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Synthesis of genistein derivatives and determination of their protective effects against vascular endothelial cell damages caused by hydrogen peroxide

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Abstract—A series of genistein derivatives, prepared by alkylation and difluoromethylation, were tested for their inhibitory effects on the hydrogen peroxide induced impairment in human umbilical vein endothelial (HUVE-12) cells in vitro. The HUVE-12 cells were pretreated with either the vehicle solvent (DMSO), genistein, or different amounts of the genistein derivatives for 30 min before exposed to 1 mM hydrogen peroxide for 24 h. Cell apoptosis was determined by flow cytometry with propidium iodide (PI) staining. Cellular injury was estimated by measuring the lactate dehydrogenase (LDH) release. Data suggested that the genistein derivatives possessed a protective effect on HUVE-12 cells from hydrogen peroxide induced apoptosis and reduced LDH release. Among these derivatives, 7-difluoromethyl-5,4′-dimethoxygenistein exhibited the strongest activity against hydrogen peroxide induced apoptosis of HUVE-12 cells.

Soybeans and soy products have been an important part of the oriental diet for centuries. In recent years, the therapeutic effects of certain compounds in the soy products have attracted increased attention among the science community. These compounds have been indicated to reduce the health risk of certain diseases, such as the cardiovascular ailments and various types of cancers.

Among these beneficiary compounds, phytochemicals were considered to play the major role. Phytochemicals are biologically active compounds in plants. Soybeans are a rich source of many phytochemicals including isoflavones, saponins, phytic acid, and phytosterols. Isoflavones are a class of phenolic compounds, which include daidzein, glycitein, and genistein. These compounds exist in soybeans either as glucosides or in free form (aglucons). Genistein is the major isoflavone in soy.

Endothelial cells are crucial in maintaining the physiological functions of the cardiovascular system and they are also involved in the development of a variety of human diseases.[1] The two leading cardiovascular diseases, hypertension and atherosclerosis, are complex processes that occur, at least in part, in response to the necrosis or apoptosis resulted vascular injury.[2,3] Increasing evidences suggested that oxidative-stress and free radicals produced during apoptosis were the major causes of endothelial damage. Therefore, pharmacological interventions targeting the endothelial remodeling have become a hot spot in the biomedical research.

Using natural products or extracts from plants for the treatment of oxidative stress-induced cell injury has been demonstrated by more and more researchers, for example, isoflavone significantly decreased the post menopause-related cardiovascular disease.[4] Genistein, an isoflavone compound, has been reported to inhibit the apoptosis in cultured endothelial cells and in animal models.[5,6] However, the low absorbance of flavonoids in intestines resulted in the low biological activities of this compound.[7,8] Therefore, designing new ready-to-absorb compounds using genistein as the lead compound is essential for the genistein bioactivity.

Keywords: Genistein derivatives; Protective effects; Vascular endothelial cell; 7-Difluoromethylyl-5,4′-dimethoxygenistein.
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Table 1. Characterization of the synthesized compounds

