Title of Invention "IMMUNOMODULATORY COMPOUND FOR TREATING CANCER"

Abstract A novel biologically active immunomodulatory compound for treating cancer having the molecular formula C21H3O2, said compound having improved bioactivity, bioavailability and solubility properties.

Full Text

FIELD OF INVENTION:

The present invention relates to a biologicaly active immunomodulatory for meeting cancer of the formula C21H3O2. Further the present invention relates to a process for isolation of a compound of formula C21H3O2 exhibiting immunomodulatory activity. The invention also relates to an immunomodulatory compound of formula C21H3O2 obtained from Piper longum, and provides formulations useful for activating the immune system.

BACKGROUND:

Due to increasing incidence of cancer, in general, there is a need to develop new therapeutic strategies. The current methods of treatment of tumors, besides radiation therapy and surgical interventions, include tumoricidal chemotherapeuric agents which act directly and are toxic to tumor cells. Such anti- tumor drugs, however, are generally associated with severe side effects as they often kill all normally dividing without discrimination. The present invention relates to an alternate strategy, referred to as immunomodulatory therapy, where instead of" directly killing the tumor cell the drug is intended to act on the immune system and activate its effector mechanisms which in turn kill tumor cells. Immunomodulatory therapy thus relates to "educating " or "activating" die immune cells to react against and kill the tumor cells ; the same strategy applies also to cells infected with parasites/ viruses. The advantage is that the immune system is selective and it normally attacks only diseased cells, ignoring the normal healthy ones.

PRIOR ART

The idea of fighting cancer by unleashing the latent powers of patient's own

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immune system has been practiced since early 20th century, when some physicians (William B.Coley) attempted this strategy by injecting patients with killed bacteria (Nauts HC THE Bibliography of Reports Concerning the Experimental Clirticall Use of Coley Toxins, Cancer Research Institute, New York, 1975).Recent advances in the field of immunology have revealed that tumor regression is carried out mainly cytotoxic T Lymphocytes (CTL) or activated macrophages. These cells recognize unique antigens displayed on the surface of tumor cells and become activated and kill the tumor cells. In addition, natural killer (NK) cells also play important role for killing tumor cells.

A) CURRENT METHODS FOR ACTIVATION OF EFFECTOR MECHANISM OF THE IMMUNE SYSTEM

Ever since the demonstration that the human disease can be treated by modulating the immune response, several immunomodulatory products have received clinical approval for therapeutic use in cancer and infections (reviewed BY Hadden JW, Trends in Pharmaceutical sciences, 14 : 169-174,1993). The list of clinically-approached immunomodulators is given in the following table :

AGENT

CHEMICAL NATURE

CLINICAL USE

Microbially derived products :

BCG
Picibani
Krestin
Lentinan
Biostim
Broncho-Vaxom

live mycobacteria Extract.strp.pyrogenes fungal polysaccharide fungal polysaccharide extract.klebsiella pneum
extract of 8 bacteria

bladder cancer gastric/other cancer gastric/other cancer gastric/other cancer chronic/recurrent infections chronic/recurrent infections

Chemically defined products:
Romurtide muramyl dipeptide(MDP)
Murabutide Ubenimex(Bestatin) Thymopentin TP- 5 Levaraisole Inosine pranobex Poly AU Ampligen

MDP derivative dipeptide pentapeptide phenylimidothiazole inosine-salt complex double -stranded poly-nucleotide of adenylic and uradylic acid mismatched poly 1C

bone marrow/ recovery cancer infection cancer
infection, cancer cancer infection breast cancer
HIV, cancei-

However, so far no plant derived immunomoduiatory compound has reached to stage of clinical testing.

B) PIPER LONGUM

The Indian medicinal plant piper longum L, (family : piperaceae) grows and cultivated in the different parts of India and other south east Asian countries and root extracts and preparations are widely used in various Indian system of medicine including its high reputation in Ayurvedic medicine for treatment of diseases of respiratory tract viz. cough, bronchitis, asthma etc: as counter-irritant and analgesic when applied locally for muscular pain and inflammation ; as snuff in coma and drowsiness and

internally as carminative; as sedative in insomnia and epilepsy; as general tonic and haematinic; as cholagogue in obstruction of bile ckicr and gall bladder; as an emmenagogue and abortifacient; and for miscellaneous

