Abstract:

Hexadecyl cw-9-tetradecenoate commonly known as Cetyl myristoleate (CMO) is being used for the treatment of osteoarthritis and other joint inflammatory diseases, cis-9- Tetradecenoic acid (cis-9-myristoleic acid) is the main precursor for the preparation of CMO. As there are limited natural plant sources for cis-9-tetradecenoic acid, the present invention aimed at the synthesis of cis-9-tetradecenoic acid methyl ester from oleic acid methyl ester. As oleic acid is not available in pure form, this has to be isolated from oleic acid-rich oils like olive oil. cis- 10-Tetradecenoic acid methyl ester, an isomer of cw-9-tetradecenoic acid was also prepared from undecenoic acid methyl ester, a derivative of castor oil. Undecenoic acid is easily available commercially in pure form. Hexadecyl cw-9-tetradecenoate and hexadecyl cis-10-tetradecenoate were prepared by enzymatic transesterification of cw-9-tetradecenoic acid methyl ester and cis-10-tetradecenoic acid methyl ester with 1-hexadecanol (cetyl alcohol) respectively. Both the isomers of cetyl myristoleate were evaluated for anti arthritis, blocking inflammation and reduction of adjuvant-induced arthritis in rats.
"A PROCESS FOR PREPARATION OF HEXADECYL cis-S-TETRADECENOATE AND HEXADECYL c/s-10-TETRADECENOATE’’

5 FIELD OF THE INVENTION
The present invention relates to a process for the preparation of two isomers of cetyl myristoleate namely hexadecyl c/s-9-tetradecenoate and hexadecyl cis-10-tetradecenoate. More particularly, the present invention also relates to evaluation of both the isomers hexadecyl c/s-9-tetradecenoate and hexadecyl c/s-10-tetradecenoate for blocking inflammation and reduction of adjuvant-induced arthritis in rats.

BACKGROUND OF THE INVENTION
Cetyl myristoleate (CMO) is the common name for hexadecyl c/s-9-myristoleate. CMO is the ester of c/s-9-tetradecenoic acid (myristoleic acid) and 1-hexadecanol (cetyl alcohol). Cetyl myristoleate is well known for its anti-arthritis properties. Hexadecyl c/s-9-tetradecenoate was found only in very selective number of species of animals including cows, whales, beavers and mice. Cetyl myristoleate was isolated from mice in 1972 by Harry W. Diehl, a researcher at the National Institutes of Health (Diehl HW, May EL. J Pharm Sci 1994; 83:296-9). Cetyl myristoleate has been used for immunizing against inflammatory rheumatoid arthritis in mammals (Diehl, US 4,049,824, Levin, WO 01/41783), treatment of rheumatoid arthritis (Diehl, US 4,113,881) and osteoarthritis (Diehl, US 5,569,676). Vegetable butter-based cetyl myristoleate was also used for treating osteoarthritis and other musculoskeletal disease conditions and injuries (Leonard, US 20030181521). Nutraceuticals containing CMO are widely used for reducing pain inflammation, and with the exception of a report suggesting a positive clinical effect of cerasomol-CMO in patients with fibromyalgia (Edwards AM. J. Nutr. Environ. Med. 2001; 11:105-11). As Swiss Albino Mice is the only natural source for CMO, Kenneth et al, synthesized pure hexadecyl cis-9-tetradecenoate by esterifying c/s-9-tertadecenoic acid (purchased from commercial source) with 1-hexadecanol by chemically and confirmed its
anti arthritic properties in a collagen-induced arthritis model in DBA / 1 Lac J mice
(Kenneth W. Hunter, Jr., Ruth A. Gault, Jeffrey S. Stehouwer, Suk-Wah Tam-Chang.
Pharmacological Research 2003; 47:43-47). c/s-9-Myristoleic acid is naturally available
as a mixture of fatty acids along with other fatty acids in beef tallow fat with c/s-9-
myristoleic acid content of 8% (Lord G, WO 00/64436) and seed fat of Pycnanthus Komb
with 20-30% of cjs-9-myristoleic acid (Leonard, US 20030181521). Literature search
revealed that there is no synthetic route reported for the preparation of c/s-9-myristoleic
acid. Since the natural availability of cis-9-myristoleic acid is scarce, the present
invention reported synthetic route for the first time from methyl oleate. As it is difficult
to isolate pure oleic acid from any vegetable oil source, the present invention reported
the synthesis of a new isomer c/s-10-myristoleic acid for the first time from commercially
available raw material namely undecenoic acid. Cetyl myristoleate was then prepared by
transesterifying both methyl c/s-10-myristoleate and c/s-9-myristoleate separately with
cetyl alcohol and evaluated for anti-inflammatory and anti-arthritis activity. The anti-
flammatory and anti-arthritis properties of the new isomer i.e. cetyl c/s-10-myristoleate
was compared with that of known CMO containing c/s-9-myristoleic acid prepared as .
described above.

