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Dihydroxybergamottin Caproate as a Potent and Stable CYP3A4 Inhibitor

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Abstract—We investigated the inhibitory activity of the furanocoumarin derivatives from grapefruit juice to the drug metabolizing enzyme, cytochrome P450 (CYP) 3A4. Although two known furanocoumarin dimers GF-I-1 (**1**) and GF-I-4 (**2**) showed potent CYP3A4 inhibition with IC_{50} value of 0.07 μM, a semi-synthetic dihydroxybergamottin caproate (**11**), which was more stable and more simple than the dimers, exhibited comparable activity against CYP3A4.

Introduction

In 1991, Baily et al. reported that concomitant oral administration of grapefruit juice increased the bioavailability of dihydropyridine-type calcium channel blockers, felodipine and nifedipine; the bioavailability of nifedipine 10 mg with grapefruit juice was 134% of that with water.[1] Although the biologically active substances in grapefruit juice were not identified, it was the first example of a pharmacokinetic interaction between a citrus juice and a drug. Subsequently, similar phenomena in the pharmacokinetics have been reported for various clinically important drugs, for example cyclosporine,[2,3] midazolam,[4] and triazolam.[5] Although no structural similarities were observed in these drugs, it was shown that compounds in grapefruit juice affected the drug metabolism in humans by inhibition of cytochrome P450 (CYP) 3A4. CYP enzymes are heme-containing monooxygenases, and majority of these enzymes have been expressed in liver microsomes and are recognized to be responsible for drug metabolism, carcinogenesis, and degradation of xenobiotics as well as biosynthesis of steroids, lipids, and other secondary metabolites.[6] CYP3A4 is present most abundantly in human liver microsomes; approximately 30% of the total CYP are suggested to be CYP3A4.[7] Since recent investigation has shown that more than 50% of clinically used drugs have been oxidized by CYP3A4,[8,9] the elucidation of the mechanism of CYP3A4 inhibition is an important subject in terms of pharmacokinetics and bioavailability of drugs.

We have reported the isolation of furanocoumarin dimers, GF-I-1 (**1**) and GF-I-4 (**2**), as specific CYP3A4 inhibitors with IC_{50} value of 0.07 μM.[10–13] Although administration of these inexpensive CYP3A4 inhibitors can reduce drug dose, which led to save cost for patients significantly, the dimers are unstable[14] and are present in grapefruit juice in the low concentration. Therefore, development of non-toxic, stable, and inexpensive CYP3A4 inhibitors have been required. In this study, we made several modifications to dihydroxybergamottin (**3**)[13] to obtain a potent and stable CYP3A4 inhibitor.

Results and Discussion

In order to find out the functional group responsible for the CYP3A4 inhibition, we first examined the IC_{50} values of dihydroxybergamottin (**3**), bergamottin (**4**), bergaptol (**5**), and 7-geranyloxycoumarin (**6**). 5-Geranyloxyfurocoumarine derivatives **3** and **4** showed moderate activity with IC_{50} values of 2.3 and 5.4 μM (Table 1), respectively, while a furocoumarine derivative without a geranyl group **5** and a coumarin derivative **3** were inactive at 10 μM. Hence, this data strongly suggests that the presence of 5-geranyloxyfurocoumarine moiety is essential for the CYP3A4 inhibition, while no contribution of two hydroxy groups was observed (Chart 1).

To investigate the effect of substituent at the terminal of the geranyl group, an alcohol **7**, an aldehyde **8**, a car-

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boxylic acid **9**, and an ester **10** were prepared from **3**. The compound **9** was inactive, while **7** and **8** showed moderate CYP3A4 inhibitory activity with IC_{50} values of 3.4 and 2.1 μM, respectively. The ester **10** showed significant activity (IC_{50} value, 0.35 μM) (Table 1).

Then, we prepared six esters **11**–**16** bearing C_6–C_{16} saturated fatty acids at C-17 positions to examine the effect of acyl groups on the CYP3A4 inhibition. Interestingly, **11** showed the most potent inhibition with IC_{50} value of 0.07 μM, which is comparable to furanocoumarin dimers **1** and **2**. The inhibitory activity of esters **11**–**16** decreased with increasing number of carbons in fatty acid moieties (Table 1). On the other hand, no increase in the activity was exhibited by acetates **17** and **18** (IC_{50} values, 0.05 and 0.18 μM, respectively) derived from **1** and **2**, respectively (Table 1).

