Bioorganic & Medicinal Chemistry Letters 20 (2010) 5349–5352

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Design, synthesis, and biological evaluation of a novel series of quercetin diacylglucosides as potent anti-MRSA and anti-VRE agents

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ARTICLE INFO

Article history:
Received 5 January 2010
Revised 10 February 2010
Accepted 13 February 2010
Available online 19 February 2010

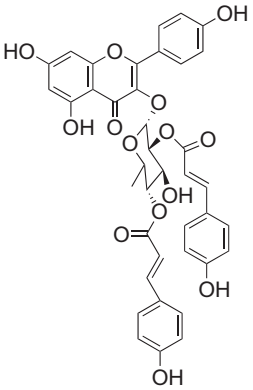
Keywords:
Antibacterials
Multi-drug resistance
Quercetin-3-glucoside

ABSTRACT

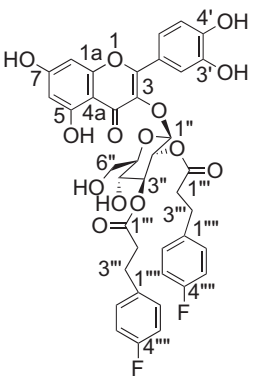
A series of novel quercetin diacylglucosides were designed and first synthesized by Steglich esterification on the basis of MRSA strains inhibiting natural compound **A**. The in vitro inhibition of different multi-drug resistant bacterial strains and *Escherichia coli* DNA gyrase B was investigated. In the series, compound **10h** was up to 128-fold more potent against vancomycin-resistant enterococci and more effective than **A**, which represents a promising new candidate as a potent anti-MRSA and anti-VRE agent.

The increasing emergence of multi-drug resistant Gram-positive bacterial pathogens, including methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci (VRE) and recently found vancomycin-intermediate resistant *S. aureus* (VISA) become a serious global clinical problem for the treatment of various nosocomial and community-acquired infections.[1] In order to overcome these bacterial resistance problems, numerous efforts have focused on discovering novel anti-MRSA, anti-VRE, and anti-VISA agents in recent decades.[2] Thus, newly disclosed antibacterial agents such as tetracycline, linezolid, quinupristin–dalforpristin, tigecycline, trimethoprim–sulfamethoxazole, and daptomycin, have all been used alone or in combination with other agents for the treatment of some MRSA, VRE or VISA infections. Notwithstanding, the limitations of these drugs have been well documented in the literature[3] and there remains a concern for the critically ill, high-risk patient population. In view of increasing resistance of Gram-positive bacteria to currently available antibacterial agents, new and potent anti-MRSA, VRE, and VISA agents are highly desired.

Flavonoids are bioactive polyphenolic compounds. They have significant inhibitions towards the growth of both Gram-positive and Gram-negative bacterial strains[4] and are prospective drug candidiates.[5] A novel kaempferol diacylrhamnoside **A**[6–9] (Fig. 1) has recently been isolated and reported as a potent anti-MRSA agent.[8,9] To assist the development of novel anti-MRSA agents, as well as to investigate structure–activity relationship for the related series of compound **A**, a new series of 2-(3′,4′-dihydroxyphenyl)-5,7-dihydroxy-3-β-D-glucosyl-4*H*-chromen-4-one 2″,3″-diesters **10a–h** commonly known as quercetin diacylglucosides was designed and synthesized for the first time. Meanwhile, the replacements of kaempferol by quercetin and 2,4-di-*O*-(4-hydroxycinnamoyl)-



A (MIC: 4 or 8 μg/mL, against VRE strains).



10h (MIC: 1 μg/mL, against VRE strains).

Figure 1. Kaempferol diacylrhamnoside **A** and quercetin diacylglucoside **10h**.

