TREATMENT FOR LIPODYSTROPHY

Field of the invention

The present invention is related to the development of therapeutic compound for prevention and treatment of lipodystrophy. In particular the invention relates to the development of therapeutic compound for prevention and treatment of lipodystrophy in HIV –infected patients (LDHIV). Specifically, the present invention further provides a suitable composition useful in the treatment or prevention or alleviation of the symptoms of lipodystrophy in HIV infected patients (LDHIV)

Background of the invention

Lipodystrophy is a very dreadful disease and has become a major global health problem. It is a disorder of fat metabolism which causes lipohypertrophy, Lipoatrophy and Metabolic abnormalities. Moreover, lipohypertrophy includes the enlargement of dorsocervical fat pad (commonly called "buffalo hump"), expansion of the circumference of the neck by 5-10 cm, hypertrophy occuring in breast, central truncal adiposity resulting from abdominal visceral fat accumulation, symmetric and asymmetric lipomatoses. A rare pattern of lipoaccumulation involves formation of band like lipomatosis tissue symmetrically from the breasts, laterally to the axillae, Suprapubic fat pads (pubic lipomas) and the development of multiple angiolipomas.

Lipoatrophy includes a temporal wasting and loss of subcutaneous fat from the cheeks (buccal fat pad) which produces an emaciated appearance with prominent nasolabial creases. Further subcutaneous tissue is depleted from the arms, shoulders, thighs, and buttocks (peripheral wasting), with prominence of the superficial veins in these sites.

Metabolic abnormalities include augmentation in cholesterol and triglyceride levels and reduced high-density lipoprotein (HDL) cholesterol levels, Insulin resistance, type 2 diabetes mellitus, and lactic academia.

Lipodystrophy is very commonly associated with the HIV patients who are being treated anti-retroviral medicines. Such medicines can include HIV-1 protease inhibitors (PIs), Nucleoside reverse transcriptase inhibitors (NRTIs), Non-nucleoside Reverse Transcriptase Inhibitors (NNRTIs), Fusion Inhibitors, Entry Inhibitors - CCR5 co-receptor antagonist, HIV integrase strand transfer inhibitors etc. These medicines improve the survival of the patient but also produce lipohypertrophy, Lipoatrophy and other Metabolic abnormalities.

HIV-1 protease inhibitors (PIs) appear to be the strongest link to lipodystrophy in HIV –infected patients LDHIV as it inhibits maturation of sterol response element binding proteins (SREBP), which affect intracellular fatty acid and glucose metabolism and adipocyte differentiation (Mallon et al, *J Infect Dis*, 2005). Furthermore, the PIs also down-regulate peroxisome proliferator-activated receptor gamma (PPAR γ), an important nuclear transcription factor that is affected by SREBPs and is necessary for adipocyte differentiation and function and fatty acid metabolism.

Other factors, such as duration of HIV infection, age, and gender, may also contribute to the risk of development of LDHIV. The molecular basis of LDHIV is still remains unknown and no specific therapy is available for LDHIV.

Reverse transcriptase inhibitors (nRTIs) like stavudine, didanosine and zidovudine may cause mitochondrial toxicity by inhibiting mitochondrial DNA polymerase-γin fat and other tissues and thus interfering with respiratory chain complexes. The result is impaired fatty acid oxidation and intracellular accumulation of triglycerides and lactate

In addition, lipodystrophy is also observed in acute HIV infection, lending support to a direct viral role as well. Potential host risk factors include age, sex, and race or ethnicity. Lipodystrophy is more common in older patients; fat accumulation is more common in women and lipoatrophy in men; and non-Hispanic black patients appear to be at lower risk of lipoatrophy. A genetic component is indicated by a recent analysis in AIDS Clinical Trials Group (ACTG) study 5005s, suggesting either predisposition or protection associated with mitochondrial DNA polymorphisms.
Hulgan et al, *J Infect Dis*, 2008 describes that patients homozygous for C/C at the HFE187 locus (n = 71) had a 0.6-kg and 12.5% loss of limb fat at weeks 48 to 64, with 37 (52%) of the 71 patients diagnosed with clinical lipoatrophy. By comparison, heterozygous patients with HFE187C/G had a 0.2-kg and 6.1% increase in limb fat, with 6 (26%) of 23 patients having clinical lipoatrophy ($P < .05$ for all comparisons).

A number of strategies for reducing central obesity have been investigated such as stopping PI treatment but it is not effective. Changes in diet and exercise have produced improvements, but adherence to a regimen of lifestyle change is difficult for most patients. Liposuction may be applied particularly with dorsocervical fat accumulation, i.e., "buffalo hump".

It is evident from the several studies that thiazolidinediones show no change in VAT (Pathogenesis and treatment of lipodystrophy, vol.16, issue 4, Oct/November,2004)

Testosterone replacement to physiologic levels reduces visceral adipose tissue (VAT), total fat, and abdominal fat and improves insulin sensitivity and lipid profile in older, non–HIV-infected men with upper body obesity and low testosterone levels. In a recent study, 88 HIV-infected men with central obesity (waist circumference >100 cm) and low testosterone levels (< 400 ng/dL) underwent randomization to testosterone as a transdermal gel at a dose of 10 g daily or placebo for 24 weeks (Bhasin et al, J Clin Endocrinol Metab, 2007). The testosterone group had statistically significant reductions in abdominal fat (–1.5% vs +4.3%), abdominal subcutaneous adipose tissue (SAT) (–7.2% vs +8.1%), trunk fat (–9.9% vs +4.6%), and limb fat (–10.1% vs +3.1%); the latter finding is of potential concern in a population predisposed to lipoatrophy. No statistically significant difference in change in VAT (+0.9% vs +2.3%) was observed, and no statistically significant differences were observed in changes in lipid levels, fasting blood glucose levels, insulin levels, or insulin resistance.

Like testosterone, growth hormone (GH) has fat-oxidizing and lipolytic properties. A substantial proportion of HIV patients with central obesity (approximately 30%-40%) have impaired GH biology, including reduced GH mass secretion, reduced response to GH releasing hormone (GHRH) and free fatty acids, and increased somatostatin tone, which suppresses GH. A number of recent studies have assessed GH treatment in HIV patients with fat accumulation. In 1 study, 325 HIV patients with increased waist: hip ratios and increased VAT measurements received.

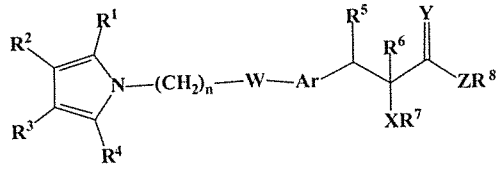
Although, the growth hormone (GH) and GH releasing hormone (GHRH) therapies show some promising result as they have fat-oxidizing and lipolytic properties however, there are limitations to their use. They are parenteral therapies and either expensive (rhGH) or not FDA-approved (tesamorelin). Thus far, there is evidence of waning durability of the reduction in VAT after their discontinuation, short-term increases in insulin resistance with rhGH, and small short-term reductions.