The first step of the synthetic route is preparation of c/s-9-myristoleic acid from oleic
acid. Oleic acid is not available in pure form, and has to be prepared from oleic acid-rich
oils like olive oil by employing methodologies like urea adducts or fractional distillation.
As the isolation of pure oleic acid from natural sources is very expensive, the cost of
cetyl myristoleate based on cis-9 myristoleic acid will also be very high. Hence, there is
a need for the identification of an alternate source of myristoleic acid for the preparation
of CMO. Surprisingly, not much work was reported in this direction either for the
chemical synthesis of cis-9 myristoleic acid or any other alternate raw material for the
preparation of CMO. Keeping these points in view, the present invention explored the
possibility of synthesis of alternative isomers of myristoleic acid, for the preparation of
Cetyl myristoleate. An attractive substrate in this direction is 10-undecenoic acid for the preparation of c/s-10 myristoleic acid. 10-Uridecenoic acid is a pyrolysis product of castor oil fatty acid methyl esters (methyl ricinoleate) and is commercially available in bulk in pure form. In the present invention, both the isomers of methyl esters of c/s-9 and cis-10 myristoleic acids (3 and 7) were prepared from oleic and undecenoic acid methyl esters and further transesterified with cetyl alcohol to obtain hexadecyl c/s-9-tetradecenoate, 4 and hexadecyl cis-10-tetradecenoate, 8. Both hexadecyl c/s-9-tetradecenoate, 4 and hexadecyl c/s-10-tetradecenoate, 8 were evaluated for anti-arthritis properties and found that hexadecyl c/s-10-tetradecenoate, 8 is comparable with that of hexadecyl c/s-9-tetradecenoate, 4 in inhibiting inflammation and effective in adjuvant-induced arthritis in rats.

OBJECTIVES OF THE INVENTION
The main objective of the present invention is to provide a process for the preparation of two isomers of cetyl myristoleate namely hexadecyl c/s-9-tetradecenoate and hexadecyl cis-10-tetradecenoate.

Still another objective of the present invention is evaluation of both the isomers hexadecyl c/s-9-tetradecenoate and hexadecyl c/s-10-tetradecenoate for blocking inflammation and reduction of adjuvant-induced arthritis in rats.

SUMMARY OF THE INVENTION
Accordingly, the present invention provides a process for the preparation of two isomers of cetyl myristoleate namely hexadecyl c/s-9-tetradecenoate and hexadecyl cis-10-tetradecenoate and evaluation of both the isomers hexadecyl c/s-9-tetradecenoate and hexadecyl c/s-10-tetradecenoate for blocking inflammation and reduction of adjuvant-induced arthritis in rats.
In an embodiment of the present invention a process for the preparation of two isomers of cetyl myristoleate namely hexadecyl c/s-9-tetradecenoate and hexadecyl cis-10-tetradecenoate having general formula 1,

\[
\text{O} \quad \text{CH}_2(\text{CH}_2)_{15}O-C-(\text{CH}_2)_n\text{-CH=CH-(CH}_2)_m\text{-CH}_3
\]

**General formula 1**

Where \(n = 7\) or \(8\)  Where \(m = 3\) or \(2\)

comprising the steps of:

a. cooling of an ester selected from the group consisting of oleic acid methyl ester and methyl undecenoate in the presence of an organic solvent at temperature ranges between -70 to -78 °C;

b. ozonolysis of cooled ester by bubbling of ozone for a period ranging between 60 - 90 min;

c. quenching the reaction with dimethyl sulphide (DMS) followed by stirring for a period ranging between 6 - 8 hr at temperature ranging between 25 - 35°C;

d. evaporating the solvent and DMS from the reaction mixture as obtained in step (c) under vacuum to get crude solid containing 1-al-methyl nonoate or 1-al-methyl deaconate depending upon the ester used in step (a);

e. dissolving crude solid containing 1-al-methylalkyloate as obtained in step (d) in dry THF (tetra hydro furan);

f. simultaneously, dissolving triphenyl phosphine- salt selected from the group consisting of n-pentyl-triphenyl phosphonium salt and n-butyl-triphenyl phosphonium salt in dry THF and cooled to a temperature ranging between 0 - 5°C and followed by addition of n-butyl lithium;

g. stirring the reaction mixture as obtained in step (f) for a period ranging between 30 - 60 min to obtain an orange solution;

h. adding solution of crude solid in dry THF as obtained in step (e) into the
solution of triphenyl phosphine salt in dry THF as obtained in step (f) followed by refluxing for a period of 3-8 hrs;

i. removing THF from the reaction mixture as obtained in step (h) under reduced pressure to get residue;

j. adding water in the residue as obtained in step (i) and followed by extraction with ether;

k. removing ether under vacuum to get the residue followed by carrying out purification using column chromatography using hexane and ethyl acetate (98:2) as eluent to get cis-9-myristoleate and cis-10-myristoleate depending upon the ester used in step (a) and triphenyl phosphine salt used in step (f);

l. transesterifying myristoleate as obtained in step (k) with cetyl alcohol in the presence of enzyme selected from the group consisting of Lipozyme TL IM and Novozyme 435 at temperature ranging between 65-70°C for a period ranging between 8 to 10 hr;

m. separating enzyme by filtration and evaporating the solvent to get the crude product having respective isomer of cetyl myristoleate;

n. purifying the crude product as obtained in step (l) by column chromatography to obtain pure cetyl myristoleate of formula 1.

