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CA 2871459 A1 2005/08/04

(21) 2 871 459

(12) DEMANDE DE BREVET CANADIEN
CANADIAN PATENT APPLICATION

(13) A1

(22) Date de dépôt/Filing Date: 2005/01/06

(41) Mise à la disp. pub./Open to Public Insp.: 2005/08/04

(62) Demande originale/Original Application: 2 547 963

(30) Priorité/Priority: 2004/01/12 (US10/754,547)

(51) Cl.Int./Int.Cl. *A61K 31/404* (2006.01),
A61P 29/00 (2006.01), *A61P 37/06* (2006.01)

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(54) Titre : PROCEDES PERMETTANT DE TRAITER UNE MALADIE DE TYPE INFLAMMATOIRE
(54) Title: METHODS OF TREATING AN INFLAMMATORY-RELATED DISEASE

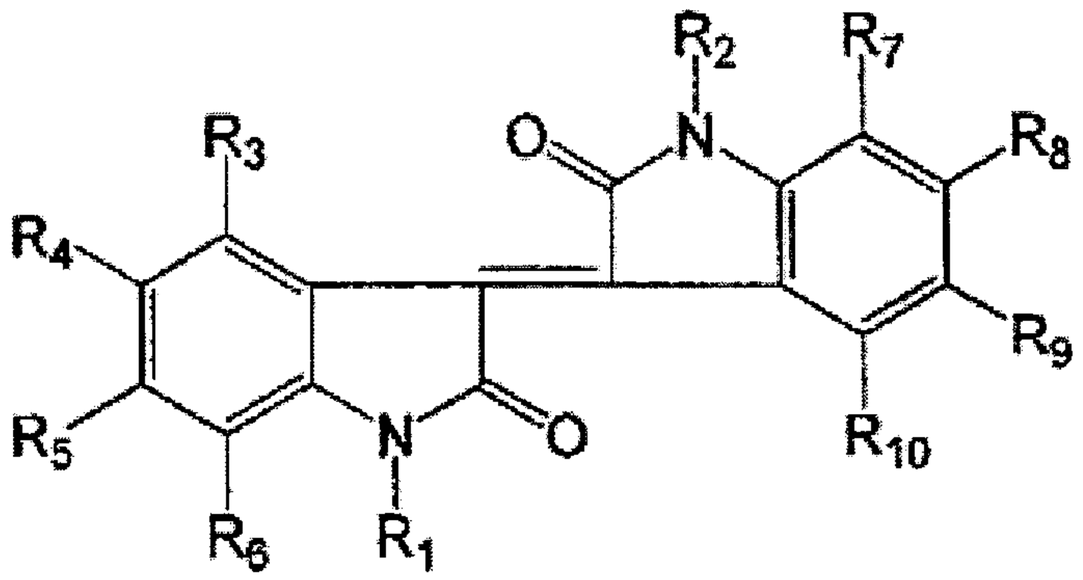
(57) Abrégé/Abstract:
The invention relates to pharmaceutical compositions and methods of treating inflammatory-related diseases associated with pro-inflammatory cytokine expression and/or reduced expression of anti-inflammatory cytokines. The method typically comprises administration of one or more compounds selected from isoindigo, indigo, indirubin, or derivatives thereof, such as, Meisoindigo and NATURA. Preferably the pharmaceutical composition comprises one or more compounds selected from isoindigo, indigo, indirubin, or derivatives thereof, an anti-inflammatory agent, and a pharmaceutically acceptable carrier.

Abstract

The invention relates to pharmaceutical compositions and methods of treating inflammatory-related diseases associated with pro-inflammatory cytokine expression and/or reduced expression of anti-inflammatory cytokines. The method typically comprises administration of one or more compounds selected from isoindigo, indigo, indirubin, or derivatives thereof, such as, Meisoindigo and NATURA. Preferably the pharmaceutical composition comprises one or more compounds selected from isoindigo, indigo, indirubin, or derivatives thereof, an anti-inflammatory agent, and a pharmaceutically acceptable carrier.

CLAIMS:

1. A pharmaceutical composition for treating inflammatory-related diseases associated with increased secretion and/or expression of at least one pro-inflammatory cytokine, comprising at least one compound in an amount sufficient to treat the inflammatory-related disease in a subject, the at least one compound being of formula (I):

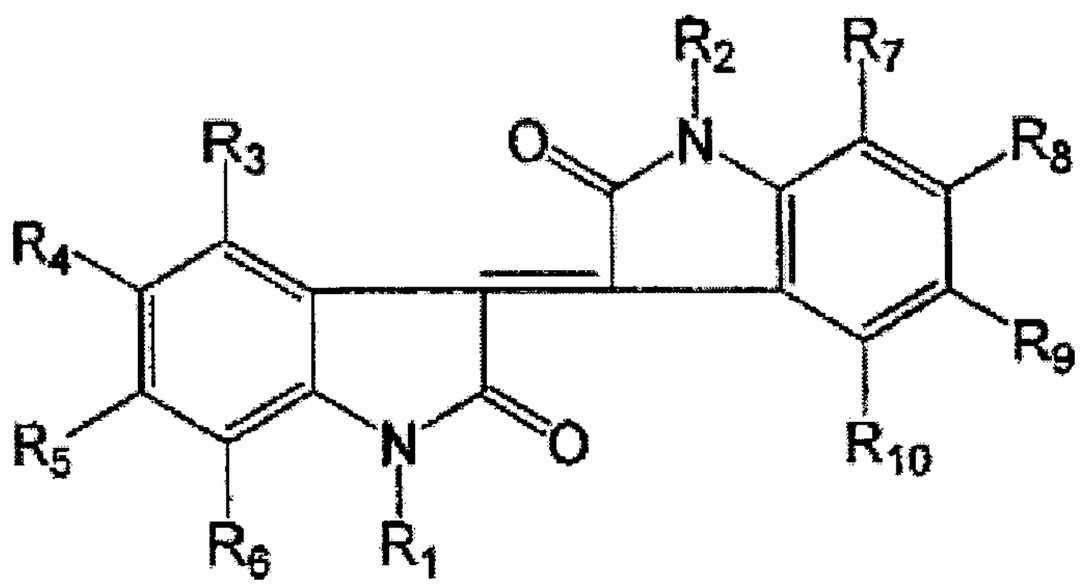


FORMULA (I)

wherein R_3, R_4, R_5, R_6, R_7, R_8, R_9, and R_{10} are the same or different and represent a hydrogen atom; a hydroxy group; a nitroso group; a nitro group; a monosaccharide; a disaccharide; a halogen atom; a hydrocarbyl group, or a functional hydrocarbyl group unsubstituted or substituted with one or more hydroxy moieties, carboxy moieties, nitroxy moieties, monosaccharides, disaccharides, amines, amides, thiols, sulfates, sulfonates, sulfonamides or halogens, wherein the hydrocarbyl has 1 to 8 carbon atoms; a $-R_{11}R_{12}$ group, wherein R_{11} and R_{12} can be the same or different and represent a hydrogen atom, a straight-chain or branched- chain alkyl group having 1 to 18 carbon atoms which can additionally carry one or more hydroxy and/or amino groups, a substituted or unsubstituted aryl group which can comprise one or more heteroatoms, or an acyl group, or R_{11} and R_{12} form together a ring having 2 to 6, optionally substituted, CH_2 groups; an azo group $-N=N-R_{13}$, wherein R_{13} represents an aromatic system which can be substituted by one or more carboxyl groups and/or phosphoryl groups, or a group selected from the group consisting of sugars, amino acids, peptides or steroid hormones; or R_1 and R_6, and R_2 and R_7, respectively, form independently from each other a ring together having 1 to 4, optionally substituted, CH_2 groups; and

