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Solid-phase synthesis and antibacterial activity of hydroxycinnamic acid amides and analogues against methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *S. aureus*

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Abstract—A library of hydroxycinnamic acid amides (HCAAs) and analogues were synthesized using solid-phase synthesis technique. These compounds were screened for antibacterial against methicillin-resistant *Staphylococcus aureus* (MRSA) (11 strains) and vancomycin-resistant *S. aureus* (VRSA) (4 strains). Dihydrocaffeoyl analogues showed activity against VRSA which were better than the reference drugs, vancomycin and oxacillin. These compounds also exhibited antibacterial activity against MRSA, which were more potent than oxacillin.

The rapid incidence of multiple drug-resistant Gram-positive bacteria requires an urgent discovery of novel active agents against these pathogens.[1] Methicillin-resistant *Staphylococcus aureus* (MRSA) is still one of the most serious issues in public health in developed countries. It does not only have a high incidence but it has also become resistant to almost all the currently available antibiotics.[2] The rapid development of pathogens resistant to vancomycin, the last resort antibiotic against MRSA, has been reported.[3] This development creates a new demand for alternative antibiotics, the structure of which differs from conventional antibiotics, against vancomycin-resistant *S. aureus* (VRSA) as well as MRSA.

Hydroxycinnamic acid amides (HCAAs) are di- and polyamines conjugated to various phenolic acids, coumaric, ferulic, and caffeic acids (Fig. 1). These classes of compounds are widely distributed in higher plants. The roles of HCAAs in plants are not clear. Many studies have suggested that these compounds might play an important role in the chemical defense of plants against fungal and bacterial pathogens.[4,5]

Our program is aimed to search for bioactive natural products as well as to investigate a natural molecular template for drug discovery to treat human diseases. HCAAs are molecules of our interest and we have hypothesized that this class of compounds might also have activity against human pathogens. The synthesis of hydroxycinnamic acid amide analogues with different polyamines has been reported. However, the synthesis of HCAAs in solution is a laborious task since it involves extensive use of protective group strategy[5] and requires tedious purification steps due to the polarity of the compounds. Solid-phase organic synthesis has emerged as a powerful technology with several advantages including simplification of reaction procedures, easy separation of supported species and products, and application to automation system.[6] We report here the solid-phase synthesis of HCAAs with different diamines and coumaroyl analogues and related aromatic carboxylic acids. The synthesized compounds have been evaluated against MRSA and VRSA strains.

Our approach to generate a HCAAs library via solid-phase parallel synthesis is shown in Scheme 1.[7] Wang resin was prepared by shaking Merrifield resin with 4-hydroxybenzyl alcohol in the presence of K_2CO_3 and KI at room temperature for overnight. Reaction of Wang resin with *p*-nitrophenyl chloroformate provided the activated carbamate **2** which was reacted with

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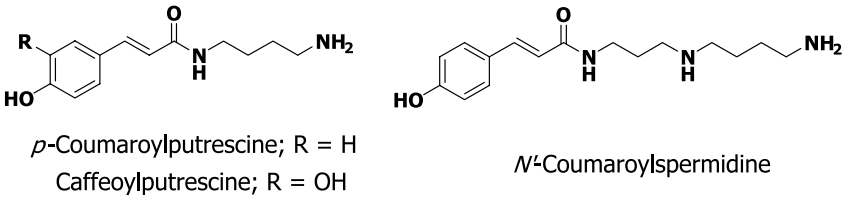
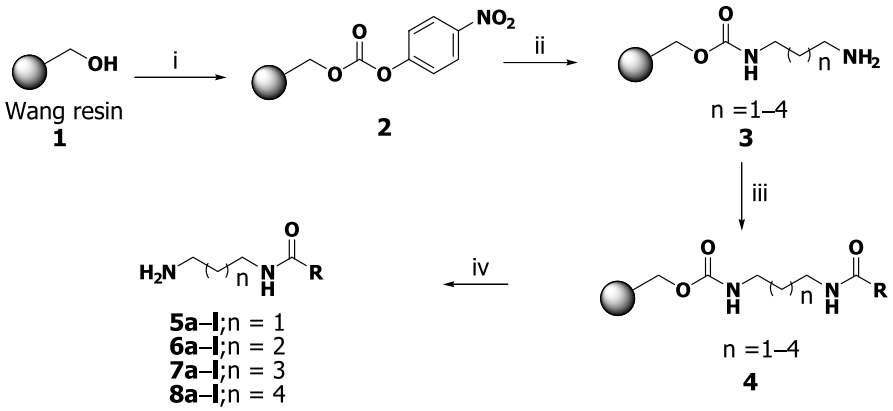


Figure 1. Chemical structure of HCAAs.



