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Prenyloxyphenylpropanoids as novel lead compounds for the selective inhibition of geranylgeranyl transferase I

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Abstract—In this study, we synthesized some natural and semisynthetic prenyloxyphenylpropanoids (e.g., coumarins and cinnamic acid derivatives) and we assessed their in vitro inhibitory activity against farnesyl transferase (FTase) and geranylgeranyl transferase I (GGTase I). No compound was an effective inhibitor of FTase, while farnesyloxycinnamic acids were shown to selectively inhibit GGTase I with IC_{50} values ranging from 28 to 39 μM.

Prenyltransferases such as farnesyltransferase (FTase) and geranylgeranyltransferase I (GGTase I) are excellent targets for designing novel anticancer drugs since the small GTPases of the Ras superfamily are involved in neoplastic transformation.[1–5] For example, Ras proteins which are farnesylated and Rho and Ral proteins which are geranylgeranylated are found persistently activated in human cancers. Furthermore, a large number of studies demonstrated the involvement of these GTPases in uncontrolled cell division, resistance to apoptosis, angiogenesis, invasion and metastasis.[1–6] The fact that post-translational modifications of small GTPases by FTase or GGTase I are required for their cancer causing activity prompted us and others to design and develop inhibitors of these two enzymes as novel anticancer drugs. FTase and GGTase I transfer the 15-carbon farnesyl and the 20-carbon geranylgeranyl, respectively, from farnesylpyrophosphate (FPP) and geranylgeranylpyrophosphate (GGPP) to the cysteine of proteins that end with CaaX sequence (C = cysteine, a = aliphatic amino acid, and X = any amino acid) at their carboxyl termini.[1–6] FTase prefers when X is methionine or serine, whereas GGTase I prefers when X is leucine or isoleucine. To date most FTase and GGTase I inhibitors have focused on the development of inhibitors that compete with the CaaX binding site, and only a few have targeted the FPP and GGPP binding sites.

In the last five years, our research group studied chemical and pharmacological properties of secondary metabolites of phenylpropanoid biosynthetic origin containing a sesquiterpenyl, monoterpenyl, and isopentenyl chains attached to a phenol group, that represents quite a rare group of natural products.[7–11] Among these the ethyl ester (**2**) of 3-(4′-geranyloxy-3′-methoxyphenyl)-2-*trans* propenoic acid (**1**), the latter isolated in 1966 from the bark of *Acronychia baueri* Schott, an Australian small plant belonging to the family of Rutaceae,[12] showed a series of interesting biological effects such as cancer chemoprevention by dietary feeding in rats and other effects closely related to cancer growth and development, that were recently reviewed.[13]

In continuation of our studies aimed to evaluate pharmacological properties of natural and semi-synthetic prenyloxyphenylpropanoids, we wish to report herein the activity of these compounds as in vitro inhibitors of prenyl transferases, namely FTase and GGTase I. In addition to compounds (**1**) and (**2**), we synthesized

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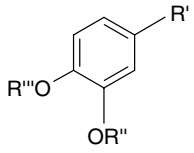
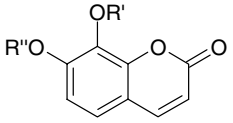
and evaluated six natural prenyloxyphenylpropanoids, namely 3-(4′-geranyloxy-3′-OH-phenyl)-2-*trans* propenoic acid (**3**), isolated from the same source of compound (**1**),[12] boropinic acid (**5**), isolated from *Boronia pinnata* Sm.,[14] valencic acid (**9**), isolated from *Citrus sinensis* L. and *Aegle marmelos* (Fam. Rutaceae),[15] 4-isopentenyloxy-3-methoxy benzoic acid (**11**), 4-geranyloxy-3-methoxy benzoic acid (**12**), both isolated as methyl esters from the liverwort *Trichocolea lanata* (Ehrh.) Dumm. (Fam. Trichocolaceae),[16] umbelliprenine (**13**), a farnesyloxycoumarin commonly found in *Ammi*, *Ruta* and *Citrus* species,[8] auraptene (**14**), the most abundant geranyloxycoumarin extracted from plants belonging to genus *Citrus*,[8] collinin (**15**), isolated from *Zanthoxylum schinifolium*,[8] 7-isopentenyloxycoumarin (**16**), extracted from plants belonging to genus *Ruta*,[8] and four semi-synthetic compounds, namely (**4**), the ethyl esters of acid (**3**), 3-(4′-isopentenyloxy-3′-OH-phenyl)-2-*trans* propenoic acid (**6**), 3-(4′-farnesyloxy-3′-OH-phenyl)-2-*trans* propenoic acid (**7**), 3-(4′-farnesyloxy-3′-methoxyphenyl)-2-*trans* propenoic acid (**8**) and finally 4′-geranyloxybenzoic acid (**10**).



