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Short Communication

Quantitatively Structure-activity Relationships to Develop Anti-asthmatic Drugs

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To develop new drugs for asthmatic therapy, lipid membrane derivatives have been studied. Based on the knowledge of the biosynthesis pathway, anti-asthmatic drugs related to the paltelet activating factors, leukotrienes and 5-lipoxygenase, are being studied.[1] Leukotrienes, types of mediators for bronchial asthma, are classified into A4, B4, C4, D4, and E4.[2-4] Among these, C4, D4, and E4 interact with the same receptor, and D4 shows the most potent binding affinity. Whenever D4 binds to its receptor, a contraction of smooth muscles occurs. Therefore, it is possible for the antagonists of Leukotriene D4 (LTD4) receptor to protect smooth muscles from being contracted, thus they could be used as anti-asthmatic drugs.[5-8]

In order to discover lead compounds as LTD4 receptor antagonists for asthmatic therapy, authors screened the extracts of higher plants. An ethylacetate extract of a mulberry tree, *Morus alba*, showed an activity against bronchial contraction caused by LTD4. After more activity guided fractionation, the final active compound was certified to be one of stilbene derivatives, resveratrol. Recently, the rational drug design has been applied for the development of new drugs from lead compounds. Studies on more stilbene derivatives, however, are necessary for the rational drug design in this work. Therefore, authors purchased 7 derivatives, dimethyl trans-stilbene-4,4'-dicarboxylate, cis-stilbene-4,4'-dicarboxylic acid, 4-amino-4'-hydroxystilbene, 4-hydroxy-4'-nitrostilbene, diethylstilbestrol, 4-benzyloxyphenol, chlorogenic acid, and synthesized 13 derivatives. The synthetic method of 13 derivatives is shown in Scheme 1, and their spectroscopic results are as follows:

CH-2B: ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.44 (dd, 1H, J = 7.9, 7.9 Hz), 7.29 (d, 1H, J = 2.0 Hz), 7.25 (m, 2H), 7.18 (d, 1H, J = 16.3 Hz), 7.00 (ddd, 1H, J = 0.8, 2.6, 8.2 Hz), 6.84 (d, 2H, J = 2.3 Hz), 6.57 (dd, 1H, J = 2.3, 2.3 Hz), 4.04 (s, 3H), 4.00 (s, 6H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 161.41 (double intensity), 160.32, 139.68, 139.01, 130.08, 129.52, 129.41, 119.74, 113.87, 112.21, 105.04 (double intensity), 100.49, 55.93 (double intensity), 55.67.

CH-2A: ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.32 (dd, 1H, J = 7.9, 7.9 Hz), 7.05 (d, 1H, J = 7.9 Hz), 7.0 (m, 1H), 6.93 (ddd, 1H, J = 0.7, 2.6, 8.1 Hz), 6.75 (d, 1H, J = 12.2 Hz), 6.70 (d, 1H, J = 12.2 Hz), 6.60 (d, 1H, J = 12.2 Hz), 6.49 (t, 1H, J = 2.3 Hz), 3.87 (s, 3H), 3.82 (s, 6H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 160.94 (double intensity), 159.80, 139.45, 139.95, 130.95, 130.84, 129.60, 121.96, 114.30, 113.72, 107.16 (double intensity), 100.33, 55.61 (double intensity), 55.50.

HJ-25B: ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.61 (dd, 1H, J = 1.7, 7.7 Hz), 7.49 (d, 1H, J = 16.5 Hz), 7.28 (ddd, 1H, J = 1.7, 7.5, 1.0 Hz), 7.08 (d, 1H, J = 16.5 Hz), 7.00 (t, 1H, J = 6.4 Hz), 6.92 (dd, 1H, J = 1.0, 8.3 Hz), 6.73 (d, 2H, J = 2.3 Hz), 6.42 (t, 1H, J = 2.3 Hz), 3.91 (s, 3H), 3.86 (s, 6H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 161.35 (double intensity), 157.39, 140.45, 129.52, 129.21, 126.95, 126.66, 124.48, 121.17, 111.38, 105.07 (double intensity), 100.21, 55.93, 55.80 (double intensity).

