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Binding of YM158, a new dual antagonist for leukotriene D_4 and thromboxane A_2 receptors, to guinea pig lung membranes

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Abstract

Arachidonic acid metabolites mediate inflammatory responses at a cellular level. The affinity of the newly synthesized compound YM158, 3-[(4-*tert*-butylthiazol-2-yl)methoxy]-5′-[3-(4-chlorobenzenesulfonyl)propyl]-2′-(1*H*-tetrazol-5-ylmethoxy)benzanilide monosodium salt monohydrate, for leukotriene D_4 and thromboxane A_2 receptors was examined in radioligand binding assays. YM158 inhibited [^{3}H]leukotriene D_4 and [^{3}H]U46619 (9,11-dideoxy-11α,9α-epoxymethanoprostaglandin $F_{2\alpha}$) binding to guinea pig lung membrane preparations, with K_i values of 0.64 ± 0.06 nM for leukotriene D_4 and 5.0 ± 0.88 nM for thromboxane A_2 receptors. The Hill coefficients (n_H) did not significantly differ from unity, indicating that this antagonism is competitive. In contrast, YM158 showed no affinity for several other receptors, including neurotransmitter-related (α_1-, α_2-, β-adrenoceptors, histamine, 5-HT, muscarinic, σ), C_{5a}, opioid, Ca^{2+} channel, K^+ channel, protein kinase C, bradykinin, endothelin, neurokinin and platelet activating factor receptors. These studies indicate that YM158 is a highly selective dual antagonist for leukotriene D_4 and thromboxane A_2 receptors, and this has potential clinical and research applications.

Keywords: Leukotriene; Thromboxane; YM158

1. Introduction

Cyclooxygenase and 5-lipoxygenase metabolites of arachidonic acid are important mediators of allergic responses in the pathogenesis of asthma (Barnes, 1992). Cysteinyl-leukotrienes (leukotriene $C_4/D_4/E_4$) (Samuelsson, 1983) are 5-lipoxygenase products of arachidonic acid metabolism. They are currently considered to be major constituents of the slow-reacting substance of anaphylaxis (Feldberg and Kellaway, 1938). Cysteinyl-leukotrienes induce airway smooth muscle contraction (Dahlén et al., 1980; Ueno et al., 1982) and increase vascular permeability (Peck et al., 1981; Rinkema et al., 1984). The potent cysteinyl-leukotriene receptor antagonists, pranlukast (Nakagawa et al., 1992) and zafirlukast (Krell et al., 1990), already in use as agents to treat bronchial asthma (Busse, 1996).

The role of thromboxane A_2 in asthma has also been widely investigated. Thromboxane A_2 contributes to the bronchoconstriction (Nagai et al., 1991; Francis et al., 1991) and airway hyperreactivity (Jones et al., 1992; Minoguchi et al., 1992; Nagai et al., 1993) commonly seen in this ailment. As a result, it is likely that thromboxane A_2 plays an important role in bronchial asthma and airway hyperreactivity. As further evidence, a thromboxane A_2 receptor antagonist, seratrodast (Fujimura et al., 1991), is already in use as an anti-asthmatic agent in Japan.

The role of cysteinyl-leukotrienes and thromboxane A_2 in asthma is thought to be different. This strongly suggests that a multi-pathway inhibitory agent, such as a broad-spectrum receptor antagonist, would prove more effective than single inhibitors for the treatment of bronchial asthma.

Consequently, attention focused on the design of a novel dual antagonist for both leukotriene D_4 and thromboxane A_2 receptors. As a result of this search, YM158 3-[(4-*tert*-butylthiazol-2-yl)methoxy]-5′-[3-(4-chlorobenzenesulfonyl) propyl]-2′-(1*H*-tetrazol-5-ylmethoxy)benzanilide monosodium salt monohydrate (Yokota et al., 1997; Fig. 1) was discovered. YM158 is a selective, dual

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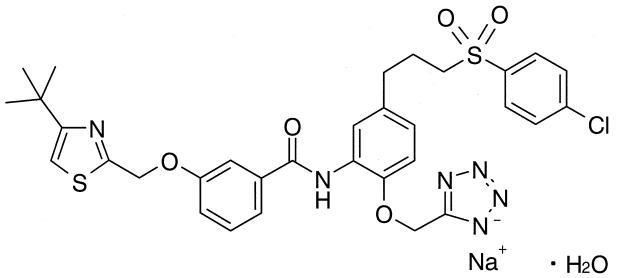