purposes as anthelmintic and in dysentery and leprosy (Atal and Ojha, Wealth of India vol.8, Ph-Re, CSIR Publication, New Delhi). The detailed investigation on the fruits of Piper longum and related species has led to identification of several pieridine alkaloids such as piperine, piplartine, piperlongumine, piperlonguminine, pipernonaline and piperundecalidine etc a few hitherto unidentified steroids and some reducing sugars and their glycosides (Desai SJ et al., Ind. J. Chem., 28B, 775, 1989, and the literature-sited therein). In our investigation on activity guided fractionation of this plant for immunomodulatory activity, we have isolated, among other compounds a biologically active compound named NII-30 as new compound from piper longum species and developed new high yielding chemical synthesis of NII-30 and its stereoisomers and analogues for improved bioactivity and bioavailability and olubility. There is one report in literature (K. Likhitwitayawuid et al, Tetrahedron, 1987, 43, 3689-3694) on isolation of similar compounds from Indonesian species piper sarmentosum but no biological investigation for any kind of activity has been reported in the scientific and patent literature.

OBJECTS OF THE INVENTION

The main objectives of the present invention are 1) isolation and identification of an immunomodulatory compound from piper longum which enhances the effector mechanism of the immune system to react against tumors, 2) provide novel synthetic process for isolation of the novel compound, generation of synthetic analogues of the said compound, and 3) development of novel immunomodulatory applications of these compounds against tumors and opportunistic infections.

STATEMENT OF INVENTION

The invention provides a novel compound biologically active immunomodulatory isolated from Piper longum having
molecular formula C21H3O2. and structural formula:
(Formula Removed)
The compound may be called as "l-(3,4-methylenedioxy-phenyl)-lE-tetradecene and for brevity referred as "NII-30".
This compound exhibits excellent immunomodulatory activity. It may be used to prepare formulations for activation of the immune system. Such formulations include the compound NII-30 in an effective amount to activate the immune system together with any pharmaceutically acceptable additive such as an anti-tumor compound, or antibiotic. The invention also provides a method for producing a formulation which comprises mixing a compound having molecular formula C21H3O2 with any other immunomodulator or anti-tumor/antibiotic compound.
Thus, the present invention describes a new method of activating the immune system to react against tumors and infections by using a formulation containing a NII-30 isolated from the fruits of Piper longum. The present invention also describes a process of isolation of NII-30 from Piper longum, a new process of synthesis of NII-30 and also its synthetic analogues with improved bioactivity and bioavailability.
Further, the invention provides a process for isolation of a compound of formula C21H3O2 exhibiting immunomodulatory activity, said process comprising:
a. treating macerated and dried fruits of Piper longum with a solvent as hereindescribed,
b. concentrating the extract below 50°C under reduced pressure,
c. solvent fractionating the concentrate of step (b) using • solvents such as a hydrocarbon, chlorinated hydrocarbon, ethyl acetate and water to obtain four fractions and identifying a biologically active fraction from the said four fractions in a manner known per se,
d. purifying the biologically active fraction by normal and reverse phase silica gel chromatography with solvent systems such as hereindescribed for elution,

e. concentrating the fractions under reduced pressure and separating the compounds therein, and

f. screening the compounds separately for immunomodulatory activity and isolating a compound of the formula C21H302 by conventional methods.

The solvent in the process is selected from methyl alcohol and water. The chlorinated solvent is selected from dichloromethane and chloroform. The hydrocarbon used is petroleum ether.

As such, in the process, solvent fractionation is effected by successively macerating the concentrate in different solvents or suspending the concentrate in different solvents. The fractions obtained in step (e) are concentrated under reduced pressure below 50°C and homogeneity of the fractions determined by thin layer chromatography technique. The solvent systems used for elution are selected from hexane, hexane-dichloromethane (7:3), hexane-dichloromethane (6:4) and dichloromethane.