Recent research publications have shown the use of two lipid-lowering classes of drugs, statins and fibrates, antiretroviral switching strategies and use of insulin-sensitising drugs as having some beneficial effect on lipodystrophy. However, no single therapy is able to reach desirable clinical end point for HIV associated lipodystrophy.

Hence it is desirable to develop a compound which can overcome the above discussed drawback associated with prior art and develop a therapy for HIV associated lipodystrophy.

Hypolipidemic agents which are PPAR modulators have been disclosed in WO 91/19702, WO 94/01420, WO 94/13650, WO 95/03038, WO 95/17394, WO 96/04260, WO 96/04261, WO 96/33998, WO 97/25042, WO 97/36579, WO 98/28534, WO 99/08501, WO 99/16758, WO 99/19313, WO99/20614, WO 00/23417, WO 00/23445, WO 00/23451, WO 01/53257.

WO 03009841 discloses compounds of the following general formula



These compounds are reported to be hypolipidaemic agents. This document also discloses sodium and calcium salts of some of the compounds disclosed therein. However, the sodium salts of the compounds of the present invention was difficult to isolate due to rapid degradation while the Calcium salt was poorly absorbed limiting its efficacy and possibility of further development. Further, the calcium salt was also found to degrade on long term storage. It has surprisingly now been found that certain compounds and their selected salts are effective in the treatment of lipohypertrophy, lipoatrophy and metabolic abnormalities in HIV patients.

Embodiments of the invention

In an embodiment the present invention provides a compound of formula (I) suitable for the treatment and prevention of lipodystrophy.

In an embodiment, the conditions associated with lipodystrophy includes the symptoms of lipohypertrophy, lipoatrophy and other metabolic abnormalities.

In another embodiment, the present invention provides a compound of formula (I) for the treatment and prevention or alleviation of symptoms of lipohypertrophy, lipoatrophy and metabolic abnormalities in HIV patient.

In yet another embodiment the present invention provides the administration of compound of formula (I) and their pharmaceutically acceptable salts alone or in combination with other suitable agents as therapeutic agent for the treatment and prevention alleviation of symptoms of lipodystrophy.

In yet another embodiment the present invention provides a suitable composition comprising the compound of formula (I) or their suitable pharmaceutical compositions suitable for the treatment and prevention alleviation of symptoms of lipodystrophy.

In another embodiment, the present invention provides for certain pharmaceutical salts of compound of formula (I).

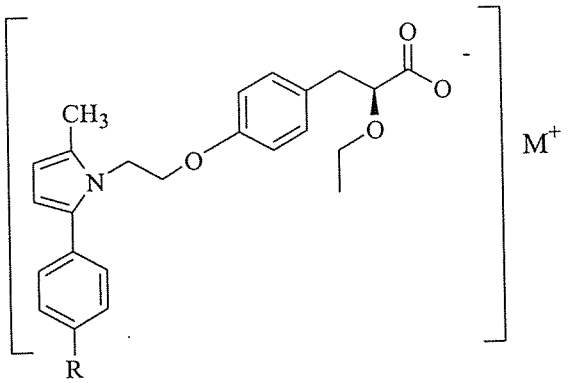
Summary of the invention

The present invention provides a compound of formula (I) and their pharmaceutically acceptable salts for the prevention and treatment or alleviation of symptoms of lipodystrophy. The present invention provides a compound of formula (I) and their pharmaceutically acceptable salts for the prevention and treatment or alleviation of symptoms of lipodystrophy caused either because of HIV infection or due to treatment with anti-retrovirals. Such anti-retrovirals can include HIV-1 protease inhibitors (PIs), Nucleoside reverse transcriptase inhibitors (NRTIs), Non-nucleoside Reverse Transcriptase Inhibitors (NNRTIs), Fusion Inhibitors, Entry Inhibitors - CCR5 co-receptor antagonist, HIV integrase strand transfer inhibitors etc. or combination therapy involving one or more anti-retrovirals. The compound of formula (I) neutralizes lipohypertrophy, lipoatrophy and metabolic abnormalities in HIV patient. Moreover, the present invention also provides a suitable composition comprising compound of formula (I) useful in the treatment or prevention or alleviation of the symptoms of lipodystrophy in HIV infected patients (LDHIV).

In a further embodiment are disclosed certain new salts corresponding to the compound of formula (I) wherein M represents K or Mg.

Description of the Invention

The present invention describes compound of formula (I) which is suitable for the treatment of lipodystrophy or HIV associated lipodystrophy.



(I)

wherein 'R' is selected from hydroxy, hydroxyalkyl, acyl, alkoxy, alkylthio, thioalkyl, aryloxy, arylthio and M^+ represents suitable metal cations such as Na^+, K^+, Ca^{+2}, Mg^{+2}, and the like.

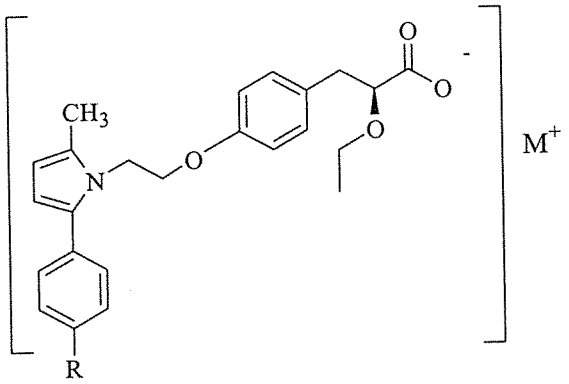
In a preferred embodiment, 'R' represents thioalkyl, alkoxy or hydroxyalkyl group; In a still preferred embodiment, 'R' represents $-SCH_3$ or $-OCH_3$ group.

In an embodiment is provided suitable pharmaceutical composition for the treatment of lipodystrophy or HIV associated lipodystrophy comprising the compound of formula (I). The pharmaceutical composition of the present invention comprises compound of formula (I) along with suitable excipients as defined hereinafter for the treatment of lipodystrophy or HIV associated lipodystrophy.

In another embodiment, the present invention provides a method of treating a subject suffering from lipodystrophy or HIV associated lipodystrophy which comprises treatment of a patient in need of such therapy, with compound of formula (I) or suitable pharmaceutical compositions containing them.

In a further embodiment the present invention provides use of the compound of formula (I) or their suitable pharmaceutical compositions for the treatment of lipodystrophy or HIV associated lipodystrophy.