Scheme 1. Reagents and conditions: (i) 4-nitrophenyl chloroformate (4 equiv), pyridine (10 equiv), CH_2Cl_2, rt, 6 h; (ii) diaminoalkane (20 equiv), CH_2Cl_2, rt, 12 h; (iii) aromatic carboxylic acid (RCOOH in Fig. 2) (4 equiv), DIC (4 equiv), DMF/CH_2Cl_2 (1:4), rt, 2 h; (iv) 50% TFA, CH_2Cl_2, rt, 2 h.

commercially available diamines to afford the resin **3**. Reaction of the resin-bound diamines **3** with various aromatic carboxylic acids using *N*,*N*′-diisopropylcarbodiimide (DIC) as the coupling agent yielded the resin **4**. Cleavage was accomplished by shaking the resin **4** with 50% TFA/CH_2Cl_2 for 2 h. The resulting resin was filtered and washed thoroughly with CH_2Cl_2. The filters were combined to afford the products by evaporation. The products **5a**–**l**, **6a**–**l**, **7a**–**l**, and **8a**–**l** (Fig. 2) were obtained in moderate to good yields (40–80%) based on the

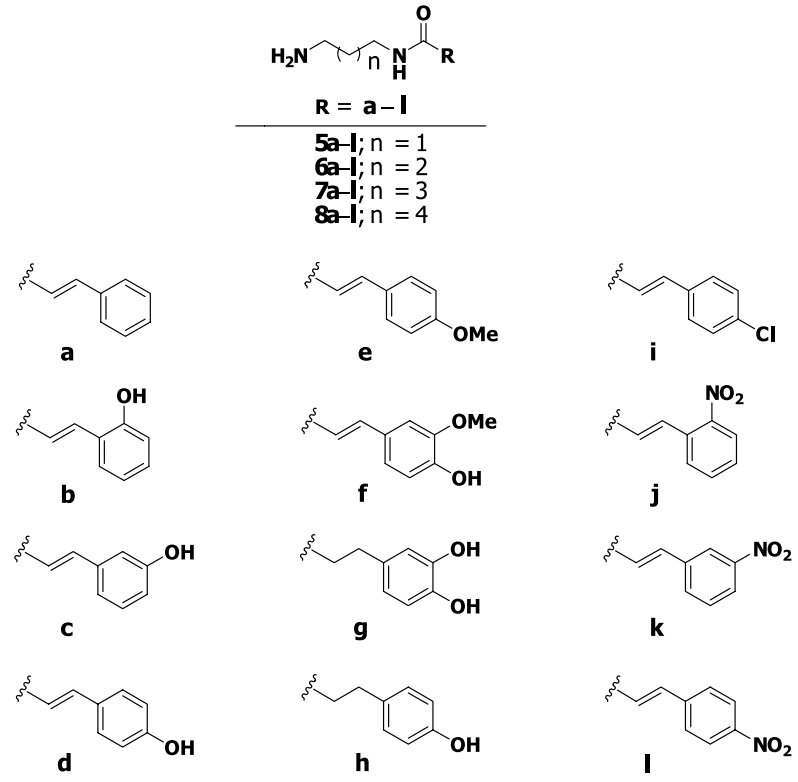


Figure 2. Structure of HCAAs and analogues.

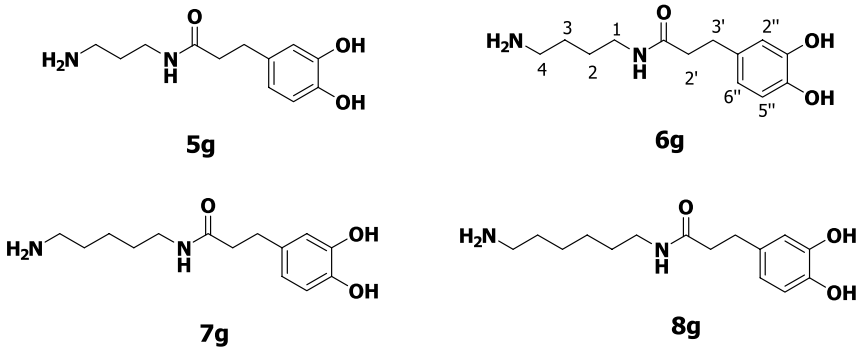


Figure 3. Structures of dihydrocaffeoyl analogues **5g**–**8g**.

original resin loading (1.11 mmol/g), and in high purity (>90%).

