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Development of LTD_4 antagonists using QSAR

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Abstract : In order to discover new Leukotriene D_4 antagonists, Quantitative Structure-Activity Relationships (QSAR) were applied based on the known data. A series of chalcone derivatives were selected for the training set. A candidate was predicted using QSAR and synthesized, and its biological activity was tested.(Received September 4, 1998; accepted October 15, 1998)

Introduction

Asthma attacks and kills more than 5000 children and adults annually in the United States. Asthma is caused by reversible airway obstruction as well as inflammation. When mast cells are activated by the attack of allergen, several inflammatory mediators are released.[1-4]

Peptidoleukotriene have been studied because of their pharmacological effects in asthma. Among peptidoleukotriene, especially leukotriene D_4 (LTD_4) shows 1000-fold stronger activity in bronchoconstrictors than histamine. There are at least two receptors for peptidoleukotriene. One of them is *CysLT1* known as LTD_4 receptor. Its activation causes bronchoconstriction, and results in asthma.[5-6] Before zafirlukast was launched in November, 1996, by Zeneca, anti-histamines were generally used against asthma. The first LTD_4 antagonist can go back to FPL 55712, which is sodium 7-3-(4-acetyl-3-hydroxy-2-propylphenoxy)-2-hydroxypropyl-4-oxo-8-propyl-4H-1-benzopyran-2-carboxylate. It was developed by Fison Co. in 1973. However, it has a weakness that is not an oral drug because of its short half-lifetime. In order to overcome this weakness, LY-171883 was developed by Eli Lilly. It also has a weakness that can be needed only for chronic asthma. Both FPL 55712 and LY-171883 belong to the first generation of LTD_4 antagonists, which has hydroxyacetophenone moiety. The compounds similar to LTD_4 structurally are classified into the second generation. Zafirlukast mentioned above belongs to the third generation which has quinolymethoxyphenyl moiety.[7-10]

Key words : LTD_4 antagonists, chalcone derivatives, QSAR
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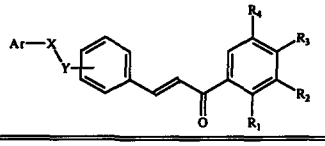
Authors tried to develop new LTD_4 antagonists belonging to the third generation by organic synthesis. A candidate obtained from QSAR was synthesized and tested as a LTD_4 antagonist in isolated strips of guinea pig trachea *in vitro*. Its activity was about 10 times less potent than a reference compound, LY-171883.

Experimental methods

QSAR

A series of chalcone derivatives published by M. Zwaagstra (J. Med. Chem. 40, 1075) were employed in this study.[11] 29 of 55 compounds described by M. Zwaagstra were used as the training set because the units of several biological data were not compatible with the others such as nM and % inhibition and activities of some data were too weak comparing to the selected set. The training set were listed in Table 1. All computational calculation were performed using MSI software (San Diego, CA) on Silicon Graphics INDY R4400 workstation. The structures of the training set were calculated using the Discover force field of InsightII packages 950 (MSI, San Diego, CA)[12] because their three dimensional structures by NMR or X-ray crystallography were not available. The initial low-energy conformations were obtained from the energy minimization with 1000 steps of steepest descent and their further conformational search was carried out using molecular dynamics with 500000 conformers. Of every tenth conformer collected during molecular dynamics, the conformer with the lowest energy was selected and used for the training set.

Table 1. The training set employed in this study. (M. Zwaagstra, J. Med. Chem. 40 1075)[11]



