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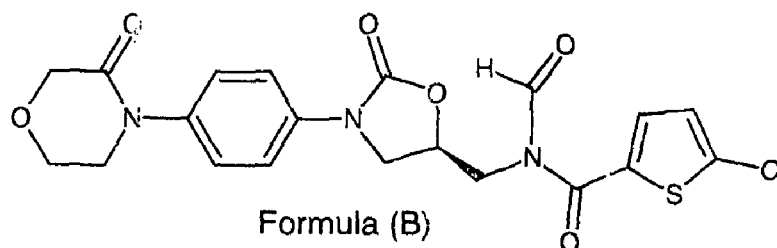
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(54) Title: ALDEHYDE DERIVATIVE OF SUBSTITUTE OXAZOLIDINONES



Formula (B)

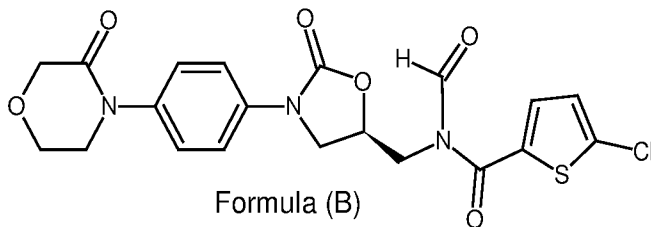
(57) Abstract: The present invention concern towards the prodrug of 5-chloro-N-((5S)-2-oxo-3-[4-(3-oxo-morpholin-4-yl)phenyl]-1,3-oxazolidin-5-yl)methylthiophene-2-carboxamide, rivaroxaban per se; processes for their preparation, and the application in treatment and/or prophylaxis of diseases, especially of thromboembolic disorders. The prodrug of compound formula (B) is chemically designated as 5-chloro-N-formyl-N-((5S)-2-oxo-3-[4-(3-oxomorpholin-4-yl)phenyl]-1,3-oxazolidin-5-yl)methylthiophene-2-carboxamide.(I)



Aldehyde derivative of substituted oxazolidinones

FIELD OF THE INVENTION

[001] The present invention mainly relates to aldehyde derivatives of substituted oxazolidinones and more particularly to a prodrug of 5-chloro-N-({(5S)-2-oxo-3-[4-(3-oxomorpholin-4-yl)phenyl]-1,3-oxazolidin-5-yl}methyl)thiophene-2-carboxamide, and a process for preparation of prodrug. The prodrug of formula (B) is chemically designated as 5-chloro-N-formyl-N-({(5S)-2-oxo-3-[4-(3-oxomorpholin-4-yl)phenyl]-1,3-oxazolidin-5-yl}methyl)thiophene-2-carboxamide, or a pharmaceutically acceptable salt or solvate or hydrate form. Further this invention relates to the use of the prodrug in treatment and/or prophylaxis of diseases, pulmonary embolism, and deep venous thrombosis, more particularly to thromboembolic disorders.



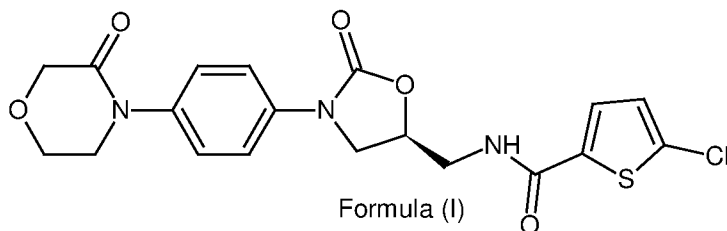
BACKGROUND OF THE INVENTION

[002] A large number of medicaments are administered as prodrugs, which exhibit an improved bioavailability by comparison with the underlying active ingredient, for example, by improving the physicochemical profile, specifically the solubility, the active or passive absorption properties or the tissue-specific distribution. In order to achieve an optimal profile of effects it is necessary for the design of the prodrug residue as well as the desired mechanism of liberation to conform very accurately to the individual active ingredient, the indication, the site of action and the administration route. The importance of the prodrug is more, when the main moiety raises concerns of solubility, stability and oral bioavailability.