Fig. 1. Chemical structure of YM158, 3-[(4-*tert*-butylthiazol-2-yl)-methoxy]-5′-[3-(4-chlorobenzenesulfonyl)propyl]-2′-(1*H*-tetrazol-5-ylmethoxy)benzanilide monosodium salt monohydrate.

antagonist for leukotriene D_4 and thromboxane A_2 receptors in trachea isolated from guinea pigs (Arakida et al., 1998). In the present study, we examined the interactions of YM158 with leukotriene D_4 and thromboxane A_2 receptors, using radioligand binding assays with lung membrane preparations, in order to determine the mechanism of the inhibitory effects of YM158 on leukotriene D_4- and U46619-induced trachial contractions.

2. Materials and methods

2.1. Animals

Male Hartley guinea pigs (Japan Charles River, Yokohama, Japan) weighing 300 to 900 g were used for the radioligand binding assay. Animals were given free access to food and water until the experiments.

2.2. Chemicals

The following drugs and chemicals were used: YM158, zafirlukast, montelukast and domitroban were synthesized by Yamanouchi Pharmaceutical (Tsukuba, Ibaraki, Japan). Pranlukast and seratrodast were purified from the commercially available formulations ONON® (Ono Pharmaceutical, Osaka, Japan) and BRONICA® (Takeda Chemical Industries, Osaka, Japan). The following compounds were purchased: leukotriene D_4, U46619, ISAP (7-[(1*R*,2*S*,3*S*,5*R*)-6,6-dimethyl-3(4-iodobenzenesulfonylamino)-bicyclo[3.1.1.]-hept-2-yl]-5(*Z*)-heptenoic acid: Saussy et al., 1992), IBOP ([1*S*(1α,2β(5*Z*),3α(1*E*,3*S*),4α)]-7-[3-(3-hydroxy-4-(4′-iodophenoxy)-1-butenyl)-7-oxabicyclo-[2.2.1]heptan-2-yl]-5-heptenoic acid: Saussy et al., 1991), SQ29548 ([1*S*[1α,2β(5*Z*),3β,4α]]-7-[3-[[2-[(phenylamino)carbonyl]hydrazino]methyl]-7-oxabicyclo[2.2.1.]hept-2-yl]-5-heptenoic acid: Saussy et al., 1991) (Cayman Chemical, Arbor, MI, USA); phenylmethylsulfonyl–fluoride, pentobarbital sodium salt, cysteine, glycine, Pipes (Nacalai Tesque, Kyoto, Japan); benzamidine (Aldrich Chemical, Milwaukee WI, USA); bacitracin (Wako Pure Chemical Industries, Osaka, Japan); soybean trypsin inhibitor (type II-L), indomethacin (Sigma, St. Louis, MO, USA); [^{3}H]leukotriene D_4, [^{3}H]U46619 (9,11-dideoxy-11α,9α-epoxymethanoprostaglandin $F_{2\alpha}$) (Du Pont NEN®, Wilmington, DE, USA).

2.3. Receptor binding studies

2.3.1. Leukotriene D_4 receptor binding studies with guinea pig lung membrane preparations

The preparation of guinea pig lung membranes and binding assays were performed according to the method of Mong et al. (1986). Briefly, male Hartley strain guinea pigs were killed by decapitation after a blow to the head. The lungs were removed, minced for 1 min in 10 vol. (W/V) of buffer A (0.25 M sucrose, 17 μM phenylmethylsulfonyl–fluoride, 155 μg/ml benzamidine, 10 μg/ml soybean trypsin inhibitor, 100 μg/ml bacitracin and 10 mM Tris–HCl, pH 7.4) with a Polytron® homogenizer (CH-6010, Kinematica, Lucerne, Switzerland), and further homogenized with 3 strokes in a glass homogenizer (model 2356, Iuchi-Seieidou, Osaka, Japan) at 4°C. Then, the homogenate was centrifuged at $1000 \times g$ for 10 min at 4°C and the supernatant fraction was centrifuged at 32,000 $\times g$ for 20 min at 4°C. The pellet was washed with buffer B (10 mM Tris–HCl (pH 7.4)) twice and suspended in buffer B. The protein content of this suspension was measured by the Bradford method (Bradford, 1976), using a Bio-Rad Protein assay kit. The protein level was adjusted to 5–11 mg/ml with buffer B and stored at −80°C.