	Ar	XY	posXY	R_1	R_2	R_3	R_4	K_D (nM)
10	2-quinolinyl	CH_2O	4	OH	H	H	CO_2H	450
15	2-quinolinyl	CH_2O	3	OH	H	H	CO_2H	609
17	2-quinolinyl	CH_2O	3	OH	H	H	CN_4H	209
25	1-[(1-ethoxyethyl)-2-benzimidazolyl]	CH_2O	4	OH	H	H	CN_4H	527
29	1-(2-thiazolyl)	CH_2O	4	OH	H	H	CN_4H	233
30	2-(7-chloroquinoline)	CH_2O	4	OH	H	H	CN_4H	196
31	2-(7-chloroquinoline)	C=C	4	OH	H	H	CN_4H	239
32	2-quinolinyl	CH_2O	4	OH	CO_2H	H	H	307
33	2-quinolinyl	CH_2O	4	OH	CO_2H	H	F	396
34	2-quinolinyl	CH_2O	4	OH	CO_2H	H	Cl	732
36	2-quinolinyl	CH_2O	3	OH	CO_2H	H	H	415
37	2-quinolinyl	CH_2O	3	OH	CO_2H	H	Cl	104
38	2-quinolinyl	CH_2O	3	OH	CO_2H	H	Br	205
40	2-quinolinyl	CH_2O	3	OH	H	CO_2H	H	928
43	2-quinolinyl	CH_2O	4	CO_2H	H	H	H	143
45	2-quinolinyl	C=C	4	OH	CO_2H	H	H	200
46	2-quinolinyl	C=C	4	OH	H	H	CO_2H	585
47	2-quinolinyl	C=C	3	OH	H	H	CN	346
50	2-quinolinyl	C=C	3	OH	CO_2H	H	F	140
52	2-quinolinyl	C=C	3	OH	CO_2H	H	Br	207
53	2-quinolinyl	C=C	3	OH	CO_2H	H	Me	105
54	2-quinolinyl	C=C	3	OH	CO_2H	H	*t*-Bu	208
55	2-quinolinyl	C=C	3	OH	CN	H	Cl	449
56	2-quinolinyl	C=C	3	OH	CN_4H	H	Cl	828
57	2-quinolinyl	C=C	3	OH	H	H	OCH_2CO	175
59	2-quinolinyl	C=C	3	OH	H	OCH_2CO	H	204
60	2-quinolinyl	CH_2O	4	OMe	H	H	CO_2H	188
61	2-quinolinyl	CH_2O	4	OMe	H	H	CO_2Me	357
63	2-quinolinyl	CH_2O	3	O-nBu	H	H	CO_2H	300

The program for QSAR calculations was Cerius2_2.3 (MSI, San Diego, CA).[13] A set of 4 descriptors of 10 descriptors supplied by Cerius2 were used, which described electronic, conformational, spatial and structural properties of the molecules. These descriptors were calculated by Cerius2. In order to generate the QSAR equation, multiple linear regression with genetic function approximation and partial least squares together were used. A candidate was predicted by Cerius2 and transferred to the organic synthetic research group.

Synthesis

Infrared spectra were recorded on a Perkin Elmer Paragon 2000 FT-IR spectrometer. NMR spectra were obtained on a Bruker DPX 400 (9.4 T) instrument in $CDCl_3$ and DMSO-d_6. Analytical thin-layer chromatography was performed by using precoated silica gel 60 F_{254} plates and the silica gel used for flash column chromatography was supplied from Merck (230~400 mesh, 60Å).

(1) Synthesis of 4-(2-quinolinylmethoxy)benzaldehyde (**3**)

To a mixture of 666 mg (3 mmol) of 2-bromomethylquinoline (**1**) and 366 mg (3 mmol) of 4-hydroxybenzaldehyde (**2**) in 15 m*l* of DMF was added 911 mg (6.6 mmol) of anhydrous K_2CO_3 and resulting suspension was stirred at 90°C for 10 h. After evaporation of solvent, 100 m*l* of ethyl acetate and 50 m*l* of water was added to the residue and extracted. Separated organic layer was washed with 1N NaOH and sat'd NaCl solution, and dried over $MgSO_4$. After filtration, the filtrate was evaporated and residue was purified by column chromatography to give 655 mg (83%) of **3**. M.P. 112~113°C; IR (KBr, cm^{-1}) 1697, 1601, 1212, 969, 823, 749; ^{1}H-NMR (400 MHz, $CDCl_3$) 5.42 (s, 2H), 7.11 (d, 2H), 7.48~7.54 (m, 1H), 7.58 (d, 1H), 7.66~7.72 (m, 1H), 7.74~7.78 (m, 1H), 7.80 (d, 2H), 8.06 (d, 1H), 8.15 (d, 1H), 9.87 (s, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$) 70.5, 114.2 (double intensity), 117.0, 125.7, 126.6, 126.7, 127.9, 128.9, 129.3, 130.9 (double intensity), 136.1, 146.5, 155.7, 162.3, 189.7

(2) Synthesis of 3-cyano-4-(2-quinolinylmethoxy)chalcone (**4**)

