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(72) Inventeurs/Inventors:
JACOBUS, KARLA M., US;
YERXA, BENJAMIN R., US;
PENDERGAST, WILLIAM, US;
BOUCHER, RICHARD C., JR., US;
...

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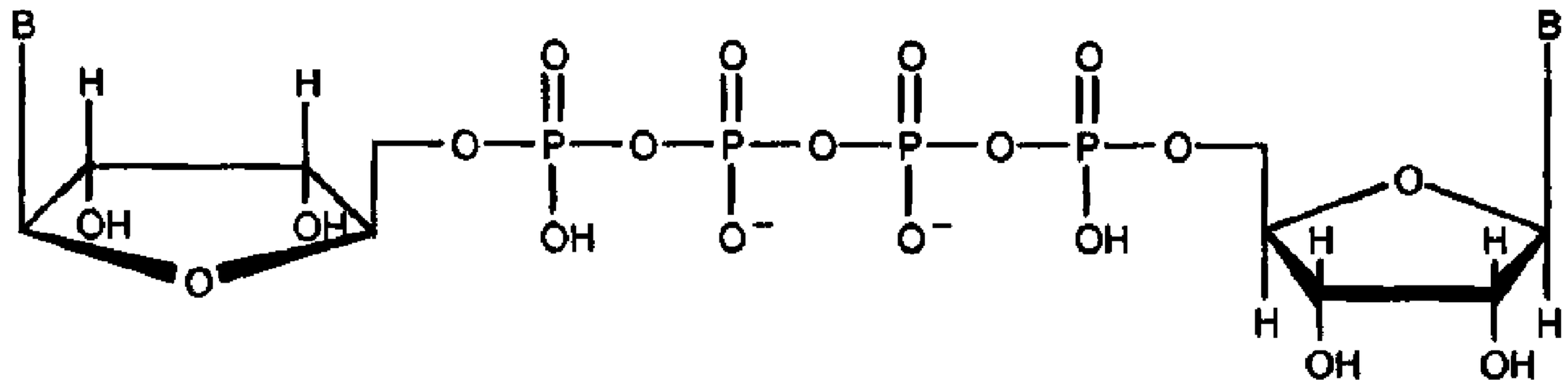
(54) Title: METHOD OF TREATING CILIARY DYSKINESIA WITH URIDINE TRIPHOSPHATES AND RELATED COMPOUNDS

Formula IV



(57) Abrégé/Abstract:
A method of stimulating ciliary beat frequency in a subject in need of such treatment is disclosed. The method comprises administering to the airways, ears, eyes, or genito-urinary tract of the subject a triphosphate nucleotide such as uridine 5'-triphosphate (UTP), an analog of UTP, or any other analog, in an amount effective to stimulate ciliary beat frequency. This method is useful for treating patients afflicted with ciliary dyskinesia, Kartagener's syndrome, or any other disease involving dysfunction of ciliary movement, such as male infertility caused by impairment of propulsion of the spermatozoa or immune deficiency caused by impairment of ciliary movement in neutrophils or macrophages. Pharmaceutical formulations and methods of making the same are also disclosed. Methods of administering the same would include any liquid suspension (including nasal spray or nasal or eye drops), oral, inhaled by nebulization, topical, injected, suppository, intra-operative by instillation or application, or ex vivo direct application to spermatozoa.

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(72) **Inventeurs(suite)/Inventors(continued)**: RIDEOUT, JANET L., US; DRUTZ, DAVID J., US; JAMES, MICHAEL K., US; STUTTS, MONROE JACKSON III, US; GEARY, CARA A., US; LAZAROWSKI, EDUARDO R., US

(73) **Propriétaires(suite)/Owners(continued)**:INSPIRE PHARMACEUTICALS, INC., US; THE UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL, US

(74) **Agent**: GOWLING LAFLEUR HENDERSON LLP

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(71) Applicant *(for all designated States except US)*: INSPIRE PHARMACEUTICALS, INC. [US/US]; Suite 470, 4222 Emperor Boulevard, Durham, NC 27703 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: JACOBUS, Karla, M. [US/US]; 200 Riverwalk Circle, Cary, NC 27511 (US). YERXA, Benjamin, R. [US/US]; 1330 Hathaway Road, Raleigh, NC 27608 (US). PENDERGAST, William [US/US]; 5815 Williamsburg Way, Durham, NC 27713 (US). BOUCHER, Richard, C., Jr. [US/US]; 735 Gimghoul Road, Chapel Hill, NC 27514 (US). RIDEOUT, Janet, L., [US/US]; 3101 Morningside Drive, Raleigh, NC 27607 (US). DRUTZ, David, J. [US/US]; 1059 Carterbury Lane, Chapel Hill, NC 27514 (US). JAMES, Michael, K. [US/US]; 4329 Basal Creek Lane, Fuquay-Varina, NC 27526. STUTTS, Monroe, Jackson [US/US]; 104 Norgan Bluff Lane, Chapel Hill, NC 27514 (US). GEARY, Cary [US/US]; 7011 Thurston-Bowles, Campus Box 7248, UNC at Chapel Hill, Chapel Hill, NC 27599 (US). LAZAROWSKI, Edwardo, R. [US/US]; Apartment #3, 212 Pinegate, Chapel Hill, NC 27514 (US).

(74) Agents: DREHKOFF, W., Dennis et al.; Banner & Witcoff, Ltd., 10 South Wacker Drive, Chicago, IL 60606 (US).

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(57) Abstract

A method of stimulating ciliary beat frequency in a subject in need of such treatment is disclosed. The method comprises administering to the airways, ears, eyes, or genito-urinary tract of the subject a triphosphate nucleotide such as uridine 5'-triphosphate (UTP), an analog of UTP, or any other analog, in an amount effective to stimulate ciliary beat frequency. This method is useful for treating patients afflicted with ciliary dyskinesia, Kartagener's syndrome, or any other disease involving dysfunction of ciliary movement, such as male infertility caused by impairment of propulsion of the spermatozoa or immune deficiency caused by impairment of ciliary movement in neutrophils or macrophages. Pharmaceutical formulations and methods of making the same are also disclosed. Methods of administering the same would include any liquid suspension (including nasal spray or nasal or eye drops), oral, inhaled by nebulization, topical, injected, suppository, intra-operative by instillation or application, or ex vivo direct application to spermatozoa.

METHOD OF TREATING CILIARY DYSKINESIA WITH URIDINE TRIPHOSPHATES AND RELATED COMPOUNDS

INTRODUCTION

Technical Field

This invention relates to a method of stimulating ciliary beat frequency to promote mucociliary or cough clearance of retained mucus secretions from the lungs, sinuses, or ears of a patient by administering certain uridine, adenosine, or cytidine triphosphates.