In another embodiment of the present invention a process, wherein the solvent used in step (a) is dichloromethane or chloroform.

In another embodiment of the present invention oleic acid methyl ester used in step (a) and n-pentyl-triphenyl phosphonium salt used in step (f) for the preparation of cis-9-tetradecenoate.

In another embodiment of the present invention methyl undecenoate used in step (a) and n-butyl-π phenyl phosphonium salt used in step (f) for the preparation of cis-10-
tetradecenoate.

In another embodiment of the present invention hexadecyl c/s-9-tetradecenoate yielded in the range of 88 - 95%.

In another embodiment of the present invention hexadecyl c/s-9-tetradecenoate purity is in the range of 92-95%.

In another embodiment of the present invention hexadecyl c/s-10-tetradecenoate yielded in the range of 80 - 90%.

In another embodiment of the present invention hexadecyl cis-10-tetradecenoate purity is in the range of 90-95%.

In another embodiment of the present invention the said isomers hexadecyl cis-9-tetradecenoate and hexadecyl c/s-10-tetradecenoate as prepared are evaluated for blocking inflammation and reduction of adjuvant-induced arthritis in rats.

In another embodiment of the present invention use of isomers hexadecyl c/s-9-tetradecenoate and hexadecyl c/s-10-tetradecenoate for blocking inflammation and reduction of adjuvant-induced arthritis.

In another embodiment of the present invention use of isomers for blocking inflammation wherein the isomers hexadecyl c/s-9-tetradecenoate and hexadecyl c/s-10-tetradecenoate are effective to reduce the inflammation and edema of the animals equally up to 36 % compared to control group at a dose 400 mg/kg body wt.

In another embodiment of the present invention use of isomers for reduction of adjuvant-
induced arthritis activity, wherein the isomers hexadecyl c/s-9-tetradecenoate and hexadecyl cis-10-tetradecenoate are effective to reduce arthritis equally up to 10 to 15% at the dose of 100 mg/kg body wt.

In another embodiment of the present invention use of isomers for anti-arthritis activity, wherein the isomer hexadecyl cis-10-tetradecenoate is effective for 21 days in 80% rats while in 20% rats it is effective for 32 days.

BRIEF DESCRIPTION OF THE ACCOMPANYING DRAWINGS

Fig. 1: Scheme 1: Preparation of Hexadecyl c/s-9-Tetradecenoate
Fig. 2: Scheme 2: Preparation of Hexadecyl cis-10-Tetradecenoate
Fig. 3: Anti-inflammatory activity of Hexadecyl cis-9-Tetradecenoate (c/s-9 CMO) and Hexadecyl c/s-10-Tetradecenoate (c/s-10 CMO)
Fig. 4: Effect of Hexadecyl c/s-9-Tetradecenoate (cis-9 CMO) and Hexadecyl cis-10-Tetradecenoate (c/s-10 CMO) on the Arthritic Rats
Fig. 5: Anti-arthritic Potential of Hexadecyl c/s-10-Tetradecenoate (c/s-10 CMO)

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to the synthesis of two isomers of cetyl myristoleate namely hexadecyl c/s-9-tetradecenoate and hexadecyl c/s-10-tetradecenoate according to Scheme 1 and Scheme 2. The main component of CMO is cis-9 myristoleic acid, and is being isolated from selective natural sources along with other fatty acids. The literature reveals that there is no source, which can provide pure c/s-9 myristoleic acid. Only solution to address this problem is its chemical synthesis starting from oleic acid. As the isolation of pure oleic acid from natural sources like olive or any other vegetable oils is very expensive, the cost of cetyl myristoleate based on c/s-9 myristoleic acid will also be very high. Hence, there is a need for the identification of an alternate source of myristoleic acid for the preparation of CMO. An attractive substrate in this direction is 10-undecenoic acid for the preparation of c/s-10 myristoleic acid. 10-Undecenoic acid is
a pyrolysis product of castor oil fatty acid methyl esters (methyl ricinoleate) and is
commercially available in pure form. Hence, in the present invention synthesis of two
isomers namely c/s-9-tetradecenoic acid methyl ester, 3 and ciWO-tetradecenoic acid
methyl ester, 7 were prepared from methyl esters of oleic and 10-undecenoic acid
respectively. The synthesis of 3 and 7 involves ozonolysis (Santiago de la Moya et al.
to get 1-al-methylnonoate, 2 and 1-al-methyldecanoate 6. These aldehydes were
Chem. 2002; 1143-1148] with triphenyl phosphine salt of n-pentyl and n-butyl bromide
prepared according to Peter et al., [Peter Vinczer, Gabor Baan, Zoltan Juvancz, Lajos
Novak, and Csaba Szantay, Synthetic Communications 1985; 15(14):1257-1270] to get
c/s-9-myristoleate, 3 and c/s-10-myristoleate, 7 and were subjected to enzymatic
transesterification with cetyl alcohol using lipozyme TL IM to obtain hexadecyl c/s-9-
tetradecenoate, 4 and hexadecyl c/s-10-tetradecenoate, 8. Both these compounds were
evaluated for anti arthritis properties and found that hexadecyl cis-10-tetradecenoate, 8
is comparable with that of hexadecyl c/s-9-tetradecenoate, 4 in inhibiting inflammation
and effective in adjuvant-induced arthritis in rats.

