Synthesis and Biological Activities of Leukotriene D4 Antagonists Predicted from Quantitatively Structure-Activity Relationships Calculation

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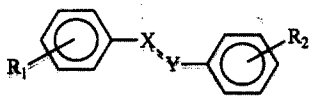
The number of asthma patients is increasing probably due to environmental pollution and extended human life span. Even though currently there exist several drugs for asthma therapy such as phosphodiesterase inhibitors (eg., theophylline), beta$_2$ adrenoceptor agonists, and corticosteroids, their efficacy is not satisfactory and their side-effects are well recognized. Because asthma is characterized by inflammatoy responses and bronchial contraction, an ideal asthma therapeutant must be efficacious for both of these symptoms. Leukotriene D4 (LTD4) antagonists have been suggested as promising anti-asthmatic candidates since they are effective against these asthma symptoms.[1-5]

In order to discover new LTD4 antagonists relationships between electron densities of stilbene moieties and leukotriene D4 antagonism were studied.[6] Varying connection bonds between two benzene rings of stilbene derivatives, different electron densities of the atoms placed at the bonds were observed. Calculated values of the electron density at the posi-

Table 1. Structures of compounds.



name	X-Y	R_1	R_2
DK-II-22	CH_2-O	H	$O_2C(CH_2)_2CH_3$
CH-IV-14	CH_2-O	4-OMe	4-OH
CH-IV-15	CH_2-O	4-OMe	3-OH
CH-IV-16	CH_2-O	4-OMe	2-OH
CH-IV-17	CH_2-O	3-OMe	4-OH
CH-IV-18	CH_2-O	3-OMe	3-OH
CH-IV-19	CH_2-O	3-OMe	2-OH
CH-IV-20	CH_2-O	2-OMe	4-OH
CH-IV-21	CH_2-O	2-OMe	3-OH
CH-IV-22	CH_2-O	2-OMe	2-OH
KM-I-12B	CH_2-O	3,4-Methylenedioxy	3-OH
KM-I-13	CH_2-O	3,4-Methylenedioxy	2-OH
KM-I-16	CH_2-O	2,3-di-OMe	4-OH
KM-I-18	CH_2-O	2,3-di-OMe	2-OH
KM-I-21	CH_2-O	3,5-di-OMe	4-OH
KM-I-23	CH_2-O	3,5-di-OMe	2-OH

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Abbreviations: LTD4, leukotriene D4.

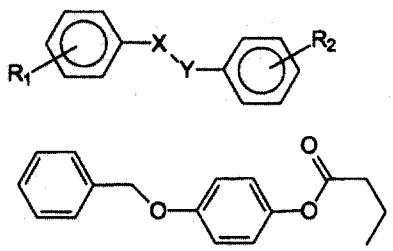

Fig. 1. The structures of stilbene moiety (top), and DK-II-22 (bottom).

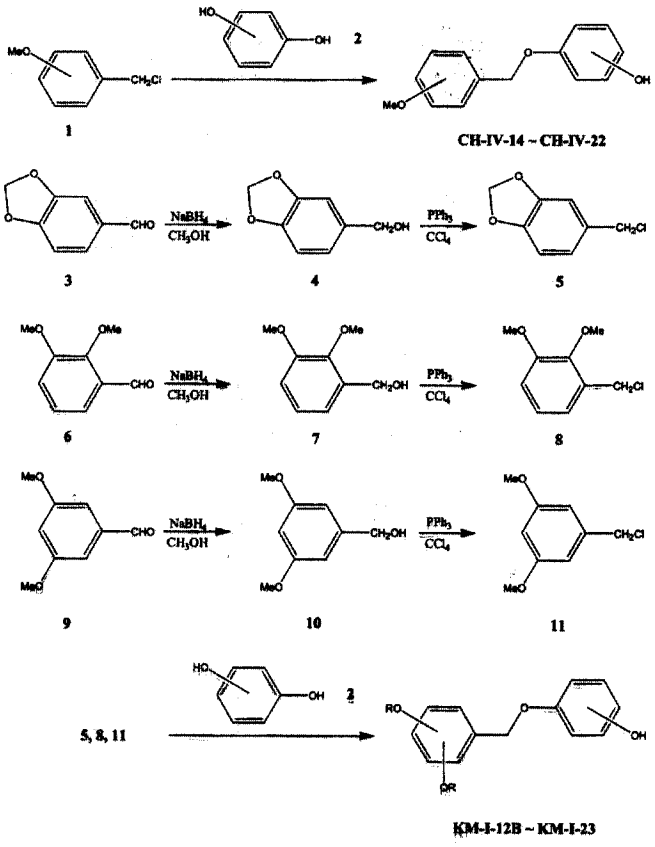

Fig. 2. The synthetic method of 15 compounds predicted by QSARs.

Table 2. ^{1}H NMR data of compounds.