HJ-25A: ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.20-7.25 (m, 2H), 6.87 (dd, 1H, J = 8.3, 0.7 Hz), 6.77 (ddd, 1H, J = 0.7, 7.4, 7.4 Hz), 6.69 (d, 1H, J = 12.2 Hz), 6.55 (d, 1H, J = 12.2 Hz), 6.39 (d, 2H, J = 2.3 Hz), 6.27 (t, 1H, J = 2.3 Hz), 3.87 (s, 3H), 3.60 (s, 6H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 160.77 (double intensity), 157.55, 139.52, 130.71, 130.61, 129.15, 126.76, 126.55, 120.61, 111.03, 107.14 (double intensity), 100.23, 55.89, 55.79 (double intensity).

HJ-22A: ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.57 (dd, 1H, J = 7.76, 1.76 Hz), 7.39 (m, 1H), 7.14 (m, 1H), 7.08 (m, 2H), 7.06 (dd, 2H, J = 4.36, 1.72 Hz), 7.03 (d, 1H, J = 1.68 Hz), 6.89 (ddd, 1H, J = 7.52, 7.52, 1.08 Hz), 6.79 (m, 4H), 6.68, (m, 3H), 6.61 (ddd, 3H, J = 7.48, 7.48, 1.08 Hz), 3.79 (s, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 157.26, 128.83, 126.82, 126.77, 124.05, 121.14, 111.29, 55.97.

HJ-22B: (minor of HJ-22A) ^{13}C-NMR (100 MHz, $CDCl_3$) δ 157.54, 130.42, 128.83, 127.51, 121.14, 110.94 (double intensity), 55.85.

HJ-23A: ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.16 (m, 2H), 6.80 (d, 1H, J = 8.28 Hz), 6.70 (m, 3H), 6.62 (d, 1H, J = 8.20 Hz), 6.48 (m, 5H), 3.73 (s, 6H), 3.46 (s, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 157.57, 148.57, 148.50, 130.64, 130.42, 130.38, 128.95, 126.93, 124.76, 122.41, 120.63, 112.00, 111.08, 111.06, 56.16, 55.88, 55.78.

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HJ-23B: ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.48 (dd, 1H, J = 7.56, 1.64 Hz), 7.24 (d, 1H, 16.44 Hz), 7.12 (m, 1H), 6.97 (m, 3H), 6.86 (m, 1H), 6.79 (dd, 1H, J = 8.28, 0.92 Hz), 6.74 (d, 1H, J = 8.24 Hz), 3.83 (s, 3H), 3.78 (s, 6H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 157.16, 149.49, 149.18, 131.55, 129.41, 128.77, 127.04, 126.65, 121.65, 121.98, 121.36, 111.59, 111.32, 109.29, 55.34, 55.32, 55.91.

HJ-24B: ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.84 (dd, 1H, J = 7.68, 1.60 Hz), 7.60 (d, 1H, J = 16.44 Hz) 7.51 (m, 1H), 7.39 (d, 1H, J = 2.0 Hz) 7.337.22 (m, 3H), 7.18 (dd, 1H, J = 8.20, 0.48 Hz), 7.07(d, 1H, J = 8.04 Hz), 4.16(s, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 157.19, 148.51, 147.54, 133.01, 129.19, 128.83, 126.93, 126.63, 122.23, 121.87, 121.17, 111.33, 108.76, 106.16, 101.49, 55.92.

HJ-24A: ^{13}C-NMR (100 MHz, $CDCl_3$) δ 157.20, 147.67, 146.92, 132.00, 131.03, 129.19, 129.01, 127.23, 121.87, 121.17, 111.33, 110.31, 108.76, 101.49, 55.64.