Background of the Invention

Mucociliary clearance is an important defense mechanism of the human airway and middle/inner ear tract. Coordinated beats of cilia in the nose, trachea, bronchi, and middle ear propel the mucous layer toward the pharynx, carrying along with it microorganisms and other particles captured in the mucus. Normal function of this system depends on the frequency and coordination of ciliary beating and the properties of mucus. There are three components of the mucociliary clearance system: (1) the mucin layer, which is formed by secretion of mucins by goblet cells, (2) cilia, which transport the overlying mucin layer by synchronous beating, and (3) the periciliary liquid layer, which surrounds the cilia and is less viscous than the mucin layer, allowing free movement of the cilia. The electrolyte and water concentration of the periciliary layer is regulated by the luminal epithelial cells. (R. Boucher, et al., Adenosine and Adenine Nucleotides: From Molecular Biology to Integrative Physiology, p. 525-32 entitled "Mechanisms and Therapeutic Actions of Uridine Triphosphates in the Lung" (L. Belardinelli, et al. ed., Alumwer Academic Publishers, Boston 1995)).

Primary ciliary dyskinesia (PCD) is a congenital disease characterized by ultrastructural defects and motility disturbances of cilia, resulting in either absent or abnormal ciliary movement. The most common clinical manifestations of PCD are chronic respiratory disease (e.g., sinusitis, rhinitis, and bronchiectasis) and otitis media. Because PCD patients have either absent or severely impaired mucociliary clearance (MCC), the only available mechanism to clear or move secretions is cough. Cough clearance may be measured in a manner similar to that previously

described for MCC. PCD also impairs the propulsion of spermatozoa, resulting in male infertility. (D. Schidlow, *Ann Alergy* 73(b), 457-68 (1995)). PCD also results in the impairment of cell motility of certain immune system cells, including neutrophils and macrophages. (N. Valerius, *Eur J Clin Invest* 13, 489-94 (1983)). PCD may be responsible for a form of hydrocephalus caused by ciliary malfunction. (M. Greenstone, *Arch Dis Child* 59, 481-82 (1984)). The incidence of PCD has been calculated to be one in 16,000 live births, and an estimated 50% of affected individuals also have situs inversus (dextrocardia). The triad of bronchiectasis, sinusitis, and situs inversus (dextrocardia) is referred to as Kartagener's syndrome. (M. Sleigh, *Lancet* ii,476 (1981)). It has been hypothesized that Kartagener's syndrome is caused by a lack of embryonic ciliary movement, resulting in the random rotation of the archenteron such that in half the cases there is situs inversus (dextrocardia) and in the other half there is normal cardia situs. (B. Afzelius *Science* 193, 317-19 (1976)). The clinical course of PCD is characterized primarily by sinus and ear infections early in life with a progressive change to lung/lower airways diseases in adulthood. Chronic airways infections can lead to chronic obstructive changes in the pulmonary tissue, progressive loss of pulmonary function, and eventually death.

A secondary and more common form of ciliary dyskinesia is the acquired form of the disease. Chronic inflammation caused by severe viral or bacterial respiratory infections, chronic smoking, severe air pollution, chemical or thermal burns to the airways, intubation and mechanical ventilation, and near drowning can result in changes in ciliary structure including disruption of the cellular membrane, loss or incorporation of microtubules, and formation of compound cilia, all of which can result in abnormal or absent ciliary function. (J. Ballenger *Ann Otol Rhinol Laryngol* 97 (3 Pt. 1), 253-58 (1988); M. Pedersen *Lung* 168 Suppl., 368-76 (1990)). Respiratory infections which often lead to secondary ciliary dyskinesia include influenza, adult respiratory distress syndrome, and ventilator-associated pneumonia (VAP) in intensive care unit (ICU) patients.In some cases acquired ciliary dyskinesia may be reversed with appropriate and timely intervention; however, permanent damage and/or sustained exposure to the above factors may render the ciliary damage irreversible. The clinical manifestations and course would likely appear similar to PCD with respect to chronic lung infections, progressive loss of pulmonary function, and obstructive pulmonary disease.

The typical mammalian respiratory epithelial cell contains about 200 cilia. Each cilium has nine peripheral microtubular doublets and two central tubules. Each peripheral doublet contains an A subunit and a B subunit, and each A subunit has a set of curved arms attached to it called the inner and outer dynein arms. These dynein arms contain ATPase--an enzyme which breaks down adenosine triphosphate (ATP), providing the energy for ciliary movement. Because the most common ultrastructural abnormality associated with primary ciliary dyskinesia is the total absence of dynein arms (B. Afzelius, et al., *J Cell Biol* 66, 225-32 (1975)), researchers began investigating whether extracellular application of ATP and ATPase could activate immotile cilia in vitro. (J. Forrest, et al., *Am Rev Resp Dis* 120, 511-15 (1979)). Although the results appeared positive, the findings have not been consistently reproduced by others. It was later discovered that extracellular application of Ca^{2+} and cAMP could increase the beat frequency of respiratory tract cilia. (A. Lansley, et al., *Am J. Physiol* 263, L232-42 (1992)). It has not been definitively established that any therapy will stimulate cilia beat in cases where complete ciliary immotility has been demonstrated. In such cases, it might be of therapeutic benefit to increase hydration of the viscous mucous secretions.

Applicant has discovered that extracellular triphosphate nucleotides, especially uridine 5'-triphosphate (UTP) modulates all three components of the mucociliary transport system. UTP stimulates ciliary beat frequency (R. Boucher, et al., *supra*); UTP stimulates mucin secretion by goblet cells (M. Lethem, et al., *Am J Respir Cell Mol Biol* 9, 315-22 (1993)); and UTP stimulates Cl^- secretion in airway epithelial cells, which increases hydration of the periciliary liquid layer (M. Knowles, et al., *N Eng J. Med* 325, 533-38 (1991)). Applicant has also demonstrated that UTP is safe and improves cough clearance in PCD patients. (P. Noone, et al., abstract submitted to the 1996 International Conference of The American Thoracic Society).

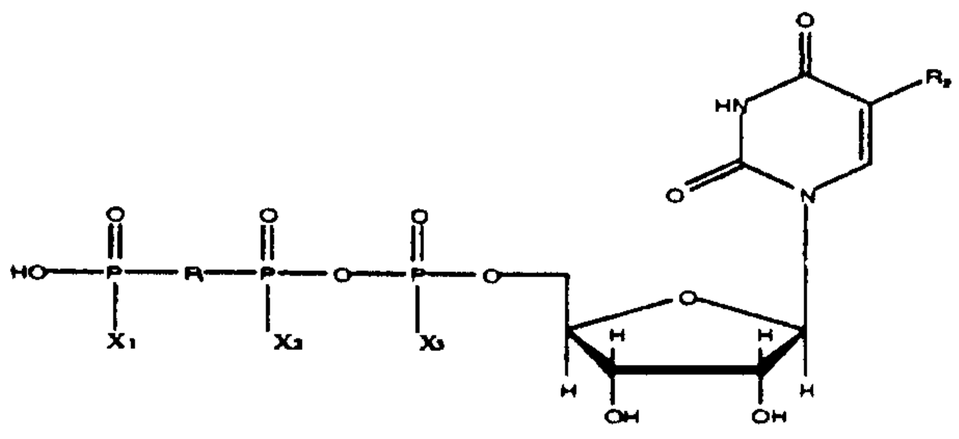
In summary, a variety of clinical manifestations of ciliary dyskinesia, such as absent or impaired mucociliary clearance in the respiratory and middle/inner ear tract, impaired propulsion of spermatozoa, and impaired motility of neutrophils and macrophages can be improved or alleviated by administering UTP and its related compounds, as well as other nucleoside phosphates such as: adenosine 5'-triphosphate (ATP); cytidine 5'-triphosphate (CTP); 1,N^6-ethenoadenosine triphosphate; adenosine 1-oxide triphosphate; 3,N^4-ethenocytidine

triphosphate; P_1,P_4-di(adenosine-5′) tetraphosphate (A_2P_4); or P_1,P_4-di(uridine-5′) tetraphosphate (U_2P_4) to the affected part of the body.

