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In vitro inhibitory activity of boropinic acid against *Helicobacter pylori*

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Abstract—In this study, we assessed in vitro minimum inhibitory concentration (MIC) values of some natural geranyloxycoumarins, geranyloxy- and isopentenyloxy acids against growth of *Helicobacter pylori*. Boropinic acid, active principle isolated from *Boronia pinnata* (Fam. Rutaceae), was seen to be the most effective compound with a MIC value of 1.62 μg/mL.

Helicobacter pylori is a highly motile, gram-negative microaerophilic bacterium thought to be an infective agent of more than 50% of the world population and can be considered the most common chronic infection for humans.[1] Moreover, *H. pylori* is now well known to be the main causal factor in the etiogenesis of chronic active or type B gastritis, peptic and duodenal ulcer, gastric carcinoma, and mucosa-associated lymphoid tumors.[2] Pharmacological treatment of *H. pylori* infections includes administration of a proton pump inhibitor and a combination of two or more antibiotics, among which the most active ones are amoxicillin, clarithromycin, metronidazole, and tetracyclines.[3] However, bacterial resistance to these antibiotics is a growing problem: for example, metronidazole is no longer effective in 10–50% of *H. pylori*-positive subjects and also amoxicillin and clarithromycin resistance have increased in the last few years.[4] For these reasons, the search for alternative therapeutic remedies for *H. pylori* infections is a field of current interest.

Secondary metabolites of phenylpropanoid biosynthetic origin containing a sesquiterpenyl, monoterpenyl, and isopentenyl chains attached to a phenol group represent quite a rare group of natural products. Among these, coumarins, cinnamic, and benzoic acids have been recently shown to exert valuable biological properties.[5,6] In continuation of our research studies aimed to evaluate pharmacological properties of this novel class of natural compounds, we wish to report here the activity of some prenyloxy-phenylpropanoids, namely, 3-(4′-geranyloxyphenyl)-2-*trans* propenoic acid **1**, 3-(4′-geranyloxy-3′-methoxyphenyl)-2-*trans* propenoic acid **2**, both isolated from *Acronychia baueri* Schott (Fam. Rutaceae),[7] boropinic acid **3**, isolated from *Boronia pinnata* Sm. (Fam. Rutaceae),[8] valencic acid **4**, isolated from *Citrus sinensis* L. and *Aegle marmelos* (Fam. Rutaceae),[9] 4-isopentenyloxy-3-methoxy benzoic acid **5**, 4-geranyloxy-3-methoxy benzoic acid **6**, both isolated as methyl esters from the liverwort *Trichocolea lanata* (Ehrh.) Dumm. (Fam. Trichocolaceae),[10] and finally auraptene, **7**, the most common geranyloxycoumarin extracted from plants belonging to genus *Citrus*,[6] as inhibitors of growth of *H. pylori* in vitro.

The synthesis of compounds **1**, **3**, **4**, **5**, and **6** was accomplished following an environment friendly route similar to that we already reported for the synthesis of compound **2**.[5] Compound **1** was obtained in 97% overall yield starting from commercially available *p*-coumaric acid that was first converted into its methyl ester by reaction in refluxing MeOH catalyzed by concd. H_2SO_4, then alkylated with geranyl bromide and hydrolyzed in a basic medium (Scheme 1).

Keywords: Boropinic acid; *Helicobacter pylori*; Prenyloxy-phenylpropanoids.
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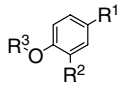
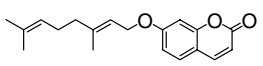


1 R^1 = -CH=CH-COOH, R^2 = -H, R^3 = geranyl
2 R^1 = -CH=CH-COOH, R^2 = $-OCH_3$, R^3 = geranyl
3 R^1 = -CH=CH-COOH, R^2 = $-OCH_3$, R^3 = isopentenyl
4 R^1 = -COOH, R^2 = -H, R^3 = isopentenyl
5 R^1 = -COOH, R^2 = $-OCH_3$, R^3 = isopentenyl
6 R^1 = -COOH, R^2 = $-OCH_3$, R^3 = geranyl



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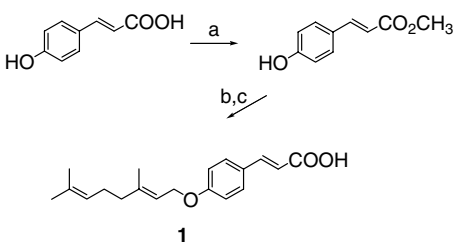
Compounds **3**, **5**, and **6** were obtained using the same reaction conditions as above in 96%, 98%, and 99% yield from ferulic acid and vanillic acid, respectively, while compound **4** was synthesized in 99% yield by one-pot alkylation-basic hydrolysis from commercially available methyl *p*-hydroxybenzoate and employing in all cases 4-bromo-2-methyl-2-butene or geranyl bromide as alkylating agents.[11] Auraptene **7** was synthesized in 95% yield as already described.[12]

Compounds **1**–**7** were assayed using metronidazole, amoxicillin, tetracycline, and clarithromycin as reference drugs. The minimum inhibitory concentration (MIC) tests were performed by the agar dilution method[12] according to guidelines provided by NCCLS.[13] MIC was defined as the lowest concentration capable of inhibiting bacterial colony formation. Results of the test on inhibition of growth against *H. pylori* by auraptene **7** and prenyloxy cinnamic acids **1**–**6** are reported in Table 1.

