Journal of Medicinal Chemistry

ARTICLE

pubs.acs.org/jmc

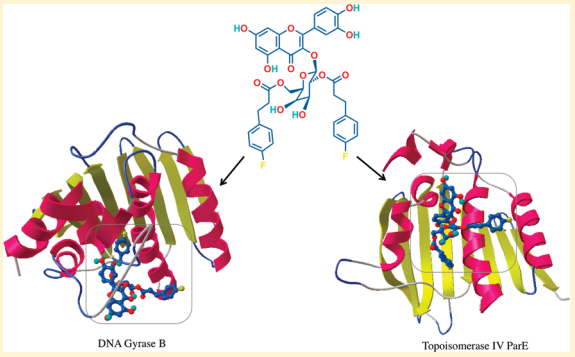
Quercetin Diacylglycoside Analogues Showing Dual Inhibition of DNA Gyrase and Topoisomerase IV as Novel Antibacterial Agents

Abugafar M. L. Hossion,*[,†] Yoshito Zamami,[†] Rafiya K. Kandahary,[†] Tomofusa Tsuchiya,[‡] Wakano Ogawa,[‡] Akimasa Iwado,[†] and Kenji Sasaki*[,†]

[†]Department of Molecular Design for Medicine and [‡]Department of Molecular Microbiology, Graduate School of Medicine, Dentistry and Pharamceutical Sciences, Okayama University, 1-1-1, Tsushima-Naka, Kita-Ku, Okayama 700-8530, Japan

Supporting Information

ABSTRACT: A structure-guided molecular design approach was used to optimize quercetin diacylglycoside analogues that inhibit bacterial DNA gyrase and topoisomerase IV and show potent antibacterial activity against a wide spectrum of relevant pathogens responsible for hospital- and community-acquired infections. In this paper, such novel 3,7-diacylquercetin, quercetin 6″-acylgalactoside, and quercetin 2″,6″-diacylgalactoside analogues of lead compound **1** were prepared to assess their target specificities and preferences in bacteria. The significant enzymatic inhibition of both *Escherichia coli* DNA gyrase and *Staphylococcus aureus* topoIV suggest that these compounds are dual inhibitors. Most of the investigated compounds exhibited pronounced inhibition with MIC values ranging from 0.13 to 128 μg/mL toward the growth of multidrug-resistant Gram-positive methicillin-resistant *S. aureus*, methicillin sensitive *S. aureus*, vancomycin-resistant enterococci (VRE), vancomycin intermediate *S. aureus*, and *Streptococcus pneumoniae* bacterial strains. Structure−activity relationship studies revealed that the acyl moiety was absolutely essential for activity against Gram-positive organisms. The most active compound **5i** was 512-fold more potent than vancomycin and 16−32-fold more potent than **1** against VRE strains. It also has realistic in situ intestinal absorption in rats and showed very low acute toxicity in mice. So far, this compound can be regarded as a leading antibacterial agent.



INTRODUCTION

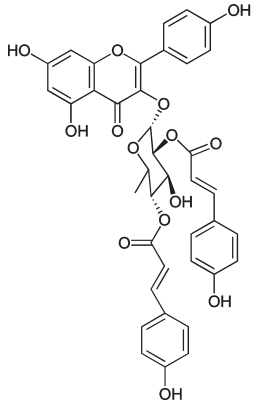
The increasing emergence of antibiotic-resistant bacteria, notably vancomycin-resistant enterococci (VRE), methicillin-resistant and vancomycin-intermediate *Staphylococcus aureus*, and penicillin-, macrolide-, and fluoroquinolone-resistant *Streptococcus pneumoniae*, is a worldwide issue,[1−6] particularly in hospitals, where antibiotics are heavily used.[7] While the rate of drug resistance among bacteria is on the rise, there have been very few examples of the development of structurally new classes of antibiotics with truly novel modes of action that can circumvent the prevailing resistance problem.[4,8] Linezolid and daptomycin are the only examples of such novel antibiotics that were developed in the last few decades. With recent reports of emerging resistance to linezolid[9,10] and daptomycin,[11] there is continuing need for the discovery and development of novel antibiotics.

DNA gyrase and topoisomerase IV (topoIV) are the two type II topoisomerases present in bacteria and are attractive targets for antibacterial drug discovery.[12−15] Each enzyme is independently essential for bacterial DNA replication. DNA gyrase is a heterotetramer comprised of two GyrA and two GyrB subunits and is primarily responsible for introducing negative supercoils into conformationally constrained DNA, whereas topoIV is a heterotetramer comprised of two ParC and two ParE subunits (GrlA and GrlB, respectively, in *S. aureus*)[16] and primarily resolves linked chromosome dimers at the conclusion of DNA replication.[17−19] The fluoroquinolone drugs, for example, norfloxacin (Chart 1) inhibits the catalytic subunits of gyrase (GyrA) and/or topoIV (ParC). The associated subunits responsible for supplying the energy necessary for catalytic turnover/resetting of the enzymes via ATP hydrolysis are GyrB (Gyrase) and ParE (topoIV), respectively. In addition, the coumarin drugs, for example, novobiocin (Chart 1), exhibit antibacterial activity via inhibition of DNA gyrase and, to a lesser extent, topoIV ATPase activities.[20−23] Nonetheless, their use has been limited due to the toxicity and to the rapid development of resistances.[24,25]

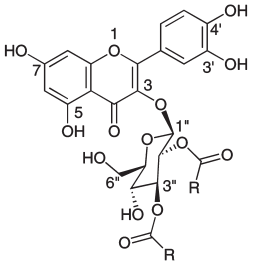
Quercetin and quercetin-3-*O*-glycosides are the most abundant flavonoids in the human diet, are associated with a myriad of biological activities, and may contribute to the prevention of human diseases.[26,27] As quercetin and quercetin-3-*O*-glycosides are widely sold as food supplements and inhibit the growth of Gram-positive and Gram-negative organisms at high concentrations,[26−29] it is interesting to expand their antibacterial activities. During the course of our initial efforts to improve the anti-MRSA (methicillin-resistant

Received: October 21, 2010
Published: May 02, 2011

Chart 1

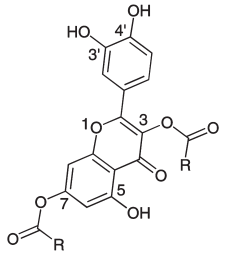


1

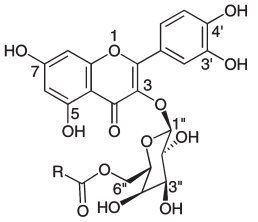


2a–h

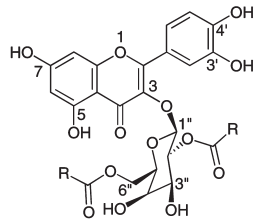
Compd	R
2a, 5a	C_6H_4(4-OEt)
2b, 5b	C_6H_4(4-^{n}Pr)
2c, 5c	C_6H_4(4-F)
2d, 5e	$CH_2C_6H_4$(4-F)
2e, 5f	$C_2H_4C_6H_4$(4-OH)
2f, 5g	$C_2H_4C_6H_4$(4-OEt)
2g, 3a, 4a, 5h	$C_2H_4C_6H_4$(4-Me)
2h, 3b, 4b, 5i	$C_2H_4C_6H_4$(4-F)
5d	$CH_2C_6H_4$(4-Me)
5j	$C_3H_6C_6H_4$(4-F)
5k	$C_4H_8C_6H_4$(4-F)



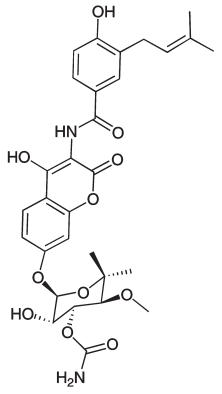
3a,b



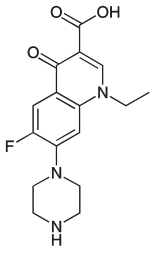
4a,b



5a–k



Novobiocin



Norfloxacin

Staphylococcus aureus) activity of lead compound (**1**),[28,30−32] we designed and synthesized novel quercetin diacylglucosides (**2a**−**h**)[28,29] (Chart 1). Compound **1** is a recently isolated natural product referred to as a kaempferol diacylrhamnoside with potent anti-MRSA activity. The significant enzymatic inhibition of both *Escherichia coli* DNA gyrase and *S. aureus* topoIV suggests that these compounds are dual inhibitors. In an attempt to clarify the mode of action (MOA) and to explore the structure−activity relationships (SARs) of this analogue, further new 3,7-diacylquercetin derivatives **3a,b**, quercetin 6″-acylgalactoside derivatives **4a,b**, and quercetin 2″,6″-diacylgalatoside derivatives **5a**−**k** have been designed and synthesized. The antibacterial activities of these compounds toward the growth of both Gram-positive and Gram-negative organisms and *E. coli* DNA supercoiling activity were investigated. Furthermore, the effects on *E. coli* and *S. aureus* topoIV decatenation activities of these compounds along with eight most potent previously synthesized compounds (**2a**−**h**)[28,29] were evaluated. To observe probable binding conformation of these ligands in the ATP binding pocket, a molecular docking study was performed, and the interaction of the most active compound with the 24 KDa fragment of DNA gyrase B and topoIV ParE was explored using the AutoDock 4.0 program. In addition, the most active compound was evaluated for its in situ intestinal absorption in rats and acute toxicity in mice.

CHEMISTRY

We first planned to incorporate 3-(4-substituted phenyl)propanoyl groups on 3-*O* and 7-*O* positions of quercetin and to incorporate various 4-substituted phenyl alkanoyl or benzoyl groups on the 6″-*O* position, as well as on both 2″-*O* and 6″-*O* positions of parent quercetin-3-*O*-galactoside as outlined in Figure 1.

The preparation of 3-(4-substituted phenyl)propanoyl groups containing quercetin derivatives **3a,b** began with the esterification of 2-(2,2-dephenylbenzo[*d*][1,3]dioxol-5-yl)-3,5,7-trihydroxy-4*H*-chromen-4-one **6**[28,33] (Scheme 1). Compound **6** was easily obtained using a previous method starting from commercially available quercetin under the action of α,α-dichlorodiphenylmethane.[28,33] Then, the 3′,4′-protected compound **6** was esterified via Steglich esterification[34] with appropriate 4-substituted cinnamic acids to provide 3,7-disubstituted derivatives **7a,b** as the major product (59−63%). The diphenylbenzo protecting group of compounds **7a,b** was removed by catalytic hydrogenation with 10% Pd/C in MeOH−EtOAc under hydrogen atmosphere to give **3a,b**. During this treatment, the carbon−carbon double bonds adjacent to carbonyl groups in the R^1 group of compounds **7a,b** were selectively hydrogenated to convert them into compounds **3a,b**. ^{1}H and ^{13}C NMR spectra of **7a,b** and **3a, b** are consistent with their molecular structures. In the ^{1}H NMR spectra of **7a** and **7b**, four vinyl protons of the R^1 group each appear as a one proton doublet at 7.66, 7.74, 8.97, and 8.99 ppm and at 7.55, 7.62, 8.87, and 8.89 ppm with J = 16.2 Hz, respectively. In contrast, compounds **3a** and **3b** each show four triplet signals at 2.61, 2.66, 3.08, and 3.13 ppm and at 2.60, 2.66, 3.09, and 3.12 ppm with J = 7.2 Hz, respectively.

Parent compounds **11** and **15** were prepared in a three-step sequence involving initial regioselective galactosylation of **6** with 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide, then removal of diphenylbenzo group, and finally the hydrolysis of the acetyl

dx.doi.org/10.1021/jm200010x |*J. Med. Chem.* 2011, 54, 3686–3703

groups (Scheme 2). The reaction between compound **6** and 1 equiv of 2,3,4,6-tetra-*O*-acetyl-α-D-galctogyranosyl bromide in the presence of anhydrous K_2CO_3 in dry *N*,*N*-dimethylformamide (DMF) or anhydrous acetone led to compound **8** as the major product (69%) along with **12** (9%). When compound **6** was treated with 2 equiv of 2,3,4,6-tetra-*O*-acetyl-α-D-galctogyranosyl bromide, compound **12** was the sole product. The diphenylbenzo protecting group of compounds **8**, **9**, and **12** was removed by catalytic hydrogenation to provide corresponding compounds **10**, **11**, and **13** according to a similar procedure as for compounds **7a**,**b** into **3a**,**b**. The removal of all acetyl groups from **8**, **10**, **12**, and **13** was achieved under the action of MeONa in MeOH−tetrahydrofuran (THF) followed by treatment with Dowex 50 (H^+) ion-exchange resin[35] to lead to the corresponding compounds **9**, **11**, **14**, and **15**. The removal of diphenylbenzo group of **14** was not successful with catalytic hydrogenation, although it was successful in the case of compound **9** (Scheme 2).

As outlined in Schemes 3 and 4, the synthesis of the intermediate 2″,6″-*O*-isopropyledene derivative **18** began from **8** and was elaborated to provide homologues **4a**,**b** and **5a**−**k**. Following a three-step sequence involving protection of free hydroxyl groups with excess benzyl bromide, followed by hydrolysis of acetyl groups, and finally protection of the 3″- and 4″-hydroxyl groups of **17** yielded intermediate 2″,6″-*O*-isopropyledene derivative **18**. First, the free hydroxyl groups of **8** were protected with excess benzyl bromide in the presence of anhydrous K_2CO_3 in dry DMF to provide compound **16**. Then, the removal of acetyl groups of **16** with the above-discussed acetyl group hydrolysis method gave compound **17**, and finally, the reaction of acetone in the presence of anhydrous copper sulfate and a catalytic amount of sulfuric acid yielded intermediate **18** in high yield (82%). The synthesis of homologues **4a**,**b** and **5a**−**k** began with selective esterification of intermediate **18** under kinetic control followed by removal of the isopropylidene protecting group and benzyl and benzophenone groups. The reaction between **18** and 1.5 equiv of the appropriate 4-substituted cinnamic acids via the previously discussed Steglich esterification method at a low temperature (−10 °C) afforded the corresponding 6″-substituted homologues **19a**,**b** as the sole products and at room temperature afforded a mixture of **19a**,**b** with corresponding diacyl derivatives **21h**,**i**. When the reaction was carried out at higher temperature (40 °C) or room temperature between **18** and 3 equiv of appropriate aliphatic or aromatic carboxylic acids, the corresponding diacyl homologues **21a**−**k** were the sole products. Deprotection of isopropylidene protecting group of **19a**,**b** and **21a**−**k** was accomplished by hydrolysis with HCl gas at 40 °C instead of 0.5 M hydrochloric acid in a mixture of MeOH and THF to provide compounds **20a**,**b** and **22a**−**k** with high yields. Heating compounds **19a**,**b** and **21a**−**k** with 0.5 M hydrochloric acid at 50 °C afforded poor yields due to decomposition, which was observed on thin-layer chromatography (TLC). Finally, the two benzyl and diphenylbenzo protecting groups of **20a**,**b** and **22a**−**k** were removed in one step by catalytic hydrogen transfer reaction to give the desired corresponding compounds **4a**,**b** and **5a**−**k**. During this treatment, the carbon−carbon double bonds adjacent to carbonyl groups in the R^1 and R^3 of compounds **20a**,**b** and **22f**−**i** also were selectively hydrogenated and were converted into compounds **4a**,**b** and **5f**−**i**, respectively. This result is similar to the cases of compounds **7a**,**b** into **3a**,**b**. The structures of all compounds were confirmed by ^{1}H NMR, ^{13}C NMR, FAB-MS, and/or elemental analysis.

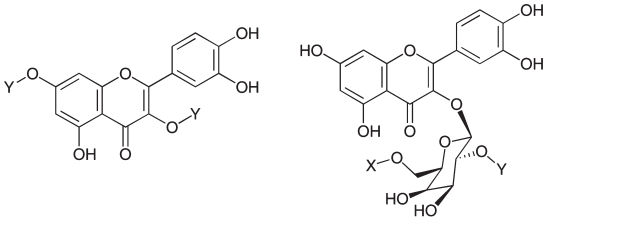
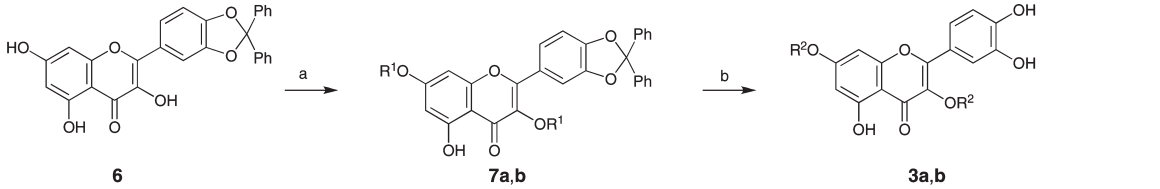


Figure 1

RESULTS

The minimum inhibitory concentrations (MICs) of the compounds **3a**,**b**, **4a**,**b**, **5a**−**k**, **10**, **11**, **13**, **15**, and quercetin against a panel of nine selected multidrug-resistant Gram-positive bacterial pathogens [MRSA OM481, OM584, N315, and COL; methicillin-sensitive *S. aureus* (MSSA) 209P; VRE FN-1 and NCTC 12201; vancomycin intermediate-resistant *S. aureus* (VISA) Mu50 and *Streptococcus pneumoniae* R6] and two Gram-negative bacterial pathogens (*Pseudomonas aeruginosa* PA01 and *E. coli* K-12) are shown in Tables 1 and 2, and their activities were compared with those of the vancomycin, norofloxacin, novobiocin, **2a**−**h**,[28,29] and **1**.[28,30−32] With the exception of **11** and **15**, all of the newly synthesized compounds, namely, **3a**,**b**, **4a**,**b**, **5a**−**k**, **10**, and **13** exhibited 2−512-fold more potent in vitro antibacterial activity than vancomycin against multidrug-resistant VRE strains, and the compound **5i** is 16−32-fold more potent than

Scheme 1[a]



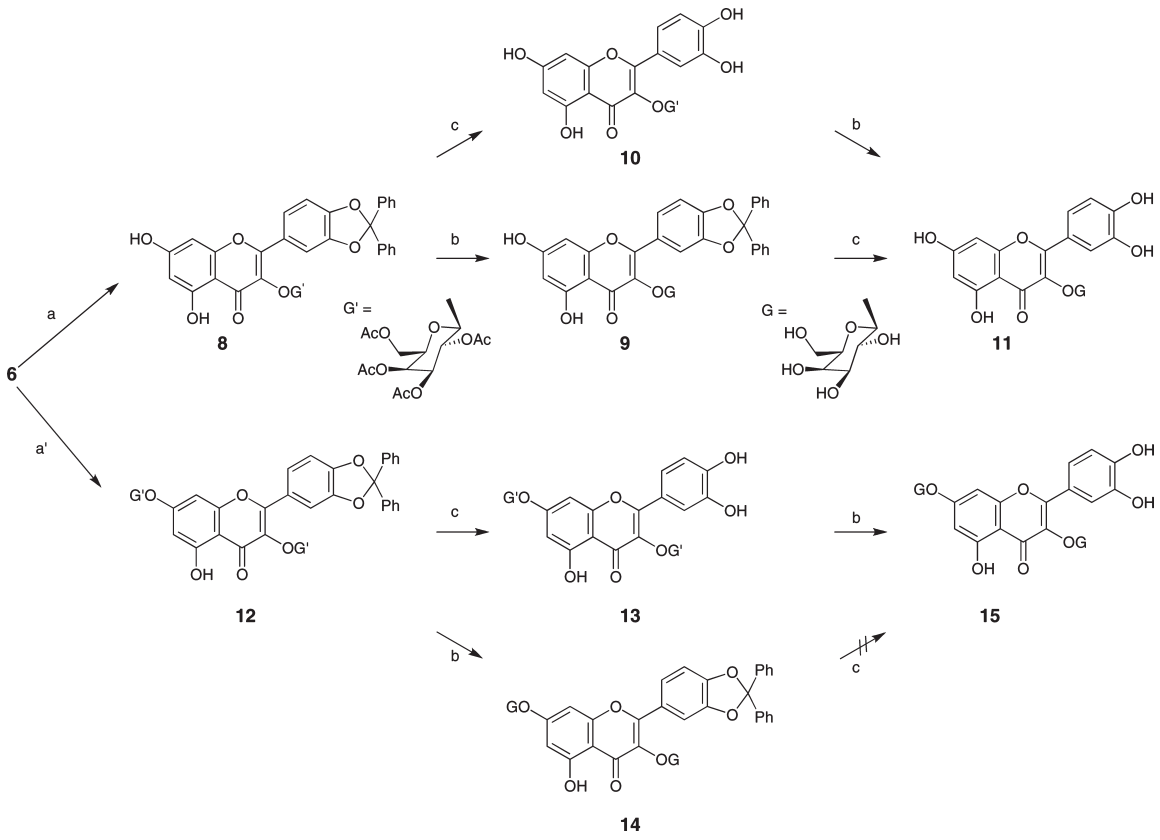
Compd	R^1
7a	$COC_2H_2C_6H_4$(4-Me)
7b	$COC_2H_2C_6H_4$(4-F)

Compd	R^2
3a	$COC_2H_4C_6H_4$(4-Me)
3b	$COC_2H_4C_6H_4$(4-F)

[a] Reagents and conditions: (a) 4-Substituted cinnamic acids, DMAP, DCC, anhydrous CH_2Cl_2, 0 °C−room temperature, 10−12 h. (b) Pd/C (10%), H_2, MeOH−EtOAc, 8−9 h.

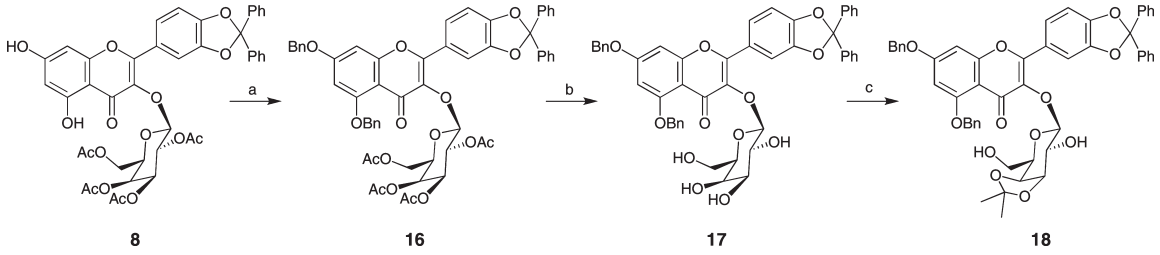
dx.doi.org/10.1021/jm200010x |*J. Med. Chem.* 2011, 54, 3686–3703

Scheme 2[a]



[a] Reagents and conditions: (a) One equiv of 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide, anhydrous K_2CO_3, anhydrous acetone or dry DMF, room temperature, 5 h. (a′) Two equiv of 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide, anhydrous K_2CO_3, anhydrous acetone or dry DMF, 40 °C, 3 h. (b) (i) MeONa, MeOH−THF, room temperature, 1.5 h; (ii) Dowex 50 (H^+) resin. (c) Pd/C (10%), H_2, MeOH−EtOAc, room temperature, 7−8 h.