at least R_1 or R_2 is a monosaccharide, a disaccharide unsubstituted or substituted with one or more hydroxy moieties or carboxy moieties; a halogen; a hydrocarbyl group, or a functional hydrocarbyl group unsubstituted or substituted with one or more hydroxy moieties, carboxy moieties, nitroxy moieties, monosaccharides, disaccharides, mines, amides, thiols, sulfates, sulfonates, sulfonarnides or halogens, wherein the hydrocarbyl has 1 to 8 carbon atoms.

2. The use of at least one compound in an amount sufficient to treat an inflammatory-related disease associated with increased secretion and/or expression of at least one pro-inflammatory cytokine in a subject, the at least one compound being of formula (I)



FORMULA (I)

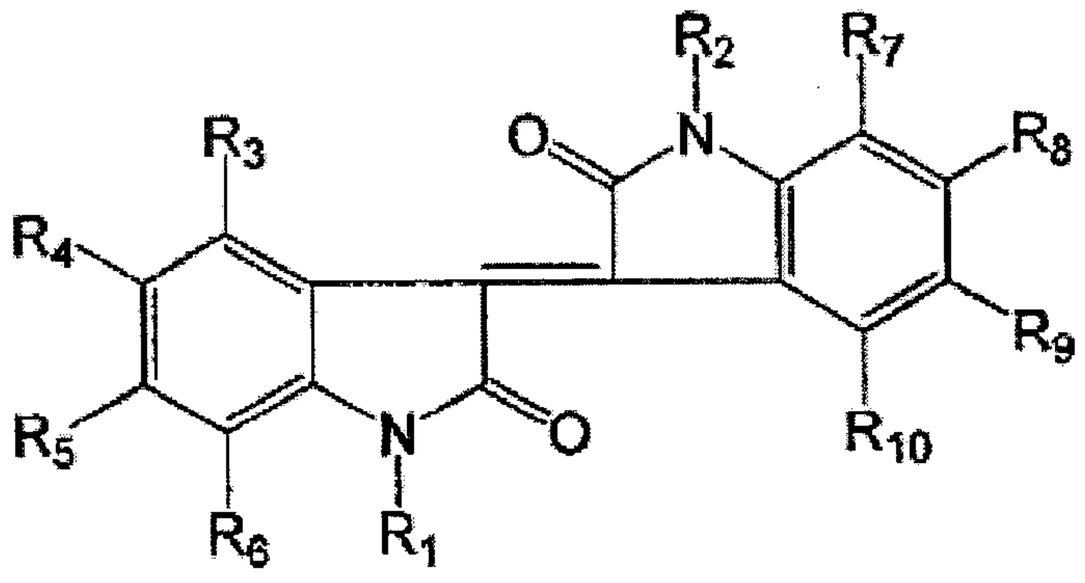
for the manufacture of a medicament for the therapeutic or prophylactic treatment of the inflammatory-related disease in the subject,

wherein R_3, R_4, R_5, R_6, R_7, R_8, R_9, and R_{10} are the same or different and represent a hydrogen atom; a hydroxy group; a nitroso group; a nitro group; a monosaccharide; a disaccharide; a halogen atom; a hydrocarbyl group, or a functional hydrocarbyl group unsubstituted or substituted with one or more hydroxy moieties, carboxy moieties, nitroxy moieties, monosaccharides, disaccharides, amines, amides, thiols, sulfates, sulfonates, sulfonamides or halogens, wherein the hydrocarbyl has 1 to 8 carbon atoms; a $-R_{11}R_{12}$ group, wherein R_{11} and R_{12} can be the same or different and represent a hydrogen atom, a straight-chain or branched- chain alkyl group having 1 to 18 carbon atoms which can additionally carry one or more hydroxy and/or amino groups, a substituted or unsubstituted aryl group which can comprise one or more heteroatoms, or an acyl group,

or R_{11} and R_{12} form together a ring having 2 to 6, optionally substituted, CH_2 groups; an azo group -N=N-R_{13}, wherein R_{13} represents an aromatic system which can be substituted by one or more carboxyl groups and/or phosphoryl groups, or a group selected from the group consisting of sugars, amino acids, peptides or steroid hormones; or R_1 and R_6, and R_2 and R_7, respectively, form independently from each other a ring together having 1 to 4, optionally substituted, CH_2 groups; and

at least R_1 or R_2 is a monosaccharide, a disaccharide unsubstituted or substituted with one or more hydroxy moieties or carboxy moieties; a halogen; a hydrocarbyl group, or a functional hydrocarbyl group unsubstituted or substituted with one or more hydroxy moieties, carboxy moieties, nitroxy moieties, monosaccharides, disaccharides, mines, amides, thiols, sulfates, sulfonates, sulfonarnides or halogens, wherein the hydrocarbyl has 1 to 8 carbon atoms.

3. The use of at least one compound in an amount sufficient to treat an inflammatory-related disease associated with increased secretion and/or expression of at least one pro-inflammatory cytokine, the at least one compound being of formula (I)



FORMULA (I)

for the therapeutic or prophylactic treatment of the inflammatory-related disease,

wherein R_3, R_4, R_5, R_6, R_7, R_8, R_9, and R_{10} are the same or different and represent a hydrogen atom; a hydroxy group; a nitroso group; a nitro group; a monosaccharide; a disaccharide; a halogen atom; a hydrocarbyl group, or a functional hydrocarbyl group unsubstituted or substituted with one or more hydroxy moieties, carboxy moieties, nitroxy moieties, monosaccharides, disaccharides, amines, amides, thiols, sulfates, sulfonates,

sulfonamides or halogens, wherein the hydrocarbyl has 1 to 8 carbon atoms; a $-R_{11}R_{12}$ group, wherein R_{11} and R_{12} can be the same or different and represent a hydrogen atom, a straight-chain or branched- chain alkyl group having 1 to 18 carbon atoms which can additionally carry one or more hydroxy and/or amino groups, a substituted or unsubstituted aryl group which can comprise one or more heteroatoms, or an acyl group, or R_{11} and R_{12} form together a ring having 2 to 6, optionally substituted, CH_2 groups; an azo group -N=N-R_{13}, wherein R_{13} represents an aromatic system which can be substituted by one or more carboxyl groups and/or phosphoryl groups, or a group selected from the group consisting of sugars, amino acids, peptides or steroid hormones; or R_1 and R_6, and R_2 and R_7, respectively, form independently from each other a ring together having 1 to 4, optionally substituted, CH_2 groups; and

at least R_1 or R_2 is a monosaccharide, a disaccharide unsubstituted or substituted with one or more hydroxy moieties or carboxy moieties; a halogen; a hydrocarbyl group, or a functional hydrocarbyl group unsubstituted or substituted with one or more hydroxy moieties, carboxy moieties, nitroxy moieties, monosaccharides, disaccharides, mines, amides, thiols, sulfates, sulfonates, sulfonarnides or halogens, wherein the hydrocarbyl has 1 to 8 carbon atoms.