HJ-15: ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.57 (m, 3H), 7.47 (d, 1H, J = 16.61 Hz), 7.34 (t, 2H, J = 7.42 Hz), 7.23 (m, 2H), 7.12 (td, 1H, J = 8.12, 1.62 Hz), 6.93 (dd, 1H, J = 8.06, 1.10 Hz), 6.83 (td, 1H, J = 7.49, 0.97 Hz); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 155.40, 138.09, 129.06 (double intensity),129.02, 128.12, 127.62, 126.86, 126.58 (double intensity), 124.12, 124.09, 119.69, 116.23.

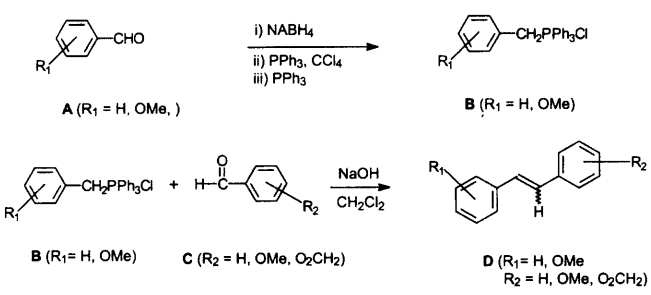
HJ-13B: ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.34 (td, 1H, J = 8.23, 1.71 Hz), 7.31(dd, 1H, J = 7.65, 1.17 Hz), 7.04 (dd, 1H, J = 8.23, 0.82 Hz), 6.96 (s, 2H), 6.946.87 (m, 4H), 4.03 (s, 6H), 4.00 (s, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 157.63, 153.13, 147.65, 132.28, 130.53, 128.91, 126.69, 126.56, 125.78, 123.74, 122.42, 120.49, 111.49, 111.01, 61.16, 56.15, 55.85.

HJ-27B: ^{1}H-NMR (400 MHz, $CDCl_3$) 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); ^{13}C-NMR (100 MHz, $CDCl_3$) 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 (double intensity).

Quantitative Structure-Activity Relationships (QSARs) calculation was carried on a Silicon Graphics INDY R4400 workstation using Cerius2 program (MSI, SanDiego, U.S.A.). NMR spectra were measured with a Bruker ARX400 NMR spectrometer. In order to test the pharmacology of LTD4 antagonism, 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 ml-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 bubbling with 95% O_2 and 5% CO_2. Inhibitory effect of the test compounds on LTD4 was examined by adding test compounds to the bath after tracheal contraction with 5 nM LTD4 was attained. Activity was expressed as the concentrationable to induce 50% relaxation (ED_{50}).

A training set for QSARs is shown in Fig. 1. 3D structures of compounds used for the calculation were obtained from Molecular Dynamics. Structural parameters and biological activities are listed in Table 1.

To obtain QSARs, a statistical method, Genetic Function Approximation (GFA) was applied. The equation showing



Scheme 1. The synthetic method of 13 compounds used for the training set of QSARs.

