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Synthesis and Bioactivity of Quercetin Aspirinates

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Hybrids of some natural compounds are promising to obtain new leads with good biological activities for drug discovery. Three quercetin aspirinates were synthesized by esterification of the 3-and 7-hydroxyl groups of quercetin with aspirin. Biological activities of these quercetin aspirinates were initially screened and showed better cytotoxic activities against tumor cell lines HL-60 and HepG2 and less scavenging activity against DPPH than the quercetin respectively.

Key Words : Quercetin, Polyphenol, Esterification, Quercetin aspirinates, Bioactivity

Introduction

Quercetin, a very efficient antioxidant,[1,2] is the widespread flavonoid in many foods and exhibits significant biological activities against many diseases such as cancer,[3,4] cardiovascular[5] and neurodegenerative diseases.[6] There has been a lot of study on synthesis,[7-9] functional elucidation,[10,11] and biological evaluation of quercetin and its derivatives[12-14] in recent years. In the development of drugs, hybrids as combinations of parts of different natural products is a new and promising approach to obtain the new leads because the biological activity of some new hybrids probably exceeds that of the parent compounds.[15] Due to the presence of multiple hydroxyl groups in the quercetin for easy esterification with carboxylic groups, as well as the similar physiological activities aganist cardiovascular diseases to the well-known medicine aspirin, hybrids of these two compounds may show better biological activities. Interested in this idea, we synthesized the quercetin aspirinates and screened their biological activities.

Experimental

General. The melting points were measured on WRS-1B digital melting points apparatus and are uncorrected. The progress of the reaction was monitored by TLC. ^{1}H NMR spectra were determined on a Brucker AVANCE 400 NMR spectrometer at 400 MHz in DMSO-d_6 using TMS as internal standard. Elemental analysis was estimated on an Elementar Vario EL-III element analyzer.

3',4'-*O*-Diphenylmethane Quercetin (2). compound **1** (302 mg, 1 mmol, 1 equiv) and dichlorodiphenylmethane (0.3 mL, 1.5 mmol, 1.5 equiv) in diphenyl ether (15 mL) were mixed and heated at 175 °C for 30 min. The residue was cooled to room temperature and petroleum ether (30 mL) was added to give a solid compound. Then solid was collected by filteration and purified by a silica gel column chromatography using petroleum ether/ethyl acetate (4:1) as eluent to afford a yellow solid 2. Yield = 66% (306 mg). R_f (petroleum ether/ethyl acetate 4:1) 0.50. mp 219-220 °C (lit.[21] 222-224 °C). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.35 (*s*, 1H), 10.79 (*s*, 1H), 9.60 (*s*, 1H), 7.81 (*d*, *J* = 1.8 Hz, 1H), 7.77 (*dd*, *J* = 8.8, 1.8 Hz, 1H), 7.58 (*m*, 4H), 7.46 (*m*, 6H), 7.23 (*d*, *J* = 8.8 Hz, 1H), 6.47 (*d*, *J* = 2.0 Hz, 1H), 6.21 (*d*, *J* = 2.0 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 178.2, 164.5, 161.4, 156.6, 155.4, 148.7, 146.8, 139.4, 137.4, 129.8, 128.8, 126.0, 124.3, 124.2, 117.5, 109.1, 108.8, 104.5, 98.9, 94.1. Anal. calcd. for $C_{28}H_{18}O_7$: C, 72.10; H, 3.89; O, 24.01; Found: C, 72.12; H, 3.88; O, 24.00.