In an embodiment the present invention provides certain new salts of compound of formula (Ia)



(Ia)

wherein 'R' is selected from hydroxy, hydroxyalkyl, acyl, alkoxy, alkylthio, thioalkyl, aryloxy, arylthio and M^+ represents suitable metal cations selected from K^+, Mg^{+2}.

In a preferred embodiment, 'R' represents thioalkyl and alkoxy or hydroxyalkyl group; In a still preferred embodiment, 'R' represents $-SCH_3$ or $-OCH_3$ group.

In another preferred embodiment, M^+ represents Mg^{+2}.

The effective amount of the said compound of formula (I) is selected from 1 mg to 500 mg preferably 1 mg to 250 mg and more preferably 4 mg to 50 mg. The compound of formula (I) or its suitable salts is administrated orally, intravenously, parentally in the subject who is in need of treatment.

In an embodiment the compound of formula (I) is useful for the treatment or prevention or alleviation of the symptoms of lipodystrophy. In a preferred embodiment the compound of formula (I) is useful in the treatment or prevention or alleviation of the symptoms of HIV associated lipodystrophy. In such embodiment the Lipodystrophy is a disorder of fat metabolism which causes lipohypertrophy lipoatrophy and metabolic abnormalities.

In an embodiment the compound of formula (1) cure or prevent or alleviate at least one symptoms of lipodystrophy including, but not limited to, acting as an agent for lowering &/or control blood glucose levels, an agent used to control lipid levels, e.g., as an agent used to lower control cholesterol, an antioxidant, an appetite suppressing agent, an anti-obesity agent, a probiotic or an anti-inflammatory agent. In another embodiment the the compound of formula (1) cure or prevent or alleviate at least one symptoms of lipodystrophy including, but not limited to triglyceride level, VLDL level and Apo B level in serum. In another embodiment the compound of formula (1) cure or prevent of lipodystrophy by improving at least one of the condition selected from HDL level, Apo A1 level, HOMA of beta cell function derived from c-peptide.

In an embodiment the present invention also provides a suitable pharmaceutical composition of compounds of formula (I) or their derivative. The pharmaceutical composition of the present invention essentially comprises of:

- the pharmaceutically active substance;
- a suitable buffering agent;
- a suitable stabilizer;
- optionally with one or more pharmaceutically acceptable excipients.

The suitable stabilizers used in pharmaceutical composition are selected from Polacrilin potassium, Potassium chloride, Sodium stearyl fumarate and preferably selected from Sodium stearyl fumarate. The suitable buffering agent are selected from

sodium acetate, ammonia solution, ammonium carbonate, sodium borate, adipic Acid, glycine, monosodium glutamate and preferably selected from ammonia solution.

The pharmaceutically acceptable excipients are selected at least one from carriers, binders, antioxidant agents, disintegrating agents, wetting agents, lubricating agents, chelating agents, surface active agents, and the like.

Diluents include, but are not limited to lactose monohydrate, lactose, polymethacrylates selected from Eudragit, potassium chloride, sulfobutylether b-cyclodextrin, sodium chloride, spray dried lactose, and preferably sulfobutyl ether b-cyclodextrin. Carriers include, but are not limited to lactose, white sugar, sodium chloride, glucose, urea, starch, calcium carbonate and kaolin, crystalline cellulose, and silicic acid. Binders include, but are not limited to carbomers selected from carbopol, gellan, gum Arabic, hydrogenated vegetable oil, polymethacrylates selected from Eudragit, xanthan, lactose and Zein. Antioxidant agents include, but are not limited to, Hypophosphorous acid, Sodium formaldehyde, sodium formaldehylde sulfoxylate, sulfur dioxide, tartaric acid, thymol and methionine. Disintegrating agents include, but are not limited to, bicarbonate salt, chitin, gellan gum, polacrillin potassium and Docusate Sodium. Wetting agents include, but are not limited to, Glycerin, lactose, Docusate Sodium and Glycine, Lubricating agents used include, but are not limited to, Glycerin behenate, hydrogenated vegetable oil, sodium stearyl fumarate and Myristic Acid. Chelating agents include, but are not limited to, Maltol and Pentetic Acid. Surface active agents include but are not limited to, Nonionic surfactant selected from alkyl polyglucosides, cocamide DEA, cocamide MBA, cocamide TEA, decyl maltoside and octyl glucoside; Anionic surfactant selected from arachidic acid and arachidonic acid; Cationic surfactant selected from cetyl trimethylammonium bromide and cetylpyridinium chloride.

In an embodiment the formulation is useful for the treatment or prevention or alleviation of the symptoms of lipodystrophy. In a preferred embodiment the said formulation is useful in the treatment or prevention or alleviation of the symptoms of HIV associated lipodystrophy.

Lipodystrophy is a disorder of fat metabolism which causes lipohypertrophy, lipoatrophy and metabolic abnormalities. Moreover, lipohypertrophy includes the enlargement of dorsocervical fat pad (commonly called "buffalo hump"), expansion of the circumference of the neck by 5-10 cm, hypertrophy occurs in breast, Central truncal

adiposity results from abdominal visceral fat accumulation, symmetric and asymmetric lipomatoses. A rare pattern of lipoaccumulation involving bandlike lipomatosis tissue symmetrically from the breasts, laterally to the axillae, suprapubic fat pads (pubic lipomas) and the development of multiple angiolipomas.

Lipoatrophy includes a temporal wasting and loss of subcutaneous fat from the cheeks (buccal fat pad) produces an emaciated appearance with prominent nasolabial creases, subcutaneous tissue is depleted from the arms, shoulders, thighs, and buttocks (peripheral wasting), with prominence of the superficial veins in these sites.

Metabolic abnormalities include augmentation in cholesterol and triglyceride levels and reduced high-density lipoprotein (HDL) cholesterol levels, Insulin resistance, type 2 diabetes mellitus, and lactic academia.

The compounds of the present invention due to their beneficial effect on lipodystrophy, will have beneficial effect on Body fat redistribution (Lioatrophy or Hypertrophy or abnormal distribution), Dyslipidemia, Glucose homeostatis, Pro-inflammatory conditions, impact on morbidity and mortality, impact on quality of life, impact on patient's reported outcomes like self perception etc

Moreover, the precise mechanisms underlying this syndrome are not well understood, several hypotheses based on in vitro and human studies may explain the pathogenesis of the changes. Some experts presently believe that HIV type 1 (HIV-1) protease inhibitors (PIs) and nucleoside reverse transcriptase inhibitors (NRTIs), especially stavudine and zidovudine, are implicated as follows:

(i) decreased production of retinoic acid and triglyceride uptake: PIs have a high affinity for the catalytic site of HIV-1 protease, which shares a 60% sequence homology with 2 proteins involved in lipid metabolism, cytoplasmic retinoic acid–binding protein type 1 (CRABP-1) and low-density lipoprotein receptor–related protein (LDLR-RP). Inhibition of CRABP-1 impairs the production of retinoic acid, leading to decreased fat storage and adipocyte apoptosis with the subsequent release of lipids into the circulation. Inhibition of LDLR-RP results in hyperlipidemia secondary to the failure of hepatic and endothelial removal of chylomicrons and triglycerides from the circulation.

