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(54) Title: PHARMACEUTICAL COMPOSITIONS AND METHODS

(57) Abstract: Pharmaceutical compositions comprising a compound of Formula (I) or a pharmaceutically acceptable salt thereof: wherein R is H or COC(CH₃)NH₂, for modulating one or more cytokines such as: Tumor necrosis factor- α (TNF- α), Interleukin-1 (IL-1), Interleukin-6 (IL-6), Interleukin-8 (IL-8), Interleukin-10 (IL-10), Interleukin-1 β (IL-1 β), Interferon-g (IFN-g), and Granulocyte-macrophage colony stimulating factor (GM-CSF), and methods for modulating one or more cytokines and their use for modulating one or more cytokines is disclosed.

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PHARMACEUTICAL COMPOSITIONS AND METHODS

RELATED PATENT APPLICATIONS

This application claims the priority to and benefit of Indian Provisional Patent Application No. 201821028333 filed on July 27, 2018; the disclosures of which are incorporated herein by reference in its entirety as if fully rewritten herein.

FIELD OF THE INVENTION

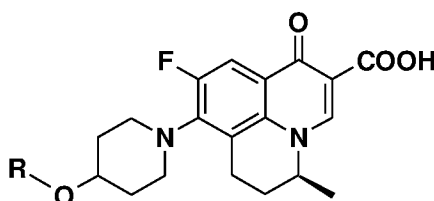
The invention relates to pharmaceutical compositions and their use in modulating one or more cytokines.

BACKGROUND OF THE INVENTION

Cytokines are important biological molecules that are involved in several biological functions. Cytokines are small proteins, and particularly play important role in the immune system. Cytokines may include chemokines, interferons, interleukins, lymphokines, and tumour necrosis factors. The modulation of cytokines, for example by increasing or decreasing their levels, can help in achieving outcome of many intended treatments (e.g. infections, allergies, inflammation).

SUMMARY OF THE INVENTION

Accordingly, there are provided pharmaceutical compositions for modulating one or more cytokines, said compositions comprising a compound of Formula (I) or a pharmaceutically acceptable salt thereof:

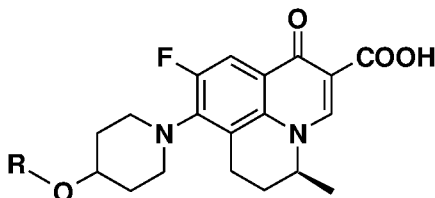


wherein R is H or $\text{COC}(\text{CH}_3)\text{NH}_2$.

In another aspect, there is provided a method for modulating one or more cytokines in a subject, said method comprising administering to the subject a

pharmaceutical composition comprising a compound of Formula (I) or a pharmaceutically acceptable salt thereof.

In another general aspect, there is provided a method for modulating one or more cytokines in a subject, said method comprising administering to the subject a compound of Formula (I) or a pharmaceutically acceptable salt thereof:



wherein R is H or $\text{COC}(\text{CH}_3)\text{NH}_2$.

The details of one or more embodiments of the invention are set forth in the description below. Other features, objects and advantages of the invention will be apparent from the following description including claims.

DETAILED DESCRIPTION OF THE INVENTION

Reference will now be made to the exemplary embodiments, and specific language will be used herein to describe the same. It should nevertheless be understood that no limitation of the scope of the invention is thereby intended. Alterations and further modifications of the inventive features illustrated herein, and additional applications of the principles of the invention as illustrated herein, which would occur to one of ordinary skills in the relevant art and having possession of this disclosure, are to be considered within the scope of the invention. It must be noted that, as used in this specification and the appended claims, the singular forms “a”, “an” and “the” include plural referents unless the content clearly dictates otherwise. All references including patents, patent applications, and literature cited in the specification are expressly incorporated herein by reference in their entirety.

The inventors have surprisingly discovered that certain compounds exhibit ability to modulate cytokines.

The term “pharmaceutically acceptable salt” as used herein refers to one or more salts of a given compound which possesses the desired pharmacological activity of the free compound and which are neither biologically nor otherwise undesirable. In general, the “pharmaceutically acceptable salts” refer to and include those salts that are suitable for use in contact with the tissues of human and animals without undue toxicity, irritation, allergic response and the like, and

are commensurate with a reasonable benefit/risk ratio. Pharmaceutically acceptable salts are well known in the art. For example, S. M. Berge, et al. (J. Pharmaceutical Sciences, 66: 1-19 (1977)), incorporated herein by reference in its entirety, describes various pharmaceutically acceptable salts in details.

The term “treat”, “treating” or “treatment” as used herein refers to administering a medicament, including a pharmaceutical composition, or one or more pharmaceutically active ingredients, for prophylactic and/or therapeutic purposes.

The term “pharmaceutically effective amount” or “therapeutically effective amount” or “effective amount” as used herein refers to an amount, which has a therapeutic effect or is the amount required to produce a therapeutic effect in a subject. The compounds and/or pharmaceutical compositions according to the invention are used in amounts that are effective in providing the desired therapeutic effect or result.

The term “administration” or “administering” includes delivery of a composition or one or more pharmaceutically active ingredients to a subject, including for example, by any appropriate methods, which serves to deliver the composition or its active ingredients or other pharmaceutically active ingredients to the desired site of action. The method of administration may vary depending on various factors, such as for example, the components of the pharmaceutical composition, nature of the pharmaceutically active or inert ingredients, the desired site of action, age and physical condition of the subject and a like. Some non-limiting examples of ways to administer a composition or a pharmaceutically active ingredient to a subject according to this invention includes oral, intravenous, topical, intra-respiratory, intra-peritoneal, intra-muscular, parenteral, sublingual, transdermal, intranasal, aerosol, intra-ocular, intra-tracheal, intra-rectal, vaginal, gene gun, dermal patch, eye drop, ear drop or mouthwash.

The term “synergistic” or “synergy” as used herein refers to the interaction of two or more agents so that their combined effect is greater than their individual effects.