4. The composition according to claim 1, or the use according to claims 2 or 3, wherein the amount sufficient to reduce, in the subject, the secretion and/or expression of at least one of IL-1β, IL-6, and TNF-α.

5. The composition according to claim 1 or 4, or the use according to any one of claims 2-4, wherein the amount is sufficient to increase IL-10 secretion and/or expression in the subject.

6. The composition according to any one of claims 1, 4 and 5, or the use according to any one of claims 2-5, wherein the inflammatory-related disease is a metabolic disease, a ophthalmological disease, an autoimmune reaction, a cardiovascular disease, a neurodegenerative disease, an infection, a liver disease, a renal disease, or post-radiotherapy-related inflammation.

7. The composition according to any one of claims 1, 4 and 5, or the use according to any one of claims 2-5, wherein the inflammatory-related disease is rheumatoid arthritis, psoriasis, multiple sclerosis, ankylosing spondylitis, Sjogren's syndrome, type I diabetes, type II diabetes, diabetes retinopathy, Alzheimer's disease, Parkinson's disease, or post-radiotherapy-related inflammation.

8. The composition or use according to any one of claims 1-7, wherein at least R1 or R2 is a methyl group.

9. The composition or use according to any one of claims 1-7, wherein R_3, R_4, R_5, R_6, R_7, R_8, R_9, and R_{10} are hydrogen and either R_1 is hydrogen and R_2 is a methyl group; R_1 is tri-acetylated monosaccharide and R_2 is a methyl group; or R_1 is tri-acetylated monosaccharide and R_2 is a hydrogen.

10. The composition or use according to any one of claims 1-9, further comprising a pharmaceutically acceptable carrier.

11. The composition or use according to claim 10, wherein the pharmaceutically acceptable carrier is an inert diluent.

12. The composition or use according to any one of claims 1 to 11, further comprising an anti- inflammatory agent.

13. The composition or use according to claim 12, wherein the anti-inflammatory agent is selected from the group consisting of: an analgesic; an antirheumatic agent; an gastrointestinal agent; a gout preparation; glucocorticoids; opthalmic preparation; respiratory agent; a nasal preparation; and a mucous membrane agent.

14. The composition or use according to claim 13, wherein the analgesic is selected from the group consisting of: naproxen, indomethacin, ibuprofen, ketorolac tromethamine,

choline magnesium trisalicylate and rofecoxib; the antirheumatic agent is selected from the group consisting of: cyclosporine, sulfasalazine, valdecoxib, penicillamine and dexamethasone; the gastrointestinal agent is selected from the group consisting of: mesalamine, balsalazide disodium and olsalazine sodium; the gout preparation is sulindac; the glucocorticoid is selected from the group consisting of: dexamethasone, dexamethasone phosphate, methylprednisolone acetate, hydrocortisone and hydrocortisone sodium phosphate; the nasal preparation is selected from the group consisting of beclomethasone dipropionate monohydrate, fluticasone propionate, triamcinolone acetonide, flunisolide, mometasone furoate monohydrate and budesonide; the opthalmic preparation is ketorolac tromethamine; the respiratory agent is nedocromil sodium; and the mucous membrane agent is selected from the group consisting of: alclometasone dipropionate, hydrocortisone butyrate, flurandrenolide, betamethasone valerate and clobetasol propionate.

15. The composition or use according to any one of claims 1-14, wherein the compound is present in an amount between about 1 mg and about 150 mg.

16. The composition or use according to claim 15, wherein the compound is present in an amount between about 5 mg and about 150 mg.

17. The composition or use according to claim 16, wherein the compound is present in an amount between about 5 mg and about 125 mg.

18. The composition or use according to claim 17, wherein the compound is present in an amount of about 25 mg.

19. The composition or use according to claim 15, wherein the compound is meisoindigo.

20. The composition or use according to claim 19, wherein the compound is present in an amount between about 5 mg and about 150 mg.

21. The composition or use according to claim 20, wherein the compound is present in an amount between about 5 mg and about 125 mg.

