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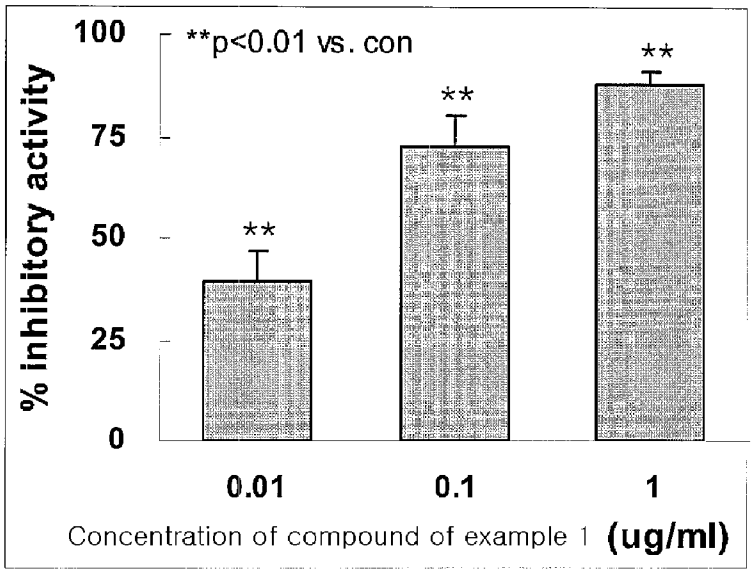
(54) Title: NEW BENZOXAZOLE DERIVATIVE, PROCESS FOR THE PREPARATION THEREOF AND PHARMACEUTICAL COMPOSITION COMPRISING THE SAME



(57) Abstract: The present invention relates to a new benzoxazole derivative having 5-lipoxygenase inhibitory activity, a process for the preparation thereof, and a pharmaceutical composition comprising the same. The benzoxazole derivative according to the present invention has excellent 5-lipoxygenase inhibitory activity, reduces the degree of airway hypersensitivity, levels of cytokines IL-4, IL-5, and IL- 13, Ova-specific IgE in serum, and degree of the peribronchial and perivascular inflammation, statistically significantly reduces the number of TRAP(+) MNCs, and significantly inhibits osteoclastogenesis in a co-culture system of bone marrow cells and osteoblasts, thereby being used to prevent and treat diseases induced by leukotriene, in particular, inflammatory diseases including asthma or osteoporosis.

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Description

NEW BENZOXAZOLE DERIVATIVE, PROCESS FOR THE PREPARATION THEREOF AND PHARMACEUTICAL COMPOSITION COMPRISING THE SAME

Technical Field

[1] The present invention relates to a new benzoxazole derivative, a process for the preparation thereof, and a pharmaceutical composition comprising the same.

Background Art

[2] Leukotriene is an oxidized polyunsaturated fatty acid having several bioactivities, and metabolized from arachidonic acid liberated from cell membrane by calcium action via multiple steps, so as to be biosynthesized as one of inflammatory mediators exerting a variety of biological activities.

[3] In the first step, an unstable epoxide intermediate, leukotriene A_4 (LTA_4) is generated from arachidonic acid by the action of 5-lipoxygenase, and LTA_4 is converted to leukotriene B_4 and leukotrienes C_4, D_4, E_4 via two different pathways, respectively.

[4] The leukotriene B_4 (LTB_4) formed through the first pathway is a bioactive compound enzymatically formed by hydration of LTA_4. The compound has chemotactic activity for inflammatory cells such as polymorphonuclear leukocyte, and causes degranulation and aggregation of inflammatory cells. Further, leukotriene B_4 increases vascular permeability, resulting in edema formation.

[5] Among leukotriene C_4 (LTC_4), leukotriene D_4 (LTD_4) and leukotriene E_4 (LTE_4) formed through the second pathway, LTC_4 is formed by the conjugation of glutathione with the epoxide of LTA_4 by LTC_4 synthase, and the peptide region of LTC_4 is metabolically transformed to form LTD_4 and LTE_4. These three leukotrienes are the major components of slow-reacting substance of anaphylaxis, and affect immediate hypersensitivity reactions. Further, the compounds cause smooth muscle contraction, and increase mucous secretion and vascular permeability.

[6] It has been known that the biological effects of leukotriene play an important role in asthma, cystic fibrosis, chronic bronchitis, gout, rheumatoid arthritis, bronchitis, allergic rhinitis, skin diseases such as psoriasis, arthritis, inflammatory bowel disease or the like, and are involved in a variety of cardiopulmonary diseases including sepsis, myocardial ischemia, heart anaphylaxis, cerebral vasospasm, and ischemic attack.

[7] Of these, asthma has been clarified to be a disease in which leukotriene plays a crucial role. Therefore, many studies on leukotriene have focused on the fields related to asthma. In particular, LTC_4 and LTD_4 exert a very strong action of bronchial

contraction on human airway, which has been known to be 1000 times stronger than the action of histamine. The compounds powerfully stimulate mucous secretion in human airway, and increase capillary permeability, which results in a high level of leukotriene in body fluid from a patient with asthma. Therefore, it can be seen that there is a close correlation between its content and disease severity.