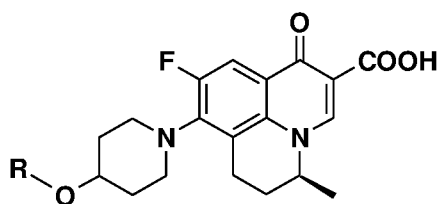
The term “pharmaceutically inert ingredient” or “carrier” or “excipient” refers to a compound or material used to facilitate administration of a compound, including for example, to increase the solubility of the compound. Typical, non-limiting examples of solid carriers include, starch, lactose, dicalcium phosphate, sucrose, and kaolin and so on. Typical, non-limiting examples of liquid carriers include sterile water, saline, buffers, non-ionic surfactants, and edible oils such as oil, peanut and sesame oils and so on. In addition, various adjuvants commonly used in the art may be included. These and other such compounds are described in the literature, for example, in the Merck Index (Merck & Company, Rahway, N.J.). Considerations for inclusion of various components in pharmaceutical compositions are described, for example, in Gilman et al. (Eds.) (1990); Goodman

and Gilman's: The Pharmacological Basis of Therapeutics, 8th Ed., Pergamon Press., which is incorporated herein by reference in its entirety.

The term “subject” as used herein refers to a vertebrate or invertebrate, including a mammal. The term “subject” includes human, animal, a bird, a fish, or an amphibian. Typical, non-limiting examples of a “subject” includes humans, cats, dogs, horses, sheep, bovine cows, pigs, lambs, rats, mice and guinea pigs.

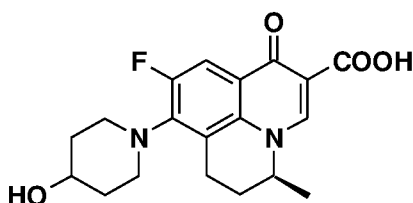
The term “modulating” or “modulation” of cytokines refers to and includes increasing or decreasing level of cytokines. The term “modulating” or “modulation” also refers to and includes stimulating cytokines.

In one general aspect, there are provided pharmaceutical compositions for modulating one or more cytokines, said composition comprising a compound of Formula (I) or a pharmaceutically acceptable salt thereof:

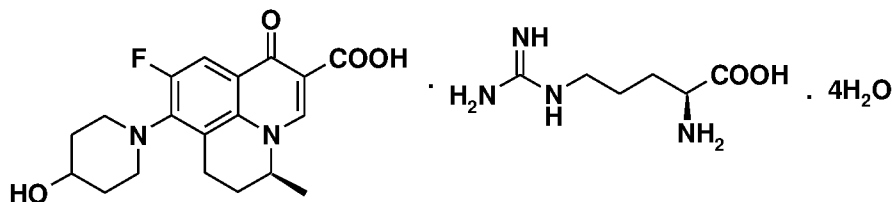


wherein R is H or $\text{COC}(\text{CH}_3)\text{NH}_2$.

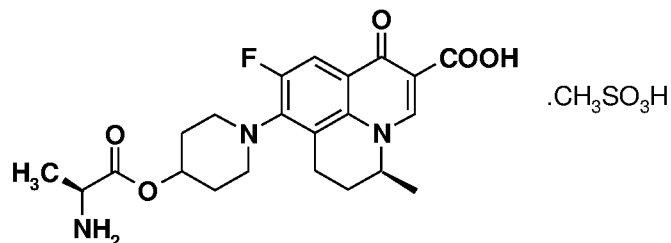
In some embodiments, the compound of Formula (I) is:



In some other embodiments, the compound of Formula (I) is:



In some other embodiments, the compound of Formula (I) is:



In some embodiments, the pharmaceutical composition comprising a compound of Formula (I) or a pharmaceutically acceptable salt thereof, for use in modulating one or more cytokines in a subject.

In some embodiments, the cytokine is one or more of Tumor necrosis factor- α (TNF- α), Interleukin-1 (IL-1), Interleukin-6 (IL-6), Interleukin-8 (IL-8), Interleukin-10 (IL-10), Interleukin-1 β (IL-1 β), Interferon-g (IFN-g), and Granulocyte-macrophage colony stimulating factor (GM-CSF).

The pharmaceutical compositions according to the invention may include one or more pharmaceutically acceptable carriers or excipients or a like. Typical, non-limiting examples of such carriers or excipients include mannitol, lactose, starch, magnesium stearate, sodium saccharine, talcum, cellulose, sodium crosscarmellose, glucose, gelatine, sucrose, magnesium carbonate, wetting agents, emulsifying agents, solubilizing agents, pH buffering agents, lubricants, stabilizing agents, binding agents and a like.

The pharmaceutical compositions according to this invention can exist in various forms. In some embodiments, the pharmaceutical composition is in the form of a powder or a solution. In some other embodiments, the pharmaceutical compositions according to the invention are in the form of a powder that can be reconstituted by addition of a compatible reconstitution diluent prior to parenteral administration. Non-limiting example of such a compatible reconstitution diluent includes water.

In some other embodiments, the pharmaceutical compositions according to the invention are in the form of a frozen composition that can be diluted with a compatible diluent prior to parenteral administration.

In some other embodiments, the pharmaceutical compositions according to the invention are in the form ready to use for parenteral administration.

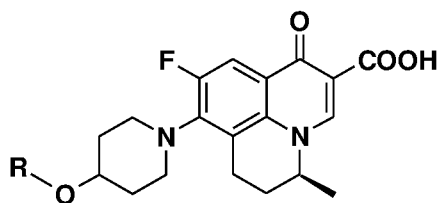
The pharmaceutical composition or the active ingredients according to the present invention may be formulated into a variety of dosage forms. Typical, non-limiting examples of dosage forms include solid, semi-solid, liquid and aerosol dosage forms; such as tablets, capsules, powders, solutions, suspensions, suppositories, aerosols, granules, emulsions, syrups, elixirs and a like.