SUMMARY OF THE INVENTION

A method of treating ciliary dyskinesia in a subject in need of such treatment is disclosed. The method comprises administering to the patient a compound of Formula I, or a pharmaceutically acceptable salt thereof, in an amount effective to stimulate ciliary beat frequency, where possible, in the luminal epithelial cells of the lung or middle/inner ear, eyes, genito-urinary tract; spermatozoa cells; or certain cells of the immune system, including neutrophils and macrophages:

Formula I



wherein:

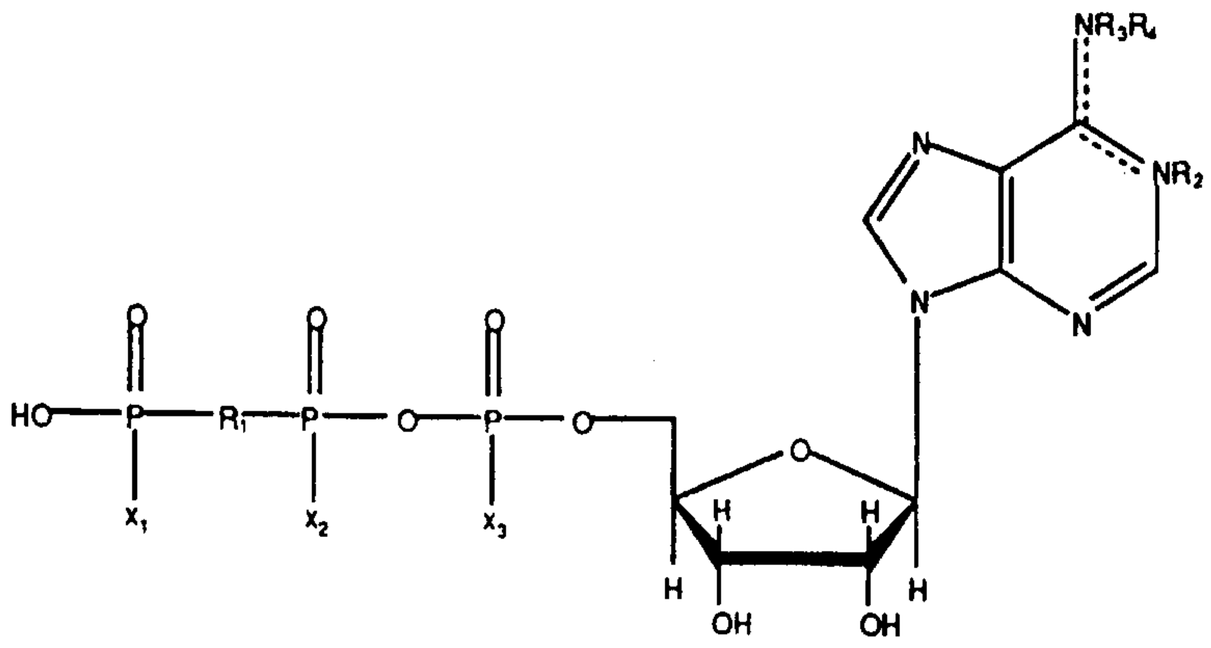
X_1, X_2, and X_3 are each independently either O^- or S^-. Preferably, X_2 and X_3 are O^-.

R_1 is O, imido, methylene, or dihalomethylene (e.g., dichloromethylene, diflouromethylene). Preferably, R_1 is oxygen or difluoromethylene.

R_2 is H or Br. Preferably, R_2 is H. Particularly preferred compounds of Formula I are uridine 5′-triphosphate (UTP) and uridine 5′-O-(3-thiotriphosphate) (UTPγS).

Formula I is the preferred embodiment of the compound, however, the method of the present invention can also include administering a compound of Formula II (adenosine 5′ triphosphate [ATP] or 1,N^6-ethenoadenosine triphosphate or adenosine 1-oxide triphosphate), or Formula III (cytidine 5′ triphosphate [CTP] or 3,N^4-ethenocytidine triphosphate), or Formula IV (P^1,P^4-di(adenosine-5′) tetraphosphate (A_2P_4) or P^1,P^4 di(uridine-5′) tetraphosphate (U_2P_4).

Formula II



wherein:

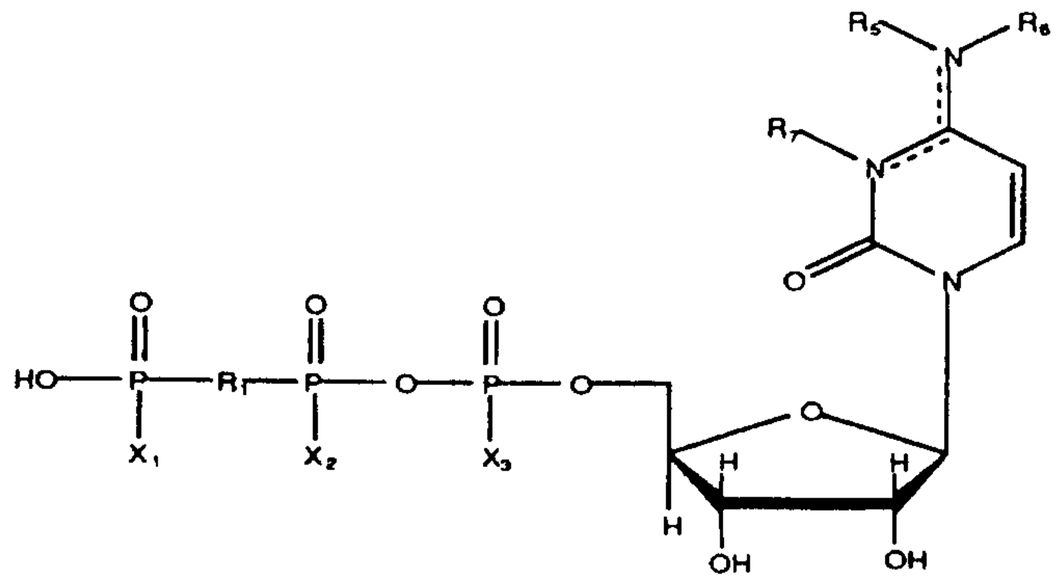
R_1, X_1, X_2, and X_3 are defined as in Formula I.

