(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
21 May 2004 (21.05.2004)

PCT

(10) International Publication Number
WO 2004/041923 A2

(51) International Patent Classification[7]: C08L

(21) International Application Number:
PCT/IN2003/000354

(22) International Filing Date:
4 November 2003 (04.11.2003)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
963/MUM/2002 5 November 2002 (05.11.2002) IN

(71) Applicant *(for all designated States except US)*: **KOPRAN RESEARCH LABORATORIES LIMITED** [IN/IN]; Parijat House, 1076, Dr E Moses Road, Worli, Mumbai 400 018, Maharashtra (IN).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **DESHPANDE, Jayant, Venkatesh** [IN/IN]; Kopran Research Laboratories Limited, D-28/2, TTC Industrial Area, MIDC. Turbhe, Navi Mumbai 400 705, Maharashtra (IN). **KADAM, Vaishali, Madhukar** [IN/IN]; Kopran Research Laboratories Limited, D-28/2, TTC Industrial Area, MIDC, Turbhe, Navi Mumbai 400 705, Maharashtra (IN). **GUPTE, Vandana, Sandeep** [IN/IN]; Kopran Research Laboratories Limited, D-28/2, TTC Industrial Area, MIDC, Turbhe, Navi Mumbai 400 705, Maharashtra (IN). **RANBHAN, Kamlesh, Jayantilal** [IN/IN]; Kopran Research Laboratories Limited, D-28/2, TTC Industrial Area, MIDC. Turbhe, Navi Mumbai 400 705, Maharashtra (IN).

(74) Agents: **JHUNJHUNWALA, Ram , N.** et al.; Khaitan & Co, Meher Chambers, 4th & 5th Floors, R K Marg, Ballard Estate, Mumbai 400 038, Maharashtra (IN).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

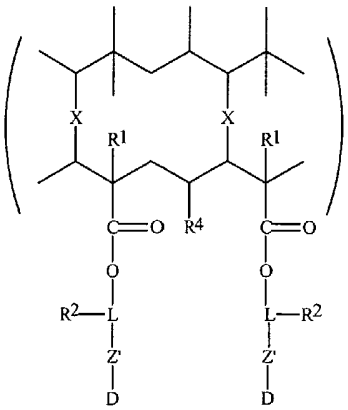
(84) Designated States *(regional)*: ARIPO patent (BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

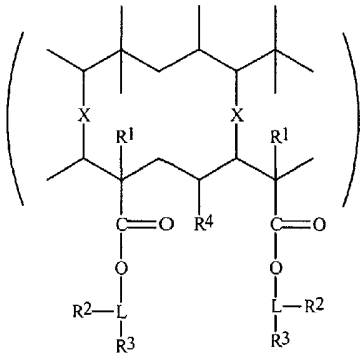
— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii)) for the following designations AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW, ARIPO patent (BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK,*

[Continued on next page]

(54) Title: CHEMOENZYMATICALLY HYDROLYSABLE BIOLOGICALLY ACTIVE COMPOUNDS



(VI)



(IV)

(57) Abstract: Chemoenzymatically hydrolysable biologically active compounds of the Formula (VI) and pharmaceutically acceptable acid addition salts and enantiomers thereof. Also process for the preparation of compounds of the Formula (VI) comprising condensation of a biologically active agent having functional groups such as Formula (1) with a reactive polymer of the Formula (IV) in polar solvent at 20-90°C and pH 2-10.

WO 2004/041923 A2

EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG)

— *of inventorship (Rule 4.17(iv)) for US only*

Published:

— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

TITLE OF INVENTION

Chemoenzymatically hydrolysable biologically active compounds

FIELD OF THE INVENTION

This invention relates to chemoenzymatically hydrolysable biologically active compounds.

This invention also relates to process for the preparation of the chemoenzymatically hydrolysable biologically active compounds.

PRIOR ART

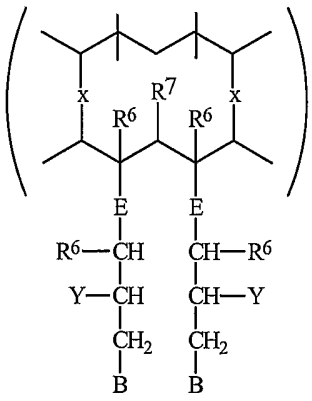
Biologically active agents substituted with polymers by covalent conjugation are reported to show therapeutic activity. For instance, US Patent No. 5162307 describes polymeric inhibitors of the enzyme elastase having the Formula P –L – R, where P is a non-biodegradable polymer, L is a covalent bond or a linker group and R is a peptide. Therapeutic peptides conjugated to polyethylene glycol chains are reported to show improved durability and reduced antigenicity (US Patent No. 5183660). Anion-binding hydrophilic epichlorohydrin and 1-(3-aminopropyl) imidazole copolymeric bile acid sequestrant and its pharmaceutical compositions are reported for use in the treatment of various ailments like diarrhoea, constipation, dumping syndrome or irritable bowel syndrome in US Patent No. 5900233. Polymer analogues of cis-dichlorodiamine platinum are reported for use as antineoplastic agents {"Organometallic polymers as drugs and drug delivery systems" by Gebelein G. G., Koblitz F. K., Biomedical and Dental Applications of polymers, New York, Plenum Press 1981, p 215]. PCT Publication No. WO 99/63940 discusses low molecular weight polymeric derivatives of benzimidazoles as antiulcer agents. The molecular weight of such polymers is generally in the range of 1000 – 10,000. Such polymeric drugs get absorbed from the gastro intestinal tract and elicit systemic activity. The above polymeric drugs are non-biodegradable in physiological fluid and are excreted from the body.

Polymer substituted biologically active compounds have been used as prodrugs. For instance US Patent No. 5372807 describes an intravenous formulation comprising an antifibrotic agent linked to a cis-4-hydroyl-L-proline polymer. US Patent No. 5622718 describes an alginate conjugated with antineoplastic agent such as daunomycin or doxorubicin via an acid labile biodegradable spacer linkage. US Patent No. 6011008 describes water-soluble conjugates of a polysaccharide and an unoxidised, oxidation-sensitive substance, conjugated via amine or

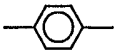
imine bonds. US Patent No. 4587046 describes biologically active drug such as catecholamine hormones coupled to carrier molecules like monodisperse peptides. US Patent Publication No. 20010031262 describes polylactide-CO-glycolide copolymers in the form of particles or a gel, lipid vesicles or liposomes, which are stabilized or targeted to enhance the delivery of antigens. US Patent No. 6254854 describes biodegradable porous particles incorporating a therapeutic agent which may be effectively aerosolized for administration to the respiratory tract to permit systemic or local delivery of the therapeutic agent. These biodegradable particles are formed of a functionalized polyester graft copolymer consisting of a linear α–hydroxy-acid polyester backbone having an amino acid group incorporated therein and polyamino acid side chain extending from an amino acid group in the polyester backbone. Chlorambucil i.e. 4-[4-(bis(2-chloroethyl)amino phenyl) butyric acid] has been bound to vinylpyrrolidone and vinylamine copolymers via an amide bond (Makromol., Chem., by Franzmann and Ringsdorf, 177, 2547, 1976). Deacetylcolchicine or daunomycin is known to be bound to polymers of N-(2-hydroxypropyl)methacrylamide. (Synthese und Untersuchung von potentiell spaltbaren spacergruppen zur Polymer-fixierung von NOR-stickstoff-LOST und den Anthracyclinen Daunomycin und Adriamycin, Ph.D Thesis, Johannes Guttenberg University Mainz, FRG 1982). Daunomycin has also been attached to polymeric carriers to form amino sugar daunosamine. (Shih et. al, 1991, Cancer Res. 51: 4192). Polymers like poly[N-2 hydroxypropyl) methacrylamide] containing hydroxyl groups activated by BrCN have been used to bind insulin (Sung Wan Kim et al in Polymeric Drug Delivery Systems, Drug Design, Volume X, Academic Press, 1980). Activated 4-alkylthioderivatives of cyclophosphamide bound to DIVEMA (divinyl ether and maleic anhydride) copolymer via the anhydride groups are reported (Hirano et. al., Cancer Res. 40:2263, 1980). Oligopeptide sequences can be incorporated into N-(2-hydroxypropyl) methacrylamide copolymers, which have been reported to serve as potential drug attachment/ release sites. Progesterone has been conjugated with aliphatic polyesters such as poly-(ε-Caprolactone), poly[ε-(+,-)-Calactone], polypivalolactone and poly –(+,-)- dilactide through an ester linkage [Biomed. Mater. Res., Pitt et al, 1979, 13, 491; "Polymer conjugates with Anticancer Activity", Advances in Polymer Science, D Putnam et al, 1995, Vol. 122, page 55 – 123, Springer Verlag Berlin]. US Patent No. 4587046 describes covalent conjugation of naturally occurring catecholamines and autocoid moieties with monodisperse amino acid polymers or peptides having an alkyl group through ester/ amide linkages. US Patent No. 5783178 describes conjugation of vinca alkaloids, mitomycins, bleomycins, fluconazole, amphotericin B, paclitaxel derivatives, cytokines, erythroprotein or polynucleotides with block copolymer of ethyleneoxy monomer or a mixture of ethyleneoxy

