Bioorganic & Medicinal Chemistry Letters 26 (2016) 2980–2983

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Caged xanthones: Potent inhibitors of global predominant MRSA USA300

Pongkorn Chaiyakunvat [a], Natthinee Anantachoke [b], Vichai Reutrakul [a], Chutima Jiarpinitnun [a,*]

[a] Department of Chemistry and Center for Innovation in Chemistry (PERCH-CIC), Faculty of Science, Mahidol University, Rama VI Road, Bangkok 10400, Thailand
[b] Department of Pharmacognosy and Center for Innovation in Chemistry (PERCH-CIC), Faculty of Pharmacy, Mahidol University, Sri-Ayudhaya Road, Bangkok 10400, Thailand

ARTICLE INFO

Article history:
Received 28 November 2015
Revised 21 April 2016
Accepted 11 May 2016
Available online 11 May 2016

Keywords:
Caged xanthones
Methicillin resistant *Staphylococcus aureus*
USA300
Bacterial invasion

ABSTRACT

Total of 22 caged xanthones were subjected to susceptibility testing of global epidemic MRSA USA300. Natural morellic acid showed the strongest potency (MIC of 12.5 μM). However, its potent toxicity diminishes MRSA therapeutic potential. We synthetically modified natural morellic acid to yield 13 derivatives (**3a**–**3m**). Synthetically modified **3b** retained strong potency in MRSA growth inhibition, yet the toxicity was 20-fold less than natural morellic acid, permitting the possibility of using caged xanthones for MRSA therapeutic.

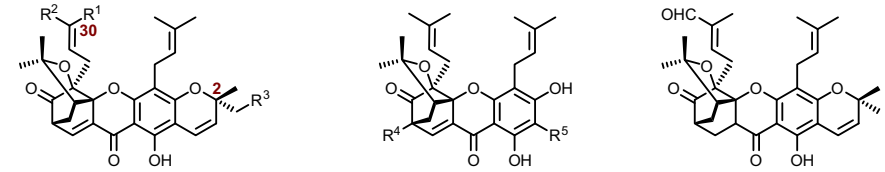
Garcinia hanburyi hook. f. belongs to Guttiferae family, commonly found in Southeast Asia and China.[1] In traditional medicine, the resin is applied as a laxative as well as therapeutic of infected wounds.[2] The prenylated caged xanthones **1**–**9** were isolated from resins and fruits of *Garcinia hanburyi hook. f.* (Fig. 1). The structures were elucidated based on spectroscopic analyses.[3] Besides their unique chemical structure, caged xanthones have received a high level of attention due to their potent cytotoxicity against various cultured mammalian cancer cell lines as well as drug-resistant cell lines at low micromolar concentration.[2–7] The mechanisms underlying the anticancer and antitumor activities were extensively studied.[8–12] Multiple targets and mechanisms were suggested to be accounting for the potent cytotoxicity of caged xanthones. These includes the induction of apoptosis via regulation of key proteins such as caspase3, Bax, and Bcl-2, suppression of telomerase activity, inhibition of cell metastasis by down-regulating the expression of metalloproteinases and integrin, and affecting angiogenesis.[13–17] In addition to potent cytotoxicity in cancer cells, naturally isolated caged xanthones from *G. hanburyi* also showed potent anti-HIV activities in reverse transcriptase assay with IC_{50} less than 50 μg/mL.[3] Antimicrobial activity of several caged xanthones were previously reported.[5,10,18–21] Caged xanthones were highlighted their potent growth inhibition in experimental staphylococcal infection in mice.[21] However, treatment of caged xanthones at high concentration resulted in side effects including the decline in cell growth and reduced blood cell count, suggesting that the potent cytotoxicity of these compounds hinders the potential of caged xanthones as anti-bacterial agent.