[8] Further, it has been recently reported that 5-lipoxygenase metabolites are formed in osteoblasts, which stimulates bone resorption, and a stromal cell line, C433 cell derived from a giant cell tumor produces 5-lipoxygenase metabolites to increase the number of osteoclast and its activity. Further, there is a report that the addition of synthetic LTB_4 stimulates bone resorption in bone tissue culture, and LTB_4 has been shown to stimulate bone resorption both in vitro and in vivo through osteoclast formation. Accordingly, the compounds having an antagonistic effect on an LTB_4 receptor have been thought to affect metabolic bone diseases, and many studies on the compounds have been made.

[9] On the other hand, the distribution of 5-lipoxygenase is limited to neutrophils, eosinophils, basophils, monocytes, macrophages, mast cells, and B lymphocytes. In contrast, LTA_4 hydrolase and LTC_4 synthase enzymes are more widely distributed in various tissues.

[10] Therefore, it is thought that a drug capable of selectively inhibiting the enzymatic action of 5-lipoxygenase effectively blocks the initial stage of leukotriene biosynthesis, thereby being used to prevent and treat diseases induced by leukotriene, in particular, inflammatory diseases including asthma or osteoporosis.

[11] As current research trends related to the present invention, every efforts have been made in a) inhibition of 5-lipoxygenase, b) inhibition of 5-lipoxygenase activating protein (FLAP), c) development of peptidyl leukotriene (LTD_4) receptor antagonist (for example, montelukast, pranlukast, zafirlukast), d) development of LTB_4 receptor antagonist, or the like.

[12] Zileuton has been recently developed as a drug to inhibit 5-lipoxygenase, and in addition, substances such as E6080, A-61443, BW-755c, A-63162, MK-886, MK-591, WY-50,295, and ZD5138 have been under development and clinical trials as a 5-lipoxygenase inhibitor.

[13] Zileuton is a drug synthesized at Abbott Laboratories, and has been applied for FDA approval in July, 1997. Zileuton is a first drug approved for sale as an agent for the treatment of asthma having the inhibitory effect on 5-lipoxygenase. Further, E6080 is a powerful 5-lipoxygenase inhibitor developed by Eisai Co. Ltd. (Japan), which has high selectivity, is orally administrable and inhibits the release of LTB_4 and LTC_4.

[14] Accordingly, it is thought that a 5-lipoxygenase inhibitory agent effectively inhibiting the leukotriene biosynthesis can be efficiently used to treat inflammatory

diseases including asthma or osteoporosis, in which leukotriene plays a major role.

Disclosure of Invention

Technical Problem

[15] Accordingly, the present inventors have developed a pharmaceutical composition having 5-lipoxygenase inhibitory activity in Korean Patent Registration No. 10-0544901. In the present invention, a new benzoxazole derivative having excellent 5-lipoxygenase inhibitory activity has been synthesized, and it was found that the compound significantly reduces the degree of airway hypersensitivity, levels of cytokines IL-4, IL-5, and IL-13, Ova-specific IgE in serum, and degree of the peri-bronchial and perivascular inflammation, statistically significantly reduces the number of TRAP(+) MNCs, and significantly inhibits osteoclastogenesis in a co-culture system of bone marrow cells and osteoblasts, thereby completing the present invention.

Technical Solution

[16] The present invention provides a new benzoxazole derivative having 5-lipoxygenase inhibitory activity, a process for the preparation thereof, and a pharmaceutical composition comprising the same for preventing and treating diseases induced by leukotriene.

Brief Description of the Drawings

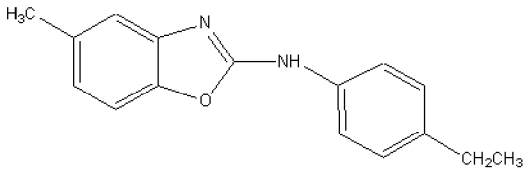
[17] Fig. 1 is a drawing showing the effect of a benzoxazole derivative of the present invention on osteoclast-inhibitory activity.

Best Mode for Carrying Out the Invention

[18] The present invention provides a benzoxazole derivative represented by the following Formula 1.

[19] **[Formula 1]**

[20]



[21] The compounds of the present invention may be prepared in the form of a pharmaceutically acceptable salt and a solvate according to the conventional method in the related art.

[22] As the pharmaceutically acceptable salt, acid addition salts produced with free acids are preferred. The acid addition salts are prepared by the conventional method, for example, a method comprising the steps of dissolving a compound in an excessive amount of acid aqueous solution, and precipitating the salt using a water-miscible organic solvent such as methanol, ethanol, acetone or acetonitrile. Acid or alcohol (for

example, glycol monomethyl ether) in the same molar amount of compound and water is heated, and the mixture is dried by evaporation or the precipitated salt can be suction-filtered. At this time, as the free acids, organic acids and inorganic acids may be used. Examples of the inorganic acids include hydrochloric acid, phosphoric acid, sulfuric acid, nitric acid, and tartaric acid, and examples of the organic acids include methanesulfonic acid, p-toluenesulfonic acid, acetic acid, trifluoroacetic acid, citric acid, maleic acid, succinic acid, oxalic acid, benzoic acid, tartaric acid, fumaric acid, mandelic acid, propionic acid, lactic acid, glycollic acid, gluconic acid, galacturonic acid, glutamic acid, glutaric acid, glucuronic acid, aspartic acid, ascorbic acid, carboxylic acid, vanillic acid, and hydroiodic acid, but are not limited thereto.