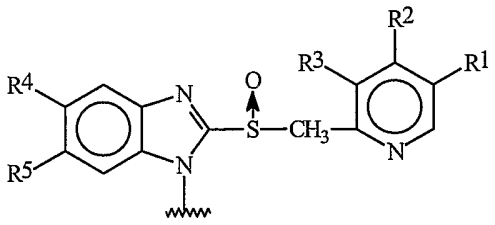
and the $-OCH(CH_3)CH_2-$ monomers through bifunctional linking group. US Patent No. 5510418 describes covalent conjugation of glycosaminoglycan with polyethylene glycol through an ether linkage and is useful for hard/ soft tissue augmentation. Biphenylamine derivatives have been conjugated with polymethacrylic acid (Baker et. at., J. Pharm. Sci. 68, 20 1979) US Patent No. 5889078 describes conjugates of cytostatic fluoro uracil with homopolymer of acrylic acids through ester or amide linkages. US Patent No. 5037883 describes conjugate of anticancer daunomycin with copolymer of N-(2-hydroxypropyl) acrylamide, N-methacrylamide, N-methacrylic acid and/ or N-methacryloylated amino acid through peptide group. US Patent No. 5976527 describes conjugates of proteins such as albumin, immunoglobulins, blood clotting factors and peptide hormones with polymethylmethacrylate or polymethacrylamide comprising reactive oxirane groups, which after immobilisation are used for interaction with biological systems. These conjugates on administration, under physiological pH and influence of enzymes, are cleaved/ hydrolysed at the point of attachment of the polymer to the biologically active agent to release the drug in the original chemical form.

There is described in our PCT Publication No. WO 01/62248, orally administrable acid stable polymer substituted antiulcer benzimidazoles of the Formula I:



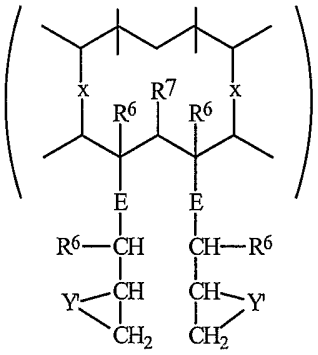
Formula I

wherein R^6 = H or CH_3, X = $-OCOCH_2COO-$,  or - $CONHCH_2NHCO-$, R^7 = H, CH_3, C_2H_5 or $CONH_2$, Y = OH or NH_2, E = -COO- and B is benzimidazole moiety of the Formula II:



Formula II

wherein each of R^1, R^2, R^3, R^4, R^5 = H, C_{1-12} alkyl, C_{6-12} (un) substituted aryl, C_{1-8} alkoxy, C_{6-12} aryloxy, C_{1-5} alkoxy carbonyl, C_{6-12} aryloxy carbonyl, C_{1-5} alkoxy alkyl, C_{6-12} alkoxyaryl, C_{1-5} haloalkyl, C_{1-5} alkyl or C_{6-12} aryl thioethers, (un)substituted amines or diamines, (un) substituted amides, halo, cyano, nitro, carboxylic acid or carbocyclic or O, N, S containing heterocyclic ring systems or enantiomers thereof. The polymeric benzimidazoles of the Formula I are formed by condensing an antiulcer benzimidazole and a biocompatible partially orally biodegradable synthetic crosslinked polymer of the Formula III:



Formula III

wherein R^6, R^7, X and E each is as defined above and Y' = O or N. On oral administration, cleavage of the polymeric benzimidazole takes place in the gastrointestinal fluid under the influence of enzymes/ chemicals, at the hydrolysable group "E" to release N-substituted benzimidazole derivatives (the benzimidazole along with a part of the polymer), which are acid stable.

OBJECTS OF INVENTION

An object of the invention is to provide chemoenzymatically hydrolysable biologically active compounds capable of undergoing rapid chemoenzymatic hydrolysis to release hydroxy alkyl derivatives thereof.

Another object of the invention is to provide chemoenzymatically hydrolysable biologically active compounds capable of undergoing rapid chemoenzymatic hydrolysis to release hydroxy alkyl derivatives thereof showing improved lipophilicity.

Another object of the invention is to provide chemoenzymatically hydrolysable biologically active compounds capable of undergoing rapid chemoenzymatic hydrolysis to release hydroxy alkyl derivatives thereof showing high polarity.

Another object of the invention is to provide chemoenzymatically hydrolysable biologically active compounds capable of undergoing rapid chemoenzymatic hydrolysis to release hydroxy alkyl derivatives thereof showing high optical purity.

Another object of the invention is to provide chemoenzymatically hydrolysable biologically active compounds capable of undergoing rapid chemoenzymatic hydrolysis to release hydroxy alkyl derivatives thereof showing improved bioavailability and bio-efficacy and reduced side effects.

Another object of the invention is to provide a process for the preparation of chemoenzymatically hydrolysable biologically active compounds capable of undergoing rapid chemoenzymatic hydrolysis to release hydroxy alkyl derivatives thereof.

Another object of the invention is to provide a process for the preparation of chemoenzymatically hydrolysable biologically active compounds capable of undergoing rapid chemoenzymatic hydrolysis to release hydroxy alkyl derivatives thereof showing improved lipophilicity.

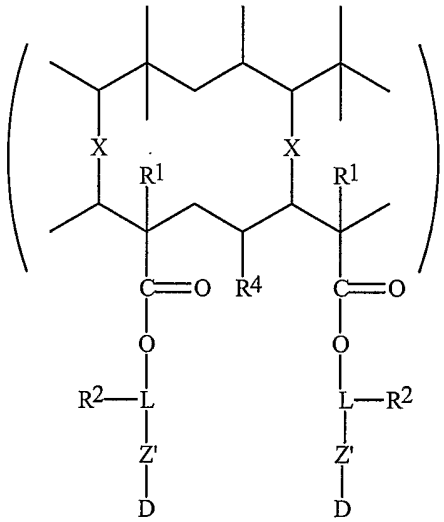
Another object of the invention is to provide a process for the preparation of chemoenzymatically hydrolysable biologically active compounds capable of undergoing rapid chemoenzymatic hydrolysis to release hydroxy alkyl derivatives thereof showing high polarity.

Another object of the invention is to provide a process for the preparation of chemoenzymatically hydrolysable biologically active compounds capable of undergoing rapid chemoenzymatic hydrolysis to release hydroxy alkyl derivatives thereof showing high optical purity.

Another object of the invention is to provide a process for the preparation of chemoenzymatically hydrolysable biologically active compounds capable of undergoing rapid chemoenzymatic hydrolysis to release hydroxy alkyl derivatives thereof showing improved bioavailability and bioefficacy and reduced side effects.