As shown in Table 1 only one among the secondary metabolites tested, namely boropinic acid (**3**), showed an appreciable activity against *H. pylori*. In fact the inhibitory activity of compound (**3**) was comparable or better than that of reference drugs with a MIC value of 1.62 μg/mL, being by far more active in respect to all other secondary metabolites, that were only slightly active (compounds **4**, **5**, and **7**) or totally inactive (compounds **1**, **2**, and **6**). Moreover, the weak activity observed for compounds **4**, **5**, and **7**, because of their lipophilic nature, is likely due to non-specific binding or aggregation effects. Data obtained by treating our *H. pylori* strain with metronidazole as reference drug



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Scheme 1. Reagents and conditions: (a) MeOH, 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) NaOH 2N, 70 °C, 1 h.

Table 1. MIC values for inhibition of growth against *Helicobacter pylori* by prenyloxy cinnamic acids **1**–**6** and auraptene **7**

Compound	MIC[a] (μg/mL)
1	>200
2	>200
3	1.62
4	100
5	50
6	>200
7	50
Metronidazole	>200
Amoxicillin	0.781
Tetracycline	4.00
Clarithromycin	1.25

[a] Values are means of three experiments.

are not significant for a comparison to those obtained from other secondary metabolites tested as this strain is clearly resistant to this antibiotic. However, these data confirm that resistance of *H. pylori* strains of different origin to metronidazole has become nowadays quite a common phenomenon and that boropinic acid could be claimed as a valuable alternative to bypass this disadvantage.

From data reported in Table 1 it is also evident that the presence of a geranyloxy side chain led to a substantial decrease of activity: in fact, with the only exception of auraptene (**7**), geranyloxy acids are virtually ineffective against growth of *H. pylori*; on the other hand, the MIC value recorded for auraptene (**7**) seems to indicate that the presence of a coumarin ring could represent another structural requirement for compounds to be active. In fact it has been recently found that differently substituted coumarins can act as antimicrobial compounds, showing inhibitory activity also against *H. pylori*.[14] The great difference of activity (from 30 to more than 120 times) between boropinic acid (**3**) and all other secondary metabolites tested seems to indicate that (**3**) could have a specific site of action inside *H. pylori* cells. Comparing structures of boropinic acid (**3**) to other compounds tested it seems that three structural requirements may be necessary for the observed activity: an isopentenyloxy side chain, an α,β-unsaturated carboxylic acid, and an (*E*) geometry for the conjugated double bond. In fact it can be seen that increasing the length of the *O*-side chain to a geranyl one (compounds **1**, **2**, and **6**) led to a complete lack of activity, while eliminating the conjugated double bond (compounds **4**, **5**, and **6**), changing the geometry of the double bond from (*E*) to (*Z*), and introducing a lactone moiety instead of a carboxylic acid one (compound **7**) led to a significative decrease of activity. These preliminary SAR considerations could be the basis to consequently modify the structure of boropinic acid (**3**) in order to obtain more active compounds against *H. pylori*.

In conclusion, the findings described herein seem to indicate boropinic acid (**3**) as a potential lead compound of a novel class of *H. pylori* inhibitors. Results obtained, considering also that (**3**) has been easily synthesized from widely available and non-toxic starting materials by a high-yielding, environment friendly, and cheap

synthetic route, prompt us to get further insights into the mechanism of action of this secondary metabolite, to perform test in vivo using a suitable animal model and in vitro and in vivo tests aimed to evaluate the activity of boropinic acid (**3**) against strains of *H. pylori* isolated from clinical patients and finally to synthesize and test both in vitro and in vivo structural analogues of (**3**).

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2006.08.043.