For the binding assay, 400 μl of buffer C (10 mM $CaCl_2$, 10 mM $MgCl_2$, 2 mM cysteine, 2 mM glycine, 10 mM Pipes buffer, pH 7.4) containing resuspended lung membranes (150–230 μg protein/ml) and [^{3}H]leukotriene D_4 (0–1.5 nM) was incubated for 30 min at 25°C. After the incubation, the mixtures were filtered through GF/C filters and rinsed 5 times with 3 ml volumes of ice-cold buffer D (100 mM NaCl, 10 mM Tris–HCl buffer, pH 7.4). Filters were removed and allowed to dry before measurement of bound radioactivity by liquid scintillation spectrophotometry (Packard TRI-CARB 2200CA) using Aquasol-2 (Packard, Japan). Specific binding was determined by subtracting the amount of radioactivity bound in the presence of 1 μM zafirlukast, from total binding in the absence of zafirlukast. The concentration of zafirlukast used was at least 3000-times higher than its K_i value on guinea pig lung membrane. For the investigation of test compounds, resuspended tissue solutions containing one of the concentrations of the test compound and 0.5 nM of [^{3}H]leukotriene D_4 were assayed by the methods previously described.

2.3.2. Thromboxane A_2 receptor binding studies with guinea pig lung membrane preparations

The preparation of guinea pig lung membranes and binding assays were performed according to the method of Saussy et al. (1991) with a slight modification. In order to

Table 1
Affinity of YM158, pranlukast, zafirlukast, montelukast and leukotriene D_4 for leukotriene D_4 receptors in guinea pig lung membrane preparations

Compound	K_i (nM)	n_H^a
YM158	0.64 ± 0.06	1.06 ± 0.02
Pranlukast	0.18 ± 0.02	1.02 ± 0.03
Zafirlukast	0.24 ± 0.05	1.06 ± 0.02
Montelukast	0.45 ± 0.04	1.16 ± 0.04
Leukotriene D_4	0.34 ± 0.03	0.97 ± 0.01

[a] The Hill coefficient.
IC_{50} value and the Hill coeficient (n_H) were obtained by nonlinear regression analysis of competitive curves for [^{3}H]leukotriene D_4 binding to guinea pig lung membranes. K_i values were calculated according to Cheng and Prusoff (1973). Data are presented as the means ± S.E.M. of 4 to 7 experiments.

remove platelets and other blood cells, the chest cavity of the guinea pig was opened after death and blood was washed out by perfusing buffer E (PBS, phosphate-buffered saline, containing 5 mM Na_4EDTA and 10 μM indomethacin) through the pulmonary artery to the left atrium. The lungs were removed, minced and homogenized for 1 min at 4°C in 5 vol. (W/V) of buffer F (10 μg/ml trypsin inhibitor, 100 μg/ml bacitracin, 10 μM phenylmethylsulfonyl-fluoride, 100 μM benzamide, 250 mM sucrose, 5 mM Na_4EDTA and 10 mM Tris–HCl buffer, pH 7.4). The supernatant fraction of this homogenate was centrifuged at $1000 \times g$ for 10 min at 4°C and re-centrifuged at $12{,}000 \times g$ for 20 min at 4°C. The supernatant fraction was ultracentrifuged at $105{,}000 \times g$ for 60 min at 4°C, and the pellets were washed and suspended with buffer G (100 mM NaCl, 5 mM $MgCl_2$ and 25 mM MOPS (3-(*N*-morpholino)propane-sulfonic acid) buffer, pH 6.5) (Saussy et al., 1991).

For the binding assay, 1 ml of buffer G containing the resuspended lung membrane preparation (360–410 μg protein/ml) and [^{3}H]U46619 (0–35 nM) was incubated for 120 min at 30°C. After the incubation, the suspension was filtered through GF/C filters and rinsed 4 times with 3-ml volumes of ice-cold 25 mM Tris–HCl buffer (pH 7.4). Then the filter-bound radioactivity was measured, and specific binding was defined as the difference between filter-bound radioactivity in the presence and absence of 1 μM ISAP. For investigation of test compounds, 5 nM of [^{3}H]U46619 was used as a ligand.