DESCRIPTION OF INVENTION

According to the invention there is provided chemoenzymatically hydrolysable biologically active compounds of the Formula VI:

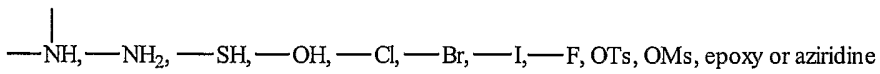


Formula VI

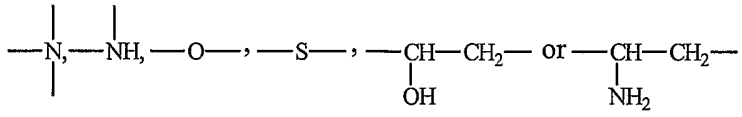
wherein R^1 = H or CH_3, R^2 = H, C_{1-8} alkyl or C_{6-12} aryl,

R^4 = $CONH_2$,–$COOR^6$ (R^6 = H or C_{1-6} alkyl) or CN

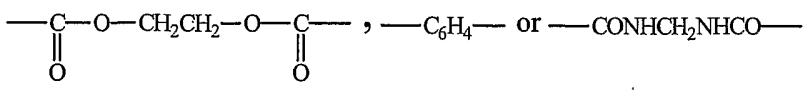
D = Biologically active agent having functional groups such as



and Z' =

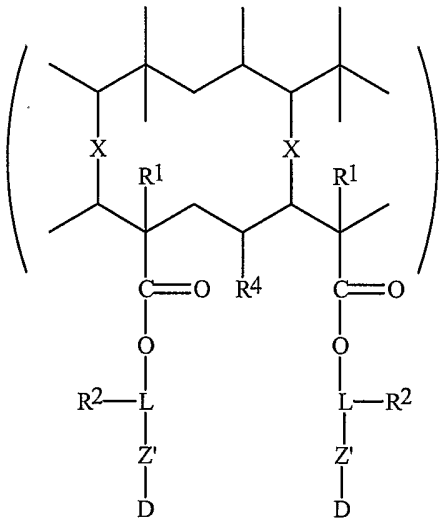


X represents a cross linking group such as


which is optional

L = spacer comprising (un)substituted alkyl, hydroxyalkyl or alkoxy alkyl (having carbon chain length with more than one carbon atom when R^3 = epoxy or aziridine) and pharmaceutically acceptable acid addition salts and enantiomers thereof.

According to the invention there is also provided a process for the preparation of chemo enzymatically hydrolysable biologically active compounds of the Formula VI:

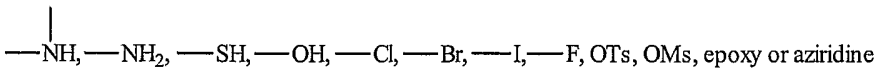


Formula VI

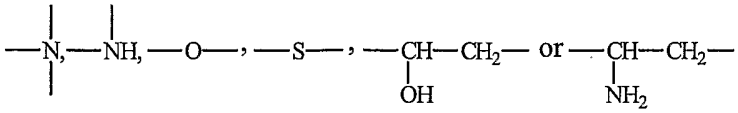
wherein R^1 = H or CH_3, R^2 = H, C_{1-8} alkyl or C_{6-12} aryl,

R^4 = $CONH_2$,–$COOR^6$ (R^6 = H or C_{1-6} alkyl) or CN

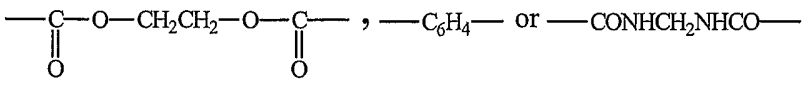
D = Biologically active agent having functional groups such as



and Z' =

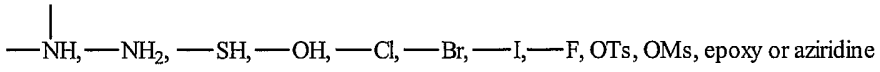


X represents a cross linking group such as

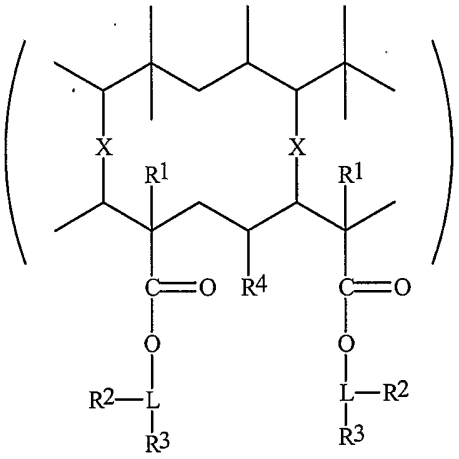

which is optional

L = spacer comprising (un)substituted alkyl, hydroxyalkyl or alkoxy alkyl (having carbon chain length with more than one carbon atom when R^3 = epoxy or aziridine) and pharmaceutically acceptable acid addition salts and enantiomers thereof, the process comprising,

condensing a biologically active agent having functional groups such as



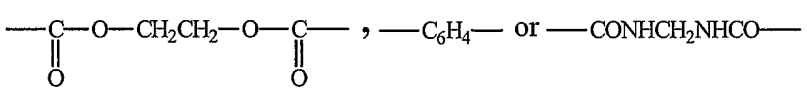
with a reactive polymer of the Formula IV



Formula IV

wherein R^1 = H or CH_3, R^2 = H, C_{1-8} alkyl or C_{6-12} aryl,

X represents a cross linking group such as



which is optional

R^3 = Cl, Br, I, F, OTs, OMs, p-nitrobenzene sulphonate, OSO_2CF_3, OH, NH_2, NHR^5 (R^5=alkyl), SH, epoxide or aziridine,

R^4 = $CONH_2$,–$COOR^6$ (R^6 = H or C_{1-6} alkyl) or CN

L = spacer comprising (un)substituted alkyl, hydroxyalkyl or alkoxy alkyl (having carbon chain length with more than one carbon atom when R^3 = epoxy or aziridine) in a polar solvent at 20-90^{0}C and pH 2-10, cooling the reaction mixture to ambient temperature, isolating the biologically active compound followed by drying and if desired, converting the resulting biologically active compound into pharmaceutically acceptable acid addition salts and enantiomers thereof by known methods.

Preferably in the formula IV R^1 is CH_3, R^2 is H and R^3 is I or OT_s and R^4 is COOH and in the formula VI R^1 is CH_3 and R^2 is H and R^4 is COOH.

The biologically active agents may be antibacterial such as, Ciprofloxacin; antiamoebic such as secnidazole; antifungal such as fluconazole or 2-mercaptobenzothiazole; antithelmintic such as albendazole; antitubercular such as ethambutol; anti-inflammatory such as mefenamic acid; anti-ulcer such as omeprazole; antiosteoporotic such as alendronate; respiratory drugs such as albuterol, astemizole, ephedrine, Montelukast, pseudoephedrine, terbutaline, fenoterol, salmeterol; antidiabetic such as metformin, Pioglitazone, rosiglitazone, troglitazone, glipizide, glimepiride, tolbutamide, gliclazide; anticoagulant such as warfarin, antimigraine such as sumatriptane, CNS drugs such as amphetamine, paroxetine, fluoxetine, sertraline, zolpidem, citalopram, risperidone, talyetant, vilazodone, lamictal, seroxat; diuretic such as Furosemide; anabolic steroids such as Trenbolone; cardiovascular such as atorvastatin, rosuvastatin, losartan, valsatran, amlodipine, atenolol, captopril, lisinopril, carvedilol, crestor, exnta, accupril; anorexic such as Fenfluramine; peristaltic stimulative agent such as Cisapride; anticancer drugs such as cycloserine, tamoxifen, gemcitabine, capecitabine, chlorambucil, methotrexate, fluorouracil, faslodex, iressa, repifermin, ethynylcytidine, epothizone; vaccines such as typhoid vaccine, polio vaccine; peptides such as Insulin; anti – HIV such as acyclovir, valacyclovir, lamivudine, stavudine, zidovudine, efavirenz, nevirapine, ziagen, EPIVIR, atazanavir or reversible proton pump inhibitors.