187 mg (0.71 mmol) of 4-(2-quinolinylmethoxy) benzal-

lehyde (**2**) and 103 mg (0.71 mmol) of 3-acetylbenzonitrile **4**) were dissolved in 6mL of ethyl alcohol to make clear olution. To the above mixture was added 1.8 mL of 25% KOH solution to give a suspension. The above suspension vas stirred for 48 h at room temperature, and 25 m*l* of THF was added to give a clear solution. To the solution vas added ice and pH was adjusted at 3 by adding 3N HCl olution. Solvent was evaporated and resulting aqueous solution vas extracted with ethyl acetate. The organic layer was lried over $MgSO_4$. After filtration, the filtrate was evaporated nd residue was purified by column chromatography to give 00 mg of chalcone **5**. Yield (72%). IR (KBr, cm^{-1}) 1650, 601, 1571, 1510, 1253, 1061, 833, 804; ^{1}H-NMR (400 MHz, $CDCl_3$) 5.45 (s, 2H), 7.07 (d, 2H, 8.5 Hz), 7.34 (d, 1H, 15.6 Hz), 7.56~7.66 (m, 5H), 7.75~7.84 (m, 4H), 8.1 (d, 1H, 8.5 Hz), 8.19~8.26 (m, 3H), ^{13}C-NMR (100 MHz, $CDCl_3$) 71.4, 13.1, 115.6 (double intensity), 118.1, 118.7, 119.1, 126.7, 27.6, 127.7, 128.9, 129.6, 130.0, 130.6 (double intensity), 32.0, 132.4, 135.4, 137.3, 139.3, 146.2, 147.5, 157.1, 160.9

(3) Synthesis of 4-(2-quinolinylmethoxy)-3-(5-tetrazolyl)-halcone (**6**)

To a mixture of 390 mg (1 mmol) of 3-cyano-4-(2-quinolinylmethoxy) chalcone **5** and 200 mg (3 mmol) of sodium azide in 7 m*l* of DMF, was added 160 mg (3 mmol) of ammonium chloride and stirred at 100°C for 28 h. After cooling, the above suspension was poured into 15 m*l* water pH was adjust at 3. Aqueous layer was extracted with ethyl acetate, and organic layer dried over $MgSO_4$. After filtration and evaporation, the residue was chromatographied to give 230 mg (53%) of **6**. ^{1}H-NMR (400 MHz, $CDCl_3$) 5.41 (s, 2H), 7.06 (d, 2H, 8.7 Hz), 7.48~7.58 (m, 4H), 7.66~7.70 t, 2H), 7.7~7.84 (m, 4H), 8.2 (d, 2H, 8.7 Hz), 8.14 (d, 2H), 9.80 (s, 1H)

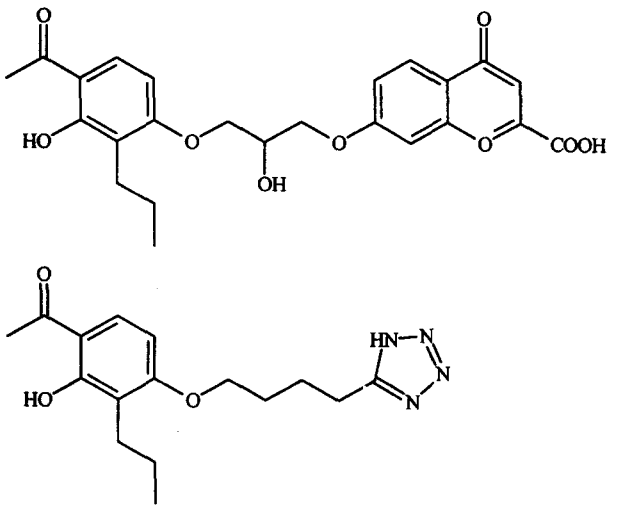


Fig. 1. The structures of FPL-55712 (top) and LY-171883 (bottom).

Biological Activity Test

Adult male Hartley guinea pigs weighing 250~350 g were killed by a sharp blow to the head and the trachea was removed. Tracheal chains were prepared by suturing two opened rings of 2~3 mm wide. The chains were placed in a water jacketed 13-m*l* organ bath and connected via silk to a force-displacement transducer. The preparations were bathed in Krebs-Henseleit solution of the following composition (mM): NaCl, 118; $MgSO_4$, 1.1; $CaCl_2$, 1.8; $NaHCO_3$, 24.9; KH_2PO_4, 1.0; and glucose 11.1. To block cyclooxygenase 5 μM of indomethacin was included in the medium. The bath was maintained at 37°C and continuously aerated with 95% O_2-5% CO_2. Muscle contraction was continuously recoded with a chart strip recorder. LTD_4 of 15 nM was added to the bath to contract the muscle and when the contraction was stabilized test substance was cumulatively added.