[003] Rivaroxaban is an orally active direct factor Xa (FXa) inhibitor drug, used for the prevention and treatment of various thromboembolic diseases, in particular pulmonary embolism, deep venous thrombosis, myocardial infarction, angina pectoris, reocclusion and restenosis after angioplasty or

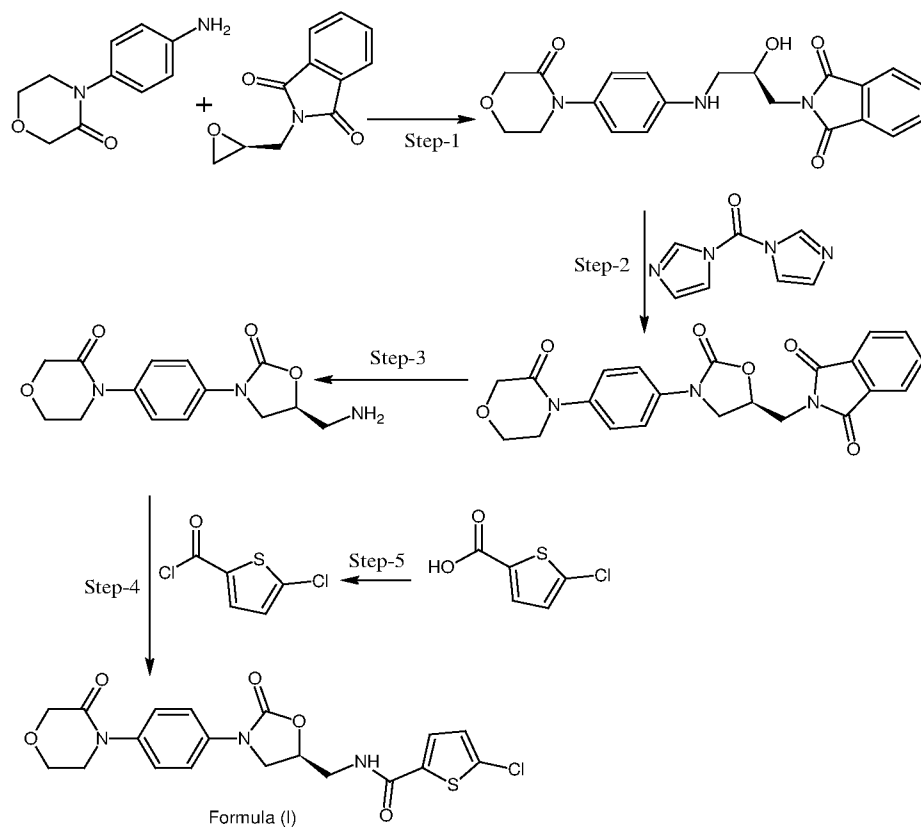
aortocoronary bypass, cerebral stroke, transitory ischemic attacks, and peripheral arterial occlusive diseases.

[004] Rivaroxaban i.e. 5-chloro-*N*-({(5*S*)-2-oxo-3-[4-(3-oxomorpholin-4-yl)phenyl]-1,3-oxazolidin-5-yl)methyl}thiophene-2-carboxamide, has a CAS number of 366789-02-8, a molecular formula of $C_{19}H_{18}ClN_3O_5S$, and the following structure:



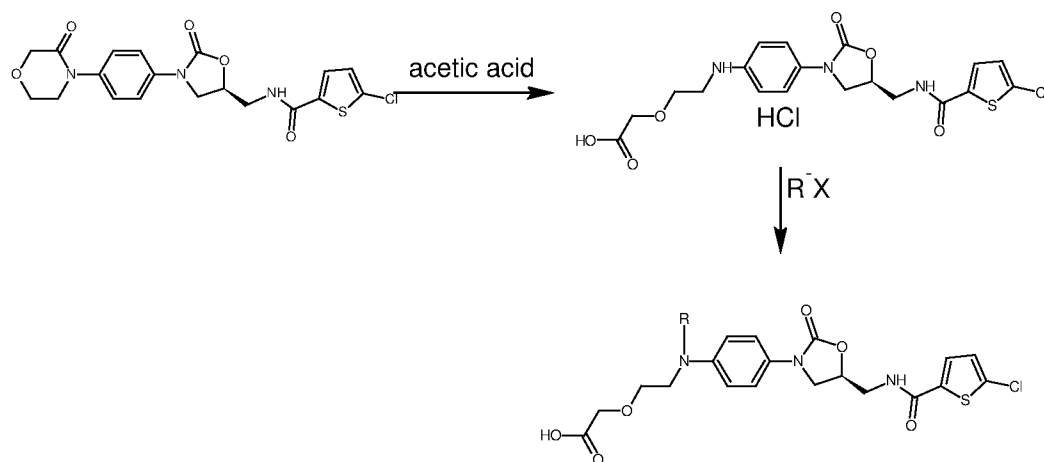
[005] Rivaroxaban, though effective for prevention and treatment of various thromboembolic diseases, often raises issue of dosage and relative bioavailability.

WO 01/47919 discloses Rivaroxaban with applications for the prevention and treatment of various thromboembolic diseases. Further this patent describes a method for preparation of Rivaroxaban of formula (I), wherein 4-(4-aminophenyl)morpholin-3-one is reacted with 2-[(2*S*)-oxiran-2-ylmethyl]-1*H*-isoindole-1,3(2*H*)-dione, in presence of solvent to obtain 2-[(2*R*)-2-hydroxy-3-{[4-(3-oxomorpholin-4-yl)phenyl]amino}propyl]-1*H*-isoindole-1,3(2*H*)-dione which is further converted to 2-({(5*S*)-2-oxo-3-[4-(3-oxomorpholin-4-yl)phenyl]-1,3-oxazolidin-5-yl)methyl}-1*H*-isoindole-1,3(2*H*)-dione by phosgene equivalent. Departing of the pthalamide group affords 4-{4-[(5*S*)-5-(aminomethyl)-2-oxo-1,3-oxazolidin-3-yl]phenyl}morpholin-3-one, which is finally coupled with 5-chlorothiophene-2-carbonyl chloride to give 5-chloro-*N*-({(5*S*)-2-oxo-3-[4-(3-oxomorpholin-4-yl)phenyl]-1,3-oxazolidin-5-yl)methyl}thiophene-2-carboxamide, i.e. Rivaroxaban of formula (I) as shown in Scheme-1:

**Scheme-I**

[006] The disclosed process involves lengthy reaction periods, excess mole ratios of reactants and reagents, use of unsafe solvents such as methanol. Moreover title compound is isolated by column chromatography which is not feasible on a commercial scale.

[007] US 7,932,278 B2 discloses the preparation of the novel 2-aminoethoxyacetic acid derivatives from Rivaroxaban by the synthesis scheme below:



The novel 2-aminoethoxyacetic acid derivatives according to the invention are suitable for use as medicaments for the treatment and/or prophylaxis of diseases in humans and animals.

[008] WO 2009/023233 discloses the compounds that are substituted oxazolidinones derivatives and pharmaceutically acceptable salts thereof. More specifically, this invention relates to novel oxazolidinones compounds that are derivatives of Rivaroxaban. The invention also provides pyrogen-free compositions comprising one or more compounds of the invention and a carrier, and the use of the disclosed compounds and compositions in methods of treating diseases and condition that are beneficially treated by administering a selective inhibitor of factor Xa, such as Rivaroxaban.