The compounds of the Formula VI may be isolated by solvent extraction and identified by LCMS (Liquid Chromatography Mass Spectra) analysis.

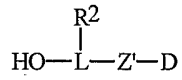
The condensation may be preferably carried out at 40 - 80^{0}C and pH 4 to 9.

The biologically active compound may be dried at 25 - 50°C.

The polar solvent may be water, alcohol such as methanol, isopropyl alcohol or mixtures thereof preferably water or water : methanol or water : isopropyl alcohol.

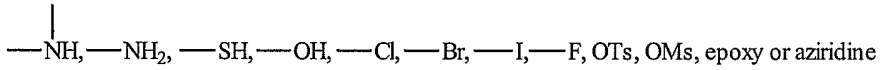
The reactive polymers of the Formula IV have been described in our Patent Application No. 962/MUM/2002.

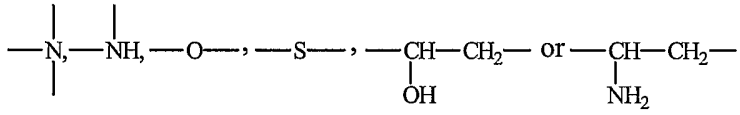
The biologically active compounds i.e. the conjugates of the invention are unreported and novel. They comprise a side chain having a hydrolysable ester group viz., -COO- attached to the polymeric backbone thereof through a spacer or linkage L. Because of the spacer the compounds of the invention have reduced steric hinderance and are capable of undergoing rapid enzymatic hydrolysis. On being chemoenzymatically hydrolysed/ cleaved at the hydrolysable group viz. –COO– group, the compounds of the invention release hydroxyalkyl derivatives thereof i.e. chemically modified biologically active compounds represented by the Formula VII.



Formula – VII

wherein R^2 = H, $C_{1\text{-}12}$ alkyl, $C_{6\text{-}12}$ aryl, or –OH and D = Biologically active agent having functional groups such as



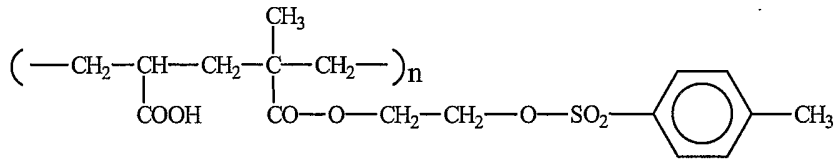
and Z' = 

L = spacer comprising (un)substituted alkyl, hydroxyalkyl or alkoxy alkyl (having carbon chain length with more than one carbon atom when R^3 = epoxy or aziridine). Since the biologically active agents are attached to the hydrolysable group through a spacer or linkage group viz. L, the hydroxy alkyl derivatives of the biologically active compounds represented by the Formula VII released therefrom also comprise the spacer or linkage which renders them highly lipophilic. Synthetic biologically active compounds of the Formula VII have been described in our Patent Application No. 964/MUM/02. Due to the hydroxyl groups they have increased polarity and better ionisation and absorption. The hydroxyalkyl derivatives of the biologically active compounds are capable of stereo selective hydrolysis to form optically pure isomers thereof. Because the hydroxyalkyl derivatives of the biologically active compounds are optically pure and highly lipophilic, their bioavailability and bioefficacy are improved. Due to the improved bioavailability and bioefficacy, the hydroxyalkyl derivatives of the biologically active compounds are effective at low doses thereby correspondingly reducing the side effects.

The following experimental examples are illustrative of the invention but not limitative of the scope thereof.

EXAMPLE - 1

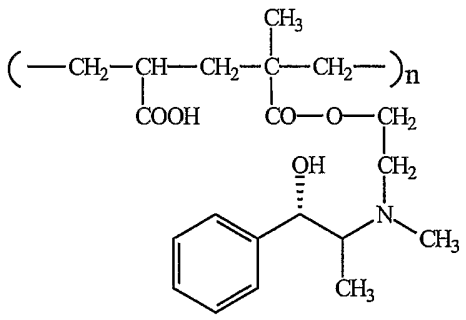
Step 1 : 45 gm of 2-(paratoluenesulphonyl) ethyl Methacrylate (prepared as per methods known in the art), 5 gm of acrylic acid and 0.6 gm of benzoyl peroxide were mixed together and added to 200 ml of ethyl acetate + Acetone (1:1) mixture. The reaction mass was agitated at 80 rpm and refluxed for 4 hours under inert conditions. The material obtained was cooled and stirred in 200 ml of ethyl acetate + acetone (1:1) mixture for one hour. The product was filtered and dried at 50^0C for 12 hours (Yield : 51 gm) to obtain polymer of the Formula VIII :



Formula VIII

Step 2 : Preparation of substituted pseudoephedrine

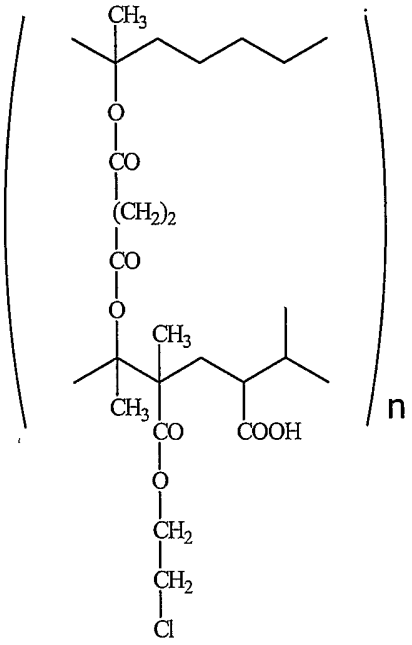
10 gm of the above reactive polymer of Formula VIII was mixed with 5 gm of pseudoephedrine hydrochloride dissolved in a solvent system of methanol + water (1: 1) at pH 9.5. The reaction mixture was refluxed for 36 hours. The product was washed with water (100 ml x 5) till free from pseudoephedrine and then dried under vacuum at 50°C for 12 hours to obtain 16.0 gm of polymer-substituted pseudoephedrine of the Formula IX.



Formula IX

EXAMPLE - 2

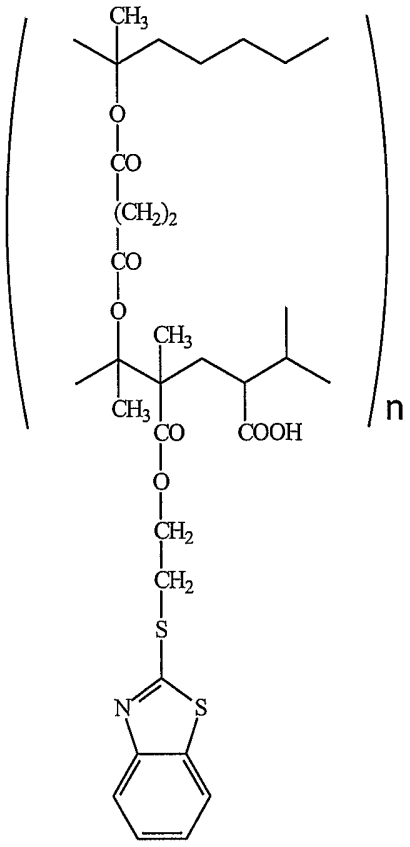
Step 1 : The copolymer was prepared in the same way as in example 1 (Step 1), except that 45 gm of 2-chloro ethyl acrylate was used in place of 2-(paratoluenesulphonyl) ethyl methacrylate and 1 g of ethylene glycol dimethacrylate was also added. 35 g of polymer of Formula X was obtained.