2.4. Other receptors binding assay

Assays for the binding of YM158 to several other receptors, namely, neurotransmitter-related (α_1-, α_2-, β-adrenoceptors, histamine H_1, H_2, H_3, 5-HT, muscarinic M_1, M_2, M_3, σ), C_{5a}, opioid, Ca^{2+} channels, K^+ channels, protein kinase C, bradykinin 2, endothelin, neurokinin NK_2, NK_3 and platelet activating factor (PAF) receptors, were performed. The membrane specimen and radioligand used are listed in Table 3. The effects of 10 μM YM158 on these binding assay systems were examined.

2.5. Statistics

All data are presented as the means ± standard error of mean (S.E.M.). In the radioligand binding assay, IC_{50} values and Hill coefficients (n_H) were obtained by nonlinear regression analysis of competition curves for [^{3}H] leukotriene D_4 and [^{3}H]U46619 binding to guinea pig lung membranes. K_i values were calculated as described previously (Cheng and Prusoff, 1973).

3. Results

3.1. Leukotriene D_4 receptor binding assay

The dissociation constant (K_d) and maximum number of binding sites (B_{max}) were 0.22 ± 0.019 nM and 375 ± 8.31 fmol/mg of protein, respectively, for [^{3}H]leukotriene D_4 binding to guinea pig lung membranes. YM158 at 0.3 to 100 nM inhibited [^{3}H]leukotriene D_4 binding to guinea pig lung membrane preparations in a concentration-dependent manner, with a K_i value of 0.64 ± 0.06 nM (Table 1). Other leukotriene D_4 receptor antagonists, pranlukast, zafirlukast and montelukast, also had an inhibitory effect on [^{3}H]leukotriene D_4 binding, with K_i values of 0.18 ± 0.02, 0.24 ± 0.05 and 0.45 ± 0.04 nM, respectively. Since the Hill coefficients of these compounds including YM158 were near unity, their inhibition was competitive.

3.2. Thromboxane A_2 receptor binding assay

The K_d and B_{max} values were 3.2 ± 0.37 nM and 94 ± 7.0 fmol/mg of protein, respectively, for [^{3}H]U46619 binding. YM158 concentration-dependently inhibited [^{3}H]U46619 binding to guinea pig lung membranes at concentrations ranging from 0.1 to 100 nM, with a K_i

Table 2
Affinity of YM158, seratrodast, domitroban and various thromboxane A_2 analogues for thromboxane A_2/prostaglandin H_2 receptors in guinea pig lung membrane preparations

Compound	K_i (nM)	n_H^b
YM158	5.0 ± 0.88	0.83 ± 0.13
Seratrodast	1800 ± 650[a]	0.35 ± 0.04[c]
Domitroban	0.31 ± 0.03	0.93 ± 0.05
IBOP	0.091 ± 0.01	1.1 ± 0.06
ISAP	0.85 ± 0.01	0.96 ± 0.08
U46619	2.9 ± 0.17	0.86 ± 0.08
SQ29548	16 ± 1.5	0.93 ± 0.04

[a] IC_{50} value, [b] the Hill coefficient (n_H), [c] significantly less than unity.
IC_{50}, K_i and n_H values were obtained by described before. Data are presented as the means ± S.E.M. of 5 experiments.

Table 3
Affinity of YM158 for receptors other than leukotriene D_4 and thromboxane A_2

