INTRODUCTION

1. General aspects of asthma

Asthma is a disease characterized by variable airway obstruction and exaggerated bronchoconstrictor response to a variety of stimuli (Barnes, 1983). Asthma has traditionally been divided into two major diagnostic categories: extrinsic atopic and intrinsic asthma. Extrinsic atopic asthma refers to the large group of patients in whom the disease is due to IgE-mediated hypersensitivity reactions to inhaled antigens commonly present in the air. Such hypersensitivity reactions or external causal factors cannot be found in patients characterized as intrinsic asthmatics (Salonen, 1985).

The pathological events which contribute to the airway narrowing are contraction of circular airway smooth muscle, edema of the bronchial mucosa, crenation of the respiratory epithelium and hypersecretion of mucus (Hogg et al., 1977) as well as mucosal inflammation (Reid, 1977). Current evidence suggests that the release of mast cells and other inflammatory cells (such as macrophages, basophils etc.)-derived chemical mediators as well as abnormalities in autonomic control of the airways interact and contribute to the pathogenesis of asthma (Barnes, 1983). The pathological changes in asthma and the mediators that may be responsible are shown in Table 1.

Pathologic change	Mediator implicated
Bronchospasm	Histamine (H_1 response)
	Leukotrienes C_4, D_4, and E_4
	Prostaglandins and thromboxane A_2
	Bradykinin
	Platelet-activating factor
	Acetylcholine (secondary action)
Mucosal edema	Histamine (H_1 response)
	Leukotrienes C_4, D_4, and E_4
	Prostaglandin E
	Bradykinin
	Platelet-activating factor
Cellular infiltration (airway hyperreactivity)	Eosinophil chemotactic factors
	Neutrophil chemotactic factors
	Inflammatory factors of anaphylaxis
	HETE
	Leukotriene B_4
Mucus secretion	Histamine (H_2 response)
	Prostaglandin-generating factor of anaphylaxis
	Prostaglandins
	HETE
	Macrophage mucus secretagogue
	Acetylcholine (secondary action)
Desquamation	O_2, H_2O_2, OH^-
	Proteolytic enzymes
Basement membrane thickening	O_2
	Proteolytic enzymes

TABLE 1. Pathologic changes in asthma and the mediators that may be responsible (Kalimer, 1985)

Mediators involved in asthma can be divided into (1) preformed granule-associated (histamine, eosinophil and neutrophil chemotactic factors) and (2) newly formed, membrane-derived (leukotrienes, prostaglandins , platelet activating factor) agents (Kay, 1982), as shown in Table 2a and 2b.

A. *Preformed mediators*

Histamine	H_1	smooth muscle contraction
		↑ vascular permeability
		stimulation of irritant receptors
		↓ cAMP
	H_2	↓ IgE mediated histamine release
		↓ lysosomal enzyme release from neutrophils
		↑ gastric acid secretion
		↑ bronchial mucus secretion
		↑ cAMP
	Both	vasodilation
Eosinophil chemotactic factor of anaphylaxis (ECF-A)		attracts eosinophils
Neutrophil chemotactic factor		attracts neutrophils
Heparin		anticoagulant
		anticomplement, against alternative pathway — C3 convertase (=anti-inflammatory)
Serotonin proteases/hydrolases		

B. *Membrane-derived mediators*

Via cycloxygenase pathway	Prostaglandins	PGD2 — bronchoconstriction
		PGE1 and 2 — bronchodilatation
		— potentiation of histamine/bradykinin
		PGF2 — potentiation of acetyl choline (bronchoconstriction blocked by atropine)
	Thromboxanes	TXA 2 and B2 — bronchoconstriction, contraction of parenchyma
	Prostacyclines	inhibit platelet aggregation
Via lipoxygenase pathway	Leukotrienes	HETE — enhances histamine release
		LTB4 — chemotactic to eosinophils
		LTC4, D4 and E4 — vasoconstriction (SRS — A) bronchoconstriction plasma exudation
Platelet activating factor (PAF)		bronchoconstriction
		platelet aggregation (and leucocyte trapping)
		extravasation of protein
		"degranulation" of platelets
		mimics IgE mediated shock

TABLE 2. Mediators involved in asthma:

a) preformed mediators and

b) membrane-derived mediators (Reiser, 1985)

In addition to various chemical mediators, asthma might be explained by an abnormality of autonomic control, which speculates that the basic defect in asthma is an imbalance between cholinergic and sympathetic nervous systems (Barnes, 1986). Several different autonomic abnormalities have been proposed, including enhanced cholinergic (Simonssen, 1967 and Reed, 1974), and adrenergic (Reed, 1974 and Szentivanyi, 1968), or noncholinergic excitatory (subtance P) mechanisms (Lundberg, 1983) or reduced β-adrenergic (Reed, 1974 and Szentivanyi, 1968), or nonadrenergic inhibitory (vasoactive intestinal peptide) mechanisms (Richardson, 1981 and Barnes, 1984).