In the methods according to the invention, the pharmaceutical composition and/or other pharmaceutically active ingredients disclosed herein may be administered by any appropriate method, which serves to deliver the composition or its constituents or the active ingredients to the desired site. The method of administration can vary depending on various factors, such as for example, the components of the pharmaceutical composition, nature of the active ingredients, age and physical condition of the subject. Some non-limiting examples of administering the composition to a subject according to this invention include oral, intravenous, topical, intra-respiratory, intra-peritoneal, intra-muscular, parenteral, sublingual, transdermal, intranasal, aerosol, intraocular, intra-tracheal, intra-rectal, vaginal, gene gun, dermal patch, eye drop, ear drop or mouthwash.

The compositions according to the invention can be formulated into various dosage forms wherein the active ingredients and/or excipients may be present either together (e.g. as an admixture) or as separate components. When the various ingredients in the composition are formulated as a mixture, such composition can be delivered by administering such a mixture. The composition or dosage form wherein the ingredients do not come as a mixture, but come as separate components, such composition/dosage form may be administered in several ways. In one possible way, the ingredients may be mixed in the desired proportions and the mixture is then administered as required. Alternatively, the components or the ingredients (active or inert) may be separately administered (simultaneously or one after the other) in appropriate proportion so as to achieve the same or equivalent therapeutic level or effect as would have been achieved by administration of the equivalent mixture.

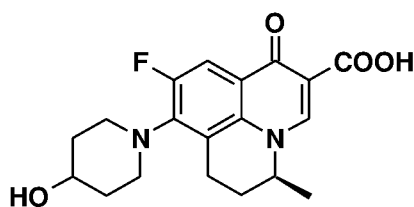
In another general aspect, there are provided methods for modulating one or more cytokines in a subject, said method comprising administering to the subject a pharmaceutical composition disclosed herein.

In another general aspect, there are provided methods for modulating one or more cytokines in a subject, said method comprising administering to the subject a compound of Formula (I) or a pharmaceutically acceptable salt thereof:

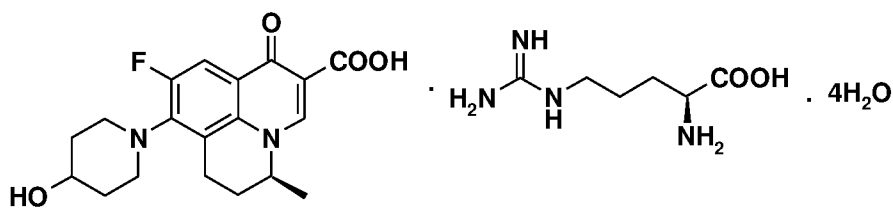


wherein R is H or $\text{COC}(\text{CH}_3)\text{NH}_2$.

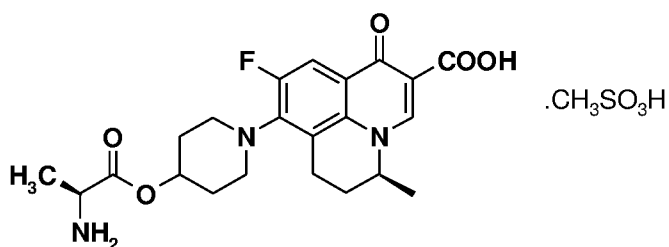
In some embodiments, the compound used in the methods according to the invention is selected from the following:



;



; or



In the methods according to the invention, the active ingredients disclosed herein may be administered to a subject in several ways depending on the requirements. In some embodiments, the active ingredients are admixed in appropriate amounts and then the admixture is administered to a subject. In some other embodiments, the active ingredients are administered separately. Since the invention contemplates that the active ingredients agents may be administered separately, the invention further provides for combining separate pharmaceutical compositions in kit form. The kit may comprise one or more separate pharmaceutical compositions, each comprising one or more active ingredients. Each of such separate compositions may be present in a separate container such as a bottle, vial, syringes, boxes, bags, and the like. Typically, the kit comprises

directions for the administration of the separate components. The kit form is particularly advantageous when the separate components are preferably administered in different dosage forms (e.g., oral and parenteral) or are administered at different dosage intervals. When the active ingredients are administered separately, they may be administered simultaneously or sequentially.

It will be readily apparent to one skilled in the art that varying substitutions and modifications may be made to the invention disclosed herein without departing from the scope and spirit of the invention. For example, those skilled in the art will recognize that the invention may be practiced using a variety of different compounds within the described generic descriptions.

EXAMPLES

The following examples illustrate the embodiments of the invention that are presently best known. However, it is to be understood that the following are only exemplary or illustrative of the application of the principles of the present invention. Numerous modifications and alternative compositions, methods, and systems may be devised by those skilled in the art without departing from the spirit and scope of the present invention. The appended claims are intended to cover such modifications and arrangements. Thus, while the present invention has been described above with particularity, the following examples provide further detail in connection with what are presently deemed to be the most practical and preferred embodiments of the invention.

Experimental Procedure

LPS induced Ex-Vivo cytokine secretion in whole blood:

The activity of test compounds in modulating one or more cytokines was evaluated using a whole blood cytokine secretion assay, using the following general procedure. Peripheral venous blood was collected with consent from healthy adult volunteers between 25 to 40 years of age and stored in tubes containing heparin. All volunteers confirmed that they did not take any concomitant medication within the last 2 weeks prior to the start of study and also that they had no known hypersensitivity to any antibacterial drugs. All blood collections were in accordance to protocols approved by the Institutional Review Board of Wockhardt Ltd, India. Written informed consent was obtained from study participants. Lipopolysaccharide (LPS) (Sigma, USA) was used as stimulating agent for immune cells. Quantikine ELISA human cytokine kits were

purchased from, R & D Systems, Inc, USA. Test compounds were prepared using known literature procedures.