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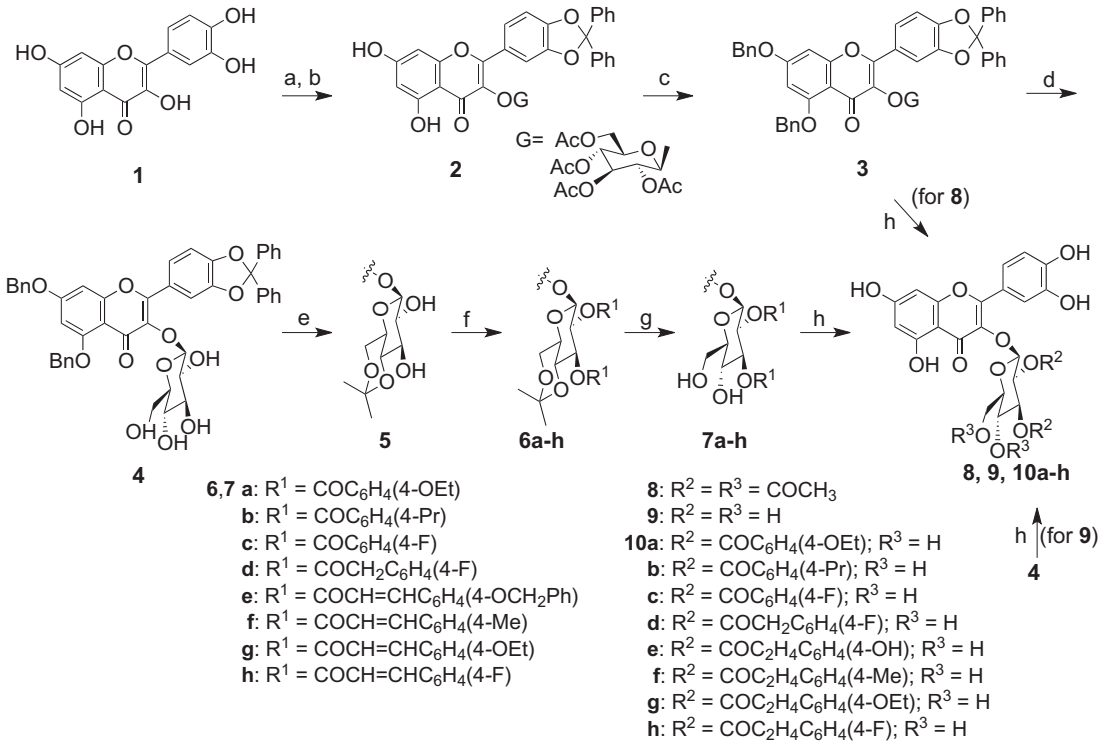
0960-894X/$ - see front matter © 2010 Published by Elsevier Ltd.
doi:10.1016/j.bmcl.2010.02.060



Scheme 1. Reagents and conditions: (a) Ph_2CCl_2, 170 °C, 8–10 min; (b) 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide, anhydrous K_2CO_3, anhydrous acetone, 40 °C, 10 h; (c) BnBr, anhydrous K_2CO_3, anhydrous DMF, rt, 4 h; (d) (i) MeONa, MeOH–THF, rt, 1.5 h, (ii) Dowex 50 (H^+) resin; (e) anhydrous acetone, concd H_2SO_4, anhydrous $CuSO_4$, rt, 24 h; (f) aliphatic or aromatic carboxylic acids, DMAP, DCC, anhydrous CH_2Cl_2, 0 °C–rt, 6–8 h; (g) 0.5 M HCl, MeOH–THF, 50–60 °C, 2–3 h; (h) Pd/C (10%), H_2, MeOH–EtOAc, rt, 8–10 h.

α-L-rhamnose by 2,3-disubstituted β-D-glucopyranose have considerably enhanced the inhibition towards the growth of bacterial strains.