3 R' =-CH=CH-COOH, R'' = -H, R''' = geranyl
4 R' = -CH=CH-COOEt, R'' = -H, R''' = geranyl
5 R' = -CH=CH-COOH, R'' = -OMe, R''' = isopentenyl
6 R' = -CH=CH-COOH, R'' = -H, R''' = isopentenyl
7 R' = -CH=CH-COOH, R'' = -H, R''' = farnesyl
8 R' = -CH=CH-COOH, R'' = -OMe, R''' = farnesyl
9 R' = -COOH, R'' = -H, R''' = isopentenyl
10 R' = -COOH, R'' = -H, R''' = geranyl
11 R' = -COOH, R'' = -OMe, R''' = isopentenyl
12 R' = -COOH, R'' = -OMe, R''' = geranyl



13 R' = -H, R'' = farnesyl
14 R' = -H, R'' = geranyl
15 R' = -OMe, R'' = geranyl
16 R' = -H, R'' = isopentenyl

Compounds (**1**), (**3**), (**5**), (**9**), (**11**), (**12**), (**14**), and (**15**) were synthesized as already reported.[11] The synthesis of compounds (**2**), (**4**), (**6**), (**7**), (**8**), (**10**), (**13**), and (**16**) was accomplished following an environmentally friendly route similar to that already described.[7,11] Ethyl esters (**2**) and (**4**) were obtained in 89% and 92% overall yield, respectively, starting from commercially available ferulic and *trans p*-coumaric acids, that were first converted into ethyl esters by reaction in refluxing EtOH under catalysis of concd H_2SO_4, alkylated with geranyl bromide in refluxing acetone using dry K_2CO_3 as base and finally purified by crystallization in *n*-hexane (Fig. 1).[18]

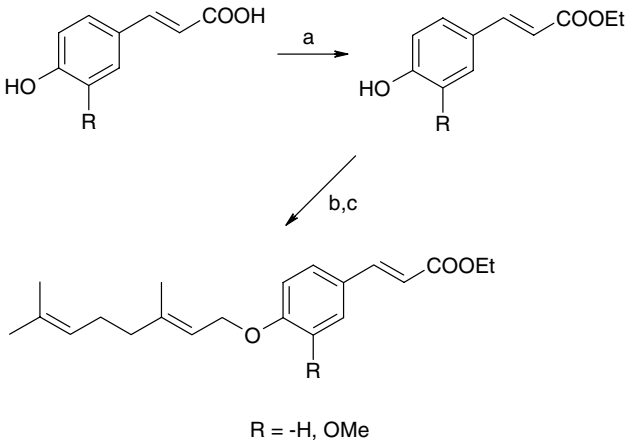


Figure 1. Reagents and conditions: (a) EtOH, concd H_2SO_4 (cat.), reflux 12 h; (b) geranyl bromide (1.2 equiv.), K_2CO_3 (1.2 equiv.), acetone, reflux 2 h; (c) crystallization.

Acids (**7**) and (**8**) were obtained by the same procedure reported for the synthesis of compounds (**1**), (**3**) and (**5**)[11] in 78% and 84% yield, respectively, and using all *trans*-farnesyl bromide as alkylating agent.[17] Finally prenyloxycoumarins (**13**) and (**16**) were synthesized in 86% and 99% yield, respectively, by the same procedure reported for the synthesis of auraptene and collinin,[7] using all *trans*-farnesyl bromide and 4-bromo-2-methyl-2-butene as alkylating agents.[16]

Compounds **1**–**16** were then evaluated for their ability to inhibit in vitro FTase and GGTase I at a concentration of 100 μM (Table 1).