LPS in concentration of 10 ng/mL was used to study the release of IL-6, TNF- α , and IL-1 β , whereas, 50 ng/mL was used for the GM-CSF. Heparinized whole blood samples (received from the human donor) were kept at room temperature and processed within 2 hrs of collection. Blood was diluted in 1:3 ratio with sterile RPMI 1640 and L-glutamine, and 25 mM HEPES (Gibco, Life Technologies; USA). A final volume of 1 mL was added to each well of 24-well tissue culture treated flat-bottom polystyrene plates (Eppendorf, Germany). The experimental plates were then incubated for 6 h except for GM-CSF release assay (incubated for 48 h) at 37 °C in a humidified incubator at 5% CO₂. Samples were collected at 6 h and 48 h time point from respective wells, centrifuged at 8000 rpm for 8 minutes at 4°C, and the supernatant was stored at -80°C until analysis.

The supernatants were analysed for IL-6, TNF- α , IL-1 β and GM-CSF by ELISA assay. The assay was performed according to the manufacturer's instructions (R & D Systems, Inc, USA) and analysed with the BioTek 96 well plate reader software.

Supernatant cytokine concentrations of samples treated with test compounds were expressed as absolute levels and percentage compared to cytokine concentrations induced by LPS alone, which were defined as 100%.

LPS induced In-Vivo cytokine production in rat:

Male Wistar rats, 10-12 weeks of age were received from the Animal House Facility, Wockhardt Research Centre, Aurangabad, India. The animals were housed in individually ventilated cages at a room temperature of 22 $\pm$ 2 °C, humidity 60 $\pm$ 10% with a 12:12 h light-dark cycle and have access to food and water ad-libitum. Animals were acclimatised at least for a week before initiation of the study. They were fasted overnight before the start of an experiment. During fasting and throughout the study, animals were kept on fasting grills not more than four animals/cages with free access to water throughout the study period. Following fasting, the blood was withdrawn from a retro-orbital puncture in micro-centrifuge tubes containing K2EDTA as anticoagulants at the concentration of 1.75 mg/mL of blood. LPS was administered at 5 (for TNF- α , IL-6, IL-1 β) and 12.5 (for GM-CSF) mg/kg as a single intraperitoneal dose for sepsis induction. Test (Drug) compound and placebo were administered subcutaneously 30 min before the LPS administration. Blood samples were collected at 0 h (before LPS), 2 and 6 h after LPS administration, centrifuged and plasma stored at -80°C until analysis.

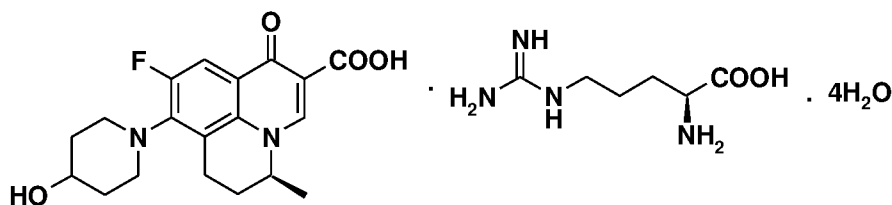
The samples were analysed for IL-6, TNF- α , IL-1 β and GM-CSF by ELISA assay. The assay was performed according to the manufacturer's instructions (R & D Systems, Inc, USA) and analysed with the BioTek 96 well plate reader software

Data Analysis:

Statistical analysis was performed using One-Way ANOVA post-hoc Tukey's test for the comparison of ex-vivo data (% inhibition of cytokines) in more than two groups. Two-Way ANOVA post-hoc Bonferroni's multiple comparison test was applied for the comparison of in-vivo study data. Paired t-test was used to compare the mean differences in ex-vivo absolute cytokine levels between two groups. The data obtained was expressed at a significance level of $p < 0.05$. All data were presented as mean $\pm$ SEM. All statistical analyses were carried out using GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego, CA, USA).

Example 1

Activity of test **Compound A**, Levofloxacin (LVFX) and Linezolid (LNZ) in modulating Lipopolysaccharide (LPS)-simulated cytokine release in human whole blood (HWB) assay and in-vivo in rat was evaluated using the above experimental procedure. In general, the activity was evaluated with respect to IL-6, TNF- α , IL-1 β and GM-CSF. The results are presented in Table 1-5, below.



Compound A

Table 1. Effect of Compound A, Levofloxacin (LVFX) and Linezolid (LNZ) on pro-inflammatory cytokines (IL-6, TNF- α , IL-1 β , GM-CSF) release in LPS-stimulated HWB								
Treatment	IL-6 (pg/mL)		TNF - α (pg/mL)		IL-1 β (pg/mL)		GM-CSF (pg/mL)	
	Absolute levels	% inhibition	Absolute levels	% inhibition	Absolute levels	% inhibition	Absolute levels	% inhibition
LPS + Dextrose	11170.5 $\pm$ 1773.1	NA	4371.6 $\pm$ 743.0	NA	4414.8 $\pm$ 892.6	NA	15.4 $\pm$ 4.0	NA
Levofloxacin (25 μ g/mL)	9093.7 $\pm$ 1684.6**	19.4 $\pm$ 3.6	3393.7 $\pm$ 659.7***	23.4 $\pm$ 1.9	1964.5 $\pm$ 506.1**	57.2 $\pm$ 3.6 ^{##}	11.7 $\pm$ 3.7	25.4 $\pm$ 9.2
Levofloxacin (100 μ g/mL)	5209.5 $\pm$ 1236.7***	55.0 $\pm$ 3.2 ⁺⁺⁺ ###, \$\$\$	1986.9 $\pm$ 456.0**	56.0 $\pm$ 2.6 ⁺⁺⁺ , \$\$\$	214.3 $\pm$ 90.9**	95.6 $\pm$ 0.9 ⁺⁺⁺ , ###, \$\$\$, @@@	4.5 $\pm$ 1.3*	68.6 $\pm$ 7.0 ⁺ , ##, \$\$
LPS + DMSO	11784.9 $\pm$ 1809.2	NA	4917.3 $\pm$ 1164.8	NA	6098.3 $\pm$ 1439.6	NA	19.4 $\pm$ 4.8	NA
Linezolid (200 μ g/mL)	9757.3 $\pm$ 1856.0***	18.6 $\pm$ 2.6	2509.0 $\pm$ 668.3**	50.0 $\pm$ 1.8 ⁺⁺⁺ , \$\$\$	3546.0 $\pm$ 804.3*	40.8 $\pm$ 2.8	17.5 $\pm$ 5.0	10.6 $\pm$ 8.8
Lipopolysaccharide (LPS)	10446.9 $\pm$ 1657.8	NA	4402.6 $\pm$ 743.7	NA	4182.0 $\pm$ 815.9	NA	14.4 $\pm$ 3.8	NA
Compound A (15 μ g/mL)	8430.3 $\pm$ 1746.7***	21.2 $\pm$ 3.1	3146.9 $\pm$ 86.2**	30.0 $\pm$ 4.6	2615.2 $\pm$ 67.3**	38.0 $\pm$ 2.1	10.8 $\pm$ 2.5	18.6 $\pm$ 6.6
Compound A (30 μ g/mL)	5714.7 $\pm$ 1415.4***	47.6 $\pm$ 3.8 ⁺⁺⁺ , ###, \$\$\$	2183.8 $\pm$ 465.4**	51.6 $\pm$ 2.3 ⁺⁺⁺ , \$\$\$	1438.9 $\pm$ 392.7**	67.0 $\pm$ 2.8 ^{\$\$\$} , ###	9.2 $\pm$ 2.3*	33.6 $\pm$ 5.9

