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Synthesis of a novel prodrug of 3-(4′-geranyloxy-3′-methoxyphenyl)-2-*trans*-propenoic acid for colon delivery

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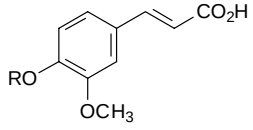
Abstract—A novel high-yielding and environment-friendly synthesis of the anticancer compound 3-(4′-geranyloxy-3′-methoxyphenyl)-2-*trans*-propenoic acid is described. This compound was conjugated to H_2N-Ala-Pro dipeptide to give a prodrug to be activated by intestinal ACE and to be used in the treatment of different forms of colon cancer. Data on the chemical and enzymatic stability of this novel prodrug are reported.

Ferulic acid (**1**) is a widespread natural compound belonging to the class of cinnamic acids.[1,2] In the last decade, ferulic acid and its derivatives have attracted the attention of many researchers due to their valuable pharmacological properties.



1 R = H
2 R = geranyl

A biosynthetically related secondary metabolite of (**1**) is 3-(4′-geranyloxy-3′-methoxyphenyl)-2-*trans*-propenoic acid (**2**), in which a geranyl chain is attached to the phenolic group. It has been isolated in 1966 from the bark of *Acronychia baueri* Schott, an Australian small tree belonging to the family of Rutaceae.[3]

Although known for four decades, only in the last five years some of the pharmacological properties of (**2**) and its synthetic derivatives began to be characterized. In particular, the ethyl ester of (**2**) showed a series of interesting biological effects such as colon and tongue cancer chemoprevention by dietary feeding in rats,[4,5] inhibition of cellular responses induced by phorbol ester and by combined lipopolysaccharide and interferon-γ,[6] and suppression of inducible nitric oxide synthase[5] and cycloxygenase-2 as such and of its promoter activities;[6,7] furthermore, some myo-inositol esters of (**2**) exerted a good inhibitory activity on phorbol ester-induced superoxide generation and Epstein–Barr virus activation.[8] These data were collected using whole cell systems so it is conceivable that, under experimental conditions, the above-reported esters could be hydrolyzed to the parent acid once inside the cell, so the true active principle exerting these biological effects would be (**2**). Then 3-(4′-geranyloxy-3′-methoxyphenyl)-2-*trans*-propenoic acid becomes a novel candidate as chemopreventive drug for the cure of various types of cancer and as anti-inflammatory compound. For these reasons the design and synthesis of biologically active new ferulic acid derivatives is a field of current and growing interest.

In the last three years, our work was devoted to the design and synthesis of natural and semisynthetic geranyloxy substituted compounds as colon cancer chemopreventive agents. One of the approaches for the treatment of local diseases of colon is the use of prodrugs which optimizes drug delivery, improves its efficacy, and lowers the absorption and release of the drug in the stomach and small intestine thereby facilitating quantitative delivery to the colon and increasing the bioavailability at the site of action. The different approaches for colonic drug delivery have been recently exhaustively reviewed.[9] Among these, a promising one is represented by the selective cleavage of prodrugs containing an oligopeptide chain by enzymes located in the external side of the intestinal brush border membrane.[10]

Keywords: Cinnamic acids; Colon delivery; Colon cancer; Ferulic acid derivatives; Prodrug.
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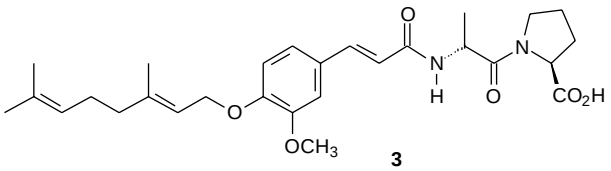
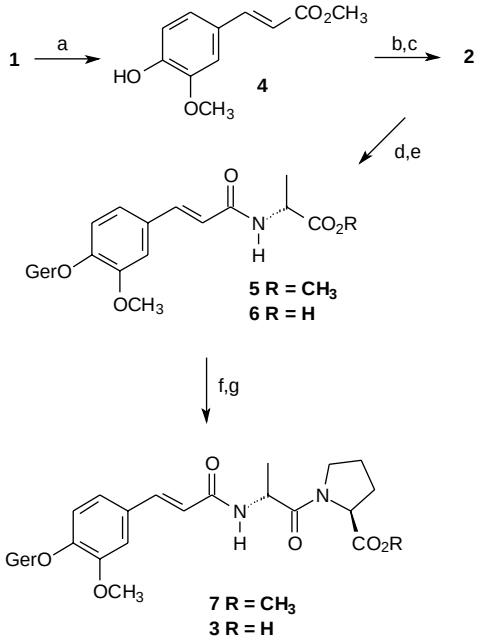
Different classes of brush border enzymes have been identified, among which are peptidases, characterized by different selectivity and distribution along the small and large intestines.[11,12] In particular, angiotensin converting enzyme (ACE, dipeptidyl carboxypeptidase, E.C. 3.4.15.1) hydrolyzes oligopeptides in which the terminal residue is proline and has alanine or glycine in the penultimate position.[13]