As shown in Table 1 a well defined and distinguished pattern of results was recorded. None of the **16** com-

Table 1. Effects of prenyloxyphenylpropanoids **1**–**16** (100 μM) on FTase and GGTase I inhibition in vitro

Compound	% Inhibition	
	FTase	GGTase I
1	13.4 ± 6.4	78.6 ± 12.8
2	5.5 ± 0.6	3.0 ± 13.4
3	12.7 ± 23.0	72.4 ± 9.4
4	−7.4 ± 22.1	7.5 ± 21.5
5	9.3 (*n* = 2)	31.0 (*n* = 2)
6	43.2 (*n* = 2)	46.4 (*n* = 2)
7	16.2 (*n* = 2)	93.5 (*n* = 2)
8	11.0 (*n* = 2)	83.9 (*n* = 2)
9	0 (*n* = 2)	0 (*n* = 1)
10	17.6 (*n* = 2)	0 (*n* = 1)
11	2.4 (*n* = 2)	0 (*n* = 1)
12	0 (*n* = 2)	0 (*n* = 1)
13	12.5 ± 4.8	13.4 ± 6.4
14	7.9 ± 9.9	18.6 ± 6.1
15	15.5 ± 15.9	34.2 ± 6.9
16	14.1 ± 14.8	−10.3 ± 15.4

Table 2. IC_{50} values for inhibition of GGTase I for compounds **1**, **3**, **7** and **8**

Compound	IC_{50} (μM)
1	55 ± 14
3	39 ± 9.5
7	28 (n = 1)
8	66 (n = 1)

pounds evaluated inhibited FTase potently. Compound (**6**) was the most potent and only inhibited FTase by 43% at 100 μM. In contrast, four compounds, (**1**), (**3**), (**7**), and (**8**) namely acids containing a geranyl or farnesyl side chain attached to the phenol group, inhibited GGTase I with values ranging from 72.4% to 93.5%. For compounds (**1**), (**3**), (**7**), and (**8**) subsequent dose response experiments were performed, and the corresponding IC_{50} values are shown in Table 2.

The most potent compound was the farnesyloxy derivative of *trans p*-coumaric acid (**7**) that inhibited GGTase I with an IC_{50} value of 28 μM. Substituting the farnesyl side chain with a geranyl one as in (**3**) (IC_{50} = 39 μM) or an isopentenyl one as in (**6**) ($IC_{50} \approx 100$ μM) decreases the ability of the prenyloxy derivative to inhibit GGTase I, suggesting that the length of the isoprenyl moiety is crucial for fully occupying the 20-carbon GGPP binding pocket of this enzyme. Addition to (**7**) of a methoxy group *ortho* to the 4-prenyloxy chain as in (**8**) decreased its potency from an IC_{50} value of 28–66 μM. Similarly, addition of a methoxy to (**3**) as in (**1**) also decreased its potency suggesting that a methoxy group is not preferred by the GGTase I binding pocket. Benzoic acids are totally inactive towards inhibition of both enzymes suggesting that an α, β-unsaturated conjugated carbon–carbon double bond is a main structural feature for compounds to be active as GGT-ase I inhibitors. Furthermore, free acids are by far better GGTase I inhibitors than the corresponding ethyl esters, indicating that the binding pocket in GGTase I requires a negatively charged carboxylate anion that probably mimics the negatively charged pyrophosphate of the GGPP molecule. Finally from data reported herein, it is evident that lactones are less efficient inhibitors of GGTase I than the corresponding cinnamic acid derivatives.