Results and Discussion

QSAR

Since the chalcone derivatives were used for the training in this study, the three dimensional structure of the chalcone moiety was calculated using molecular dynamics. It contains three rotational bonds. From the molecular dynamics calculation, 17 conformers whose energy were ranged between 180 and 190 kcal/mol, were chosen and aligned in Fig. 2. And their geometric data were listed in Table 2. According to the same method, the three dimensional structures of other

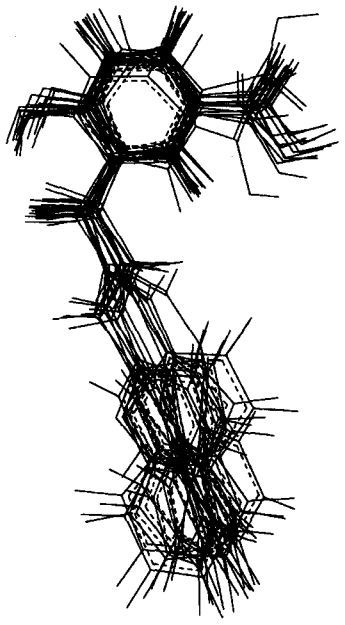
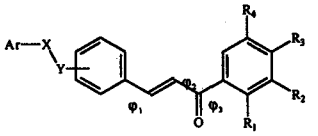


Fig. 2. Alignments of 17 conformers obtained from the molecular dynamics calculation, whose energy were ranged between 180 and 190 kcal/mol.

Table 2. The geometric data of the training set employed in this study



No.	φ_1*	φ_2*	φ_3*	Energy#
1	174.24	43.22	177.84	181.31
2	18.96	-56.01	176.83	196.85
3	-95.36	-43.21	148.62	184.92
4	-112.79	-97.45	-159.80	185.69
5	21.54	78.68	-167.01	187.38
6	150.88	-165.52	-151.67	182.93
7	-39.50	-20.49	152.54	189.33
8	-43.62	-49.20	177.20	184.39
9	87.79	-170.94	138.25	187.20
10	139.50	117.96	175.16	187.55
11	-139.34	172.85	-165.85	187.66
12	119.87	-121.69	-174.93	189.02
13	172.96	82.14	166.63	183.97
14	41.71	-74.45	-170.38	187.02
15	57.76	-160.85	-165.23	185.07
16	-32.86	149.08	169.52	185.28
17	-131.04	-111.56	163.59	185.33

*: dihedral angles.
#: total energy (kcal/mol).

28 compounds were obtained.

The best equation is the following

ED_{50}=228.875+0.272884 * <"Apol" -19368.9> + 0.14221 * <2076.9-"PMI-Z" > + 216.684 * <-10.9207-"HOMO" > - 1.11415 * <"PMI-X" -449.044> + 359.859 * <1-"Hbond donor" > - 27.3824 * <"Dipole-X" + 10.3351> - 0.505498 * <18124-"Apol" > + 2.3568 * <"MW" -449.468> + 176.103 * <"Hbond donor" -1> - 5.84959 * <443.43-"MW" > - 81.3957 * <"Dipole-Z" -0.375667> + 232.887 * <"Dipole-mag" -14.6796> - 135.69 * <-12.3767-"Dipole-X" > + 24.9266 * <439.442-"MW" > (r^2=0.955)

Apol : The electronic descriptor that computes the sum of atomic polarizabilities.

PM1 : The spatial descriptor that calculates the principal moments of inertia about the principal axes of a molecule.

HOMO : The electronic descriptor that computes Highest Occupied Molecular Orbital energy.

H-bond donor : The structural descriptor that counts the number of hydrogen bond donors.

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

MW : The structural descriptor that calculates the molecular weights with molecular structures.

A comparison of experimental activities and predicted activities is listed in Table 3, and graphically shown in Fig.

Table 3. A comparison of experimental activities and predicte activities

	Experimental activities	Predicted activities
10	450	441
15	609	502
17	209	186
25	527	546
29	233	229
30	196	196
31	239	269
32	307	298
33	396	421
34	732	694
35	415	467
37	104	173
38	205	165
40	928	890
43	143	166
45	200	198
46	585	620
47	346	379
50	140	162
52	207	197
53	105	171
54	208	143
55	449	330
56	828	892
57	175	177
59	204	188
60	188	241
61	357	360
63	300	269