The synthesis of hexadecyl c/s-9-tetradecenoate, 4 was carried out as shown in Scheme
1, comprising ozonolysis of oleic acid methyl ester, 1 [prepared by esterification of oleic
acid (>98% purity) with 2% sulfuric acid/methanol] using dichloromethane as solvent, at
78 degree C for 1 hr. The reaction was quenched with dimethyl sulphide (DMS) and
continued the stirring for 6 hr. The solvent and DMS from the reaction mixture was
evaporated and dried under vacuum. The product thus obtained was rich in
1-10-methylnonoate, 2 along with 1-nonanal (C-9 aldehyde) and the same was subjected to
Wittig reaction with triphenyl phosphine salt of n-pentyl bromide in n-BuLi/THF to get
c/s-9-myristoleate, 3 in 55-65% yield with 66-70% purity. The c/s-9-myristoleate, 3 was
transesterified with cetyl alcohol using either 1,3-specific or non-specific lipases like
Lipozyme TL IM (Thermomyces lanuginosa) and Novozyme 435 (Candida antarctica) at 65 °C to 70 °C for 8 hr to get hexadecyl c/s-9-tetradecanoate, 4 in 88-95% yield with 92-95% purity. The purity of all the products was checked by GC and the structures were confirmed by GC-MS, IR and 1H NMR.

The synthesis of hexadecyl cis-10-tetradecenoate, 8 was carried out as shown in Scheme 2, ozonolysis of 10-undecenoic acid methyl ester, 5 (>99% pure) using dichloromethane as solvent at -78 °C for 1 hr. The reaction was quenched with dimethyl sulphide (DMS) and continued the stirring for 6 hr. The solvent and DMS from the reaction mixture was evaporated and dried under vacuum to get 1-ahnethyldecanoate, 6 in 90% yield. The crude aldehyde, 6 was subjected to Wittig reaction with triphenyl phosphine salt of n-butyl bromide in n-BuLi/THF to get c/s-10-myristoleate, 7 in 65-68% yield with 75-78% purity. The c/s-10-myristoleate, 7 was transesterified with cetyl alcohol using Lipozyme TL IM at 65 °C to 70 °C for 8 hr to get hexadecyl c/s-10-tetradecanoate, 8 in 88-90% yield with 90-95% purity. The purity of all the products was checked by GC and the structures were confirmed by GC-MS, IR and 1H NMR.

The anti-inflammatory activity potential of both the isomers of the cetyl myristoleate (4 & 8) was evaluated initially in carrageenan induced rat paw edema model employing the method of Winter et al (Winter CA, Risley EA and Nuss GW, J. Pharmacol. Exp. Ther. 1963; 141:369-376). The arthritis developed by injecting Freund's Complete Adjuvant in Wistar rats is almost similar to the rheumatoid arthritis of the human beings. Hence, further studies on anti-arthritis activity were done employing the method of Pearson et al (Pearson CM, Proc. Soc. Exper. Biol. Med. 1953; 91:95-101). The studies of anti-arthritis properties were evaluated for both the isomers of cetyl myristoleates and found that hexadecyl c/s-10-tetradecenoate, 8 was also comparable with that of hexadecyl cis-9-tetradecenoate, 4 in inhibition of inflammation and reduction of adjuvant-induced arthritis in rats.
EXAMPLE: 1