In conclusion, the findings described in this paper indicate that farnesyloxy- and geranyloxycinnamic acids are potential lead compounds of a novel class of selective GGTase I inhibitors. It is noteworthy that the interest towards molecules having this kind of mechanism of inhibition as potential cancer therapeutic agents has greatly increased over the past few years, and some compounds having GGTase I inhibitory effects have been reported.[6] Considering that all compounds tested have been easily synthesized from widely available and non-toxic starting materials by a high-yielding, environmentally friendly and cheap synthetic route, these results provide further insights into the mechanism of action and help to better define the pharmacological profile of these secondary metabolites and related semi-synthetic derivatives.

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17. Experimental. For the synthesis of compounds **1**–**12** the same general procedure as reported previously was followed (see Refs. [7,11]). 3-(4′-Geranyloxy-3′-methoxyphenyl)-2-*trans* propenoic acid (**1**). White solid; yield 96%; analytical data are in full agreement with those reported in the literature.[9] Ethyl 3-(4′-geranyloxy-3′-methoxyphenyl)-2-*trans* propenoate (**2**). White solid; yield 89%; analytical data are in full agreement with those reported in the literature.[16] 3-(4′-Geranyloxyphenyl)-2-*trans* propenoic acid (**3**). White solid; yield 97%; analytical data are in full agreement with those reported in the literature.[11] Ethyl 3-(4′-Geranyloxyphenyl)-2-*trans* propenoate (**4**). White solid; yield 92%; mp: 121–124 °C; IR (KBr): 1685 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$ δ): 1.33 (t, 3 H, J = 7.2 Hz), 1.59 (s, 3H), 1.66 (s, 3H), 1.72 (s, 3H), 2.09–2.25 (m, 4H), 4.25 (q, 2H, J = 7.2 Hz), 4.45–4.48 (m, 2H), 5.05–5.12 (m, 1H), 5.46–5.51 (m, 1H), 6.41 (d, 1H, J = 3.6 Hz), 6.86–6.91 (m, 2H), 7.39–7.44 (m, 2H), 7.62 (d, 1H, J = 3.6 Hz); ^{13}C NMR (50 MHz, $CDCl_3$ δ) 14.9, 16.1, 17.5, 25.6, 26.2, 39.4, 60.9, 64.9, 115.0, 118.8, 119.7, 123.8, 127.9, 130.1, 131.3, 141.6, 144.7, 156.9, 167.4; Anal. Calcd for $C_{21}H_{28}O_3$: C, 76.79; H, 8.59; O, 14.61. Found C, 76.77; H, 8.60; O, 14.59. Boropinic acid (**5**). White solid; yield 96%; analytical data are in full agreement with those