[009] The present invention relates to a prodrug of Rivaroxaban. The compounds according to the invention are selective inhibitors of blood coagulation factor Xa, which act in particular as anticoagulants, with favorable physicochemical properties, advantageous in therapeutic applications such as the treatment and/or prophylaxis of thromboembolic disorders, as an inhibitor of factor Xa, and/or the treatment and/or prophylaxis of thromboembolic complications.

SUMMARY

[010] In its main aspect, the present invention discloses a compound of formula (B), chemically designated as 5-chloro-N-formyl-N-((5S)-2-oxo-3-[4-(3-oxomorpholin-4-yl)phenyl]-1,3-oxazolidin-5-yl)methylthiophene-2-carboxamide.

In another aspect, of the present invention discloses the use of the compounds of formula (B), for treatment and/or prophylaxis of disorders, such as thromboembolic disorders; inhibitor of factor Xa, and/or thromboembolic complications. The “thromboembolic disorders” include in the context of the present invention disorders such as myocardial infarction with ST segment elevation (STEMI) and without ST segment elevation (non-STEMI), stable angina pectoris, unstable angina pectoris, reocclusions and restenoses following coronary interventions such as angioplasty or aortocoronary bypass, peripheral arterial occlusive diseases, pulmonary embolisms, deep venous thromboses and renal vein thromboses, transient ischaemic attacks, and thrombotic and thromboembolic stroke.

[011] In yet another aspect, the present invention discloses a use of the compound of formula (B) for prevention and treatment of cardiogenic thromboembolisms, such as, for example, cerebral ischaemias, stroke and systemic thromboembolisms and ischaemias, in patients with acute, intermittent or persistent cardiac arrhythmias such as, for example, atrial fibrillation, and those undergoing cardioversion, also in patients with heart valve diseases or with artificial heart valves. The compound according to the invention is additionally suitable for the treatment of disseminated intravascular coagulation (DIC).

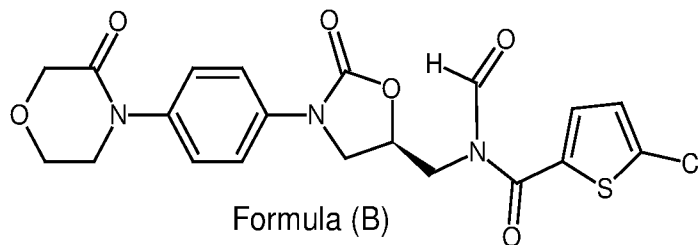
[012] Another aspect of the present invention is to provide the process for the preparation of the compound of formula (B) which is substantially free from impurities.

[013] Yet another aspect of the present invention is to provide compound of formula (B) in crystalline or amorphous form.

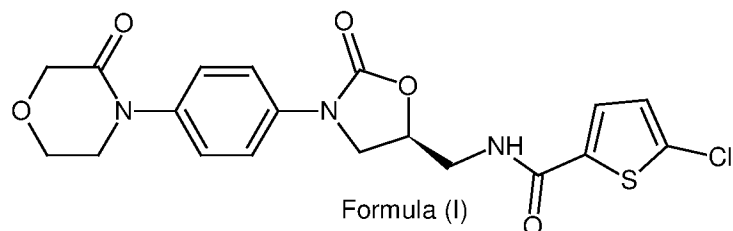
[014] Yet another aspect, the present invention discloses a compound of formula (B) in a pharmaceutically accepted salt or hydrate form or solvate form of compound of formula (B).