Scheme 3[a]



[a] Reagents and conditions: (a) BnBr, anhydrous K_2CO_3, dry DMF, room temperature, 7 h. (b) (i) MeONa, MeOH−THF, room temperature, 0.5 h; (ii) Dowex 50 (H^+) resin. (c) Anhydrous acetone, concentrated H_2SO_4, anhydrous $CuSO_4$, room temperature, 12 h.

lead compound **1** against VRE strains. In contrast, against Gram-negative strains, the in vitro antibacterial activities of these compounds were 4−512-fold less potent than novobiocin and norfloxacin.

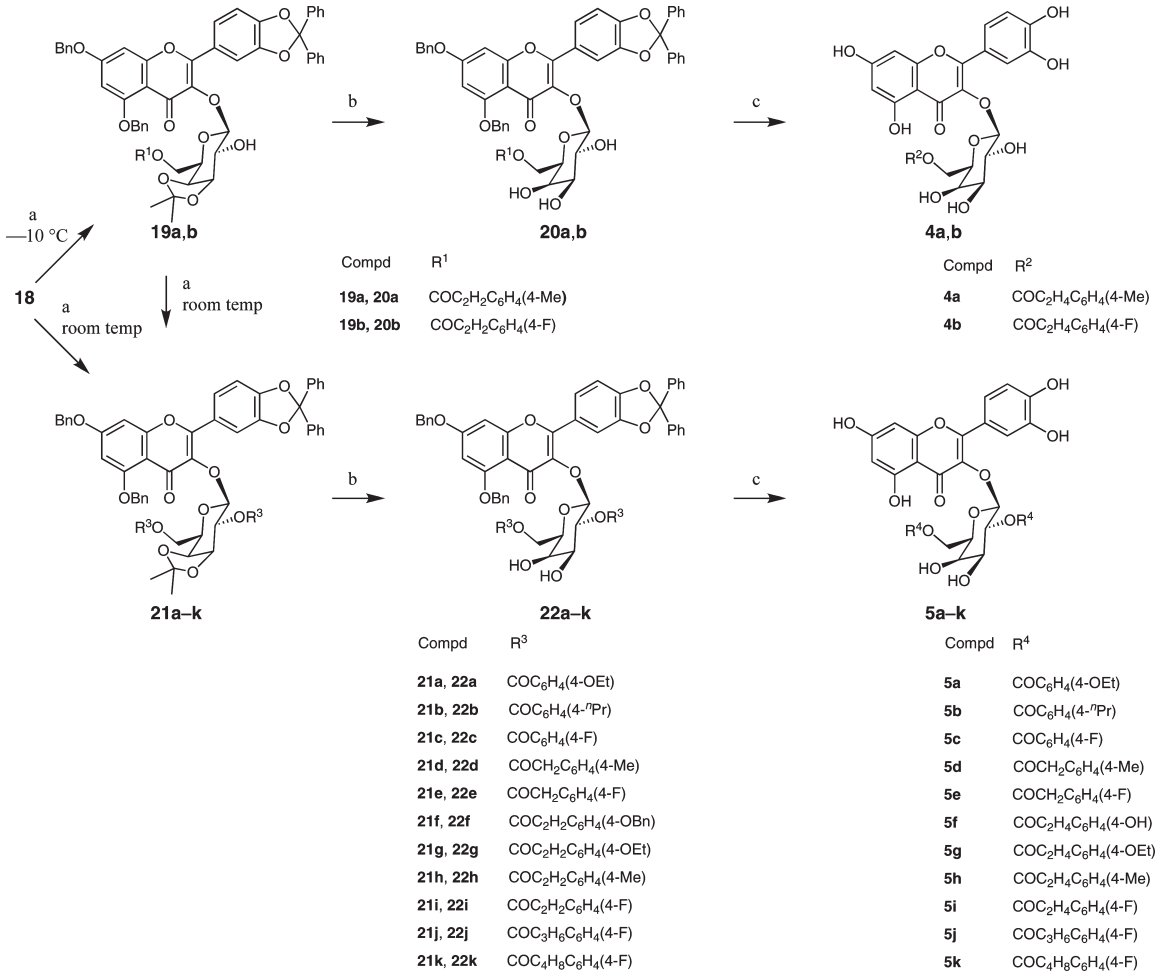
Compounds **5a**−**k** were shown in Table 1 to have a relatively good antibacterial activity against MRSA, MSSA, VRE, VISA, and *S. pneumoniae* strains (MIC ranging 0.13−16 μg/mL), and among these 2″,6″-diacyl compounds, the lowest MIC values were shown by compound **5i** (MIC: 0.13−0.5 μg/mL). Compounds **3a**,**b**, **4a**,**b**, **10**, and **13** were found to have moderate antibacterial activity, but quercetin, **11**, and **15** did not show antibacterial activity toward the growth of those Gram-positive organisms.

On the basis of the known ability of quercetin to inhibit DNA supercoiling and the binding of quercetin to the ATP binding site of DNA gyrase B,[37−39] inhibition of *E. coli* DNA gyrase supercoiling activity by **3a**,**b**, **4a**,**b**, **5a**−**k**, **10**, **11**, **13**, and **15** along with those of quercetin, norfloxacin, novobiocin, and **1**[28,30−32] were tested as shown in Table 2. Most of these compounds exhibited strong inhibition of DNA supercoiling activity (IC_{50}: 0.10 ± 0.06 to 5.39 ± 0.28 μM).

As DNA gyrase and topoIV share 36−40% amino acid sequence homology,[40] these compounds also were screened for the inhibition of enzymatic activities of *E. coli* topoIV along with eight previously synthesized compounds **2a**−**h**[28,29] as

dx.doi.org/10.1021/jm200010x |*J. Med. Chem.* 2011, 54, 3686–3703

Scheme 4[a]



[a] Reagents and conditions: (a) Aliphatic or aromatic carboxylic acids, DMAP, DCC, anhydrous CH_2Cl_2, −10 °C−room temperature, 5−12 h. (b) HCl gas, MeOH−THF, 40 °C, 4−5 h. (c) Pd/C (10%), H_2, MeOH−EtOAc, room temperature, 9−11 h.

shown in Tables 2 and 3. Unfortunately, these investigated compounds showed moderate and ca. 11 times reduced inhibition of topoIV decatenation activity (IC_{50}: 3.07 ± 0.18 to >12 μM) as compared to DNA supercoiling activity.

As shown in Table 1, the Gram-positive organisms were strongly susceptible to most tested compounds, consistent with the probability that these compounds may inhibit *S. aureus* topoIV in Gram-positive bacteria. Encouraged by this observation, these compounds along with **2a**−**h**[28,29] and **1**[28,30−32] were screened against enzyme *S. aureus* topoIV as shown in Tables 2 and 3. All of the investigated compounds exhibited pronounced inhibition of *S. aureus* topoIV decatenation activity (IC_{50}: 0.07 ± 0.08 to 4.05 ± 0.21 μM). Among the tested compounds, **5i** was the most active of this series with pronounced DNA supercoiling inhibition of *E. coli* gyrase (IC_{50}: 0.78 ± 0.03 μM), topoIV decatenation activity from *S. aureus* (IC_{50}: 0.22 ± 0.06 μM), and ca. 40-fold lower decatenation activity of *E. coli* topoIV (IC_{50}: 9.16 ± 0.19 μM) that were measured as a function of concentration as shown in Figure 2. These data suggest that the MOAs of the tested compounds are due to specific inhibition of DNA gyrase and topoIV in a fluoroquinolone-like manner, where the primary target in *S. aureus* is topoIV and in *E. coli* is DNA gyrase.[16,41−43] However, remarkably reduced DNA supercoiling and topoIV decatenation activities were observed for compounds **5g** and **5k**.

AutoDock-Modeled Binding of the Most Potent Compound 5i to the 24 kDa Fragment of *E. coli* DNA Gyrase B and TopoIV ParE. The availability of a high-quality crystal structure[21] has made it possible to perform AutoDock modeling[44] to fit the ligand in the ATP binding pocket of the 24 kDa fragment of the DNA gyrase B and topoIV ParE from *E. coli* (PDB accession codes 1AJ6 and 1S14, respectively). For each protein−ligand complex, less than 100 possible docking configurations were discovered. We observed that most compounds were successfully fit inside the ATP binding pocket of both enzymes. Nevertheless, increasing K_i (inhibition constant) values were predicted for compounds **5g** and **5k** with bulky substituents at the 2″- and 6″-positions, relative to unbranched (**11**) or less bulky (**5a**−**f**, **5h**−**j**) compounds (data are in the Supporting Information). The preferential mode of binding of the most potent compound **5i** for the best configuration to DNA gyrase B was characterized by three H-bonds formed between the 3′-, 4′-, and 7-hydroxyl groups with ASN46, VAL120, and LYS103, respectively (left side in Figure 3). Similarly, the preferential mode of binding of compound **5i** for best configuration to topoIV ParE was characterized by one H-bond formed

dx.doi.org/10.1021/jm200010x |*J. Med. Chem.* 2011, 54, 3686–3703

Table 1. Antibacterial Activity (MICs) against Gram-Positive Bacterial Strains for 1, 2a−h, 3a,b, 4a,b, 5a−k, 10, 11, 13, 15, Quercetin, Vancomycin, Norfloxacin, and Novobiocin[a]

	MIC[b] (μg/mL, Gram-positive)								
	VRE		VISA	MRSA				MSSA	*S. pneumoniae*
compd	FN-1[c]	NCTC 12201[d]	Mu50[e]	OM481[f]	OM584[g]	N315[h]	COL[i]	209P[j]	R6[k]
3a	64	64	16	32	32	32	32	64	32
3b	64	32	32	16	16	16	32	64	32
4a	32	32	16	16	16	16	32	16	32
4b	16	16	32	16	16	16	32	16	32
5a	8	8	4	4	4	8	16	4	4
5b	8	4	4	4	4	2	16	2	2
5c	4	4	2	2	2	8	8	4	4
5d	8	4	4	2	2	8	16	4	2
5e	0.5	0.5	1	1	1	1	8	1	1
5f	4	4	2	1	1	2	4	2	4
5g	16	16	8	4	4	2	8	8	8
5h	0.5	0.5	0.5	0.25	0.25	0.25	1	0.25	0.25
5i	0.25	0.25	0.5	0.13	0.13	0.13	0.5	0.13	0.13
2h	1	1	1	2	2	0.25	1	0.25	0.50
5j	8	8	4	2	2	4	16	4	4
5k	16	16	16	4	4	2	16	4	8
10	32	32	64	32	32	64	128	64	64
11	>128	>128	>128	>128	>128	>128	>128	>128	>128
13	64	64	128	128	128	128	>128	128	128
15	>128	>128	>128	>128	>128	>128	>128	>128	>128
quercetin	>128	>128	>128	>128	>128	>128	>128	>128	>128
vancomycin	>128	>128	8	0.25	0.25	0.25	0.25	0.25	0.25
norfloxacin	>128	4	>128	64	128	2	1	0.5	0.25
novobiocin	>128	2	8	0.25	0.25	0.13	0.25	0.13	0.25
1	8	4	NT	1	2	1	1	0.5	NT
2a−g	2−16	4−16	1−16	2−32	2−32	1−8	4−16	1−8	1−8

[a] NT, not tested. [b] Microdilution method,[36] MIC determined after 24 h. [c] VRE FN-1. [d] VRE NCTC 12201. [e] VISA Mu50. [f] MRSA OM481. [g] MRSA OM584. [h] MRSA N315. [i] MRSA COL. [j] MSSA 209P. [k] *Streptococcus pneumoniae* R6.

between 3′-hydroxyl group with VAL2119 (right side in Figure 3). Figure 3 also represents the superposition of original X-ray structure (native ligand novobiocin) and the docked ligand **5i** inside the ATP binding pocket of DNA gyrase B and topoIV ParE. On the basis of the in vitro antibacterial activity, the inhibition of bacterial enzymes, and the molecular docking study, we propose the SARs for quercetin diacylglycoside analogues as shown in Figure 4.

Absorption Property of Compound 5i in Rats. The most potent compound **5i** was selected as a representative compound with which to explore absorption property and acute toxicity of quercetin diacylglycoside analogues in rats and mice, respectively. The required concentration of **5i** for in situ intestinal absorption and acute toxicity testing was achieved with 50% polyoxyethyleneglycol (PEG) in saline. The absorption property was studied by in situ prefusion technique[45] in rats (n = 4). The in situ intestinal absorption studies in rats demonstrated realistic absorption of **5i** from small intestine, 76.6 ± 0.60%.

Acute Toxicity of 5i in Mice. Intervenous doses of **5i** in 50% PEG via the tail vein were well tolerated by mice (n = 4) at 10 (for 4 days, total dose was 40 mg/kg, total dose volume was 20 mL/kg) and 50 mg/kg/day (for 4 days, total dose was 200 mg/kg, total dose volume was 20 mL/kg). At 100 mg/kg/day (for 4 days, total dose was 400 mg/kg, total dose volume was 20 mL/kg), the mice showed a transient, seizurelike reaction to a fourth injection after ca. 10 min, but all animals appeared normal after 1 day.

DISCUSSION

In this paper, the discovery of quercetin diacylgalctoside analogues that inhibit both bacterial DNA gyrase and topoIV enzymes and display potent antibacterial activity against Gram-positive bacteria has been described. We have not been the first group to identify potent antibacterial DNA gyrase and topoIV inhibiting agents. Many research groups have published extensively on the identification of DNA gyrase and topoIV inhibiting agents that display potent antibacterial activity.[46−51] However, a number of groups have noted that there is no correlation between DNA gyrase or topoIV inhibition and antibacterial activity,[49,52,53] while some researchers have proposed that cell penetration differences account for this lack of correlation.

The two factors that contribute to antibacterial potency are the kinetics of drug uptake and the ability to inhibit DNA gyrase

Table 2. Antibacterial Activity (MICs) against Gram-Negative Bacterial Strains and DNA Gyrase Supercoiling and TopoIV Decatenation Activities for 1, 3a,b, 4a,b, 5a−k, 10, 11, 13, 15, Quercetin, Norfloxacin, and Novobiocin[a]

	MIC[b] (μg/mL, Gram-negative)		supercoiling inhibition, IC_{50}[e] (μM)	decatenation inhibition, IC_{50}[e] (μM)	
compd	PA01[c]	K-12[d]	*E. coli* DNA gyrase	*E. coli* topoIV	*S. aureus* topoIV
3a	64	64	1.66 ± 0.07	8.59 ± 0.11	0.44 ± 0.17
3b	64	64	0.72 ± 0.05	6.76 ± 0.17	0.29 ± 0.11
4a	>128	>128	1.79 ± 0.20	11.99 ± 0.09	2.57 ± 0.12
4b	>128	>128	1.40 ± 0.06	11.52 ± 0.13	2.99 ± 0.03
5a	>128	>128	0.43 ± 0.08	11.33 ± 0.06	1.78 ± 0.18
5b	>128	>128	0.77 ± 0.07	10.89 ± 0.11	1.93 ± 0.25
5c	>128	>128	0.38 ± 0.12	11.02 ± 0.04	0.52 ± 0.04
5d	>128	>128	2.21 ± 0.20	10.52 ± 0.10	2.69 ± 0.11
5e	>128	>128	1.05 ± 0.15	10.21 ± 0.17	0.46 ± 0.02
5f	>128	>128	2.39 ± 0.09	10.86 ± 0.13	1.59 ± 0.09
5g	>128	>128	5.39 ± 0.28	>12	2.99 ± 0.13
5h	>128	>128	2.09 ± 0.16	9.46 ± 0.11	1.49 ± 0.16
5i	>128	>128	0.78 ± 0.03	9.16 ± 0.19	0.22 ± 0.06
5j	>128	>128	2.98 ± 0.09	10.74 ± 0.31	2.94 ± 0.10
5k	>128	>128	5.02 ± 0.06	>12	4.05 ± 0.21
10	>128	>128	NT	NT	NT
11	32	16	0.10 ± 0.06	3.07 ± 0.18	0.07 ± 0.08
13	>128	>128	NT	NT	NT
15	32	32	0.26 ± 0.08	5.34 ± 0.22	0.20 ± 0.01
quercetin	>128	64	0.14 ± 0.04	6.42 ± 0.07	NT
norfloxacin	0.25	0.25	0.09 ± 0.01	NT	3.50 ± 0.02
novobiocin	8	8	0.05 ± 0.01	0.11 ± 0.05	NT
1	>128	>128	3.11 ± 0.11	NT	2.70 ± 0.23

[a] NT, not tested. [b] Microdilution method,[36] MIC determined after 24 h. [c] *Pseudomonas aeruginosa* PA01. [d] *E. coli* K-12; IC_{50}. [e] The concentration of compounds that inhibits 50% of supercoiling (*E. coli* DNA gyrase) and decatenation (*E. coli* and *S. aureus* topoIV) activities.

or topoIV.[54] The physicochemical properties (e.g., relative hydrophobicity, charge, or molecular mass) of antibacterial compounds are essential for penetration into the bacterial cell and have a different role in Gram-positive and Gram-negative organisms. Compounds with antibacterial activity against Gram-positive bacteria have less limitation in high molecular weight (MW); the most likely reason for this property is the different cell architecture of bacteria that greatly affects permeability and efflux of compounds.[55] Thus, the 2″,6″-diacyl homologues **5a**−**k** except **5g** and **5k** showed comparatively better activity against Gram-positive organisms tested than those of 6″-acyl homologues **4a**,**b** and 3,7-diacyl homologues **3a**,**b** with pronounced *S. aureus* topoIV decatenation activity, although **3a**,**b** and **4a**,**b** showed almost equal or better inhibition to the same enzyme. Furthermore, the antibacterial activity of 2″,6″-diacyl homologues is ca. four times more potent than previously reported 2″,3″-diacyl homologues **2a**−**h**. Maybe the acyl substituents give these 2″,6″-diacyl homologues comparatively good properties to enter into Gram-positive bacterial organisms across the periplasmic space or cytoplasm. Nevertheless, the investigated compounds appeared to show moderate inhibition of *E. coli* topoIV decatenation as compared to *E. coli* DNA supercoiling and *S. aureus* decataenation. These data indicate that the MOA of these compounds are fluoroquinolone-like, but in an entirely new way, and therefore exemplify a new class of antibacterial agents. Among the 2″,6″-diacyl homologues, fluorine-containing **5c**, **5e**, **5i**, and **5j** except **5k** displayed comparatively greater inhibition toward the growth of Gram-positive organisms. Maybe the fluorine modulates the electronic effects on phenyl rings and also influences the steric characteristics and the hydrophilic−hydrophobic balance of these molecules.[28]

On the other hand, compounds having comparatively high MW and bulky side chains did not inhibit the growth of Gram-negative organisms, even at high concentrations, despite showing pronounced *E. coli* DNA gyrase supercoiling inhibition. The reason for these results is not clear, but hindrance of penetration into Gram-negative organism through the porin channel might be involved.[53] Compounds with activity against Gram-negative organisms must overcome further barriers to function, namely, the penetration of the outer lipid membrane and evasion of efflux pumps.[56,57] Both parameters are partially believed to be derived by the properties of cylinder-shaped porin proteins (β-barrels) that serve as a major entry pathway in Gram-negative bacteria.[55] The required activation energy for lipophilic molecules to pass this hydrophilic channel is too high,[55] and these molecules are consequently prevented from crossing the outer membrane and subsequently entering the relevant target containing compartment of either the periplasmic space or the cytoplasm.

Comparatively low MW and highly polar quercetin, **11** and **15** showed Gram-negative organism resistance at high concentrations with good *E. coli* DNA gyrase supercoiling activity. Nonetheless, these three compounds did not inhibit the growth of Gram-positive organisms tested, despite showing significant *S. aureus* topoIV inhibition. The cause may be less favorable accumulation

of these three compounds in Gram-positive bacterial membrane.[53,56,58,59] Therefore, for an antibacterial agent to be effective, it must penetrate the bacterial cell first to reach its target enzymes.

However, compounds **5g** and **5k** with comparatively bulky substituents showed dramatically reduced enzyme inhibition and antibacterial activity. Molecular docking studies also demonstrated least favorable binding due to the relative bulky substituents on the 2″,6″-positions of **5g** and **5k**. So, it is clear that size and lipophilicity of the substituent on quercetin diacylglycosides **5a**−**k** are the key factors in determining and enhancing its antibacterial activity.

CONCLUSION

We have previously synthesized and tested a series of quercetin diacylglucosides, which showed interesting biological activity against a panel of selected multidrug-resistant Gram-positive bacterial pathogens. In the present paper, we have successfully developed a compound **5i** as a representative of quercetin diacylglycoside analogues that showed ca. four times better activity than previous compounds, as well as minimum two times better efficacy than vancomycin and norfloxacin against a wide range of antibiotic-resistant bacterial pathogens including MRSA, MSSA, VRE, VISA, and *Streptococcus pneumoniae* and exhibited realistic absorption in rats and very low toxicity in mice. Our mechanistic studies revealed that although quercetin diacylglycosides show modest and dual inhibition toward *E. coli* DNA gyrase and *S. aureus* topoIV, their antibacterial activity is not related only to their effect on these enzymes. The two factors that contribute to their antibacterial potency are the ability to penetrate the bacterial cell and their potentiality to inhibit these enzymes. The presence of suitable sizes of liphophilic acyl groups on the sugar moiety of quercetin diacylglycoside is required for activity against multidrug-resistant Gram-positive organisms. In summary, novel quercetin diacylglycosides represent one of the most exiciting recent chemical advances in the antibacterial field. As a novel chemical class that addresses the multidrug resistance problem by their novel mode of action, the representative compound **5i** is an attractive candidate for further investigation to provide new antibacterial agents for use against nosocomial multidrug-resistant Gram-positive infection.

Table 3. Decatenation Activity for Compounds 1, 2a−h, Norfloxacin, and Novobiocin[a]

	decatenation inhibitions, IC_{50}[b] (μM)	
compd	*E. coli* topoIV	*S. aureus* topoIV
2a	10.98 ± 0.12	1.98 ± 0.07
2b	11.83 ± 0.09	1.83 ± 0.19
2c	10.79 ± 0.14	1.21 ± 0.11
2d	10.09 ± 0.18	1.45 ± 0.06
2e	11.79 ± 0.08	1.98 ± 0.08
2f	>12	3.02 ± 0.14
2g	11.57 ± 0.11	2.02 ± 0.21
2h	10.40 ± 0.13	0.69 ± 0.03
norfloxacin	NT	3.50 ± 0.02
novobiocin	0.11 ± 0.05	NT
1	NT	2.70 ± 0.23

[a] NT, not tested. [b] The concentration of compounds that inhibits 50% decatenation (*E. coli* and *S. aureus* topoIV) activity.

EXPERIMENTAL SECTION

Mass spectra were recorded at 70 eV ionizing voltage with FAB ionization using VG-70SE spectrometer and 3-nitrobenzyl alcohol as a matrix. ^{1}H NMR and ^{13}C NMR spectra were recorded on a VXR 300, 400, and 600 MHz (300, 400, and 600 MHz for ^{1}H and 150 MHz for ^{13}C) spectrometer. ^{1}H chemical shifts are expressed in parts per million (ppm) based on internal TMS in $CDCl_3$ or DMSO-d_6. ^{13}C chemical shifts are expressed in parts per million (ppm) based on solvent signal of DMSO-d_6. The four-bond coupling in aromatic system is expressed by ^4J. Elemental analyses were measured by a Yanako CHN Corder MT-5 apparatus and confirmed purity of compounds at ≥95%. All reagents were of commercial quality from freshly opened containers and were used without further purification. Reaction progress was monitored by analytical TLC on precoated glass plates (silica gel 60 F_{254} Merck and 70 FM Plate-Wako), and products were visualized by UV light. Column chromatography was accomplished on silica gel 60 (spherical, 50−60 mm, Kanto Chemical Co. Inc.). The reaction temperatures are indicated as the temperature of the oil bath. Anhydrous DMF was stored over activated 4 Å molecular sieves, and all other solvents were dried and freshly distilled prior to use.