Conclusion

In natural and synthetic furanocoumarines, furanocoumarin dimers, GF-I-1 (**1**) and GF-I-4 (**2**), and dihydroxybergamottin caproate (**11**) showed the prominent CYP3A4 inhibition with IC_{50} value of 0.07 μM. A 5-geranyloxyfuranocoumarine moiety was found to be essential for the CYP3A4 inhibition. Furthermore, substitution at the C-17 or C-18 position with suitable size increased the activity.

The inhibition of the drug metabolizing enzyme result is an important consequence in pharmacokinetics and bioavailability of drugs. In the cases of **1** and **2**, their instability and their low concentration in the grapefruit juice hampered the possibility of being an inexpensive alternative which will reduce a drug dose. Dihydroxybergamottin caproate (**11**) seems to be a good candidate as an alternative as well as for study of a mechanism of the effect.

Table 1. CYP3A4 inhibition of furanocoumarine derivatives

Compound	IC_{50} (μM)	Compound	IC_{50} (μM)	Compound	IC_{50} (μM)
1	0.07	**7**	3.4	**13**	0.30
2	0.07	**8**	2.1	**14**	0.93
3	2.3	**9**	>10	**15**	>10
4	5.4	**10**	0.35	**16**	>10
5	>10	**11**	0.07	**17**	0.05
6	>10	**12**	0.12	**18**	0.18
Ketoconazole[a]	0.11				

[a]A typical CYP3A4 inhibitor.[15]

Experimental

General methods

UV spectra were measured on a Shimadzu UV-1600 UV–visible spectrophotometer. IR spectra were recorded on a Shimadzu IR-460 infrared spectrophotometer. NMR spectra in $CDCl_3$ were recorded on a JEOL GSX500 NMR spectrometer. All chemical shifts were reported with respect to $CDCl_3$ (δ_H 7.26, δ_C 77.0). Mass spectra were measured on a JEOL SX-102 mass spectrometer.

Chemicals

Nifedipine was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Glucose-6-phosphate, β-$NADP^+$, and glucose-6-phosphate dehydrogenase were purchased from Oriental Yeast Co., Ltd. (Tokyo, Japan). CYP3A4 was purchased from Gentest Co. (Woburn, MA, USA). Grapefruit juice was purchased from Kirin Co., Ltd. (Tokyo, Japan).

CYP inhibition assay

CYP activity was based on nifedipine oxidation. Various amounts (0–10 μM, final concentration) of samples in 1 μL of DMSO were added to 192 μL of solution containing 100 mM phosphate buffer (pH 7.4) containing 50 μM nifedipine, 5 mM glucose-6-phosphate, 0.5 mM β-$NADP^+$, 0.5 mM $MgCl_2$, and 4.3 μg/mL glucose-6-phosphate dehydrogenase and incubated at 37 °C for 5 min. CYP3A4 was also preincubated at 37 °C for 5 min in 7 μL of the buffer and added to the sample solution. After the incubation at 37 °C for 1 h, the reaction was terminated by the addition of 100 μL of MeOH. After adding 3.7 μg of 6-methoxycarbonyl-5-

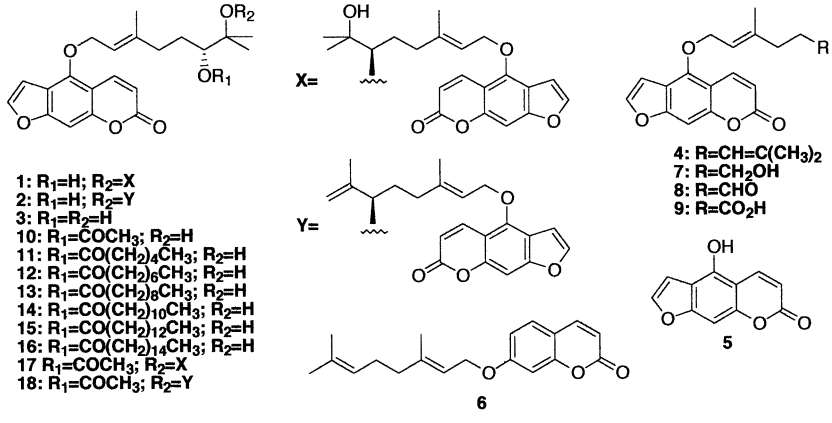

Chart 1.

methyl-7-(2-nitrophenyl)-4,7-dihydrofuro[3,4-b]pyridin-1-(3*H*)-one in 1 μL of DMSO as an internal standard, the reaction mixture was extracted with 1 mL of ether, and the ether layer was evaporated. The residue was dissolved in 100 μL of MeOH, and an aliquot (20 μL) was analyzed by reverse phase HPLC (column, TSK-gel ODS-120T, 4.6 mm i.d. × 150 mm; mobile phase, 64% MeOH-H_2O; flow rate, 1.0 mL/min; detection, UV 254 nm); retention times: 2.9 min for the internal standard, 4.0 min for the nifedipine metabolite (nifedipine pyridine), and 5.5 min for nifedipine.