Scheme 1 depicts a convenient route for the synthesis of compounds **8**, **9**, and **10a–h**. Quercetin **1** was reacted with α,α-dichlorodiphenylmethane to protect the 3′- and 4′-hydroxyl groups. Then, the 3′,4′-protected compound was regioselectively glucosylated with one equiv. of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide in the presence of 1 equiv of K_2CO_3 in dry DMF to give the compound **2**[10] as the major product (61%). Since the higher reactivity at position 3 of starting compound allows the selective glucosylation, trace amounts of 3,7-di-glucosylated quercetin was detected and ignored. The free hydroxyl groups of compound **2** were then protected with excess benzyl bromide in the presence of anhydrous K_2CO_3 in dry DMF to give compound **3**. The removal of acetyl group of **3** with MeONa in MeOH followed by treatment with Dowex 50 (H^+) ion exchange resin gave compound **4**. Reaction of **4** with acetone in the presence of anhydrous copper sulfate and a catalytic amount of sulfuric acid gave a key intermediate **5**. The 2″,3″-dihydroxy groups of compound **5** were esterified by Steglich esterification[11] with different aliphatic or aromatic carboxylic acids in the presence of DMAP and DCC to obtain compounds **6a–h**. The isopropylidene protecting group of **6a–h** was removed by hydrolysis with 0.5 M hydrochloric acid in a mixture of MeOH and THF to give compounds **7a–h**. Finally, the benzyl and diphenylbenzo protecting groups of **7a–h**, **3**, and **4** were removed in one step by catalytic hydrogenation with 10% Pd/C in MeOH–EtOAc under hydrogen atmosphere to give the compounds **10a–h**, **8**, and **9**. During this treatment, the carbon–carbon double bond adjacent to carbonyl group in the R^1 of compounds **7e–h** were selectively hydrogenated and were converted into compounds **10e–h**. The structure of all compounds were confirmed by ^1H NMR, ^{13}C NMR, FAB-MS, and/ or elemental analysis.

Synthesized compounds **8**, **9**, **10a–h**, and quercetin were screened for their potential antibacterial activity in vitro against eight selected multi-drug resistant Gram-positive and two Gram-negative bacterial strains, including MRSA, MSSA, VRE, VISA, *Pseodomonas aeruginosa* and *E. coli* strains (Table 1). Norfloxacin, vancomycin, and novobiocin were used as reference strains standards. Encouraged by promising inhibition by quercetin of *E. coli* DNA gyrase B,[13] compounds **9** and **10a–h** bearing quercetin moiety also were investigated for in vitro inhibitions towards same enzyme DNA gyrase B from *E. coli* (Table 1). Furthermore, AutoDock modeling[14] was performed (Table 1) to explore the probable binding conformation inside the ATP binding site of 24 kDa fragment of the DNA gyrase B subunit from *E. coli* (PDB accession code 1AJ6). Most of the tested compounds showed pronounced inhibitions of *E. coli* DNA gyrase B. Among those, compound **10h** showed the most pronounced inhibition (IC_{50}: 0.19 μM) that was increased concentration-dependently as shown in Figure 2. Docking simulation also supports the inhibition and the superposition on native ligand (novobiocin)[15] as shown in Figure 3 where the carbonyl group of 3-(4-fluorophenyl)propanoyloxy at 3″-position and the hydroxyl group at 4″-position of compound **10h** could form H-bonds with ASN46 and SER121 of ATP binding site in the 24 kDa of *E. coli* DNA gyrase B, respectively. On the contrary, screened compounds did not show reasonable inhibitions towards the growth of Gram-negative bacterial strains, which may be caused by difficulty in outer-membrane permeability of Gram-negative organisms. Compounds bearing 3-(4-substitutedphenyl)propanoyloxy groups (e.g., **10e–h**) and (4-fluorophenyl)acetyloxy groups (e.g., **10d**) at 2″,3″-positions tend to show comparatively enhanced activity against the growth of Gram-positive bacterial strains. Among these compounds, 3-(4-fluorophenyl) propanoyloxy derivative **10h** has the most pronounced inhibition (0.25–1 μg/mL) towards the growth of VRE, VISA, MRSA or MSSA strains and was

Table 1
Antibacterial activity (MICs) against multi-drug resistant Gram-positive and Gram-negative bacterial strains and DNA gyrase inhibition (IC_{50}) against DNA gyrase supercoiling kit from *E. coli* and the best docking results on the inhibitions constants (K_i)