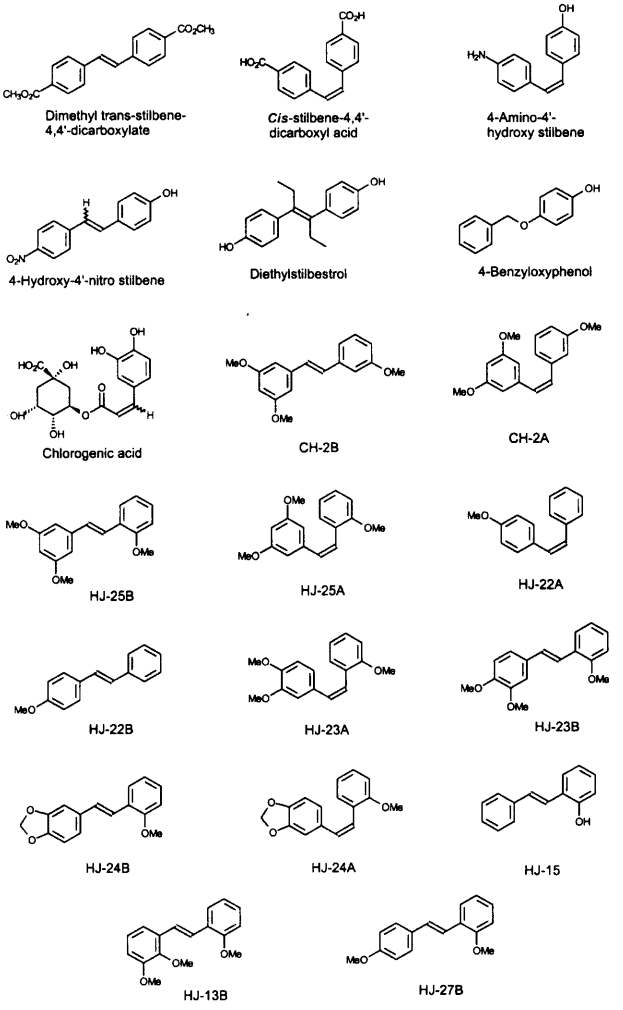


Fig. 1. Structures used for the training set of QSARs.

Table 1. Structural parameters and biological activities used for QSARs.

Molecule	Apol	Dipole-mag	Dipole-X	Dipole- Y	Dipole- Z	HOMO	LUMO	MW	Rad of Gyration	Density	Vm	AlogP	Area	Log (Biological activity*)
Dimethyl trans-stilbene-4.4-dicarboxylate	1.24E+04	0.165	-0.053	-0.013	0.056	-11.3877	1.0989	296.322	5.495	1.09	272.477	3.72	353.828	1.48
HJ-24B	1.07E+04	4.549	3.422	1.671	2.487	-10.5634	2.1862	254.285	4.275	1.09	233.203	3.70	296.853	3.00
HJ-25A	1.14E+04	3.122	2.094	-1.098	-2.038	-11.1175	2.3808	270.327	3.965	1.04	260.629	3.51	333.395	3.00
HJ-22A	9.58E+03	2.429	-2.001	1.366	0.164	-10.9280	2.5834	210.275	3.350	1.01	208.638	4.01	270.094	3.00
HJ-15	9.06E+03	1.947	-1.149	1.103	1.120	-10.8869	2.2207	196.248	3.789	1.02	191.872	3.98	245.909	1.38
CH-2B	1.14E+04	5.096	4.229	2.338	1.620	-12.2904	2.0294	270.327	4.618	1.04	260.720	3.51	336.482	3.00
HJ-23B	1.14E+04	3.298	2.765	-1.333	1.208	-10.5601	2.1227	270.327	4.451	1.04	261.002	3.51	325.671	2.36
HJ-24A	1.18E+04	1.821	1.056	0.239	-1.464	-10.5637	2.5729	282.338	4.075	1.06	266.218	4.33	345.765	3.00
HJ-25B	1.14E+04	3.220	2.501	-1.123	1.689	-11.0516	2.1125	270.327	4.489	1.04	260.726	3.51	341.495	3.00
HJ-27B	1.05E+04	2.793	1.788	-2.061	-0.598	-10.6706	2.4135	240.301	4.374	1.02	234.842	3.76	304.577	2.48
HJ-22B	9.58E+03	2.436	-1.598	1.510	-1.051	-10.7656	2.1928	210.275	4.302	1.00	209.451	4.01	263.467	3.00
Cis-srilbene-4.4-dicarboxylic acid	1.14E+04	6.664	6.101	-2.648	0.417	-11.5453	1.3257	268.268	3.789	1.12	239.054	3.66	303.863	1.93
CH-2A	1.14E+04	1.808	1.676	0.272	-0.621	-11.3606	2.4188	270.327	3.951	1.04	259.898	3.51	342.585	3.00
4-Amino-4-hydroxystilbene	9.54E+03	3.481	-1.993	-2.833	0.344	-10.2987	2.8259	211.263	3.452	1.04	203.202	3.20	267.996	2.54
4-Hydroxy-4-nitrostilbene	1.01E+04	7.462	6.615	1.954	-2.845	-11.3579	0.1361	241.246	4.210	1.12	215.780	3.93	270.294	1.08
Diethylstibestrol	1.16E+04	1.002	0.050	-0.060	0.999	-10.7405	3.2287	268.355	3.770	1.00	267.983	4.79	357.067	-0.70
4-(Benzyloxy)phenol	8.68E+03	4.235	-3.906	-1.541	-0.547	-11.5674	3.6117	200.237	3.851	1.06	189.303	3.29	245.166	-1.00
Chlorogenic acid	1.25E+04	6.317	-2.030	4.793	3.579	-11.5905	1.6013	354.313	4.813	1.08	300.097	0.15	375.498	3.00
HJ-13B	1.14E+04	3.186	2.526	1.664	1.001	-10.9420	2.2373	270.327	4.009	1.04	260.167	3.51	333.748	3.00
HJ-23A	1.14E+04	2.961	2.084	-0.992	-1.856	-10.7786	2.4555	270.327	3.807	1.04	260.881	3.51	337.155	3.00