Extraction and isolation

Grapefruit juice (30 L) was extracted with hexane/AcOEt (1:1, 10 L × 3). The organic layer was concentrated under reduced pressure, and an oily residue (9 g) was subjected to silica gel chromatography with hexane/AcOEt (3:1, 1:1, 1:2) and AcOEt as eluents to afford four fractions. The first (3.5 g) and second (0.4 g) fractions were each purified by reverse phase HPLC with 82% MeOH–H_2O to afford 5-geranyloxypsoraren (**4**, 45 mg) and GF-I-4 (**2**, 4 mg), respectively. The third fraction (1.1 g) was purified by reverse phase HPLC with 82% MeOH–H_2O to afford GF-I-1 (**1**, 14 mg) and a fraction containing dihydroxybergamottin (**3**), which was purified by reverse-phase HPLC with 60% MeOH–H_2O to afford **3** (115 mg).

Preparation of 8. To the solution of **3** (6.2 mg) in THF (90 μL), water (90 μL) and sodium metaperiodate (4.7 mg) were added. The mixture was stirred at room temperature for 3 h and extracted with $CHCl_3$. The $CHCl_3$ layer was dried over Na_2SO_4 and evaporated under reduced pressure. The residue was subjected to silica gel chromatography with hexane/AcOEt (3:1) to afford **8** (3.3 mg) as a colorless oil.

8: UV (CH_3CN) λ_{max} (log ε) 308 (4.8), 251 nm (4.1); IR (KBr) ν_{max} 1734, 1622, 1582, 1456 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.71 (s, 3H), 2.40 (t, 2H, J = 7.6 Hz), 2.59 (t, 2H, J = 9.8 Hz), 4.94 (d, 2H, J = 6.8 Hz), 5.56 (t, 1H, J = 6.8 Hz), 6.28 (dd, 1H, J = 1.0, 9.8 Hz), 6.98 (dd, 1H, J = 1.0, 2.4 Hz), 7.17 (s, 1H), 7.60 (dd, 1H, J = 1.0, 2.5 Hz), 8.13 (d, 1H, J = 9.8 Hz), 9.78 (d, 1H, J = 1.0 Hz); FABMS m/z 313 $[M+H]^+$; HRFABMS m/z 313.1061 ($C_{18}H_{17}O_5$, Δ −1.5 mmu).

Preparation of 7. The aldehyde **8** was obtained from 4.9 mg of **3** in the same manner described above. To the solution of **8** in MeOH (100 μL), $NaBH_4$ (1.2 mg) was added and the mixture was allowed to stand at room temperature for 1 h. The mixture was added with ice and extracted with ether. The organic extract was dried in vacuo, and the residue was purified by silica gel chromatography with hexane/AcOEt (3:1) to afford **7** (2.0 mg) as a colorless oil.

7: UV (MeOH) λ_{max} (log ε) 310 (4.0), 251 (4.1), 225 nm (4.3); IR (KBr) ν_{max} 1732, 1718, 1622, 1580, 1543, 1458 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.70 (m, 2H), 1.71 (s, 3H), 2.16 (t, 2H, J = 7.6 Hz), 3.64 (t, 2H, J = 6.3 Hz), 4.95 (d, 2H, J = 7.3 Hz), 5.58 (t, 1H, J = 6.8 Hz), 6.27 (d, 1H, J = 9.8 Hz), 6.95 (d, 1H, J = 2.4 Hz), 7.16 (s, 1H), 7.60 (d, 1H, J = 2.4 Hz), 8.15 (d, 1H, J = 9.8 Hz); FABMS m/z 315 $[M+H]^+$; HRFABMS m/z 315.1241 ($C_{18}H_{19}O_5$, Δ +0.9 mmu).