Receptor	Species	Region or cell line	[^{3}H] ligand	IC_{50} (nM)
Neurotransmitter related				
α_1-adrenoceptor	Rat	Forebrain	[^{3}H] 7-MeOxy-Prazosin	> 10,000
α_2-adrenoceptor	Rat	Cortex	[^{3}H] RX821002	> 10,000
β-adrenoceptor	Rat	Cortex	[^{3}H] DHA	> 10,000
Histamine (H_1)	Bovine	Cellebellum	[^{3}H] Pyrilamine	> 10,000
Histamine (H_2)	Guinea pig	Striata	[^{3}H] Tiotidine	> 10,000
Histamine (H_3)	Rat	Forebrain	[^{3}H] *N*-a-Methylhistamine	> 10,000
5-HT	Rat	Cortex	[^{3}H] LSD	> 10,000
Muscarine (M_1)	Bovine	Striata	[^{3}H] Pirenzepine	> 10,000
Muscarine (M_2)	Rat	Heart	[^{3}H] AF-DX 384	> 10,000
Muscarine (M_3)	Guinea pig	Ileum	[^{3}H] Scopolamine	> 10,000
σ	Guinea pig	Whole brain	[^{3}H] DTG	> 10,000
Other				
C_{5a}	Human	U937	[^{125}I] BH-rC_{5a}	> 10,000
Opioid	Rat	Forebrain	[^{3}H] Naloxone	> 10,000
Ca^{2+} channel	Rat	Cortex	[^{3}H] Conotoxin GVIA	> 10,000
	Rat	Cortex	[^{3}H] Nitrendipine	> 10,000
K^+ channel	Rat	Cortex	[^{3}H] Glibenclamide	> 10,000
	Rat	Forebrain	[^{125}I] Apamin	> 10,000
	Rat	Brain	[^{125}I] Charybdotoxin	> 10,000
Protein kinase C	Mouse	Whole brain	[^{3}H] PDBu	> 10,000
Bradykinin 2	Guinea pig	Ileum	[^{3}H] Bradykinin	> 10,000
Endothelin	Human	cDNA/CHO	[^{125}I] Endothelin	> 10,000
Neurokinin (NK_2)	Human	cDNA/CHO	[^{3}H] NKA	> 10,000
Neurokinin (NK_3)	Rat	Cortex	[^{3}H] Eledoisin	> 10,000
PAF	Rabbit	Platelets	[^{3}H] Hexadecyl, PAF	> 10,000

value of 5.0 ± 0.88 nM (Table 2). The Hill coefficient (0.83 ± 0.13) was near unity, indicating that the inhibitory effect of YM158 was competitive. Although seratrodast showed a concentration-dependent inhibition of [^{3}H]U46619 binding, it was a non-competitive antagonist since the Hill coefficient (0.35 ± 0.04) was different from unity. The other thromboxane A_2 analogues (agonists or antagonists), domitroban, IBOP, ISAP and SQ29548, showed competitive inhibition against [^{3}H]U46619 binding, with K_i values of 0.31 ± 0.03, 0.091 ± 0.01, 0.85 ± 0.01 and 16 ± 1.5 nM, respectively.

3.3. Other receptors binding assay

YM158 at a concentration of 10 μM showed no affinity for any of the following receptor types: neurotransmitter-related (α_1-, α_2-, β-adrenoceptors, histamine, 5-HT, muscarinic, σ), C_{5a}, opioid, Ca^{2+} channels, K^+ channels, protein kinase C, bradykinin, endothelin, neurokinins or PAF (Table 3).

4. Discussion

YM158 shows potent inhibitory effects against leukotriene D_4- or U46619-induced contraction of trachea isolated from guinea pigs (Arakida et al., 1998). The present study demonstrated that YM158 had high affinity for both leukotriene D_4 and thromboxane A_2 receptors, indicating that the inhibitory effects of YM158 on leukotriene D_4- or U46619-induced contractions of isolated tracheas are due to the direct interaction of this compound with leukotriene D_4 and thromboxane A_2 receptors.

The K_i values of YM158 obtained from the present study were 0.64 nM for leukotriene D_4 and 5.0 nM for thromboxane A_2 receptors, respectively. Based on the K_i value, it appears that the affinity of YM158 for the leukotriene D_4 receptor is approximately 8-times higher than that for the thromboxane A_2 receptor, which is in good agreement with the K_i value of each agonist used as a radioligand. The ratio of K_i values of YM158 and leukotriene D_4 (0.64 nM/0.34 nM = 1.88), and that of YM158 and U46619 (5.0 nM/2.9 nM = 1.72) were almost equal (see in Tables 1 and 2). Therefore, YM158 is a dual antagonist that has almost the same binding potency for both leukotriene D_4 and thromboxane A_2 receptors, as was shown by its inhibitory effects on leukotriene D_4- and U46619-induced contraction of isolated trachea in vitro (Arakida et al., 1998).