R_3 and R_4 are H while R_2 is nothing and there is a double bond between N-1 and C-6 (adenine), or

R_3 and R_4 are H while R_2 is O and there is a double bond between N-1 and C-6 (adenine 1-oxide), or

R_3, R_4 and R_2 taken together are -CH=CH-, forming a ring from N-6 to N-1 with a double bond between N-6 and C-6 ($1,N^6$-ethenoadenine).

Formula III



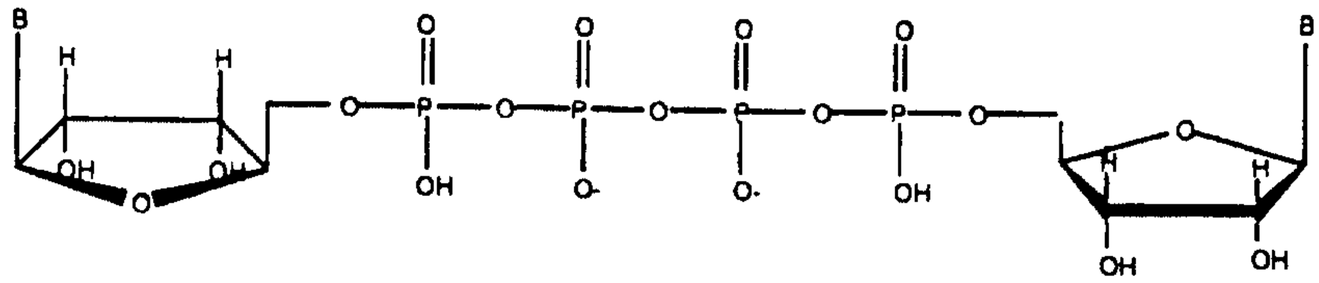
wherein:

R_1, X_1, X_2, and X_3 are defined as in Formula I.

R_5 and R_6 are H while R_7 is nothing and there is a double bond between N-3 and C-4 (cytosine), or,

R_5, R_6 and R_7 taken together are -CH=CH-, forming a ring from N-3 to N-4 with a double bond between N-4 and C-4 (3,N^4-ethenocytosine).

Formula IV



wherein:

B is adenine or uracil.

A second aspect of the present invention is a pharmaceutical formulation containing the compound of Formula I, II, III, or IV in an amount effective to stimulate ciliary beat frequency in: the epithelial cells of the lungs or middle and inner ears; the mucous clearance defense system of the eyes or genito-urinary tract; spermatozoa cells; the ovaries or fallopian tubes; or certain cells of the immune system, including neutrophils and macrophages, in a pharmaceutically acceptable carrier.

A third aspect of the present invention is the use of the active compounds disclosed herein for the manufacture of a medicament for the therapeutic stimulation of ciliary beat frequency in: the epithelial cells of the lungs or middle and inner ears; the mucous clearance defense system of the eyes or genito-urinary tract; spermatozoa cells; the ovaries or fallopian tubes; or certain cells of the immune system, including neutrophils and macrophages, of a patient in need of such treatment.

BRIEF DESCRIPTION OF THE DRAWINGS

These and other features of the invention will become more apparent from the following description in which reference is made to the appended drawings wherein:

FIGURE 1 shows the results of a randomized, double-blind, placebo-controlled, crossover study in 12 patients with primary ciliary dyskinesia (PCD), ages 10 and above. The cough clearance (%/min) at 30 minute time intervals of PCD patients following inhalation of a saline control (vehicle=V) or inhalation of 10^{-2} M uridine 5'-triphosphate (UTP=U) are shown;

FIGURE 2 shows the results of a blinded cross-over design study in 8 patients with PCD. The volume of sputum produced (ml/min) in a unit of time by PCD patients following inhalation of a saline vehicle (column 1) or inhalation of UTP (column 2).

DESCRIPTION OF THE SPECIFIC EMBODIMENTS

The method of the present invention may be used to stimulate ciliary beat frequency in a subject in need of such treatment for any reason, including (but not limited to) increasing the mucociliary clearance of retained secretions in the lungs, sinuses, or middle and inner ears. The method of the present invention may also be used to treat primary ciliary dyskinesia, secondary ciliary dyskinesia, Kartagener's syndrome, otitis media, cystic fibrosis, diseases involving the dysfunction of the ocular or genito-urinary mucociliary clearance defense system caused by impairment of ciliary movement, diseases of the immune system caused by impairment of ciliary movement of neutrophils and macrophages, hydrocephalus caused by impairment of ciliary movement, male infertility caused by impairment of the ciliary propulsion of the spermatozoa, female infertility caused by impairment of ciliary movement on the luminal epithelial cells of the ovaries or fallopian tubes, or any other disease caused by an impairment of ciliary movement. The present invention increases mucociliary clearance in three ways: (1) by increasing the ciliary beat frequency of cilia on the surface of luminal epithelia cells, (2) by increasing the secretions of mucins by goblet cells, and (3) by increasing the secretion of water into the periciliary liquid layer by luminal epithelial cells. The mucins secreted by goblet cells form a layer on top of the cilia and captures foreign particles, including viruses and bacteria; the mucin layer is transported by the wave-like action of cilia, and the movement of cilia is facilitated by the composition and hydration of the periciliary liquid layer surrounding the cilia. Although the primary aspect of the present invention is to increase ciliary beat frequency in patients afflicted with ciliary dyskinesia, in patients whose cilia are permanently incapable of any movement regardless of treatment, the active compounds of the present invention can still facilitate the clearance of retained mucous secretions by increasing the secretion of water into the periciliary liquid layer and by increasing the secretion of mucins by goblet cells.

Additionally, because of the well-demonstrated ability of the active compounds of the present invention to enhance lung mucociliary clearance in normal subjects, the active compounds of the present invention can accelerate the clearance of any type of inhaled foreign materials from the airways. This would prove beneficial in a number of

situations--biological warfare, e.g. the chemical warfare agent ricin; smoke inhalation; industrial exposure to inhaled toxins (resulting in e.g., silicosis, anthracosis, and the gamut of so-called pneumoconioses); and allergic reaction to inhaled particles such as pollen.

Furthermore, the ability of the active compounds of the present invention to increase lung clearance would also prove beneficial in the diagnosis of lung disease--specifically, to improve the quality of radioisotopic scans of the lungs by removing the secretions that might otherwise obscure the visualization of ventilated portions of the lung. In radioisotopic lung scanning, the mismatch of ventilated versus perfused lung is used to identify areas of pulmonary infarction. As a result of improved aeration of the lungs after administering the active compounds of the present invention, the ventilated portions of the scan would be more distinct, and the diagnostician would be in a better position to clearly identify true mismatches.

The present invention is concerned primarily with the treatment of human subjects, but may also be employed for the treatment of other mammalian subjects, such as dogs and cats, for veterinary purposes.