[23] Further, a pharmaceutically acceptable metal salt can be prepared using a base. An alkali metal salt and alkaline earth metal salt can be obtained by a method, for example, the method comprising the steps of dissolving a compound in an excessive amount of alkali metal hydroxide or alkaline earth metal hydroxide solution, filtering the undissolved salt, and then evaporating and drying the filtrate. In respects to metal salts, sodium, potassium, or calcium salt is pharmaceutically preferable, and the corresponding silver salt is obtained by reacting alkali metal salt or alkaline earth metal salt with a suitable silver salt (e.g. silver nitrate).

[24] A pharmaceutically acceptable salt of the compound represented by Formula 1 includes salts of acidic or basic groups, which can be present in the compound of Formula 1, as long as particular mention is not made. For example, the pharmaceutically acceptable salt includes sodium salt, calcium salt, and potassium salt of hydroxy group, and other pharmaceutically acceptable salt of amino group includes hydrobromide, sulfate, hydrogen sulfate, phosphate, hydrogen phosphate, dihydrogen phosphate, acetate, succinate, citrate, tartrate, lactate, mandelate, methanesulfonate (mesylate), and p-toluenesulfonate (tosylate). Further, the salts can be prepared by a preparation method or preparation process thereof known in the related art.

[25]

[26] Further, the present invention provides a process for the preparation of the benzoxazole derivative represented by Formula 1, comprising the steps of:

[27] 1) reacting 2-amino-4-methylphenol with 4-ethylphenyl isocyanate in an organic solvent to prepare an N-(2-hydroxy-5-methylphenyl)-N'-(4-ethylphenyl)thiourea compound, and

[28] 2) reacting the N-(2-hydroxy-5-methylphenyl)-N'-(4-ethylphenyl)thiourea compound with potassium peroxide to prepare 8-methyl-2-[N-(4-ethylphenyl)] aminobenzoxazole.

[29] In the reaction, 2-amino-4-methylphenol used as a starting material can be prepared by reduction reaction of 4-methyl-2-nitrophenol with Pd/C or purchased from com-

mercially available sources.

[30] In the step 1), 2-amino-4-methylphenol and 4-ethylphenyl isocyanate are dissolved in an organic solvent, and then reacted for about 24 hours to prepare a N-(2-hydroxy-5-methylphenyl)-N'-(4-ethylphenyl)thiourea compound.

[31] Examples of the organic solvent used in the step include methanol, ethanol, and ether, most preferably methanol.

[32] In the step 2), about 5 equivalent weights of potassium peroxide is preferably used based on 1 equivalent weight of the N-(2-hydroxy-5-methylphenyl)-N'-(4-ethylphenyl)thiourea compound.

[33]

[34] Further, the present invention provides a pharmaceutical composition for preventing and treating diseases induced by leukotriene, comprising the benzoxazole derivative represented by Formula 1 or a pharmaceutically acceptable salt thereof.

[35] Examples of diseases induced by leukotriene include asthma, pertussis, psoriasis, arthritis, inflammatory bowel disease, cystic fibrosis, chronic bronchitis, allergic rhinitis, gout, rheumatoid arthritis, sepsis, myocardial ischemia, heart anaphylaxis, cerebral vasospasm, ischemic attack, osteoporosis, breast cancer, pancreatic cancer, or pain.

[36] The benzoxazole derivative according to the present invention has IC_{50} values of 0.12 μM in BMMC (Bone-marrow mast cell), and remarkably excellent 5-lipoxygenase inhibitory activity, as compared to a known 5-lipoxygenase inhibitor.

[37] Further, the benzoxazole derivative according to the present invention significantly reduces the degree of airway hypersensitivity, levels of cytokines IL-4, IL-5, and IL-13, Ova-specific IgE in serum, and degree of the peribronchial and perivascular inflammation.

[38] Further, as a concentration of the benzoxazole derivative according to the present invention increases, the number of TRAP(+) MNCs is statistically significantly reduced (**$p<0.01$ compared to a control), and osteoclastogenesis is significantly inhibited in a co-culture system of bone marrow cells and osteoblasts.

[39] Accordingly, the benzoxazole derivative according to the present invention can be used to prevent and treat diseases induced by leukotriene, in particular, inflammatory diseases including asthma or osteoporosis.

[40] The composition of the present invention may contain one or more active ingredients having the effects of preventing and treating diseases induced by leukotriene in addition to the benzoxazole derivative of Formula 1.