**Preparation of Hexadecyl cis-9-Tetradecenoate:** Preparation of hexadecyl cis-9-tetradecenoate, 4 was carried out according to the Scheme 1. Oleic acid methyl ester, 1 (12.0 g, 0.0314 mol) in dichloromethane (100 ml) was cooled to -78 degree. C and ozone gas was bubbled into the reaction mixture for 1 hr. After reaction, the reaction was quenched by adding dimethyl sulphide (DMS, 8 ml) and was stirred for 6 hr at 25°C. The solvents were removed under vacuum and the residue 2 thus obtained (4.25 g, 0.022 mol) was used directly for the preparation of cis-9-myristoleate, 3. n-Pentyl-triphenyl phosphonium salt (11.16 g, 0.027 mol) was taken in 50 ml of dry THF and cooled to 0°C. To this slurry, n-butyl lithium (17.0 ml, 1.6 M in hexane) was added, stirred the reaction mixture for 0.5 hr to obtain an orange solution. 1-Al-methyl nonanoate containing crude product 2 (5.0 g, 0.027 mol) dissolved in dry THF (20 ml) was added to the above contents slowly and allowed the reaction mixture to reach to 25 degree. C and then heated to reflux temperature to reflux for 4 hr. The reaction was monitored by TLC and after completion of the reaction, THF was removed from the reaction mixture under reduced pressure, and to the residue distilled water 825 ml) was added and extracted with ether (25 ml X 3 times). The combined ether layer was dried over anhydrous sodium sulphate and solvent was removed and dried under vacuum to get the residue and was purified by column chromatography using hexane and ethyl acetate (98:2) as eluent to get cis-9-myristoleate, 3 (4.2 g, 0.0175 mol) in 65% yield with 66% purity by GC. cis-9-Myristoleate, 3 (4.2 g, 0.0175 mol) was enzymatically transesterified with cetyl alcohol (5.08 g, 0.021 mol) in the presence- of Lipozyme TL-IM- (0.930 g, 10 wt% of the total substrate) at 68°C for 8 hr. The reaction was monitored by TLC and after completion of the reaction, hexane (50 ml) was added and the lipase was separated by filtration and the solvent was evaporated to get the crude product and was purified by column chromatography to obtain hexadecyl cis-9-tetradecenoate, 4 (7.48 g, 0.0166 mol) in 95% yield with 92% purity by GC. The structure of hexadecyl cis-9-tetradecenoate, 4 was confirmed by 1H NMR, IR, and GC-MS.
Spectral data:

\(^1\text{H} \text{NMR: (600 MHz, CDCl}_3\): } \delta 5.36-5.33 \ (m, \ 2H, J = 3 \text{ Hz, } -\text{CH} \equiv \text{CH-}), \ 4.01 \ (t, \ 2H, -\text{CH}_2\text{O}\text{OC}_7\text{H}_4\text{O}) , \ 2.25 \ (t, \ 2H, -\text{C}-\text{CH}_2\text{O}) , \ 1.99 \ (m, \ 4H, -\text{CH}_2\text{=CH}-\text{CH}-\text{CH}_2\text{)}, \ 1.60 \ (m, \ 4H, -\text{CH}_2\text{=CH}-\text{CH}_2\text{)}, \ 1.30 - 1.20 \ (br, \ d, \ 38H, -\text{CH}_2\text{=CH}-\text{CH}_2\text{)}, \ 0.90 \ (q, \ 6H, -\text{CH}_2\text{CH}_3) .

\text{IR (neat/NaCl): } 2926, \ 1738, \ 1654, \ 1242, \ 721 \text{ cm}^{-1}.

\text{GC-MS: } m/z: C_{30}H_{58}O_2 (M^+): 450.

\text{EXAMPLE 2}

\text{Preparation of Hexadecyl cis-r-9-Tetradecenoate: } c/5-9-Myristoleate, 3 (4.2 g, 0.0175 mol) was enzymatically transesterified with cetyl alcohol (5.08 g, 0.021 mol) in the presence of Novozyme 435 (0.930 g, 10 wt\% of the total substrate) at 68 ^\circ \text{C} for 8 hr. The reaction was monitored by TLC and after completion of the reaction, hexane (50 ml) was added and the lipase was separated by filtration and the solvent was evaporated to get the crude product and was purified by column chromatography to obtain hexadecyl cis-9-tetradecenoate, 4 (7.48 g, 0.0166 mol) in 90% yield with 90% purity by GC. The structure of hexadecyl c/s-9-tetradecenoate, 4 was confirmed by \(^1\text{H} \text{NMR, IR, and GC-MS.}

\text{Spectral data:}

\(^1\text{H} \text{NMR: (600 MHz, CDCl}_3\): } \delta 5.36-5.33 \ (m, \ 2H, J = 3 \text{ Hz, } -\text{CH} \equiv \text{CH-}), \ 4.01 \ (t, \ 2H, -\text{CH}_2\text{O}\text{OC}_7\text{H}_4\text{O}) , \ 2.25 \ (t, \ 2H, -\text{C}-\text{CH}_2\text{O}) , \ 1.99 \ (m, \ 4H, -\text{CH}_2\text{=CH}-\text{CH}_2\text{)}, \ 1.60 \ (m, \ 4H, -\text{CH}_2\text{=CH}-\text{CH}_2\text{)}, \ 1.30 - 1.20 \ (br, \ d, \ 38H, -\text{CH}_2\text{=CH}-\text{CH}_2\text{)}, \ 0.90 \ (q, \ 6H, -\text{CH}_2\text{CH}_3) .