We are interested in developing antibacterial agents for global epidemic methicillin-resistant *Staphylococcus aureus* (MRSA), particularly MRSA PFGE strain type USA300 (SF8300). USA300 is known as community-acquired MRSA; its rapid transmission and resistance towards antibiotics lead to global healthcare problem.[22,23] Natural caged xanthones **1**–**9** isolated from *G. hanburyi hook. f.* were first quantitatively screened for their ability to inhibit growth of MRSA USA300 strain SF8300 via disk diffusion assay. Methicillin susceptible *S. aureus* (MSSA) ATCC 25923 was used as control strain. Out of 9 caged xanthones, 5 compounds (**2**–**5**, **8**) exhibited inhibition zones against both MSSA and MRSA strains while beta-lactam antibiotics such as ampicillin and oxacillin, as anticipated did not inhibit MRSA USA300. These 5 caged xanthones (**2**–**5**, **8**) were further subjected to microdilution assay to quantitatively access their minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). Among the natural caged xanthones assayed, morellic acid (**3**) was the most potent MSSA and MRSA growth inhibitors with MIC of 12.5 μM (Table 1).

Gambogic acid (**4**) inhibited growth with comparable extent. The results indicated that prenyl moiety at C-2 position was not significant for growth inhibition. The potency of isomorellinol (**2**), 2-isoprenylforbesione (**5**) and forbesione (**8**) were dramatically decrease (MIC > 200 μM) while the other caged adducts (**1**, **6**–**7**, **9**) tested could not inhibit growth of neither MRSA USA300 strain SF8300 nor MSSA control strain. Deoxymorellin (**1**) and morellic acid (**3**) share similarity in their structures with only minor

* Corresponding author.

http://dx.doi.org/10.1016/j.bmcl.2016.05.030
0960-894X/



1: R^1 = -CH_3, R^2 = -CH_3, R^3 = -H
2: R^1 = -CH_3, R^2 = -CH_2OH, R^3 = -H
3: R^1 = -COOH, R^2 = -CH_3, R^3 = -H
4: R^1 = -COOH, R^2 = -CH_3, R^3 = Prenyl

5: R^4 = -H, R^5 = Prenyl
6: R^4 = -H, R^5 = Geranyl
7: R^4 = Prenyl, R^5 = -H
8: R^4 = -H, R^5 = H

9

Figure 1. Natural isolated caged xanthones (**1**–**9**).

Table 1
Antibacterial activities of caged-xanthones and their synthetic derivatives determined by disk diffusion[a] and microdilution susceptibility test[b]

Compounds	Diameter of clear zones (mm)		MIC (μM)		MBC (μM)		IC_{50} (μM)
	ATCC25923	SF8300	ATCC29213	SF8300	ATCC 29213	SF8300	
Ampicillin (10 μg)	16.97 ± 1.19[c]	6	1.56	>100	1.56	>100	–
Oxacillin (1 μg)	28.86 ± 3.68[c]	6	0.62	>19.9	1.25	>19.9	–
Deoxymorellin (**1**)	6	6	–	–	–	–	1.42
Isomorellinol (**2**)	7.75 ± 0.13	8.57 ± 0.35	–	–	–	–	2.69
Morellic acid (**3**)	19.23 ± 0.35	19.52 ± 0.49	12.5	12.5	12.5	25	3.50
Gambogic acid (**4**)	16.59 ± 0.51	17.29 ± 0.77	12.5	25	25	50	3.40
2-Isoprenylforbesione (**5**)	7.56 ± 0.12	7.20 ± 0.53	400	>400	>400	>400	–
Deoxygamboginin (**6**)	6	6	–	–	–	–	–
Hanburin (**7**)	6	6	–	–	–	–	–
Forbesione (**8**)	7.86 ± 0.12	7.97 ± 0.11	200	>400	200	>400	–
Dihydroisomorellin (**9**)	6	6	–	–	–	–	–
Methyl morellate (**3a**)	6	6	–	–	–	–	–
3b	19.99 ± 0.43	22.24 ± 2.06	12.5	25	12.5	25	29.8
3c	17.07 ± 2.03	16.27 ± 2.33	12.5	25	12.5	25	12.2
3d	6.53 ± 0.92	6.27 ± 0.46	–	–	–	–	–
3e	6	6	–	–	–	–	–
3f	7.09 ± 1.13	9.53 ± 1.13	–	–	–	–	–
3g	16.52 ± 2.49	18.34 ± 2.43	25	25	25	50	15.5
3h	6.09 ± 0.18	6.28 ± 0.56	–	–	–	–	–
3i	15.91 ± 0.79	19.35 ± 1.60	25	25	25	50	24.5
3j	13.08 ± 0.37	15.91 ± 1.60	50	100	100	>100	21.1
3k	6.89 ± 1.19	8.21 ± 1.60	–	–	–	–	–
3l	7.50 ± 0.45	9.09 ± 0.45	–	–	–	–	–