Synthesis and Characterization of 2-(2,2-Diphenylbenzo[*d*]-[1,3]dioxol-5-yl)-5,7-dihydroxy-3-β-D-tetraacetylgalacto-syl-4*H*-chromen-4-one (8). A mixture of 2-(2,2-dephenylbenzo[*d*][1,3]dioxol-5-yl)-3,5,7-trihydroxy-4*H*-chromen-4-one **6**[28,33] (0.47 g, 1 mmol), 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (0.62 g, 0.97 mmol), and anhydrous K_2CO_3 (0.19 g, 0.87 mmol) in dry DMF or anhydrous acetone (10 mL) was stirred at 0 °C for 5 h under argon. The resulting reaction mixture was poured into cold water (20 mL). The deposited pale yellow solid mass was collected by filtration. Collected crude product was purified by flash column chromatography on silica gel, eluted with a mixture of EtOAc and *n*-hexane (1.25:2), to afford colorless powder of **8** (0.54 g, 69%). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.90, 2.00, 2.01, and 2.14 (each s, each 3H, 4 × CH_3CO), 3.84−3.91 (m, 3H, 3″-H, 4″-H, 5″-H), 5.09

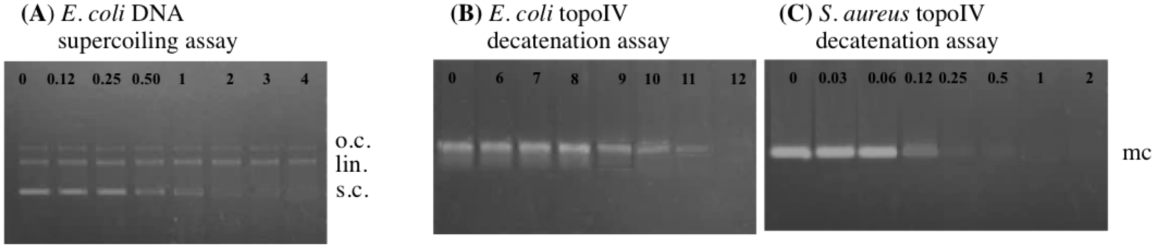


Figure 2. DNA gyrase and topoIV activity assays of compound **5i**: (A) DNA supercoiling assay by gyrase from *E. coli* strains JMtacA and JMtacB,[60] (B) decatenation assay by topoIV from *E. coli* ParC and ParE subunits, and (C) decatenation assay by topoIV from *S. aureus*.[61] Gels were run in the absence of ethidium bromide or chloroquine. o.c., open-circular DNA; lin., linear DNA; s.c., supercoiled DNA; and mc, decatenated mini circles.

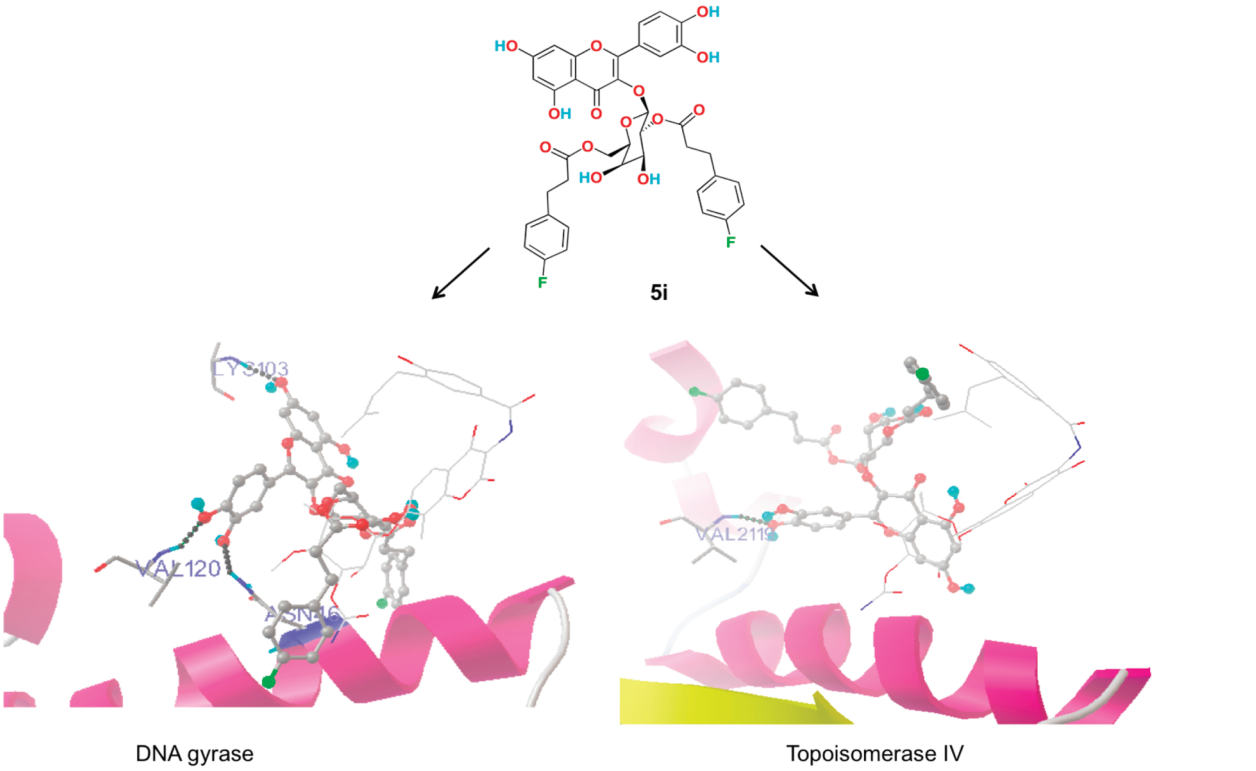
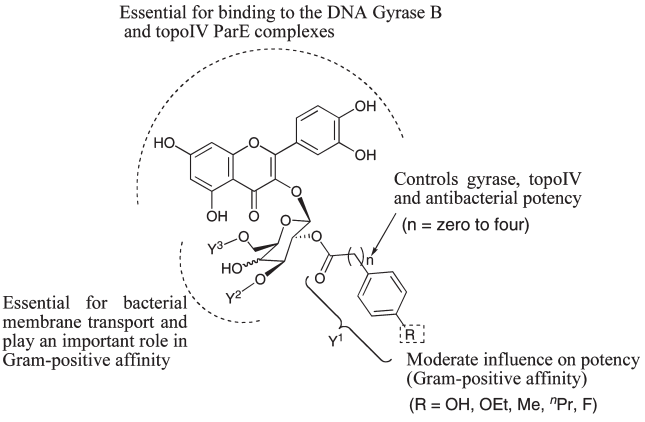


Figure 3. AutoDock-modeled binding of **5i** at the ATP binding site of DNA gyrase B and topoIV ParE. Superposition of native ligand novobiocin (lines) and **5i** (sticks and balls). H-bonds are displayed as spheres.

(dd, $J_{5'',6''Hb}$ = 3.0 Hz, J_{gem} = 10.8 Hz, 1H, 6″-H_b), 5.35 (d, $J_{5'',6''Ha}$ = 3.6 Hz, 1H, 6″-H_a), 5.41 (dd, $J_{1'',2''}$ = 7.8 Hz, $J_{2'',3''}$ = 7.2 Hz, 1H, 2″-H), 5.53 (d, $J_{1'',2''}$ = 7.8 Hz, 1H, 1″-H), 6.25 (d, ^4J = 1.8 Hz, 1H, 8-H), 6.35 (d, ^4J = 2.4 Hz, 1H, 6-H), 6.96 (d, J = 8.1 Hz, 1H, 5′-H), 7.37−7.42 (m, 6H, PhH), 7.58−7.61 (m, 4H, PhH), 7.67 (dd, J = 8.4 Hz, ^4J = 2.4 Hz, 1H, 6′-H), 7.70 (d, ^4J = 2.4 Hz, 1H, 2′-H), 12.52 (s, 1H, 5-OH, D_2O exch.). FAB-MS m/z $[M + H]^+$ ion = 797.

Synthesis and Characterization of 2-(2,2-Diphenylbenzo[*d*]-[1,3]dioxol-5-yl)-5,7-dihydroxy-3-β-D-galactosyl-4*H*-chromen-4-one (9). To a solution of **8** (0.80 g, 1 mmol) in EtOAc and MeOH (1:1, 20 mL) was added MeONa (0.10 g, 1.80 mmol), and the solution was stirred at room temperature for 1.5 h. After the reaction was completed, the solution was neutralized by passage down through a Dowex 50 (H^+) ion-exchange resin column. The resin was filtered, and the filtrate was concentrated to afford **9** (0.49 g, 78%) as a colorless powder. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 4.13, 4.18, 4.80, and 5.10 (each br s, each 1H, 2″-OH, 3″-OH, 4″-OH, and 6″-OH, D_2O exch.), 4.21−4.30 (m, 3H, 3″-H, 4″-H, 5″-H), 4.61 (dd, $J_{5'',6''Hb}$ = 3.0 Hz, J_{gem} = 10.8 Hz, 1H, 6″-H_b), 4.91 (d, $J_{5'',6''Ha}$ = 3.6 Hz, 1H, 6″-H_a), 4.95 (dd, $J_{1'',2''}$ = 7.8 Hz, $J_{2'',3''}$ = 7.2 Hz, 1H, 2″-H), 5.10 (d, $J_{1'',2''}$ = 7.8 Hz, 1H, 1″-H), 6.24 (d, ^4J = 1.8 Hz, 1H, 8-H), 6.36 (d, ^4J = 2.4 Hz, 1H, 6-H), 6.97 (d, J = 8.1 Hz, 1H, 5′-H), 7.39−7.43 (m, 6H, PhH), 7.57−7.60 (m, 4H, PhH), 7.67 (dd, J = 8.4 Hz, ^4J = 2.4 Hz, 1H, 6′-H), 7.72 (d, ^4J = 2.4 Hz, 1H, 2′-H), 10.79 (s, 1H, 7-OH, D_2O exch.), 12.72 (s, 1H, 5-OH, D_2O exch.). FAB-MS m/z $[M + H]^+$ ion = 629.

Synthesis and Characterization of 2-(3′,4′-Dihydroxyphenyl)-5,7-dihydroxy-3-β-D-tetraacetylgalactosyl-4*H*-chromen-4-one (10). To a solution of **8** (0.80 g, 1.00 mmol) in EtOAc−EtOH (1:1, 30 mL), 10% Pd/C (1 equiv) was added and vigorously stirred at 0 °C to room temperature for 8 h under hydrogen pressure (balloon). After the Pd/C was removed by filtration, the filtrate was concentrated under reduced pressure at 30 °C, and the residue was purified by flash column



Compd	Y^1, Y^2, Y^3 (Attached aliphatic or aromatic carbonoyl groups/ Hydrogen)	n (no. of linker CH_2 groups)
2a–h	$Y^1 = Y^2 = COC_6H_4(R)$, $CO(CH_2)_1C_6H_4(R)$, $CO(CH_2)_2C_6H_4(R)$; Y^3 = Hydrogen	zero to two
4a–b	$Y^3 = CO(CH_2)_2C_6H_4(R)$; $Y^1 = Y^2$ = Hydrogen	two
5a–k	$Y^1 = Y^3 = COC_6H_4(R)$, $CO(CH_2)_1C_6H_4(R)$, $CO(CH_2)_2C_6H_4(R)$, $CO(CH_2)_3C_6H_4(R)$, $CO(CH_2)_4C_6H_4(R)$; Y^2 = Hydrogen	zero to four

Figure 4. Overview of SAR of quercetin diacylglycoside analogues.

chromatography using a mixture of EtOAc and *n*-hexane (1:1) as eluting solvent to give **10** (0.43 g, 68%) as pale yellow powders. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 1.77, 1.91, 2.02, and 2.11 (each s, each 3H, 4 × CH_3CO), 3.81−3.86 (m, 2H, 4″-H, 5″-H), 4.16 (dd, $J_{2'',3''}$ = 7.2 Hz,

$J_{3'',4''}$ = 6.6 Hz, 1H, 3''-H), 5.16 (dd, $J_{1'',2''}$ = 7.8 Hz, $J_{2'',3''}$ = 7.2 Hz, 1H, 2''-H), 5.19−5.25 (m, 2H, 6''-H), 5.61 (d, $J_{1'',2''}$ = 7.8 Hz, 1H, 1''-H), 6.18 (d, ^4J = 1.8 Hz, 1H, 8-H), 6.38 (d, ^4J = 2.4 Hz, 1H, 6-H), 6.83 (d, J = 8.1 Hz, 1H, 5'-H), 7.44 (d, ^4J = 2.4 Hz, 1H, 2'-H), 7.50 (dd, J = 8.4 Hz, ^4J = 2.4 Hz, 1H, 6'-H), 9.15 (s, 1H, 3'-OH, D_2O exch.), 9.80 (s, 1H, 4'-OH, D_2O exch.), 10.87 (s, 1H, 7-OH, D_2O exch.), 12.56 (s, 1H, 5-OH, D_2O exch.). FAB-MS m/z $[M + H]^+$ ion = 633. Anal. calcd for $C_{29}H_{28}O_{16}$: C, 55.07; H, 4.46. Found: C, 55.29; H, 4.77.

Synthesis and Characterization of 2-(3′,4′-Dihydroxyphenyl)-5,7-dihydroxy-3-β-D-galactosyl-4*H*-chromen-4-one (11). Compound **11** in 64−67% was prepared using similar procedures to that for **9** or **10**. ^{1}H NMR (DMSO, 400 MHz): δ 3.44 (br s, 2H, 5''-H, 4''-H), 3.53−3.58 (m, 2H, 3''-H, 6''-H_b), 3.64 (br s, 2H, 2''-H, 6''-H_a), 4.20, 4.23, 4.85, and 5.13 (each br s, each 1H, 2''-OH, 3''-OH, 4''-OH, 6''-OH, each D_2O exch.), 5.38 (d, $J_{1'',2''}$ = 7.5 Hz, 1H, 1''-H), 6.18 (d, ^4J = 1.8 Hz, 1H, 8-H), 6.39 (d, ^4J = 1.8 Hz, 1H, 6-H), 6.81 (d, J = 9.0 Hz, 1H, 5'-H), 7.51 (d, ^4J = 2.4 Hz, 1H, 2'-H), 7.67 (dd, J = 8.4 Hz, ^4J = 2.4 Hz, 1H, 6'-H), 9.15 (s, 1H, 3'-OH, D_2O exch.), 9.72 (s, 1H, 4'-OH, D_2O exch.), 10.86 (s, 1H, 7-OH, D_2O exch.), 12.63 (s, 1H, 5-OH, D_2O exch.). FAB-MS m/z $[M + H]^+$ ion = 465. Anal. calcd for $C_{21}H_{20}O_{12}$: C, 54.31; H, 4.34. Found: C, 54.09; H, 4.16.

Synthesis and Characterization of 2-(2,2-Diphenylbenzo[*d*]-[1,3]dioxol-5-yl)-5-hydroxy-3,7-di-(β-D-tetraacetylgalactosyl)-4*H*-chromen-4-one (12). Compound **12** (0.86 g, 76%) was prepared from compound 6 (0.47 g, 1 mmol) using a similar procedure to that for **8**. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 1.91, 2.00, 2.01, 2.02, 2.07, 2.08, 2.12, and 2.19 (each s, each 3H, 8 × CH_3CO), 3.83−3.84 (m, 1H, 5''-H), 3.88−3.89 (m, 2H, 3''-H, 4''-H), 4.10−4.14 (m, 1H, 5'''-H), 4.20−4.23 (m, 2H, 3'''-H, 4'''-H), 5.091 (dd, $J_{5'',6''Hb}$ = 3.6 Hz, J_{gem} = 10.8 Hz, 1H, 6''-H_b), 5.13 (dd, $J_{5''',6'''Hb}$ = 3.6 Hz, J_{gem} = 12.0 Hz, 1H, 6'''-H_b), 5.35 (d, $J_{5'',6''Ha}$ = 3.6 Hz, 1H, 6''-H_a), 5.41 (dd, $J_{1'',2''}$ = 7.8 Hz, $J_{2'',3''}$ = 7.2 Hz, 1H, 2''-H), 5.48 (d, $J_{5''',6'''Ha}$ = 3.6 Hz, 1H, 6'''-H_a), 5.52 (dd, $J_{1''',2'''}$ = 8.4 Hz, $J_{2''',3'''}$ = 7.8 Hz, 1H, 2'''-H), 5.54 (d, $J_{1'',2''}$ = 7.8 Hz, 1H, 1''-H), 5.56 (d, $J_{1''',2'''}$ = 7.8 Hz, 1H, 1'''-H), 6.43 (d, ^4J = 1.8 Hz, 1H, 8-H), 6.56 (d, ^4J = 2.4 Hz, 1H, 6-H), 6.98 (d, J = 8.1 Hz, 1H, 5'-H), 7.26−7.42 (m, 5H, PhH), 7.57−7.61 (m, 5H, PhH), 7.69 (dd, J = 8.4 Hz, ^4J = 2.4 Hz, 1H, 6'-H), 7.71 (d, ^4J = 2.4 Hz, 1H, 2'-H), 12.49 (s, 1H, 5-OH, D_2O exch.). FAB-MS m/z $[M + H]^+$ ion = 1127.

Synthesis and Characterization of 2-(3′,4′-Dihydroxyphenyl)-5-hydroxy-3,7-di-(β-D-tetraacetylgalactosyl)-4*H*-chromen-4-one (13). Compound **13** (0.77 g, 80%) was prepared from **12** (1.13 g, 1 mmol) using a similar procedure to that for **10**. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 1.78, 1.93, 1.95, 1.98, 2.03, 2.04, 2.13, and 2.14 (each s, each 3H, 8 × CH_3CO), 3.82−3.88 (m, 2H, 3''-H, 5''-H), 4.07−4.13 (m, 2H, 3'''-H, 5'''-H), 4.18 (dd, $J_{1'',2''}$ = 7.2 Hz, $J_{2'',3''}$ = 6.0 Hz, 1H, 2''-H), 4.51 (dd, $J_{1''',2'''}$ = 7.2 Hz, $J_{2''',3'''}$ = 6.0 Hz, 1H, 2'''-H), 5.18−5.22 (m, 3H, 4''-H, 6''-H), 5.23−5.27 (m, 2H, 4'''-H, 6'''-H_a), 5.36 (br s, 1H, 6'''-H_b), 5.63 (d, $J_{1'',2''}$ = 7.2 Hz, 1H, 1''-H), 5.68 (d, $J_{1''',2'''}$ = 7.2 Hz, 1H, 1'''-H), 6.42 (d, ^4J = 1.8 Hz, 1H, 8-H), 6.73 (d, ^4J = 2.4 Hz, 1H, 6-H), 6.86 (d, J = 8.1 Hz, 1H, 5'-H), 7.50 (d, ^4J = 1.8 Hz, 1H, 2'-H), 7.51 (dd, J = 8.4 Hz, ^4J = 2.4 Hz, 1H, 6'-H), 9.15 (s, 1H, 3'-OH, D_2O exch.), 9.92 (s, 1H, 4'-OH, D_2O exch.), 12.62 (s, 1H, 5-OH, D_2O exch.). FAB-MS m/z $[M + H]^+$ ion = 963. Anal. calcd for $C_{43}H_{46}O_{25}$: C, 53.46; H, 4.82. Found: C, 53.72; H, 5.03.

Synthesis and Characterization of 2-(2,2-Diphenylbenzo[*d*]-[1,3]dioxol-5-yl)-5-hydroxy-3,7-di-(β-D-galactosyl)-4*H*-chromen-4-one (14). Compound **14** (0.55 g, 69%) was prepared from **12** (1.13 g, 1 mmol) using a similar procedure to that for **9**. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 3.80−3.82 (m, 1H, 5''-H), 3.85−3.87 (m, 2H, 3''-H, 4''-H), 4.09−4.12 (m, 1H, 5'''-H), 4.20−4.24 (m, 2H, 3'''-H, 4'''-H), 4.40, 4.51, 4.85, 4.88, 5.01, and 5.21 (each d, each 1H, each J = 5.4 Hz, 2''-OH, 3''-OH, 4''-OH, 2'''-OH, 3'''-OH, and 4'''-OH, D_2O exch.), 4.38 and 4.61 (each dd, each J = 5.4 Hz, J = 6.0 Hz, 6''-OH, 6'''-OH, D_2O exch.), 5.07 (dd, $J_{5'',6''Hb}$ = 3.6 Hz, J_{gem} = 10.8 Hz, 1H, 6''-H_b), 5.09 (dd, $J_{5''',6'''Hb}$ = 3.6 Hz, J_{gem} = 12.0 Hz, 1H, 6'''-H_b), 5.31 (d, $J_{5'',6''Ha}$ = 3.6 Hz, 1H, 6''-H_a), 5.38 (dd, $J_{1'',2''}$ = 7.8 Hz, $J_{2'',3''}$ = 7.2 Hz, 1H, 2''-H), 5.46 (d, $J_{5''',6'''Ha}$ = 3.6 Hz, 1H, 6'''-H_a), 5.49 (dd, $J_{1''',2'''}$ = 8.4 Hz, $J_{2''',3'''}$ = 7.8 Hz, 1H, 2'''-H), 5.51 (d, $J_{1'',2''}$ = 7.8 Hz, 1H, 1''-H), 5.53 (d, $J_{1''',2'''}$ = 7.8 Hz, 1H, 1'''-H), 6.40 (d, ^4J = 1.8 Hz, 1H, 8-H), 6.47 (d, ^4J = 2.4 Hz, 1H, 6-H), 6.99 (d, J = 8.1 Hz, 1H, 5'-H), 7.26−7.43 (m, 5H, PhH), 7.56−7.60 (m, 5H, PhH), 7.68 (dd, J = 8.4 Hz, ^4J = 2.4 Hz, 1H, 6'-H), 7.77 (d, ^4J = 2.4 Hz, 1H, 2'-H), 12.36 (s, 1H, 5-OH, D_2O exch.). FAB-MS m/z $[M + H]^+$ ion = 791.