Compound	MIC[a] (μg/mL, Gram-positive)								IC_{50}[j] (μM, *E. coli* DNA gyrase B inhibitions)	K_i[k] (μM, inhibitions constants)	MIC[a] (μg/mL, Gram-negative)	
	VRE		VISA	MRSA				MSSA			PA01[l]	K-12[m]
	FN-1[b]	NCTC 12201[c]	Mu50[d]	OM481[e]	OM584[f]	N315[g]	COL[h]	209P[i]				
8	64	64	128	128	128	128	>128	128	nt	nc	>128	>128
9	>128	>128	>128	>128	>128	>128	>128	>128	0.10	0.05×10^{-3}	64	32
10a	16	16	16	32	32	8	>128	8	1.10	6.61	>128	>128
10b	8	8	16	32	32	8	16	8	0.34	2.87	>128	>128
10c	8	8	2	16	16	2	16	2	0.21	1.48	>128	>128
10d	8	8	2	8	8	2	8	2	0.28	2.64	>128	>128
10e	8	8	4	4	4	4	8	4	1.21	40.08	>128	>128
10f	8	8	4	8	4	2	8	2	1.23	46.96	>128	>128
10g	2	4	1	2	2	1	4	1	0.46	2.77	>128	>128
10h	1	1	1	2	2	0.25	1	0.25	0.19	1.93	>128	>128
Quercetin	>128	>128	>128	>128	>128	>128	>128	>128	0.14	0.18×10^{-3}	>128	64
Norfloxacin	nt	nt	nt	64	128	2	1	0.5	0.09	0.09×10^{-3}	nt	0.25
Vancomycin	>128	>128	8	0.25	0.25	0.25	0.25	0.25	nt	nc	>128	nt
Novobiocin	nt	nt	nt	0.25	0.25	0.125	0.25	0.125	0.05	nc	16	8
A	8	4	nt	1	2	1	1	0.5	nt	nc	>128	nt

nt, not tested; nc, not calculated.
[a] Microdilution method,[12] MIC determined after 24 h.
[b] Vancomycin-resistant enterococci FN-1.
[c] Vancomycin-resistant enterococci NCTC 12201.
[d] Vancomycin intermediate-resistant *Staphylococcus aureus* Mu50.
[e] Methicillin-resistant *S. aureus* OM481.
[f] Methicillin-resistant *S. aureus* OM584.
[g] Methicillin-resistant *S. aureus* N315.
[h] Methicillin-resistant *S. aureus* COL.
[i] Methicillin sensitive *S. aureus* 209P.
[j] IC_{50} the concentration of the drugs that inhibits 50% of supercoiling activity.
[k] K_i values were calculated by computer aided AutoDock 4.0 soft.
[l] *Pseodomonas aeruginosa* PA01.
[m] *Escherichia coli* K-12.

up to 128 times more potent than vancomycin as well as eight times more potent than **A**. On the other hand, compounds bearing comparatively shorter 4-substituted benzoyloxy groups (e.g., **10a–c**) on the same positions have reduced activity against the same bacterial strains. Moreover, most recently Tsuchiya and co-workers reported, the site of action of compound **A**[8,9] might be at DNA topoisomerase IV and/or DNA gyrase, and the primary target would be DNA topoisomerase IV. Correspondingly, the site of action of compounds **10a–h** would be similar to compound **A** in *S. aureus* (e.g., Gram-positive). Therefore, it seems that compounds **10a–h** have divergent modes of action in Gram-negative and Gram-positive organisms.

For an antibacterial agent to be effective, it must penetrate the bacterial cell to reach its target. The biological evaluation revealed that the size and lipophilicity of the substituent on quercetin-3-β-D-glucoside should be considered the key factors in determining its antibacterial activity. Physical properties including relative hydrophobicity and molecular mass are important for penetration into the bacterial cell and have a different role in Gram-negative and Gram-positive bacteria. Thus, increasing molecular mass and bulkiness of substituent at 2″,3″-positions hinder penetration of quercetin diacylglucosides into Gram-negative organisms through the porin channels.[16] The accumulation of compound in Gram-positive bacteria (e.g., *S. aureus*) is thought to take place by simple diffusion across the cytoplasmic membrane.[17] Accordingly, screened compounds **10a–h** having high molecular mass and bulky side chains at 2″,3″-positions were accumulated in Gram-positive organisms more favorably than Gram-negative organisms. Furthermore, halogen such as fluorine is very useful to modulate the electronic effects on phenyl rings of compounds **10c**, **10d**, **10h** and may also influence the steric characteristics and the hydrophilic–hydrophobic balance of the molecules.