\text{IR (neat/NaCl): } 2926, \ 1738, \ 1654, \ 1242, \ 721 \text{ cm}^{-1}.

\text{GC-MS: } m/z: C_{30}H_{58}O_2 (M^+): 450.
EXAMPLE: 3

**Preparation of Hexadecyl cis-10-Tetradecanoate:** Preparation of hexadecyl cis-10-tetradecanoate, 8 was carried out according to the Scheme 2. Methyl undecenoate, 5 (10.21 g, 0.0515 mol) in dichloromethane (150 ml) was cooled to -78 °C and ozone gas was bubbled into the reaction mixture for 1 hr. The reaction was quenched by adding dimethyl" sulphide (DMS, 5 ml) and was stirred for 6 hr at 25°C. The solvents were removed under vacuum and the residue 6 thus obtained was used directly further preparation of ds-10-myristoleate, 7. n-Butyl-triphenyl phosphonium bromide salt (10.0 g, 0.0251 mol) was taken in dry THF (50 ml) and cooled to 0 °C. To this slurry, n-Butyl lithium (17.0 ml, 1.6 M in hexane) was added and stirred for 0.5 hr. 1-Al-methyl deaconate containing crude product 6 (5.01 g, 0.025 mol) dissolved in dry THF (50 ml) was added slowly to the above contents and allowed the reaction mixture to warm to room temperature and then heated to reflux temperature to reflux for 4 hr. THF was removed from the reaction product under reduced pressure, and to the residue distilled water (25 ml) was added and extracted with ether (25 ml X 3 times). The combined ether layer was dried over anhydrous sodium sulphate and solvent was evaporated and dried under vacuum to get the product and was further purified by column chromatography using hexane and ethyl acetate (98:2) as eluent to obtain cis-10-myristoleate, 7 (3.35 g, 0.014 mol) in 56% yield with 97% purity by GC. cis-10-Myristoleate, 7 (3.35 g, 0.014 mol) was enzymatically transesterified with cetyl alcohol (4.07 g, 0.0168 mol) in the presence of Lipozyme TL IM (0.745 g, 10 wt% of the total substrate), at 65 °C for 8 hr. The reaction was monitored by TLC and after completion of reaction, hexane (50 ml) was added and the lipase was separated by filtration and the solvent was evaporated to get the crude product and was purified by column chromatography to obtain hexadecyl m-10-tetradecenoate, 8 (5.55 g, 0.0123) in 88% yield with 95% purity by GC. The structure of hexadecyl cw-10-tetradecenoate, 8 was confirmed by 1H NMR, IR, and GC-MS.

Spectral data:

1H NMR (600 MHz, CDCl3): 5.30 (m, 2H, -CH=CH-), 4.01 (t, 2H, -CH2O- C-),
2.25 (t, 2H, -C-CH₂⁻), 2.0 (m, 4H, -CH₂⁻CH=CH-CH⁻), 1.60 (m, 4H, -CH₂⁻CH⁻CH=CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH⁻CH'article
EXAMPLE 5

**Evaluation Studies of Hexadecyl c/s-9-Tetradecenoate and Hexadecyl cis-10-Tetradecenoate for Anti-inflammatory Potential:** Hexadecyl c/s-9-tetradecenoate, 4 and hexadecyl cis-10-tetradecenoate, 8 produced synthetically exemplified above in Scheme 1 and Scheme 2 were assessed for the anti-inflammatory potential against carrageenan induced paw edema model. Rats were divided into 4 groups. Hexadecyl c/s-9-tetradecenoate, 4 and hexadecyl c/s-10-tetradecenoate, 8 were administered intraperitoneal^ to rats in two different groups at a dose of 400 mg/kg. Another test group received indomethacin at a dose of 10 mg/kg, which is used as reference standard. Control group received vehicle alone. One hour after the administration of test compounds carrageenan (1%, 0.1 ml) was injected into the sub plantar region of animals in all the groups. Paw volumes were measured using plethysmometer before injecting carrageenan. Paw volumes were again measured 3 hr after the carrageenan administration. The anti-inflammatory potential of hexadecyl c/s-9-tetradecenoate, 4 and hexadecyl cis-10-tetradecenoate, 8 were assessed by measuring the inhibition of the paw volumes with reference to mean paw volume in control group. Both hexadecyl cis-9-tetradecenoate, 4 and hexadecyl c/s-10-tetradecenoate, 8 were found to reduce the inflammation and edema of the animals equally up to 36 % compared to control group (Fig. 3).