Synthesis and Characterization of 2-(3′,4′-Dihydroxyphenyl)-5-hydroxy-3,7-di-(β-D-galactosyl)-4*H*-chromen-4-one (15). Compound **15** (0.44 g, 70%) was prepared from **13** (0.96 g, 1 mmol) using a similar procedure to that for **9**. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 3.25−3.31 (m, 1H, 5''-H), 3.34−3.37 (m, 2H, 3''-H, 4''-H), 3.40−3.44 (m, 1H, 5'''-H), 3.45−3.50 (m, 2H, 3'''-H, 4'''-H), 3.52−3.61 (m, 3H, 2''-H, 6''-H), 3.63−3.700 (m, 3H, 2'''-H, 6'''-H), 4.42 (d, J = 4.2 Hz, 1H, 2''-OH, D_2O exch.), 4.45 (dd, J = 4.8 Hz, J = 6.0 Hz, 1H, 6''-OH, D_2O exch.), 4.55 (d, J = 4.8 Hz, 1H, 2'''-OH, D_2O exch.), 4.68 (dd, J = 5.4 Hz, J = 6.0 Hz, 1H, 6'''-OH, D_2O exch.), 4.86 and 4.91 (each d, each 1H, J = 6.0 Hz, 2H, 3''-OH, 3'''-OH, D_2O exch.), 5.02 (d, $J_{1'',2''}$ = 7.8 Hz, 1H, 1''-H), 5.12 and 5.25 (each d, each 1H, each J = 5.4 Hz, 2H, 4''-OH, 4'''−OH, D_2O exch.), 5.40 (d, $J_{1''',2'''}$ = 7.8 Hz, 1H, 1'''-H), 6.43 (d, ^4J = 1.8 Hz, 1H, 8-H), 6.73 (d, ^4J = 2.4 Hz, 1H, 6-H), 6.82 (d, J = 8.1 Hz, 1H, 5'-H), 7.53 (d, ^4J = 1.8 Hz, 1H, 2'-H), 7.67 (dd, J = 8.4 Hz, ^4J = 2.4 Hz, 1H, 6'-H), 9.14 (s, 1H, 3'-OH, D_2O exch.), 9.75 (s, 1H, 4'-OH, D_2O exch.), 12.49 (s, 1H, 5-OH, D_2O exch.). ^{13}C NMR (DMSO, 150 MHz): δ 60.67, 60.88, 68.45, 68.6, 70.57, 71.69, 73.53, 73.61, 76.26, 76.43, 94.10, 99.92, 100.97, 102.24, 106.15, 115.80, 116.78, 121.66, 122.65, 134.40, 134.79, 145.28, 149.05, 156.61, 157.47, 163.55, 178.24. FAB-MS m/z $[M + H]^+$ ion = 627. Anal. calcd for $C_{27}H_{30}O_{17}$: C, 51.76; H, 4.83. Found: C, 52.07; H, 4.94.

Synthesis and Characterization of 5,7-Dibenzyloxy-2-(2,2-diphenylbenzo[*d*][1,3]dioxol-5-yl)-3-β-D-tetraacetylgalactosyl-4*H*-chromen-4-one (16). To a mixture of **8** (0.80 g, 1 mmol) and anhydrous K_2CO_3 (3 mmol) in anhydrous DMF (10 mL), benzyl bromide (3.0 mmol) was added at room temperature, and the mixture was stirred at room temperature for 7 h. After the reaction was completed, cold water was added to the reaction mixture. The deposited solid mass was collected by filtration, washed with water, and dried. The resulting crude product was recrystallized from the mixture of EtOAc and *n*-hexane to afford **16** (0.85 g, 87%) as a colorless powder. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.90, 2.00, 2.15, and 2.19 (each s, each 3H, 4 × CH_3CO), 3.84−3.90 (m, 1H, 5''-H), 4.09−4.18 (m, 2H, 6''-H), 5.09 (dd, 3H, $J_{2'',3''}$ = 8.4 Hz, $J_{3'',4''}$ = 6.6 Hz, 1H, 3''-H), 5.10 and 5.27 (each s, each 2H, 2 × CH_2Ph), 5.33−5.51 (m, 2H, 2''-H, 4''-H), 5.72 (d, $J_{1'',2''}$ = 7.8 Hz, 1H, 1''-H), 6.41 (d, ^4J = 2.4 Hz, 1H, 8-H), 6.62 (d, ^4J = 2.4 Hz, 1H, 6-H), 6.97 (d, J = 8.4 Hz, 1H, 5'-H), 7.37−7.43 (m, 14H, PhH), 7.56−7.61 (m, 6H, PhH), 7.68 (d, ^4J = 2.4 Hz, 1H, 2'-H), 7.70 (dd, J = 8.4 Hz, ^4J = 2.4 Hz, 1H, 6'-H). FAB-MS m/z $[M + H]^+$ ion = 977.

Synthesis and Characterization of 5,7-Dibenzyloxy-2-(2,2-diphenylbenzo[*d*][1,3]dioxol-5-yl)-3-β-D-galactosyl-4*H*-chromen-4-one (17). Compound **17** (0.63 g, 78%) was prepared from **16** (1.0 g, 1.20 mmol) using a similar procedure to that for **9**. ^{1}H NMR (DMSO, 300 MHz): δ 3.31−3.66 (m, 5H, 3''-H, 4''-H, 5''-H, 6''-H), 3.66, 4.45, 6.71, and 6.98 (each br s, 4H, 2''-OH, 3''-OH, 4''-OH, 6''-OH, each D_2O exch.), 5.24 and 5.27 (each s, each 2H, 2 × CH_2Ph), 5.26−5.30 (m, 2H, 2''-H, 1''-H), 7.13 (d, ^4J = 2.1 Hz, 1H, 8-H), 7.16 (d, ^4J = 2.1 Hz, 1H, 6-H), 7.30 (d, J = 8.1 Hz, 1H, 5'-H), 7.32−7.48 (m, 14H, PhH), 7.55−7.61 (m, 6H, PhH), 7.85 (dd, J = 8.4 Hz, ^4J = 2.4 Hz, 1H, 6'-H), 7.92 (d, ^4J = 2.4 Hz, 1H, 2'-H). FAB-MS m/z $[M + H]^+$ ion = 809. Anal. calcd for $C_{48}H_{40}O_{12}$: C, 71.28; H, 4.98. Found: C, 71.56; H, 4.84.

Synthesis and Characterizations of 5,7-Dibenzyloxy-2-(2,2-diphenylbenzo[*d*][1,3]dioxol-5-yl)-3-β-D-(3′′,4′′-*O*-isopropylidene)galactosyl-4*H*-chromen-4-one (18). A mixture of **17** (0.81 g, 1 mmol), anhydrous acetone (50 mL), and anhydrous copper sulfate (2.0 g) was taken in a 300 mL flask, and to it was added concentrated H_2SO_4 (1 drop). Then, the flask was sealed and kept at room temperature

for 12 h. The reaction mixture was shaken several times with in this period. After the reaction was complete, copper sulfate was filtered off, and filtrate was concentrated under reduced pressure to ca. 3 mL. After the addition of *n*-hexane into the solution, solid product was deposited and was collected by filtration to give **18** (0.70 g, 82%) as colorless powdery crystals. ^{1}H NMR (DMSO-d_6, 600 MHz): δ 1.27 and 1.44 (each s, each 3H, 2 × CH_3), 3.36−3.41 (m, 1H, 5″-H), 3.39−3.72 (m, 2H, 2″-H, 3″-H), 3.73 (ddd, $J_{5'',6''Hb}$ = 5.4 Hz, $J_{6''Hb,6''OH}$ = 4.8 Hz, J_{gem} = 9.6 Hz, 1H, 6″-H_b), 4.08 (dd, $J_{3'',4''}$ = 6.6 Hz, $J_{3'',4''}$ = 5.4 Hz, 1H, 4″-H), 4.13 (dd, 1H, $J_{5'',6''Ha}$ = 5.4 Hz, J_{gem} = 9.6 Hz, 6″-Ha) 4.65 (t, J = 4.8 Hz, 1H, 6″-OH, D_2O exch.), 5.23 and 5.24 (each s, each 2H, 2 × CH_2Ph), 5.46 (d, $J_{1'',2''}$ = 7.8 Hz, 1H, 1″-H), 5.72 (d, J = 4.8 Hz, 1H, 2″-OH, D_2O exch.), 6.71 (d, ^4J = 2.4 Hz, 1H, 8-H), 6.96 (d, ^4J = 2.4 Hz, 1H, 6-H), 7.17 (d, J = 7.8 Hz, 1H, 5′-H), 7.42−7.49 (m, 14H, PhH), 7.53−7.56 (m, 6H, PhH), 7.73 (dd, J = 8.4 Hz, ^4J = 2.4 Hz, 1H, 6′-H), 7.96 (d, ^4J = 2.4 Hz, 1H, 2′-H). FAB-MS m/z $[M + H]^+$ ion = 849. Anal. calcd for $C_{51}H_{44}O_{12}$: C, 72.16; H, 5.22. Found: C, 71.89; H, 5.06.

General Procedure and Characterizations for Compounds 7a,b, 19a,b, and 21a−k. A mixture of **6** (0.47 g, 1 mmol) (for **7a,b**) or **18** (0.85 g, 1 mmol) (for **21a−k**), aliphatic or aromatic carboxylic acid (3 mmol), *N,N′*-dicyclohexylcarbidiimide (DCC, 0.62 g, 3 mmol), 4-dimethylaminopyridine (DMAP, 0.36 g, 3 mmol), except for **19a,b**, **18** (0.85 g, 1 mmol), and 4-substituted cinnamic acid (1.0 mmol), DCC (1.0 mmol), and DMAP (0.8 mmol) were added in anhydrous dichloromethane (10 mL) and were stirred under argon at −10 °C for 1 h and then at 0 °C to room temperature for 5−12 h. After the reaction was completed, white precipitate (dicyclohexylurea) was removed by filtration through a fritted Büchner funnel (G3), and the filtrate was washed twice with 50 mL portions of 0.5 M citric acid and twice with 50 mL of 0.5 M sodium bicarbonate solution. In the case of **7a,b** and **19a,b**, the filtrate was washed with water instead of 0.5 sodium bicarbonate solution. During this procedure, some additional dicyclohexylurea was precipitated, which was removed by filtration. The organic solution was dried over anhydrous sodium sulfate and evaporated under reduced pressure at 30 °C. The residue was purified by flash column chromatography using a mixture of EtOAc and *n*-hexane (1:4 or 1.25:4) as eluting solvent to give corresponding products **7a,b**, **21a−k**, and **19a,b** as colorless needles.

2-(2,2-Diphenylbenzo[d][1,3]dioxol-5-yl)-5-hydroxy-3,7-di-(4-methylcinnamoyloxy)-4H-chromen-4-one (***7a***). Yield, 0.45 g (59%). ^{1}H NMR ($CDCl_3$, 600 MHz): δ 3.49 and 3.50 (each s, each 3H, 2 × CH_3Ph), 7.66 and 7.74 (each d, each 1H, each J_{trans} = 16.2 Hz, 2 × PhCH═C*H*−), 7.76 (d, ^4J = 1.8 Hz, 1H, 8-H), 8.28 (d, ^4J = 2.4 Hz, 1H, 6-H), 8.34 (d, J = 8.4 Hz, 1H, 5′-H), 8.45−8.50 (m, 14H, PhH), 8.57−8.60 and 8.63−8.65 (each m, each 3H, PhH), 8.97 and 8.99 (each d, each 1H, each J_{trans} = 16.2 Hz, 2 × PhC*H*═CH−). FAB-MS m/z $[M + H]^+$ ion = 755.

2-(2,2-Diphenylbenzo[d][1,3]dioxol-5-yl)-5-hydroxy-3,7-di-(4-fluorocinnamoyloxy)-4H-chromen-4-one (***7b***). Yield, 0.48 g (63%). ^{1}H NMR ($CDCl_3$, 600 MHz): δ 7.55 and 7.62 (each d, each 1H, each J_{trans} = 16.2 Hz, 2 × PhCH═C*H*−), 7.67 (d, ^4J = 1.8 Hz, 1H, 8-H), 7.93 (d, ^4J = 2.4 Hz, 1H, 6-H), 7.99 (d, J = 8.4 Hz, 1H, 5′-H), 8.05−8.16 (m, 14H, PhH), 8.17−8.19 (m, 6H, PhH), 8.87 and 8.89 (each d, each 1H, each J_{trans} = 16.2 Hz, 2 × PhC*H*═CH−). FAB-MS m/z $[M + H]^+$ ion = 763.

5,7-Dibenzyloxy-2-(2,2-diphenylbenzo[d][1,3]dioxol-5-yl)-3-β-D-[6″-O-(4-methylcinnamoyl)-3″,4″-O-isopropylidene]galactosyl-4H-chromen-4-one (***19a***). Yield, 0.68 g (69%). ^{1}H NMR ($CDCl_3$, 600 MHz): δ 1.37 and 1.60 (each s, each 3H, 2 × CH_3), 2.35 (s, 3H, CH_3Ph), 3.87 (dd, $J_{2'',3''}$ = 7.2 Hz, $J_{3'',4''}$ = 5.4 Hz, 1H, 3″-H), 3.93 (ddd, $J_{4'',5''}$ = 4.8 Hz, $J_{5'',6''Ha}$ = 4.2 Hz, $J_{5'',6''Hb}$ = 3.6 Hz, 1H, 5″-H), 4.13 (dd, $J_{3'',4''}$ = 5.4 Hz, $J_{4'',5''}$ = 4.8 Hz, 1H, 4″-H), 4.22 (dd, $J_{1'',2''}$ = 7.8 Hz, $J_{2'',3''}$ = 7.2 Hz, 1H, 2″-H), 4.23 (dd, $J_{5'',6''Hb}$ = 3.6 Hz, J_{gem} = 11.4 Hz, 1H, 6″-H_b), 4.42 (dd, $J_{5'',6''Ha}$ = 4.2 Hz, J_{gem} = 11.4 Hz, 1H, 6″-H_a), 4.85 (d, $J_{1'',2''}$ = 7.8 Hz, 1H, 1″-H), 5.07 and 5.24 (each s, each 2H, 2 × CH_2Ph), 6.17 (d, 1H, J_{trans} = 15.6 Hz, PhCH═C*H*−), 6.47 (d, ^4J = 2.4 Hz, 1H, 8-H), 6.57 (d, ^4J = 2.4 Hz, 1H, 6-H), 6.96 (d, J = 8.4 Hz, 1H, 5′-H), 7.12 and 7.25 (each d, each 2H, each J = 8.4 Hz, 2‴′-H, 3‴′-H, 5‴′-H, 6‴′-H), 7.33−7.42 (m, 16H, PhH), 7.47 (d, 1H, J_{trans} = 16.2 Hz, PhC*H*═CH−), 7.56−7.58 (m, 4H, PhH), 7.80 (dd, J = 8.4 Hz, ^4J = 1.8 Hz, 1H, 6′-H), 7.81 (d, ^4J = 1.8 Hz, 1H, 2′-H). FAB-MS m/z $[M + H]^+$ ion = 993. Anal. calcd for $C_{61}H_{52}O_{13}$: C, 73.78; H, 5.28. Found: C, 73.77; H, 5.26.

5,7-Dibenzyloxy-2-(2,2-diphenylbenzo[d][1,3]dioxol-5-yl)-3-β-D-[6″-O-(4-fluorocinnamoyl)-3″,4″-O-isopropylidene]galactosyl-4H-chromen-4-one (***19b***). Yield, 0.67 g (67%). ^{1}H NMR ($CDCl_3$, 600 MHz): δ 1.37 and 1.60 (each s, each 3H, 2 × CH_3), 3.87 (dd, $J_{2'',3''}$ = 7.2 Hz, $J_{3'',4''}$ = 5.4 Hz, 1H, 3″-H), 3.93−3.95 (m, 1H, 5″-H), 4.13 (dd, $J_{3'',4''}$ = 5.4 Hz, $J_{4'',5''}$ = 2.4 Hz, 1H, 4″-H), 4.24 (dd, $J_{1'',2''}$ = 8.4 Hz, $J_{2'',3''}$ = 7.2 Hz, 1H, 2″-H), 4.28 (dd, $J_{5'',6''Hb}$ = 4.8 Hz, J_{gem} = 11.4 Hz, 1H, 6″-H_b), 4.45 (dd, $J_{5'',6''Ha}$ = 3.6 Hz, J_{gem} = 11.4 Hz, 1H, 6″-H_a), 4.86 (d, $J_{1'',2''}$ = 8.4 Hz, 1H, 1″-H), 5.06/5.09 (AB system, J_{AB} = 15.4 Hz, 2H, CH_2Ph), 5.24 (s, 2H, CH_2Ph), 6.10 (d, 1H, J_{trans} = 16.2 Hz, PhCH═C*H*−), 6.47 (d, ^4J = 2.4 Hz, 1H, 8-H), 6.54 (d, ^4J = 2.4 Hz, 1H, 6-H), 6.94 (d, J = 8.4 Hz, 1H, 5′-H), 6.95 and 6.97 (each t, each 2H, each J = 8.4 Hz, 2‴′-H, 3‴′-H, 5‴′-H, 6‴′-H), 7.34−7.41 (m, 16H, PhH), 7.42 (d, 1H, J_{trans} = 16.2 Hz, PhC*H*═CH−), 7.56−7.57 (m, 4H, PhH), 7.81 (d, ^4J = 1.8 Hz, 1H, 2′-H), 7.82 (br s, 1H, 6′-H). FAB-MS m/z $[M + H]^+$ ion = 997. Anal. calcd for $C_{60}H_{49}O_{13}F$: C, 72.28; H, 4.95. Found: C, 72.63; H, 5.13.

5,7-Dibenzyloxy-2-(2,2-diphenylbenzo[d][1,3]dioxol-5-yl)-3-β-D-[2″,6″-di-O-(4-ethoxybenzoyl)-3″,4″-O-isopropylidene]galactosyl-4H-chromen-4-one (***21a***). Yield, 0.76 g (66%). ^{1}H NMR ($CDCl_3$, 600 MHz): δ 1.32 and 1.60 (each s, each 3H, 2 × CH_3), 1.36 and 1.41 (each t, each 3H, J = 7.2 Hz, 2 × CH_3CH$_2$), 3.90 and 4.03 (each q, J = 7.2 Hz, each 2H, 2 × CH_3CH_2), 4.16−4.19 (m, 1H, 5″-H), 4.24 (dd, $J_{3'',4''}$ = 5.4 Hz, $J_{4'',5''}$ = 2.4 Hz, 1H, 4″-H), 4.38 (dd, $J_{5'',6''Hb}$ = 5.4 Hz, J_{gem} = 11.4 Hz, 1H, 6″-H_b), 4.38 (dd, $J_{2'',3''}$ = 7.2 Hz, $J_{3'',4''}$ = 5.4 Hz, 3″-H), 4.38 (dd, $J_{5'',6''Ha}$ = 5.4 Hz, J_{gem} = 11.4 Hz, 1H, 6″-H_a), 5.05 (s, 2H, CH_2Ph), 5.20/5.24 (AB system, each d, J_{AB} = 12.6 Hz, 2H, CH_2Ph), 5.45 (dd, $J_{1'',2''}$ = 7.8 Hz, $J_{2'',3''}$ = 7.2 Hz, 1H, 2″-H), 5.98 (d, $J_{1'',2''}$ = 7.8 Hz, 1H, 1″-H), 6.38 (d, ^4J = 2.4 Hz, 1H, 8-H), 6.62 (d, ^4J = 2.4 Hz, 1H, 6-H), 6.62 and 6.84 (each d, each 2H, each J = 8.4 Hz, 2 × 3‴′-H, 2 × 5‴′-H), 6.95 (d, J = 8.4 Hz, 1H, 5′-H), 7.32−7.41 (m, 14H, PhH), 7.59−7.62 (m, 7H, 2′-H, PhH), 7.71 (dd, J = 8.4 Hz, ^4J = 2.4 Hz, 1H, 6′-H), 7.74 and 8.10 (each d, each 2H, each J = 9.0 Hz, 2 × 2‴′-H, 2 × 6‴′-H). FAB-MS m/z $[M + H]^+$ ion = 1145. Anal. calcd for $C_{69}H_{60}O_{16}$: C, 72.37; H, 5.28. Found: C, 72.06; H, 5.19.

5,7-Dibenzyloxy-2-(2,2-diphenylbenzo[d][1,3]dioxol-5-yl)-3-β-D-[2″,6″-di-O-(4-propylbenzoyl)-3″,4″-O-isopropylidene]galactosyl-4H-chromen-4-one (***21b***). Yield, 0.80 (70%). ^{1}H NMR ($CDCl_3$, 600 MHz): δ 0.89−0.92 (each t, each 3H, J = 7.2 Hz, 2 × CH_3CH$_2$), 1.34 and 1.60 (each s, each 3H, 2 × CH_3), 1.55−1.58 (m, 4H, 2 × CH_3CH_2CH$_2$), 2.54 and 2.60 (each t, each 2H, J = 7.2 Hz, 2 × CH_3CH$_2$CH_2), 4.17 (ddd, $J_{4'',5''}$ = 2.4 Hz, $J_{5'',6''Ha}$ = 4.8 Hz, $J_{5'',6''Hb}$ = 5.4 Hz, 1H, 5″-H), 4.26 (dd, $J_{3'',4''}$ = 5.4 Hz, $J_{4'',5''}$ = 1.8 Hz, 1H, 4″-H), 4.39 (dd, $J_{5'',6''Hb}$ = 5.4 Hz, J_{gem} = 11.4 Hz, 1H, 6″-H_b), 4.43 (dd, $J_{2'',3''}$ = 7.2 Hz, $J_{3'',4''}$ = 5.4 Hz, 3″-H), 4.39 (dd, $J_{5'',6''Ha}$ = 4.8 Hz, J_{gem} = 11.4 Hz, 1H, 6″-H_a), 5.05 (s, 2H, CH_2Ph), 5.20/5.22 (AB system, each d, J_{AB} = 12.6 Hz, 2H, CH_2Ph), 5.47 (dd, $J_{1'',2''}$ = 7.8 Hz, $J_{2'',3''}$ = 7.2 Hz, 1H, 2″-H), 6.10 (d, $J_{1'',2''}$ = 7.8 Hz, 1H, 1″-H), 6.39 (d, ^4J = 2.4 Hz, 1H, 8-H), 6.52 (d, ^4J = 2.4 Hz, 1H, 6-H), 6.94 (d, J = 8.4 Hz, 1H, 5′-H), 7.00 and 7.19 (each d, each 2H, each J = 8.4 Hz, 2 × 3‴′-H, 2 × 5‴′-H), 7.33−7.41 (m, 14H, PhH), 7.59−7.62 (m, 7H, 2′-H, PhH), 7.71 (dd, J = 8.4 Hz, ^4J = 2.4 Hz, 1H, 6′-H), 7.74 and 8.08 (each d, each 2H, each J = 8.4 Hz, 2 × 2‴′-H, 2 × 6‴′-H). FAB-MS m/z $[M + H]^+$ ion =1141. Anal. calcd for $C_{71}H_{64}O_{14}$: C, 74.72; H, 5.65. Found: C, 74.72; H, 5.35.