Preparation of 9. The aldehyde **8** was obtained from 4.2 mg of **3** in the same manner described above. To the solution of **8** in *t*-BuOH (10 μL), 2-methyl-2-butene (20 μL), THF (200 μL), $NaClO_2$ (1.7 mg), $NaH_2PO_4 \cdot H_2O$ (1.6 mg), and H_2O (30 μL) were added, and the mixture was left stirring at room temperature for 19 h. The reaction mixture was extracted with $CHCl_3$. The organic layer was washed with 0.1 M $NaHSO_4$ and water, dried over $MgSO_4$, and evaporated under the reduced pressure. The residue was purified by reverse phase HPLC with 90% MeOH–H_2O to afford **9** (2.0 mg) as a colorless oil.

9: UV (MeOH) λ_{max} (log ε) 310 (4.0), 268 (4.0), 260 (4.0), 251 (4.1), 226 nm (4.2); IR (KBr) ν_{max} 1734, 1717, 1701, 1684, 1653, 1634, 1624, 1558, 1541, 1508, 1458 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.72 (s, 3H), 2.42 (t, 2H, J = 7.6 Hz), 2.51 (t, 2H, J = 7.8 Hz), 4.94 (d, 2H, J = 6.8 Hz), 5.59 (t, 1H, J = 6.1 Hz), 6.28 (d, 1H, J = 9.8 Hz), 6.93 (d, 1H, J = 2.0 Hz), 7.16 (s, 1H), 7.59 (d, 1H, J = 2.0 Hz), 8.13 (d, 1H, J = 9.8 Hz); FABMS m/z 329 $[M+H]^+$; HRFABMS m/z 329.1016 ($C_{18}H_{17}O_6$, Δ −0.9 mmu).

Preparation of 10. The solution of **3** (5.0 mg) containing a mixture of pyridine (100 μL) and acetic anhydride (100 μL) was stirred at room temperature for 12 h. The mixture was dried in vacuo, and the residue was purified by silica gel chromatography with hexane/AcOEt (3:1) to afford **10** (3.9 mg) as a colorless oil.

10: UV (MeOH) λ_{max} (log ε) 311 (4.1), 268 (4.2), 261 (4.2), 251 (4.1), 225 nm (4.3); IR (KBr) ν_{max} 1736, 1718, 1701, 1655, 1624, 1560, 1541, 1458 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.19 (s, 3H), 1.20 (s, 3H), 1.69 (s, 3H), 1.75 (m, 2H), 2.07 (m, 2H), 2.12 (s, 3H), 4.80 (dd, 1H, J = 2.4, 10.3 Hz), 4.94 (d, 2H, J = 6.8 Hz), 5.54 (t, 1H, J = 6.8 Hz), 6.27 (d, 1H, J = 9.8 Hz), 6.95 (dd, 1H, J = 1.0, 2.4 Hz), 7.16 (s, 1H), 7.59 (d, 1H, J = 2.4 Hz), 8.17 (d, 1H, J = 9.8 Hz); FABMS m/z 415 $[M+H]^+$; HRFABMS m/z 415.1789 ($C_{23}H_{27}O_7$, Δ +3.2 mmu).

Preparation of 11–16. To the solution of **3** (5.9 mg) in dry ether (500 μL), pyridine (10 μL), *n*-caproyl chloride (10 μL), and DMAP (1.0 mg) was added, and the mixture was stirred at room temperature for 16 h. The solution was evaporated under reduced pressure, and the residue was purified by silica gel chromatography with hexane/AcOEt (3:1) and reverse phase HPLC with 90% MeOH–H_2O to afford **11** (2.8 mg) as a colorless oil.

11: UV (MeOH) λ_{max} (log ε) 312 (3.9), 251 (4.0), 224 nm (4.1); IR (KBr) ν_{max} 1734, 1718, 1653, 1636, 1624, 1456 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.89 (3H, J = 6.8 Hz), 1.19 (s, 3H), 1.20 (s, 3H), 1.31–1.34 (m, 6H), 1.69 (s, 3H), 1.77 (m, 2H), 2.06 (m, 2H), 2.36 (t, 2H, J = 8.0 Hz), 4.80 (dd, 1H, J = 1.5, 9.8 Hz), 4.93 (d, 2H, J = 6.8 Hz), 5.54 (t, 1H, J = 6.8 Hz), 6.27 (dd, 1H, J = 1.5, 9.8 Hz),

6.95 (d, 1H, $J=2.4$ Hz), 7.16 (s, 1H), 7.59 (dd, 1H, $J=1$, 2 Hz), 8.17 (d, 1H, $J=9.8$ Hz). FABMS m/z 471 $[M+H]^+$; HRFABMS m/z 471.2426 ($C_{23}H_{35}O_7$, Δ +4.4 mmu).