Compound	MS (EI, 70 eV)	IRυ_{max} (cm^{-1}, KBr)	^{1}H NMR (300 MHz, $CDCl_3$)	^{19}F NMR (282 MHz)
2	*m*/*z*: 332	1448, 1519, 1662 (C═O), 3319, 3528 (OH)	6.599 (1H, d, *J* = 2.4 Hz), 6.820 (1H, t, *J* = 12.0 Hz), 6.867 (1H, d, *J* = 2.4 Hz), 7.371 (2H, d, *J* = 12.0 Hz), 7.427 (1H, t, *J* = 72.9 Hz), 8.244 (1H, s), 9.550 (1H, s), 12.994 (1H, s)	−84.372 (d, *J* = 72.9 Hz)
3	*m*/*z*: 370	1430, 1448, 1498, 1653 (C═O), 3074 (OH)	6.651 (1H, t, *J* = 73.8 Hz), 6.590 (1H, d, *J* = 2.4 Hz), 6.638 (1H, t, *J* = 72.3 Hz), 6.684 (1H, d, *J* = 2.4 Hz), 7.229 (2H, d, *J* = 9.0 Hz), 7.551 (2H, d, *J* = 9.0 Hz), 7.975 (1H, s)	−80.953 (d, *J* = 73.8 Hz), −82.414 (d, *J* = 72.3 Hz)
4a	*m*/*z*: 348	1430, 1469, 1512, 1580, 1612, 1637 (C═O)	3.834 (3H, s),3.970 (3H, s), 6.540 (1H, d, *J* = 2.1 Hz), 6.642 (1H, t, *J* = 72.3 Hz), 6.724 (1H, d, *J* = 2.1 Hz), 6.940 (1H, d, *J* = 8.7 Hz), 7.472 (1H, d, *J* = 8.7 Hz), 7.811 (1H, s)	−82.150 (d, *J* = 72.3 Hz)
4b	*m*/*z*: 376	1476, 1581, 1613 (C═O), 1645, 2874, 2984	1.386–1.572 (6H, m), 4.023–4.181 (4H, m), 6.525 (1H, d, *J* = 2.4 Hz), 6.628 (1H, t, *J* = 72.6 Hz), 6.699 (1H, d, *J* = 2.4 Hz), 6.931 (2H, d, *J* = 8.4 Hz), 7.436 (2H, d, *J* = 8.4 Hz)	−82.085 (d, *J* = 72.6 Hz)
4c	*m*/*z*: 375	1469, 1513, 1581, 1614, 1651 (C═O), 2880, 2968	0.972–1.120 (4H, m), 1.157 (3H, t, *J* = 7.5 Hz), 1.223 (3H, t, *J* = 7.5 Hz), 3.987–4.164 (4H, m), 6.643 (1H, d, *J* = 2.4 Hz), 6.761 (1H, t, *J* = 72.6 Hz), 6.807 (1H, d, *J* = 2.4 Hz), 7.053 (2H, d, *J* = 9.0 Hz), 7.547 (2H, d, *J* = 9.0 Hz)	−82.142 (d, *J* = 72.6 Hz).
4d	*m*/*z*: 500	1454, 1513, 1579, 1614, 1647 (C═O), 2926	4.636 (4H, s), 7.314–7.342 (18H, m)	−82.161 (d, *J* = 72.2 Hz)
4e	*m*/*z*: 431, 517 (M^+)	1433, 1469, 1581, 1614, 1652 (C═O), 2859, 2956	0.906–2.016 (26H, m), 3.950 (2H, t, *J* = 6.6 Hz), 4.047 (2H, t, *J* = 6.6 Hz), 6.512 (1H, d, *J* = 2.1 Hz), 6.648 (1H, d, *J* = 2.1 Hz), 6.764 (1H, t, *J* = 72.9 Hz), 9.893 (2H, d, *J* = 9.0 Hz), 7.412 (2H, d, *J* = 9.0 Hz), 7.759 (1H, s)	−82.897 (d, *J* = 72.9 Hz)
4f	*m*/*z*: 544	1435, 1467, 1512, 1569, 1611, 1651 (C═O), 2858, 2929	0.860–1.218 (30H, m), 3.985–4.046 (4H, m), 6.378 (1H, d, *J* = 2.1 Hz), 6.423 (1H, d, *J* = 2.1 Hz), 6.531 (1H, t, *J* = 74.1 Hz), 7.155 (2H, d, *J* = 8.7 Hz), 7.543 (2H, d, *J* = 8.7 Hz), 7.748 (1H, s)	−80.555 (d, *J* = 74.1 Hz)
4g	*m*/*z*: 473, 600 (M^+)	1658 (C═O)	0.848–1.783 (38H, m), 3.966 (2H, t, *J* = 6.6 Hz), 4.040 (2H, t, *J* = 6.6 Hz), 6.513 (1H, d, *J* = 2.1 Hz), 6.627 (1H, t, *J* = 72.3 Hz), 6.674 (1H, d, *J* = 2.1 Hz), 6.925 (2H, d, *J* = 8.4 Hz), 7.423 (2H, d, *J* = 8.4 Hz), 7.753 (1H,s)	−82.052 (d, *J* = 72.3 Hz)
4h	*m*/*z*: 432	1472, 1513, 1579, 1628, 1650 (C═O), 2876, 2963	0.809–1.057 (12H, m), 2.176–2.198 (2H, m), 3.759 (2H, t, *J* = 6.6 Hz), 3.783 (2H, t, *J* = 6.6 Hz), 6.568 (1H, d, *J* = 2.4 Hz), 6.627 (1H, t, *J* = 72.9 Hz), 6.656 (1H, d, *J* = 2.4 Hz), 6.984 (2H, d, *J* = 9.0 Hz), 7.446 (2H, d, *J* = 9.0 Hz), 7.941 (1H, s)	−82.348 (d, *J* = 72.9 Hz)