5,7-Dibenzyloxy-2-(2,2-diphenylbenzo[d][1,3]dioxol-5-yl)-3-β-D-[2″,6″-di-O-(4-fluorobenzoyl)-3″,4″-O-isopropylidene]galactosyl-4H-chromen-4-one (***21c***). Yield, 0.78 g (71%). ^{1}H NMR ($CDCl_3$, 600 MHz): δ 1.35 and 1.57 (each s, each 3H, 2 × CH_3), 4.17 (ddd, $J_{4'',5''}$ = 2.4 Hz, $J_{5'',6''Ha}$ = 5.4 Hz, $J_{5'',6''Hb}$ = 4.8 Hz, 1H, 5″-H), 4.25 (dd, $J_{3'',4''}$ = 5.4 Hz, $J_{4'',5''}$ = 2.4 Hz, 1H, 4″-H), 4.43 (dd, $J_{2'',3''}$ = 7.2 Hz, $J_{3'',4''}$ = 5.4 Hz, 3″-H), 4.45−4.47 (m, 2H, 6″-H), 5.06 and 5.21 (each s, each 2H, 2 × CH_2Ph), 5.44 (dd, $J_{1'',2''}$ = 7.8 Hz, $J_{2'',3''}$ = 7.2 Hz, 1H, 2″-H), 5.91

(d, $J_{1'',2''}$ = 7.8 Hz, 1H, 1''-H), 6.42 (d, ^4J = 2.4 Hz, 1H, 8-H), 6.49 (d, ^4J = 2.4 Hz, 1H, 6-H), 6.83 and 7.04 (each t, each 2H, J = 9.0 Hz, 2 × 3'''-H, 2 × 5'''-H), 6.94 (d, J = 8.4 Hz, 1H, 5'-H), 7.35−7.43 (m, 14H, PhH), 7.58−7.62 (m, 7H, 2'-H, PhH), 7.67 (dd, J = 8.4 Hz, ^4J = 2.4 Hz, 1H, 6'-H), 7.78 and 8.19 (each dd, each 2H, J = 8.4 Hz, J = 9.0 Hz, 2 × 2'''-H, 2 × 6'''-H). FAB-MS m/z $[M + H]^+$ ion =1093. Anal. calcd for $C_{65}H_{50}O_{14}F_2$: C, 71.42; H, 4.61. Found: C, 71.71; H, 4.56.

*5,7-Dibenzyloxy-2-(2,2-diphenylbenzo[d][1,3]dioxol-5-yl)3-β-D-[2'',6''-di-O-(4-methylphenylacetyl)-3'',4''-O-isopropylidene]galactosyl-4H-chromen-4-one (**21d**).* Yield, 0.80 g (72%). ^{1}H NMR ($CDCl_3$, 600 MHz): δ 1.25 and 1.48 (each s, each 3H, 2 × CH_3), 2.23 and 2.26 (each s, each 3H, 2 × CH_3Ph), 3.38/3.41 (AB system, each d, J_{AB} = 15.6 Hz, 2H, $PhCH_2CO$), 3.72/3.79 (AB system, each d, J_{AB} = 16.2 Hz, 2H, $PhCH_2CO$), 3.85 (ddd, $J_{4'',5''}$ = 2.4 Hz, $J_{5'',6''Ha}$ = 5.4 Hz, $J_{5'',6''Hb}$ = 4.8 Hz, 1H, 5''-H), 3.99 (dd, $J_{3'',4''}$ = 5.4 Hz, $J_{4'',5''}$ = 2.4 Hz, 1H, 4''-H), 4.09 (dd, $J_{5'',6''Hb}$ = 4.8 Hz, J_{gem} = 11.4 Hz, 1H, 6''-H_b), 4.14 (dd, $J_{5'',6''Ha}$ = 5.4 Hz, J_{gem} = 11.4 Hz, 1H, 6''-H_a), 4.23 (dd, $J_{2'',3''}$ = 7.2 Hz, $J_{3'',4''}$ = 5.4 Hz, 1H, 3''-H), 5.06 and 5.27 (each s, each 2H, 2 × CH_2Ph), 5.20 (dd, $J_{1'',2''}$ = 7.8 Hz, $J_{2'',3''}$ = 6.6 Hz, 1H, 2''-H), 5.66 (d, $J_{1'',2''}$ = 7.8 Hz, 1H, 1''-H), 6.44 (d, ^4J = 2.4 Hz, 1H, 8-H), 6.57 (d, ^4J = 2.4 Hz, 1H, 6-H), 6.90 (d, J = 8.4 Hz, 1H, 5'-H), 6.97−7.00 (m, 4H, 2 × 3''''-H, 2 × 5''''-H), 7.05 and 7.20 (each d, J = 8.4, each 2H, 2 × 2''''-H, 2 × 6''''-H), 7.34−7.40 (m, 14H, PhH), 7.57−7.59 (m, 6H, PhH),, 7.64 (d, ^4J = 2.4 Hz, 1H, 2'-H), 7.67 (dd, J = 8.4 Hz, ^4J = 1.8 Hz, 1H, 6'-H). FAB-MS m/z $[M + H]^+$ ion = 1113. Anal. calcd for $C_{69}H_{60}O_{14}$: C, 74.45; H, 5.43. Found: C, 74.61; H, 5.82.

*5,7-Dibenzyloxy-2-(2,2-diphenylbenzo[d][1,3]dioxol-5-yl)3-β-D-[2'',6''-di-O-(4-fluorophenylacetyl)-3'',4''-O-isopropylidene]galactosyl-4H-chromen-4-one (**21e**).* Yield, 0.78 g (70%). ^{1}H NMR ($CDCl_3$, 600 MHz): δ 1.27 and 1.51 (each s, each 3H, 2 × CH_3), 3.37/3.40 (AB system, each d, J_{AB} = 15.6 Hz, 2H, $PhCH_2CO$), 3.73/3.81 (AB system, each d, J_{AB} = 16.2 Hz, 2H, $PhCH_2CO$), 3.88 (ddd, $J_{4'',5''}$ = 2.4 Hz, $J_{5'',6''Ha}$ = 5.4 Hz, $J_{5'',6''Hb}$ = 4.8 Hz, 1H, 5''-H), 4.03 (dd, $J_{3'',4''}$ = 5.4 Hz, $J_{4'',5''}$ = 2.4 Hz, 1H, 4''-H), 4.11 (dd, $J_{5'',6''Hb}$ = 4.8 Hz, J_{gem} = 11.4 Hz, 1H, 6''-H_b), 4.17 (dd, $J_{5'',6''Ha}$ = 5.4 Hz, J_{gem} = 11.4 Hz, 1H, 6''-H_a), 4.24 (dd, $J_{2'',3''}$ = 7.2 Hz, $J_{3'',4''}$ = 5.4 Hz, 1H, 3''-H), 5.08 and 5.26 (each s, each 2H, 2 × CH_2Ph), 5.19 (dd, $J_{1'',2''}$ = 7.8 Hz, $J_{2'',3''}$ = 7.2 Hz, 1H, 3''-H), 5.64 (d, $J_{1'',2''}$ = 7.8 Hz, 1H, 1''-H), 6.46 (d, ^4J = 2.4 Hz, 1H, 8-H), 6.58 (d, ^4J = 2.4 Hz, 1H, 6-H), 6.83 and 6.91 (each t, each J = 9.0 Hz, 4H, 2 × 3''''-H, 2 × 5''''-H), 6.90 (d, J = 8.4 Hz, 1H, 5'-H), 7.03 and 7.27 (each dd, J = 8.4, J = 9.0 Hz, each 2H, 2 × 2''''-H, 2 × 6''''-H), 7.35−7.41 (m, 14H, PhH), 7.56−7.59 (m, 6H, PhH), 7.63 (d, ^4J = 2.4 Hz, 1H, 2'-H), 7.67 (dd, J = 8.4 Hz, ^4J = 1.8 Hz, 1H, 6'-H). FAB-MS m/z $[M + H]^+$ ion = 1121. Anal. calcd for $C_{67}H_{54}O_{14}F_2$: C, 71.78; H, 4.85. Found: C, 71.59; H, 4.96.

*5,7-Dibenzyloxy-2-(2,2-diphenylbenzo[d][1,3]dioxol-5-yl)-3-β-D-[2'',6''-di-O-(4-benzyloxycinnamoyl)-3'',4''-O-isopropylidene]galactosyl-4H-chromen-4-one (**21f**).* Yield, 0.81 g (61%). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 1.33 and 1.55 (each s, each 3H, 2 × CH_3), 4.09 (ddd, $J_{4'',5''}$ = 2.4 Hz, $J_{5'',6''Ha}$ = 5.4 Hz, $J_{5'',6''Hb}$ = 4.8 Hz, 5''-H), 4.21 (dd, $J_{3'',4''}$ = 5.4 Hz, $J_{4'',5''}$ = 2.4 Hz, 1H, 4''-H), 4.29−4.34 (m. 2H, 6''-H), 4.36 (dd, $J_{2'',3''}$ = 7.2 Hz, $J_{3'',4''}$ = 5.4 Hz, 1H, 3''-H), 4.94/4.97 (AB system, each d, J_{AB} = 11.4 Hz, 2H, CH_2Ph), 5.00, 5.07, and 5.18 (each s, each 2H, 3 × CH_2Ph), 5.34 (dd, $J_{1'',2''}$ = 7.8 Hz, $J_{2'',3''}$ = 7.2 Hz, 1H, 2''-H), 5.89 (d, $J_{1'',2''}$ = 7.8 Hz, 1H, 1''-H), 6.11 and 6.38 (each d, each 1H, each J_{trans} = 16.2 Hz, 2 × PhCH=C*H*-), 6.31 (d, ^4J = 1.8 Hz, 1H, 8-H), 6.46 (d, ^4J = 1.8 Hz, 1H, 6-H), 6.88 and 6.92 (each d, each 2H, each J = 8.4 Hz, 2 × 3''''-H, 2 × 5''''-H), 6.98 (d, J = 9.0 Hz, 1H, 5'-H), 7.31−7.46 (m, 24H, PhH), 7.60−7.63 (m, 8H, 2 × 2''''-H, 2 × 6''''-H, PhH), 7.65 and 7.71 (each d, each 1H, each J_{trans} = 16.2 Hz, 2 × PhC*H*=CH−), 7.67 (d, ^4J = 1.8 Hz, 1H, 2'-H), 7.74 (dd, J = 8.4 Hz, ^4J = 1.8 Hz, 1H, 6'-H). FAB-MS m/z $[M + H]^+$ ion = 1321. Anal. calcd for $C_{83}H_{68}O_{16}$: C, 75.44; H, 5.19. Found: C, 75.78; H, 5.12.

*5,7-Dibenzyloxy-2-(2,2-diphenylbenzo[d][1,3]dioxol-5-yl)-3-β-D-[2'',6''-di-O-(4-ethoxycinnamoyl)-3'',4''-O-isopropylidene]galactosyl-4H-chromen-4-one (**21g**).* Yield, 0.81 g (68%). ^{1}H NMR ($CDCl_3$, 600 MHz): δ 1.32 and 1.55 (each s, each 3H, 2 × CH_3), 1.36 and 1.41 (each t, each 3H, J = 7.2 Hz, 2 × CH_3CH_2), 3.95 and 4.04 (each q, each 2H, J = 7.2 Hz, 2 × CH_3CH_2), 4.08 (ddd, $J_{4'',5''}$ = 2.4 Hz, $J_{5'',6''Ha}$ = 5.4 Hz, $J_{5'',6''Hb}$ = 4.8 Hz, 1H, 5''-H), 4.22 (dd, $J_{3'',4''}$ = 5.4 Hz, $J_{4'',5''}$ = 2.4 Hz, 4''-H), 4.30−4.34 (m, 2H, 6''-H), 4.37 (dd, $J_{2'',3''}$ = 7.2 Hz, $J_{3'',4''}$ = 5.4 Hz, 1H, 3''-H), 4.92/5.00 (AB system, each d, J_{AB} = 11.4 Hz, 2H, CH_2Ph), 5.20 (s, 2H, CH_2Ph), 5.35 (dd, $J_{1'',2''}$ = 7.8 Hz, $J_{2'',3''}$ = 7.2 Hz, 1H, 2''-H), 5.90 (d, $J_{1'',2''}$ = 7.8 Hz, 1H, 1''-H), 6.10 and 6.37 (each d, each 1H, each J_{trans} = 16.2 Hz, 2 × PhCH=C*H*−), 6.32 (d, ^4J = 2.4 Hz, 1H, 8-H), 6.44 (d, ^4J = 2.4 Hz, 1H, 6-H), 6.78 and 6.83 (each d, each 2H, each J = 9.0 Hz, 2 × 3''''-H, 2 × 5''''-H), 6.96 (d, J = 8.4 Hz, 1H, 5'-H), 7.32−7.43 (m, 18H, PhH), 7.60−7.63 (m, 6H, PhH, 2 × 2''''-H, 2 × 6''''-H), 7.64 and 7.71 (each d, each 1H, each J_{trans} = 16.2 Hz, 2 × PhC*H*=CH−), 7.67 (d, ^4J = 1.8 Hz, 1H, 2'-H), 7.74 (dd, J = 8.4 Hz, ^4J = 1.8 Hz, 1H, 6'-H). FAB-MS m/z $[M + H]^+$ ion =1197. Anal. calcd for $C_{73}H_{64}O_{16}$: C, 73.23; H, 5.39. Found: C, 73.19; H, 5.35.

*5,7-Dibenzyloxy-2-(2,2-diphenylbenzo[d][1,3]dioxol-5-yl)-3-β-D-[2'',6''-di-O-(4-methylcinnamoyl)-3'',4''-O-isopropylidene]galactosyl-4H-chromen-4-one (**21h**).* Yield, 0.86 g (76%). ^{1}H NMR ($CDCl_3$, 600 MHz): δ 1.34 and 1.56 (each s, each 3H, 2 × CH_3), 2.31 and 2.35 (each s, each 3H, 2 × CH_3Ph), 4.08 (ddd, $J_{4'',5''}$ = 2.4 Hz, $J_{5'',6''Ha}$ = 4.8 Hz, $J_{5'',6''Hb}$ = 5.4 Hz, 1H, 5''-H), 4.22 (dd, $J_{3'',4''}$ = 5.4 Hz, $J_{4'',5''}$ = 2.4 Hz, 1H, 4''-H), 4.29−4.36 (m, 2H, 6''-H), 4.37 (dd, $J_{2'',3''}$ = 7.2 Hz, $J_{3'',4''}$ = 5.4 Hz, 1H, 3''-H), 4.94/4.98 (AB system, each d, J_{AB} = 11.4 Hz, 2H, CH_2Ph), 5.19/5.21 (AB system, each d, J_{AB} = 11.4 Hz, 2H, CH_2Ph), 5.35 (dd, $J_{1'',2''}$ = 7.8 Hz, $J_{2'',3''}$ = 7.2 Hz, 1H, 2''-H), 5.89 (d, $J_{1'',2''}$ = 7.8 Hz, 1H, 1''-H), 6.21 and 6.46 (each d, each 1H, each J_{trans} = 16.2 Hz, 2 × PhCH=C*H*−), 6.33 (d, ^4J = 2.4 Hz, 1H, 8-H), 6.47 (d, ^4J = 2.4 Hz, 1H, 6-H), 6.96 (d, J = 8.4 Hz, 1H, 5'-H), 7.11 and 7.14 (each d, each 2H, each J = 7.8 Hz, 2 × 2''''-H, 2 × 6''''-H), 7.26−7.43 (m, 18H, PhH), 7.60−7.63 (m, 6H, PhH, 2 × 3''''-H, 2 × 5''''-H), 7.66 and 7.73 (each d, each 1H, each J_{trans} = 16.2 Hz, 2 × PhC*H*=CH−), 7.68 (d, ^4J = 1.8 Hz, 1H, 2'-H), 7.74 (dd, J = 8.4 Hz, ^4J = 1.8 Hz, 1H, 6'-H). FAB-MS m/z $[M + H]^+$ ion = 1137. Anal. calcd for $C_{71}H_{60}O_{14}$: C, 74.99; H, 5.32. Found: C, 74.77; H, 5.38.

*5,7-Dibenzyloxy-2-(2,2-diphenylbenzo[d][1,3]dioxol-5-yl-3-β-D-[2'',6''-di-O-(4-fluorocinnamoyl)-3'',4''-O-isopropylidene]galactosyl-4H-chromen-4-one (**21i**).* Yield, 0.82 g (72%). ^{1}H NMR ($CDCl_3$, 600 MHz): δ 1.35 and 1.58 (each s, each 3H, 2 × CH_3), 4.09 (ddd, $J_{4'',5''}$ = 6.0 Hz, $J_{5'',6''Ha}$ = 2.4 Hz, $J_{5'',6''Hb}$ = 1.8 Hz, 1H, 5''-H), 3.58 (dd, $J_{3'',4''}$ = 5.4 Hz, $J_{4'',5''}$ = 2.4 Hz, 1H, 4''-H), 4.35 (br d, J_{gem} = 11.4 Hz, 2H, 6''-H), 4.37 (dd, $J_{2'',3''}$ = 7.2 Hz, $J_{3'',4''}$ = 5.4 Hz, 1H, 3''-H), 4.95/4.98 (AB system, each d, J_{AB} = 12.0 Hz, 2H, CH_2Ph), 5.20 (s, 2H, CH_2Ph), 5.35 (dd, 2H, $J_{1'',2''}$ = 7.8 Hz, $J_{2'',3''}$ = 7.2 Hz, 2''-H), 5.88 (d, $J_{1'',2''}$ = 7.2 Hz, 1H, 1''-H), 6.16 and 6.43 (each d, each 1H, each J_{trans} = 16.2 Hz, 2 × PhCH=C*H*−), 6.34 (d, ^4J = 2.4 Hz, 1H, 8-H), 6.45 (d, ^4J = 2.4 Hz, 1H, 6-H), 6.92 (d, J = 8.4 Hz, 1H, 5'-H), 6.98 and 7.01 (each t, each 2H, J = 8.4 Hz, 2 × 3''''-H, 2 × 5''''-H), 7.32−7.47 (m, 18H, PhH), 7.59−7.62 (m, 6H, 2 × 2''''-H, 2 × 6''''-H, PhH), 7.65 and 7.72 (each d, each 1H, each J_{trans} = 16.2 Hz, 2 × PhC*H*=CH−), 7.66 (d, ^4J = 1.8 Hz, 1H, 2'-H), 7.73 (dd, J = 8.4 Hz, ^4J = 1.8 Hz, 1H, 6'-H). FAB-MS m/z $[M + H]^+$ ion = 1145. Anal. calcd for $C_{69}H_{54}O_{14}F_2$: C, 72.37; H, 4.75. Found: C, 72.05; H, 4.59.

*5,7-Dibenzyloxy-2-(2,2-diphenylbenzo[d][1,3]dioxol-5-yl-3-β-D-{2'',6''-di-O-[4'''-(4-fluorophenylbutanoyl)]-3'',4''-O-isopropylidene}galactosyl-4H-chromen-4-one (**21j**).* Yield, 0.87 g (74%). ^{1}H NMR ($CDCl_3$, 600 MHz): δ 1.32 and 1.55 (each s, each 3H, 2 × CH_3), 1.61 and 2.10 (each quintet, 4H, 2 × $PhCH_2CH_2CH_2CH_2$), 2.38 and 2.40 (each t, each 2H, J = 7.2 Hz, 2 × CH_2CH_2CO), 2.48 and 2.51 (each t, each 2H, J = 7.2 Hz, 2 × $PhCH_2CH_2$), 3.95 (ddd, $J_{4'',5''}$ = 2.4 Hz, $J_{5'',6''Ha}$ = 5.4 Hz, $J_{5'',6''Hb}$ = 4.8 Hz, 1H, 5''-H), 4.13 (dd, $J_{3'',4''}$ = 5.4 Hz, $J_{4'',5''}$ = 2.4 Hz, 1H, 4''-H), 4.15−4.18 (m, 2H, 6''-H), 4.26 (dd, $J_{2'',3''}$ = 7.2 Hz, $J_{3'',4''}$ = 5.4 Hz, 1H, 3''-H), 5.01/5.10 (AB system, each d, J_{AB} = 11.4 Hz, 2H, CH_2Ph), 5.21−5.25 (m, 3H, 2''-H, CH_2Ph), 5.75 (d, $J_{1'',2''}$ = 7.8 Hz, 1H, 1''-H), 6.41 (d, ^4J = 2.4 Hz, 1H, 8-H), 6.49 (d, ^4J = 2.4 Hz, 1H, 6-H), 6.85 and 6.94 (each t, each 2H, J = 9.0 Hz, 2 × 3''''-H, 2 × 5''''-H), 6.95 (d, J = 8.4 Hz, 1H, 5'-H), 7.06 and 7.08 (each d, J = 8.4, each 2H, 2 × 2''''-H,

2 × 6‴′-H), 7.28−7.41 (m, 14H, PhH), 7.59−7.62 (m, 6H, PhH), 7.65 (d, ^4J = 2.4 Hz, 1H, 2′-H), 7.71 (dd, J = 8.4 Hz, ^4J = 1.8 Hz, 1H, 6′-H). FAB-MS m/z $[M + H]^+$ ion = 1177. Anal. calcd for $C_{71}H_{62}O_{14}F_2$: C, 72.44; H, 5.31. Found: C, 72.19; H, 5.35.

*5,7-Dibenzyloxy-2-(2,2-diphenylbenzo[d][1,3]dioxol-5-yl-3-β-D-{2″, 6″-di-O-[5‴-(4-fluorophenylpentanoyl)]-3″,4″-O-isopropylidene]galactosyl}-4H-chromen-4-one (**21k**).* Yield, 0.84 g (70%). ^{1}H NMR ($CDCl_3$, 600 MHz): δ 1.31 and 1.55 (each s, each 3H, 2 × CH_3), 1.55−1.63 (m, 4H, 2 × $PhCH_2CH_2CH_2$), 1.70 and 2.14 (each quintet, each 2H, J = 7.2 Hz, 2 × $CH_2CH_2CH_2CH_2$), 2.41 and 2.45 (each t, each 2H, J = 7.2 Hz, 2 × CH_2CH_2CO), 2.49 and 2.52 (each t, each 2H, J = 7.2 Hz, 2 × $PHCH_2CH_2$), 3.92 (ddd, $J_{4'',5''}$ = 2.4 Hz, $J_{5'',6''Ha}$ = 5.4 Hz, $J_{5'',6''Hb}$ = 4.8 Hz, 1H, 5″-H), 4.11 (dd, $J_{3'',4''}$ = 5.4 Hz, $J_{4'',5''}$ = 2.4 Hz, 1H, 4″-H), 4.13−4.19 (m, 2H, 6″-H), 4.26 (dd, $J_{2'',3''}$ = 7.2 Hz, $J_{3'',4''}$ = 5.4 Hz, 1H, 3″-H), 4.99/5.04 (AB system, each d, J_{AB} = 11.4 Hz, 2H, CH_2Ph), 5.22 (dd, $J_{1'',2''}$ = 7.8 Hz, $J_{2'',3''}$ = 7.2 Hz, 1H, 2″-H), 5.24 (s, 2H, CH_2Ph), 5.69 (d, $J_{1'',2''}$ = 7.8 Hz, 1H, 1″-H), 6.41 (d, ^4J = 2.4 Hz, 1H, 8-H), 6.52 (d, ^4J = 2.4 Hz, 1H, 6-H), 6.79 and 6.89 (each t, each 2H, J = 9.0 Hz, 2 × 3‴′-H, 2 × 5‴′-H), 6.95 (d, J = 8.4 Hz, 1H, 5′-H), 6.98 and 7.00 (each d, J = 8.4, each 2H, 2 × 2‴′-H, 2 × 6‴′-H), 7.35−7.41 (m, 14H, PhH), 7.59−7.61 (m, 6H, PhH), 7.65 (d, ^4J = 2.4 Hz, 1H, 2′-H), 7.71 (dd, J = 8.4 Hz, ^4J = 1.8 Hz, 1H, 6′-H). FAB-MS m/z $[M + H]^+$ ion = 1205. Anal. calcd for $C_{73}H_{66}O_{14}F_2$: C, 72.74; H, 5.52. Found: C, 72.58; H, 5.28.