Compounds illustrative of the compounds of Formula I above include: (a) uridine 5'-triphosphate (UTP); (b) uridine 5'-O-(3-thiotriphosphate) (UTPγS); and (c) 5-bromo-uridine 5'-triphosphate (5-BrUTP). These compounds are known or may be made in accordance with known procedures, or variations thereof which will be apparent to those skilled in the art. See generally N. Cusack and S. Hourani, *Annals N.Y. Acad. Sci.* 603, 172-81 (entitled "Biological Actions of Extracellular ATP"). For example, UTP may be made in the manner described in Kenner, et al., *J. Chem. Soc.* 1954, 2288; or Hall and Khorana, *J. Am. Chem. Soc.* 76, 5056 (1954). See Merck Index, Monograph No. 9795 (11th Ed. 1989). UTPγS may be made in the manner described in R. S. Goody and F. Eckstein, *J. Am. Chem. Soc.* 93, 6252 (1971).

For simplicity, Formulae I-IV herein illustrate the active compounds in the naturally occuring D-configuration, but the present invention also encompasses compounds in the L-configuration, and mixtures of compounds in the D- and L- configurations, unless otherwise specified. The naturally occuring D-configuration is preferred.

Compounds illustrative of the compounds of Formula II above include (a) adenosine 5'-triphosphate (ATP) and (b) 1,N^6-

ethenoadenosine triphosphate. Compounds illustrative of the compounds of Formula III above include (a) cytidine 5′-triphosphate and (b) 3,N^4-ethenocytidine triphosphate. These compounds can be made in accordance with known procedures, or variations thereof which will be apparent to those skilled in the art. For example, phosphorylation of nucleosides by standard methods such as D. Hoard and D. Ott, *J. Am. Chem. Soc.* 87, 1785-1788 (1965); M. Yoshikawa, et al., *Tetrahedron Lett.* 5065-68 (1967) and *idem., Bull. Chem. Soc. (Jpn)* 42, 3505-08 (1969); J. Moffatt and H. Khorana, *J. Am. Chem. Soc.* 83, 649-59 (1961); and B. Fischer, et al., *J. Med. Chem.* 36, 3937-46 (1993) and references therein. Etheno derivatives of cytidine and adenosine are prepared by known methods such as: N. Kotchetkov, et al., *Tetrahedron Lett.* 1993 (1971); J. Barrio, et al., *Biochem. Biophys. Res. Commun.* 46, 597 (1972); J. Secrist, et al., *Biochemistry* 11, 3499 (1972); J. Bierndt, et al., *Nucleic Acids Res.* 5, 789 (1978); K. Koyasuga-Mikado, et al., *Chem. Pharm. Bull. (Tokyo)* 28, 932 (1980). Derivatives with alpha, beta and gamma thiophosphorus groups can be derived by the following or by adapting methods of: J. Ludwig and F. Eckstein, *J. Org. Chem.* 54, 631-35 (1989); F. Eckstein and R. Goody, *Biochemistry* 15, 1685 (1976); R. Goody and F. Eckstein, *J. Am. Chem. Soc.* 93, 6252 (1971).

Compounds of Formulas I, II, or III where R_1 is CCl_2 and CF_2 can be prepared by methods similar to that described in G. Blackburn, et al., *J. Chem. Soc. Perkin Trans.* I, 1119-25 (1984). Compounds of Formula I, II, III where R_1 is CH_2 can be prepared by methods similar to that described in T. Myers, et al., *J. Am. Chem. Soc.* 85, 3292-95 (1963).

Compounds illustrative of the compounds of Formula IV include (P^1,P^4-di(adenosine-5′) tetraphosphate (A_2P_4) or P^1,P^4-di(uridine-5′) tetraphosphate (U_2P_4). These compounds can be made in accordance with known procedures, or variations thereof which will be described by: P. Zamecnik, et al., *Proc. Natl. Acad. Sci. USA* 89, 838-42 (1981); and K. Ng and L. E. Orgel, *Nucleic Acids Res.* 15 (8), 3572-80 (1987).

In addition, UTP, ATP, CTP, A_2P_4, 3,N^4-ethenocytidine triphosphate, 1,N^6-ethenoadenine triphosphate, adenosine 1-oxide triphosphate, ATPγS, ATPβS, ATPαS, $AMPPCH_2P$, AMPPNHP, N^4-ethenocytidine and 1,N^6-ethenoadenosine are commercially available, for example, from Sigma Chemical Company, PO Box 14508, St. Louis, MO 63178.

The active compounds of Formulae I - IV may be administered by themselves or in the form of their pharmaceutically acceptable salts, e.g., an alkali metal salt such as sodium or potassium, an alkaline earth metal salts such as manganese, magnesium and calcium or an ammonium and tetraalkyl ammonium salts, NX_4^+ (wherein X is C_{1-4} alkyl group). Pharmaceutically acceptable salts are salts that retain the desired biological activity of the parent compound and do not impart undesired toxicological effects.

The active compounds disclosed herein may be administered to the lungs, sinuses, ears, eyes, spermatozoa, ovaries or fallopian tubes, or genito-urinary tract by a variety of suitable means, but are preferably administered by administering a nebulized form of the active compound into their respiratory tract, such that the active compound enters the lungs and reaches the area of the body afflicted with impaired ciliary movement either directly or via systemic absorption and circulation. The active compound can be aerosolized in a variety of forms, such as, but not limited to, dry powder inhalants, metered dose inhalants, or liquid/liquid suspensions. In dry powder delivery, the UTP may be formulated alone or in combination with diluent or carrier, such as sugars (e.g., lactose, sucrose, trehalose, mannitol) where the compounds may be intimately incorporated in the matrix through glassification or simply admixed with the carrier, or other acceptable excipients for lung or airway delivery. The dry powder may be obtained by methods known in the art, such as spray-drying, milling, freeze-drying, super-critical fluid manufacturing or via controlled crystallization or precipitation

The dosage of active compound to stimulate ciliary beat frequency will vary depending on the condition being treated and the state of the subject, but generally an effective amount is the amount sufficient to achieve concentrations of active compound on the lungs, sinuses, ears, eyes, or genito-urinary surfaces of the subject of from about 10^{-7} to about 10^{-1} Moles/liter, and more preferably from about 10^{-6} to about 10^{-1} Moles/liter.

Depending upon the solubility of the particular formulation of active compound administered, the daily dose to promote fluid drainage may be divided among one or several unit dose administrations. Preferably, the daily dose is no more than four times per day.

Another means of administering the active compound to the lungs, sinuses, ears, eyes, or genito-urinary tract of the patient to promote

fluid/secretion drainage may include any oral form of the active compound, administered to the patient either by means of a liquid suspension of the active compound which is poured into the mouth of the patient, or by means of a pill form swallowed by the patient.

Another means of administering an effective amount of the active compound to the lungs, sinuses, eyes, or middle and inner ears would involve administering a liquid/liquid suspension (either a nasal spray of respirable particles which the subject inhales, or nasal drops of a liquid formulation, or eye drops of a liquid formulation) comprised of the active compound. Liquid pharmaceutical compositions of the active compound for producing a nasal spray or nasal or eye drops may be prepared by combining the active compound with a suitable vehicle, such as sterile pyrogen free water or sterile saline by techniques known to those skilled in the art.

Another means of administering the active compound to the middle ear or eye would include any topical form of the active compound, administered as a cream or gel to the outer ear or eye, which would subsequently permeate through the tympanic membrane or cornea into the middle ear or lens of the patient.