*the 50% inhibitory concentration of the test compounds on LTD4 (μg/ml)

APol: Electronic descriptor that computes the sum of atomic polarizabilites.

Dipole: Electronic descriptor that indicates the strength and orientation behavior of a molecule in an electrostatic field.

HOMO: Electronic descriptor that computes highest occupied molecular orbital energy.

LUMO: Electronic descriptor that computes lowest unoccupied molecular orbital energy.

MW: Structural descriptor that calculates the molecular weights with molecular structure.

Rad of Gyration: Spatial descriptor that calculates the molecular radius of gyration.

Density: Spatial descriptor that calculates the molecular density.

Vm: Spatial descriptor that calculates the molecular volume.

AlogP: Thermodynamic descriptor that calculates log of the partition coefficient.

Area: Spatial descriptor that calculates the molecular surface area.

Table 2. A comparison of the experimental values with the calculated values.

Molecule	Log (experimental activity*)	Log (calculated activity*)
Dimethyl trans-stilbene-4.4-dicarboxylate	1.480	1.733
HJ-24B	3.000	2.786
HJ-25A	3.000	3.022
HJ-22A	3.000	3.087
HJ-15	1.380	1.409
CH-2B	3.000	2.684
HJ-23B	2.360	2.774
HJ-24A	3.000	2.995
HJ-25B	3.000	2.764
HJ-27B	2.480	2.989
HJ-22B	3.000	2.712
Cis-srilbene 4.4-dicarboxylic acid	1.930	1.971
CH-2A	3.000	3.059
4-Amino-4 hydroxystilbene	2.540	2.035
4-Hydroxy-4nitrostilbene	1.080	0.926
Diethylstibestrol	-0.700	-0.470
4-(Benzyloxy)phenol	-1.000	-1.040
Chlorogenic acid	3.000	3.135
HJ-13B	3.000	2.884
HJ-23A	3.000	3.094

*the 50% inhibitory concentration of the test compounds on LTD4 (μg/ml).

the relation between structures and activities is as follows:

$$\text{(Biological Activity)} = -0.26 \times (\text{AlogP}) + 0.96 \times (\text{LUMO}) + 0.095 \times (\text{MW}) - 18.42 \quad (r^2 = 0.959)$$

where AlogP, LUMO, and MW denote log of the partition coefficient, the lowest unoccupied orbital energy, and molecular weights, respectively. That is to say, even though ten structural descriptors were applied to obtain QSARs (Table 1), only three parameters showed major contributions. A comparison of the experimental values with the calculated values is listed in Table 2. The cross validated value of the comparison, 0.90, shows that the QSARs equation can express the relation between the biological activity and the structural parameters well.

In conclusion, to obtain a compound with a high activity, methoxy group should be hydrolyzed because the parameter related to a partition coefficient is proportional to the biological activity negatively in the QSARs equation. Moreover the conformation between two phenyl rings is independent on the activity.

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