Formula X

Step 2 : Preparation of substituted 2-mercaptobenzothiazole

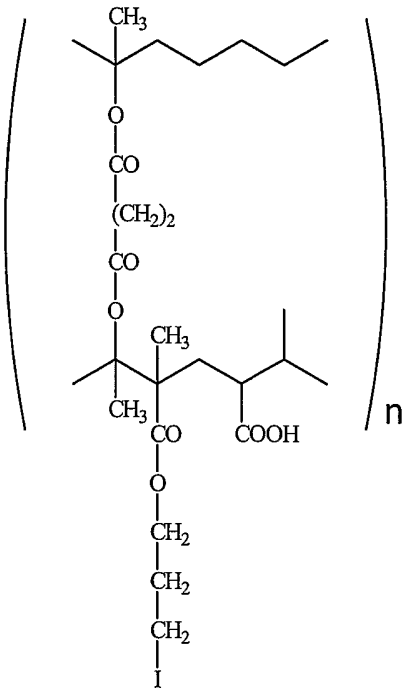
5 g of the polymer of step 1 (Formula X) was mixed with 2.5 g of 2-mercaptobenzothiazole dissolved in a solvent system of methanol + water (9 : 1) at pH 9.3. The reaction mixture was refluxed at 65°C for 36 hours. The resultant product was washed with the methanol (100 ml x 4) will free from 2-mercaptobenzothiazole and dried under vacuum at 50°C for 12 hours to obtain 6.0 g of the polymer substituted 2-mercaptobenzothiazole of the Formula XI.



Formula XI

EXAMPLE - 3

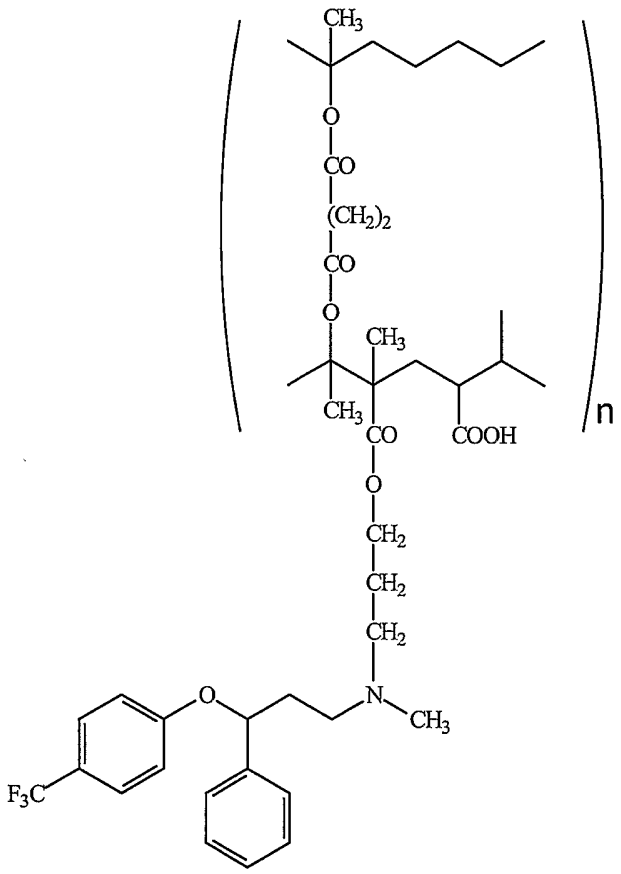
Step 1 : The copolymer was prepared in the same way as in example 1 (Step 1), except that 45 gm of 3-iodopropyl Methacrylate was used in place of 2-(paratoluenesulphonyl) ethyl methacrylate and 1 g of ethylene glycol dimethacrylate was also added. 37 g of polymer of Formula XII was obtained



Formula XII

Step 2 : Preparation of substituted fluoxetine

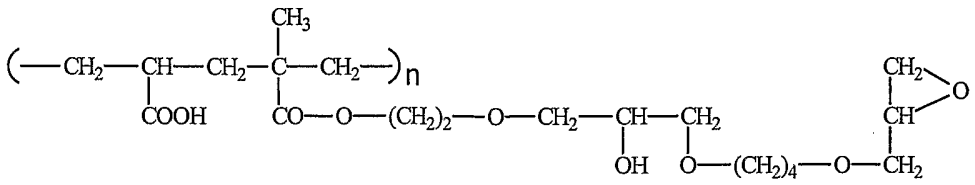
3.5 g of this reactive polymer of Formula XII was mixed with 1.75 g of fluoxetine dissolved in aqueous medium at pH 9.5. The reaction mixture was stirred at 70°C for 24 hours. The resultant product was washed with water (100 ml x 5) till free from fluoxetine and dried under vacuum at 45°C for 12 hours to obtain 4.9 g of polymer-substituted fluoxetine of the Formula XIII.



Formula XIII

EXAMPLE - 4

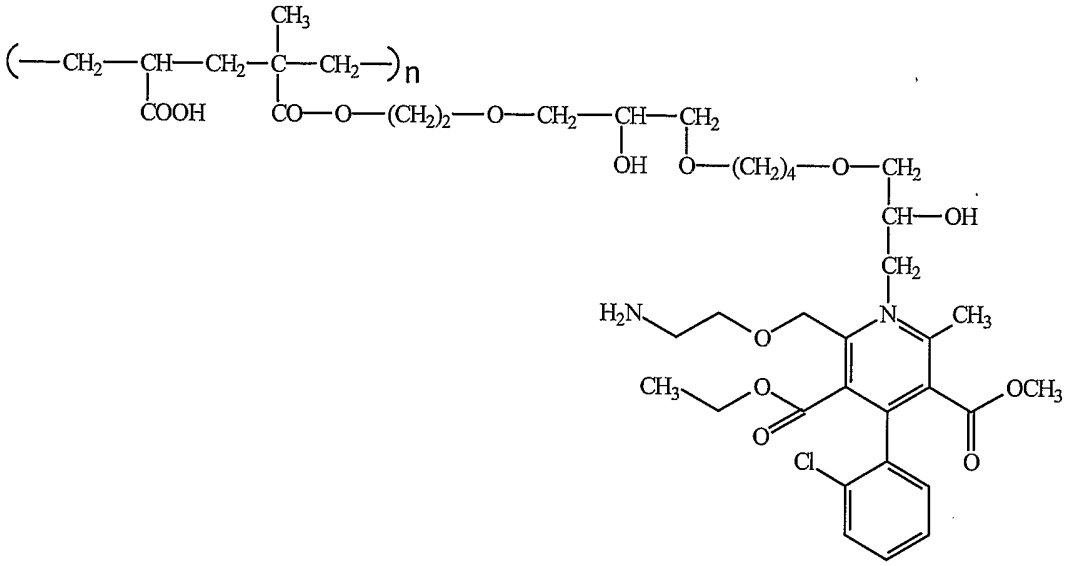
Step 1 (a) : The copolymer was prepared in the same way as Example 1 (step 1), except that 45 g of 3-[Epoxy-(2-methacrylate)]-1-[n-butoxy-(4-glycidylether)]-2-propanol (prepared as per methods known in the art) was used in place of 2-(paratoluenesulphonyl) ethyl methacrylate. 46 g polymer of Formula XIV was obtained.



Formula XIV

Step 2 : Preparation of substituted amlodipine

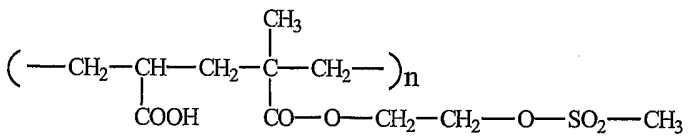
10.0 g of this reactive polymer of formula XIV was mixed with 5.0 g of Amlodipine base dissolved in a solvent of isopropyl alcohol + water (1 : 1) at pH 9.5. The reaction mixture was stirred at 30^{0}C for 72 hours. The product was washed with the same solvent system (100 ml x 5) till free from Amlodipine and then dried under vacuum at 45°C for 12 hours to obtain 13.8 g of polymer-substituted Amlodipine of the Formula XV.



Formula XV

EXAMPLE - 5

Step 1 : The copolymer was prepared in the same way as in Example 1 (Step 1), except that 45 gm of 2-(methanesulphonyl) ethyl methacrylate (prepared as per methods known in the art) was used in place of 2-(paratoluenesulphonyl) ethyl methacrylate. 43 gm of the polymer of the Formula XVI was obtained.