Another means of administering the active compound to the middle ear, genito-urinary tract, or eye would involve an injected form of the active compound, injected from the outer ear directly through the tympanic membrane into the middle ear, injected into the genito-urinary tract, or injected into the eye. This could involve a patch containing UTP which would be applied directly to the tympanic membrane.

Another means of administering the active compound to the lungs, sinuses, middle and inner ears, eyes, or genito-urinary tract would involve a suppository form of the active compound, such that a therapeutically effective amount of the compound reaches the lungs, sinuses, middle ear, eye, genito-urinary tract, or male or female reproductive systems via systemic absorption.

Another means of administering the active compound would involve intra-operative instillation of a gel, cream, or liquid suspension form of the active compound such that a therapeutically effective amount reaches the lungs, sinuses, middle and inner ears, eyes, or genito-urinary tract.

An additional means of administering the active compound would involve ex vivo administration of the active compound to the

spermatozoa by means of a topical, injection, or immersion form of the compound, such that a therapeutically effective amound of said compound contacts the spermatozoa having impaired impaired ciliary movement.

An additional means of administering the active compound would involve administration of the active compound via a transdermal patch, in which the active compound would be delivered to the affected are via local absorption or systemic absorption and circulation.

UTP and compounds for Formulae I - IV also have therapeutic benefit when used in combination with other agents used to treat ciliary dyskinesia, such as, but not limited to: antibiotics; vaccines; decongestants, mucolytic agents; nonsteroidal antiinflammatory agents; steroids; antiviral agents; and bronchodilators. UTP may also be used in combination with other treatments under development, such as gene therapy. UTP may also be used in combination with the recently discovered therapeutic protein defensin.

The present invention is explained in greater detail in the Examples which follow. These examples are intended as illustrative of the invention, and are not to be taken as limiting thereof.

EXPERIMENTAL

Example 1

In Vitro Stimulation of Ciliary Beat Frequency

10^{-4} M UTP was applied to isolated airway epithelial cells from normal subjects. Within four minutes, ciliary beat frequency (CBF) increased by 76 ± 17% as compared to baseline (from 9.3 ± 0.23 to 16.12 ± 0.92 Hz, n = 7, $p < 0.0001$). Similar results were obtained when 10^{-4} M UTP was applied to isolated airway epithelial cells from patients afflicted with cystic fibrosis. CBF increased by 56 ± 17% as compared to baseline (from 11.25 ± 0.56 Hz to 16.1 ± 1.45 Hz).

Example 2

Treatment of Primary Ciliary Dyskinesia

Uridine 5'-triphosphate (UTP) was administered to patients diagnosed with primary ciliary dyskinesia (PCD) (verified by electron microscopy analysis of ciliary ultrastructure defect from nasal biopsy). The efficacy of UTP was determined by measuring the clearance of an inhaled radiolabeled particle from the lung by radionuclide scanning techniques using a gamma camera. Each subject inhaled an aerosol of iron oxide labeled with Technetium 99m (^{99}Tc-Fe_2O_3). Subjects inhaled the aerosol for approximately 5 minutes. Subjects were then seated in front of a gamma camera, and for the next 20 minutes subjects randomly inhaled either a saline control (0.12% saline), or 10^{-2} M UTP for approximately 20 minutes. After this inhalation, subjects remained seated in front of the gamma camera for the next 30 minutes to measure clearance of the radiolabeled iron oxide. The efficacy of aerosolized UTP in treating primary ciliary dyskinesia was demonstrated by an improvement in cough clearance of Tecnetium 99m as compared to the saline vehicle alone.

In the same study, the amount of sputum induced by the inhalation of UTP versus placebo was measured. Subjects inhaled either placebo or UTP for 20 minutes according to the method above. For the next 30 minutes, subjects performed 60-90 controlled coughs, and matched sputum samples were collected from 8 of the 12 patients. Total volume of sputum was measured.

Safety data was collected by monitoring heart rate, ECT rhythm strip-Lead II, blood pressure, oxyhemoglobin saturation by pulse oximetry prior to, during, and after inhalation for all dosing periods. All patients during all phases of the study were monitored for any adverse reactions during each dosing period, beginning with inhalation of study drug and ending after 30-minute scan at 24 hours.

Results are shown in Figure 1 which shows the cough clearance (%/min) of patients with PCD following inhalation of the saline Vehicle (V) vs. UTP (U).

This was a randomized, double-blind, placebo-controlled, crossover study in 12 patients with PCD, ages 10 and above. Figure 1 summarizes data demonstrating that UTP (U) significantly enhanced cough clearance at the 60-minute ($p<0.05$) and 120-minute ($p<0.05$) time points, as compared to placebo (vehicle=V); this improvement also approaches significance at the 30-minute time point. These data are particularly compelling, given that the effect was observed following only a single dose of UTP. With the data in Figure 2, these data suggest that there is a strong correlation between measures of cough clearance and sputum expectoration.

Figure 2 shows the volume of sputum produced per unit time in PCD patients.

Figure 2 shows data demonstrating that the volume of sputum produced per unit time is also enhanced in PCD patients by the inhalation of UTP. The study was a blinded cross-over design and involved 8 patients. Column 1 shows that approximately 0.2 ml of sputum were expectorated per minute following inhalation of saline vehicle. In contrast, Column 2 shows that inhalation of UTP approximately doubled the amount of sputum expectorated in the same time period ($p<0.01$). Based on data from patient-completed questionnaires (completed in a blinded fashion), UTP appeared to enhance the ease of expectoration relative to vehicle. Several patients stated that sputum was thinner and easier to expectorate following UTP than following vehicle. These data are particularly compelling given that the effect was observed following only a single dose of UTP. With the data in Figure 2, these data indicate that there is a strong correlation between measures of cough clearance and sputum expectoration.

Other Embodiments

Other embodiments will be evident to those of skill in the art.

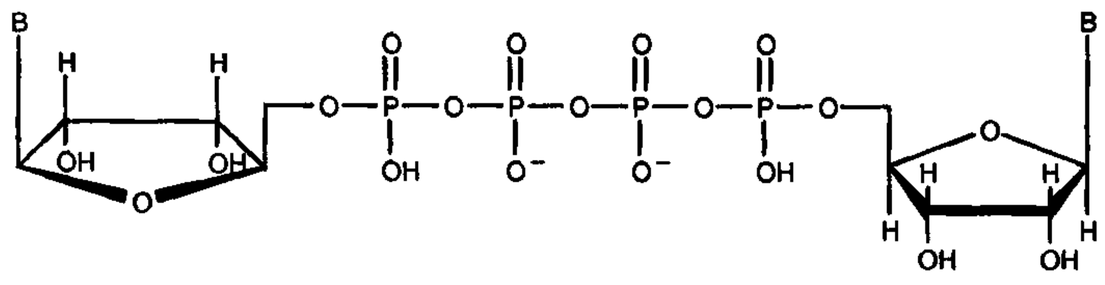
Although the invention has been shown and described with respect to an --illustrative embodiment thereof, it should be appreciated that the foregoing and various other changes, omissions, and additions in the

form and detail thereof may be made without departing from the spirit and scope of the invention as delineated in the claims.