**EXAMPLE: 6**

Effect of Hexadecyl cisf-9-Tetradecenoate and Hexadecyl c/s^10-Tetradecenoate on the Arthritic Rats: The hexadecyl c/s-9-tetradecenoate, 4 and hexadecyl cis-10-tetradecenoate, 8 produced synthetically given above in Scheme 1 and 2 were subjected to evaluation for anti-arthritic potential in Freund’s complete adjuvant induced arthritis model in rats. Male Wistar rats were divided into 4 groups each containing 6 rats. Arthritis was induced in rats in all the groups by administering Complete Freund’s Adjuvant (CFA) at the dose of 1 mg per rat by injecting at sub plantar region of the right hind paw. Paw volumes were measured for both paws on every alternate day till 14 days
and till full arthritis was developed. Hexadecyl αs-9-tetradecenoate, 4 (100 rag/kg/day), hexadecyl cis-10-tetradecenoate, 8 (100 mg/Kg/day) and indomethacin (2.5 mg/Kg/day) were administered from 14th day till 22nd day. Paw volumes were again measured daily from 15th day to 22nd day for all rats in all groups. The percent inhibition of arthritis in the treated groups was calculated with reference to control group (Fig. 4). It was found that hexadecyl c/s-9-tetradecenoate, 4 and hexadecyl c/s-10-tetradecenoate, 8 reduced the paw volumes and the symptoms associated with the arthritis equally up to 10 to 15% at the dose of 100 mg/kg and this inhibition was dose dependent when dose was increased from 100 mg/kg to 300 mg/kg, which indicates that hexadecyl c/s-9-tetradecenoate, 4 and hexadecyl c/y-10-tetradecenoate, 8 are useful in alleviating the symptoms associated with the established arthritis.

EXAMPLE: 7

Evaluation of Hexadecyl c/sr-10-Tetradecenoate for Anti-arthritic Potential in Rats: The hexadecyl cis-10-tetradecenoate, 8 produced synthetically exemplified above in Scheme 2 was administered parenterally, particularly, by intraperitoneal route, either alone or in a compatible pharmaceutical carrier such as vegetable oils namely groundnut, sesame and cottonseed [for adjusting the dose volume of the active compound(s)] to male rats ranging in weight from 150 to 180 g. It should be noted that use of the mineral oil is not mandatory as the active compounds are oils themselves. The procedure as described in (Vogel’s Drug Discovery and Evaluation Pharmacological assays, 2nd ed, 2002, Springer Verlag, pages 802-803) was followed. Male Wistarrats were divided into one control and two treated groups. All the animals in three groups received Freund's complete adjuvant (Img/rat). This day was designated as day 1. After a period of 24 hr, hexadecyl ciWO-tetradecenoate, 8 was administered at a dose of 300 mg/kg to one treated group and another treated group received 2.5 mg/kg of indomethacin (used as standard drug). Dosing was continued for 15 days (Fig. 3). Control group received the vehicle (mineral oil) without any drug. Paw volumes (left and right) were measured on every alternate day.
All the rats in the control group developed severe polyarthritis as indicated by the increase in the paw volumes. Whereas, rats treated with hexadecyl c/s-10-tetradecenoate, 8 were protected from the polyarthritis. Around 80% of the animals in the hexadecyl c/s-10-tetradecenoate treated group were protected from developing arthritis in a period of 21 days. The remaining 20% rats were protected from arthritis in a period of 32 days. It was also found that the effective range for protection of arthritis by hexadecyl c/s-10-tetradecenoate, 8 was 0.1 to 1.0 gm/kg body weight of the animal. However doses less or more than this range were found to protect developments of the Freund’s Complete Adjuvant induced poly arthritis in rats.
We Claim:


\[
\text{General formula 1:}
\begin{align*}
O \\
\text{CH}_3(\text{CH}_2)_{16}-\text{O}-\text{CH}-(\text{CH}_2)_n-\text{CH}=	ext{CH}-(\text{CH}_2)_m-\text{CH}_3
\end{align*}
\]

Where \( n = 7 \) or 8  
Where \( m = 3 \) or 2

comprising the steps of:

a. cooling of an ester selected from the group consisting of oleic acid methyl ester and methyl undecenoate in the presence of an organic solvent at temperature ranges between -70 to -78°C;
b. ozonolysis of cooled ester by bubbling of ozone for a period ranging between 60 - 90 min;
c. quenching the reaction with dimethyl sulphide (DMS) followed by stirring for a period ranging between 6 - 8 hr at temperature ranging between 25 - 35°C;
d. evaporating the organic solvent and DMS from the reaction mixture as obtained in step (c) under vacuum to get a crude solid containing 1-al-methyl nonoate or 1-al-methyl deaconate depending upon the ester used in step (a);
e. dissolving crude solid containing 1-al-methyl nonoate or 1-al-methyl deaconate as obtained in step (d) in dry THF (tetrahydrofuran);
f. simultaneously, dissolving triphenyl phosphine salt selected from the group consisting of n-pentyl-triphenyl phosphonium salt and n-butyl-triphenyl phosphonium salt in dry THF and cooled to a temperature ranging between 0 - 5°C and followed by addition of n-butyl lithium;
g. stirring the reaction mixture as obtained in step (f) for a period ranging
between 30 - 60 min to obtain an orange solution;
h. adding solution of crude solid in dry THF as obtained in step (e) into the solution of triphenyl phosphine salt in dry THF as obtained in step (f) followed by refluxing for a period of 3 - 8 hrs;
i. removing THF from the reaction mixture as obtained in step (h) under reduced pressure to get residue;
j. adding water in the residue as obtained in step (i) and followed by extraction with ether;
k. removing ether under vacuum to get the residue followed by carrying out purification using column chromatography using hexane and ethyl acetate (98:2) as eluent to get cis-9- myristoleate and cis-10- myristoleate depending upon the ester used in step(a) and triphenyl phosphine salt used in step (f);
l. transesterifying myristoleate as obtained in step (k) with cetyl alcohol in the presence of enzyme selected from the group consisting of Lipozyme TL IM and Novozyme 435 at temperature ranging between 65- 70°C for a period ranging between 8 to 10 hr;
m. separating enzyme by filtration and evaporating the solvent to get the crude product having respective isomer of cetyl myristoleate;
n. purifying the crude product as obtained in step (l) by column chromatography to obtain pure cetyl myristoleate of formula 1.