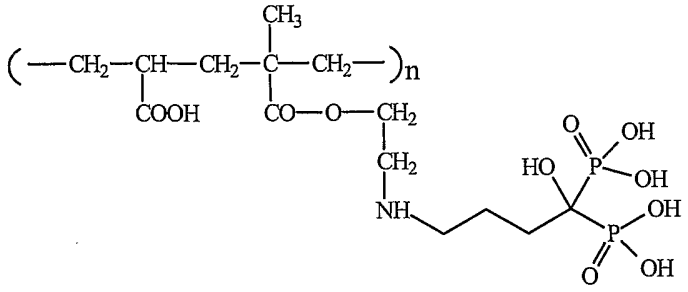


Formula XVI

Step 2 : Preparation of substituted Alendronate

5.0 g of the above reactive polymer of formula XVI was mixed with 2.5 g of Alendronate sodium dissolved in a aqueous medium at pH 9.5. The reaction mixture was stirred at 30°C for 24 hours. The resultant product was washed with water (100 ml x 7) and dried under vacuum

at 50°C for 12 hours to obtain 6.8 g of the polymer-substituted Alendronate of the formula XVII.



Formula XVII

BIOLOGICAL ACTIVITY

ANTI-DEPRESSANT ACTIVITY

Principle

It is known that mice or rats forced to swim in a restricted space from which they cannot escape are induced to characteristic behaviour of immobility. This behaviour reflects a state of despair, which can be reduced by several agents, which are therapeutically effective in human depression.

Materials & Methods

Animals

Swiss albino mice of either sex.

Weight of animals : 30 - 40 g

Drugs : Dose (mg/kg)

1. Compound of formula XIII : 42mg/kg
2. Fluoxetine (Standard) : 20mg/kg

Method

Swiss albino mice of either sex weighing about 30 – 40 g were used. They were brought to the laboratory and acclimatized for 7 days. Mice were individually forced to swim inside a vertical Plexiglas cylinder; mice placed in cylinders for the first time were initially highly active, after 2 – 3 min activity began to subside and phases of immobility or floating increased. Mice were immobilized approximately for 80% of the time. They were again placed in the cylinder 24 hr later and total duration of immobility was measured during a 5 min test. Floating behaviour during this 5 min period has been found to be reproducible in different groups of mice. An animal was judged to be immobile whenever it remains floating passively in water. The drugs were administered one hour prior to testing.

Evaluation

Duration of immobility was measured in controls and drug treated animals. Significance was calculated using 't' test.

Result and discussion

Table – 1 : Effect of test and standard drug on swimming model

Group	Dose (mg/kg)	Immobility Time (Sec)
Vehicle control	--	179.83 ± 22.44
Fluoxetine[+]	20	26.17 ± 5.93*
Compound of formula XIII	42	57.67 ± 10.03*

N=6

*P<0.05 significant as compared to control

Results are in Mean ± SEM

[+] Fluoxetine manufactured by Zydus Cadila, India

Standard drug fluoxetine and test drug Compound of formula XIII showed less immobility time (sec) when compared to control group.

Conclusion

Standard drug fluoxetine and test compound Compound of formula XIII showed significant antidepressant activity when compared to control group.

ANTI-ASTHMATIC ACTIVITY

Principle : Exposure of spasmogen like Acetylcholine chloride or Histamine causes contraction of bronchial smooth muscle. This method permits the evaluation of bronchodilator drugs by measuring time required to produce convulsion after exposure to spasmogens.

Materials and methods :

Animals : Adult guinea pigs of either sex.

Weight of animals : 300 to 350gms.

Materials : Aerosol chamber with 2 compartments and with a central spout for introduction of atomized histamine.

Drugs : Histamine, Test and standard drug.

Experimental procedure : Protection against Histamine Aerosol induced Bronchospasm. Experimental bronchial asthma was induced in guinea pigs by exposing them to 10% histamine under constant pressure in an aerosol chamber.The animals exposed to histamine aerosol showed progressive dyspnoea. The end point preconvulsive dyspnoea (PCD) was determined from the time of aerosol exposure to the onset of dyspnoea leading to the appearance of

convulsion. As soon as PCD was commenced the animals were removed from the chamber and placed in fresh air. This PCD was taken as T_1 Guinea pigs were administered with test and standard drugs, two hours after the dose administration the time for the onset of PCD was recorded as T_2 and protection offered by the treatment was calculated by following formula

$$\% \text{ Protection} = 1 - \frac{T_1}{T_2} \times 100$$

Results

Table 1 : Effect on histamine aerosol – induced bronchospasm in guinea pigs

No.	Group	Dose (mg / kg)	Preconvulsion time (secs.)	Protection (%)
1	Pseudoephedrine (std.)+	10		
	Before treatment		111.20 ±10.28	
	After treatment		296.36 ± 11.56*	63.0
2	Compound of formula IX	65		
	Before treatment		116.66 ± 11.81	
	After treatment		256.88 ± 11.58*	54.59

N=6,

* P<0.005 as compared to control

+ Pseudoephedrine manufactured by Avon Organics Ltd., India

Conclusion

Test and standard drug significantly prolonged the latent period of convulsion as compared to control following exposure to histamine aerosol.

ANTI-HYPERTENSIVE ACTIVITY

Principle

Ischemia of kidneys causes elevation of blood pressure by activation of renin-angiotensin system. This principle can be used for inducing acute renal hypertension by clamping the left renal artery. The protease renin catalyses the first and rate limiting step in the formation of angiotensin-II leading to acute hypertension. This test was used to evaluate antihypertensive activities of drugs.

Procedure

Male Sprague-Dawley rats weighing about 200 – 250 g were anesthetized by anaesthetic ether. The fur was shaved and the skin was disinfected. In left lumbar area a flank incision was made parallel to long axis of the rat. The renal pedicel was exposed with the kidney retracted to abdomen. The artery was dissected clean and a U-shaped silver clip was clipped around it near the aorta, using special forcep. The size of the clip was adjusted so that internal gap ranges

form 0.25 to 0.38 mm. The right kidney was removed through a flank incision after tying off renal pedicle.

The skin incisions were closed by wound clips. Four to five weeks after clipping, the blood pressure was measured and rats with higher than 150 mm Hg selected for the experiments. Blood pressure reading was taken at 1, 2, 3 and 4 hrs after drug treatment.

Drug treatment schedule

The animals were divided into 3 groups.

Group I received 25mg/kg of compound of Formula XV

Group II received 0.9 mg/kg of Amlodipine (Manufactured by Kopran Ltd.)

Group III were the hypertensive controls

All the compounds were administered personally between 3 pm – 4 pm

Expression of results and statistics

The results were analysed statistically using Student's 't' test. The value of P less than 5% ($P < 0.05$) was considered to be statistically significant.

Table 1 : Effect of compound of Formula XV on hypertensive rats at different time intervals

Compounds	Mean blood pressure (mmHg) at hours			
	1	2	3	4
Compound of Formula XV	77.0 ± 0.86*	75.0 ± 5.31*	67.5 ± 0.91*	63.0 ± 2.68*
Amlodipine BP+	79.1 ± 2.15*	78.67 ± 5.1*	69.65 ± 1.13*	66.66 ± 2.71*
Control	122.0 ± 3.63	122.25 ± 3.97	119.0 ± 4.73	117.25 ± 4.92

P < 0.05 Significant*

N = 4

+ Amlodipine British Pharmacopoeial grade manufactured by Kopran Limited, India

Results

In the present investigation, potent antihypertensive effect was observed with the test compound of the invention. This anti-hypertensive effect was comparable to amlodipine. The test compound was statistically significant anti-hypertensive compound.

ANTI FUNGAL ACTIVITY

Principle

Inhibition of microbial growth under standardized conditions may be utilized for demonstrating the therapeutic efficacy of antibiotics. The microbiological assay is based upon the comparison of inhibition of growth of microorganisms by measured concentration of antibiotics to be examined with that produced by known concentration of the antibiotic having

known activity. For such screening cylinder plate (or cup-plate) method and turbidimetric (or tube assay) methods are used.

