmol), guanidine carbonate (1.3 g, 7 mmol), and *trans,trans*-farnesyl bromide (1.7 g, 6 mmol) were mixed overnight in 75 mL of dry acetone at room temperature. After the acetone had evaporated, chloroform was added together with a few drops of 2 N HCl. The mixture was washed with water and the organic phase collected and dried on magnesium sulfate and then evaporated. The product was purified on a silica gel column with ethyl acetate and mixtures of ethyl acetate:methanol (4:1–1:4) as eluants (32% yield). The product, FTN, was characterized by TLC, mass spectrometry, and NMR. FTN (**8**): IUPAC name, 3′-(3,7,11-trimethyldodeca-2,6,10-trienyl)thiopyridine-2′-carboxylic acid; appearance, white solid; MS *m/e* 359 (M^+); TLC (silica gel, chloroform/ethyl acetate, 1:1) $R_f = 0.33$; mp 86 °C; ^{1}H-NMR ($CDCl_3$, TMS) δ 1.55 (s, 3H), 1.57 (s, 3H), 1.60 (s, 3H), 1.67 (s, 3H), 2.05 (m, 4H), 2.10 (m, 4H), 3.75 (d, 2H), 5.1 (m, 2H), 5.4 (m, 1H), 7.0 (m, 1H), 8.25 (m, 1H), 8.5 (m, 1H), 9.6 (m, H).

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