DETAILED DESCRIPTION OF THE INVENTION

[016] In its main embodiment, the present invention relates to a compound of Formula (B), chemically designated as 5-chloro-N-formyl-N-((5S)-2-oxo-3-[4-(3-oxomorpholin-4-yl)phenyl]-1,3-oxazolidin-5-yl)methylthiophene-2-carboxamide, having the structure:



or its pharmaceutically acceptable salt or solvate or hydrate form, which acts as a prodrug of the compound of formula (I), chemically designated as 5-chloro-N-({(5S)-2-oxo-3-[4-(3-oxomorpholin-4-yl)phenyl]-1,3-oxazolidin-5-yl}methyl)thiophene-2-carboxamide, having the structure:



[017] The word “prodrug” refers to a compound which may be pharmacologically active or inactive, but on ingestion is enzymatically or hydrolytically converted by the body in to the active compound. The present invention focuses on these concerns. The compound of formula (B), as disclosed in *in vitro* and *in vivo* animal studies has shown superior solubility and stability as is explicitly reflected in the examples, and hence encourages clinical trials.

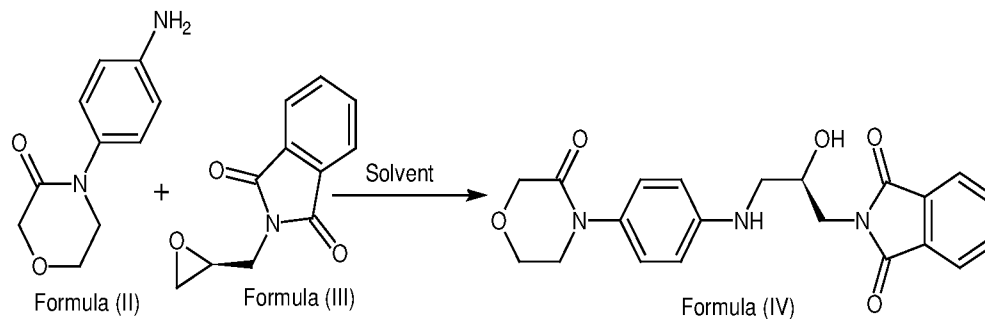
[018] The present compound of formula (B) exists in stereoisomeric forms (enantiomers, diastereomers). Accordingly, the invention further relates to the enantiomers or diastereomers and their respective mixtures. From such mixtures of enantiomers and/or diastereomers, it is possible to isolate the stereoisomerically uniform components in a known manner. If the compounds according to the invention can be present in tautomeric forms, the present invention also relates to all tautomeric forms.

[019] The present invention carried out a detailed study on the solubility, stability and liberation behavior of the invented compound (i.e., the compound of formula (B)). Further *in vitro* and *in vivo* studies for the compound of formula (B) were carried out in order to establish the selective activity such as In Vitro Liver Microsomal Stability Assay, In Vitro Stability in Rat, Mouse and Human Plasma, CYP Inhibition Assay, Plasma Protein Binding Intravenous and Oral Pharmacokinetics in Wistar Rats, Suspension for Intravenous Administration and Solution for Oral Administration, Anticoagulant activity and Antithrombotic activity, wherein these studies are well exemplified or illustrated with best mode in the examples section/example (B).

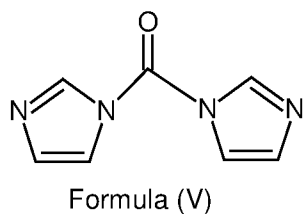
[020] In an important embodiment, the present invention provides a process for the preparation of a compound of formula (B) comprising:

- a) reacting 4-(4-aminophenyl)morpholine-3-one of formula (II) with 2-[(2S)-oxiran-2-