3',4'-*O*-Diphenylmethane-3 (or 7)-quercetin aspirinate (3). A mixture of compound **2** (466 mg, 1 mmol), asprin (360 mg , 2 mmol), 4-dimethyl-aminopyridine (DMAP, 24.0 mg, 0.2 mmol, 20 mol %), were added in anhydrous CH_2Cl_2 (5 mL) and were stirred under argon, cooled in an ice bath to 0 °C while *N*,*N'*-dicyclohexylcarbodiimide (DCC, 412 mg, 2 mmol) was added and then at 0 °C to room temperature for 12 h. After the reaction was completed, white precipitate (dicyclohexylurea) was removed by filtration and the filtrate was cooled in fridge for 5 h, some additional dicyclohexylurea was precipitated, which was removed by filtration. The organic solution was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure at 32 °C. The residue was purified by column chromatography on silica gel (hexane/ethyl acetate = 8:1) to afford a yellow oil mixture of **3a**, **3b**, **3c** (522 mg). These three compounds were collected together to react in next step without isolation. To a solution of **3a**, **3b**, **3c** in ethyl acetate/ethanol (1.5:1, 15 mL) Palladium on charcoal (162 mg, 10%, 1.5 mmol) was added and vigorously stirred at 0 °C to room temperature for 8-9 h under hydrogen pressure (balloon). After the Pd/C was removed by filtration, the filtrate was concentrated under reduced pressure at 32 °C, and the residue was purified by column chromategraphy on silica gel using a mixture of $CHCl_3$/methanol (100:1) as the eluting solvent to give the corresponding products **4a**, **4b**, and **4c** as pale yellow powders.

3-(7-*O*-Acyl) quercetin aspirinate (4a). Yield = 31% (155 mg, from **2** to **4a**). R_f ($CHCl_3$/methanol 20:1) 0.68. mp 207-208 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.17 (*s*,

1H), 9.99 (*s*, 1H), 9.45 (*s*, 1H), 8.26 (*d*, *J* = 7.7 Hz, 1H), 7.83 (*m*, 1H), 7.55 (*m*, 1H), 7.41 (*d*, *J* = 2.1 Hz, 1H), 7.37 (*d*, *J* = 7.7 Hz, 1H), 7.32 (*dd*, *J* = 8.4, 2.1 Hz, 1H), 7.11 (*d*, *J* = 2.0 Hz, 1H), 6.89 (*d*, *J* = 8.4 Hz, 1H), 6.73 (*d*, *J* = 2.0 Hz, 1H), 2.32 (*s*, 3H), 2.19 (*s*, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 175.1, 168.9, 168.3, 160.9, 160.3, 157.6, 156.1, 155.4, 151.0, 149.8, 145.6, 135.6, 132.0, 130.1, 126.7, 124.7, 120.9, 120.8, 119.2, 115.9, 115.3, 107.8, 105.4, 101.7, 20.9, 20.5. Anal. calcd. for $C_{26}H_{18}O_{11}$: C, 61.66; H, 3.58; O, 34.75; Found: C, 61.65; H, 3.56; O, 34.78.

7-(3-*O*-Acyl) quercetin aspirinate (4b). Yield = 35% (175 mg, from **2** to **4b**). R_f ($CHCl_3$/methanol 20:1) 0.57. mp 203-205 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.31 (*s*, 1H), 9.87 (*s*, 1H), 9.60 (*s*, 1H), 8.20 (*d*, *J* = 7.6 Hz, 1H), 7.81 (*m*, 1H), 7.52 (*m*, 1H), 7.43 (*d*, *J* = 2.1 Hz, 1H), 7.39 (*d*, *J* = 7.7 Hz, 1H), 7.35 (*dd*, J = 8.4, 2.1 Hz, 1H), 7.19 (*d*, *J* = 2.0 Hz, 1H), 6.96 (*d*, *J* = 8.4 Hz, 1H), 6.82 (*d*, *J* = 2.0 Hz, 1H), 2.40 (*s*, 3H), 2.29 (*s*, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 175.4, 169.1, 167.8, 161.7, 160.5, 157.1, 155.6, 155.4, 150.5, 149.8, 145.5, 135.4, 131.9, 130.1, 126.5, 124.2, 121.6, 120.9, 119.2, 116.1, 115.2, 108.1, 105.3, 101.7, 20.7, 20.3. Anal. calcd. for $C_{26}H_{18}O_{11}$: C, 61.66; H, 3.58; O, 34.75; Found: C, 61.68; H, 3.55; O, 34.76.