The affinity of seratrodast was very weak and its mode of action was non-competitive in the present thromboxane A_2 binding assay system. Seratrodast is well known as a potent thromboxane A_2 receptor antagonist, and there are many reports of its effectiveness (Ashida et al., 1989; Imura et al., 1990; Fukumoto et al., 1992; Zhang et al., 1996). Seratrodast has affinity for the thromboxane A_2

receptor in the binding assay system with thromboxane A_2 receptor-expressing Chinese hamster ovary (CHO) cells (Kurokawa et al., 1994) and washed guinea pig platelets (Imura et al., 1990), but not lung membrane preparations. In contrast, the K_i values of domitroban and thromboxane A_2 analogues in the present study were similar to those previously described (Saussy et al., 1992). Therefore, the binding assay system for the thromboxane A_2 receptor used in this study seems to be appropriate, although the reason why seratrodast did not show high affinity for thromboxane A_2 receptor is at present unknown.

It is well known that the two lipid mediators, leukotriene D_4 and thromboxane A_2, have several important roles in the pathogenesis of bronchial asthma. Leukotriene D_4 induces potent bronchoconstriction and hypersecretion, and increases vascular permeability, whereas thromboxane A_2 induces bronchoconstriction and hyperreactivity in both asthmatic animal models and human patients. A leukotriene D_4 receptor antagonist, a thromboxane A_2 receptor antagonist and a thromboxane A_2 synthetase inhibitor have each proven to be effective in patients with bronchial asthma. In the light of these results, YM158, which interacts with both leukotriene D_4 and thromboxane A_2 receptors and exhibits similar affinity for both, shows promise as a leading candidate for a novel group of therapeutic agents against lipid mediator-related diseases and disorders, such as bronchial asthma, rhinitis, and possibly anaphylaxis.

References

Arakida, Y., Suwa, K., Ohga, K., Yokota, M., Miyata, K., Yamada, T., Honda, K., 1998. In vitro pharmacologic profile of YM158, a new dual antagonist for LTD_4 and TXA_2 receptors. J. Pharmacol. Exp. Ther., in press.

Ashida, Y., Matsumoto, T., Kuriki, H., Shiraishi, M., Kato, K., Terao, S., 1989. A novel anti-asthmatic quinone derivative, AA-2414 with a potent antagonistic activity against a variety of spasmogenic prostanoids. Prostaglandins 38, 91–112.

Barnes, P.J., 1992. New drugs for asthma. Eur. Respir. J. 5, 1126–1136.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254.

Busse, W.W., 1996. The role of leukotrienes in asthma and allergic rhinitis. Clin. Exp. Allergy 26, 868–879.

Cheng, Y.C., Prusoff, W.H., 1973. Relationship between the inhibition constant (K_1) and the concentration of inhibitor which causes 50% inhibition (I_{50}) of an enzymatic reaction. Biochem. Pharmacol. 22, 3099–3108.

Dahlén, S.E., Hedqvist, P., Hammarström, S., Samuelsson, B., 1980. Leukotrienes are potent constrictors of human bronchi. Nature 288, 484–486.

Feldberg, W., Kellaway, C.H., 1938. Liberation of histamine and formation of lysocithin-like substances by cobra venom. J. Physiol. 94, 187–226.

Francis, H.P., Greenham, S.J., Patel, U.P., Thompson, A.M., Gardiner, P.J., 1991. Bay u3405 an antagonist of thromboxane A_2- and prostaglandin D_2-induced bronchoconstriction in the guinea-pig. Br. J. Pharmacol. 104, 596–602.

Fujimura, M., Sakamoto, S., Saito, M., Miyake, Y., Matsuda, T., 1991. Effect of thromboxane A_2 receptor antagonist (AA-2414) on bronchial hyperresponsiveness to methacholine in subjects with asthma. J. Allergy Clin. Immunol. 87, 23–27.

Fukumoto, S., Shiraishi, M., Terashita, Z., Ashida, Y., Inada, Y., 1992. Synthesis and thromboxane A_2/prostaglandin H_2 receptor antagonistic activity of phenol derivatives. J. Med. Chem. 35, 2202–2209.

Imura, Y., Terashita, Z., Shibouta, Y., Inada, Y., Nishikawa, K., 1990. Antagonistic action of AA-2414 on thromboxane A_2/prostaglandin endoperoxide receptor in platelets and blood vessels. Jpn. J. Pharmacol. 52, 35–43.

