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Resveratrol Derivatives Showing the Leukotriene D4 Antagonism

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In order to discover a potent active compound, an iterative process of organic syntheses and biological tests of derivatives is required. Recently, quantitatively structure-activity relationships (QSARs) have been applied to improve the efficiency. During the last decade, steroid derivatives, beta2-adrenoceptor agonists, and muscarinic receptor antagonists have been used for anti-asthmatic therapy but development of new drug types is crucial due to the side-effects and the limited effects of the present drugs.[1-4] To find a new potent drug for anti-asthmatic therapy, compounds showing the leukotriene D4 (LTD4) antagonism are being searched.[5] From a previous work, it was clarified that resveratrol isolated from *Morus alba* showed the LTD4 antagonism in guinea pig ileum.[6] Because resveratrol has low activity, however, organic syntheses and biological tests of derivatives are required. A study on a series of resveratrol derivatives was carried out in this research.

Based on the following equation obtained from a previous work,[6] 13 candidates were predicted.

(Biological Activity) =
$-0.26 \times (\text{AlogP}) + 0.96 \times (\text{LUMO}) + 0.095 \times (\text{MW}) + 18.42$

where AlogP, LUMO, and MW denote log of the partition coefficient, the lowest unoccupied orbital energy, and molecular weights, respectively. To obtain the above equation, a statistical method, genetic function approximation (GFA) was applied. Its correlation factor r^2 was 0.959. The structures of the candidates are shown in Table 1.

The synthetic method of 13 candidates is shown in scheme

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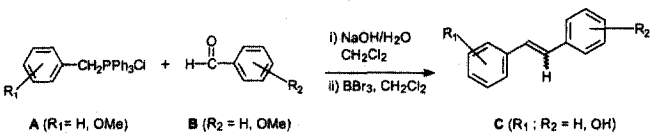
Abbreviations: QSARs, quantitatively structure-activity relationships; LTD4, leukotriene D4; GEA, genetic function approximation



Scheme 1. Synthetic method of 13 candidates predicted by QSARs.

1. General methods for the preparation of phosphonium chloride **A** (scheme 1) were described in a previous work.[7] Wittig reaction under a two-phase solvent system gave polymethoxylated stilbenes, which were transformed into the corresponding polyhydroxylated stilbenes **C** using boron tribromide. Spectroscopic data of polyhydroxylated stilbenes **C** are as follows:

1, resveratrol: m.p. 263°C; ^{1}H-NMR (400 MHz, CD_3OD) δ 7.53 (d, 2 H, J = 11.4 Hz), 7.15 (d, 1 H, J = 16.3 Hz), 6.94-7.01 (m, 3 H), 6.67 (d, 2 H, J = 2.1 Hz), 6.39 (t, 1 H, J = 2.1 Hz); ^{13}C-NMR (100 MHz, CD_3OD) δ 160.00 (double intensity), 158.68, 142.83, 130.89, 129.92, 129.32 (double intensity), 127.44, 116.96 (double intensity), 106.32 (double intensity), 103.11.

2: m.p. 191°C; ^{1}H-NMR (400 MHz, CD_3OD) δ 7.39 (dd, 1 H, J = 1.4, 7.6 Hz), 7.26 (d, 1 H, J = 16.5 Hz), 6.95 (ddd, 1 H, J = 1.6, 7.8 Hz), 6.88 (d, 1 H, J = 16.5 Hz), 6.68-6.72 (m, 2 H), 6.38 (d, 2 H, J = 2.1 Hz), 6.07 (t, 1 H, J = 2.1 Hz); ^{13}C-NMR (100 MHz, CD_3OD) δ 160.07 (double intensity), 155.56, 142.05, 129.88, 129.84, 127.88, 126.14, 125.14, 121.21, 117.02, 105.39 (double intensity), 103.24.

3: m.p. 51°C; ^{1}H-NMR (400 MHz, CD_3OD) δ 6.93 (t, 1 H, J = 8.1 Hz), 6.61 (m, 2 H), 6.50 (ddd, 1 H, J = 0.9, 2.5, 8.1 Hz), 6.33 (dd, 2 H, J = 12.3, 15.8 Hz), 6.10 (d, 2 H, J = 2.1 Hz), 6.00 (t, 1 H, J = 2.1 Hz); ^{13}C-NMR (100 MHz, CD_3OD) δ 159.73 (double intensity), 158.56, 141.07, 140.42, 131.80, 131.51, 130.58, 121.97, 117.05, 115.53, 108.81 (double intensity), 102.98.