Compounds	Phenyl	CH_2-O	OCH_3 or O-CH_2-O
CH-IV-14	δ 7.20-7.19 (m, 2H), 6.78-6.76 (m, 2H), 6.70-6.66 (m, 2H), 6.59-6.56 (m, 2H)	4.74 (s, 2H)	3.64 (s, 3H)
CH-IV-15	δ 7.16-7.02 (m, 3H), 6.78-6.71 (m, 4H), 6.32-6.28 (m, 1H)	4.70 (s, 2H)	3.70 (s, 3H)
CH-IV-16	δ 7.44-7.35 (m, 3H), 7.03-6.90 (m, 5H)	5.10 (s, 2H)	3.89 (s, 3H)
CH-IV-17	δ 7.13 (t, 1H, J = 8.06 Hz), 6.85-6.84 (m, 2H), 6.71 -6.69 (m, 3H), 6.60-6.57 (m, 2H),	4.80 (s, 2H)	3.65 (s, 3H)
CH-IV-18	δ 7.43 (m, 1H), 7.31-7.26 (m, 1H), 7.11 (t, 1H, J = 8.14 Hz) 6.98-6.86 (m, 2H) 6.59 (dd, 1H, J = 2.37 Hz), 6.49 (t, 1H, J = 2.35 Hz), 6.40-6.38 (m, 1H)	5.07 (s, 2H)	3.83 (s, 3H)
CH-IV-19	δ 7.32-7.23 (m, 1H), 7.00-6.82 (m, 7H)	5.06 (s, 2H)	3.79 (s, 3H)
CH-IV-20	δ 7.24 (dd, 1H, J = 6.02 Hz), 7.15 (td, 1H, J = 5.92, 1.62 Hz), 6.84-6.80 (m, 2H) 6.70-6.68 (m, 2H), 6.60-6.57 (m, 2H)	4.86 (s, 2H)	3.70 (s, 3H)
CH-IV-21	δ 7.31-7.23 (m, 1H), 7.10 (t, 1H, J = 8.21Hz), 6.98-6.83 (m, 3H) 6.58 (dd, 1H, J = 8.23, 2.33Hz), 6.49-6.48 (m, 1H), 6.42-6.38 (m, 1H)	5.03 (s, 2H)	3.83 (s, 3H)
CH-IV-22	δ 7.36-7.32 (m, 2H), 7.04-6.99 (m, 2H), 6.97-6.92 (m, 3H) 6.84-6.80 (m, 1H)	5.08 (s, 2H)	3.88 (s, 3H)
KM-I-12B	δ 6.91 (t, 1H, J = 8.08 Hz), 6.70-6.58 (m, 3H), 6.34 (ddd, 1H, J = 8.28, 2.35, 0.82 Hz), 6.25-6.20 (m, 2H)	4.70 (s, 2H)	5.74 (s, 2H)
KM-I-13	δ 6.85-6.71 (m, 7H)	4.90 (s, 2H)	5.88 (s, 2H)
KM-I-16	δ 7.01-6.94 (m, 2H), 6.82-6.75 (m, 3H), 6.70-6.68 (m, 2H)	4.98 (s, 2H)	3.88 (s, 3H) 3.81 (s, 3H)
KM-I-18	δ 6.90-6.86 (m, 2H), 6.74-6.72 (m, 4H), 6.51 (dd, 1H, J = 7.58, 0.85 Hz)	4.66 (s, 2H)	3.86 (s, 3H) 3.78 (s, 3H)
KM-I-21	δ 6.77-6.74 (m, 2H), 6.68-6.65 (m, 2H), 6.50(d, 2H, J = 1.91 Hz), 6.33 (t, 1H, J = 2.12 Hz)	4.85 (s, 2H)	3.71 (s, 6H)
KM-I-23	δ 6.90-6.89 (m, 1H), 6.68-6.83 (m, 2H), 6.79-6.76 (m, 1H), 6.51 (d, 2H, J = 2.31 Hz), 6.39 (t, 1H, J = 2.30 Hz)	4.62 (s, 2H)	3.85 (s, 3H) 3.79 (s, 3H)

tion Y (Fig. 1a) obtained by MOPAC[7] showed an order such as ethylene oxide group>diazene group>ethenyl group. Because the higher the electron density, the higher the activity of LTD4 antagonism (EC_{50}[8]), stilbene derivatives connected with ethylene oxide group were expected to be synthesized. Fifteen derivatives except DK-II22 (Fig. 1b) were predicted based on the quantitatively structure-activity relationships (QSARs) calculation in a previous work[6], and in this work they were synthesized and their activities were measured. The structures are listed in Table 1.

The synthetic method of 15 compounds is shown in Fig. 2.