Preparation of antibiotic solution

To prepare a stock solution, 200 mg of the standard (2-mercaptobenzothiazole), was dissolved in 1 ml of Dimethylformamide (DMF), which was used as solvent. This stock was then diluted serially to get the concentrations of 5 mg/ml and 1 mg/ml. These concentrations were selected so as to determine the range at which the compound is effective against the selected organism. Once the range is determined, further dilutions within the range are tested to determine the minimum inhibitory concentration. Preparation of the test compound is same as the standard.

Determination of antifungal activity using agar cup method

0.1 ml of standardized inoculum of Asp. niger was plated on to muller hinter agar plate, using surface spread method. Cups upto 8 mm in diameter were bored in the inoculated agar with a sterile borer. In one plate 3 cups were made for application of standard solution of 2-mercaptobenzothiazole, compound of Formula XI and DMF as a control respectively, of the same concentration. After application of above mentioned solution to the plate, plates were kept in a refrigerator for prediffusion of compound, for 1 hr. Plates were removed from the refrigerator after an hour and incubated for 3 days at 30°C. Results were noted after 24 hrs, 48 hrs and 72 hrs.

Minimum Inhibitory Concentration values of compounds tested against Aspergillus niger

Concentration (mg/ml)	Compound of formula XI (Zone diameter)	2- Mercaptobenzo-thiazole (Zone diameter)	Dimethyl formamide (DMF) Control (Zone diameter)
1.0	27 mm	26 mm	15 mm
5.0	40 mm	38 mm	15 mm

[+] 2-Mercaptobenzothiazole manufactured by Loba Chemie Ltd, India

Results

Significant anti-fungal activity was observed with the test compound and the activity was comparable to 2-mercaptobenzothiazole.

ANTI-OSTEOPOROTIC ACTIVITY

Principle

Parathyroid Hormone (PTH) increases plasma calcium by stimulating bone resorption mediated through osteoclastic activity and reabsorption of calcium by the kidney. Hypercalcemia induced by PTH were reduced by drug like alendronate, so this model is used to test the antiosteoporotic activity of the test compound.

Procedure

PTH induced hypercalcemia

To establish experimental hypercalcemia, PTH was administered (30 μg/kg) orally to 7 weeks old male rats. At 5th day, first dose of standard and test drug was administered. Blood was collected from fundus oculi at 1, 2, 3, 4, 6 and 9 days after the single dose of drugs. The results were analysed statistically using student's 't' test.

Result

Table 1 : Effect of alendronate and test compound on plasma calcium concentration in rats treated with bPTH

Treatment	Plasma Calcium concentration (mg/dl)					
	1 Day	2 Day	3 Day	4 Day	6 Day	9 Day
Control (+PTH)	11.07 ± 0.14	11.34 ± 0.13	11.53 ± 0.31	10.59 ± 0.57	10.90 ± 0.33	10.93 ± 0.12
Alendronate^{+} (+PTH) (1.25 mg/kg)	10.39 ± 0.26*	9.92 ± 0.46*	9.72 ± 0.24*	10.28 ± 0.23	10.56 ± 0.14	10.68 ± 0.15*
Compound of formula XVII (+PTH) (5.2 mg/kg)	10.67 ± 0.08*	10.20 ± 0.34*	10.14 ± 0.19*	10.76 ± 0.46	10.60 ± 0.10	10.59 ± 0.30

* P < 0.05 Significant

N = 5

$^{+}$ Alendronate manufactured by Nivedita Chemicals Ltd., India

Conclusion

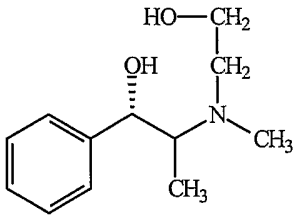
Plasma calcium was significantly increased above normal by intravenous injection of bPTH. One hour after injection of bPTH, the plasma calcium level of bPTH injected animals was increased above the normal range at each time point. Standard drug alendronate and test drug reduces the increment of plasma calcium level induced by bPTH.

IN-VITRO RELEASE STUDIES

a) 1.0 g of compound of Formula IX was accurately weighed and suspended in 100 ml of 0.1N sodium hydroxide in a round bottom flask at room temperature. Temperature of reaction mass was increased and maintained at 37°C ± 1°C. The contents were stirred mechanically at 37°C ± 1°C for 5 hrs. At the end of reaction the reaction mass was

extracted twice with 50 ml of methylene chloride. The two extracts of methylene chloride were mixed and methylene chloride evaporated under vacuum at room temperature.

The residue was analysed by LCMS and found to have molecular ion peak of 209.23 nm. It has a chemical structure of the following Formula XVIII.



Formula - XVIII

This suggests cleavage of compound of Formula IX at hydrolysable ester link and formation of a chemically modified pseudoephedrine i.e. hydroxyalkyl derivative of pseudoephedrine with an ethanol moiety.

b) Synthesis of the compound of Formula XVIII

25 ml Methanol, 7 g pseudoephedrine hydrochloride and 8.89 g of potassium carbonate were stirred mechanically for 30 min at 30°C. 7.04 g of chloroethanol was added dropwise to the reaction mass at 30°C over a period of 20 min. The reaction mass was refluxed for further 24 hours and then poured slowly in 25ml of water. The product was extracted twice with 20 ml methylene chloride, the solvent was removed under vacuum at 40^0C. The solid product obtained was purified by LCMS (Liquid Chromatography Mass Spectra) as Pseudoephedrine with an ethanol moiety showing molecular ion peak at 209.23 nm.

This shows that the compound of Formula XVIII as released from the compound of Formula IX as well as that synthesized from pseudoephedrine have the same structure and properties, thereby establishing their chemical identity.

Biological activities of compound of Formula XVIII as obtained from compound of Formula IX and compound of Formula XVIII as synthetically obtained from pseudoephedrine were compared with pseudoephedrine as below.

ANTI-ASTHMATIC ACTIVITY

Principle : Exposure of spasmogen like Acetylcholine chloride or Histamine causes contraction of bronchial smooth muscle. This method permits the evaluation of bronchodilator drugs by measuring time required to produce convulsion after exposure to spasmogens.

Materials and methods :

Animals : Adult guinea pigs of either sex.

Weight of animals : 300 to 350gms.

Materials : Aerosol chamber with 2 compartments and with a central spout for introduction of atomized Histamine.

Drugs : Histamine, Test and standard drug.

Experimental procedure : Protection against Histamine Aerosol induced Bronchospasm. Experimental bronchial asthma was induced in guinea pigs by exposing them to 10% histamine under constant pressure in an aerosol chamber.The animals exposed to histamine aerosol showed progressive dyspnoea. The end point preconvulsive dyspnoea (PCD) was determined from the time of aerosol exposure to the onset of dyspnoea leading to the appearance of convulsion. As soon as PCD was commenced the animals were removed from the chamber and placed in fresh air. This PCD was taken as T_1 Guinea pigs were administered with test and standard drugs, two hours after the dose administration the time for the onset of PCD was recorded as T_2 and protection offered by the treatment was calculated by following formula

$$\% \text{ Protection} = 1 - \frac{T_1}{T_2} \times 100$$

Results

Table 1 : Effect on histamine aerosol – induced bronchospasm in guinea pigs

No.	Group	Dose (mg / kg)	Preconvulsion time (secs.)	Protection (%)
1	Pseudoephedrine (std.)$^+$	10		
	Before treatment		111.20 ±10.28	
	After treatment		296.36 ± 11.56*	63.0
2	Compound of formula IX	65		
	Before treatment		116.66 ± 11.81	
	After treatment		256.88 ± 11.58*	54.59
3	Synthetic compound of Formula XVIII	10		
	Before treatment		119.58 ± 11.21	
	After treatment		287.67 ± 11.58*	58.43

N=6,

* P<0.005 as compared to control

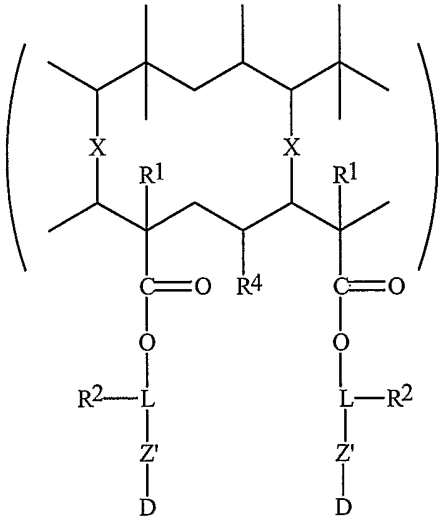
$^+$ Pseudoephedrine manufactured by Avon Organics Ltd., India

Conclusion

Test and standard drug significantly prolonged the latent period of convulsion as compared to control following exposure to histamine aerosol.