2. Airway hyperresponsiveness and inflammatory mediators.

Asthma has been taken as an example of a lung disease in which inflammation of airways is present and may be a certain mechanism in the pathophysiology of the disease. Many different cell types are present and evidence exists that various inflammatory mediators with relevant biological activities are also present (Barnes, 1983). The followings are mediators which are suggested to play roles in the inflammatory changes of asthma.

2.1 Histamine

Histamine was the first allergic mediator to be implicated in asthma. Histamine levels have been shown to increase in peripheral blood following antigen challenge, although it is not know whether the histamine is derived from mast cells or basophils (Durham et al., 1984). The

physiologic and pharmacologic actions of histamine are exerted through interaction with two types of membrane receptors, H_1 and H_2, and the resultant effects reflect the ratio of these receptors on a given cell or tissue. Via H_1 actions, histamine causes bronchial and intestinal smooth muscle contraction, increases vascular permeability by causing venular endothelial disconnections (Majno et al., 1961), induces pulmonary vasoconstriction, and stimulates nasal mucus production (Shelhamer et al., 1980). In addition, histamine stimulates lung irritant receptors (Mills et al., 1969), opens up endothelial "tight junctions" (Majno, 1964), thus increasing vascular permeability and promoting the inflammatory response. Histamine is also found to be chemotactic for eosinophils (Clark et al., 1975).

2.2 Prostaglandins (PGs)

Products of arachidonic acid metabolism via a cyclo-oxygenase-dependent pathway constitute the majority of prostaglandins in humans. During the course of human lung anaphylaxis different prostaglandins are generated (Platshon and Kaliner, 1978). Prostaglandins have several effects on the lung. $PGF_{2\alpha}$, and PGD_2 are potent bronchoconstrictors, asthmatic patients showing greater effects with these mediators as with other spasmogens (Hardy et al., 1984). PGE_1 and thromboxane B_2 cause bronchospasm while PGE_2 are bronchodilators. It is conceivable that a balance between those opposing influences may affect bronchial tone and play a role in allergic bronchospasm (Thomas and Marom, 1983). Anyhow,

PGE_2, which produces bronchodilation in normal subjects *in vivo*, can provoke bronchoconstriction in asthmatic patients because of its irritant effect (Hyman *et al.*, 1978). Prostaglandins also stimulate mucous secretion from human airways (Shelhamer *et al*, 1982) and chemotactic attraction of eosinophils or neutrophils (Wasserman, 1980).

2.3 Leukotrienes

Leukotrienes C_4, D_4 and E_4 collectively account for the biological activity of SRS-A, which has long been proposed as an important mediator of allergic bronchoconstriction in man (Piper and Samhoun, 1987). The leukotrienes are generated by a variety of cells, including mast cells, macrophages and eosinophils. Leukotrienes are synthesized from arachidonic acid by the 5-lipoxygenase pathway. The potent effects of these mediators in causing bronchoconstriction, mucous secretion and airway edema suggest a role in asthma (Barnes, 1985). The leukotrienes are at least 1000 times more potent than histamine as constrictors of human or guinea-pig airway smooth muscle (Dahlén *et al.*, 1980; Hedqvist *et al.*, 1982).

2.4 Platelet-activating factor (PAF)

PAF (acetyl-glycero-phosphorylcholine) is generated from membrane phospholipids by phospholipase A_2 in a variety of cells, including macrophages, neutrophils and eosinophils, although there is some doubt about whether human lung mast cells can secrete PAF (Barnes, 1985; Vergaffig and Braquet, 1987). PAF has very potent effects in the airways,

causing bronchoconstriction, mucosal edema, mucous hypersecretion and inflammatory cell chemotaxis (Morley et al., 1984). Recent studies of inhaled PAF-acether suggest that aerosolised PAF-acether is about 50 times more potent than methacholine, although tachyphylaxis develops (Rubin, et al., 1986; Cuss et al., 1986).