22. The composition or use according to claim 21, wherein the compound is present in an amount of about 25 mg.

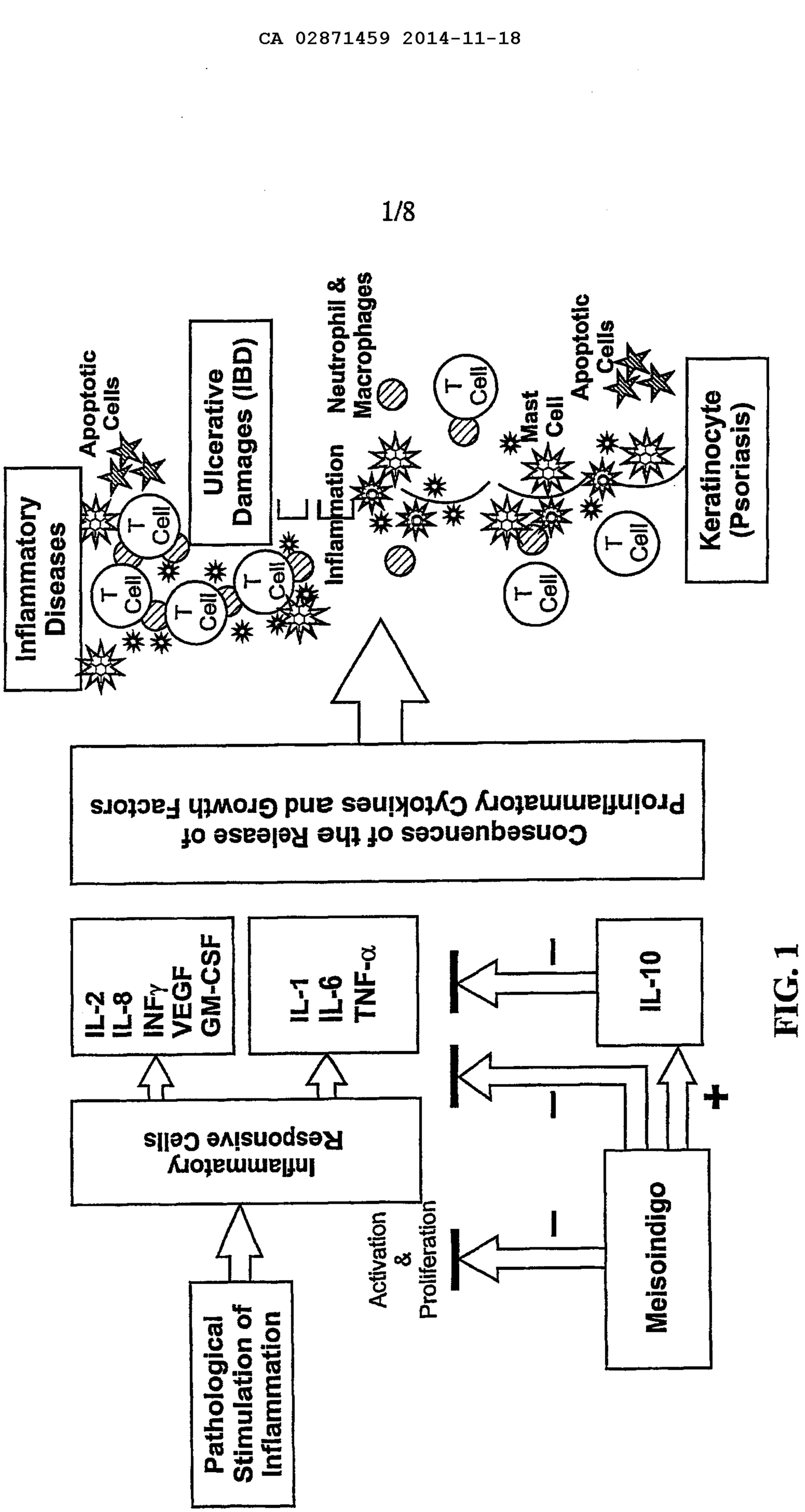

Pathological Stimulation of Inflammation
Activation & Proliferation
Inflammatory Responsive Cells
IL-2 IL-8 INFγ VEGF GM-CSF
IL-1 IL-6 TNF-α
IL-10
Meisoindigo
+
−
Consequences of the Release of Proinflammatory Cytokines and Growth Factors
Inflammatory Diseases
T Cell
Apoptotic Cells
Ulcerative Damages (IBD)
Inflammation
Neutrophil & Macrophages
Mast Cell
Keratinocyte (Psoriasis)

FIG. 1

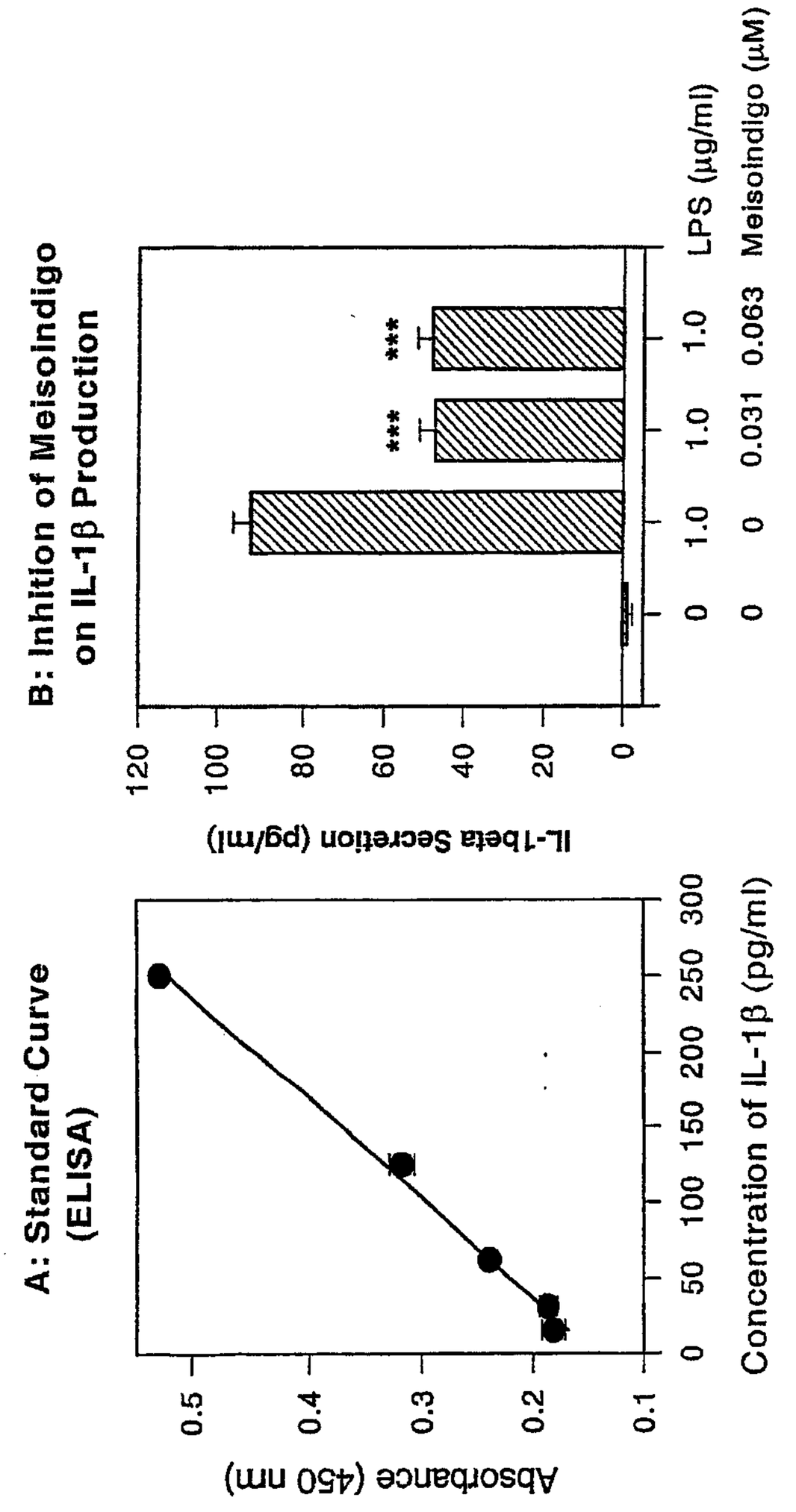

A: Standard Curve
(ELISA)
Absorbance (450 nm)
0.5
0.4
0.3
0.2
0.1
0
50
100
150
200
250
300
Concentration of IL-1β (pg/ml)
B: Inhition of Meisoindigo
on IL-1β Production
IL-1beta Secretion (pg/ml)
120
100
80
60
40
20
0
***
***
0
1.0
1.0
1.0
LPS (μg/ml)
0
0
0.031
0.063
Meisoindigo (μ