The antibacterial activity of all synthesized compounds was determined by disk diffusion method[8] against several clinically isolated MRSA and VRSA strains. The standard antibacterial agents, vancomycin and oxacillin, were also screened under identical conditions for comparison. It has been observed that cinnamoyl analogues with different substituted groups (OH, OMe, Cl, and NO_2) were resistant to MRSA and VRSA at the concentration of 50 μg/mL (data not shown). Dihydrocaffeoyl analogues (**5g**–**8g**)[9] (Fig. 3) exhibited activity against test organisms (Table 1). These compounds were more potent against MRSA than oxacillin. Only compound **6g** was more active than vancomycin against MRSA 20631 strain. It is interesting to note that these compounds were active against VRSA strains at the MIC range of 25–100 μg/mL. It should be noted that the dihydrocinnamoly analogues (**5h**–**8h**), deoxy analogues of **5g**–**8g**, were resistant to both MRSA and VRSA strains (data not shown). These HCAAs were evaluated for antifungal activity but negative results were obtained.

Table 1. Antibacterial activity of compounds **5g**–**8g** against MRSA and VRSA strains

Bacterial strain	MIC (μg/mL)					
	Vancomycin	Oxacillin	**5g**	**6g**	**7g**	**8g**
MRSA	2	R[a]	50	25	50	100
MRSA 20628	2	R[a]	50	50	R[c]	50
MRSA 20630	1.5	R[a]	50	50	50	50
MRSA 20631	48	R[a]	50	25	50	50
MRSA 20632	3	R[a]	50	25	50	50
MRSA 20633	1	R[a]	50	25	R[c]	50
MRSA 20635	8	R[a]	25	12.5	25	25
MRSA 20636	1.5	R[a]	25	25	R[c]	50
MRSA 20652	0.25	R[a]	R[b]	R[b]	R[c]	R[d]
MRSA 20653	2	R[a]	25	25	R[c]	25
MRSA 20654	1.5	R[a]	50	25	R[c]	25
VRSA 20622	R[a]	R[a]	100	100	50	100
VRSA 20623	R[a]	R[a]	50	25	25	50
VRSA 20624	R[a]	R[a]	50	25	50	50
VRSA 21083	R[a]	R[a]	25	25	25	25

[a] Resistant at 256 μg/mL.
[b] Resistant at 50 μg/mL.
[c] Resistant at 25 μg/mL.
[d] Resistant at 100 μg/mL.

In summary, we have synthesized a library of hydroxycinnamic acid amides and analogues using solid-phase synthesis. Dihydrocaffeoyl analogues (**5g**–**8g**) were active against MRSA and VRSA, supporting our hypothesis that this class of compounds also has activity against human pathogen. It should be noted that these compounds exhibited no cytotoxicity (IC_{50} > 100 μg/mL) against human gingival cells.

Acknowledgments

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References and notes

1. Taylor, P. W.; Stapleton, P. D.; Luzio, J. P. *Drug Discovery Today* **2002**, *7*, 1086.
2. Deresinski, S. *Clin. Infect. Dis.* **2005**, *40*, 562.
3. Tenover, F. C. *Clin. Microbiol. Newslett.* **2005**, *27*, 35.
4. Walters, D. R. *Phytochemistry* **2003**, *64*, 97.
5. Jacyn Baker, C.; Whitaker, B. D.; Roberts, D. P.; Mock, N. M.; Rice, C. P.; Deahl, K. L.; Aver'yanov, A. A. *Physiol. Mol. Plant Pathol.* **2005**, *66*, 90.
6. Dolle, R. E. *J. Comb. Chem.* **2005**, *7*, 739.
7. Procedure for the preparation of HCCAs: Merrifield resin (3.13 g, 3.47 mmol, 1% cross-linked, 200–400 mesh, loading = 1.11 meq Cl/g) was added K_2CO_3 (1.44 g, 10.41 mmol), KI (58 mg, 0.35 mmol), and a solution of 4-hydroxybenzyl alcohol (1.72 g, 13.88 mmol) in DMF (20 mL). The suspension was shaken at room temperature overnight. The resin was washed with DMF, H_2O, MeOH, and CH_2Cl_2 (3×20 mL each), and dried under reduced pressure to dryness. The resulting resin **1** (1.28 g, 1.23 mmol) was added a solution of 4-nitrophenyl chloroformate (1.38 g, 3.07 mmol) in CH_2Cl_2 (10 mL) and pyridine (1 mL, 12.3 mmol). The mixture was shaken for 6 h. After being washed with DMF and CH_2Cl_2 (3×5 mL each), the resin **2** was obtained. To the resin **2** was added a solution of excess diamines in CH_2Cl_2 (5 mL) and shaken for 12 h. The resin was washed with DMF, MeOH, and CH_2Cl_2 (3× 5 mL each), and dried under reduced pressure to afford resin **3**. The resulting resin **3** was divided to small