The aim of our study was to synthesize a properly structured prodrug of (**2**) that could be selectively cleaved by intestinal ACE so reaching in high concentration the large bowel. Basing on the selectivity of this enzyme (**2**) must be incorporated to H_2N-Ala-Pro dipeptide, such as in (**3**).



3

The synthesis of (**3**) was accomplished using commercially available ferulic acid (**1**) as starting material (Scheme 1). Initially, the acid (**1**) was easily transformed into the corresponding methyl ester (**4**) by treatment with refluxing MeOH in the presence of catalytic amount of concd H_2SO_4. The 4′-geranyloxy derivative (**2**) was obtained by a two-step one-pot procedure: geranylation of (**4**) with geranyl bromide and anhydrous K_2CO_3 in acetone at 60 °C and subsequent treatment in the same vessel with a basic medium. The described procedure represents a new entry to the synthesis of (**2**) and other prenyloxy-substituted cinnamic acid derivatives by means of a short and environment-friendly procedure, simple purification after standard work-up, not needing chromatographic separation, and very high yield with respect to those reported in the literature.[3,5,14] Coupling of (**2**) with commercially available L-alanine methyl ester hydrochloride in the presence of 1,3-dicyclohexylcarbodiimide (DCC) and triethyl amine in Et_2O at room temperature gave ester (**5**), which was subsequently transformed into 3-(4′-geranyloxy-3′-methoxyphenyl)-2-*trans*-propenoyl-L-alanine (**6**) by hydrolysis with hydroalcoholic NaOH 0.5 N. The same two-step reaction sequence repeated for the coupling of acid (**6**) with commercially available L-proline methyl ester hydrochloride and subsequent hydrolysis gave the desired 3-(4′-geranyloxy-3′-methoxyphenyl)-2-*trans*-propenoyl-L-alanyl-L-proline (**3**).[15] The overall yield of the 'ferulic acid to (**3**)' process was 56.6%.



Scheme 1. Reagents and conditions: (a) MeOH, concd H_2SO_4, reflux, overnight, 98% yield; (b) K_2CO_3, geranyl bromide, acetone, 60 °C, 2 h; (c) NaOH 2 N, 60 °C, 3 h, 99% yield; (d) L-AlaOMe·HCl, DCC, Et_3N, Et_2O, rt, 6 h, 91% yield; (e) NaOH 0.5 N, EtOH/H_2O 1:1 30 min, 88% yield; (f) L-ProOMe·HCl, DCC, Et_3N, Et_2O, rt, 6 h, 81% yield; (g) NaOH 0.5 N, EtOH/H_2O 1:1 30 min, 90% yield.

The chemical stability of compound (**3**) was assayed by incubating it at 37 °C for 2 h in aqueous solutions at different pH values ranging from 0.5 to 9.5:[16] at any pH value tested (**3**) was absolutely stable and has been recovered in quantitative yield from all reaction media. The degree of enzymatic hydrolysis by large bowel exopeptidases and commercially available purified ACE was assessed using the method reported by Kim and co-workers[13] after incubation of (**3**) for 30 min at 37 °C with isolated intestinal brush border membranes or purified ACE, the parent acid (**2**) was detected as the only hydrolytic product.

In conclusion, the sequence described here provides a new high-yielding synthesis of a novel prodrug of 3-(4′-geranyloxy-3′-methoxyphenyl)-2-*trans*-propenoic acid, a compound subject of more growing interest as potential drug for cancer treatment. Moreover this is, to the best of our knowledge, the first example of prodrug activation by intestinal ACE, so that it could be a novel approach of enzyme targeting for drug specific colon delivery. Its mechanism of activation would ensure chemical and enzymatic stability while passing through the stomach and small intestine and a selective release in near proximity of the large bowel. Results obtained from in vitro tests prompted us to carry out in vivo pharmacological studies on (**3**) as a novel colon cancer chemopreventive agent by dietary feeding in rats that are now in progress.