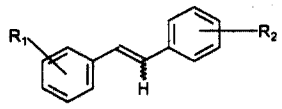
4: m.p. 235°C; ^{1}H-NMR (400 MHz, CD_3OD) δ 7.06 (t, 1 H, J = 7.8 Hz), 6.816.90 (m, 4 H), 6.58 (ddd, 1 H, J = 0.8, 2.4, 8.0 Hz), 6.39 (d, 2 H, J = 2.1 Hz), 6.01 (t, 1 H, J = 2.1 Hz); ^{13}C-NMR (100 MHz, CD_3OD) δ 160.14 (double intensity), 159.15, 141.18, 140.60, 131.06, 130.25, 129.97, 119.66, 116.10, 114.21, 106.49 (double intensity), 103.59.

5: m.p. 139°C; ^{1}H-NMR (400 MHz, CD_3OD) δ 7.05(t, 2 H, J = 7.8 Hz), 6.926.85 (m, 6 H), 6.59 (ddd, 2 H, J = 8.07, 2.46, 0.91 Hz); ^{13}C-NMR (100 MHz, CD_3OD) δ 159.16, 140.61, 131.06, 130.06, 119.67, 116.13, 114.24 (all double intensity).

6: m.p. 172°C; ^{1}H-NMR (400 MHz, CD_3OD) δ 7.26 (dt, 2 H, J = 9.48, 2.83 Hz), 7.01 (t, 1 H, J = 7.85 Hz), 6.84 (dd, 2 H, J = 2.58, 16.32 Hz), 6.866.81 (m, 2 H), 6.66 (dt, 2 H, J = 9.42, 2.78 Hz), 6.54 (ddd, 1 H, J = 8.03, 2.45, 0.9 Hz); ^{13}C-NMR (100 MHz, CD_3OD) δ 159.09, 158.79, 141.16, 130.99, 130.88, 129.91, 129.25 (double intensity), 127.27, 119.38, 116.92 (double intensity), 115.56, 113.96.

7: m.p. 102°C; ^{1}H-NMR (400 MHz, CD_3OD) δ 7.42 (dd,

Table 1. Predicted structures of the candidates based on the QSARs calculation, and a log scale comparison of experimental biological activities with calculated values.



Compound	Double bond configuration	R_1	R_2	Log (experimental activity[a])	Log (calculated activity[a])	$\|\Delta\|^b$
1	*trans*	4-OH	3,5-OH	0.71	0.95	0.24
2	*trans*	2-OH	3,5-OH	1.49	1.16	0.33
3	*trans*	3-OH	3,5-OH	1.98	1.18	0.80
4	*cis*	3-OH	3,5-OH	0.80	1.32	0.52
5	*trans*	3-OH	3-OH	1.06	1.18	0.12
6	*trans*	3-OH	4-OH	1.11	0.94	0.17
7	*trans*	3-OH	2-OH	1.15	1.11	0.04
8	*trans*	2-OH	2-OH	1.19	1.12	0.07
9	*trans*	2-OH	4-OH	1.26	0.97	0.29
10	*trans*	4-OH	4-OH	0.46	0.72	0.26
11	*cis*	3-OH	H	1.19	1.34	0.15
12	*trans*	3-OH	H	1.25	1.11	0.14
13	*trans*	H	3,4-OH	0.15	1.07	0.92

[a]50% inhibitory concentration of the test compounds on LTD4 (μg/m*l*)
[b]absolute value of the difference between the experimental activity and the calculated activity

1 H, J = 4.74, 1.38 Hz), 7.33 (d, 1 H, J = 16.51 Hz), 7.11-6.85 (m, 5 H), 6.72 (m, 2 H), 6.57 (ddd, 1 H, J = 8.04, 2.44, 0.80 Hz) ; ^{13}C-NMR (100 MHz, CD_3OD) δ 159.09, 156.59, 141.56, 133.03, 131.01, 129.93, 129.70, 127.91, 126.17, 125.21, 121.24, 119.61, 117.04, 115.77, 114.10.

8: m.p. 198°C; ^{1}H-NMR (400 MHz, CD_3OD) δ 7.64 (dd, 1 H, J = 7.70, 1.54 Hz), 7.54 (s, 1 H), 7.13 (m, 1 H), 6.90 (m, 2 H); ^{13}C-NMR (100 MHz, CD_3OD) δ 156.34, 129.58, 127.60, 127.02, 124.53, 121.27, 117.01 (all double intensity).

9: m.p. 186°C; ^{1}H-NMR (400 MHz, CD_3OD) δ 7.35 (m, 1 H), 7.23-7.12 (m, 3 H), 6.92 (m, 2 H), 6.64 (m, 4 H); ^{13}C-NMR (100 MHz, CD_3OD) δ 158.46, 158.29, 131.72, 129.59, 129.34, 129.08 (double intensity), 127.55, 126.69, 122.35, 121.24, 116.97, 116.86 (double intensity).