*p<0.05, ***p<0.001 vs. Levofloxacin (25 μ g/mL), ##p<0.01, ###p<0.001 vs. Linezolid, \$\$p<0.01, \$\$\$p<0.001 vs. Compound A (15 μ g/mL), @@@ vs. Compound A (30 μ g/mL) by One Way ANOVA post-hoc Tukey's Multiple Comparison test; *p<0.05, **p<0.01, ***p<0.001 vs. respective control group calculated by paired t-test. LPS (lipopolysaccharide), IL-6 (interleukin-6), TNF- α (tumour necrosis factor - α), IL-1 β (interleukin-1 β), GM-CSF (granulocyte-macrophage-colony-stimulating factor)

Effect on IL-6:

Compound A 15 and 30 μ g/mL concentration showed significant reduction in IL-6 levels 8430.3 $\pm$ 1746.7 pg/mL (p<0.001) and 5714.7 $\pm$ 1415.4 pg/mL (p<0.001) as compared to LPS control (10446.9 $\pm$ 1657.8 pg/mL) in HWB assay, respectively. Similarly, Levofloxacin (25,100 μ g/mL) and Linezolid (200 μ g/mL) demonstrated significant reduction in IL-6 levels (p<0.001) as compared to respective control, respectively.

Compound A caused 21.2 $\pm$ 3.1% and 47.6 $\pm$ 3.8% inhibition of IL-6 release at 15 and 30 μ g/mL, respectively, while Levofloxacin showed 19.4 $\pm$ 3.6% and 55 $\pm$ 3.2% inhibition of IL-6 release at 25 and 100 μ g/ml concentrations, respectively. Thus, Compound A was found to be almost three times more effective compared to Levofloxacin. Linezolid at 200 μ g/mL concentration

demonstrated $18.6 \pm 2.6\%$ inhibition of IL-6 release. These results indicate that Compound A is 10 times more effective compared to Linezolid in inhibiting IL-6 release ($p < 0.001$, Table 1). Compound A and Levofloxacin inhibited the production of IL-6 in a dose-dependent fashion.

Effect on TNF- α :

Compound A at doses 15 and 30 $\mu\text{g/mL}$ caused significant reduction in TNF- α levels 3146.9 ± 686.2 pg/mL ($p < 0.01$) and 2183.8 ± 465.4 pg/mL ($p < 0.01$) as compared to LPS control (4402.6 ± 743.7 pg/mL) in HWB assay, respectively. Similarly, Levofloxacin (25 and 100 $\mu\text{g/mL}$) and Linezolid (200 $\mu\text{g/mL}$) showed significant reduction in TNF- α levels as compared to control, respectively.

Compound A at 15 and 30 $\mu\text{g/mL}$ demonstrated $30 \pm 4.6\%$ and $51.6 \pm 2.3\%$ inhibition, whereas, Levofloxacin at 25 and 100 $\mu\text{g/mL}$ concentrations showed $23.4 \pm 1.9\%$ and $56 \pm 3\%$ inhibition, respectively. Linezolid at 200 $\mu\text{g/mL}$ concentration demonstrated $50 \pm 2\%$ inhibition of TNF- α release. Overall, Compound A was found to be three times more effective than Levofloxacin and six times more effective than Linezolid in inhibiting TNF- α release (Figure 1). Compound A and Levofloxacin inhibited the production of TNF- α in a dose-dependent fashion.

Effect on IL-1 β :

Compound A (15 and 30 $\mu\text{g/mL}$) demonstrated significant reduction in IL-1 β levels ($p < 0.01$) compared to LPS control. Similarly, Levofloxacin and Linezolid showed significant reduction in IL-1 β levels ($p < 0.01$) as compared to respective control. Compound A showed $38 \pm 2.1\%$ and $67 \pm 2.8\%$ inhibition of IL-1 β release at 15 and 30 $\mu\text{g/mL}$ concentrations, respectively. Levofloxacin exhibited $57.2 \pm 3.6\%$ and $95.6 \pm 0.9\%$ inhibition of IL-1 β release at 25 and 100 $\mu\text{g/mL}$ concentrations, respectively. Linezolid at 200 $\mu\text{g/mL}$ concentration demonstrated $40.8 \pm 2.8\%$ inhibition of IL-1 β release. Thus, Compound A was 10 times more effective compared to Linezolid (Table 1).