2.5 Bradykinin

Bradykinin is a 9-amino acid peptide enzymatically cleaved by kallikrein and kininogenase from high molecular weight kininogen which circulates in plasma (Barnes, 1985). Bradykinin is a potent bronchoconstrictor when given by inhalation (Fuller et al, 1985 b), but has little effect on human airways in vitro, and there is evidence that a cholinergic reflex is involved. In several tissues bradykinin produces its effects by the release of prostaglandins, but the bronchoconstrictor effect of inhaled bradykinin is unaffected by aspirin (Fuller et al., 1985 b). In animals, bradykinin selectively activates afferent nerve endings in the airways, so it is possible that some of the constrictor effect of bradykinin is due to the release of sensory neuropeptides, via an axon reflex. Bradykinin is also a potent inducer of bronchial edema and mucous secretion (Barnes, 1985).

2.6 Chemotactic factors

Several products released in inflammatory reactions are found to possess chemotactic activity and attract inflammatory cells such as

neutrophils, eosinophils and monocytes from the circulation. These cells, when activated, then release other mediators which may contribute to the bronchoconstrictor response or bronchial hyperreactivity (Barnes, 1985). Chemotactic mediators include histamine, LTB_4, PGD_2, 5-HETE and PAF, as mentioned above, but, in addition, several large molecular weight products have been identified. These include various eosinophil chemotactic factors and a high molecular weight neutrophil chemotactic factor, which has been detected in plasma in a variety of bronchial challenges in asthmatic subjects (Kay and Lee, 1982), although the protein has not yet been isolated. The complement factor $C5_a$ is a potent chemotactic factor and may also be involved in inflammatory reactions in the airways (Barnes, 1985).

2.7 Adenosine

Adenosine is produced under conditions of hypoxia and its concentration increases in plasma after antigen challenge (Mann et al., 1983). Furthermore, a release of adenosine in the venous blood can be demonstrated in asthmatic subjects when exposed to allergens and when inhaled, adenosine elicits bronchoconstriction in asthmatic but not in non-asthmatic subjects (Church and Holgate, 1986). The mechanism of adenosine-induced bronchoconstriction is as yet unclear but possibilities include interaction of adenosine with the A_2 subtype of P_2 purinoceptors, enhancement of mediator secretion from mast cells and contraction of airway smooth muscle by either a direct action or indirectly by stimulation

of neuronal reflexes. Another possibility of adenosine-induced tracheal contraction seems to be caused by a release of 5-HT, presumably from mast cells (Church and Holgate, 1986).

2.8 Acetylcholine

Acetylcholine is a neurotransmitter at autonomic ganglia, neuromuscular junctions and postganglionic nerves of the parasympathetic division of the autonomic system. Acetylcholine release from postganglionic nerves activates muscarinic cholinergic receptors on smooth muscle cells in the airways, which are blocked by atropine and related drugs such as ipratropium bromide (Barnes, 1987). Activation of muscarinic receptors in airway smooth muscle causes smooth muscle contraction by stimulating the breakdown of membrane phosphoinositides, which results in the release of calcium ions from intracellular stores, and there is a closed relationship between muscarinic receptor occupation and stimulation of phosphoinositide turnover (Grandordy et al., 1986). Activation of muscarinic receptors also inhibits adenylate cyclase, leading to a reduction in the concentration of cAMP (Madison et al., 1985). Cyclic AMP is found to be an important final intracellular messenger for relaxation of various types of smooth muscles (Barnes, 1986).

The schematic presentation of the concept of the pathogenesis of asthma is shown in Fig. 1. This scheme combines the effects of reflex, allergic and nonallergic stimuli, their common effector systems that cause mediator release, and specific effects on tissues, all of which