(ii) inhibition of mitochondrial DNA (mtDNA) polymerase gamma: NRTIs inhibit mtDNA polymerase gamma, leading to mtDNA depletion, respiratory chain dysfunction, and reduced energy production, which, in turn, causes insulin

resistance and secondary dyslipidemia. Interestingly, mtDNA is depleted only at normal oxygen levels—hypoxic adipocytes do not take up triglycerides and are resistant to mtDNA-induced damage, except after treatment with NRTIs.

(iii) inhibition of lipid metabolism: Some PIs, particularly ritonavir, inhibit cytochrome P450 3A, a key enzyme in lipid metabolism.

(iv) prevention of the development of adipocytes: Saquinavir, ritonavir, and nelfinavir (all PIs) directly inhibit the development of adipocytes from stem cells and increase the metabolic destruction of fat in existing adipocytes.

In an embodiment the compound of formula (I) or pharmaceutical composition containing the compound of formula (I) cure or prevent or alleviate at least one symptoms of lipodystrophy including, but not limited to, acting as an agent for lowering &/or an agent used to control blood glucose levels, an agent used to control lipid levels, e.g., as an agent used to lower control cholesterol, an antioxidant, an appetite suppressing agent, an anti-obesity agent, an antibiotic/ probiotic or an anti-inflammatory agent.. In another embodiment the pharmaceutical composition cure or prevent or alleviate at least one symptoms of lipodystrophy including, but not limited to triglyceride level, VLDL level and Apo B level in serum. In another embodiment the pharmaceutical composition cure or prevent of lipodystrophy by improving at least one of the condition selected from HDL level, Apo A1 level, HOMA of beta cell function derived from c-peptide.

In another embodiment the compounds according to Formula (I) can be used alone or in combination e.g., as an adjunct therapy, with at least one other therapeutic agent. Compound according to formula (I) can be co-administered with a therapeutic agent used to reduce one or more of the symptoms of lipodystrophy including, but not limited to, an agent used to control blood glucose levels, an agent used to control lipid levels, e.g., an agent used to lower control cholesterol, an antioxidant, an appetite suppressing agent, an anti-obesity agent an antibiotic/ probiotic or an anti-inflammatory agent. Such combination treatment may be adjunct to anti-retroviral therapy. In a preferred embodiment the compound of formula (I) administrated alone or in combination for the treatment of lipohypertrophy, lipoatrophy and Metabolic abnormalities in HIV patient.

The compound of the present invention when M+ represents K, Mg can be prepared by the processes disclosed herein below along with suitable modifications known to a skilled person.

Example 1

Preparation of (S)-α-Ethoxy-4-[2-[-methyl-5-[4-(methylthio)phenyl]-1*H*-pyrrol-1-yl]ethoxy]benzene-propanoic acid ethyl ester

In a dry, 5 L round bottom flask 2.1 L toluene was taken under nitrogen. To this 366.1 g ethyl (S)- α-2-ethoxy-3-(4-hydroxyphenyl)propionate was added at room temperature.

The reaction mixture was stirred under heating, using Dean-stark apparatus, to remove water azeotropically. The reaction mixture was cooled to 50 ^{0}C. To this was added 319 g anhydrous potassium carbonate and stirred at 90-92 ^{0}C for 1 hr. Cooled to 65 ^{0}C and added 500 g 2-(2-methyl-5-(4-(methylthio)phenyl)-1H-pyrrol-1-yl)ethyl methanesulfonate and 22 g tetra butyl ammonium bromide. Reaction mixture was heated to 87-92 ^{0}C and stirred for 46 hrs. Cooled to 70-75 ^{0}C, added 1.5 L toluene, charcoalised using 75 g charcoal and cooled to room temperature. Filtrate washed with alkaline solution, washed with water, dried over sodium sulfate and concentrated under vacuum to obtain (S)-α-Ethoxy-4-[2-[-methyl-5-[4-(methylthio)phenyl]-1H-pyrrol-1-yl]ethoxy]benzene-propanoic acid ethyl ester.

Yield: 650 g, HPLC purity: 84.10 %; % Yield 76.0 %.

Example 2

Preparation of (S)-α-Ethoxy-4-[2-[-methyl-5-[4-(methylthio)phenyl]-1H-pyrrol-1-yl]ethoxy] benzenepropanoic acid magnesium salt.

In a dry, 250 mL round bottom flask 80 mL methanol was taken. To this 20 g (S)-α-ethoxy-4-[2-[-methyl-5-[4-(methylthio)phenyl]-1*H*-pyrrol-1-yl]ethoxy]benzene-propanoic acid ethyl ester was added at room temperature, under nitrogen. To this 1.89 g sodium hydroxide dissolved in 20 mL water was added and stirred at room temperature for 3 hours to complete hydrolysis. Solvent was removed under reduced pressure. 150 mL water was added to concentrate the material. Impurity was removed by solvent washing. To aqueous layer was added 5 g magnesium acetate tetra hydrate (dissolved in 20 mL water) and stirred with for 15 min. Sticky material was extracted with dichloromethane and subsequently add n-heptane to precipitate (S)-α-ethoxy-4-

[2-[-methyl-5-[4-(methylthio)phenyl]-1*H*-pyrrol-1-yl]ethoxy]benzenepropanoic acid magnesium salt. Solid was filtered, and dried.

Yield: 10.3 g; HPLC Purity: 98.32 %; Chiral purity: 97.64 %.

Following the process similar to those described in Examples 1 & 2 the following batches of)-α-Ethoxy-4-[2-[-methyl-5-[4-(methylthio)phenyl]-1H-pyrrol-1-yl]ethoxy] benzenepropanoic acid magnesium salt were prepared.

No.	Batch no.	Input	Output	% Yield	HPLC purity	Chiral purity
1	Example 3	10 g	5.02 g	61.21 %	98.22 %	98.58 %
2	Example 4	10 g	4.97 g	60.68 %	97.91 %	-
3	Example 5	15 g	7.34 g	61.94 %	98.20 %	-
4	Example 6	15 g	8.38 g	67.50 %	99.05 %	
Similar reaction carried out using Magnesium chloride						
5	Example 7	10 g	6.5 g	79.25 %	98.53 %	99.32 %
Similar reaction carried out using Magnesium sulfate						
6	Example 8	10 g	6.8 g	82.91 %	98.5 %	

The present invention further discloses use of said compound of formula (I) or their suitable pharmaceutical compositions for the treatment of lipohypertrophy, lipoatrophy and metabolic abnormalities in HIV patient.