Effect on GM-CSF:

Compound A (30 $\mu\text{g/mL}$) demonstrated significant reduction in GM-CSF levels compared to LPS control (9.2 vs. 14.4 pg/mL, $p < 0.05$). Similarly, Levofloxacin 100 $\mu\text{g/mL}$ showed significant reduction in GM-CSF levels compared to LPS+Dextrose control (4.5 vs. 15.4 pg/mL, $p < 0.05$). Compound A at 15 and 30 $\mu\text{g/mL}$ concentrations demonstrated $18.6 \pm 6.6\%$ and $33.6 \pm 5.9\%$ inhibition, whereas, Levofloxacin at 25 and 100 $\mu\text{g/mL}$ concentrations showed $25.4 \pm 9.2\%$ and $68.6 \pm 7\%$ inhibition, respectively. Linezolid at 200 $\mu\text{g/mL}$ concentration demonstrated $10.6 \pm 8.8\%$ inhibition of GM-CSF release. Overall, Compound A was found to be equally effective as Levofloxacin and twenty times more effective than Linezolid in inhibiting GM-CSF release (Table 1).

Table 2. Effect of Compound A and Levofloxacin on TNF-α release in LPS-induced sepsis rat model						
Treatment	Tumour necrosis factor-α (TNF-α)					
	Absolute levels (pg/mL)			% Inhibition		
	0 h	1 h	2 h	0 h	1 h	2 h
Placebo control	0.7 $\pm$ 0.3	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	-	-	-
LPS Control (5mg/kg)	1.3 $\pm$ 0.4	7827.0 $\pm$ 1906.7 ^{###}	11114.1 $\pm$ 4413.4 ^{###}	-	-	-
Levofloxacin 50 mg/kg	0.7 $\pm$ 0.1	5295.2 $\pm$ 1064.3	3641.9 $\pm$ 364.6 ^{***}	-	32.4 $\pm$ 13.6	67.2 $\pm$ 3.3
Compound A 50 mg/kg	0.5 $\pm$ 0.2	4668.1 $\pm$ 599.8	3765.4 $\pm$ 776.7 ^{***}	-	40.4 $\pm$ 7.7	66.1 $\pm$ 7.0

###p<0.001 vs. placebo control, ***p<0.001 vs. LPS control by Two Way ANOVA post-hoc Bonferroni's Multiple comparison test

Table 3. Effect of Compound A and Levofloxacin on IL-6 release in LPS-induced sepsis rat model						
Treatment	Interleukin 6 (IL-6)					
	Absolute levels (pg/mL)			% Inhibition		
	0 h	2 h	6 h	0 h	2 h	6 h
Placebo control	18.3 $\pm$ 3.2	288.2 $\pm$ 57.5	303.9 $\pm$ 111.1	-	-	-
LPS Control (5mg/kg)	21.6 $\pm$ 4.4	46990.2 $\pm$ 3096.1 ^{###}	73649.9 $\pm$ 12428.8 ^{###}	-	-	-
Levofloxacin 50 mg/kg	16.3 $\pm$ 2.1	42578.3 $\pm$ 4267.2	47330.9 $\pm$ 7501.7 ^{**}	-	9.4 $\pm$ 9.1	35.7 $\pm$ 10.2
Compound A 50 mg/kg	16.6 $\pm$ 3.0	36949.4 $\pm$ 3787.2	54128.6 $\pm$ 15871.1 [*]	-	21.4 $\pm$ 8.1	31.7 $\pm$ 18.5

###p<0.001 vs placebo control, *p<0.05, **p<0.01, vs LPS control by Two Way ANOVA post hoc Bonferroni's Multiple comparison test

Table 4. Effect of Compound A and Levofloxacin on IL-1β release in LPS-induced sepsis rat model						
Treatment	Interleukin-1β (IL-1β)					
	Absolute levels (pg/mL)			% Inhibition		
	0 h	2 h	6 h	0 h	2 h	6 h
Placebo control	14.4 $\pm$ 2.2	172.2 $\pm$ 49.8	214.4 $\pm$ 47.6	-	-	-
LPS Control (5mg/kg)	18.7 $\pm$ 6.8	1450.4 $\pm$ 85.3 ^{***}	1565.4 $\pm$ 232.1 ^{***}	-	-	-
Levofloxacin 50 mg/kg	12.0 $\pm$ 3.8	1241.1 $\pm$ 150.3	1288.2 $\pm$ 215.2	-	14.4 $\pm$ 10.4	17.7 $\pm$ 13.7
Compound A 50 mg/kg	21.4 $\pm$ 5.8	1388.8 $\pm$ 181.5	1402.6 $\pm$ 278.8	-	4.2 $\pm$ 12.5	10.4 $\pm$ 17.8

***p<0.001 vs placebo control by Two Way ANOVA post hoc Bonferroni's Multiple comparison test

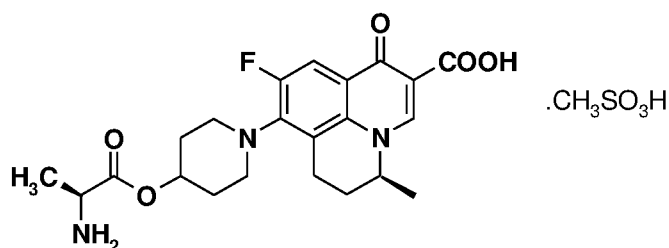
Table 5. Effect of Compound A and Levofloxacin on GM-CSF release in LPS-induced sepsis rat model.				
Treatment	Granulocyte-macrophage colony-stimulating factor (GM-CSF)			
	Absolute levels (pg/mL)		% Inhibition	
	0 h	2 h	0 h	2 h
Placebo control	0.0 ± 0.0	0.0 ± 0.0 ^{***}	-	-
LPS Control (12.5 mg/kg)	0.0 ± 0.0	3.4 ± 0.9 ^{###}	-	-
Levofloxacin 50 mg/kg	0.0 ± 0.0	1.2 ± 0.1 ^{***}	-	65.8 ± 2.3
Compound A 50 mg/kg	0.0 ± 0.0	1.1 ± 0.3 ^{**}	-	68.2 ± 7.2

###p<0.001 vs. placebo control, ***p<0.001 vs LPS control by Two Way ANOVA post hoc Bonferroni's Multiple comparison test.