Claims:

1. A composition for treating ciliary dyskinesia in the respiratory tract in a subject, said composition comprising a compound of Formula IV, or a pharmaceutically acceptable salt thereof, in a pharmaceutical carrier having an amount of said compound effective to increase ciliary beat frequency in the respiratory tract;

Formula IV



wherein B is adenine or uracil, and at least one B is uracil.

2. The composition according to Claim 1, wherein said composition is in the form of a liquid or a liquid suspension.

3. The composition according to Claim 2, wherein said composition is in the form of nasal drops or a nasal spray.

4. The composition according to Claim 1, wherein said composition is the form of an aerosol suspension.

5. The composition according to Claim 1, wherein said composition is in an oral form.

6. The composition according to Claim 1, wherein said composition is in a topical form.

7. The composition according to Claim 1, wherein said composition is in an injection form.

8. The composition according to Claim 1, wherein said composition is in a suppository form.

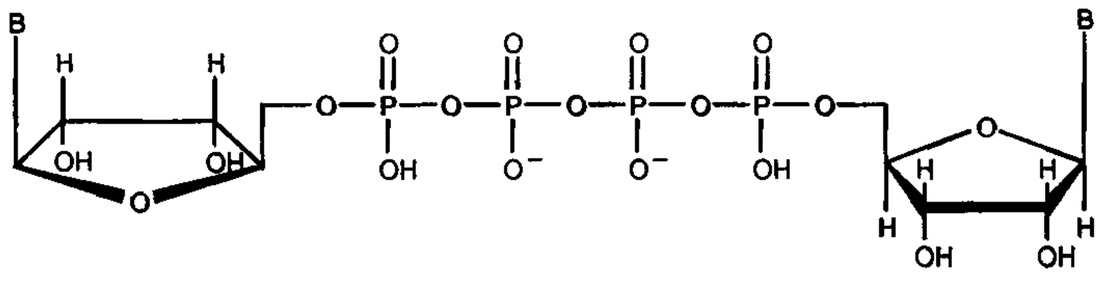
9. The composition according to Claim 1, wherein said composition is in the form of a gel or cream.

10. The composition according to Claim 1, wherein said composition is in the form of a transdermal patch.

11. The composition according to any one of Claims 1 to 10, wherein said compound of Formula IV is P^1, P^4-di(uridine-5') tetraphosphate.

12. A composition for stimulating expectoration in a subject having ciliary dyskinesia, said composition comprising a compound of Formula IV, or a pharmaceutically acceptable salt thereof, in a pharmaceutical carrier having an amount of said compound effective to increase ciliary beat frequency in the subject

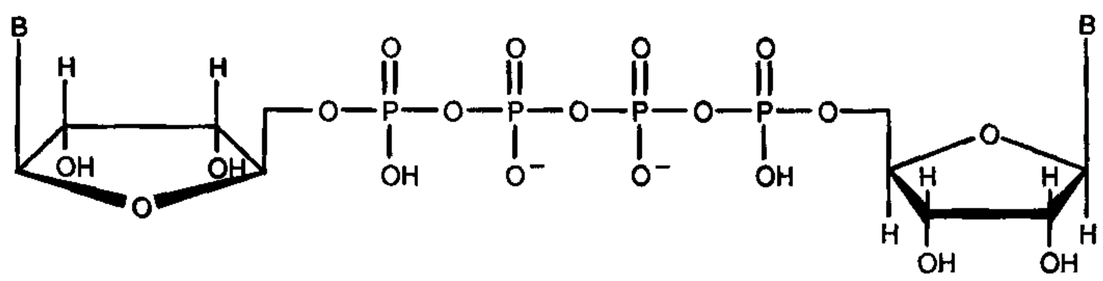
Formula IV



wherein B is adenine or uracil, and at least one B is uracil.

13. Use of a composition for treating ciliary dyskinesia in the respiratory tract in a subject, said composition comprising a compound of Formula IV, or a pharmaceutically acceptable salt thereof, in a pharmaceutical carrier having an amount of said compound effective to increase ciliary beat frequency in the respiratory tract;

Formula IV



wherein B is adenine or uracil, and at least one B is uracil.

14. Use according to Claim 13, wherein said composition is in the form of a liquid or a liquid suspension.

15. Use according to Claim 13, wherein said composition is in the form of nasal drops or a nasal spray.

16. Use according to Claim 13, wherein said composition is the form of an aerosol suspension.

17. Use according to Claim 13, wherein said composition is in an oral form.

18. Use according to Claim 13, wherein said composition is in a topical form.

19. Use according to Claim 13, wherein said composition is in an injection form.

20. Use according to Claim 13, wherein said composition is in a suppository form.

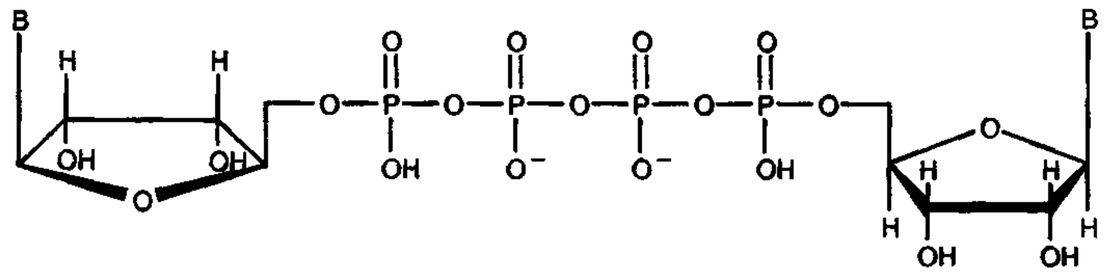
21. Use according to Claim 13, wherein said composition is in the form of a gel or cream.

22. Use according to Claim 13, wherein said composition is in the form of a transdermal patch.

23. Use according to any one of Claims 13 to 22, wherein said compound of Formula IV is P^1, P^4-di(uridine-5') tetraphosphate.

24. Use of a composition for stimulating expectoration in a subject having ciliary dyskinesia, said composition comprising a compound of Formula IV, or a pharmaceutically acceptable salt thereof, in a pharmaceutical carrier having an amount of said compound effective to increase ciliary beat frequency in the subject

Formula IV



wherein B is adenine or uracil, and at least one B is uracil.