In conclusion, we have discovered a novel series of quercetin diacylglucosides that have remarkable and acute antibacterial properties. A range of diverse substituents at 2″,3″-positions of quercetin-3-β-D-glucoside modulate antibacterial activity, and makes it worthy for consideration to be developed as a drug candidate for nosocomial infections caused by multi-drug resistant Gram-positive pathogens. Compound **10h** appears to be an attractive candidate for further investigations to provide a new antibacterial agent for use against nosocomial multi-drug resistant

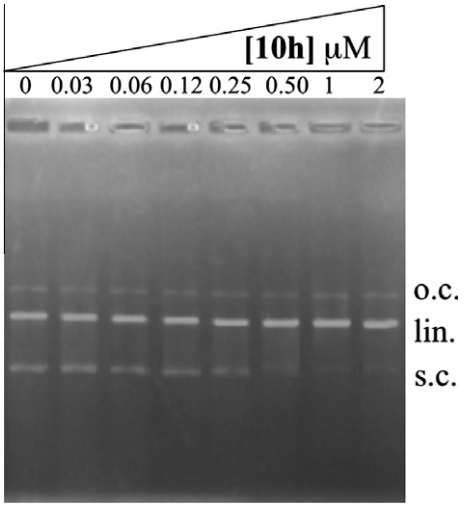


Figure 2. DNA supercoiling assay by gyrase from *E. coli* strains JMtacA and JMtacB (Hallett et al., 1990) of compound **10h**. o.c., open-circular DNA; lin., linear DNA; s.c., supercoiled DNA.

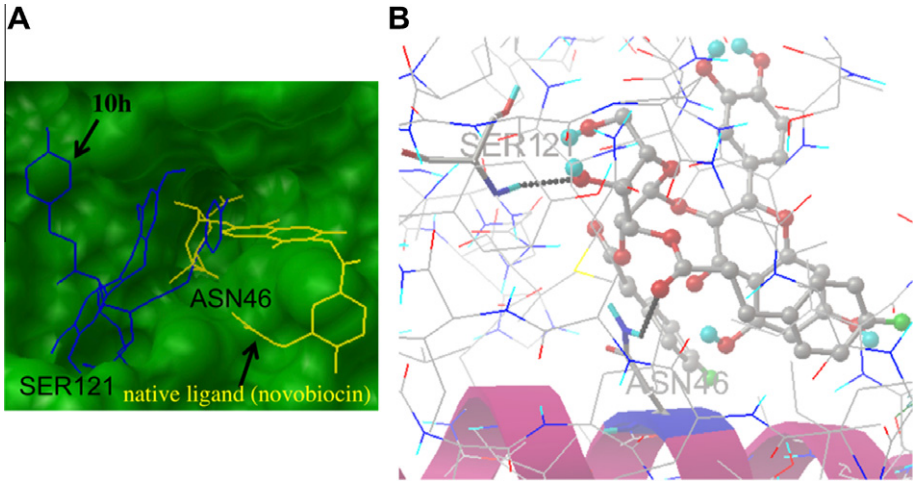


Figure 3. (A) Superposition of native ligand (yellow color) and **10h** (Blue color) in the complex with 24 kDa fragment of the DNA gyrase B by using the AutoDock 4.0 software tool. (B) AutoDock-modeled binding of **10h** inside ATP binding site of DNA gyrase B. H-Bonds are displayed as sphere.

Gram-positive infections, in particular VRE and VISA infections. Further studies on antibacterial properties of series related to quercetin diacylglucosides are currently in progress to obtain more potent inhibitions towards the growth of Gram-positive bacterial strains.

Acknowledgements

We thank Professor Adrian L. Schwan of University of Guelph for discussion and critical reading of the manuscript, and are grateful to the SC-NMR Laboratory of Okayama University for NMR experiments.

Supplementary data

Supplementary data (experimental details, spectroscopic data, FAB-MS, elemental analysis, biological procedures and molecular modeling) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.02.060.

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