Example 9

(S)-α-Ethoxy-4-[2-[-methyl-5-[4-(methylthio)phenyl]-1H-pyrrol-1-yl]ethoxy] benzenepropanoic acid potassium salt.

In a dry, 250 mL round bottom flask 72 mL ethyl acetate was taken. To this 10 g (S)-(-)α-1-phenylethylamine salt of (S)- α-ethoxy-4-[2-[-methyl-5-[4-(methylthio) phenyl]-1H-pyrrol-1-yl]ethoxy]benzene -propanoic acid was added at room temperature and subsequently 50 mL water and 4.8 mL dilute hydrochloric acid (water 1: 1:35% HCl) was added and stirred at room temperature till solid was dissolved. Layer was separated and organic layer was washed with water, dried over sodium sulfate and solvent removed. 9.2 g oily mass obtained. To this was added 50 mL methanol and stirred under nitrogen. To this was added 1.81 g potassium t-butoxide and was stirred at room temperature for 15 min. Solvent removed and added n-Hexane. Again n-hexane was removed and added methanol. Solvent removed under vacuum.

Hygroscopic material obtained. Dried it under vacuum to get (S)- α-ethoxy-4-[2-[-methyl-5-[4-(methylthio)phenyl]-1H-pyrrol-1-yl]ethoxy]benzenepropanoic acid potassium salt.

Yield- 7.6 g, (92.77 %), HPLC Purity 98.60 %, Chiral purity 99.56 %

Example 10

Title of Study: A Prospective, Multi-Centric, Open-Label, Single Arm Study to Evaluate the Safety and Efficacy of 4 mg of compound of formula (I) in Hypertriglyceridemia in HIV Associated Lipodystrophy.

Objectives: The objective of this study was to evaluate the safety and efficacy of 4 mg of compound of formula (I) in hypertriglyceridemia in HIV associated lipodystrophy.

Methodology: This was a prospective, multi-centric, open-label, single arm study to evaluate the safety and efficacy of 4 mg of compound of formula (I) in hypertriglyceridemia in HIV associated lipodystrophy.

After obtaining informed written consent, subjects with hypertriglyceridemia in HIV associated lipodystrophy, on treatment with HAART for at least 18 months and satisfying the inclusion and exclusion criteria were enrolled in the study. The subjects received 4 mg of compound of formula (I) tablet orally, once daily for a period of 12 weeks. During this 12-week program, safety parameters were assessed at weeks 2, 6, and 12 and the efficacy was evaluated at week 6 and 12.

Number of patients: Planned: 50, Analyzed: 50

Test product: Compound of formula (I)

Dose 4 mg

Duration of treatment: 12 weeks

Mode of administration: Oral

Batch number: EMK328

Criteria for evaluation: **Efficacy**:

The primary efficacy endpoint was to assess the percent change in TG levels from baseline to Week 6 and Week 12. The secondary efficacy endpoint was the assessment of LDL, VLDL, HDL, Non HDL cholesterol, Total cholesterol, Apo A1, Apo B, and C-peptide and fasting insulin for HOMA beta and HOMA IR.

Safety:

Clinical examination and recording of adverse events (AEs) was done on all visits. Electrocardiogram was recorded at screening visit and at Week 12. Urine pregnancy test was conducted at screening visit

Haematological examination included haemoglobin, haematocrit, red blood cell (RBC) count, white blood cell (WBC) count with differential (neutrophils, lymphocytes, monocytes, eosinophils and basophils) and platelet count.

Biochemistry tests included AST, ALT, ALP, total bilirubin, serum proteins, total albumin and globulin, γ- GTT, BUN, Serum creatinine, serum uric acid, CPK, and urine R/Ms (including microalbuminuria and ketonuria).

All laboratory parameters were evaluated at enrolment visit (Week 0) and at Weeks 2, 6, and 12.

Statistical methods

For the efficacy endpoints, treatment effect was evaluated using an analysis of variance (ANOVA) model with factors for baseline and treatment. Treatment effects were estimated using the least-square means (LSM) and 95% confidence intervals (CIs) from the ANOVA model. Statistical significance was defined as a two-sided p-value <0.05. All other secondary endpoints were analyzed using appropriate statistical methods.

For safety analysis the frequency tabulations of abnormal physical examination and abnormal clinical laboratory parameters were presented for each visit. Summary statistics for clinical laboratory parameters and vital signs were presented for each visit. A list of concomitant medications taken during the study period was summarised.

Adverse events were coded using the Medical Dictionary for Regulatory Activities (MedDRA) (Version 14). Adverse events and SAEs were summarized overall, by system organ class (SOC) and by MedDRA preferred term for treatment emergent adverse events (TEAEs). All AEs, including those arising before or after treatment was included in the listings. Separate listings were provided for SAEs and AEs leading to discontinuation from the study.

STUDY DESIGN

This was a safety and efficacy study to evaluate 4 mg of compound of formula (I) in hypertriglyceridemia in HIV associated lipodystrophy. This was exploratory proof of concept study designed to assess the proof of safety and efficacy in intended population. The results of compound of formula (I) from phase II studies in

Dyslipidemia subjects demonstrated that compound of formula (I) 4 mg is well tolerated and effective at once daily dosing. Phase I study demonstrated food significantly affects absorption of compound of formula (I), so drug was recommended to be consumed preferably in fasting condition. Based upon these observations 4 mg once daily in fasted condition was selected for present study

SELECTION OF STUDY POPULATION

Inclusion Criteria

Subjects who satisfied all of the following criteria were eligible for enrolment in the study:

1. Males and females aged 18- 65 years.
2. Confirmed diagnosis of HIV1 and on HAART for at least 18 months.
3. On stable ART regimen for at least 8 weeks prior to inclusion in the study and ART regimen not expected to change in next 3 months.
4. Subjects clinically diagnosed as HIV lipodystrophy (at least 1 moderate or severe lipodystrophy feature identified by doctor and patient, except isolated abdominal obesity)
5. Triglycerides >200 to 500 mg %.
6. CD4 count of >50/mm^3
7. Subject who had given informed consent for participation in this trial.

TREATMENTS

Treatments Administered

The study had a single arm. Subjects received 4 mg of compound of formula (I) orally once daily in the morning before breakfast, for a period of 12 weeks.