[41] For administration, the composition of the invention can be prepared including at least one pharmaceutically acceptable carrier, in addition to the active ingredients as described above. Examples of the pharmaceutically acceptable carrier include saline

solution, sterile water, Ringer's solution, buffered saline solution, dextrose solution, maltodextrin solution, glycerol, ethanol and a mixture of two or more thereof. If necessary, the composition may also contain other conventional additives, such as antioxidants, buffers, and bacteriostatic agents. Moreover, the composition may additionally contain diluents, dispersants, surfactants, binders, and lubricants in order to formulate it into injectable formulations, such as aqueous solution, suspension, and emulsion, pills, capsules, granules and tablets. Furthermore, the composition may preferably be formulated depending on particular diseases and its components, using the method described in Remington's Pharmaceutical Science (latest edition), Mack Publishing Company, Easton PA., which is a suitable method in the relevant field of art.

[42] The composition of the invention may be administered orally or parenterally (for example, intravein, subcutaneous, intraperitoneal, or topical application) depending on the purpose of the invention, and the dosage of the composition can vary depending on various factors, including patient's weight, age, sex, health condition, and diet, and administration time, administration route, secretion rate, disease severity, etc.. The compound of Formula 1 is administered at a daily dosage of about 10 to 1000 mg/kg, more preferably once or several times.

[43] The composition of the invention may be used alone or in combination with surgical operations, hormone therapies, chemical therapies, and other methods using biological reaction regulators in order to prevent and treat diseases induced by leukotriene.

Mode for the Invention

[44] Hereinafter, the preferred Examples are provided for better understanding. However, these Examples are for the illustrative purpose only, and the invention is not intended to be limited by these Examples.

[45] **Example 1 : Preparation of 8-methyl-2-[N-(4-ethylphenyl)]aminobenzoxazole**

[46] 1. 2-amino-4-methylphenol

[47] A Pd/C catalyst was added to 4-methyl-2-nitrophenol, and air was completely removed by using a pump. Then, methanol was injected thereto to dissolve reagents, and connected to a hydrogen-filled balloon to be substituted with hydrogen. After strongly stirring for 5 hours at room temperature, the resultant was filtered to completely remove Pd/C, and the filtrate was concentrated under reduced pressure to obtain 2-amino-4-methylphenol.

[48] 2. N-(2-hydroxy-5-methylphenyl)-N'-(4-ethylphenyl)thiourea

[49] 132.6 □ (0.812 mmol) of 4-ethylphenyl isothiocyanate was added to 100 □ (0.812 mmol) of 2-amino-4-methylphenol prepared in 1, 25 □ of methanol was added thereto

to be completely dissolved and stirred for 24 hours. the precipitate formed with time was filtered under reduced pressure to obtain 100 □ of N-(2-hydroxy-5-methylphenyl)-N'-(4-ethylphenyl)thiourea as a brown powder (yield: 43%).

[50] ^{1}H NMR (Acetone-d_6, 400MHz) δ 1.178(t, J=8.0Hz, 3H), 2.586(q, J=8.0Hz), 6.879(m, 1H), 7.178~7.204(m, 4H), 7,711~7.738(m, 2H)

[51] 3. 8-methyl-2-[N-(4-ethylphenyl)]aminobenzoxazole

[52] 100 □ (0.35 mmol) of N-(2-hydroxy-5-methylphenyl)-N'-(4-ethylphenyl)thiourea prepared in 2 was mixed with 123.9 □ (1.75 mmol) of potassium peroxide, and 5 □ of acetonitrile was added to the mixture under nitrogen gas. Then, while being strongly stirred, the mixture reacted for 12 hours at room temperature, and diluted with cooling water. The mixture was extracted from dichloromethane, and dried over $MgSO_4$. then, the solvent was concentrated under reduced pressure to obtain 62.5 □ of 8-methyl-2-[N-(4-ethylphenyl)]aminobenzoxazole as a white power (yield: 71%).

[53] Melting point: 184~186.2℃

[54] ^{1}H NMR (Acetone-d_6, 400MHz) δ 1.364(t, J=6.8Hz, 3H), 2.383(s, 3H), 4.041(q, J=6.8Hz, 2H), 6.895~6.918(m, 1H), 6.933~6.965(m, 2H), 7.199~7.219(m, 2H), 7.722~7.762(m, 2H), 9.210(s, NH),

[55] FABHRMS (m/z): 253.1345 (M^++1, $C_{16}H_{17}N_2O$requires 253.1341)

[56] **Experimental Example 1 : Test on 5-lipoxygenase inhibitory activity**

[57] In order to confirm the 5-lipoxygenase inhibitory activity of the benzoxazole derivative of Formula 1 according to the present invention, experiments were performed as follows.

[58] 1. Preparation of BMMC (Bone-marrow mast cell)

[59] Two female BALB/c mice (5~6 weeks old) were sterilized with 71% ethanol, and femurs were separated from the mice. A 25G needle was put into the femur, and then bone-marrow was flushed out with 25 □ of RPMI to be centrifuged (1000 rpm, 5 mins). The supernatant was discarded, and the bone-marrow mast cells were cultured in RPMI 1640/10% FBS. After about 1 day, the cells were observed to be subcultured. After 90% or more of cell growth was confirmed, the following experiments were performed with BMMC.