portions (ca. 120 mg) and reacted with a solution of carboxylic acid (4 equiv), DIC (4 equiv) in CH_2Cl_2/DMF (4:1, 1.5 mL). The mixture was allowed to shake for 2 h and was washed with DMF and CH_2Cl_2 (3× 2 mL each) to give resin **4**. To the resin **4** was added 50% TFA/CH_2Cl_2 (1.5 mL) and shaken for 2 h. The mixture was filtered and the resin was washed with CH_2Cl_2. The washings were combined with the filtrate, concentrated to dryness to give the products **5a**–**l**, **6a**–**l**, **7a**–**l**, and **8a**–**l** (Fig. 2).

8. Bauer, A. W.; Kirby, M. D. K.; Sherris, J. C.; Turck, M. *Am. J. Clin. Pathol.* **1966**, *45*, 493.
9. ^{1}H NMR and mass spectral characterizations of active compounds. Compound **5g** (400 MHz, $CDCl_3$+CD_3OD, 40:1) δ 1.53 (m, 2H, H-2), 2.34 (t, J = 6.8 Hz, 2H, H-2′), 2.42 (m, 2H, H-3), 2.68 (t, J = 6.8 Hz, 2H, H-3′), 3.06 (m, 2H, H-1), 6.42 (d, J = 8.0 Hz, 1H, H-5″), 6.55 (s, 1H, H-2″), 6.62 (d, J = 8.0 Hz, 1H, H-6″); MS (ES^+): *m*/*z* (%): 239 (100) $[M+H]^+$. Compound **6g** (400 MHz, $CDCl_3$+CD_3OD, 40:1) δ 1.19 (br s, 4H, H-2 and H-3), 2.28 (t, J = 6.9 Hz, 2H, H-2′), 2.65 (m, 3H, H-4 and H-3′), 2.95 (br s, 1H, H-1), 6.42 (d, J = 8.0 Hz, 1H, H-5″), 6.53 (s, 1H, H-2″), 6.61 (d, J = 8.1 Hz, 1H, H-6″); MS (ES^+): *m*/*z* (%): 253 (100) $[M+H]^+$. Compound **7g** (400 MHz, $CDCl_3$+CD_3OD, 40:1) δ 0.88 (m, 2H, H-3), 1.15 (m, 2H, H-4), 1.38 (m, 2H, H-2), 2.31 (t, J = 6.6 Hz, 2H, H-2′), 2.68 (m, 4H, H-5 and H-3′), 2.97 (t, J = 6.0 Hz, 2H, H-1), 6.44 (d, J = 7.7 Hz, 1H, H-5″), 6.55 (s, 1H, H-2″), 6.64 (d, J = 7.7 Hz, 1H, H-6″); MS (ES^+): *m*/*z* (%): 267 (100) $[M+H]^+$. Compound **8g** (400 MHz, $CDCl_3$+CD_3OD, 40:1) δ 0.88 (m, 2H, H-3), 1.13 (m, 4H, H-4 and H-5), 1.42 (m, 2H, H-2), 2.32 (t, J = 6.8 Hz, 2H, H-2′), 2.68 (t, J = 6.8 Hz, 2H, H-3′), 2.70 (m, 2H, H-6), 3.00 (t, J = 6.3 Hz, 2H, H-1), 6.43 (d, J = 7.9 Hz, 1H, H-5″), 6.56 (s, 1H, H-2″), 6.63 (d, J = 7.9 Hz, 1H, H-6″); MS (ES^+): *m*/*z* (%): 281 (100) $[M+H]^+$.