General methods for the preparation of methoxybenzyl chloride **1** (Fig. 2) were described in a previous work.[9] Using the same methods, poly-substituted benzylchloride (**5**, **8**, **11**) were prepared from the corresponding benzaldehydes (**3**, **6**, **9**). The substituted benzylchloride (**1**, **5**, **8**, **11**) were coupled with hydroxyphenols **2** to give the desired 15 products. Spectroscopic data of the final 15 compounds are listed in Tables 2 and 3.

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 298 K. 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 various concentrations, and the results were expressed as 50% effective concentration (EC_{50} in mg/m*l*).[10]

The calculated values of EC_{50} and experimental values are listed in Table 4. Their correlation factor is 0.76. The reason of a bad correlation factor can be explained by small number of the training set used for QSARs which may select poor structural parameters and experimental errors occurred in the biological tests. Synthesis and biological tests of more compounds are expected to give better results.

Table 3. ^{13}C NMR data of compounds.

Molecule	Phenyl	CH_2-O	OCH_3 or O-CH_2-O
CH-IV-14	δ 161.24, 153.96, 152.87, 131.35, 130.78 (double intensity), 117.55 (double intensity), 117.17 (double intensity), 115.18 (double intensity)	72.07	56.07
CH-IV-15	δ 158.61, 153.87, 153.53, 132.01, 129.94 (double intensity), 129.17, 119.95, 114.50 (double intensity), 108.61, 108.09		55.71
CH-IV-16	δ 159.70, 145.89, 129.68 (double intensity), 129.45, 121.73, 120.09, 114.67, 114.05 (double intensity), 113.64, 112.20	70.82	55.31
CH-IV-17	δ 161.68, 153.92, 152.94 141.08, 130.90, 121.10, 117.51 (double intensity), 117.22 (double intensity), 114.63, 114.33	72.10	56.04
CH-IV-18	δ 160.24, 156.77, 156.69, 130.07, 128.97, 128.64, 125.31, 120.57, 110.24, 107.82, 107.28, 102.37	65.06	55.36
CH-IV-19	δ 159.81, 145.83, 145.72, 137.90, 129.78, 121.83, 120.11, 119.92, 114.74, 113.73, 113.25, 112.18	70.91	55.25
CH-IV-20	δ 158.84, 154.06, 152.83, 130.49, 130.20, 127.32, 121.83, 117.40 (double intensity), 117.18 (double intensity), 111.78	67.36	56.27
CH-IV-21	δ 160.23, 156.76, 155.30, 130.04, 129.37, 128.94, 125.13, 120.55, 110.21, 108.27, 107.80, 102.98	65.02	55.34
CH-IV-22	δ 157.46, 147.06, 145.93, 129.88, 129.81, 124.52, 122.61, 120.68, 120.02, 114.88, 112.85, 110.52	68.42	55.71
KM-I-12B	δ 160.44, 157.06, 148.28, 147.82, 131.04, 130.57, 121.66, 108.57, 108.68, 108.50, 107.79, 102.91	70.40	101.49
KM-I-13	δ 148.40, 148.15, 146.39, 146.13, 130.54, 122.30, 122.09, 120.50, 115.20, 112.79, 108.94, 108.76	71.54	101.62
KM-I-16	δ 148.52, 124.28, 123.21, 121.02, 119.99, 115.96, 114.95 (double intensity), 114.89 (double intensity), 111.06, 109.93	64.69	60.13, 54.74
KM-I-18	δ 151.75, 150.01, 146.70, 145.92, 133.28, 131.60, 129.19, 124.73, 122.90, 120.95, 111.53, 109.48	60.41	59.50, 54.71
KM-I-21	δ 161.32 (double intensity), 153.21, 150.13, 140.06, 116.44 (double intensity), 116.41, 105.61, (double intensity), 100.24 (double intensity)	71.08	55.80 (double intensity)
KM-I-23	δ 161.45 (double intensity), 146.26, 146.14, 139.14, 122.30, 120.56, 115.18, 112.67, 105.91 (double intensity), 100.45	71.46	55.81, 55.75

Table 4. The calculated values and experimental values of EC_{50} (μg/ml).

Compounds	Experimental values	Calculated values
DK-II-22	1.6	0.71
CH-IV-14	1.8	0.15
CH-IV-15	1.2	0.11
CH-IV-16	0.73	0.13
CH-IV-17	0.88	0.30
CH-IV-18	1.1	0.11
CH-IV-19	1.4	0.26
CH-IV-20	2.8	0.21
CH-IV-21	2.2	0.21
CH-IV-22	1.6	0.30
KM-I-12B	2.0	0.30
KM-I-13	2.7	0.32
KM-I-16	3.2	0.40
KM-I-18	3.4	0.56
KM-I-21	6.7	3.65
KM-I-23	3.5	0.56

Even though activities of CH-IV-16 and CH-IV-17 are not satisfied with the goal of authors comparing to drugs on the market, the activities will be increased by hydrolysis of methoxy group.[8] This is remained for the future works.

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