3-Quercetin aspirinate (4c). Yield = 20% (91 mg, from **2** to **4c**). R_f ($CHCl_3$/methanol 20:1) 0.45. mp 178-180 °C (lit.[22] 175.6-177.8 °C). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.16 (*s*, 1H), 10.93 (*s*, 1H), 9.54 (*s*, 1H), 9.53 (*s*, 1H), 8.24 (*d*, *J* = 8.0 Hz, 1H), 7.82 (*m*, 1H), 7.54 (*m*, 1H), 7.37 (*d*, *J* = 2.0 Hz, 1H), 7.35 (*d*, *J* = 8.0 Hz, 1H), 7.27 (*dd*, *J* = 8.4, 2.0 Hz, 1H), 6.88 (*d*, *J* = 8.4 Hz, 1H), 6.53 (*d*, *J* = 2.0 Hz, 1H), 6.30 (*d*, *J* = 2.0 Hz, 1H), 2.18 (*s*, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 174.6, 168.9, 164.7, 161.1, 161.0, 156.7, 156.4, 151.0, 149.4, 145.6, 135.5, 132.0, 129.6, 126.6, 124.6, 121.1, 120.6, 119.5, 115.8, 115.1, 103.5, 99.2, 94.2, 20.5. Anal. calcd. for $C_{24}H_{16}O_{10}$: C, 62.07; H, 3.47; O, 34.45; Found: C, 62.04; H, 3.45; O, 34.50.

Cytotoxicity Assays. 3×103 HepG2, and 2×104 HL-60 were seeded in 96 well tissue culture plates and treated with the tested compounds or vehicle (0.1% DMSO) at various concentrations and incubated for 48 h followed by MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay at 570 nm. All treatments were performed in triplicate. The IC_{50} was defined as the concentration of the test compound resulting in a 50% reduction of absorbance compared to untreated cells in the MTT assay. The compound **4a-c** were tested for their cytotoxic ability against HL-60, and HepG2 tumor cell lines by MTT-assay with doxorubicin as the positive control (Table 1).

Table 1. Cytotoxicities of **4a-c** compounds

Compound	Cell lines (IC_{50}/μM)	
	HL-60	HepG2
4a	68.71	54.22
4b	69.29	38.49
4c	> 100	55.80
Doxorubicin	1.73	0.50
Quercetin	> 100	> 100

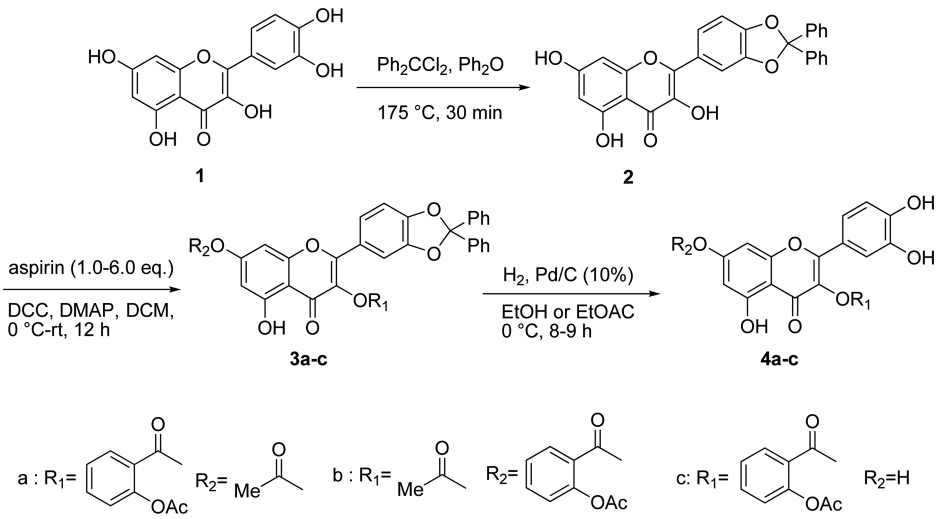
Table 2. DPPH scavenging activity of **4a-c** compounds