Esters **12**–**16** were also prepared in the same manner as that of **11**.

12: UV (MeOH) λ_{max} (log ε) 311 (3.9), 268 (4.0), 251 (4.0), 224 nm (4.2); IR (KBr) ν_{max} 1734, 1717, 1699, 1684, 1653, 1634, 1558, 1539, 1506, 1456 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.87 (t, 3H, $J=7.0$ Hz), 1.19 (s, 3H), 1.20 (s, 3H), 1.26–1.43 (m, 10H), 1.69 (s, 3H), 1.77 (m, 2H), 2.07 (m, 2H), 2.36 (t, 2H, $J=7.5$ Hz), 4.81 (dd, 1H, $J=2.4$, 10.3 Hz), 4.94 (d, 2H, $J=6.8$ Hz), 5.54 (dd, 1H, $J=5.4$, 6.8 Hz), 6.27 (d, 1H, $J=9.8$ Hz), 6.95 (dd, 1H, $J=1$, 2.4 Hz), 7.16 (s, 1H), 7.60 (d, 1H, $J=2.4$ Hz), 8.17 (d, 1H, $J=9.8$ Hz); FABMS m/z 499 $[M+H]^+$; HRFABMS m/z 499.2694 ($C_{29}H_{39}O_7$, Δ −0.2 mmu).

13: UV (MeOH) λ_{max} (log ε) 311 (4.0), 268 (4.1), 251 (4.1), 226 nm (4.2); IR (KBr) ν_{max} 1734, 1624, 1458 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.87 (t, 3H, $J=7.0$ Hz), 1.19 (s, 3H), 1.20 (s, 3H), 1.25–1.34 (m, 14H), 1.69 (s, 3H), 1.77 (m, 2H), 2.06 (m, 2H), 2.36 (t, 2H, $J=7.5$ Hz), 4.82 (dd, 1H, $J=2.4$, 10.3 Hz), 4.93 (d, 2H, $J=6.3$ Hz), 5.54 (t, 1H, $J=6.8$ Hz), 6.27 (d, 1H, $J=9.8$ Hz), 6.95 (dd, 1H, $J=1$, 2.4 Hz), 7.16 (s, 1H), 7.59 (d, 1H, $J=2.4$ Hz), 8.17 (d, 1H, $J=9.8$ Hz); FABMS m/z 527 $[M+H]^+$; HRFABMS m/z 527.3012 ($C_{31}H_{43}O_7$, Δ +0.3 mmu).

14: UV (MeOH) λ_{max} (log ε) 313 (4.0), 268 (4.2), 251 (4.2), 226 nm (4.3); IR (KBr) ν_{max} 1734, 1718, 1701, 1686, 1655, 1560, 1541, 1508, 1458 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.88 (t, 3H, $J=6.8$ Hz), 1.19 (s, 3H), 1.20 (s, 3H), 1.25–1.35 (m, 18H), 1.69 (s, 3H), 1.77 (m, 2H), 2.06 (m, 2H), 2.36 (m, 2H), 4.81 (dd, 1H, $J=2.4$, 10.3 Hz), 4.94 (d, 2H, $J=6.8$ Hz), 5.55 (t, 1H, $J=6.8$ Hz), 6.28 (d, 1H, $J=9.8$ Hz), 6.96 (d, 1H, $J=2.4$ Hz), 7.16 (s, 1H), 7.60 (d, 1H, $J=2.4$ Hz), 8.17 (d, 1H, $J=9.8$ Hz); FABMS m/z 555 $[M+H]^+$; HRFABMS m/z 555.3330 ($C_{33}H_{47}O_7$, Δ +0.8 mmu).

15: UV (MeOH) λ_{max} (log ε) 312 (3.9), 268 (4.0), 251 (4.1), 226 nm (4.2); IR (KBr) ν_{max} 1734, 1624, 1456 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.87 (t, 3H, $J=6.8$ Hz), 1.19 (s, 3H), 1.20 (s, 3H), 1.24–1.33 (m, 22H), 1.69 (s, 3H), 1.77 (m, 2H), 2.06 (m, 2H), 2.36 (m, 2H), 4.80 (dd, 1H, $J=2.4$, 9.8 Hz), 4.94 (d, 2H, $J=6.8$ Hz), 5.54 (t, 1H, $J=6.8$ Hz), 6.27 (d, 1H, $J=9.8$ Hz), 6.95 (d, 1H, $J=2.4$ Hz), 7.16 (s, 1H), 7.59 (d, 1H, $J=2.0$ Hz), 8.17 (d, 1H, $J=9.8$ Hz); FABMS m/z 583 $[M+H]^+$; HRFABMS m/z 583.3634 ($C_{35}H_{51}O_7$, Δ −0.1 mmu).