10: m.p. 205°C; ^{1}H-NMR (400 MHz, CD_3OD) δ 7.11 (td, 1 H, J = 9.52, 2.89 Hz), 6.65 (s, 2 H), 6.53 (td, 4 H, J = 9.52, 2.88 Hz); ^{13}C-NMR (100 MHz, CD_3OD) δ 158.29, 131.40, 128.83, 127.15, 116.84, 116.87, 108.21 (all double intensity).

11: ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.23 (m, 2 H), 7.15 (m, 3 H), 7.04 (t, 1 H, J = 7.83 Hz), 6.80 (d, 1 H, J = 7.64 Hz), 6.65 (m, 1 H), 6.59 (dd, 1 H, J = 7.96, 2.15 Hz), 6.51 (dd, 2 H, J = 8.85, 12.24 Hz); ^{13}C-NMR (100 MHz, CD_3OD) δ 157.00, 140.76, 139.12, 132.54, 131.78, 131.54, 131.15 (double intensity), 130.23 (double intensity), 129.14, 123.66, 117.46, 116.23.

12: mp 123°C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.47 (m, 2 H), 7.32 (m, 2 H), 7.21 (m, 3 H), 7.05 (m, 1 H), 7.02 (d, 2H, J = 3.08 Hz), 6.95 (m, 1 H), 6.71 (dd, 1 H, J = 7.89, 2.35 Hz); ^{13}C-NMR (100 MHz, CD_3OD) δ 157.62, 140.96, 139.01, 131.78, 131.06, 130.58 (double intensity), 130.13, 129.62, 128.47 (double intensity), 121.36, 116.65, 114.93.

13: ^{1}H-NMR (400 MHz, CD_3OD) δ 7.35 (m, 2 H), 7.19 (m, 2 H), 7.07 (m, 1 H), 6.92-6.77 (m, 4 H), 6.65 (d, 1 H, J = 8.14 Hz); ^{13}C-NMR (100 MHz, CD_3OD) δ 146.99, 146.93, 139.71, 131.52, 130.27, 130.04 (double intensity), 128.39, 127.56 (double intensity), 127.17, 120.68, 116.86, 114.31.

The melting points were measured on a Digital Melting Point Apparatus IA9000 Series (Electrothermal, USA), and NMR data were collected on a Bruker DPX 400 (9.4 T) instrument in a 5 mm tube at 298K. The QSARs calculation was carried out on a Silicon Graphics INDY R4400 workstation using Cerius 2 software (MSI, SanDiego, CA, USA). In order to test biological activities of the compounds, male Hartley guinea pigs weighing 400-500 g were sacrificed by a sharp blow to the head, and the trachea was removed. The trachea was opened by cutting along the ventral side, and two strips containing three cartilages each were sutured in parallel. The preparation was bathed in a jacketed 13 m*l* organ bath filled with Krebs-Henseleit buffer (in mM: NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.6, $NaHCO_3$ 24.9, KH_2PO_4 1.2, glucose 11.0, pH 7.4 at 37°C). Contractile change was monitored by connecting the preparation to an isometric transducer and recorded on a chart-strip recorder. The bath was saturated by a continuous supply of 95% O_2 and 5% CO_2. Inhibitory effect of the test compounds on LTD4 was examined by adding the test compounds to the bath after tracheal contraction with 5 nM LTD4 was attained. Activity was expressed as the relaxant action induced by a test substance at fixed concentrations. The results were expressed as 50% inhibitory concentration

(EC_{50}). The values of biological activities of the compounds were calculated using the equation mentioned above, and the experimental values are listed in Table 1. The result of cross-validated analyses gave 0.916.

While resveratrol derivatives used for the QSARs calculation in the authors previous paper showed an average EC_{50} of 604 mg/m*l*, the compounds studied in this work showed 9.77 mg/m*l*.[6] The structural difference between the resveratrol derivatives used for the QSARs calculation in the authors previous paper and those of this work is the presence of the hydrolysis of methoxy group. While the previous compounds contain methoxy groups, the current ones do not; that is, all methoxy groups were hydrolyzed. This can be explained by the fact that LTD4 has several hydroxyl group. In addition, even though all compounds include stilbene moiety contained in resveratrol and have *trans* conformation except compounds **4** and **11**, the difference between *trans* and *cis* conformations does not result in a big difference in their activities. Therefore, to improve LTD4 antagonism of resveratrol derivatives, they should have hydroxyl groups instead of methoxy groups. Detailed experiments on the relationships between the electron densities of the compounds and their activities remain for future works.

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