[a] 30 μg of test compounds in DMSO were used for disk diffusion testing. At least three independent experiments were performed and the average diameters of complete inhibition zones were reported with standard errors. Whatman disk has a diameter of 6 mm, therefore, average inhibition zone of 6 mm indicates inability to inhibit growth.
[b] Each compound was 2-fold diluted based on its stock concentration.
[c] The number indicates the diameter of visually complete inhibition zone. However, the compound also exhibited incomplete zone of inhibition.

exception at C-30. Interestingly, the anti-bacterial activities of these compounds were drastically different, highlighting the importance of carboxyl functionality at C-30 position. To verify the significance of carboxylic acid moiety, morellic acid **3** was methylated to the methyl ester (**3a**). The modified ester **3a** resulted in no inhibition activity. Hence, the carboxylic functionality is crucial for anti-bacterial activity. Even though the natural morellic acid (**3**) and gambogic acid (**4**) possessed potent antibacterial activities, their high cytotoxicity hinder their potentials as bacterial infection therapeutics. The reported IC_{50} of morellic acid (**3**) and gambogic acid (**4**) are 3.5 μM and 3.4 μM, respectively. These IC_{50} values are above MIC and MBC values, suggesting that these compounds potentially would be toxic to treated cells (see Supporting information). We, therefore, modified morellic acid (**3**) in an effort to acquire the derivative of caged xanthones that are potent antibacterial activities with low toxicity. Based on our SAR analysis, the key structure for retaining anti-bacterial activity is carboxylic functionality at C-30. To determine whether carboxylic functionality at C-30 is required for toxicity of the caged xanthones, deoxymorellin **1** and isomorellinol **2** were evaluated in comparison to morellic acid **3** and gambogic acid **4** as natural analogues **1** and **2** lack of –COOH substituent while **3** and **4** do contain –COOH at C-30. All compounds—deoxymorellin **1**, isomorellinol **2**, morellic acid **3** and gambogic acid **4** exhibited high cytotoxicity; however, deoxymorellin **1**, isomorellinol **2** were slightly more toxic than morellic acid **3** and gambogic acid **4**. These results along the susceptibility experiments suggested that replacing polar COOH substituent with less polar groups could drastically alter the antibacterial activity and could perhaps increase the cytotoxicity. Interestingly, N-(carboxymethyl) gambogiamide, GA3, was previously reported as potential candidate in cancer therapy.[24] GA3 is a modification of –COOH functional group with natural amino acid glycine; this alteration in structure showed slightly reduce in cytotoxicity of gambogic acid yet enhanced solubility in aqueous solution. The declining trend in cytotoxicity with glycine conjugation at C-30 led us to further explore other amino acids conjugate at this particular position. We envisioned that amino acid conjugation analogues **3b**–**m**, which still possess the carboxylic functionality, could perhaps retain potent antibacterial activity against MSSA and MRSA strains with less toxicity (Fig. 2).

The synthetically modified morellic (**3b**–**3m**) were achieved via solid-phase synthesis and subjected to susceptibility testing with MRSA: USA300 and MSSA strains. The results showed that compound **3b**, **3c**, **3g**, **3i**, and **3j** could retain growth inhibitory activity in both strains (Table 1). Interestingly, all of the morellic derivatives that inhibit bacterial growth were those that modified