Identity of Investigational Product(s)

Compound of formula (I) is divalent magnesium salt of carboxylic acid in the form of white, amorphous powder, which is freely soluble in dimethyl sulfoxide, dichloromethane, slightly soluble in methanol and insoluble in water. The drug was supplied as uncoated tablets of 4 mg of the active ingredient.

Supply from batch no EMK328 was used during the study. The study drug was manufactured and packaged in cGMP facility.

Primary Efficacy Variable(s)

The primary efficacy endpoint was to determine the percent change in TG levels from baseline to Week 6 and Week 12.

Secondary Efficacy Variables

The secondary efficacy endpoint was to determine the percent change in LDL, VLDL, HDL, total cholesterol, non-HDL Cholesterol (measured value), Apo A1, and Apo B, C-peptide and fasting insulin for HOMA beta and HOMA IR levels from baseline to Week 6 and Week 12.

STATISTICAL METHODS PLANNED IN THE PROTOCOL AND DETERMINATION OF SAMPLE SIZE

Statistical and Analytical Plans

The demographic and baseline characteristics were summarized for compound of formula (I) 4 mg treatment arm. For continuous measurements such as age, the mean, median, standard deviation (SD) and range were tabulated. For categorical measurements such as gender, the frequencies were computed.

Efficacy Analyses:

The primary efficacy variable was the reduction in TG at Week 6 and Week 12 of the treatment period compared with baseline. The change from baseline was determined as the difference between the means for the treatment period (Weeks 6 / Weeks 12) and the baseline.

For the efficacy endpoints, treatment effect was evaluated using an analysis of variance (ANOVA) model with factors for baseline and treatment. Treatment effects were estimated using the least-square means (LSM) and 95% confidence intervals (CIs) from the ANOVA model. Statistical significance was defined as a two-sided p-value <0.05.

All other secondary endpoints were analyzed using appropriate statistical methods.

Intent-to-treat (ITT) and/or Per Protocol (PP) analysis were carried out for the study. The PP analysis was considered definitive while the ITT analysis was considered supportive during the trial analysis.

EFFICACY RESULTS AND TABULATIONS OF INDIVIDUAL PATIENT DATA

Analysis of Efficacy

One subject identified as EHT004 in the study, a 35-year-old male, was reported with abnormally low levels of HDL (3.95 mg/L) and LDL (6.25 mg/L) at Visit 1. Though this subject completed the study and was assessable for efficacy, it was decided to exclude this subject from the efficacy analyses. Therefore a total of 49 subjects were analyzed for efficacy.

Primary Endpoints

The percent change from baseline in serum TG levels at Week 6 and Week 12 following compound of formula (I) 4 mg was statistically significant (-40.98±4.89 and -45.11±3.60, respectively [p-value: <0.0001, each]) (Table 1).

Table 1:-Analysis of change in Triglyceride (mg/dL) from baseline by visit

Laboratory Test (Unit)	**Visit**		**COMPOUND OF FORMULA (I) 4 mg (N=49)**
TG (mg/dL)	Visit 1	n	49
		Mean ± SD	301.68 ± 86.99
		Median	275.45
		Minimum	200.10
		Maximum	481.42
	Visit 3(Week 6)	n	49
		Mean ± SD	172.81 ± 106.30
		Median	147.68
		Minimum	42.61
		Maximum	631.08
		Change from Visit 1 (LS Mean ± SE)	-128.87±14.96
		p-values	<0.0001
		% Change from Visit 1 (LS Mean ± SE)	-40.98±4.89
		p-values	<0.0001

Table 1:-Analysis of change in Triglyceride (mg/dL) from baseline by visit			
Laboratory Test (Unit)	**Visit**		**COMPOUND OF FORMULA (I) 4 mg (N=49)**
	Visit 4(Week 12)	n	49
		Mean ± SD	166.97 ± 89.17
		Median	145.91
		Minimum	46.88
		Maximum	387.69
		Change from Visit 1 (LS Mean ± SE)	-134.71±10.78
		p-values	<0.0001
		% Change from Visit 1 (LS Mean ± SE)	-45.11±3.60
		p-values	<0.0001

Key to abbreviations: LSM = least square means; N = number of subjects in the treatment group; n = number of subjects having non-missing baseline and post-baseline values; SD = standard deviation; SE = standard error; TG = triglycerides.

Note: p–values <0.05 indicates significant and from ANOVA model

Secondary Endpoints

HDL Cholesterol:

There was an increase in the HDL cholesterol levels following administration of compound of formula (I) 4 mg. The percent change from baseline in HDL cholesterol following compound of formula (I) 4 mg at Week 6 and Week 12 was statistically significant (29.92±5.73 and 34.56±6.13, respectively [p-value: <0.0001 each]) (Table 2).

Table 2: Analysis of change in HDL Cholesterol (mg/dL) from baseline by visit			
Laboratory Test (Unit)	**Visit**		**COMPOUND OF FORMULA(I) 4 mg (N=49)**
HDL Cholesterol (mg/dL)	Visit 1	n	49
		Mean ± SD	35.27 ± 7.85
		Median	34.52
		Minimum	22.23
		Maximum	49.90
	Visit 3(Week 6)	n	49
		Mean ± SD	44.44 ± 14.04
		Median	43.36
		Minimum	20.13
		Maximum	73.50
		Change from Visit 1 (LS Mean ± SE)	9.17±1.99
		p-values	<0.0001
		% Change from Visit 1 (LS Mean ± SE)	29.92±5.73
		p-values	<0.0001
	Visit 4(Week 12)	n	49
		Mean ± SD	46.14 ± 14.84
		Median	47.70
		Minimum	17.61
		Maximum	82.89
		Change from Visit 1 (LS Mean ± SE)	10.87±2.08
		p-values	<0.0001
		% Change from Visit 1 (LS Mean ± SE)	34.56±6.13

Table 2: Analysis of change in HDL Cholesterol (mg/dL) from baseline by visit			
Laboratory Test (Unit)	**Visit**		**COMPOUND OF FORMULA(I) 4 mg (N=49)**
		p-values	<0.0001
Key to abbreviations: LSM = least square means; N = number of subjects in the treatment group; n = number of subjects having non-missing baseline and post-baseline values; SD = standard deviation; SE = standard error; HDL = high density lipoprotein. Note: p–values <0.05 indicates significant and from ANOVA model			

C-peptide HOMA of Insulin Resistance:

There was an increase in insulin resistance after treatment with compound of formula (I). The percent change in HOMA IR from baseline following administration of compound of formula (I) 4 mg at Week 6 and Week 12 was statistically significant (27.87±4.22 and 58.29±5.74 respectively[p-value: <0.0001 each]) (Table 3).