reported in the literature.[11] 3-(4′-Isopentenyloxyphenyl)-2-*trans* propenoic acid (**6**). White solid; yield 97%; mp: 164-165 °C; IR (KBr): 1690 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$ δ): 1.71 (s, 3H), 1.77 (s, 3H), 4.54–4.56 (m, 2H), 5.44–5.47 (m, 1H), 6.38 (d, 1H, J = 3.7 Hz), 6.79–6.82 (m, 2H), 7.58–7.61 (m, 2H), 7.74(d, 1H, J = 3.7 Hz); ^{13}C NMR (50 MHz, $CDCl_3$ δ) 18.3, 25.9, 65.1, 115.3, 117.6, 119.9, 128.3, 129.3, 138.4, 144.2, 157.7, 168.8; Anal. Calcd for $C_{14}H_{16}O_3$: C, 72.39; H, 6.94; O, 20.63. Found C, 72.41; H, 6.93; O, 20.61. 3-(4′-Farnesyloxyphenyl)-2-*trans* propenoic acid (**7**). White solid; yield 78%; mp: 199–202 °C; IR (KBr): 1688 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$ δ):1.60 (s, 3H), 1.62 (s, 3H), 1.67 (s, 3H), 1.80 (s, 3H), 2.03–2.14 (m, 8H), 4.47–4.50 (m, 2H), 5.06–5.13 (m, 2H), 5.47–5.52 (m, 1H), 6.40 (d, 1H, J = 3.6 Hz), 6.78 – 6.82 (m, 2H), 7.57–7.61 (m, 2H), 7.73 (d, 1H, J = 3.6 Hz); ^{13}C NMR (50 MHz, $CDCl_3$ δ) 13.7, 16.1, 17.7, 25.7, 25.9, 26.7, 39.6, 64.9, 115.1, 117.4, 119.8, 123.9, 124.3, 128.5, 129.2, 131.3, 135.3, 141.6, 144.9, 157.2, 168.7; Anal. Calcd for $C_{24}H_{32}O_3$: C, 78.22; H, 8.75; O, 13.02. Found C, 78.24; H, 8.73; O, 13.00. 3-(4′-Farnesyloxy-3′-methoxyphenyl)-2-*trans* propenoic acid (**8**). White solid; yield 84%; mp: 216–218 °C; IR (KBr): 1687 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$ δ): 1.61 (s, 3H), 1.63 (s, 3H), 1.67 (s, 3H), 1.81 (s, 3H), 2.02–2.14 (m, 8H), 3.82 (s, 3H), 4.70–4.74 (m, 2H), 5.07–5.13 (m, 2H), 5.47–5.51 (m, 1H), 6.42 (d, 1H, J = 3.6 Hz), 6.73–6.76 (m, 1H), 7.02–7.06 (d, 1H, J = 3.6 Hz), 7.19–7.22 (m, 2H); ^{13}C NMR (50 MHz, $CDCl_3$ δ) 13.7, 16.2, 17.6, 25.5, 25.8, 26.4, 39.8, 55.8, 65.9, 110.8, 114.6, 117.1, 119.8, 121.0, 123.8, 124.1, 127.9, 129.2, 131.2, 135.4, 141.9, 144.3, 145.3, 150.9, 168.1; Anal. Calcd for $C_{25}H_{34}O_4$: C, 75.34; H, 8.60; O, 16.06. Found C, 75.33 H, 8.62; O, 16.04. Valencic acid (**9**): White solid; yield 94%; analytical data are in full agreement with those reported in the literature.[11] 4′-geranyloxybenzoic acid (**10**): White solid; yield 92%; analytical data are in full agreement with those reported in the literature.[19] 4-isopentenyloxy-3-methoxy benzoic acid (**11**): White solid; yield 97%; analytical data are in full agreement with those reported in the literature.[11] 4-geranyloxy-3-methoxy benzoic acid (**12**): White solid; yield 95%; analytical data are in full agreement with those reported in the literature.[11] Umbelliprenine (**13**). White solid; yield 86%; analytical data are in full agreement with those reported in the literature.[8] Auraptene (**14**). White solid; yield 99%; analytical data are in full agreement with those reported in the literature.[8] Collinin (**15**). White solid; yield 74%; analytical data are in full agreement with those reported in the literature.[8] 7-Isopentenyloxycoumarin (**16**). White solid; yield 99%; analytical data are in full agreement with those reported in the literature.[8] *Prenyltransferase activity assays.* Inhibition studies were performed by determining the ability of prenyloxyphenylpropanoids to inhibit the transfer of [^{3}H]farnesyl and [^{3}H]geranylgeranyl from [^{3}H]farnesylpyrophosphate ([^{3}H]FPP, Amersham Biosciences, Piscataway, NJ) and [^{3}H]geranyl-geranylpyrophosphate ([^{3}H]GGPP, Perkin Elmer, Wellesley, MA) to recombinant H-Ras-CVLL and H-Ras-CVLS, respectively, as described previously.[19] Briefly, 60,000*g* supernatants of human Burkitt's lymphoma (Daudi) cells (American Type Culture Collection, Rockville, MD) were incubated for 30 min at 37 °C in the presence of either vehicle control or prenyloxyphenylpropanoids, H-Ras substrate, and 0.5 μCi/sample of [^{3}H]-FPP or [^{3}H]GGPP. Samples were denatured with SDS and precipitated with TCA then filtered onto glass fiber filters. Unbound [^{3}H]FPP or [^{3}H]GGPP was washed through the filters. Samples were counted on a scintillation counter and activity of compounds were compared to vehicle control to obtain prenyltransferase inhibition values and IC_{50}s.

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