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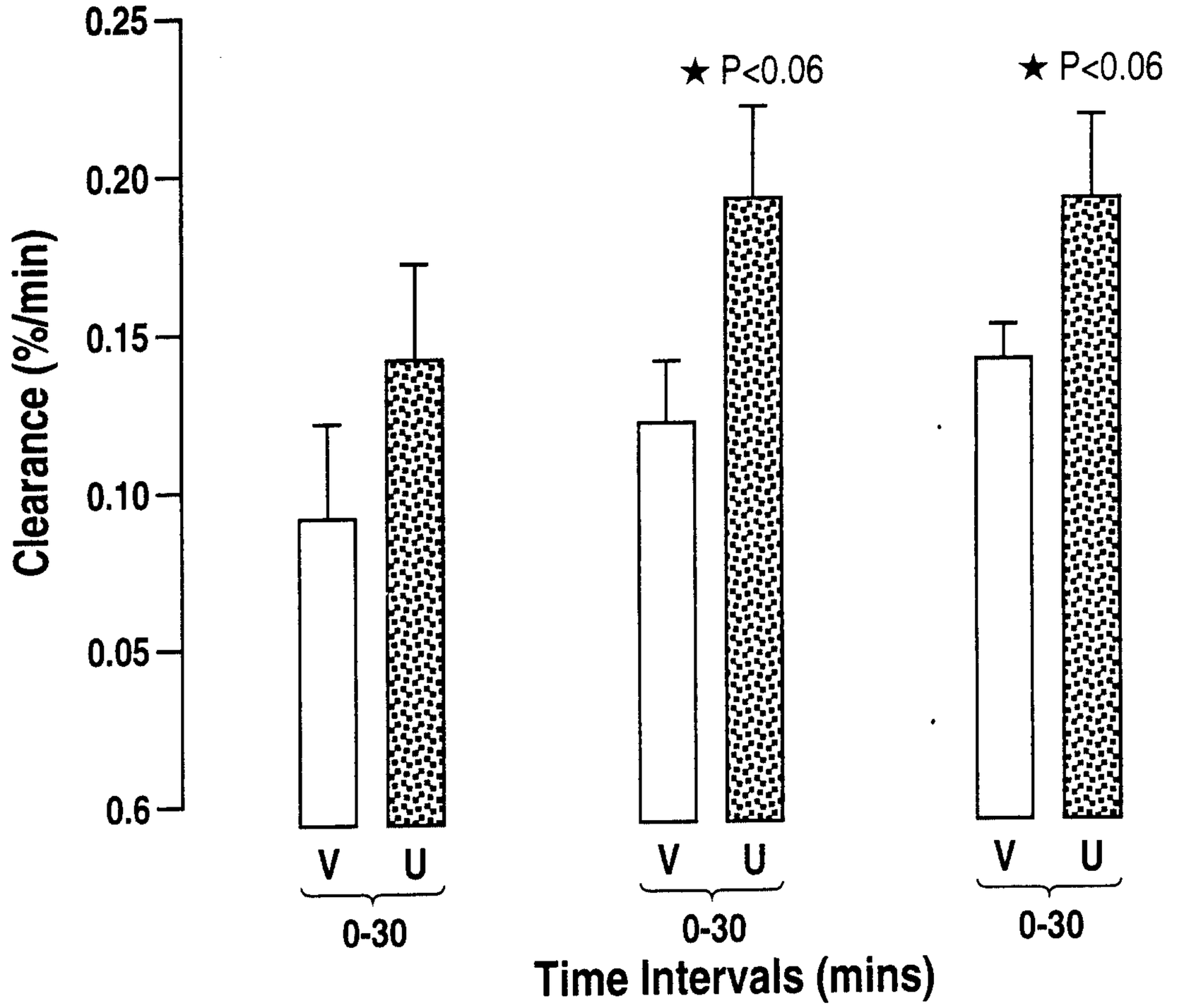

Fig.1

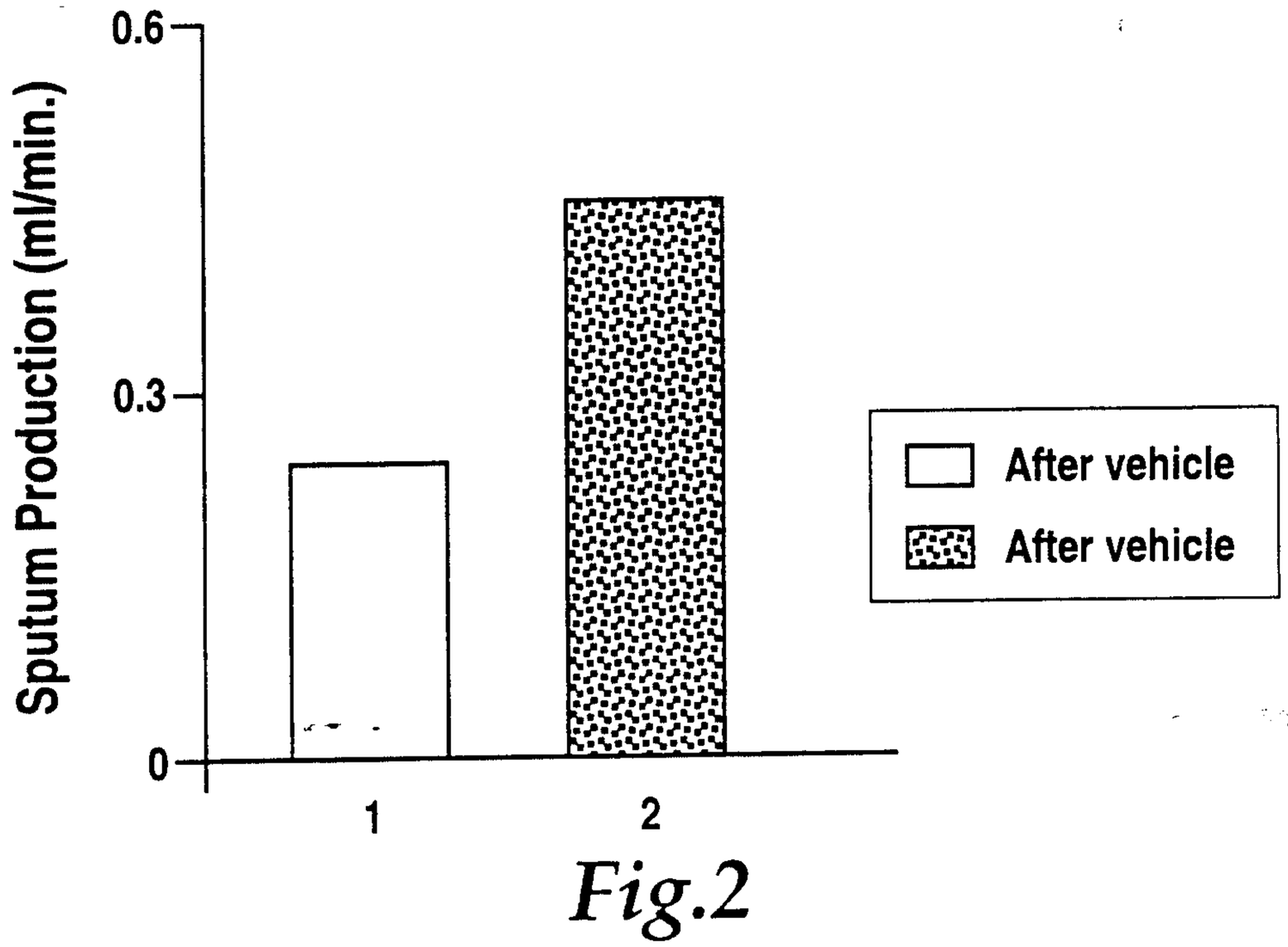

Fig.2

Formula IV