Table 3: Analyses of change in HOMA of insulin resistance for C-Peptide from baseline by visit			
Laboratory Test (Unit)	**Visit**		**COMPOUND OF FORMULA(I) 4 mg (N=49)**
Homa of Insulin Resistance for C-Peptide	Visit 1	n	49
		Mean ± SD	1.59 ± 0.82
		Median	1.40
		Minimum	0.50
		Maximum	3.80
	Visit 3(Week 6)	n	49
		Mean ± SD	1.86 ± 0.77
		Median	1.70
		Minimum	0.90
		Maximum	3.60

Table 3: Analyses of change in HOMA of insulin resistance for C-Peptide from baseline by visit			
Laboratory Test (Unit)	**Visit**		**COMPOUND OF FORMULA(I) 4 mg (N=49)**
		Change from Visit 1 (LS Mean ± SE)	0.27±0.05
		p-values	<0.0001
		% Change from Visit 1 (LS Mean ± SE)	27.87±4.22
		p-values	<0.0001
	Visit 4(Week 12)	n	49
		Mean ± SD	2.15 ± 0.62
		Median	2.10
		Minimum	1.10
		Maximum	3.60
		Change from Visit 1 (LS Mean ± SE)	0.56±0.05
		p-values	<0.0001
		% Change from Visit 1 (LS Mean ± SE)	58.29±5.74
		p-values	<0.0001

Key to abbreviations: HOMA: homeostasis model assessment, IR: insulin resistance, LSM = least square means; N = number of subjects in the treatment group; n = number of subjects having non-missing baseline and post-baseline values; SD = standard deviation; SE = standard error

Note: p–values <0.05 indicates significant and from ANOVA model

Insulin (fasting):

There was an increase in insulin resistance after treatment with compound of formula (I). The percent change in Insulin from baseline following administration of

compound of formula (I) 4 mg at Week 6 and Week 12 was statistically significant (23.71±3.55 and 47.10±4.21 respectively [p-value: <0.0001 each]) (Table 4).

Table 4: Analyses of change in Insulin (fasting) from baseline by visit			
Laboratory Test (Unit)	**Visit**		**COMPOUND OF FORMULA (I) 4 mg (N=49)**
Insulin (fasting) µu/mL	Visit 1	n	49
		Mean ± SD	9.21 ± 6.26
		Median	7.40
		Minimum	2.65
		Maximum	28.06
	Visit 3(Week 6)	n	49
		Mean ± SD	10.42 ± 5.74
		Median	8.35
		Minimum	2.14
		Maximum	26.82
		Change from Visit 1 (LS Mean ± SE)	1.21±0.22
		p-values	<0.0001
		% Change from Visit 1 (LS Mean ± SE)	23.71±3.55
		p-values	<0.0001
	Visit 4(Week 12)	n	49
		Mean ± SD	11.40 ± 4.45
		Median	10.18
		Minimum	5.93
		Maximum	24.29
		Change from Visit 1 (LS Mean ± SE)	2.20±0.21
		p-values	<0.0001
		% Change from Visit 1 (LS Mean ± SE)	47.10±4.21
		p-values	<0.0001

Table 4: Analyses of change in Insulin (fasting) from baseline by visit			
Laboratory Test (Unit)	**Visit**		**COMPOUND OF FORMULA (I) 4 mg (N=49)**
Key to abbreviations: LSM = least square means; N = number of subjects in the treatment group; n = number of subjects having non-missing baseline and post-baseline values; SD = standard deviation; SE = standard error Note: p–values <0.05 indicates significant and from ANOVA model			

Insulin HOMA of Beta-cell Function:

There was an increase in HOMA of Beta-cell function derived from Insulin after treatment with compound of formula (I). The percent change in the HOMA of Beta-cell function derived from Insulin from baseline at Week 6 and Week 12 was statistically significant (52.50±14.94 and 45.64±6.22, respectively [p-value: 0.0010 and <0.0001, respectively])(Table 5).

Table 5: Analyses of change in HOMA of Beta Cell Function for Insulin from baseline by visit			
Laboratory Test (Unit)	**Visit**		**COMPOUND OF FORMULA(I) 4 mg (N=49)**
HOMA of Beta Cell Function for Insulin	Visit 1	n	48
		Mean ± SD	107.82 ± 52.85
		Median	97.25
		Minimum	10.20
		Maximum	234.50
	Visit 3(Week 6)	n	49
		Mean ± SD	136.41 ± 76.00
		Median	116.50
		Minimum	34.90
		Maximum	348.00
		Change from Visit 1 (LS Mean ± SE)	29.55±8.76
		p-values	0.0015
		% Change from Visit 1 (LS Mean ± SE)	52.50±14.94

Table 5: Analyses of change in HOMA of Beta Cell Function for Insulin from baseline by visit			
Laboratory Test (Unit)	**Visit**		**COMPOUND OF FORMULA(I) 4 mg (N=49)**
		p-values	0.0010
	Visit 4(Week 12)	n	49
		Mean ± SD	137.56 ± 46.11
		Median	125.60
		Minimum	9.80
		Maximum	273.30
		Change from Visit 1 (LS Mean ± SE)	30.78±4.25
		p-values	<0.0001
		% Change from Visit 1 (LS Mean ± SE)	45.64±6.22
		p-values	<0.0001

Key to abbreviations: HOMA: homeostasis model assessment, LSM = least square means; N = number of subjects in the treatment group; n = number of subjects having non-missing baseline and post-baseline values; SD = standard deviation; SE = standard error

Note: p–values <0.05 indicates significant and from ANOVA model

Efficacy Conclusions

Primary endpoint:

- There was a statistically significant reduction from baseline in serum TG levels at Week 6 and Week 12 following compound of formula (I) 4 mg (percent change of -40.98±4.89 and -45.11±3.60, respectively [p value: <0.0001, each])

Secondary Endpoints:

- There was no statistically significant change in the non-HDL cholesterol levels from baseline following administration of compound of formula (I) 4 mg at Week 6 and Week 12 (p-values: 0.3963 and 0.4646, respectively)
- There was a statistically significant increase in the HDL cholesterol levels from baseline following administration of compound of formula (I) 4 mg at Week 6

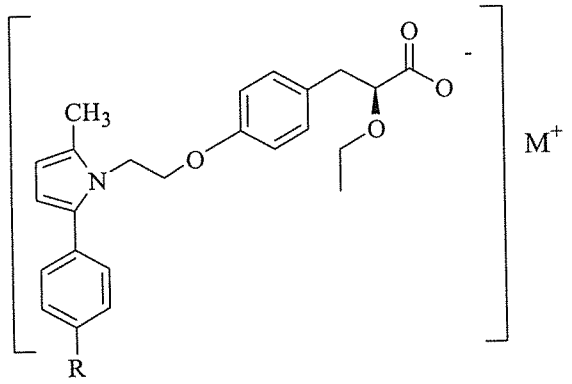
and Week 12 (percent change: 29.92±5.73 and 34.56±6.13, respectively [p-value: <0.0001 each]).