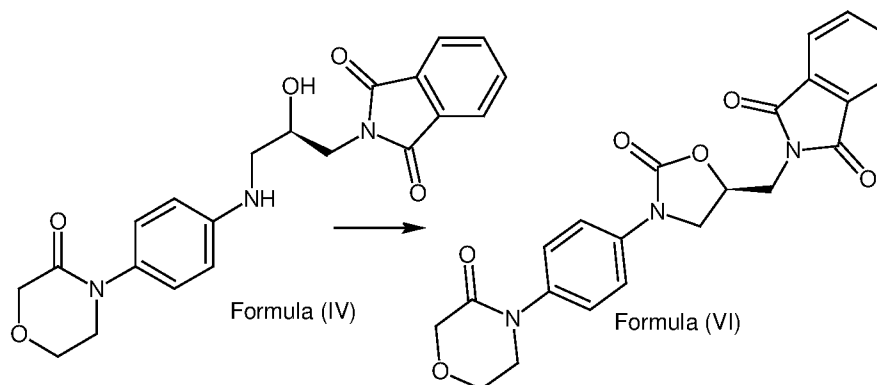
ylmethyl]-1H-isoindole-1,3(2H)-dione of formula (III) in a suitable solvent to obtain 2-[(2*R*)-2-hydroxy-3-{[4-(3-oxomorpholin-4-yl)phenyl]amino}propyl]-1*H*-isoindole-1,3(2*H*)-dione of formula (IV):



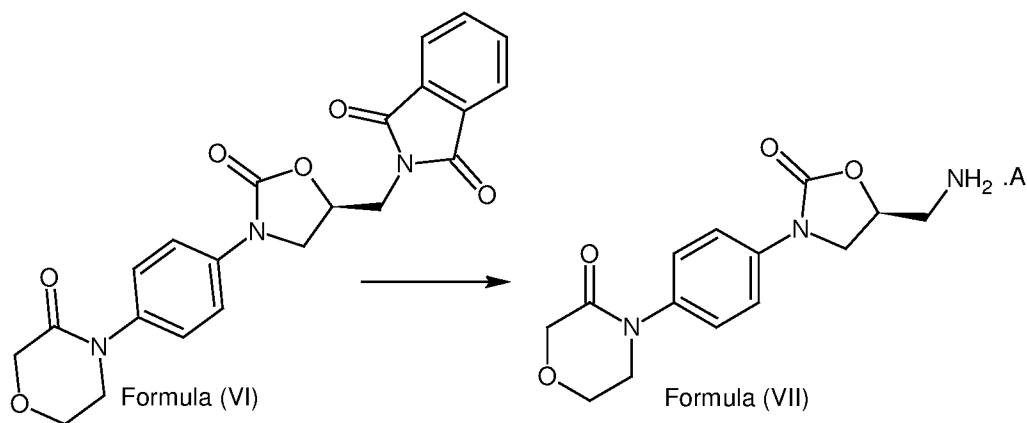
- b) preparing a compound of formula (VI) by reacting a compound of formula (IV) with di-1*H*-imidazol-1-ylmethanone of formula (V):



In the alternative, in a suitable solvent, a compound of formula (IV) is converted to a compound of formula (VI) in the presence of a base:

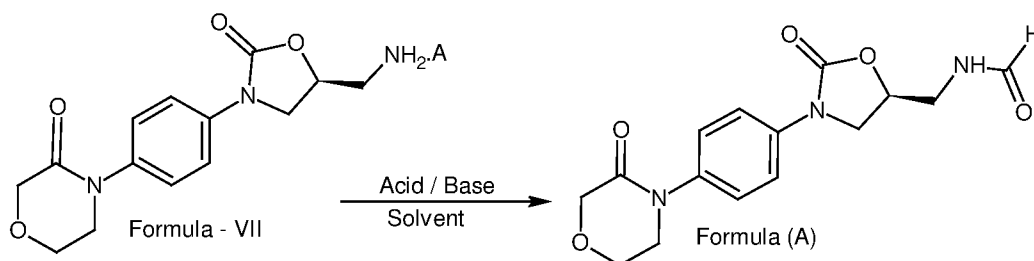


- c) eliminating the phthalamide group from the compound of formula (VI) by using a suitable de-protecting agent and an acid in a suitable solvent to obtain the acid addition salt of 4-{4-[(5*S*)-5-(aminomethyl)-2-oxo-1,3-oxazolidin-3-yl]phenyl}morpholin-3-one of formula (VII).