In line with our ex-vivo observations, we found a selective down-modulation of TNF- α , IL-6 and GM-CSF levels in the course of endotoxin triggered sepsis, whereas, IL-1 β cytokine remained unaffected by the treatment (Table 2-5). Compound A and Levofloxacin showed significant reduction in TNF- α levels compared to LPS control at 2 h. Overall, Compound A was equally effective as Levofloxacin at 50 mg/kg in LPS-induced sepsis rat model (Table 2). Compound A and Levofloxacin demonstrated significant reduction in IL-6 levels 54128.6 pg/mL (p<0.05) and 47330.9 pg/mL (p<0.01) compared to LPS control (73649.9 pg/mL) at 6 h, respectively. The observed reductions in IL-6 levels were 31.7% and 35.7% in Compound A and Levofloxacin group, respectively. Thus, no significant difference between Compound A and Levofloxacin was observed on IL-6 production (Table 3). Compound A and Levofloxacin did not show significant reduction in IL-1 β levels compared to LPS control (Table 4). Significant reduction in GM-CSF levels were observed with Compound A and Levofloxacin (1.1 and 1.2 pg/mL, p<0.001) compared to LPS control (3.4 pg/mL) at 6h, respectively.

Example 2

Activity of test **Compound B** and Linezolid in modulating LPS-simulated cytokine release in whole blood assay was evaluated using the above experimental procedure. In general, the activity was evaluated with respect to Interleukin-6 (IL-6) and Tumor necrosis factor α (TNF- α). The results are presented in Table 6 and 7, below.



Compound B

Table 6. Effect of Compound B and Linezolid on Interleukin-6 (IL-6) release in whole blood assay after 6 hours of incubation

Treatment	Conc. ($\mu\text{g/mL}$)	IL-6 (n=1)	
		Absolute levels (pg/mL)	% Inhibition vs. positive control
LPS	0.01	5909	-
Compound B	25	2452	42
LPS + Dimethyl sulfoxide	0.01	7158	-
Linezolid	200	6721	6

As can be seen, Compound B showed significant inhibition of LPS-stimulated IL-6 release. The data also indicates that Compound B is more potent compared to Linezolid.

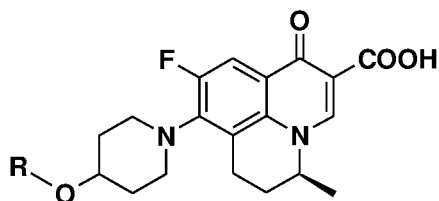
Table 7. Effect of Compound B and Linezolid on Tumor necrosis factor α (TNF- α) release in whole blood assay after 6 hours of incubation

Treatment	Conc. ($\mu\text{g/mL}$)	TNF- α (n=1)	
		Absolute levels (pg/mL)	% Inhibition vs. positive control
LPS	0.01	2579	-
Compound B	25	1267	51
LPS + Dimethyl sulfoxide	0.01	2996	-
Linezolid	200	2886	3.6

As can be seen, Compound B showed significant inhibition of LPS-stimulated TNF- α release. The data also indicates that Compound B is potent compared to Linezolid.

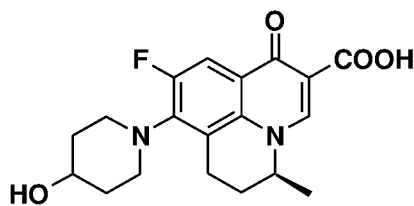
Claims

1. A pharmaceutical composition for modulating one or more cytokines, said composition comprising a compound of Formula (I) or a pharmaceutically acceptable salt thereof:

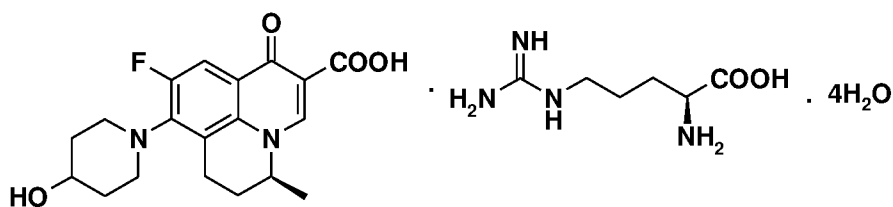


wherein R is H or $\text{COC}(\text{CH}_3)\text{NH}_2$.

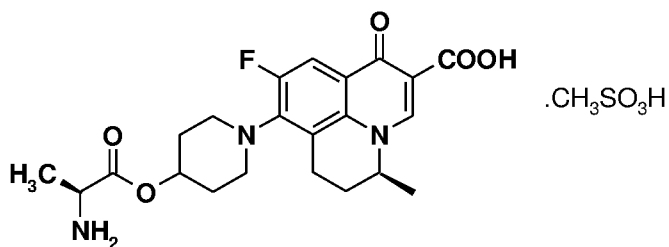
2. The pharmaceutical composition according to Claim 1, wherein the compound of formula (I) is selected from:



;



; or



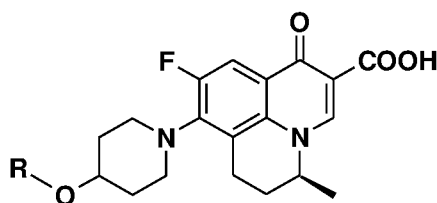
3. The pharmaceutical composition according to Claim 1, wherein the cytokine is one or more of Tumor necrosis factor- α (TNF- α), Interleukin-1 (IL-1), Interleukin-6 (IL-6), Interleukin-8 (IL-8), Interleukin-10 (IL-10), Interleukin-1 β

(IL-1 β), Interferon-g (IFN-g), and Granulocyte-macrophage colony stimulating factor (GM-CSF).