FIG. 2

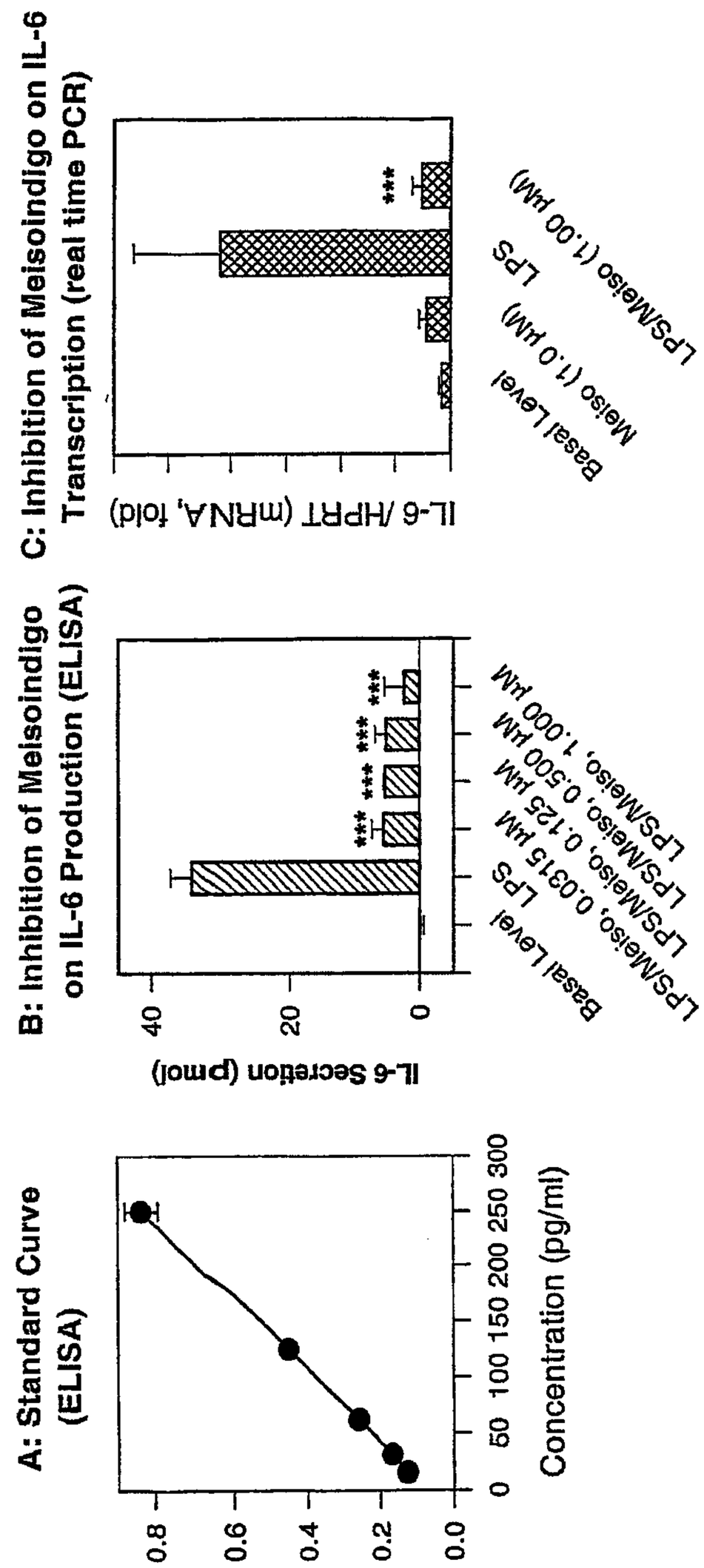
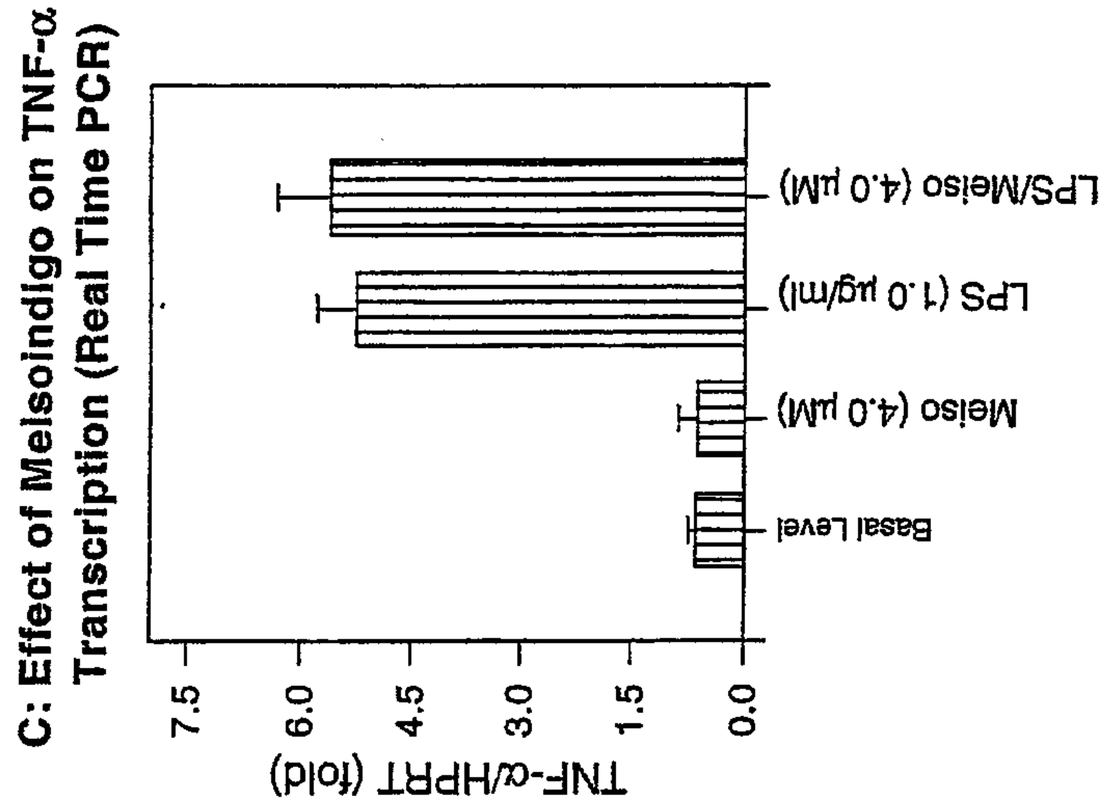
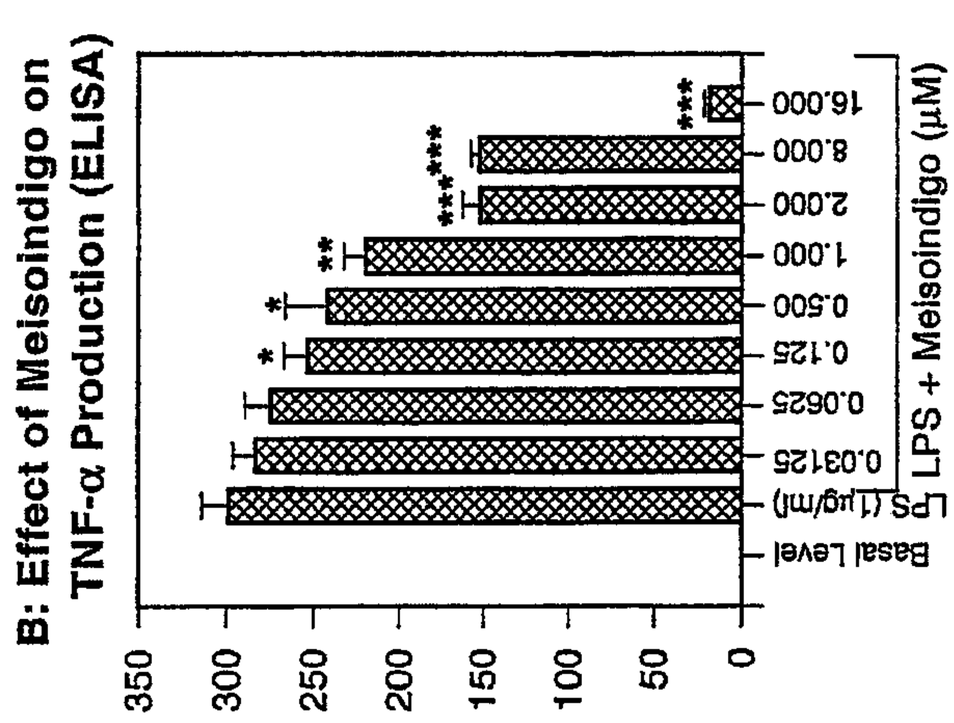