Acknowledgments

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Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bmcl.2005.07.088.

References and notes

1. Clifford, M. N. *J. Sci. Food Agric.* **1999**, *79*, 362.
2. Kroon, P. A.; Garcia-Conesa, M. T.; Fillingham, I. J.; Hazlewood, G. P.; Williamson, G. *J. Sci. Food Agric.* **1999**, *79*, 428.
3. Prager, R. H.; Thregold, H. M. *Aust. J. Chem.* **1966**, *19*, 451.
4. Han, B. S.; Park, C. B.; Takasuka, N.; Naito, A.; Sekine, K.; Nomura, E.; Taniguchi, H.; Tsuno, T.; Tsuda, H. *Jpn. J. Cancer Res.* **2001**, *92*, 404.
5. Tanaka, T.; Kohno, H.; Nomura, E.; Taniguchi, H.; Tsuno, T.; Tsuda, H. *Oncology* **2003**, *64*, 166, and references cited herein.
6. Murakami, A.; Kadota, M.; Takahashi, D.; Taniguchi, H.; Nomura, E.; Hosoda, A.; Tsuno, T.; Maruta, Y.; Ohigashi, H.; Koshimizu, K. *Cancer Lett.* **2000**, *157*, 77.
7. Hosoda, A.; Ozaki, Y.; Kashiwada, A.; Mutoh, M.; Wakabayashi, K.; Mizuno, K.; Nomura, E.; Taniguchi, H. *Bioorg. Med. Chem.* **2002**, *10*, 1189.
8. Hosoda, A.; Nomura, E.; Murakami, A.; Koshimizu, K.; Ohigashi, H.; Mizuno, K.; Taniguchi, H. *Bioorg. Med. Chem. Lett.* **2002**, *10*, 1855.
9. Sinha, V. R.; Kumria, R. *Pharm. Res.* **2001**, *18*, 557.
10. Pellicciari, R.; Garzon-Aburbeh, A.; Natalini, B.; Marinozzi, M.; Clerici, C.; Gentili, G.; Morelli, I. *J. Med. Chem.* **1993**, *36*, 4201, and references cited herein.
11. Skowbjerg, H. *Clin. Chim. Acta* **1981**, *112*, 205.
12. Auricchio, S.; Greco, L.; DeVizia, B.; Buonocore, V. *Gastroenterology* **1978**, *75*, 1073.
13. Yoshioka, M.; Erickson, R. H.; Kim, Y. S. *J. Clin. Invest.* **1988**, *81*, 1090.
14. Hosoda, A.; Miyake, Y.; Nomura, E.; Mizuno, K.; Taniguchi, H. *ITE Lett. Batt. New Tech. Med.* **2001**, *2*, 659.
15. **Experimental.** *Methyl ferulate (**4**).* Ferulic acid (**1**) (15.0 g, 77.2 mmol) was dissolved in 300 mL of dry MeOH and to this solution few drops of concd H_2SO_4 were added. The resulting mixture was stirred at reflux overnight, then basified with aq 5% $NaHCO_3$ and extracted with EtOAc (3× 200 mL). The collected organic phases were dried over Na_2SO_4 and evaporated to dryness giving (**4**) (16.0 g, 98% yield) as brownish solid. This compound was identified by comparison with a commercially available sample.