General Procedure and Characterizations for Compounds 20a,b and 22a−k. A solution one of **19a,b** and **22a−k** (1.0 mmol) in a mixture of MeOH (25 mL) was heated at 40 °C for 4−5 h under HCl gas. After it was cooled to room temperature, the solution was neutralized with triethylamine and evaporated in vacuo. Finally, coevaporation of the solution with EtOH to complete dryness was achieved. The dry residue was treated with absolute ethanol, and the insoluble material was filtered off. The filtrate was evaporated in vacuo, and the residue was purified by flash column chromatography using a mixture of EtOAc and *n*-hexane (1:1) as the eluting solvent to give the corresponding products **20a,b** and **22a−k** as a colorless powder. Characterizations for compounds **20a,b** and **22a−k** are in the Supporting Information.

General Procedure and Characterizations for Compounds 3a,b, 4a,b, and 5a−k. To a solution of one of **7a,b**, **20a,b**, and **22a−k** (1.00 mmol) in EtOAc−EtOH (1.5:1, 40 mL) 10% Pd/C (1.5 equiv) was added and vigorously stirred at 0 °C to room temperature for 8−11 h under hydrogen pressure (balloon). After the Pd/C was removed by filtration, the filtrate was concentrated under reduced pressure at 30 °C, and the residue was purified by flash column chromatography using a mixture of EtOAc and *n*-hexane (1:0.25, 1:0.5, or 1:1) as the eluting solvent to give the corresponding products **3a,b**, **4a,b**, and **5a−k** as pale yellow powders

*2-(3′,4′-Dihydroxyphenyl)-5-hydroxy-3,7-di-[3″-(4-methylphenylpropanoyloxy)]-4H-chromen-4-one (**3a**).* Yield, 0.41 g (69%). ^{1}H NMR (DMSO-d_6, 600 MHz): δ 2.61 and 2.66 (each t, each 2H, J = 7.2 Hz, 2 × $PhCH_2CH_2CO$), 3.08 and 3.13 (each t, each 2H, J = 7.8 Hz, 2 × $PhCH_2CH_2CO$), 3.37 and 3.39 (each s, each 3H, 2 × CH_3Ph), 6.45 (d, ^4J = 1.8 Hz, 1H, 8-H), 6.61 (d, ^4J = 2.4 Hz, 1H, 6-H), 6.73 (d, J = 8.4 Hz, 1H, 5′-H), 7.01−7.22 (m, 8H, PhH), 7.93 (dd, J = 8.4 Hz, ^4J = 2.4 Hz, 1H, 6′-H), 7.98 (d, ^4J = 2.4 Hz, 1H, 2′-H), 9.59 (s, 1H, 3′-OH, D_2O exch.), 10.99 (s, 1H, 4′-OH, D_2O exch.), 12.34 (s, 1H, 5-OH, D_2O exch.). ^{13}C NMR (DMSO-d_6, 150 MHz): δ 22.02, 22.15, 31.71, 31.82, 37.34, 37.82, 99.98, 101.72, 103.21, 115.73, 117.23, 121.89, 123.61, 129.73, 129.98, 131.26, 131.79, 134.40, 134.63, 134.99, 135.01, 136.12, 136.44, 146.28, 148.01, 155.06, 158.77, 163.99, 169.13, 170.01, 177.12. FAB-MS m/z $[M + H]^+$ ion = 595. Anal. calcd for $C_{35}H_{30}O_9$: C, 70.70; H, 5.09. Found: C, 70.66; H, 4.98.

*2-(3′,4′-Dihydroxyphenyl)-5-hydroxy-3,7-di-[3″-(4-fluorophenylpropanoyloxy)-4H-chromen-4-one (**3b**).* Yield, 0.40 g (66%). ^{1}H NMR (DMSO-d_6, 600 MHz): δ 2.60 and 2.66 (each t, each 2H, J = 7.2 Hz, 2 × $PhCH_2CH_2CO$), 3.09 and 3.12 (each t, each 2H, J = 7.8 Hz, 2 × $PhCH_2CH_2CO$), 6.46 (d, ^4J = 1.8 Hz, 1H, 8-H), 6.61 (d, ^4J = 2.4 Hz, 1H, 6-H), 6.72 (d, J = 8.4 Hz, 1H, 5′-H), 7.00−7.22 (m, 8H, PhH), 7.92 (dd, J = 8.4 Hz, ^4J = 2.4 Hz, 1H, 6′-H), 7.99 (d, ^4J = 2.4 Hz, 1H, 2′-H), 9.57 (s, 1H, 3′-OH, D_2O exch.), 10.99 (s, 1H, 4′-OH, D_2O exch.), 12.33 (s, 1H, 5-OH, D_2O exch.). ^{13}C NMR (DMSO-d_6, 150 MHz): δ 33.11, 33.29, 36.07, 36.19, 100.01, 100.97, 103.04, 116.44, 116.86, 117.01, 117.88, 121.03, 121.27, 131.05, 131.18, 134.77, 135.99, 137.44, 137.79, 146.38, 149.83, 156.11, 157.97, 162.93, 163.01, 164.45, 168.55, 168.99, 178.44. FAB-MS m/z $[M + H]^+$ ion = 603. Anal. calcd for $C_{33}H_{24}F_2O_9$: C, 65.78; H, 4.01. Found: C, 68.90; H, 4.38.

*2-(3′,4′-Dihydroxyphenyl)-5,7-dihydroxy-3-β-D-{6″-O-[3‴-(4-methylphenylpropanoyl)]}galactosyl-4H-chromen-4-one (**4a**).* Yield, 0.49 g (80%). ^{1}H NMR (DMSO-d_6, 600 MHz): δ 2.21 (s, 3H, CH_3Ph), 2.30 and 2.52 (each t, each 2H, J = 7.8 Hz, $PhCH_2CH_2CO$ and $PhCH_2CH_2CO$), 3.35−3.40 (m, 1H, 5″-H), 3.54−3.58 (m, 3H, 2″-H, 3″-H, 4″-H), 3.93 (dd, 1H, $J_{5'',6''Hb}$ = 4.2 Hz, J_{gem} = 11.4 Hz, 1H, 6″-H_b), 4.03 (dd, 1H, $J_{5'',6''Ha}$ = 4.2 Hz, J_{gem} = 11.4 Hz, 1H, 6″-H_b), 4.71 (d, J = 4.2 Hz, 1H, 2″-OH, D_2O exch.), 4.96 (d, J = 5.4 Hz, 1H, 3″-OH, D_2O exch.), 5.23 (d, J = 4.2 Hz, 1H, 4″-OH, D_2O exch.), 5.39 (dd, $J_{1'',2''}$ = 7.8 Hz, 1H, 1″-H), 6.14 (d, ^4J = 2.4 Hz, 1H, 8-H), 6.32 (d, ^4J = 1.8 Hz, 1H, 6-H), 6.81 (d, J = 8.4 Hz, 1H, 5′-H), 6.88 and 6.97 (each d, each 2H, each J = 8.4 Hz, 2‴′-H, 3‴′-H, 5‴′-H, 6‴′-H), 7.49 (d, ^4J = 2.4 Hz, 1H, 2′-H), 7.65 (dd, J = 8.4 Hz, ^4J = 2.4 Hz, 1H, 6′-H), 9.15 (s, 1H, 3′-OH, D_2O exch.), 9.75 (s, 1H, 4′-OH, D_2O exch.), 10.81 (s, 1H, 7-OH, D_2O exch.), 12.62 (s, 1H, 5-OH, D_2O exch.). ^{13}C NMR (DMSO-d_6, 150 MHz): δ 20.82, 29.91, 35.33, 63.57, 68.48, 71.14, 73.04, 73.09, 93.63, 98.86, 101.66, 103.98, 115.96, 116.11, 121.23, 122.12, 128.10, 128.29, 128.71, 128.91, 133.53, 135.03, 136.99, 137.32, 145.07, 148.69, 156.43, 161.36, 164.29, 171.90, 177.63. FAB-MS m/z $[M + H]^+$ ion = 611. Anal. calcd for $C_{31}H_{30}O_{13}\cdot 1/3H_2O$: C, 60.39; H, 5.01. Found: C, 60.10; H, 5.00.

*2-(3′,4′-Dihydroxyphenyl)-5,7-dihydroxy-3-β-D-{6″-O-[3‴-(4-fluorophenylpropanoyl)]}galactosyl-4H-chromen-4-one (**4b**).* Yield, 0.49 g (79%). ^{1}H NMR (DMSO-d_6, 600 MHz): δ 2.31 and 2.53 (each t, each 2H, J = 7.8 Hz, $PhCH_2CH_2CO$ and $PhCH_2CH_2CO$), 3.38−3.40 (m, 1H, 5″-H), 3.55−3.61 (m, 3H, 2″-H, 3″-H, 4″-H), 3.93 (dd, 1H, $J_{5'',6''Ha}$ = 4.2 Hz, J_{gem} = 11.4 Hz, 1H, 6″-H_a), 4.03 (dd, 1H, $J_{5'',6''Hb}$ = 3.6 Hz, J_{gem} = 11.4 Hz, 1H, 6″-H_b), 4.71 (br s, 1H, 2″-OH, D_2O exch.), 4.97 (br s, 1H, 3″-OH, D_2O exch.), 5.22 (d, J = 4.2 Hz, 1H, 4″-OH, D_2O exch.), 5.40 (d, $J_{1'',2''}$ = 7.8 Hz, 1H, 1″-H), 6.11 (d, ^4J = 2.4 Hz, 1H, 8-H), 6.30 (d, ^4J = 1.8 Hz, 1H, 6-H), 6.81 (d, J = 8.4 Hz, 1H, 5′-H), 6.97 (t, J = 9.0 Hz, 2H, 3‴′-H, 5‴′-H), 7.02 (dd, J = 8.4 Hz, J = 9.0 Hz, 2H, 2‴′-H, 6‴′-H), 7.49 (d, ^4J = 2.4 Hz, 1H, 2′-H), 7.64 (dd, J = 8.4 Hz, ^4J = 2.4 Hz, 1H, 6′-H), 9.15 (s, 1H, 3′-OH, D_2O exch.), 9.75 (s, 1H, 4′-OH, D_2O exch.), 10.80 (s, 1H, 7-OH, D_2O exch.), 12.62 (s, 1H, 5-OH, D_2O exch.). ^{13}C NMR (DMSO-d_6, 150 MHz): δ 29.47, 35.30, 63.67, 68.48, 71.12, 73.03, 73.08, 93.60, 98.84, 101.56, 103.94, 114.95, 115.00, 115.36, 116.01, 121.22, 122.15, 129.96, 130.02, 133.48, 136.54, 145.07, 148.69, 156.41, 161.64, 164.31, 171.75, 177.63. FAB-MS m/z $[M + H]^+$ ion = 615. Anal. calcd for $C_{30}H_{27}O_{13}F$: C, 58.63; H, 4.43. Found: C, 58.59; H, 4.10.

*2-(3′,4′-Dihydroxyphenyl)-5,7-dihydroxy-3-β-D-[2″,6″-di-O-(4-ethoxybenzoyl)]galactosyl-4H-chromen-4-one (**5a**).* Yield, 0.62 g (81%). ^{1}H NMR (DMSO-d_6, 600 MHz): δ 1.32 and 1.34 (each t, each 3H, J = 7.8 Hz, 2 × CH_3CH_2), 3.81 (dd, $J_{3'',4''}$ = 4.8 Hz, $J_{4'',5''}$ = 3.6 Hz, 1H, 4″-H), 3.84−3.87 (m, 1H, 5″-H), 3.91 (dd, $J_{2'',3''}$ = 8.4 Hz, $J_{3'',4''}$ = 4.8 Hz, 1H, 3″-H), 4.02 and 4.09 (each q, each H, J = 7.2 Hz, 2 × CH_3CH_2), 4.21 (dd, $J_{5'',6''Hb}$ = 3.0 Hz, J_{gem} = 11.4 Hz, 1H, 6″-H_b), 4.26 (dd, $J_{5'',6''Ha}$ = 4.2 Hz, J_{gem} = 11.4 Hz, 1H, 6″-H_a), 5.13 (d, J = 4.8 Hz, 3″-OH, D_2O exch.), 5.24 (d, J = 6.6 Hz, 4″-OH, D_2O exch.), 5.34 (dd, $J_{2'',3''}$ = 8.4 Hz, $J_{1'',2''}$ = 7.8 Hz, 1H, 2″-H), 5.82(d, $J_{1'',2''}$ = 7.8 Hz, 1H, 1″-H), 6.16 (d, ^4J = 2.4 Hz, 1H, 8-H), 6.32 (d, ^4J = 2.4 Hz, 1H, 6-H), 6.73 and 7.02 (each d, each 2H, each J = 8.4 Hz, 2 × 3‴-H, 2 × 5‴-H), 6.80 (d, J = 8.4 Hz, 1H, 5′-H), 7.38 (d, ^4J = 2.4 Hz, 1H, 2′-H), 7.58 and 7.94 (each d, each 2H, each J = 9.0 Hz, 2 × 2‴-H, 2 × 6‴-H), 7.63 (dd, J = 8.4 Hz, ^4J = 1.8 Hz, 1H, 6′-H), 9.14 (s, 1H, 3′−OH, D_2O exch.), 9.80 (s, 1H, 4′-OH, D_2O exch.), 10.84 (s, 1H, 7-OH,

D_2O exch.), 12.60 (s, 1H, 5-OH, D_2O exch.). ^{13}C NMR (DMSO-d_6, 150 MHz): δ 14.68, 14.71, 63.55, 63.67, 63.71, 68.70, 71.08, 73.01, 73.41, 93.63, 98.65, 98.81, 103.92, 114.16, 114.40, 115.34, 115.73, 121.02, 121.68, 122.28, 122.40, 131.01, 132.80, 145.17, 148.77, 156.37, 156.50, 161.41, 162.47, 162.57, 164.26, 165.03, 165.17, 177.27. FAB-MS m/z $[M + H]^+$ ion = 761. Anal. calcd for $C_{39}H_{36}O_{16}\cdot 1/4H_2O$: C, 61.22; H, 4.81 Found: C, 61.11; H, 4.68.

*2-(3′,4′-Dihydroxyphenyl)-5,7-dihydroxy-3-β-D-[2″,6″-di-O-(4-propylbenzoyl])]galactosyl-4H-chromen-4-one (**5b**).* Yield, 0.59 g (79%). ^1H NMR (DMSO-d_6, 600 MHz): δ 0.86 and 0.88 (each t, each 3H, J = 7.2 Hz, 2 × CH_3CH_2), 1.531.63 (m, 4H, 2 × $CH_3CH_2CH_2$), 2.55 and 2.62 (each t, each 2H, J = 7.2 Hz, 2 × $CH_3CH_2CH_2$), 3.81 (br s, 1H, 4″-H), 3.86−3.89 (m, 1H, 5″-H), 3.93 (dd, $J_{2'',3''}$ = 8.4 Hz, $J_{3'',4''}$ = 4.8 Hz, 1H, 3″-H), 4.23 (dd, $J_{5'',6''Hb}$ = 3.0 Hz, J_{gem} = 11.4 Hz, 1H, 6″-H_b), 4.29 (dd, $J_{5'',6''Ha}$ = 4.2 Hz, J_{gem} = 11.4 Hz, 1H, 6″-H_a), 5.15 (d, J = 4.2 Hz, 3″-OH, D_2O exch.), 5.27 (d, J = 6.6 Hz, 4″-OH, D_2O exch.), 5.36 (dd, $J_{1'',2''}$ = 7.8 Hz, $J_{2'',3''}$ = 8.4 Hz, 1H, 2″-H), 5.84 (d, $J_{1'',2''}$ = 7.8 Hz, 1H, 1″-H), 6.16 (d, ^4J = 2.4 Hz, 1H, 8-H), 6.30 (d, ^4J = 2.4 Hz, 1H, 6-H), 6.80 (d, J = 8.4 Hz, 1H, 5′-H), 7.05 and 7.34 (each d, each 2H, each J = 8.4 Hz, 2 × 3‴-H, 2 × 5‴-H), 7.39 (d, ^4J = 2.4 Hz, 1H, 2′-H), 7.56 and 7.94 (each d, each 2H, each J = 9.0 Hz, 2 × 2‴-H, 2 × 6‴-H), 7.62 (dd, J = 8.4 Hz, ^4J = 2.4 Hz, 1H, 6′-H), 9.14 (s, 1H, 3′-OH, D_2O exch.), 9.80 (s, 1H, 4′-OH, D_2O exch.), 10.85 (s, 1H, 7-OH, D_2O exch.), 12.59 (s, 1H, 5-OH, D_2O exch.). ^{13}C NMR (DMSO-d_6, 150 MHz): δ 13.74, 13.78, 23.79, 24.01, 37.33, 63.75, 68.69, 71.04, 73.23, 73.34, 93.65, 98.62, 98.86, 103.94, 115.36, 115.75, 121.02, 122.36, 127.24, 127.82, 128.58, 128.76, 128.98, 129.01, 129.70, 132.79, 145.18, 148.08, 148.18, 148.79, 156.34, 161.41, 164.34, 165.37, 165.51, 177.26. FAB-MS m/z $[M + H]^+$ ion = 757. Anal. calcd for $C_{41}H_{40}O_{14}$: C, 65.07; H, 5.33. Found: C, 65.01; H, 5.48.

*2-(3′,4′-Dihydroxyphenyl)-5,7-dihydroxy-3-β-D-[2″,6″-di-O-(4-fluorobenzoyl)]galactosyl-4H-chromen-4-one (**5c**).* Yield, 0.53 g (75%). ^1H NMR (DMSO-d_6, 600 MHz): δ 3.82 (br s, 1H, 4″-H), 3.86−3.89 (m, 1H, 5″-H), 3.93 (dd, $J_{2'',3''}$ = 8.4 Hz, $J_{3'',4''}$ = 4.2 Hz, 1H, 3″-H), 4.26 (dd, $J_{5'',6''Hb}$ = 4.2 Hz, J_{gem} = 11.4 Hz, 1H, 6″-H_b), 4.34 (dd, $J_{5'',6''Ha}$ = 3.0 Hz, J_{gem} = 11.4 Hz, 1H, 6″-H_a), 5.17 (d, J = 4.2 Hz, 3″-OH, D_2O exch.), 5.31 (d, J = 6.6 Hz, 1H, 4″-OH, D_2O exch.), 5.34 (dd, $J_{1'',2''}$ = 7.8 Hz, $J_{2'',3''}$ = 8.4 Hz, 1H, 2″-H), 5.74 (d, $J_{1'',2''}$ = 7.8 Hz, 1H, 1″-H), 6.13 (d, ^4J = 2.4 Hz, 1H, 8-H), 6.28 (d, ^4J = 2.4 Hz, 1H, 6-H), 6.78 (d, J = 8.4 Hz, 1H, 5′-H), 7.09 and 7.36 (each t, each 2H, J = 9.0 Hz, 2 × 3‴-H, 2 × 5‴-H), 7.37 (d, ^4J = 2.4 Hz, 1H, 2′-H), 7.58 (dd, J = 8.4 Hz, ^4J = 2.4 Hz, 1H, 6′-H), 7.73 and 8.07 (each dd, each 2H, J = 8.4 Hz, J = 9.0 Hz, 2 × 2‴-H, 2 × 6‴-H), 9.14 (s, 1H, 3′-OH, D_2O), 9.79 (s, 1H, 4′-OH, D_2O exch.), 10.84 (s, 1H, 7-OH, D_2O exch.), 12.54 (s, 1H, 5-OH, D_2O exch.). ^{13}C NMR (DMSO-d_6, 150 MHz): δ 63.85, 68.59, 70.96, 73.29, 73.56, 93.61, 98.84, 99.47, 103.87, 115.33, 115.64, 115.76, 115.79, 115.84, 115.98, 120.94, 122.29, 126.20, 126.80, 131.73, 131.80, 132.43, 132.88, 145.13, 148.77, 156.31, 164.52, 164.95, 161.32, 164.28, 164.44, 164.49, 177.17. FAB-MS m/z $[M + H]^+$ ion = 709. Anal. calcd for $C_{35}H_{26}O_{14}F_2$: C, 59.33; H, 3.70. Found: C, 58.12; H, 4.01.

*2-(3′,4′-Dihydroxyphenyl)-5,7-dihydroxy-3-β-D-[2″,6″-di-O-(4-methylphenylacetyl)]galactosyl-4H-chromen-4-one (**5d**).* Yield, 0.58 g (79%). ^1H NMR (DMSO-d_6, 600 MHz): δ 2.20 and 2.21 (each s, each 3H, 2 × CH_3Ph), 3.30/3.37 (each AB system, each d, each J_{AB} = 15.6 Hz, 2H, $PhCH_2CO$), 3.66 (br s, 2H, $PhCH_2CO$), 3.69−3.72 (m, 2H, 4″-H, 5″-H), 3.97−4.04 (m, 3H, 3″-H, 6″-H), 5.00 (d, J = 4.8 Hz, 1H, 3″-OH), 5.105 (dd, $J_{1'',2''}$ = 7.8 Hz, $J_{2'',3''}$ = 6.6 Hz, 1H, 2″-H), 5.18 (d, J = 6.0 Hz, 1H, 4″-OH), 5.54 (d, $J_{1'',2''}$ = 7.8 Hz, 1H, 1″-H), 6.20 (d, ^4J = 2.4 Hz, 1H, 8-H), 6.40 (d, ^4J = 1.8 Hz, 1H, 6-H), 6.78 (d, J = 8.4 Hz, 1H, 5′-H), 6.90 and 6.96 (each d, each 2H, J = 8.4 Hz, 2 × 3‴-H, 2 × 5‴-H), 7.04 and 7.14 (each d, each 2H, J = 8.4 Hz, 2 × 2‴-H, 2 × 6‴-H), 7.45 (d, ^4J = 2.4 Hz, 1H, 2′-H), 7.61 (dd, J = 8.4 Hz, ^4J = 2.4 Hz, 1H, 6′-H), 9.13 (s, 1H, 3′-OH, D_2O exch.), 9.78 (s, 1H, 4′-OH, D_2O exch.), 10.87 (s, 1H, 7-OH, D_2O exch.), 12.70 (s, 1H, 5-OH, D_2O exch.). ^{13}C NMR (DMSO-d_6, 150 MHz): δ 20.80, 20.82, 38.77, 38..28, 63.80, 68.60, 70.64, 72.90, 72.97, 93.73, 98.88, 99.25, 104.06, 115.30, 115.80, 120.96, 122.41, 128.92, 128.95, 129.16, 129.48, 131.15, 131.53, 133.24, 133.79, 135.82, 135.94, 145.13, 150.66, 156.56, 161.51, 164.40, 170.88, 171.01, 177.24. FAB-MS m/z $[M + H]^+$ ion = 729. Anal. calcd for $C_{39}H_{36}O_{14}$: C, 64.28; H, 4.98. Found: C, 64.46; H, 4.71.