A: Standard Curve (ELISA)
Absorbance (450 nM)
0.0
0.2
0.4
0.6
0.8
0
50
100
150
200
250
300
Concentration (pg/ml)
B: Inhibition of Meisoindigo on IL-6 Production (ELISA)
IL-6 Secretion (pmol)
0
20
40
Basal Level
LPS
LPS/Meiso, 0.0315 μM
LPS/Meiso, 0.125 μM
LPS/Meiso, 0.500 μM
LPS/Meiso, 1.000 μM
***
C: Inhibition of Meisoindigo on IL-6 Transcription (real time PCR)
IL-6 /HPRT (mRNA, fold)
Basal Level
Meiso (1.0 μM)
LPS
LPS/Meiso (1.00 μM)
***

FIG. 3


C: Effect of Melsoindigo on TNF-α
Transcription (Real Time PCR)
TNF-α/HPRT (fold)
7.5
6.0
4.5
3.0
1.5
0.0
Basal Level
Meiso (4.0 μM)
LPS (1.0 μg/ml)
LPS/Meiso (4.0 μM)


B: Effect of Melsoindigo on
TNF-α Production (ELISA)
TNF-α Secretion (pg/ml)
350
300
250
200
150
100
50
0
Basal Level
LPS (1μg/ml)
0.03125
0.0625
0.125
0.500
1.000
2.000
8.000
16.000
LPS + Melsoindigo (μM)
*
*
**
***
***

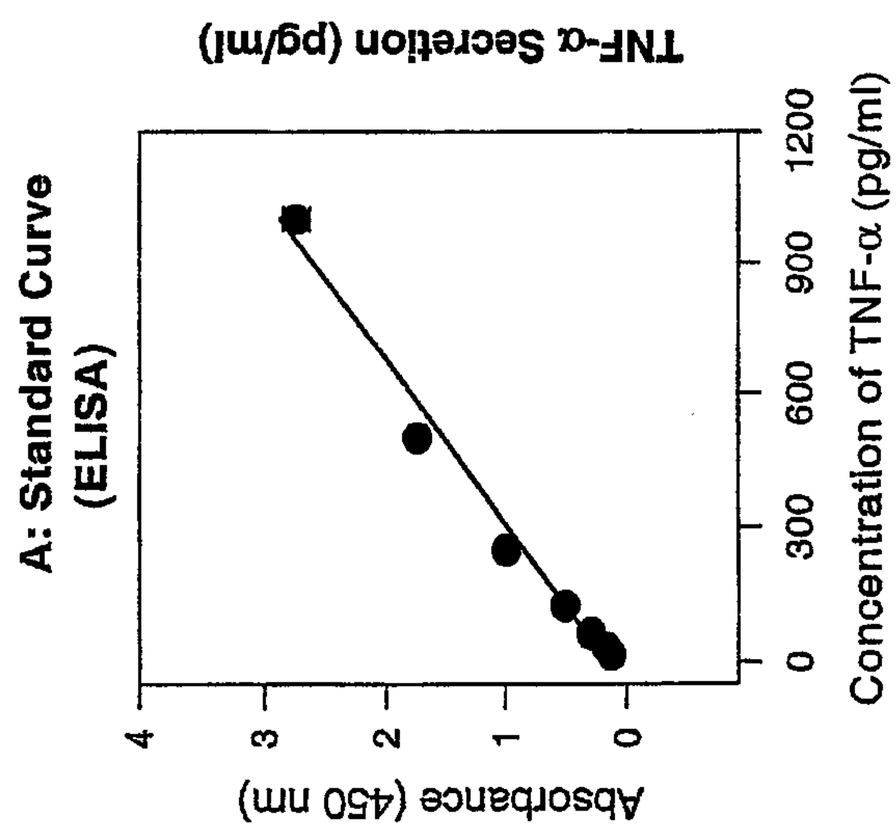

A: Standard Curve
(ELISA)
Absorbance (450 nm)
4
3
2
1
0
0
300
600
900
1200
Concentration of TNF-α (pg/ml)