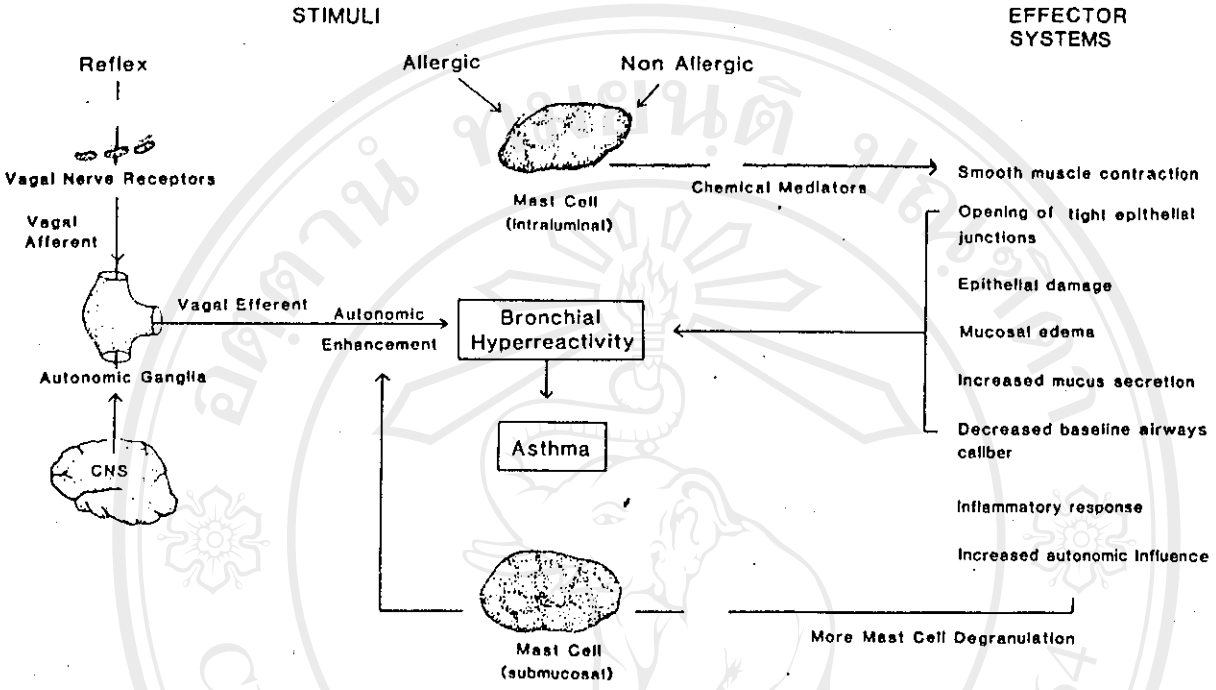


Fig. 1 Concept of the pathogenesis of asthma

may enhance the development of bronchial hyperreactivity. Afferent and efferent nerve stimulation by various stimuli will also increase bronchial hyperreactivity. When bronchial hyperreactivity exceeds a critical stage, clinical asthma develops (Bernstin, 1985).

3. Reference drugs used in this study

Bronchodilators which relieve airway obstruction are aimed at reversing airway obstruction and by definition bronchodilators should produce rapid reversal of airway obstruction which implies an action on airway smooth muscle contraction rather than the other pathological events (Barnes, 1986). New approaches to improved bronchodilators are in the direction of reduced side effects and improve compliance (Reed, 1986). In an attempt to evaluate the bronchodilator activity of any compound, its activity should be compared with various reference drugs. In this study the following reference drugs were used:

3.1 Beta adrenergic agonists (e.g. isoproterenol)

Beta-adrenergic agonists are the most commonly used bronchodilators. They are believed to raise cAMP levels in bronchial smooth muscle by stimulation of adenlate cyclase (Svedmyr, 1977). Although direct relaxation of airway smooth muscle is probably the major mechanism of bronchodilatation, beta-adrenergic agonists may also produce bronchodilation indirectly, both by inhibiting cholinergic tone and reducing mediator released from airway mast cells (Barnes, 1984). In addition

they may speed the clearance of viscous mucus from the airways (Barnes, 1986). Isoproterenol, the most potent sympathomimetic amine acting on beta-receptors, is a powerful relaxant of bronchial smooth muscle. Because of its equal $beta_1$ and $beta_2$ actions, the main side effects of isoproterenol are cardiac, and it can readily give rise to arrhythmias (Lockett, 1965). The new agents such as metaproterenol, albuterol, fenoteral which improve $beta_2$-receptor selectivity have been synthesized and should be the first choice of drugs in this group.