*2-(3′,4′-Dihydroxyphenyl)-5,7-dihydroxy-3-β-D-[2″,6″-di-O-(4-fluorophenylacetyl)]galactosyl-4H-chromen-4-one (**5e**).* Yield, 0.57 g (77%). ^1H NMR (DMSO-d_6, 600 MHz): δ 3.37/3.45 (each AB system, each d, J_{AB} = 15.6 Hz, 2H, $PhCH_2CO$), 3.67 (br s, 1H, 4″-H), 3.70−3.74 (m, 4H, $PhCH_2CO$, 3″-H, 5″-H), 3.99 (dd, $J_{5'',6''Hb}$ = 4.2 Hz, J_{gem} = 11.4 Hz, 1H, 6″-H_b), 4.04 (dd, $J_{5'',6''Ha}$ = 3.0 Hz, J_{gem} = 11.4 Hz, 1H, 6″-H_a), 5.01 (br s, 3″-OH, D_2O exch.), 5.11 (dd, $J_{1'',2''}$ = 7.8 Hz, $J_{2'',3''}$ = 7.2 Hz, 1H, 2″-H), 5.20 (br s, 4″-OH, D_2O exch.), 5.50 (d, $J_{1'',2''}$ = 7.8 Hz, 1H, 1″-H), 6.19 (d, ^4J = 2.4 Hz, 1H, 8-H), 6.40 (d, ^4J = 2.4 Hz, 1H, 6-H), 6.76 (d, J = 8.4 Hz, 1H, 5′-H), 6.99 and 7.04 (each t, each 2H, J = 9.0 Hz, 2 × 3‴-H, 2 × 5‴-H), 7.07 and 7.30 (each dd, each 2H, J = 8.4 Hz, J = 9.0 Hz, 2 × 2‴-H, 2 × 6‴-H), 7.45 (d, ^4J = 2.4 Hz, 1H, 2′-H), 7.60 (dd, J = 8.4 Hz, ^4J = 2.4 Hz, 1H, 6′-H), 9.14 (s, 1H, 3′-OH, D_2O exch.), 9.79 (s, 1H, 4′-OH, D_2O exch.), 10.88 (s, 1H, 7-OH, D_2O exch.), 12.68 (s, 1H, 5-OH, D_2O exch.). ^{13}C NMR (DMSO-d_6, 150 MHz): δ 31.36, 31.72, 63.97, 68.70, 70.84, 73.10, 73.22, 93.95, 99.10, 99.54, 104.22, 115.21, 115.35, 115.49, 116.01, 121.14, 122.58, 130.64, 131.04, 131.48, 131.33, 131.72, 131.77, 133.48, 134.23, 145.33, 149.02, 156.63, 160.69, 161.66, 162.30, 164.63, 170.90, 171.04, 177.45. FAB-MS m/z $[M + H]^+$ ion = 737. Anal. calcd for $C_{37}H_{30}O_{14}F_2$: C, 60.33; H, 4.10. Found: C, 60.05; H, 4.15.

*2-(3′,4′-Dihydroxyphenyl)-5,7-dihydroxy-3-β-D-{2″,6″-di-O-[3‴-(4-hydroxyphenylpropanoyl)]}galactosyl-4H-chromen-4-one (**5f**).* Yield, 0.60 g (79%). ^1H NMR (DMSO-d_6, 600 MHz): δ 2.29 and 2.46 (each t, each 2H, J = 7.8 Hz, 2 × $PhCH_2CH_2CO$), 2.55 and 2.73 (each t, each 2H, J = 7.8 Hz, 2 × $PhCH_2CH_2CO$), 3.66−3.70 (m, 3H, 3″-H, 4″-H, 5″-H), 3.95 (dd, $J_{5'',6''Hb}$ = 4.2 Hz, J_{gem} = 11.4 Hz, 1H, 6″-H_b), 4.01 (dd, $J_{5'',6''Ha}$ = 4.8 Hz, J_{gem} = 11.4 Hz, 1H, 6″-H_a), 4.99 (d, J = 4.2 Hz, 3″-OH, D_2O exch.), 5.11 (dd, $J_{1'',2''}$ = 7.8 Hz, $J_{2'',3''}$ = 7.2 Hz, 1H, 2″-H), 5.14 (d, J = 4.2 Hz, 4″-OH, D_2O exch.), 5.52 (d, $J_{1'',2''}$ = 7.8 Hz, 1H, 1″-H), 6.14 (d, ^4J = 1.8 Hz, 1H, 8-H), 6.34 (d, ^4J = 1.8 Hz, 1H, 6-H), 6.57 and 6.60 (each d, each 2H, each J = 8.4 Hz, 2 × 3‴-H, 2 × 5‴-H), 6.79 (d, J = 8.4 Hz, 1H, 5′-H), 6.81 and 6.99 (each d, each 2H, each J = 8.4 Hz, 2 × 2‴-H, 2 × 6‴-H), 7.44 (d, ^4J = 2.4 Hz, 1H, 2′-H), 7.61 (dd, J = 8.4 Hz, ^4J = 2.4 Hz, 1H, 6′-H), 9.10 and 9.13 (each s, each 1H, 2 × OHPh, D_2O exch.), 9.28 (s, 1H, 3′-OH, D_2O exch.), 9.79 (s, 1H, 4′-OH, D_2O exch.), 10.83 (s, 1H, 7-OH, D_2O exch.), 12.65 (s, 1H, 5-OH, D_2O exch.). ^{13}C NMR (DMSO-d_6, 150 MHz): δ 29.57, 29.61, 35.65, 36.02, 63.21, 68.54, 70.61, 72.54, 73.02, 93.68, 95.77, 99.12, 104.01, 115.20, 115.22, 115.33, 115.81, 121.02, 122.41, 129.12, 129.28, 130.52, 130.87, 133.22, 145.15, 148.79, 155.70, 155.89, 156.52, 161.42, 164.36, 171.85, 171.96, 177.30. FAB-MS m/z $[M + H]^+$ ion = 761. Anal. calcd for $C_{39}H_{36}O_{16}$: C, 61.58; H, 4.77. Found: C, 61.52; H, 4.97.

*2-(3′,4′-Dihydroxyphenyl)-5,7-dihydroxy-3-β-D-{2″,6″-di-O-[3‴-(4-ethoxyphenylpropanoyl)]}galactosyl-4H-chromen-4-one (**5g**).* Yield, 0.68 g (83%). ^1H NMR (DMSO-d_6, 600 MHz): δ 1.21 and 1.29 (each t, each 3H, J = 7.8 Hz, 2 × CH_3CH_2), 2.30 and 2.52 (each t, each 2H, J = 7.8 Hz, 2 × $PhCH_2CH_2CO$), 2.59 and 2.78 (each t, each 2H, J = 7.8 Hz, 2 × $PhCH_2CH_2CO$), 3.65−3.70 (m, 3H, 3″-H, 4″-H, 5″-H), 3.82 and 3.92 (each q, each 2H, J = 7.2 Hz, 2 × CH_3CH_2), 3.95 (dd, $J_{5'',6''Hb}$ = 4.2 Hz, J_{gem} = 11.4 Hz, 1H, 6″-H_b), 4.01 (dd, $J_{5'',6''Ha}$ = 4.8 Hz, J_{gem} = 11.4 Hz, 1H, 6″-H_a), 5.00 (d, J = 4.2 Hz, 1H, 3″-OH, D_2O exch.), 5.11 (dd, $J_{1'',2''}$ = 8.4 Hz, $J_{2'',3''}$ = 7.8 Hz, 1H, 2″-H), 5.16 (d, J = 5.4 Hz, 1H, 4″-OH, D_2O exch.), 5.55 (d, $J_{1'',2''}$ = 8.4 Hz, 1H, 1″-H), 6.14 (d, ^4J = 1.8 Hz, 1H, 8-H), 6.31 (d, ^4J = 1.8 Hz, 1H, 6-H), 6.66 and 6.70 (each d, each 2H, each J = 9.0 Hz, 2 × 3‴-H, 2 × 5‴-H), 6.80 (d, J = 8.4 Hz, 1H, 5′-H), 6.89 and 7.08 (each d, each 2H, each J = 9.0 Hz, 2 × 2‴-H, 2 × 6‴-H), 7.43 (d, ^4J = 2.4 Hz, 2′-H), 7.62 (dd, J = 8.4 Hz, ^4J = 2.4 Hz, 1H, 6′-H), 9.14 (s, 1H, 3′-OH, D_2O exch.), 9.81 (s, 1H, 4′-OH, D_2O exch.), 10.82 (s, 1H, 7-OH, D_2O), 12.65 (s, 1H, 5-OH, D_2O). ^{13}C NMR (DMSO-d_6, 150 MHz): δ

14.83, 14.92, 29.53, 29.79, 35.61, 35.81, 62.88, 62.98, 63.34, 68.57, 70.71, 72.54, 73.05, 93.63, 98.85, 98.98, 103.98, 114.18, 114.26, 115.32, 115.75, 121.01, 122.46, 129.16, 129.37, 132.13, 132.46, 133.16, 134.99, 145.15, 148.79, 156.35, 156.92, 156.98, 161.40, 164.33, 171.83, 171.90, 177.25. FAB-MS m/z $[M + H]^+$ ion = 817. Anal. calcd for $C_{43}H_{44}O_{16}$: C, 63.23; H, 5.43. Found: C, 63.34; H, 5.50.

*2-(3′,4′-Dihydroxyphenyl)-5,7-dihydroxy-3-β-D-{2″,6″-di-O-[3‴-(4-methylphenylpropanoyl)]}galactosyl-4H-chromen-4-one (**5h**).* Yield, 0.59 g (78%). ^{1}H NMR (DMSO-d_6, 600 MHz): δ 2.15 and 2.22 (each s, each 3H, 2 × CH_3Ph), 2.35 and 2.55 (each t, each 2H, J = 7.8 Hz, 2 × PhCH$_2$CH_2CO), 2.61 and 2.81 (each t, each 2H, J = 7.8 Hz, 2 × PhCH_2CH$_2$CO), 3.66−3.70 (m, 3H, 3″-H, 4″-H, 5″-H), 3.97 (dd, $J_{5'',6''Hb}$ = 4.2 Hz, J_{gem} = 11.4 Hz, 1H, 6″-H$_b$), 4.02 (dd, $J_{5'',6''Ha}$ = 4.2 Hz, J_{gem} = 11.4 Hz, 1H, 6″-H$_a$), 5.00 (d, J = 3.6 Hz, 1H, 3″-OH, D$_2$O exch.), 5.11 (dd, $J_{1'',2''}$ = 8.4 Hz, $J_{2'',3''}$ = 7.8 Hz, 1H, 2″-H), 5.15 (d, J = 5.4 Hz, 1H, 4″-OH, D$_2$O exch.), 5.52 (d, $J_{1'',2''}$ = 8.4 Hz, 1H, 1″-H), 6.15 (d, ^4J = 1.8 Hz, 1H, 8-H), 6.32 (d, ^4J = 1.8 Hz, 1H, 6-H), 6.90 and 6.96 (each d, each 2H, each J = 8.4 Hz, 2 × 2‴′-H, 2 × 6‴′-H), 6.95 (d, J = 8.4 Hz, 1H, 5′-H), 6.99 and 7.08 (each d, each 2H, each J = 8.4 Hz, 2 × 3‴′-H, 2 × 5‴′-H), 7.44 (d, ^4J = 2.4 Hz, 1H, 2′-H), 7.61 (dd, J = 8.4 Hz, ^4J = 2.4 Hz, 1H, 6′-H), 9.14 (s, 1H, 3′-OH, D$_2$O exch.), 9.80 (s, 1H, 4′-OH, D$_2$O exch.), 10.82 (s, 1H, 7-OH, D$_2$O exch.), 12.65 (s, 1H, 5-OH, D$_2$O exch.). ^{13}C NMR (DMSO-d_6, 150 MHz): δ 21.27, 21.37, 30.50, 30.53, 35.92, 36.21, 63.86, 69.08, 71.26, 73.11, 73.56, 94.18, 96.15, 99.69, 104.57, 115.86, 116.34, 121.56, 122.96, 128.62, 128.83, 129.50, 129.54, 133.77, 134.98, 135.50, 135.60, 137.86, 138.21, 145.68, 149.32, 156.97, 161.96, 164.86, 172.33, 172.43, 177.80. FAB-MS m/z $[M + H]^+$ ion =757. Anal. calcd for $C_{41}H_{40}O_{14}$: C, 65.07; H, 5.33. Found: C, 64.87; H, 5.08.

*2-(3′,4′-Dihydroxyphenyl)-5,7-dihydroxy-3-β-D-{2″,6″-di-O-[3‴-(4-fluorophenylpropanoyl)]}galactosyl-4H-chromen-4-one (**5i**).* Yield, 0.58 g (76%). ^{1}H NMR (DMSO-d_6, 600 MHz): δ 2.36 and 2.57 (each t, each 2H, J = 7.8 Hz, 2 × PhCH$_2$CH_2CO), 2.64 and 2.86 (each t, each 2H, J = 7.8 Hz, 2 × PhCH_2CH$_2$CO), 3.67−3.72 (m, 3H, 3″-H, 4″-H, 5″-H), 3.97 (dd, 1H, $J_{5'',6''Hb}$ = 4.2 Hz, J_{gem} = 11.4 Hz, 1H, 6″-H$_b$), 4.03 (dd, 1H, $J_{5'',6''Ha}$ = 3.6 Hz, J_{gem} = 11.4 Hz, 1H, 6″-H$_a$), 5.01 (d, J = 4.8 Hz, 1H, 3″-OH, D$_2$O exch.), 5.12 (dd, $J_{1'',2''}$ = 7.8 Hz, $J_{2'',3''}$ = 7.2 Hz, 1H, 2″-H), 5.16 (d, J = 6.0 Hz, 1H, 4″-OH, D$_2$O exch.), 5.54 (d, $J_{1'',2''}$ = 7.8 Hz, 1H, 1″-H), 6.13 (d, ^4J = 2.4 Hz, 1H, 8-H), 6.31 (d, ^4J = 2.4 Hz, 1H, 6-H), 6.81 (d, J = 8.4 Hz, 1H, 5′-H), 6.96 and 6.98 (each t, each 2H, J = 9.0 Hz, 2 × 3‴′-H, 2 × 5‴′-H), 7.05 and 7.24 (each dd, each 2H, J = 9.0 Hz, J = 8.4 Hz, 2 × 2‴′-H, 2 × 6‴′-H), 7.44 (d, ^4J = 2.4 Hz, 1H, 2′-H), 7.61 (dd, J = 8.4 Hz, ^4J = 2.4 Hz, 1H, 6′-H), 9.14 (s, 1H, 3′-OH, D$_2$O exch.), 9.80 (s, 1H, 4′-OH, D$_2$O exch.), 10.81 (s, 1H, 7-OH, D$_2$O), 12.64 (s, 1H, 5-OH, D$_2$O exch.). ^{13}C NMR (DMSO-d_6, 150 MHz): δ 30.06, 30.09, 35.87, 36.11, 63.92, 69.10, 71.28, 73.15, 73.57, 94.15, 99.38, 99.61, 104.53, 115.46, 115.33, 115.60, 115.66, 115.87, 116.32, 121.54, 122.93, 130.52, 130.57, 130.76, 130.82, 133.71, 134.99, 137.40, 137.42, 145.69, 149.33, 156.87, 160.62, 161.93, 162.20, 164.86, 172.28, 172.34, 177.82. FAB-MS m/z $[M + H]^+$ ion = 765. Anal. calcd for $C_{39}H_{34}O_{14}F_2$: C, 61.26; H, 4.48. Found: C, 60.84; H, 4.60.

*2-(3′,4′-Dihydroxyphenyl)-5,7-dihydroxy-3-β-D-{2″,6″-di-O-[4‴-(4-fluorophenylbutanoyl)]}galactosyl-4H-chromen-4-one (**5j**).* Yield, 0.62 g (78%). ^{1}H NMR (DMSO-d_6, 600 MHz): δ 1.54 and 1.80 (each quintet, each 2H, 2 × PhCH$_2$CH_2CH$_2$), 1.98 and 2.02 (each t, each 2H, J = 7.2 Hz, 2 × CH$_2$CH_2CO), 2.33 and 2.55 (each t, each 2H, J = 7.2 Hz, 2 × PhCH_2CH$_2$), 3.67−3.74 (m, 3H, 3″-H, 4″-H, 5″-H), 3.93 (dd, 1H, $J_{5'',6''Hb}$ = 3.6 Hz, J_{gem} = 11.4 Hz, 1H, 6″-H$_b$), 4.09 (dd, 1H, $J_{5'',6''Ha}$ = 3.0 Hz, J_{gem} = 11.4 Hz, 1H, 6″-H$_a$), 5.00 (br s, 1H, 3″−OH, D$_2$O exch.), 5.11 (dd, $J_{1'',2''}$ = 7.8 Hz, $J_{2'',3''}$ = 8.4 Hz, 1H, 2″-H), 5.14 (br s, 1H, 4″−OH, D$_2$O exch.), 5.55 (d, $J_{1'',2''}$ = 7.8 Hz, 1H, 1″-H), 6.17 (d, ^4J = 2.4 Hz, 1H, 8-H), 6.33 (d, ^4J = 2.4 Hz, 1H, 6-H), 6.79 (d, J = 8.4 Hz, 1H, 5′-H), 6.99 and 7.01 (each t, each 2H, J = 9.0 Hz, 2 × 3‴′-H, 2 × 5‴′-H), 7.05 and 7.16 (each dd, each 2H, J = 9.0 Hz, J = 8.4 Hz, 2 × 2‴′-H, 2 × 6‴′-H), 7.42 (d, ^4J = 2.4 Hz, 1H, 2′-H), 7.63 (dd, J = 8.4 Hz, ^4J = 2.4 Hz, 1H, 6′-H), 9.14 (s, 1H, 3′-OH, D$_2$O exch.), 9.80 (s, 1H, 4′-OH, D$_2$O exch.), 10.85 (s, 1H, 7-OH, D$_2$O), 12.59 (s, 1H, 5-OH, D$_2$O exch.). ^{13}C NMR (DMSO-d_6, 150 MHz): δ 26.17, 26.43, 31.03, 31.15, 36.79, 36.87, 63.07, 68.38, 70.51, 72.21, 72.84, 93.41, 98.61, 98.81, 103.76, 113.99, 114.36, 114.76 114.89, 115.10, 115.56, 120.75, 122.18, 129.91, 129.96, 129.99, 130.04, 132.90, 134.99, 137.23, 137.61, 144.94, 148.60, 156.16, 159.76, 161.21, 161.36, 164.20, 171.95, 172.10, 177.01. FAB-MS m/z $[M + H]^+$ ion = 793. Anal. calcd for $C_{41}H_{38}O_{14}F_2 \cdot 1/5H_2O$: C, 61.84; H, 4.86. Found: C, 61.84; H, 4.97.

*2-(3′,4′-Dihydroxyphenyl)-5,7-dihydroxy-3-β-D-{2″,6″-di-O-[5‴-(4-fluorophenylpentanoyl)]}galactosyl-4H-chromen-4-one (**5k**).* Yield, 0.66 g (80%). ^{1}H NMR (DMSO-d_6, 600 MHz): δ 1.22−1.30 (m, 4H, 2 × PhCH$_2$CH_2CH$_2$), 1.33 and 1.52 (each quintet, each 2H, J = 7.2 Hz, 2 × CH$_2$CH$_2$CH_2CH$_2$), 1.58 and 2.02 (each t, each 2H, J = 7.2 Hz, 2 × CH$_2$CH_2CO), 2.35 and 2.52 (each t, each 2H, J = 7.2 Hz, 2 × PHCH_2CH$_2$), 3.65−3.71 (m, 3H, 3″-H, 4″-H, 5″-H), 3.92 (dd, 1H, $J_{5'',6''Hb}$ = 4.2 Hz, J_{gem} = 11.4 Hz, 1H, 6″-H$_b$), 4.09 (dd, 1H, $J_{5'',6''Ha}$ = 3.6 Hz, J_{gem} = 11.4 Hz, 1H, 6″-H$_a$), 5.00 (d, J = 4.2 Hz, 1H, 3″-OH, D$_2$O exch.), 5.09 (dd, $J_{1'',2''}$ = 7.8 Hz, $J_{2'',3''}$ = 8.4 Hz, 1H, 2″-H), 5.13 (d, J = 6.0 Hz, 1H, 4″-OH, D$_2$O exch.), 5.54 (d, $J_{1'',2''}$ = 7.8 Hz, 1H, 1″-H), 6.17 (d, ^4J = 2.4 Hz, 1H, 8-H), 6.36 (d, ^4J = 2.4 Hz, 1H, 6-H), 6.79 (d, J = 8.4 Hz, 1H, 5′-H), 6.92 and 7.04 (each t, each 2H, J = 9.0 Hz, 2 × 3‴′-H, 2 × 5‴′-H), 7.07 and 7.11 (each dd, each 2H, J = 9.0 Hz, J = 8.4 Hz, 2 × 2‴′-H, 2 × 6‴′-H), 7.42 (d, ^4J = 2.4 Hz, 1H, 2′-H), 7.62 (dd, J = 8.4 Hz, ^4J = 2.4 Hz, 1H, 6′-H), 9.14 (s, 1H, 3′-OH, D$_2$O exch.), 9.81 (s, 1H, 4′-OH, D$_2$O exch.), 10.85 (s, 1H, 7-OH, D$_2$O), 12.63 (s, 1H, 5-OH, D$_2$O exch.). ^{13}C NMR (DMSO-d_6, 150 MHz): δ 23.88, 24.38, 30.30, 30.39, 31.18, 33.18, 33.80, 34.11, 63.16, 68.57, 70.73, 72.36, 73.10, 93.60, 98.82, 98.88, 103.97, 114.78, 114.92, 114.97, 115.10, 115.33, 115.72, 120.96, 122.41, 129.86, 129.92, 130.06, 130.11, 133.04, 134.99, 138.10, 138.24, 145.17, 148.79, 156.37, 159.91, 161.39, 161.51, 164.37, 172.37, 172.49, 177.21. FAB-MS m/z $[M + H]^+$ ion = 821. Anal. calcd for $C_{43}H_{42}O_{14}F_2 \cdot 1/4H_2O$: C, 62.58; H, 5.19. Found: C, 62.29; H, 5.00.

Determination of Antibacterial Activity[36]. *In Vitro Susceptibility Test.* MICs of antimicrobial agents were determined by broth dilution techniques, according to the instructions of the Clinical and Laboratory Standard Institute (CLSI; formerly NCCLS). The MIC determinations were made in triplicate on separate occasions. Broth MIC testing was performed in 96-well microtiter trays with an inoculums of about 10^5 CFU in 100 μL of Mueller−Hinton broth (Difco) supplemented with 0.85% NaCl. The bacteria were diluted in the broth to a concentration of 10^5 in 100 μL of test compounds in water to various concentrations from 25.6 mg/mL stocks. The final concentrations tested were 0.062, 0.125, 0.5, 1, 2, 4, 8, 16, 32, 64, and 128 μg/mL (2-fold serial dilutions of test compounds). The MIC was the lowest of these concentrations, at which each of the triplicate wells was clear after incubation of the plate for 24 h at 35 °C. The MIC values corresponded to three independent determinations. To be considered valid, identical MIC values had to be obtained in at least two determinations; otherwise, experiments were repeated.