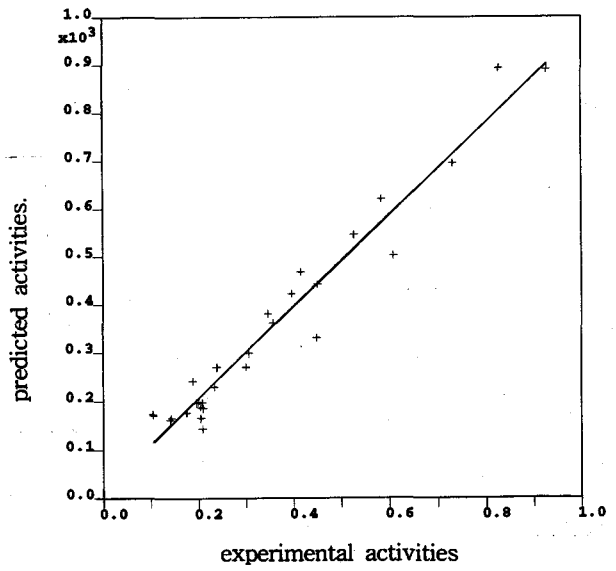
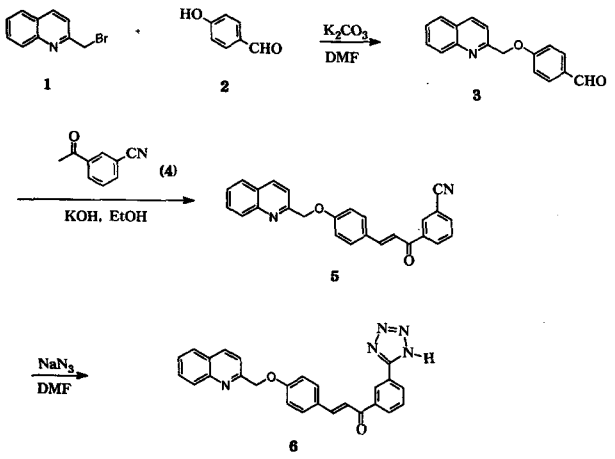


Fig. 3. A comparison of experimental activities and predicted activities.

3. Using the equation mentioned above, the activity of LY 171883 was calculated to be 1 which could be considered to show the best activity comparing to the training set because its EC_{50} value was out of range. The activity of the compound, **6**, as shown in scheme 1 was calculated to be 68.



Scheme 1. Synthesis of the compound 6.

Synthesis

The target compound (**6**) was synthesized as shown in Scheme 1. Alkylation of 4-hydroxybenzaldehyde (**2**) with 2-bromethylquinoline (**1**) in the presence of base (K_2CO_3, DMF) gave 4-(2-quinolinylmethoxy benzaldehyde (**3**) in 86% yield. Chalcone (**5**) was prepared by Claisen-Schmidt[4] condensation of aldehyde (**3**) with 3-cyanoacetophenone (**4**). Reaction of equimolar amount of the aldehyde and the acetophenone, in a mixture of ethanol and water containing sodium hydroxide, at room temperature for two days gave the chalcone **5** in 72% yield after chromatography. Finally, the cyano group in the chalcone **5** was converted to desired tetrazole ring with sodium azide at elevated temperature to give target compound **6**.

Biological test

The pharmacological activity of **6** was assessed in isolated strips of guinea pig trachea *in vitro*. After contraction of the organ preparation with 15 nM LTD_4, **6** was added to the organ bath containing Krebs-Henseleit solution (pH 7.2, 37°C) in a cumulative manner and relaxing activity was monitored with a physiography (Letica). The ED_{50} value of **6** was 5.8±1.1 μM, which was about 10 times less potent than a reference compound LY 171883 (0.4±0.2 μM). As mentioned above, because the calculated value of LY 171883 was out of range, the experimental values could not be compared with the calculated values directly. But the order coincided with each other. Even though **6** did not show stronger activity than LY 171883, the synthesis of the compound belonged to the third generation based on the QSAR calculations was meaningful.

Acknowledgements

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구조-활성간 연구를 통한 LTD4 antagonists의 개발

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초 록 : 새로운 Leukotriene D_4 antagonists를 찾기 위해 구조-활성간 연구를 수행하였다. 이미 알려진 chalcone 유도체의 구조와 생물학적 활성 자료를 이용하여 구조-활성간 계산을 수행한 결과 새로운 화합물을 발견하였고, 이를 합성하여 효과를 측정한 결과를 보고하고자 한다.

찾는말 : LTD_4 antagonists, chalcone 유도체, 구조-활성 관계

*연락저자