16: UV (MeOH) λ_{max} (log ε) 311 (3.9), 251 (4.1), 225 nm (4.3); IR (KBr) ν_{max} 1736, 1718, 1686, 1655, 1624, 1560, 1541, 1508, 1458 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.88 (t, 3H, $J=6.8$ Hz), 1.19 (s, 3H), 1.20 (s, 3H), 1.24–1.33 (m, 26H), 1.69 (s, 3H), 1.77 (m, 2H), 2.06 (m, 2H), 2.36 (t, 2H, $J=7.5$ Hz), 4.80 (dd, 1H, $J=2.9$, 10.3 Hz), 4.94 (d, 2H, $J=6.8$ Hz), 5.54 (t, 1H, $J=6.8$ Hz), 6.27 (d, 1H, $J=9.8$ Hz), 6.95 (dd, 1H, $J=1.0$, 2.4 Hz), 7.16 (s, 1H), 7.59 (d, 1H, $J=2.4$ Hz), 8.17 (d, 1H, $J=9.8$ Hz); FABMS m/z 611 $[M+H]^+$; HRFABMS m/z 611.3975 ($C_{37}H_{55}O_7$, Δ +2.7 mmu).

Preparation of 17 and 18. Esters **17** and **18** were also prepared from **1** and **2**, respectively, in the same manner described for **10**.

17: UV (MeOH) λ_{max} (log ε) 311 (3.9), 251 (4.1), 227 nm (4.2); IR (KBr) ν_{max} 1734, 1624, 1580, 1456 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.15 (s, 3H), 1.17 (s, 3H), 1.19 (s, 3H), 1.20 (s, 3H), 1.50–1.82 (m, 4H), 1.68 (s, 3H), 1.69 (s, 3H), 2.03–2.26 (m, 4H), 2.09 (s, 3H), 3.34 (m, 1H), 4.92 (d, 2H, $J=6.4$ Hz), 4.93 (d, 1H, $J=6.8$ Hz), 5.53 (t, 1H, $J=6.8$ Hz), 5.54 (m, 1H), 6.26 (d, 1H, $J=9.8$ Hz), 6.27 (d, 1H, $J=9.8$ Hz), 6.94 (d, 1H, $J=2.4$ Hz), 6.95 (d, 1H, $J=2.9$ Hz), 7.15 (s, 1H), 7.16 (s, 1H), 7.59 (d, 1H, $J=2.0$ Hz), 7.60 (d, 1H, $J=2.0$ Hz), 8.14 (d, 1H, $J=9.8$ Hz), 8.17 (d, 1H, $J=9.8$ Hz); FABMS m/z 769 $[M+H]^+$; HRFABMS m/z 769.3218 ($C_{44}H_{49}O_{12}$, Δ −0.6 mmu).

18: UV (MeOH) λ_{max} (log ε) 311 (3.9), 251 (4.1), 223 nm (4.2); IR (KBr) ν_{max} 1736, 1657, 1626, 1580, 1558, 1458 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.07 (s, 3H), 1.10 (s, 3H), 1.28 (s, 3H), 1.40–1.73 (m, 4H), 1.66 (s, 3H), 1.69 (s, 3H), 1.90–2.09 (m, 4H), 2.07 (s, 3H), 3.90 (t, 1H, $J=5.8$ Hz), 4.80 (s, 1H), 4.85 (s, 1H), 4.92 (m, 4H), 5.36 (m, 1H), 5.55 (m, 1H), 6.25 (d, 1H, $J=9.8$ Hz), 6.26 (d, 1H, $J=9.8$ Hz), 6.93 (d, 1H, $J=2.4$ Hz), 6.94 (d, 1H, $J=2.9$ Hz), 7.13 (s, 1H), 7.14 (s, 1H), 7.58 (d, 1H, $J=2.4$ Hz), 7.59 (d, 1H, $J=2.0$ Hz), 8.14 (d, 1H, $J=9.8$ Hz), 8.16 (d, 1H, $J=9.8$ Hz); FABMS m/z 751 $[M+H]^+$; HRFABMS m/z 751.3106 ($C_{44}H_{47}O_{11}$, Δ −1.2 mmu).

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