References and notes

1. Dunn, B. E.; Cohen, H.; Blase, M. J. *Clin. Microbiol. Rev.* **1997**, *10*, 720.
2. Lee, J. H.; Shim, J. S.; Lee, J. S.; Kim, M. K.; Chung, M. S.; Kim, H. K. *Carbohydr. Res.* **2006**, *341*, 1154.
3. Graham, D. Y.; Lew, G. M.; Klein, P. D. *Ann. Int. Med.* **1992**, *116*, 705.
4. Sorberg, M. H.; Hanberger, H.; Nilsson, M.; Bjorkman, A.; Nilsson, L. E. *Antimicrob. Agents Chemother.* **1998**, *42*, 1222.
5. Curini, M.; Epifano, F.; Genovese, G. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 5049, and references cited herein.
6. Curini, M.; Cravotto, G.; Epifano, F.; Giannone, G. *Curr. Med. Chem.* **2006**, *13*, 199.
7. Prager, R. H.; Thregold, H. M. *Aust. J. Chem.* **1966**, *19*, 451.
8. Ito, C.; Itoigawa, M.; Otsuka, T.; Tokuda, H.; Nishino, H.; Furukawa, H. *J. Nat. Prod.* **2000**, *63*, 1344.
9. Ali, M. S.; Pervez, M. K. *Nat. Prod. Res.* **2004**, *18*, 141.
10. Perry, N. B.; Foster, L. M.; Lorimer, S. D.; May, B. C.; Weavers, R. T. *J. Nat. Prod.* **1996**, *59*, 729.
11. *Experimental.* Synthesis of compounds **1**–**7**. For the synthesis of compounds (**1**)–(**6**) the same general procedure as reported previously was followed (see Ref. 5). Auraptene (**7**) was synthesized as already reported.[12] 3-(4′-Geranyloxyphenyl)-2-*trans* propenoic acid (**1**). White solid; yield: 97%; mp: 156–157 °C; IR (KBr): 3550, 1690 cm^{-1}; ^1H NMR: Ref. 7 ^{13}C NMR (100 MHz $CDCl_3$ δ): 16.1, 17.5, 25.6, 26.2, 39.4, 64.9, 115.3, 117.6, 119.8, 123.8, 128.3, 129.3, 131.4, 141.6, 144.2, 157.7, 168.9; Anal. Calcd for $C_{19}H_{24}O_3$: C, 75.97; H, 8.05; O, 15.98. Found C, 75.96; H, 8.07, O, 15.99.
3-(4′-Geranyloxy-3′-methoxyphenyl)-2-*trans* propenoic acid (**2**). White solid; yield: 96%; analytical data are in full agreement with those reported in the literature.[5]
Boropinic acid (**3**). White solid; yield: 96%; analytical data are in full agreement with those reported in the literature.[8]
Valencic acid (**4**). White solid; yield: 99%; mp: 131–132 °C; IR: Ref. 15; ^1H NMR: Ref. 15; ^{13}C NMR (100 MHz $CDCl_3$ δ): 18.7, 26.2, 66.5, 116.0, 120.9, 122.1, 132.2, 139.1, 162.7, 170.5; Anal. Calcd for $C_{12}H_{14}O_3$: C, 69.89; H, 6.84; O, 23.27. Found C, 69.88; H, 6.82, O, 23.26.
4-Isopentenyloxy-3-methoxy benzoic acid (**5**). White solid; yield: 98%; analytical data are in full agreement with those reported in the literature.[10]
4-Geranyloxy-3-methoxy benzoic acid (**6**). White solid; yield: 98%; analytical data are in full agreement with those reported in the literature.[10]
Auraptene (**7**). White solid; yield: 95%; analytical data are in full agreement with those reported in the literature.[12]
Microbiological tests. Strain of *H. pylori* (DSMZ 4867, originated from human gastric samples) was cultured on Columbia agar (Difco, Italy) with 4% horse blood (Difco, Italy). Before tests, *H. pylori* plates were prepared by subculturing onto Mueller–Hinton Agar supplemented with 5% defibrinated horse blood and incubated for 48 h microaerobically. An evaluation of the activity of each compound with reference drugs was made by comparison of bacterial growth degree in each plate of *H. pylori*. Auraptene **7** and acids **1**–**6** were dissolved in dimethylsulfoxide (DMSO). By serial double diluitions of antimicrobial agents, they were diluted in melted Mueller–Hinton Agar supplemented with 5% defibrinated horse blood to give concentrations ranging from 200 to 0.781 μg/mL. They were inoculated with 5 μL of bacterial suspension (10^7 CFU/mL) and incubated at 37 °C for 3 days under microaerobic conditions. An antimicrobic-free plate and plates with corresponding dilutions of DMSO were used as negative controls to ensure bacterial viability and no contaminants in inoculums.
12. Curini, M.; Epifano, F.; Maltese, F.; Prieto Gonzales, S. P.; Rodriguez, J. C. *Aust. J. Chem.* **2003**, *59*, 59.
13. National Committee for Clinical Laboratory Standards, Performance standards for antimicrobial susceptibility testing. V informational supplement. M100S9 National Committee for Clinical Laboratory Standards, Villanova, PA.
14. Chimenti, F.; Bizzarri, B.; Bolasco, A.; Secci, D.; Cimenti, P.; Carradori, S. *Eur. J. Med. Chem.* **2006**, *41*, 208.
15. Takemura, Y.; Kawaguchi, H.; Maki, S.; Juichi, M.; Omura, M.; Ito, C.; Furukawa, H. *Chem. Pharm. Bull* **1996**, *44*, 804.