3.2 Methylxanthines (e.g. aminophylline)

Aminophylline (theophylline ethylendiamine) is the most widely used of the soluble theophylline salts. Theophylline compounds are useful as prophylactic drugs and are valuable adjuncts in the treatment of prolonged attacks and in the management of status asthmaticus. Theophylline was originally thought to act by increasing cAMP levels in smooth muscle through inhibition of phosphodiesterase. However, it has subsequently been shown that phosphodiesterase is not significantly inhibited at tissue concentrations achieved with usual therapeutic doses. Alternative mechanisms have been proposed including alterations in smooth muscle calcium ion concentration, inhibition of the effects of prostaglandins on smooth muscle and of the release of histamine and leukotrienes from mast cells (Isles et al., 1982). In addition, theophylline at therapeutic concentrations is a potent antagonist of adenosine receptors, which suggests another possible mechanism for its therapeutic effects (Fredholm, 1980).

3.3 Antimuscarinic agents (e.g. atropine)

The anticholinergic, antimuscarinic compounds are potent and hitherto neglected bronchodilators (Gross and Skordin, 1984). The bronchodilator effect of antimuscarinic agents is due to competitive inhibition of cholinergic muscarinic receptors on bronchial smooth muscle, antagonizing the action of acetylcholine at its membrane-bound receptor site, and thereby blocking the bronchoconstrictor action of vagal efferent impulses (Su and Bevan, 1976). The prototype is atropine. A few antimuscarinic agents that have been extensively studied for their bronchodilator activity are atropine, its sulfate and methonitrate salt as well as the synthetic agent ipratropium bromide (Gross and Skorodin, 1984). Atropine is well absorbed from the gastrointestinal tract as well as from mucosa and therefore produces undesired systemic side effects. The synthetic quaternary ammonium congeners of atropine, such as ipratropium bromide and atropine methonitrate, are poorly absorbed. When given by inhalation, they are as effective bronchodilators as atropine is, but longer acting and much less prone to side effects.

3.4 Calcium channel blockers (e.g.verapamil)

Because calcium ion flux through the membrane is necessary for activation of cells (in asthma, for example, mast cells and bronchial smooth muscle), the calcium channel blockers introduced for treating heart disease have been tested in asthma (Reed, 1986). The calcium channel blocking agents have recently received considerable attention

based upon the fact that mediator release and smooth muscle spasm are dependent upon the entry of calcium into the mast cells and the muscle cells, respectively (George and Payne, 1984). There are two types of calcium antagonists: those that block entry of calcium ions into the cell, and those that affect the intracellular sites of calcium action. Some examples of the first are nifedipine, verapamil, diltiazem. The aminoindenes and mechinarone seem to have their primary action at intracellular sites (McFadden, 1981). At present, it seems unlikely that the currently available calcium channel blockers will have a major role in the treatment of acute bronchospasm (Fanta and Drazen, 1983).

3.5 Spasmolytic agents (e.g. papaverine)

Papaverine is currently considered as the prototype of nonspecific spasmolytic agents (Ferrari, 1974), and is often used as a standard drug in the screening of the activity of new antispasmodics (Nickerson, 1975). Inhibition of phosphodiesterase, leading to accumulation of cAMP , was proposed as the mechanism of action of papaverine.

4. Experimental models used for investigation of bronchodilators.

A perfect animal model of human asthma is hard to find, especially as the disease itself is still uncompletely defined and characterized (Salonen at al., 1985). However, the isolated guinea-pig trachea was found to exhibit a relevant model of human large and central

airways and is a suitable preparation to screen compounds with potential efficacy in allergic disorders of the respiratory tract, such as asthma (Muccitelli et al., 1987). In addition, Burns and Doe (1978) as well as Lulich and Paterson (1980) have used the rat tracheal, bronchial and lung strip in vitro preparation for study of various drugs on central and peripheral airways. The tracheal muscles of guinea-pigs, dogs and human are sensitive to histamine whereas those of cats, rabbits and rats are not (Akcasu, 1959; Jamieson, 1962). Acetylcholine and serotonin are proved to be potent bronchoconstrictive inducers in rat, guinea-pig (Jamieson, 1962) and human airway (Muccitelli et al., 1987).