4. A method for modulating one or more cytokines in a subject, said method comprising administering to the subject a pharmaceutical composition according to any one of the Claims 1-2.

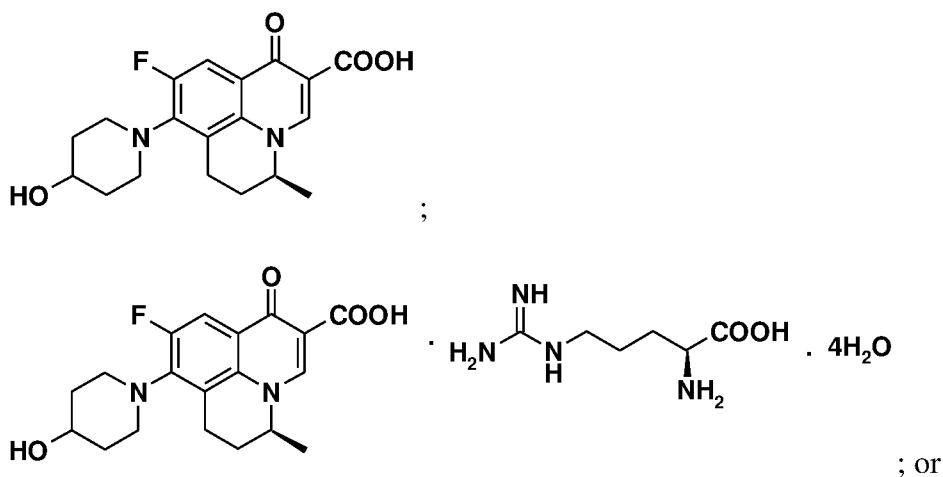
5. The method according to Claim 4, wherein the cytokine is one or more of Tumor necrosis factor- α (TNF- α), Interleukin-1 (IL-1), Interleukin-6 (IL-6), Interleukin-8 (IL-8), Interleukin-10 (IL-10), Interleukin-1 β (IL-1 β), Interferon-g (IFN-g), and Granulocyte-macrophage colony stimulating factor (GM-CSF).

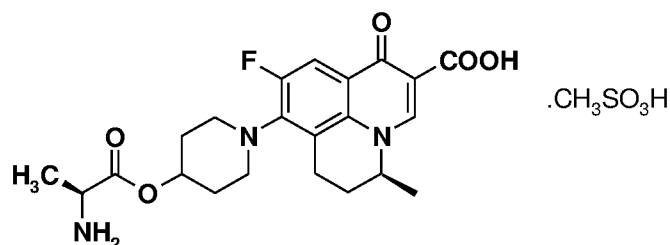
6. A method for modulating one or more cytokines in a subject, said method comprising administering to the subject a compound of Formula (I) or a pharmaceutically acceptable salt thereof:



wherein R is H or $\text{COC}(\text{CH}_3)\text{NH}_2$.

7. The method according to Claim 6, wherein the compound of formula (I) is selected from:





8. The method according to Claim 6 or 7, wherein the cytokine is one or more of Tumor necrosis factor- α (TNF- α), Interleukin-1 (IL-1), Interleukin-6 (IL-6), Interleukin-8 (IL-8), Interleukin-10 (IL-10), Interleukin-1 β (IL-1 β), Interferon-g (IFN-g), and Granulocyte-macrophage colony stimulating factor (GM-CSF).

9. The pharmaceutical composition according to any one of the claims 1-3, for use in modulating one or more cytokines in a subject, wherein the cytokine is one or more of Tumor necrosis factor- α (TNF- α), Interleukin-1 (IL-1), Interleukin-6 (IL-6), Interleukin-8 (IL-8), Interleukin-10 (IL-10), Interleukin-1 β (IL-1 β), Interferon-g (IFN-g), and Granulocyte-macrophage colony stimulating factor (GM-CSF).

10. The pharmaceutical composition for modulating one or more cytokines, comprising a compound of Formula (I) or a pharmaceutically acceptable salt thereof according to any one of claims 1 to 2 and one or more pharmaceutically acceptable excipients.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IN2019/050547

A. CLASSIFICATION OF SUBJECT MATTER
A61K9/00 Version=2019.01

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)

A61K 31/435

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)

TotalPatent One, IPO Internal Database, SureChEMBL Open Patent Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO/2016/181276 WOCHKHARDT LIMITED 17.11.2016 17 November 2016 [abstarct, page 2 para 2-5 , claims 1-2,10,11]	1-2,10
A	[abstarct, page 2 para 2-5 , claims 1-2,10,11] -----	3,9
X	WO/2008/053295 WOCHKHARDT RESEARCH CENTRE 08.05.2008 08 May 2008 [abstarct, page 4 lines5-30, claims 1-2,5-6,9]	1-2,10
A	[abstarct, page 4 lines5-30, claims 1-2,5-6,9]	3,9

☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"D" document cited by the applicant in the international application

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

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

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"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

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

21-10-2019

Date of mailing of the international search report

21-10-2019

Name and mailing address of the ISA/

Indian Patent Office
Plot No.32, Sector 14, Dwarka, New Delhi-110075
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Authorized officer

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/IN2019/050547

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 4-8
because they relate to subject matter not required to be searched by this Authority, namely:
The subject matter of claims 4-8 relates to a method for treatment of
the human by administering a pharmaceutical composition, which
does not require an international search by the International
Searching Authority in accordance with PCT Article 17(2)(a)(i) and
[Rule 39.1(iv)] 1.
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an
extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable
claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of
additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers
only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted
to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the
payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest
fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/IN2019/050547

Citation	Pub.Date	Family	Pub.Date
WO 2016/181276 A1	17-11-2016	AU 2016259897 A1	17-08-2017
		EP 3236965 A1	01-11-2017
		JP 2018510197 A	12-04-2018
		BR 112017016393 A2	27-03-2018