DETAILED DESCRIPTION OF THE INVENTION: a. ISOLATION OF NII-30:

The well characterized Piper longum fruits were dried, macerated by solvent extraction (MeOH:water 9:1) in a 10 litre capacity aspirator. The extract was drained out after 24 hours and fresh solvent was added and the process was repeated about five times. The extract was then concentrated below 50°C under reduced pressure, in a rotary evaporator. The concentrated extract (10%) of the total weight of the dried starting plant material) was solvent fractionated into hydrocarbon solvent (petrleum ether fraction boiling point 40-60 and 60 to 80°C), chlorinated hydrocarbons such as dichloromethane and chloroform, ethyl acetate and water soluble fractions respectively. The biologically active fraction amongst all the fractions made was further purified by purifying the biologically active fraction by chromatographic methods on normal phase reverse phase silica gel columns. The normal phase silica gel column chromatography of active fraction with hexane, hexane-dichloromethane (7:3), hexane-dichloromethane (6:4) and dichloromethane, respectively. The fractions were concentrated under reduced pressure and homogeneity of the compounds

determined by thin layer chromatography of fractions in different solvent systems. The repeated chromatography of biologically active fraction led to purification of four pure compounds which were separately screened for immunomodulatoiA activities. This led to identification of NII-30 as the most active pure compound. Total 70 mg (0116 %) could be isolated from one kilogram dry weight of the plnni material. The NII-30 was recrystallised from ethanol-hexane (9.9:1), mp 34-3ftV.

STRUCTURAL CHARACTERISATION

NII-30 is a low melting solid, mp 34-36 °C with molecular formula C21H2O2 as determined by mass spectormetric data(M= at m/z 316) The UV : y max (EtOH) 215, 220,260 and 305 nm which indicated the presence of an aromatic ring in the compound. The IR max cm-1 3019, 2937,2953,1550,1540,1200, 798 and 750 were the typical characteristic absorption bands of an aromatic moiety and it was clearly devoid of any free phenolic or carboxylic group. The 1H NMR (300 mhz] spectrum of the NII-30 exhibited typical charateristic peak of a methylen-dioxy group in a benzene ring at 5 5.89 (2H, s), two olefinic protons at 8 6.45 and 6.01 (each 1H, d, J = 16 Hz), three aromatic protons at 6 6.858 (1H, d, J-1.5 Hz) and 6.7 (2H, ill resolved multiple:). These chemical, shifts altogether indicated that the compound contains one central benzene ring, two positions of which are substituted by a methylene dioxy group and one of the two meta positions of tins group is associated with an alkenyl side chain. The coupling constant value (J = l6

HZ) confirmed the trans orientation of the olefinic double bond. In the upfiied

region peak at 8 0.85 (3H, apparent t,) and 6 2.125 (2H, q) showed a ethyl moiety

attached with a saturated hydrocarbon long chain . A broad signal at 8 1.21 (18H. m) and S 1.39 (2H, m) also supported the presence of the long hydrocarbon chain. but the actual length of the chain can only be confirmed by the mass spectral

studies and chemical synthesis of the compound. The El mass spectrum of the compound showed the molecular ion peak at m/z 316, It also showed intense peaks at m/z 288,161,135,131 and 103 which corresponds to the following ion fragments. The above mass spectral data clearly suggests the presence of a 3.4 methylenedioxy-phenyl moiety (C-7 unit) conjugated to an alkenyl side chain (C-14). The most convincing evidence in favour of the structure was obtained by NMR studies and DEPT experiment of the compound. The three quatemaiy carbons at dc 148.0, 146,5, and 133.0 could be

attributed to C-3, C-4, and (C1 respectively. Out of the five protonated carbon atoms, three aromatic carbons appeared at dc 105.3, 108.2 and 120.1 corresponding to C-2, C-5 and C-6 and two olefinic carbons at 8c 129.5 and 129.0 could be placed at C-1' and C-2' respectively. A sharp methylinic carbon at 8c 101 confirmed the presence of methylenedioxy group and dc 23.5 (CH2) and 14.5 (CH3) suggested a terminal ethyl group in the side chain. The proton and carbon chemical shifts assignments were unambiguously confirmed by two-dimensional homo- and heteronuclear correlation NMR experiments. The spectral data discussed led to structure of NII-30 as 1- (3,4-methylenedioxy-phenyl)- IE- tetradecene (1).