Determination of DNA Gyrase Supercoiling Activity[60]. *E. coli DNA Gyrase Inhibition (IC_{50}).* DNA gyrase from *E. coli* was purchased (Gyrase Supercoiling assay kit: K0001) from John Innes Enterprises. One unit of DNA gyrase was incubated with 0.5 μg of relaxed pBR322 DNA in a 30 μL reaction at 37 °C for 30 min under a condition of 35 mM Tris HCl (pH 7.5), 24 mM KCl, 4 mM $MgCl_2$, 2 mM dithiotheritol, 1.8 mM spermidine, 1 mM ATP, 6.5% (w/v) glycerol, and 0.1 mg/mL albumin (BSA). Each reaction was stopped by the addition of 8 μL of Stop dye [40% sucrose, 100 mM Tris HCl (pH 7.5), 1 mM EDTA, and 0.5 mg/mL bromophenol blue]. Agarose gels (1.0%) were run in TAE (40 mM Tris acetate, 2 mM EDTA). The concentration of compounds that inhibits 50% of supercoiling activity (IC_{50}) was determined using densitometry and NIH image, and finally, the values were applied to the following equation.

$$IC_{50} = \hat{1}0[\hat{L}OG(A/B) \times (50 - C)/(D - C) + LOG(B)]$$

where *A* is 50% highest inhibition, *B* is 50% lowest inhibition, *C* is the concentration of lowest inhibition, and *D* is the concentration of highest inhibition. The determinations were performed at least three times.

Determination of Decatenation Activity[61]. *E. coli TopoIV Inhibition (IC_{50}). E. coli* topoIV was purchased (*E. coli* topoIV decatenation kit: D4001) from John Innes Enterprises. One unit of topoIV was incubated with 200 ng of kDNA in a 30 μL reaction at 37 °C for 30 min under a condition of 50 mM HEPES−KOH (pH 7.6), 5 mM magnesium acetate, 100 mM potassium glutamate, 10 mM dithiotheritol, 1 mM ATP, and 40% (w/v) glycerol. Each reaction was stopped by the addition of 8 μL of Stop dye [40% sucrose, 100 mM Tris HCl (pH 7.5), 1 mM EDTA, and 0.5 mg/mL bromophenol blue]. Agarose gels (1.0%) were run in TAE (40 mM Tris acetate, 2 mM EDTA). The concentration of compounds that inhibits 50% of decatenating activity (IC_{50}) was determined using densitometry and NIH image, and finally, the values were applied to the above equation similar to DNA gyrase inhibition. The determinations were done at least three times.

S. aureus TopoIV Inhibition (IC_{50}). S. aureus topoIV was purchased (*S. aureus* topoIV decatenation kit: SAD4001) from John Innes Enterprises. One unit of topoIV was incubated with 200 ng of kDNA in a 30 μL reaction at 37 °C for 30 min under a condition of 50 mM Tris HCl (pH 7.5), 5 mM $MgCl_2$, 350 mM potassium glutamate, 5 mM dithiotheritol, 1.5 mM ATP, and 40% (w/v) glycerol. Each reaction was stopped by the addition of 8 μL of Stop dye [40% sucrose, 100 mM Tris HCl (pH 7.5), 1 mM EDTA, and 0.5 mg/mL bromophenol blue]. Agarose gels (1.0%) were run in TAE (40 mM Tris acetate, 2 mM EDTA). The concentration of compounds that inhibits 50% of decatenating activity (IC_{50}) was determined with using densitometry and NIH image, and finally, the values were applied to the above equation similar to DNA gyrase inhibition. The determinations were conducted at least three times.

In Situ Absorption Study. The in situ absorption was studied in male Wister rats ($n = 4$) weighing from 200 to 300 g. The rats were fasted with ad libitum access to water 18 h and anesthetized by intraperitoneal injection of pentobarbital at 50 mg/kg 30 min prior to sugery. The in situ perfusion technique described in the original publication[45] was used. After the cannula was set up and the segment under study was cleaned, 1 mg/mL of **5i** was injected into the small intestine. After 1 h, 10 mL of saline was pumped through the intestine all the way to the distal cannula and drained back into the intestine to ensure uniform sample concentration throughout the gut segment, and subsquently, this 10 mL saline solution along with unabsorbed **5i** was collected. The concentration of the collected solution gave amounts of absorption from small intestine by subtraction from injected cocentration. Thus, the amount absorbed was determined with a three-step sequence involving initial centrifugation of the intestinal samples at 1500 rpm for 10 min to provide 0.1 mg/mL sample solution, then extraction with chloroform followed by removal of the solvent under reduced pressure. Finally, the resulting sample was dissolved in 10 mL of methanol, and the absorbance of the solution was measured in a UV spectrophotometer at 340 nm.

Acute Toxicity Study. The acute toxicity of **5i** was studied in male mice ($n = 4$) weighing from 15 to 30 g, and animals were purchased from Charles River Co. Ltd. The study was conducted according to internationally accepted principles of laboratory animals. Only water was provided ad libitum during the 12 h before experimentation. After intervenous injection of **5i**, food and water were provided ad libitum. The weight gain of the animals as well as the mobility and the mortality in animals were observed up to 14 days as an indicator of toxicity. The mortality was zero, and the mobility in animals was normal within the period of test.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information. Multidrug-resistant strains (clinical isolates), characterizations of intermediate compounds **20a,b**, **21a−k**, molecular modeling, and table of the best docking result on the inhibition constants K_i. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Fax: 81-086-251-8021. E-mail: chem.lokman@gmail.com (A.M.L.H.) or ksasaki@pheasant.pharm.okayama-u.ac.jp (K.S.).

■ ACKNOWLEDGMENT

We thank Professor Adrian L. Schwan of University of Guelph for a thorough reading and critique of this manuscript and are grateful to the SC-NMR Laboratory of Okayama University for NMR experiments. We are also grateful to Associate Professor Tsutomu Inokuchi of Okayama University for his support of this project. We also thank Mrs. Ayako Sato and Professor Masayuki Sato of School of Pharmaceutical Sciences University of Shizuoka for providing elemental analysis data.

■ ABBREVIATIONS USED

E. coli, *Escherichia coli*; *S. aureus*, *Staphylococcus aureus*; topoIV, topoisomerase IV; MRSA, methicillin-resistant *S. aureus*; MSSA, methicillin-sensitive *S. aureus*; VRE, vancomycin-resistant enterococci; VISA, vancomycin intermediate *S. aureus*; MIC, minimum inhibitory concentration; DMAP, 4-dimethylaminopyridine; DCC, *N*,*N*′-dicyclohexylcarbidiimide; DMF, *N*,*N*-dimethylformamide; THF, tetrahydrofuran; TLC, thin-layer chromatography

■ REFERENCES

(1) Center for disease control and prevention, public health dispatch: Vancomycin-resistant *Staphylococcus aureus-Pennsylvania*. *MMWR Morb. Mortal. Wkly. Rep.* **2002**, *51*, 902−902.

(2) Levy, S. B.; Marshall, B. Antibacterial resistance worldwide: Cause, challenges and responses. *Nat. Med.* **2004**, *10*, S122–S129.

(3) Aspa, J.; Rajas, O.; Rodriguez de Castro, F.; Blanquer, J.; Zalacain, R.; Fenoll, A.; de Celis, R.; Vargas, A.; Salvanes, F. R.; Espana, P. P.; Rello, J.; Torres, A. Drug-resistant *pneumococcal pneumonia*: Clinical relevance and related factors. *Clin. Infect. Dis.* **2004**, *38*, 787–798.

(4) Barrett, C. T.; Barrett, J. F. Antibacterials: are the new entries enough to deal with the emerging resistance problems?. *Curr. Opin. Biotechnol.* **2003**, *14*, 621–626.

(5) Livermore, D. M. The need for new antibiotics. *Clin. Microbial. Infect.* **2004**, *10* (Suppl. 4), 1–9.

(6) Jacobs, M. R. Worlwide trends in antimicrobial resistance among common respiratory tract pathogens in children. *Pediatr. Infect. Dis. J.* **2003**, *22*, S109–S119.

(7) Häbich, D.; von Nussbaum, F. Platensimycin, a new antibiotic and "superbug challenger" from nature. *ChemMedChem* **2006**, *1*, 951–954.

(8) Thomson, C. J.; Power, E.; Ruebsamen-Waigmann, H.; Labischinski, H. Antibacterial research and development in the 21(st) century—an industry perspective of the challenges. *Curr. Opin. Microbiol.* **2004**, *7*, 445–450.

(9) Mutnick, A. H.; Enne, V.; Jones, R. N. Linezolid resistance since 2001. SENTRY Antimicrobial Surveillance Program. *Ann. Pharmacother.* **2003**, *37*, 909–911.

(10) Hachem, R. Y.; Hicks, K.; Huen, A.; Raad, I. Myelosupperssion and serotonin syndrome associated with concurrent use of linezolid and selective serotonin reuptake inhibitora in bone marrow transplant recipients. *Clin. Infect. Dis.* **2003**, *37*, e8–e11.

(11) Hidron, A. I.; Schuetz, A. N.; Nolte, F. S.; Gould, C. V.; Osborn, M. K. Daptomycin resistance in enterococcus faecalis prosthetic valve endocarditis. *Antimicrob. Chemother.* **2008**, *61*, 1394–1396.

(12) Maxwell, A. DNA gyrase as a drug target. *Trends Microbiol.* **1997**, *5*, 102–109.

(13) Maxwell, A.; Lawson, D. M. The ATP binding site of type II topoisomerases as a target for antibacterial drugs. *Curr. Top. Med. Chem.* **2003**, *3*, 283–303.

(14) Drlica, K.; Zhao, X. DNA gyrase, topisomerase IV and the 4-quinolones. *Microbiol. Mol. Biol. Rev.* **1997**, *61*, 377–392.

(15) Heisig, P. Inhibitors of bacterial topoisomerases: Mechanisms of action and resistance and clinical aspects. *Planta Med.* **2001**, *67*, 3–12.

(16) Ferrero, L.; Cameron, B.; Manse, B.; Lagneaux, D.; Crouzet, J.; Famechon, A.; Blanche, F. Cloning and primary structure of *Staphylococcus aureus* DNA topoisomerase IV: A primary target of fluoroquinolones. *Mol. Microbiol.* **1994**, *13*, 641–653.

(17) Kato, J.-I.; Nishimura, Y.; Imamura, R.; Niki, H.; Hiraga, S.; Suzuki, H. New topoisomerases essential for chromosome segregation in *E. coli*. *Cell* **1990**, *63*, 393–404.

(18) Deibler, R. W.; Rahmati, S.; Zechiedrich, E. L. Topoisomerase IV, alone, unknots DNA in *E. coli*. *Genes Dev.* **2001**, *15*, 748–761.

(19) Zechiedrich, E. L.; Khodrusky, A. B.; Bachellier, S.; Schneider, R.; Chen, D.; Lilley, D. M. J.; Cozzarelli, N. R. Roles of topoisomerases in maintaining steady-state DNA supercoiling in *Escherichia coli*. *J. Biol. Chem.* **2000**, *275*, 8103–8113.

(20) Chen, A. Y.; Liu, L. F. DNA Topoisomerases: Essential enzymes and lethal targets. *Annu. Rev. Pharmacol. Toxicol.* **1994**, *34*, 191–218.

(21) Bellon, S.; Parsons, J. D.; Wei, Y.; Haykawa, K.; Swenson, L. L.; Charifson, P. S.; Lippke, J. A.; Aldape, R.; Gross, C. H. Crystal structures of *Escherichia coli* topoisomerase IV ParE subunit (24 and 43 kDas): A single residue dictates differences in novobiocin potency against topoisomerase IV and DNA gyrase. *Antimicrob. Agents Chemother.* **2004**, *48*, 1856–1864.

(22) Gross, C. H.; Parsons, J. D.; Grossman, T. H.; Charifson, P. S.; Bellon, S.; Jernee, J.; Dwyer, M.; Chambers, S. P.; Markland, W.; Botfield, M.; Raybuck, S. A. Active-site residues of *Escherichia coli* DNA gyrase required in coupling ATP hydrolysis to DNA supercoiling and amino acid substitutions leading to novobiocin resistance. *Antimicrob. Agents Chemother.* **2003**, *47*, 1037–1046.

(23) Muñoz, R.; Bustamante, M.; de la Campa, A. G. Ser-127-to-Leu substitution in the DNA gyrase B subunit of *Streptococcus pneumoniae* is implicated in nivobiocin resistance. *J. Bacteriol.* **1995**, *177*, 4166–4170.

(24) Booker, B. M.; Smith, P. F.; Forrest, A.; Bullock, J.; Kelchlin, P.; Bhavnani, S. M.; Jones, R. N.; Ambrose, P. G. Application of an *in vitro* infection model and simulation for reevaluation of fluoroquinolone breakpoints for *Salmonella enterica* Serotype Typhi. *Antimicrob. Agents Chemother.* **2005**, *49*, 1775–1781.

(25) Jones, R. N. Should novobiocin be clinically re-evaluated?. *Diagn. Microbiol. Infect. Dis.* **1989**, *12*, 363–365.

(26) Kaldas, M. I.; Walle, K.; van der Woude, H.; McMillan, J. M.; Walle, T. Covalent binding to the flavonoid quercetin to human serum albumin. *J. Agric. Food. Chem.* **2005**, *53*, 4194–4197.

(27) Castillo-Muñoz, N.; Gómez-Alonso, S.; García-Romero, E.; Gómez, M. V.; Velders, A. H.; Hermosín-Gutiérrez, I. Flavonol 3-O-glycosides series of *Vitis vinifera* CV. Petit Verdot red wine grapes. *J. Agric. Food. Chem.* **2009**, *57*, 209–219.

(28) Hossion, A. M. L.; Otsuka, N.; Kandahary, R. K.; Tsuchiya, T.; Ogawa, W.; Iwado, A.; Zamami, Y.; Sasaki, K. Design, synthesis and biological evaluation of a novel series of quercetin diacylglucosides as potent anti-MRSA and anti-VRE agents. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 5349–5352.

(29) Sasaki, K.; Tsuchiya, T.; Hossion, A. M. L. Novel flavanone derivative. PCT Int. Appl. WO 2011013735, 2011; National University Corporation Okayama University: Japan; http://www.sumobrain.com/patents/wipo/Novel-flavanone-derivative/WO2011013735.html.

(30) Bloor, S. J. An antimicrobial kaempferol-diacyl-rhamnoside from *Pentachondra pumila*. *Phytochemistry* **1995**, *38*, 1033–1035.

(31) Otsuka, N.; Liu, M.-H.; Shiota, S.; Ogawa, W.; Kuroda, T.; Hatano, T.; Tsuchiya, T. Anti-methicillin resistant *Staphylococcus aureus* (MRSA) compounds isolated from *Laurus nobilis*. *Biol. Pharm. Bull.* **2008**, *31*, 1794–1797.

(32) Liu, M, -H.; Otsuka, N.; Noyori, K.; Shiota, S.; Ogawa, W.; Kuroda, T.; Hatano, T.; Tsuchiya, T. Synergistic effect of kaempferol glycosides purified from *Laurus nobilis* and fluloroquinolones on methicillin-resistant *Staphylococcus aureus*. *Biol. Pharm. Bull.* **2009**, *32*, 489–492.

(33) Chen, L.; Li, J.; Luo, C.; Xu, W.; Chen, G.; Liew, O. W.; Zhu, W.; Puah, C. M.; Shen, X.; Jiang, H. Binding interection of quercetin-3-β-galctoside and its synthetic derivatives with SARS-CoV $3CL^{pro}$: Structure-activity relationship studies reveal salient pharmacophore features. *Bioorg. Med. Chem.* **2006**, *14*, 8295–8306.

(34) Neises, B.; Steglich, W. Esterification of carboxylic acids with diacylcyclohexylcarbodiimide/4-dimethylaminopyridine: *tert*-Butyl ethyl formate. *Organic Syntheses*; Wiley & Sons: New York, 1990; Vol. 7, pp 93−93.

(35) Bauman, W. C.; Skidmore, J. R.; Osmun, R. H. DOWEX 50—A new high capacity cation exchange resisn. *Ind. Eng. Chem.* **1948**, *40*, 1350–1355.

(36) Clinical and Laboratory Standard Institute (CLSI). *Methods for Dilution Antibacterial Susceptibility Test for Bacteria That Grow Aerobically*, 7th ed.; Approved Standard (MA7-A7); Clinical and Laboratory Standard Institute: Wayne, 2007; Vol. 27, p 133.

(37) Plaper, A.; Golob, M.; Hafner, I.; Oblak, M.; Solmajer, T.; Jerela, R. Characterization of quercetin binding site on DNA gyrase. *Biochem. Biophys. Res. Commun.* **2003**, *306*, 530–536.

(38) Marcu, M. G.; Chadli, A.; Bouchouche, I.; Catelli, M.; Neckers, L. M. The heat shock protein 90 antagonist novobiocin interacts with a previously unrecognized ATP-binding domain in the carboxyl terminus of the chaperone. *J. Biol. Chem.* **2000**, *275*, 37181–37186.

(39) Gradišar, H.; Pristovsek, P.; Plaper, A.; Jerala, R. Green tea catechins inhibit bacterial DNA gyrase by interection with its ATP binding site. *J. Med. Chem.* **2007**, *50*, 264–271.

(40) Levine, C.; Hiasa, H.; Marians, K. J. DNA gyrase and topoisomerase IV: biochemical activities, physiological roles during chromosomes replication, and drug sensitivities. *Biochim. Biophys. Acta* **1998**, *1400*, 29–43.

(41) Gellert, M.; Mizuuchi, K.; O'Dea, M. H.; Nash, H. A. DNA gyrase: An enzyme that introduces superhelical turns into DNA. *Proc. Natl. Acad. Sci. U.S.A.* **1976**, *73*, 3872–3876.

(42) Ferrero, L.; Cameron, B.; Crouzet, J. Analysis of *gyrA* and *grlA* muatations in stepwise-selected ciprofloxacin-resistant mutants of *Staphylococcus aureus*. *Antimicrob. Agents Chemother.* **1995**, *39*, 1554–1558.

(43) Yamagishi, J. -I.; Kojima, T.; Oyamada, Y.; Fujimoto, K.; Hattori, H.; Nakamura, S.; Inoue, M. Alternations in the DNA topoisomerase IV *grlA* gene responsible for quinolone resistance in *Staphylococcus aureus*. *Antimicrob. Agents Chemother.* **1996**, *40*, 1157–1163.

(44) Rosenfeld, R. J.; Goodsell, D. S.; Musah, R. A.; Morris, G. M.; Goodin, D. B.; Olson, A. J. Automated docking of ligands to an artificial active site: Augmenting crystallographic analysis with computer modeling. *J. Comput.-Aided Mol. Des.* **2003**, *17*, 525–536.

(45) Doluisio, J. T.; Billups, N. F.; Dittert, L. W.; Sugita, E. T.; Swintosky, J. V. Drug absorption I: an *in situ* rat gut technique yielding realistic absorption rates. *J. Pharm. Sci.* **1969**, *58*, 1196–1200.

(46) Belland, R. J.; Morrison, S. G.; Ison, C.; Huang, W. M. *Neisseria gonorrhoeae* acquires mutations in analogous regions of gyrA and parC in fluoroquinolone-resistant isolates. *Mol. Microbiol.* **1994**, *14*, 371–380.

(47) Bürli, R. W.; Ge, Y.; White, S.; Baird, E. E.; Touami, S. M.; Taylor, M.; Kaizerman, J. A.; Moser, H. E. DNA binding ligands with excellent antibiotic potency against drug-resistant Gram-positive bacteria. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2591–2594.

(48) Kaizerman, J. A.; Gross, M. I.; Ge, Y.; White, S.; Hu, W.; Duan, J. X.; Baird, E. E.; Johnson, K. W.; Tanaka, R. D.; Moser, H. E.; Bürli, R. W. DNA binding ligands targeting drug-resistant bacteria: Structure, activity, and pharmacology. *J. Med. Chem.* **2003**, *46*, 3914–3929.

(49) Khalaf, A. I.; Waigh, R. D.; Drummond, A. J.; Pringle, B.; McGroarty, I.; Skellern, G. G.; Suckling, C. J. Distamycin analogues with enhanced lipophilicity: synthesis and antimicrobial activity. *J. Med. Chem.* **2004**, *47*, 2133–2156.

(50) Hu, W.; Bürli, R. W.; Kaizerman, J. A.; Johnson, K. W.; Gross, M. I.; Iwamoto, M.; Jones, P.; Lofland, D.; Difuntorum, S.; Chen, H.; Bozdogan, B.; Appelbaum, P. C.; Moser, H. E. DNA binding ligands with improved *in vitro* and *in vivo* potency against drug-resistant *Staphylococcus aureus*. *J. Med. Chem.* **2004**, *47*, 4352–4355.

(51) Grossman, T. H.; Bartels, D. J.; Mullin, S.; Gross, C. H.; Parsons, J. D.; Liao, Y.; Grillot, A.-L.; Stamos, D.; Olson, E. R.; Charifson, P. S.; Mani, N. Dual targeting of GyrB and ParE by a novel aminobenzimidazole class of antibacterial compounds. *Antimicrob. Agents Chemother.* **2007**, *51*, 657–666.

(52) Anthony, N. G.; Breen, D.; Clarke, J.; Donoghue, G.; Drummond, A. J.; Ellis, E. M.; Gemmell, C. G.; Helebeux, J. J.; Hunter, I. S.; Khalaf, A. I.; Mackay, S. P.; Parkinson, J. A.; Suckling, C. J.; Waigh, R. D. Antimicrobial lexitropsins containing amide, amidine, and alkene linking groups. *J. Med. Chem.* **2007**, *50*, 6116–6125.

(53) Piddock, L. J. V.; Jin, Y. F.; Griggs, D. J. Effect of hydrophobicity and molecular mass on the accumulation of fluoroquinolones by *Staphylococcus aureus*. *J. Antimicrob. Chemother.* **2001**, *47*, 261–270.

(54) Hooper, D. C. Mechanisms of action and resistance of older and newer fluoroquinolones. *Clin. Infect. Dis.* **2000**, *31* (Suppl. 2), S24–28.

(55) O'Shea, R.; Moser, H. E. Physicochemical properties of antibacterial compounds: Implifications for drug discovery. *J. Med. Chem.* **2008**, *51*, 2871–2878.

(56) Bryan, L. E.; Bedard, J. Impemeability to quinolones in Gram-positive and Gram-negative bacteria. *Eur. J. Clin. Microbiol. Infect. Dis.* **1991**, *10*, 232–239.

(57) Walle, T. Absorption and metabolism of flavonoids. *Free Radical Biol. Med.* **2004**, *36*, 829–837.

(58) Krolicka, A.; Szpitter, A.; Gilgenast, E.; Romanik, G.; Kaminski, M.; Lojkowska, E. Stimulation of antibacterial naphthoquinones and flavonoids accumulation in carnivorous plants grown *in vitro* by addition of elicitors. *Enzyme Microb. Technol.* **2008**, *42*, 216–221.

(59) Biasutto, L.; Marotta, E.; De March, U.; Zoratti, M.; Paradisi, C. Ester based precursors to increase the bioavailability of quercetin. *J. Med. Chem.* **2007**, *50*, 241–253.

(60) Hallett, P.; Grimshaw, A. J.; Wigley, D. B.; Maxwell, A. Cloning of the DNA gyrase genes under *tac* promoter control: Overproduction of the gyrase A and B proteins. *Gene* **1990**, *93*, 139–142.

(61) Peng, H.; Marians, K. J. Overexpression and purification of bacterial topoisomerase IV. *Methods Mol. Biol.* **1999**, *94*, 163–169.