2. A process as claimed in step (a) claim 1, wherein the organic solvent used in step (a) is dichloromethane or chloroform.

3. A process as claimed in claim 1, wherein oleic acid methyl ester used in step (a) and n-pentyl-triphenyl phosphonium salt used in step (f) for the preparation of cis-9-tetradecenoate.

4. A process as claimed in claim 1, wherein methyl undecenoate used in step (a) and n-butyl-triphenyl phosphonium salt used in step (f) for the preparation of c/s-10-tetradecenoate.
5. A process as claimed in claim 1, wherein hexadecyl c/s-9-tetradecenoate yielded in the range of 88 - 95%.

6. A process as claimed in claim 1, wherein hexadecyl c/s-9-tetradecenoate purity is in the range of 92-95%.

7. A process as claimed in claim 1, wherein hexadecyl cis-10-tetradecenoate yielded in the range of 80 - 90%.

8. A process as claimed in claim 1, wherein hexadecyl cis-10-tetradecenoate purity is in the range of 90-95%.

9. A process as claimed in claim 1 wherein, the isomers hexadecyl cis-9-tetradecenoate and hexadecyl c/s-10-tetradecenoate as prepared are evaluated for blocking inflammation and reduction of adjuvant-induced arthritis in rats.

10. Use of isomers hexadecyl c/s-9-tetradecenoate and hexadecyl c/s-10-tetradecenoate as claimed in claim 1 for blocking inflammation and reduction of adjuvant-induced arthritis.

11. Use of isomers for blocking inflammation as claimed in claim 10, wherein the isomers hexadecyl c/s-9-tetradecenoate and hexadecyl c/s-10-tetradecenoate are effective to reduce the inflammation and edema of the animals equally up to 36% compared to control group at a dose 400 mg/kg body wt.

12. Use of isomers for reduction of adjuvant-induced arthritis activity as claimed in claim 10, wherein the isomers hexadecyl c/s-9-tetradecenoate and hexadecyl c/s-10-tetradecenoate are effective to reduce arthritis equally up to 10 to 15% at the dose of 100 mg/kg body wt.

13. Use of isomers for anti-arthritic activity as claimed in claim 10, wherein the isomer hexadecyl c/s-10-tetradecenoate is effective for 21 days in 80% rats while in 20% rats it is effective for 32 days.
CH₃O·C·(CH₂)₇·CH=CH·(CH₂)₇·CH₃
  i) O₂ / DCM, -78°C, 1 hr
   \[ \text{CH₃O·C·(CH₂)₇·CHO + CH₃·(CH₂)₇·CHO} \]

CH₃·(CH₂)₃·CH₂Ph₃P⁺Br⁻
  n-BuLi/THF, 0°C, 4 hr
  \[ \text{CH₃O·C·(CH₂)₇·CH = CH·(CH₂)₃·CH₃} \]

Cetyl Alcohol
Lypozyrne TL 1M, 65-70°C, 8 hr
CH₃(CH₂)₁₅·O·C·(CH₂)₇·CH=CH·(CH₂)₃·CH₃

Figure 1
Figure 2
Figure 3
Figure 4
Figure 5
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

INV. A61P19/02 A61P29/00 C07C67/08 C07C67/333 C07C67/343
C07C69/533 C07C69/716 A61K31/231 C12P7/62

ADD.

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)
A61Q C07C C12P A61K

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 practical, search terms used)

EPO-Internal , WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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| A        |                                                                                     | 1-8 |

Further documents are listed in the continuation of Box C

See patent family annex

Date of the actual completion of the international search

27 May 2010

Date of mailing of the international search report

04/06/2010

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Authorized officer

Patteux, Claudine

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### INTERNATIONAL SEARCH REPORT

**International application No**: PCT/IN2010/000021

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