FIG. 4

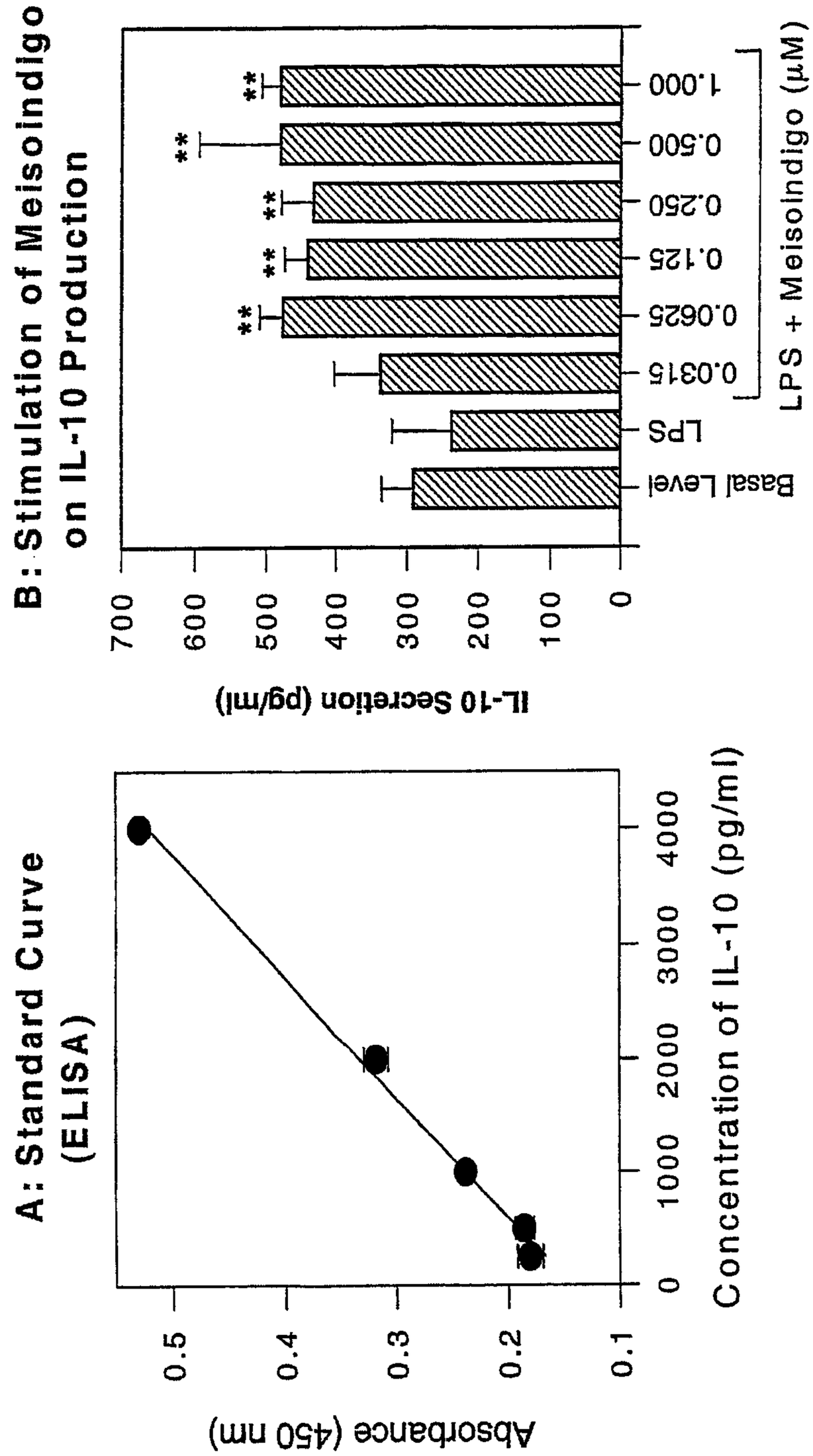

A: Standard Curve
(ELISA)
Concentration of IL-10 (pg/ml)
0
1000
2000
3000
4000
Absorbance (450 nm)
0.1
0.2
0.3
0.4
0.5
B: Stimulation of Meisoindigo
on IL-10 Production
IL-10 Secretion (pg/ml)
0
100
200
300
400
500
600
700
Basal Level
LPS
LPS + Meisoindigo (μM)
0.0315
0.0625
0.125
0.250
0.500
1.000
**

FIG. 5

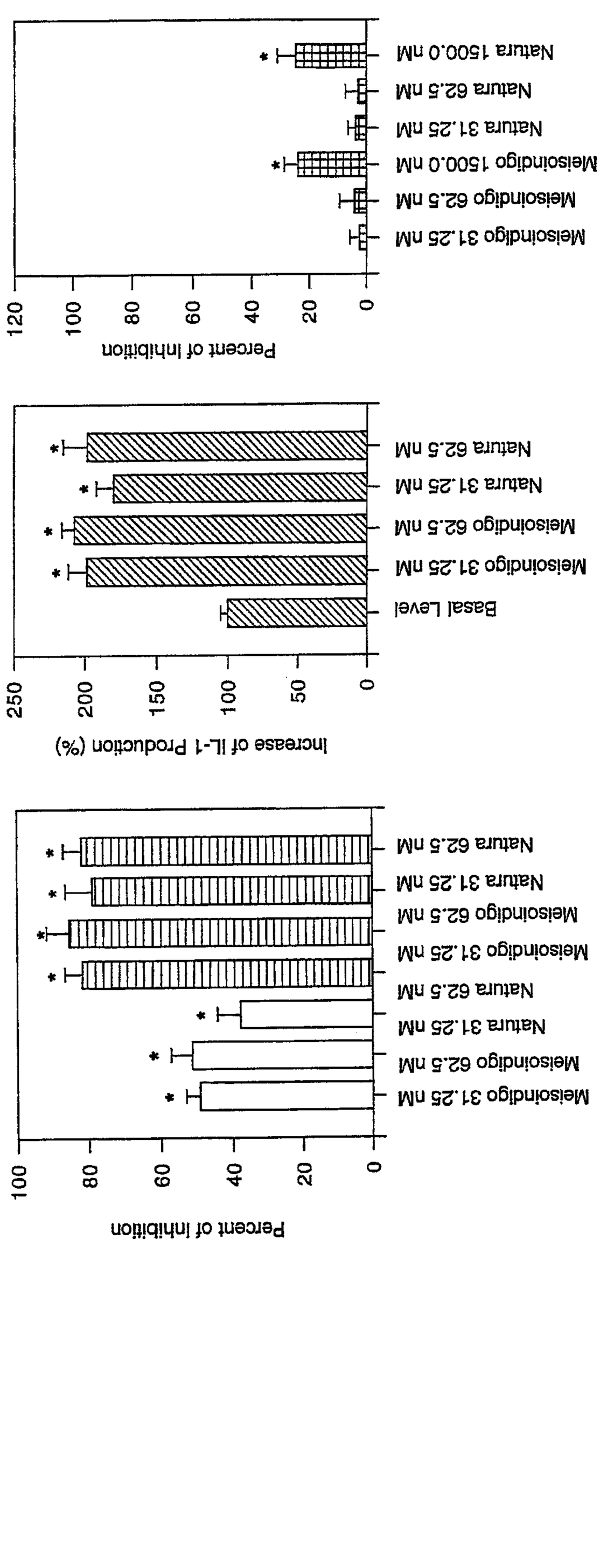

A: Inhibition of IL-1β and IL-6
Percent of Inhibition
100
80
60
40
20
0
Increase of IL-1 Production (%)
250
200
150
100
50
0
Meisoindigo 31.25 nM
Meisoindigo 62.5 nM
Natura 31.25 nM
Natura 62.5 nM
Meisoindigo 31.25 nM
Meisoindigo 62.5 nM
Natura 31.25 nM
Natura 62.5 nM
B: Stimulation of IL-10
Basal Level
Meisoindigo 31.25 nM
Meisoindigo 62.5 nM
Natura 31.25 nM
Natura 62.5 nM
C: Inhibition of Cdk2
Percent of Inhibition
120
100
80
60
40
20
0
Meisoindigo 31.25 nM
Meisoindigo 62.5 nM
Meisoindigo 1500.0 nM
Natura 31.25 nM
Natura 62.5 nM
Natura 1500.0 nM