CLAIMS

1. Chemoenzymatically hydrolysable biologically active compounds of the Formula VI:

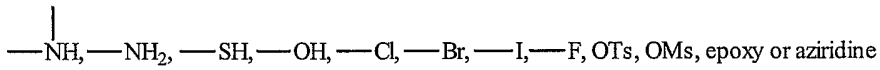


Formula VI

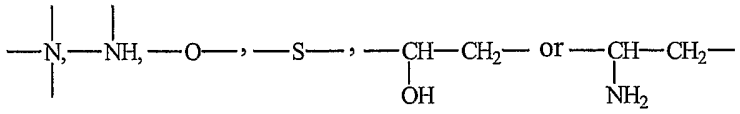
wherein R^1 = H or CH_3, R^2 = H, C_{1-8} alkyl or C_{6-12} aryl,

R^4 = $CONH_2$,–$COOR^6$ (R^6 = H or C_{1-6} alkyl) or CN

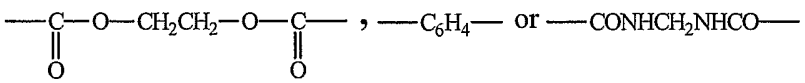
D = Biologically active agent having functional groups such as



and Z' =



X represents a cross linking group such as

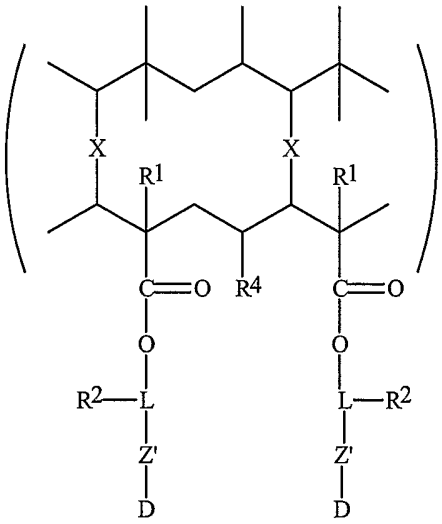


which is optional

L = spacer comprising (un)substituted alkyl, hydroxyalkyl or alkoxy alkyl (having carbon chain length with more than one carbon atom when R^3 = epoxy or aziridine) and pharmaceutically acceptable acid addition salts and enantiomers thereof.

2. Biologically active compounds of the formula VI as claimed in claim 1, wherein R^1 is CH_3 and R^2 is H and R^4 is COOH.

3. Process for the preparation of chemoenzymatically hydrolysable biologically active compounds of the Formula VI:

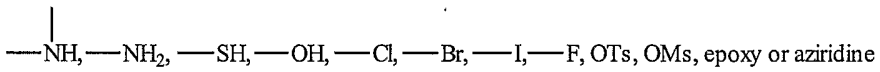


Formula VI

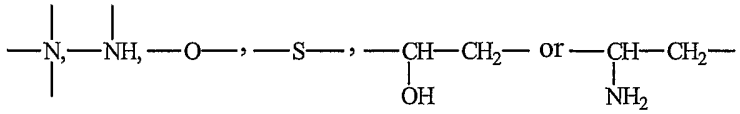
wherein R^1 = H or CH_3, R^2 = H, C_{1-8} alkyl or C_{6-12} aryl,

R^4 = $CONH_2$,–$COOR^6$ (R^6 = H or C_{1-6} alkyl) or CN

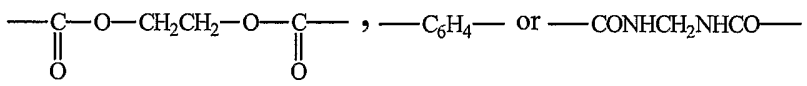
D = Biologically active agent having functional groups such as



and Z' =



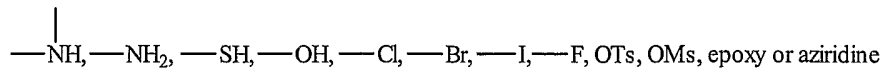
X represents a cross linking group such as



which is optional

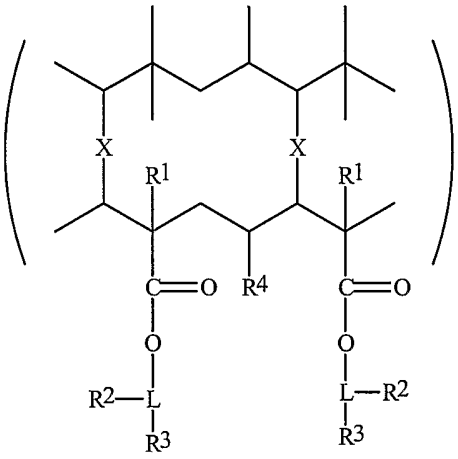
L = spacer comprising (un)substituted alkyl, hydroxyalkyl or alkoxy alkyl (having carbon chain length with more than one carbon atom when R^3 = epoxy or aziridine) and pharmaceutically acceptable acid addition salts and enantiomers thereof, the process comprising,

condensing a biologically active agent having functional groups such as



—NH, —NH_2, —SH, —OH, —Cl, —Br, —I, —F, OTs, OMs, epoxy or aziridine

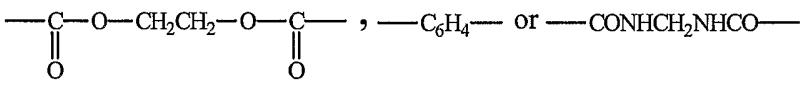
with a reactive polymer of the Formula IV



Formula IV

wherein R^1 = H or CH_3, R^2 = H, C_{1-8} alkyl or C_{6-12} aryl,

X represents a cross linking group such as



—C(=O)—O—CH_2CH_2—O—C(=O)— , —C_6H_4— or —CONHCH$_2$NHCO— which is optional

R^3 = Cl, Br, I, F, OTs, OMs, p-nitrobenzene sulphonate, OSO_2CF_3, OH, NH_2, NHR^5 (R^5=alkyl), SH, epoxide or aziridine,

R^4 = $CONH_2$,–$COOR^6$ (R^6 = H or C_{1-6} alkyl) or CN

L = spacer comprising (un)substituted alkyl, hydroxyalkyl or alkoxy alkyl (having carbon chain length with more than one carbon atom when R^3 = epoxy or aziridine) in polar solvent at 20-90^{0}C and pH 2-10, cooling the reaction mixture to ambient temperature, isolating the biologically active compound followed by drying and if desired, converting the resulting biologically active compound into pharmaceutically acceptable acid addition salts and enantiomers thereof by known methods.

4. Process as claimed in claim 3, wherein in the formula IV R^1 is CH_3, R^2 is H and R^3 is I or OT_s and R^4 is COOH and in the formula VI R^1 is CH_3, R^2 is H and R^4 is COOH.

5. Process as claimed in claim 3, wherein the condensation is carried out at 40 - 80°C and pH 4 to 9.

6. Process as claimed in claim 3, wherein the polar solvent is water or water : methanol or water : isopropyl alcohol.