Compound	DPPH (SC_{50}/μM)
4a	40.58
4b	29.56
4c	53.65
quercetin	21.68

DPPH Scavenging Assay. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) is a free radical, stable at room temperature, which produces a violet solution in ethanol. In presence of antioxidant compounds, the DPPH is reduced producing a non-color ethanol solution. The free radical scavenging activities of compound **4a-c** were evaluated with the DPPH. The solution containing different amounts of compound **4a-c** was added into freshly prepared DPPH solution (1.3×10^{-4} mol/L, in ethanol). Absorbance at 517 nm was measured by a spectrophotometer on the 30 minutes after starting the reaction.[23] All experiments were repeated three times and the mean effective scavenging concentrations (SC_{50}) were calculated. As to the bleaching of the DPPH, this test is carried out in ethanol solution. Thus the test is useful to determine the free radical scavenging effect of the compounds (Table 2).

Results and Discussion

In our synthetic route (Scheme 1), to obtain the mono-acylated derivatives, selective protection of the catechol group of quercetin **1** with dichlorodiphenylmethane referring to the corresponding procedures was performed in the first step.[16] This method was probably the most effective for protection of the catechol group, while other ones of protection with 1,2-dibromomethane or acetone according to the reported literatures[17,18] were not successful in our repeated experiments. Esterification of compound **2** under the common condition with aspirin and the sequent deprotection of **3a-c** with H_2 and Pd/C[19] gave three products **4a-c** what were confirmed by NMR and mass spectroscopy to be acyl and diacyl compounds at the 3-*O* and 7-*O* positions of quercetin. Surprisingly, the 3-and 7-OH groups of quercetin were easily esterified not only by acetylsalicylic acid but also by acetic acid. In our initial experiments, the deprotection reaction was performed in ethyl acetate and the solvent might provide acetyl group for esterification, then the deprotection reaction was performed in THF again and gave the same result. Obviously, the acetyl group probably came not from the solvent but from aspirin. To obtain a single product in the esterification reaction, the ratio of aspirin to quercetin was varied from 1:1 to 6:1. However, the reaction showed no regioselective and the three quercetin aspirinates were always generated in almost equal yields whatever the ratio was. It should be noted that in order to establish the structures of **4a-c** in detailed, not only the 1D-NMR but also the 2D-NMR data was obtained. The **4a** and **4b** could be determined by



Scheme 1. Synthetic route of the quercetin aspirinates.

the different signals of H-6 and H-8 aroused by the acetylation of the C-7 phenolic alcohol with acetyl or acetylsalicylic group. The chemical shifts of H-6 and H-8 would move to lower fields if the acetylation of C-7 phenolic with acetylsalicylic group rather than acetyl group. The **4c** was also indicated by the signal of 7-OH at 10.93 ppm of the ^{1}H spectrum because only the signal of 7-OH should appear at 10-11 in the quercetin according to the reported literatures,[20] and the signal of 3-OH would be 9-10 if it not be esterified.

The biological activities of quercetin aspirinates were screened as shown as Table 1 and 2. All these hybrids showed more potent cytotoxic activities against tumor cell lines HL-60 and HepG2 of than quercetin but less than doxorubicin, and their scavenging activity against DPPH was less active than that of quercetin too.

Conclusion

Three quercetin aspirinates were synthesized by esterification of the 3-and 7-hydroxyl groups of quercetin with aspirin. Biological activities of these quercetin aspirinates showed better cytotoxic activities against tumor cell lines HL-60 and HepG2 and less scavenging activity against DPPH than the quercetin respectively.

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Supporting Information. The NMR spectral data of compounds **4a-c** are available as Supporting Information.