*3-(4′-Geranyloxy-3′-methoxyphenyl)-2-trans-propenoic acid (**2**).* Methyl ferulate (**4**) (1.26 g, 3.0 mmol) was dissolved in dry acetone (15 mL) and to this solution were added anhydrous K_2CO_3 (0.50 g, 3.6 mmol) and geranyl bromide (0.65 mL, 3.3 mmol). The resulting mixture was stirred at 60 °C for 2 h, then 2 N NaOH (15 mL) was added and the reaction mixture was stirred at 90 °C for additional 3 h. The cooled solution was diluted with H_2O (50 mL) and extracted twice with Et_2O (30 mL). The aqueous phase was acidified to pH 2 with 10% HCl and extracted with Et_2O (3× 50 mL). The collected organic phases were dried over Na_2SO_4 and evaporated to dryness giving (**2**) as a white solid (0.33 g, 99% yield). Mp: 60–61 °C; IR (cm^{-1}): 3500 (br), 1710, ^1H NMR (200 MHz, $CDCl_3$, δ) 1.63 (s, 3H), 1.70 (s, 3H), 1.77 (s, 3H), 2.04–2.24 (m, 4H), 3.94 (s, 3H), 4.68–4.72 (m, 2H), 5.06– 5.12 (m, 1H), 5.50–5.56 (m, 1H), 6.35 (d, 1H, J = 16.2 Hz), 6.88–7.30 (m, 3H), 7.76 (d, 1H, J = 16.2 Hz); ^{13}C NMR (50 MHz, $CDCl_3$, δ) 16.7, 17.7, 25.7, 26.2, 39.5, 55.9, 65.8, 109.9, 112.5, 114.7, 119.1, 123.0, 123.7, 126.8, 131.8, 141.2, 147.0, 149.5, 150.8, 172.7; HRMS (EI) Calcd for, $C_{20}H_{26}O_4$: 330.1831. Found: 330.1828.

*3-(4′-Geranyloxy-3′-methoxyphenyl)-2-trans-propenoyl-L-alanine methyl ester (**5**).* 3-(4′-Geranyloxy-3′-methoxyphenyl)-2-*trans*- propenoic acid (**2**) (1.87 g, 5.7 mmol) was dissolved in 57 mL of anhydrous Et_2O and to this solution were added triethyl amine (0.87 mL, 6.2 mmol) and L-AlaOMe·HCl (0.87 g, 6.2 mmol). A solution of DCC (1.29 g, 6.2 mmol) in Et_2O (25 mL) was then added dropwise over a period of 30 min and the resulting suspension was stirred at room temperature for 6 h and filtered under vacuum. The ethereal solution was washed in turn with 5% aq $NaHCO_3$ (2× 50 mL) and 5% aq citric acid (2× 50 mL), dried over Na_2SO_4, and evaporated to dryness giving (**5**) as a pale yellow solid (2.15 g, 91% yield). [α] (c 1, CH_2Cl_2) = +21.5; mp: 95–97 °C; IR (cm^{-1}): 1710, 1670; ^1H NMR (200 MHz, $CDCl_3$, δ) 1.49 (d, 3H, J = 7.1 Hz), 1.61 (s, 3H), 1.68 (s, 3H), 1.75 (s, 3H), 1.95–2.12 (m, 4H), 3.79 (s, 3H), 3.90 (s, 3H), 4.64–4.67 (m, 2H), 4.81 (q, 1H, J = 7.1 Hz) 5.04–5.17 (m, 1H), 5.48–5.55 (m, 1H), 6.40 (d, 1H, J = 16.0 Hz), 6.83–7.18 (m, 3H), 7.58 (d, 1H, J = 16.0 Hz); ^{13}C NMR (50 MHz, $CDCl_3$, δ) 16.7, 17.6, 18.3, 24.9, 25.3, 41.4, 48.1, 52.4, 56.5, 65.7, 109.8, 112.5, 117.8, 122.2, 123.7, 127.0, 131.7, 141.0, 141.6, 149.3, 149.8, 166.2, 173.8; HRMS (EI) Calcd for, $C_{24}H_{33}NO_5$: 415.2358. Found: 415.2352.