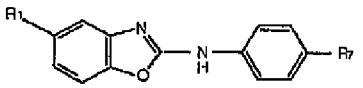
[60] 2. Formation assay for leukotriene C_4

[61] BMMC was cultured for 3 weeks or more, and then transferred to a 50 □ falcon tube to be centrifuged (1000 rpm, 5 min, 4℃). The supernatant was discarded, and RPMI 1640 was added thereto. 50 □ of supernatant sample, 50 □ of LTC_4AChE tracer, and 50 □ of LTC_4 antiserum were aliquoted into a well, and an EIA buffer solution was used for dilution. 100 □ of EIA buffer solution, 50 □ of LTC_4AChE tracer, and 50 □ of LTC_4 antiserum were aliquoted into a NSB well. After culturing at room temperature for 16

to 20 hours, the cells were washed with 200 □ of wash buffer about 5 times. 200 □ of Ellman's reagent was added to each well, and cultured for 60 minutes. Then, the plate was read at 405 □.

[62] An IC_{50} value of benzoxazole derivative, which was used as an active ingredient in a pharmaceutical composition for the inhibition of 5-lipoxygenase in Korean Patent Registration No. 10-0544901, was described below.

[63]



No. of compound	R_1	R_7	IC_{50} (uM)
4	-H	-N=N-⬡	6.28
5	-H	-H	8.91
6	-H	$-C_2H_5$	1.21
7	-Cl	-N=N-⬡	12.61
8	-Cl	-H	4.50
9	-Cl	$-C_2H_5$	0.95
10	$-NO_2$	-N=N-⬡	28.37
11	$-NO_2$	$-C_2H_5$	23.88

[64] As a result of the experiments, it was found that the IC_{50} value of benzoxazole derivative was 0.12 μM, which is 7.91~236.4 times better than that of benzoxazole derivative described in Korean Patent Registration No. 10-0544901, thereby having excellent inhibitory activity.

[65]

[66] **Experimental Example 2 : Measurement of inhibitory effect on asthma (in vivo)**

[67] 1. Induction of asthma

[68] The sterilized 6 week-old healthy female BALB/c mice (Korean Research Institute of Chemistry Technology, Daejeon, Korea) were bred in laminar flow cabinets. The BALB/c mice were divided into 3 groups, each group with 6 mice: group I, sham-sensitization plus challenge with phosphate-buffered saline (PBS; ipNeb); group II, sensitization plus challenge with OVA (ovalbumin: Sigma A5503; Sigma, St. Louis, MO) (ipNeb); group III, sensitization with OVA (ip) plus challenge with OVA (Neb) and samples (po). On the first day, 20 □ of ovalbumin / 4 □ of adjuvant aluminum hydroxide were intraperitoneally administered. On the 11th day, OVA/alum was intraperitoneally administered and the mice were exposed to 1% ovalbumin antigen in

the form of spray for 20 minutes, and on the 21th, 22th, and 23th days, exposed to 1% ovalbumin antigen in the form of spray. On the 25th day, the mice were finally exposed to ovalbumin antigen in the form of spray to induce inflammation. A drug was orally administered every day on day from 21th to 25th (50 □/□ weight/mouse). The animals were evaluated at 24 hours after the last ovalbumin antigen challenge.

[69]

[70] 2. Measurement of airway hypersensitivity

[71] The measurement of airway hypersensitivity was performed at 24 hours after the last challenge while all mice were conscious and unstrained in a barometric plethysmography chamber. The average was calculated with the measured values for 3 minutes to be used as the baseline value of airway hypersensitivity. As the concentration of methacholine was gradually increased from 0 □/□ to 30 □/□, mice were exposed to each concentration of methacholine by inhalation for 3 minutes. Then, the degree of airway hypersensitivity was measured at each concentration. The degree of airway hypersensitivity was represented as the increased ratio, by comparing an enhanced pause (Penh) value ($Penh_{MCH}$), which was measured after administration of each concentration of methacholine, with a Penh value ($Penh_{PBS}$), which was measured after inhalation of saline solution. The calculation formula of Penh value was represented by the following Mathematical Formula 1. As a positive control, zileuton was used.

[72] **[Mathematical Formula 1]**

[73] $Penh_{PBS}$ = [Expiratory time (Te) / Relaxation time (RT)-1] x [Peak expiratory flow (PEF) / Peak inspiratory flow (PIF)]

[74] The results are shown in Table 1.

[75] Table 1

	Methacholine concentration (□/□)				
	0	5	10	20	30
Negative control group	0.656	1.376	2.418	2.878	3.277
Positive control group (zileuton)	0.567	1.673	2.344	2.588	2.866
Example 1	0.383	0.756	1.339	1.863	2.724

[76]

[77] As shown in Table 1, the degree of airway hypersensitivity of benzoxazole derivative according to the present invention was significantly reduced as compared to the negative control and positive control.