Jones, G.L., Saroea, H.G., Watson, R.M., O'byrne, P.M., 1992. Effect of an inhaled thromboxane mimetic (U46619) on airway function in human subjects. Am. Rev. Respir. Dis. 145, 1270–1274.

Krell, R.D., Aharony, D., Buckner, C.K., Keith, R.A., Kusner, E.J., Snyder, D.W., Bernstein, P.R., Matassa, V.G., Yee, Y.K., Brown, F.J., Hesp, B., Giles, R.E., 1990. The preclinical pharmacology of ICI 204,219: a peptide leukotriene antagonist. Am. Rev. Respir. Dis. 141, 978–987.

Kurokawa, T., Matsumoto, T., Ashida, Y., Sasada, R., Iwasa, S., 1994. Antagonism of the human thromboxane A_2 receptor by an anti-asthmatic agent AA-2414. Biol. Pharm. Bull. 17, 383–385.

Minoguchi, K., Adachi, M., Kobayashi, T., Takahashi, T., 1992. The mechanism of airway hyperresponsiveness following immediate asthmatic response in ovalbumine-sensitized guinea-pigs. J. Lipid Mediators 5, 169–172.

Mong, S., Wu, H.L., Stadel, J.M., Clark, M.A., Crooke, S.T., 1986. Solubilization of [^{3}H]leukotriene D_4 receptor complex from guinea pig lung membranes. Mol. Pharmacol. 29, 235–243.

Nagai, H., Tsuji, F., Inagaki, N., Kitagaki, K., Fukutomi, O., Koda, A., Daikoku, M., 1991. The effect of ONO-3708, a novel TXA_2 receptor antagonist, on U-46619-induced contraction of guinea pig and human tracheal strips in vitro and on bronchoconstriction in guinea pigs in vivo. Prostaglandins 41, 375–382.

Nagai, H., Tsuji, F., Goto, S., Koda, A., 1993. Pharmacological modulation of antigen-induced airway hyperresponsiveness by thromboxane A_2 inhibitors in guinea pigs. Biol. Pharm. Bull. 16, 1099–1103.

Nakagawa, N., Obata, T., Kobayashi, T., Okada, Y., Nambu, F., Terawaki, T., Aishita, H., 1992. In vivo pharmacologic profile of ONO-1078: a potent, selective and orally active peptide leukotriene (LT) antagonist. Jpn. J. Pharmacol. 60, 217–225.

Peck, M.J., Piper, P.J., Williams, T.J., 1981. The effect of leukotrienes C_4 and D_4 on the microvasculature of guinea-pig skin. Prostaglandins 21, 315–321.

Rinkema, L.E., Bemis, K.G., Fleisch, J.H., 1984. Production and antagonism of cutaneous vascular permeability in the guinea pig in response to histamine, leukotrienes and A23187. J. Pharmacol. Exp. Ther. 230, 550–557.

Samuelsson, B., 1983. Leukotriene: mediators of immediate hypersensitivity reactions and inflammation. Science 220, 568–575.

Saussy, D.L. Jr., Mais, D.E., Pube, G.P., Magee, D.E., Brune, K.A., Kurtz, W.L., Williams, C.M., 1991. Characterization of a thromboxane A_2/Prostaglandin H_2 receptor in guinea pig lung membranes using a radioiodinated thromboxane mimetic. Mol. Pharmacol. 39, 72–78.

Saussy, D.L. Jr., Clark, P.D., Gunn, D.L., Mais, D.E., Froelich, L.L., 1992. Binding of a novel radioiodinated thromboxane A_2/prostaglandin H_2 antagonist to guinea pig lung membranes. Eicosanoids 5, 1–4.

Ueno, A., Tanaka, K., Katori, M., 1982. Possible involvement of thromboxane in bronchoconstrictive and hypertensive effects of LTC_4 and LTD_4 in guinea pigs. Prostaglandins 23, 865–880.

Yokota, M., Nagaoka, H., Akane, H., Arakida, Y., Isomura, Y., 1997. Synthesis and biological activities of the dual receptor antagonists of thromboxane A_2 and peptidoleukotrienes. Abst. Pap. Am. Chem. Soc. 213 Meet., 2, MEDI 075.

Zhang, R., Ogletree, M.L., Moreland, S., 1996. Characterization of thromboxane A_2/prostaglandin endoperoxide receptors in aorta. Eur. J. Pharmacol. 317, 91–96.