*3-(4′-Geranyloxy-3′-methoxyphenyl)-2-trans-propenoyl-L-alanine (**6**).* 3-(4′-Geranyloxy-3′-methoxyphenyl)-2-*trans*-propenoyl-L-alanine methyl ester (**5**) (0.68 g, 1.6 mmol) was dissolved in EtOH (5 mL) and to this solution 5 mL of 1 N aq NaOH was added. The resulting mixture was stirred for 1 h at room temperature, acidified to pH 1 with 10% HCl, and extracted with Et_2O (3× 30 mL). The collected organic phases were dried over Na_2SO_4 and evaporated to dryness giving (**6**) as a pale yellow solid (0.57 g, 88% yield). [α] (c 1, CH_2Cl_2) = −50.6; mp: 113–115 °C; IR (cm^{-1}): 3500 (br), 1710, 1670; ^1H NMR (200 MHz, $CDCl_3$, δ) 1.52 (d, 3H, J = 7.1 Hz), 1.61 (s, 3H), 1.69 (s, 3H), 1.74 (s, 3H), 2.02–2.12 (m, 4H), 3.84 (s, 3H), 4.61–4.64 (m, 2H), 4.75 (q, 1H, J = 7.1 Hz) 4.99–5.09 (m, 1H), 5.45–5.52 (m, 1H), 6.43 (d, 1H, J = 15.8 Hz), 6.80–7.15 (m, 3H), 7.74 (d, 1H, J = 15.8 Hz); ^{13}C NMR (50 MHz, $CDCl_3$, δ) 16.7, 17.7, 18.0, 25.7, 26.2, 39.5, 48.6, 55.8, 65.7, 109.8, 111.8, 117.2, 119.2, 122.3, 123.7, 127.3, 131.8, 141.2, 142.3, 149.3, 150.1, 166.9, 176.0; HRMS (EI) Calcd for, $C_{23}H_{31}NO_5$: 401.2202. Found: 401.2209.

*3-(4′-Geranyloxy-3′-methoxyphenyl)-2-trans-propenoyl-L-alanyl-L-proline methyl ester (**7**).* The same procedure to obtain compound (**5**) was followed. (81% yield). [α] (c 1, CH_2Cl_2) = −15.2; mp: 143–146 °C; IR (cm^{-1}): 1725, 1710, 1670; ^1H NMR (200 MHz, $CDCl_3$, δ) 1.48 (d, 3H, J = 7.1 Hz), 1.60 (s, 3H), 1.68 (s, 3H), 1.73 (s, 3H), 1.88–2.37 (m, 10H), 3.53–3.61 (m, 1H), 3.72 (s, 3H), 3.91 (s, 3H), 4.60–4.63 (m, 1H), 4.65–4.68 (m, 2H), 4.99– 5.02 (m, 1H), 5.47–5.51 (m, 1H), 6.39 (d, 1H,J = 15.9 Hz), 6.82–7.18 (m, 3H), 7.51 (d, 1H, J = 15.9 Hz); ^{13}C NMR (50 MHz, $CDCl_3$, δ) 16.5, 17.7, 18.1, 24.6, 25.2, 25.6, 26.1, 33.0, 39.0, 47.4, 49.0, 54.8, 55.8, 65.8, 112.0, 119.1, 121.5, 122.0, 122.4, 123.7, 127.6, 131.2, 133.7, 141.0, 141.2, 149.4, 157.1, 166.2, 173.9; HRMS (EI) Calcd for, $C_{29}H_{40}N_2O_6$: 512.2886. Found: 512.2880.

*3-(4′-Geranyloxy-3′-methoxyphenyl)-2-trans-propenoyl-L-alanyl-L-proline (**3**).* The same procedure to obtain compound (**6**) was followed (90% yield). Pale yellow solid; [α] (c 1, CH_2Cl_2) = −38.8; mp: 62–63 °C; IR (cm^{-1}): 3500 (br), 1739, 1658, 1655; ^1H NMR (200 MHz, $CDCl_3$, δ) 1.41 (d, 3H, J = 6.9 Hz), 1.61 (s, 3H), 1.68 (s, 3H), 1.74 (s, 3H), 1.89–2.39 (m, 10H), 3.52–3.64 (m, 1H), 3.90 (s, 3H), 4.63–4.66 (m, 1H), 4.67–4.70 (m, 2H), 4.98–5.01 (m, 1H), 5.48–5.52 (m, 1H), 6.38 (d, 1H, J = 15.9 Hz), 6.83–7.30 (m, 3H), 7.55 (d, 1H,

J = 15.9 Hz); ^{13}C NMR (50 MHz, $CDCl_3$, δ) 16.6, 17.8, 18.2, 24.8, 25.4, 25.6, 26.2, 33.1, 39.4, 47.5, 49.4, 55.8, 65.8, 112.4, 119.2, 121,4, 122.0, 122.6, 123.7, 127.6, 131.5, 133.9, 141.0, 141.3, 149.4, 157.7, 166.8, 174.1; HRMS (EI) Calcd for, $C_{28}H_{38}N_2O_6$: 498.2729. Found: 498.2721. For experiments aimed to evaluate the chemical and enzymatic stability of compound (**3**), see Refs. 13, 16.

16. Landon, M. *Methods Enzymol.* **1977**, *47*, 145.