Salonen et al. (1985) described that whole animal models are commonly regarded as more relevent than in vitro experiments, because pathophysiological changes similar to human asthma (e.g. acute bronchoconstriction, mucosal edema, and hypersecretion of mucus) can be induced. A major advantage is that various, control mechanisms (e.g. neural reflexes and adrenal secretion) are intact as well. Knösett and Rösler (1940) were the first to introduce a reliable, objective method for measuring bronchoconstriction in whole animals. Since that time other workers have modified this method e.g. McCulloch et al. (1967), Amdur and Mead (1958) and Drazen (1975). Andersson and Persson (1977) have later modified this technique to anesthetized, mechanically ventilated small animals as well. Another alternative for investigating the antiasthmatic action of drugs in whole animals is to induce airway obstruction by pharmacological means. Most often some humoral mediator, e.g. acetylcholine or histamine, has been administered intravenously at one moderate bronchoconstrictor dose and the effects of antiasthmatic drugs on this challenge have been studied (Salonen, 1985).

5. Purpose of the study.

Five derivatives of phenylalkane, investigated for bronchodilator activity in this study, i.e. 1-(3, 4-dimethoxyphenyl)-but-1-ene (PA-1), 1-(3, 4-dimethoxyphenyl),-but-4-01 (PA-2), 1-(3, 4-dimethoxyphenyl)-1, 4-butanediol (PA-3), 1-(3, 4-dichlorophenyl)-but-1-ene-4-ol (PA-4) and 1-(4-chlorophenyl)-but-1-ene-4-01 (PA-5) were synthesized by Dr. Pittaya Tuntiwachwuttikul and her colleagues (Department of Chemistry, Faculty of Sciences, Silapakorn University, Nakornpathom). Their chemical structures are shown in Table 3. These phenylalkane derivatives were derived by the structural modification of compound D (3, 4-dimethoxyphenyl butenol) (Fig. 2) which was one of the biologically active constituents isolated from hexane extract of the commonly used Thai medicinal plant "Plai" (Zingiber caussumunar Roxb.).

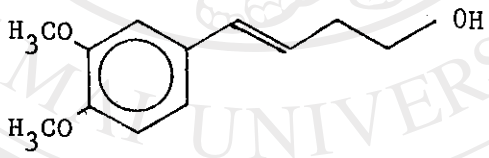
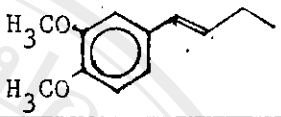
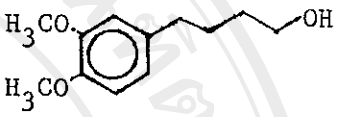
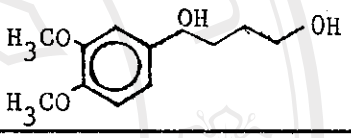
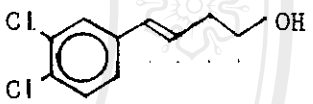
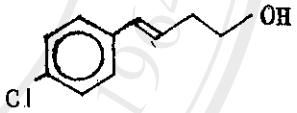


Fig. 2 Compound D (3, 4-dimethoxyphenyl butenol)

Compound D was found to possess smooth muscle relaxant activity including bronchodilator activity. Kiatyingungsulee et al. (1979) and Soparat (1984) reported that compound D could inhibit histamine-induced contraction of isolated guinea-pig tracheal smooth muscle. According to folklore remedies, "Plai" is effective in relieving bronchial

Table 3 Chemical structures of phenylakane derivatives.

	Phenylalkane derivatives	Structure
PA-1	1-(3,4-dimethoxyphenyl)-but-1-ene	
PA-2	1-(3,4-dimethoxyphenyl)-but-4-ol	
PA-3	1-(3,4-dimethoxyphenyl)-1,4-butanediol	
PA-4	1-(3,4-dichlorophenyl)-but-1-ene-4-ol	
PA-5	1-(4-chlorophenyl)-but-1-ene-4-ol	

asthma (Pongseboonrod, 1965), and the clinical study carried out to evaluate the efficacy and tolerability by Tuchinda et al. (1984) supports this reputed effect. Asthmatic children (age ranged from 9 - 14 years) receiving 2 capsules, containing Plai powder 130 mg/capsule, twice daily, exhibited significant improvement of respiratory rate and all respiratory functions.

The purpose of this study was therefore to evaluate the bronchodilator activity of the five phenylalkane derivatives mentioned above in comparison with compound D and reference drugs including isoproterenol, aminophylline, atropine, verapamil and papaverine. The potency of tested drugs was determined and only one potent and suitable phenylalkane derivative was selected as a representative for detail bronchodilator study. General effects of the compound selected were also evaluated in conscious animals using Hippocratic screen.