[78]

[79] 3. Measurement of cytokines IL-4, IL-5, IL-13 and Ova-specific IgE in serum

[80] At 24 hours after the measurement of airway hypersensitivity, the mice were sacrificed using an excessive amount of pentobarbital, and then a tracheotomy was performed. The chests of mice were excised to expose trachea, and the upper portion of the trachea was excised. Then, a tube was carefully inserted to be ligatured. 0.5 □ of cold PBS was injected to trachea-lung, and the recovered bronchoalveolar lavage fluid (BALF) was collected to be centrifuged at 4℃. The supernatant was separately stored at -70℃. The levels of cytokines IL-4, IL-5 (BD Biosciences Pharmingen Inc., San Dieogo, CA, USA), and IL-13 (Pierce Endogen, Inc., Woburn, Massachusetts, USA) were quantified and examined in the supernatant of bronchoalveolar lavage fluid by an ELISA method.

[81] On the other hand, the plasma was obtained by a cardiac puncture after performing the tracheotomy. The level of Ova-specific IgE in serum (BD Biosciences Pharmingen Inc., San Dieogo, CA, USA) was quantified and examined by the ELISA method.

[82] The measured levels of cytokines IL-4, IL-5, and IL-13 are shown in Tables 2, 3, and 4 respectively.

[83] Table 2

Level of cytokine IL-4

IL-4 Level	1	2	3	4	5	Mean	Standard deviation
Ovalbumin	422.03	476.73	316.4	424.01	470.2	421.872	64.1715
Zileuton	274.43	321.74	358.3	266.8	204.9	285.234	58.2696
Example 1	147.6	234.42	218.2	154.17	237.3	198.34	43.9897

[84] Table 3

Level of cytokine IL-5

IL-5 Level	1	2	3	4	5	Mean	Standard deviation
Ovalbumin	248.82	227.21	219.52	215.05	323	246.717	44.5694
Zileuton	113.31	161.01	181.44	219.27	214.3	177.864	43.3198
Example 1	174.05	109.83	112.81	86.353	101.6	116.923	33.5457

[85] Table 4

Level of cytokine IL-13

IL-13 Level	1	2	3	4	5	Mean	Standard deviation

Ovalbumin	128.45	123.16	105.09	191.87	165.9	142.89	35.1914
Zileuton	124.37	101.3	83.84	85.45	104.3	99.842	16.4802
Example 1	75.23	48.19	41.873	80.2	76.02	64.303	17.8335

[86]

[87] As shown in Tables 2 to 4, it can be seen that the levels of cytokines IL-4, IL-5, and IL-13 of the benzoxazole derivative according to the present invention were significantly reduced as compared to the negative control group and positive control group, and the level of Ova-specific IgE in serum was reduced by the benzoxazole derivative according to the present invention.

[88]

[89] 4. Histological analysis of trachea and lung

[90] The trachea and lungs of mice were filled intratracheally with a fixative (0.8% formalin, 4% acetic acid) through the inserted tube. Then, the lung parenchyma and trachea tissues were removed, and fixed in 10% neutral formaldehyde solution for 20~24 hours to prepare a paraffin embedded tissue section. For histological analysis, the paraffin embedded tissue was sectioned in a thickness of 4 □ using a Leica Microsystems (Nussloch GmbH, Nussloch, Germany), and then deparaffinized. An H-E (hematoxylin; Sigma MHS-16 and eosin, Sigma HT110-1-32) staining and Dako-mounting medium (Dakocytomation; Denmark Carpinteria CA) staining were performed.

[91] The degree of the peribronchial and perivascular inflammation was evaluated by a objective scale of 0~3. A value of 0 was assigned when no inflammation was detectable, a value of 1 was assigned for occasional cuffing with inflammatory cells, a value of 2 was assigned for most bronchi or vessels surrounded by a thin layer (one to five cells), and a value of 3 was assigned for most bronchi or vessels surrounded by a thick layer (more than five cells thick). Total lung inflammation was defined as the average of the peribronchial and perivascular inflammation scores.

[92] The results are shown in Table 5.

[93] Table 5

	Ovalbumin	Zileuton	Example 1
Mean	2.225	1.835	1.2427
Deviation	0.224	0.195	0.232

[94]

[95] As shown in Table 5, it can be seen that the degree of the peribronchial and perivascular inflammation was significantly reduced by the benzoxazole derivative

according to the present invention.

[96]

[97] **Experimental Example 3 : Inhibitory effect of osteoclast differentiation**

[98] In order to confirm the inhibitory effect of thebenzoxazole derivative of Formula 1 according to present invention on the formation and differentiation of osteoclast, the experiments are performed through a co-culture method as follows.

[99] 1. Reagent and laboratory animal

[100] α-MEM (Minimal essential medium alpha modification) medium, FBS (fetal bovine serum), penicillin, streptomycin and trypsin-EDTA used for cell culture were purchased from Gibco-BRL. In order to study osteoclast, 1,25(OH)$_2$D$_3$, dexamethasone, TRAP (Tartrate-resistant acid phosphatase) staining kit and other chemical reagents were purchased from Sigma-Aldrich. New born ICR mice were purchased from Biolinks Co. (Korea), and 6 week-old ddY mice were purchased from Charles River Japan Lab (Japan). The compounds prepared in Example 1 were dissolved in DMSO, and diluted with culture medium containing a 0.1% solvent.