Synthesis of NII-30 and its analogues:

Keeping in view very limited amounts that could be isolated from Piper longum and pressing requirement for various in-vitro and in-vivo immunomodulatory. antitumor and anti-infective activities, the applicants decided to chemically synthesise NII-30 and its analogues from commercially available and cheap starting materials. The other objective of developing synthetic method was to enable us with" the strategy for synthesis of non-natural cis- stereoisomer of NII-30 and modified analogues with enhanced biological activity, solubility and bioavailability. Therefore, a new high yielding Witting olefination based chemical

synthesis was successfully carried out using piperonal (heliotropin. 14-methylenedioxybenzaldehyde) and 1-tridecanol. This synthetic strategy is suitable for synthesis of NII-30 and analogues having varying hydrocarbon chain length and structural and stereochemical modification at olefin and phenyl ring. The Osmium tetraoxide/Sodium chlorate and Os04/N-methyhnorpholinoxide mediate I cis-dihydroxylation of olefinic bond of NII-30 led to synthesis of l-(3.4-methylenedioxyphenyl)-l,2-dmydroxy-tetradecane. In order to prepare water soluble analogues of NII-30, synthetic l-(3,4-methylenedioxyphenyl)-l.2-dihydroxy-tetradecane was monoglycosylated at benzylic hydroxyl position wirh various tetra-acetylglycosyl halides under Koeing-Knorr coupling conditions followed by deacetylation to provide water .soluble glycosides of NII-30. Tins strategy could be used for preparation of various hexose and pentose 0-glycosides.

Formulation:

NII-30 can be used alone or in combination with any other immunomodulaioi or anti-tumor/antibiotic compound. Compound can also be combined with pharmaceutically acceptable additives. The composition comprising NII-30 and pharmaceutically acceptable additives show synergistic properties. In other word the composition comprising NII-30 and pharmaceutically acceptable additives is a synergistic admixture and such synergistic composition can be administered orally or injected i.e., s.c. or i.v. or can be applied topically in the form of powder, cream, jelly or spray.

Examples of Immunotherapeutic applications:

The present invention is illustrated by way of the following experimental studies and such experiments should not be construed as limiting the scope of the

invention. P-815 tumor implant in DBA/2J mice was used as an animal model this invention. P-815 tumor is known for its sensitivity to cytotoxic killing b> activated lymphocytes and macrophages.

Brief of the accompanying drawings

Fig. 1 shows the result of the treatment with NII-30 significantly arresting the tumor growth.

Fig. 2 shows the anti-tumor effect of NII-30 on the immuno-competent cells. Fig.3 shows the improved bioactivity and bioavailability of synthetic NII-30.

Example 1

Inbred DBA/2J mice were inoculated sub-cutaneously with P-815 tumor cells (4 X 106 at a single site) on day-0 and animals were treated daily or on alternate days with NII-30, with a dose range of 0.1 to 10 mg/kg body weight (preferably 1 mg kig body weight) starring day 1 post-tumor inoculation. Treatment in each group was given daily for 21 days and the tumor size was recorded at weekly intervals. The results showed that treatment with NII-30 significantly arrested the tumor growth (Fig. 1).

Example 2

Inbred DBA/2J mice were treated with NII-30, at a dose of 1mg/kg body weight) for 7 days. On day 8, splenocytes were removed from these animals and were injected intravenously into DBA/IT mice inoculated sub-cutaneously with P-815

tumor cells. No treatment was given to tumor bearing mice. The results showed that adoptive transfer of splenocytes from NII-30 treated mice to tumor-bearing mice caused significant reduction in tumor size, indicating that the anti-tumor effect of NII-30 mediated by the immuno-competent cells (Fig.2).
Example 3
P-815 tumor cells were cultured in vitro in the presence of various concentration of NII-30. After 48 hours. MTT assay was carried out and the OD was recorded. Results showed that NII-30, even at a dose of 50 ug/ml did not significantly affect the viability and metabolic activity of P-815 tumor cells (Fig. 3), whereas the effective dose of this compound in animals is only 20 ug-animal per day.

We claim:
1. A novel biologically active immunomodulatory compound for treating cancer having the molecular formula C21H3O2, said compound having improved bioactivity, bioavailability and solubility properties.
2. A novel biologically active compound as claimed in claim 1 wherein the compound is isolated from Piper longum species.
3. A novel biologically active compound substantially as herein described with reference to the drawings and examples.

Documents:

http://ipindiaonline.gov.in/documents/911-DEL-2002/911-del-2002-abstract.pdf
(http://ipindiaonline.gov.in/documents/911-DEL-2002/911-del-2002-abstract.pdf)

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« Previous Patent (http://www.allindianpatents.com/patents/210536)
Next Patent » (http://www.allindianpatents.com/patents/210538)

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