FIG. 6

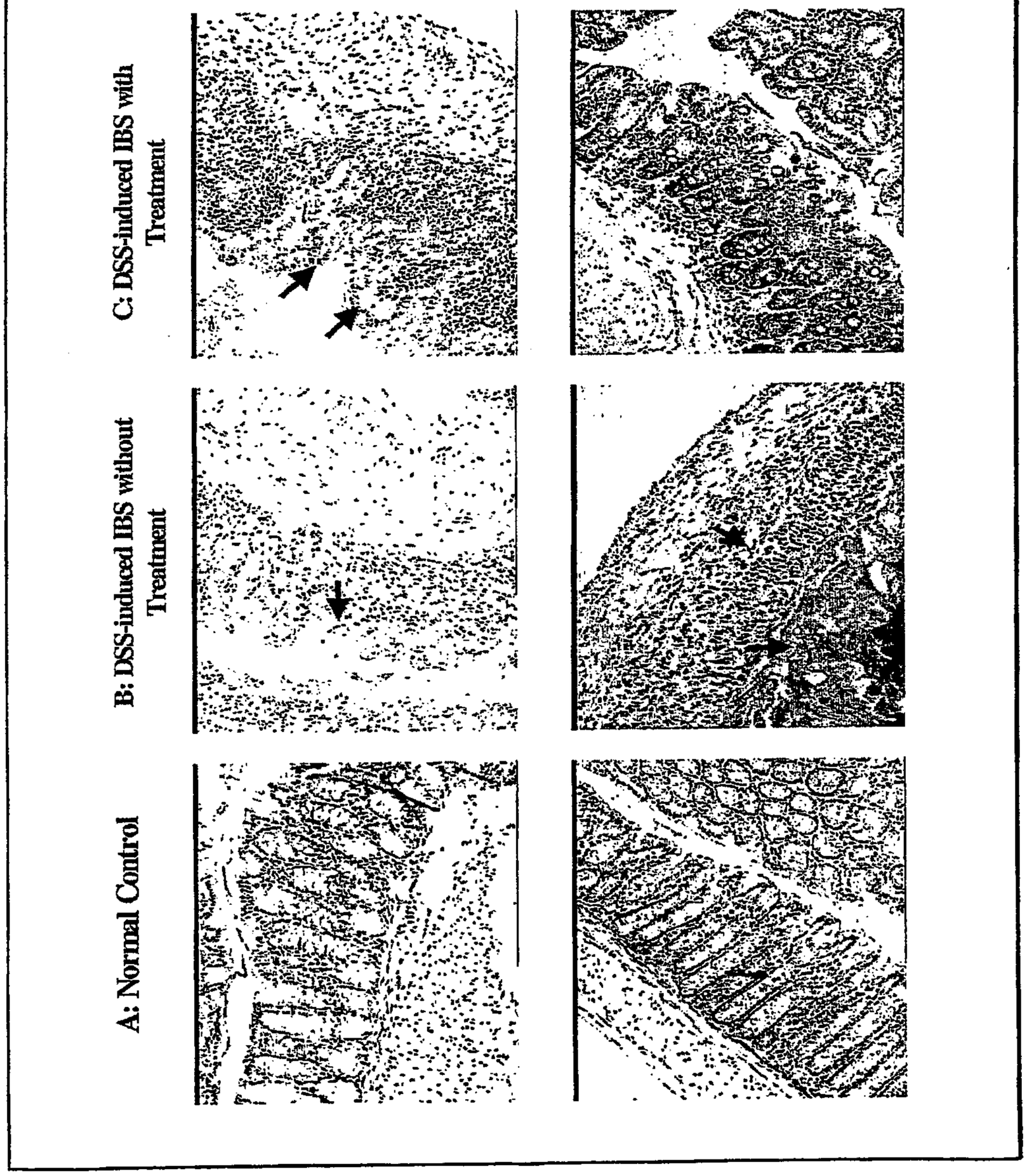

A: Normal Control
B: DSS-induced IBS without Treatment
C: DSS-induced IBS with Treatment

FIG. 7

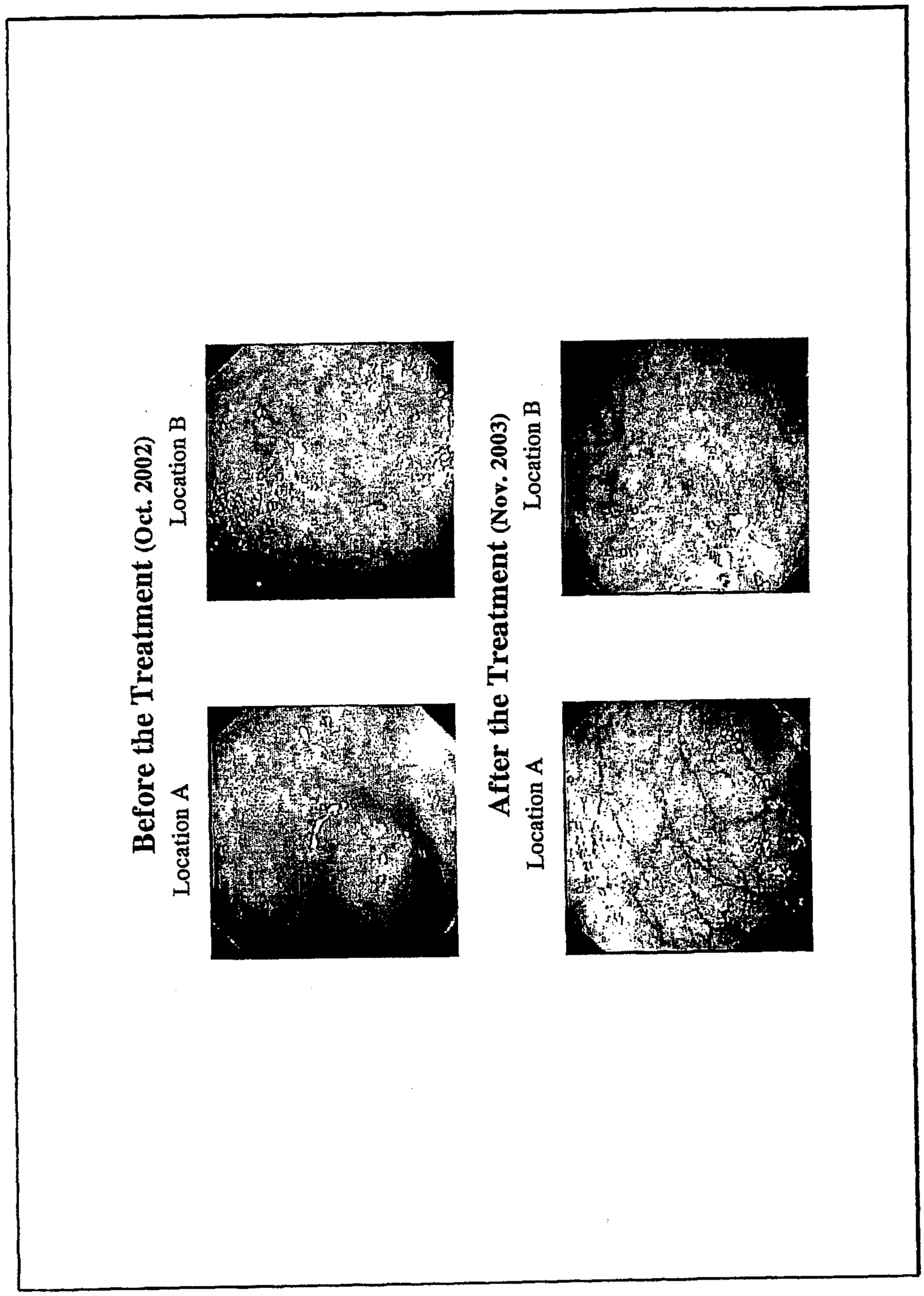

Before the Treatment (Oct. 2002)
Location A
Before the Treatment (Oct. 2002)
Location B
After the Treatment (Nov. 2003)
Location A
After the Treatment (Nov. 2003)
Location B

FIG. 8