[101]

[102] 2. Osteoclast formation assay (co-culture)

[103] The osteoclast formation index was measured by counting the number of TRAP-positive multinucleated osteoclasts (TRAP(+) MNCs). Bone marrow cells (2.5×10^5 cells/□) and calvaria-derived osteoblasts (4×10^4cells/□) were co-cultured in α-MEM containing the compounds prepared in Example 1 (0.01, 0.1 and 1 □/□) and 10% FBS in the presence of 1,25(OH)$_2$D$_3$ (10^{-8}M) and dexamethasone (10^{-7}M) for 7 days. Bone marrow cells were isolated from the femora of 6 week-old ddY mice, and osteoblasts were isolated from calvaria of new born ICR mice by sequential digestions with 0.2% collagenase. The media were changed with fresh media every two days. On the 6th day, the cells were fixed with 10% formaldehyde, and stained for TRAP. The number of TRAP(+) MNCs containing 6~7 nuclei was observed using an electron microscope (ZEISS Axiovert 25, Swiss).

[104] The numbers of TRAP(+) MNCs are shown in Table 6, and the inhibitory effect of the benzoxazole derivative according to the present invention on osteoclast is shown in Fig. 1.

[105] Table 6

	Number of TRAP(+) MNCs / well	
	First experiment	Second experiment
Control group	85±4.8	119±3.2

Compound of Example 1	0.01 □/□	49±6.7**	78±7.2**
	0.1 □/□	18±1.4**	41±3.1**
	1 □/□	8±0.8**	18±0.6**

[106]

[107] As shown in Table 6, as the concentration of the benzoxazole derivative of the present invention increased, the number of TRAP(+) MNCs was statistically significantly decreased (**p<0.01 compared to a control).

[108] Further, as shown in Fig. 1, the benzoxazole derivative of the present invention showed the inhibitory effects of osteoclast differentiation of 39%, 73% and 88% at the concentrations of 0.01, 0.1 and 1 □/□ respectively.

[109] Accordingly, it can be seen that the benzoxazole derivative of the present invention significantly inhibits osteoclastogenesis in the co-culture system of bone marrow cells and osteoblasts, thereby being an effective inhibitor of osteoclast differentiation.

[110]

[111] Hereinbelow, Formulation Examples for the composition of the present invention will be illustrated.

[112] **Formulation Example 1 : Preparation of liquid injectable formulation**

[113] An injectable liquid formulation containing 10 mg of the active ingredient was prepared as the following method.

[114] 1 g of the compound of the formula 1, 0.6 g of sodium chloride, and 0.1 g of ascorbic acid were dissolved in distilled water to be 100 ml. The solution was put into a bottle, and heated to be sterilized at 20℃ for 30 minutes.

[115] The composition of the injectable liquid formulation is as follows.

[116] Compound of formula 1 1 g

[117] Sodium chloride 0.6 g

[118] Ascorbic acid 0.1 g

[119] Distilled water predetermined amount

[120] **Formulation Example 2 : Preparation of syrup formulation**

[121] A syrup formulation containing the compound of the formula 1 as an active ingredient (2%, weight/volume) was prepared as the following method.

[122] The compound of the formula 1, saccharin, and sugar were dissolved in 80 g of warm water. The solution was cooled, and a solution containing glycerin, saccharin, flavor, ethanol, sorbic acid, and distilled water was added thereto. Water was added to the mixture to be 100 ml.

[123] The composition of the syrup formulation is as follows.

[124] Compound of formula 1 2 g

[125] Saccharin 0.8 g

[126] Sugar 25.4 g

[127] Glycerin 8.0 g

[128] Flavor 0.04 g

[129] Ethanol 4.0 g

[130] Sorbic acid 0.4 g

[131] Distilled water Predetermined amount

[132] **Formulation Example 3 : Preparation of tablet formulation**

[133] A tablet formulation containing 15 mg of the active ingredient was prepared as the following method.

[134] 250 g of the compound of the formula 1 was mixed with 175.9 g of lactose, 180 g of potato starch, and 32 g of colloidal silicic acid. 10% Gelatin solution was added to the mixture, and then pulverized to pass through a 14-mesh sieve. The mixture was dried. Then, 160 g of potato starch, 50 g of talc, and 5 g of magnesium stearate were added thereto to prepare a tablet.

[135] The composition of the tablet formulation is as follows.

[136] Compound of formula 1 250 g

[137] Lactose 175.9 g

[138] Potato starch 180 g

[139] Colloidal silicic acid 32 g

[140] 10% Gelatin solution

[141] Starch 160 g

[142] Talc 50 g

[143] Magnesium stearate 5 g

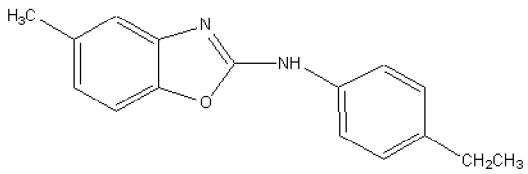
Industrial Applicability

[144] The benzoxazole derivative according to the present invention has excellent 5-lipoxygenase inhibitory activity, reduces the degree of airway hypersensitivity, levels of cytokines IL-4, IL-5, and IL-13, Ova-specific IgE in serum, and degree of the peribronchial and perivascular inflammation, statistically significantly reduces the number of TRAP(+) MNCs, and significantly inhibits osteoclastogenesis in a co-culture system of bone marrow cells and osteoblasts, thereby being used to prevent and treat diseases induced by leukotriene, in particular, inflammatory diseases including asthma or osteoporosis.