References

1. Conquer, J. A.; Maiani, G.; Azzini, E.; Raguzzini, A.; Holub, B. J. *J. Nutr.* **1998**, *128*, 593.
2. Lee, E.-J; Choi, E.-J.; Choi, C.-I.; Park, J.-S.; Sung, M.-K. *FASEB J.* **2007**, *21*, A1092.
3. Ferry, D. R.; Smith, A.; Malkhandi, J.; Fyfe, D. W.; Takats, P. G.; Anderson, D.; Baker, J.; Kerr, D. J. *Clin. Cancer Res.* **1996**, *2*, 659.
4. Choi, J. A.; Kim, J. Y.; Lee, J. Y.; Kang, C. M.; Kwon, H. J.; Yoo, Y. D.; Kim, T. W.; Lee, Y. S.; Lee, S. J. *Int. J. Oncol.* **2001**, *19*, 837.
5. Yoshizumi, M.; Tsuchiya, K.; Kirima, K.; Kyaw, M.; Suzaki, Y.; Tamaki, T. *Mol. Pharmacol.* **2001**, *60*, 656.
6. Schroeter, H.; Spencer, J. P. E.; Rice-Evans, C.; Williams, R. J. *Biochem. J.* **2001**, *358*, 547.
7. Li, M.; Han, X. W.; Yu, B. *Tetrahedron Lett.* **2002**, *43*, 9467.
8. Bouktaib, M.; Atmani, A.; Rolando, C. *Tetrahedron Lett.* **2003**, *44*, 6263.
9. Du, Y.-G.; Wei, G.-H.; Linhardt, R. J. *J. Org. Chem.* **2004**, *69*, 2206.
10. Needs, P. W.; Kroon, P. A. *Tetrahedron* **2006**, *62*, 6862.
11. Biasutto, L.; Marotta, E.; Marchi, U. D.; Zoratti, M.; Paradisi, C. *J. Med. Chem.* **2007**, *50*, 241.
12. Enomoto, S.; Okada, Y.; Güvenc, A.; Erdurak, C. S.; Coşkun, M.; Okuyama, T. *Biol. Pharm. Bull.* **2004**, *27*, 1140.
13. Chen, Z.-W.; Hu, Y.-Z.; Wu, H.-H.; Jiang, H.-D. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3949.
14. Gatto, M. T.; Falcocchio, S.; Grippa, E.; Mazzanti, G.; Battinelli, L.; Nicolosi, G.; Lambusta, D.; Saso, L. *Bioorg. Med. Chem.* **2002**, *10*, 269.
15. Tietze, L. F.; Bell, H. P.; Chandrasekhar, S. *Angew. Chem. Int. Ed.* **2003**, *42*, 3996.
16. Mattarei, A.; Biasutto, L.; Marotta, E.; Marchi, U. D.; Sassi, N.; Garbisa, S.; Zoratti, M.; Paradisi, C. *ChemBioChem*. **2008**, *9*, 2633.
17. Soloshonok, V. A.; Ueki, H. *Synthesis* **2008**, *5*, 693.
18. Luo, W.; Li, Y.-P.; He, Y.; Huang, S.-L.; Tan, J.-H.; Ou, T.-M.; Li, D.; Gu, L.-Q.; Huang, Z.-S. *Bioorg. Med. Chem.* **2011**, *19*, 763.
19. Hossion, A. M. L.; Zamami, Y.; Kandahary, R. K.; Tsuchiya, T.; Iwado, W. O. A.; Sasaki, K. *J. Med. Chem.* **2011**, *54*, 3686.
20. Hasana, A.; Sadiqa, A.; Abbasa, A.; Mughala, E.; Khanb, K. M.; Alib, M. *Nat. Prod. Res*. **2010**, *24*, 995.
21. Bouktaib, M.; Lebrun, S.; Atmani, A.; Rolando, C. *Tetrahedron* **2002**, *58*, 10001.
22. Zhai, G.-Y.; Zhu, W.; Qu, W.-T.; Li, H.-F.; Zhou, L. *Faming Zhuanli Shenqing* 2012, CN 102659735 A 20120912.
23. Blois, M. S. *Nature* **1958**, *181*, 1199.