- There was a statistically significant increase in the HOMA of Beta-cell function derived from C-peptide from baseline following administration of compound of formula (I) 4 mg at Week 6 and Week 12 (68.25±25.58 and 71.67±16.20, respectively [p-value: 0.0104 and <0.0001, respectively]).
- There was a statistically significant increase in the HOMA of insulin resistance derived from insulin from baseline after treatment with compound of formula (I) at Week 6 and Week 12 (percent change: 29.10±3.94 and 42.65±3.79, respectively [p-value: <0.0001 each]).

Therefore, the compound of the present invention including pharmaceutical compositions containing the same was found to be useful for the treatment of lipohypertrophy, lipoatrophy and Metabolic abnormalities in HIV patients.

We claim,

1. A method for the treatment of lipodystrophy which comprises administering a compound of formula (I)



(I)

wherein 'R' is selected from hydroxy, hydroxyalkyl, acyl, alkoxy, alkylthio, thioalkyl, aryloxy, arylthio and M^+ represents suitable metal cations selected from Na^+, K^+, Ca^{+2}, Mg^{+2} to a patient in need thereof.

2. The method as claimed in claim 1, wherein 'R' represents thioalkyl, alkoxy or hydroxyalkyl group.
3. The method as claimed in claim 1 wherein 'R' represents $-SCH_3$ or $-OCH_3$ group.
4. The method as claimed in claim 1 wherein M^+ represents Mg^{+2}.
5. The method as claimed in claim 1 wherein the lipodystophy is HIV associated lipodystrophy.
6. The method as claimed in any one of the preceding claims wherein the HIV associated lipodystrophy causes lipohypertrophy or lipoatrophy or metabolic abnormality.
7. Use of the compound of formula (I) as claimed in any one of the preceding claims for the preparation of a medicament for the treatment of HIV associated lipodystrophy.
8. Use of the compound of formula (I) as claimed in any one of the preceding claims for the preparation of a medicament for the treatment of lipohypertrophy or lipoatrophy or metabolic abnormality.
9. Use of the compound of formula (I) as claimed in any one of the preceding claims wherein the compound is administrated in a daily dosage range selected from 1 mg to 500 mg given singly or in multiple divided dosage.

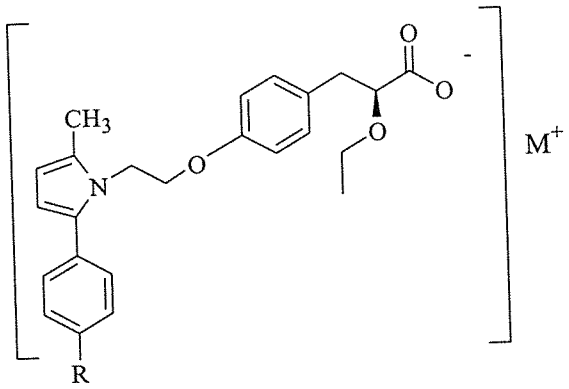
10. The daily dosage as claimed in claim 9 which is in the range of 4 mg to 250 mg given singly or in multiple divided dosage.
11. The daily dosage as claimed in claim 9 & 10 which is in the range of 4 mg to 50 mg.
12. The method of treatment or use of compound of formula (I) as claimed in preceding claims which is administrated in combination with other suitable therapeutic agent.
13. A pharmaceutical composition for the treatment of lipodystophy comprising

a) The compound of formula (I) or its salt thereof as claimed in claim 1;
b) a suitable stabilizer;
c) a suitable buffering agent;
d) optionally, with one or more pharmaceutically acceptable excipients.

14. The pharmaceutical composition as claimed in claim 13 wherein the suitable stabilizer is selected from Polacrilin potassium, Potassium chloride and Sodium stearyl fumarate.
15. The pharmaceutical composition as claimed in claim 13 wherein the suitable stabilizer is preferably selected from Sodium stearyl fumarate.
16. The pharmaceutical composition as claimed in claim 13 wherein the suitable buffering agents are selected from Sodium acetate, Ammonia solution, Ammonium carbonate, Sodium borate, Adipic Acid, Glycine, monosodium glutamate.
17. The pharmaceutical composition as claimed in claim 16 wherein the suitable buffering agent is preferably selected from Ammonia solution.
18. The method for treating HIV associated lipodystrophy in a patient in need of such treatment comprising providing to such a patient the pharmaceutical composition as claimed in claim 13.
19. The method as claimed in claim 19 wherein the HIV associated lipodystrophy causes lipohypertrophy or lipoatrophy or metabolic abnormality.
20. Use of the pharmaceutical composition as claimed in any one of the preceding claims for the preparation of medicament for the treatment of HIV associated lipodystrophy.
21. Use of the pharmaceutical composition as claimedin any one of the preceding claims for the treatment of medicament for the treatment of lipohypertrophy or lipoatrophy or metabolic abnormality

22. The pharmaceutical composition as claimed in any one of the preceding claims in combination with another suitable agent selected from an agent used to control blood glucose levels, an agent used to control lipid levels, an agent used to lower or control cholesterol, an antioxidant, an appetite suppressing agent, an anti-obesity agent a probiotic or an anti-inflammatory agent.

23. The compound of formula (Ia)



(Ia)

wherein 'R' is selected from hydroxy, hydroxyalkyl, acyl, alkoxy, alkylthio, thioalkyl, aryloxy, arylthio and M^+ represents suitable metal cations selected from K^+, Mg^{+2}.

24. The compound as claimed in claim 23, wherein 'R' represents thioalkyl, alkoxy or hydroxyalkyl group.

25. The compound as claimed in claim 23 wherein 'R' represents $-SCH_3$ or $-OCH_3$ group.

26. The compound as claimed in claim 23 wherein M^+ represents Mg^{+2}.

27. The compound as claimed in claims 23-26 for the treatment lipodystrophy.

28. The compound as claimed in claim 27 wherein the lipodystrophy is HIV associated lipodystrophy.

29. The compound as claimed in any one of the preceding claims which reduces the concentration of triglyceride, Very low density lipoprotein, Apo B level.

30. The compound as claimed in any one of the preceding claims which increases high density lipoprotein, Apo A1 level.

31. A pharmaceutical composition comprising compound of formula (Ia) for the treatment of lipodystrophy.

32. The pharmaceutical composition as claimed in claim 31 wherein the lipodystrophy is HIV associated lipodystrophy.