Claims

[1] A benzoxazole derivative represented by the following Formula 1.

<Formula 1>



[2] A process for the preparation of the benzoxazole derivative of claim 1, comprising the steps of:

1) reacting 2-amino-4-methylphenol with 4-ethylphenyl isocyanate in an organic solvent to prepare an N-(2-hydroxy-5-methylphenyl)-N'-(4-ethylphenyl)thiourea compound, and

2) reacting the N-(2-hydroxy-5-methylphenyl)-N'-(4-ethylphenyl)thiourea compound with potassium peroxide to prepare 8-methyl-2-[N-(4-ethylphenyl)] aminobenzoxazole.

[3] A pharmaceutical composition for preventing and treating diseases induced by leukotriene including asthma, pertussis, psoriasis, arthritis, inflammatory bowel disease, cystic fibrosis, chronic bronchitis, allergic rhinitis, gout, rheumatoid arthritis, sepsis, myocardial ischemia, heart anaphylaxis, cerebral vasospasm, ischemic attack, osteoporosis, breast cancer, pancreatic cancer or pain comprising the benzoxazole derivative of claim 1 or a pharmaceutically acceptable salt thereof.

[4] The pharmaceutical composition according to claim 3, wherein the disease induced by leukotriene is asthma.

[5] The pharmaceutical composition according to claim 3, wherein the disease induced by leukotriene is osteoporosis.

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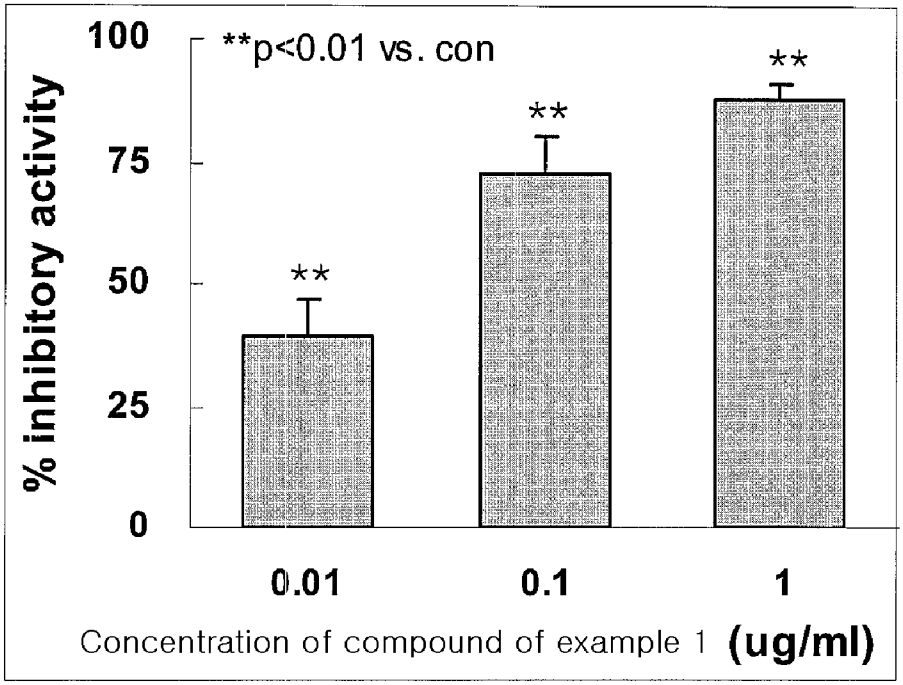
[Fig. 1]



INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR2007/004681

A. CLASSIFICATION OF SUBJECT MATTER

C07D 263/58(2006.01)i, A61K 31/145(2006.01)i

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 8 : C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

eKIPASS(KIPO internal), CAS(ON LINE), DELPHION

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	KR 1020050007056 A1 (EWHA HAKDANG INCORPORATED EDUCATIONAL INSTITUTION) 17 January 2005	1, 3-5
Y	See the abstract, example 4, claims 1-5.	2
X	KR 1020040086698 A1 (EWHA HAKDANG INCORPORATED EDUCATIONAL INSTITUTION) 12 October 2004	2
Y	See the abstract, example 7, claims 1-7.	2
A		1, 3-5
A	WO 2004/063177 A1 (F. HOFFMANN-LA ROCHE AG) 29 July 2004 See the whole document.	1-5
A	WO 02/051821 A1 (ASTRAZENECA AB) 04 July 2002 See the whole document.	1-5

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents:

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"P" document published prior to the international filing date but later than the priority date claimed

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
13 NOVEMBER 2007 (13.11.2007)	**13 NOVEMBER 2007 (13.11.2007)**

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

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