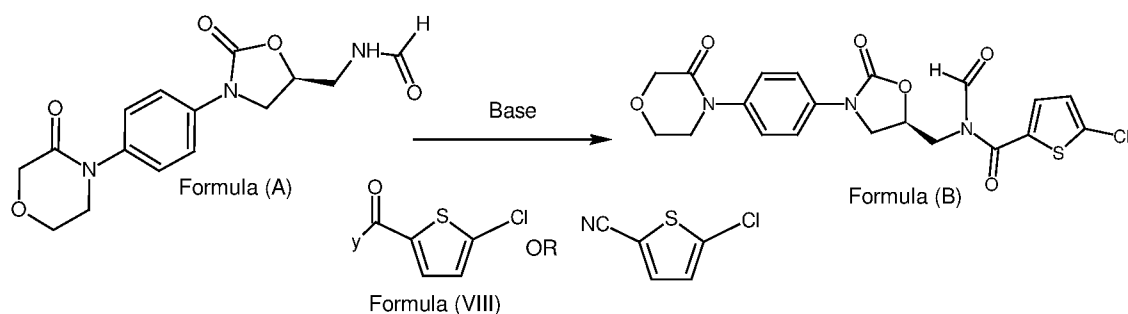
where A is an acid addition salt. In the alternative, a compound of formula (VII) can also be isolated as a free base. The synthesis of a compound of formula (VII), either as an acid addition salt or a free base, is known and hence not claimed. A compound of formula (VII) may be made by any known method.

- d) the compound of formula (VII) with acid addition salt is reacted in the presence of a base with formic acid to obtain a novel intermediate formula (A), *N*-({(5*S*)-2-oxo-3-[4-(3-oxomorpholin-4-yl)phenyl]-1,3-oxazolidin-5-yl)methyl}formamide:



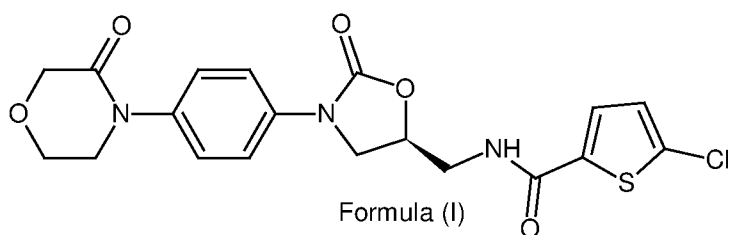
In the alternative, where the compound of formula (VII) is a free base, it is directly reacted with formic acid to give a novel intermediate formula (A), *N*-({(5*S*)-2-oxo-3-[4-(3-oxomorpholin-4-yl)phenyl]-1,3-oxazolidin-5-yl)methyl}formamide.

- e) reacting the compound of formula (A) with the compound of formula (VIII) or 5-chlorothiophene-2-carbonitrile in a suitable solvent and base to obtain the compound of formula (B), optionally in the presence of catalyst and/or activating agents:

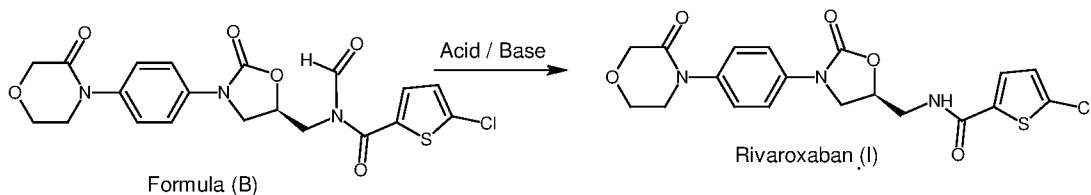


wherein Y may be alkyl sulfonyl, aryl sulfonyl, imidazole, triazole, tetrazole, alkoxy, substituted alkoxy, N-oxy-succinimide, hydroxy, primary amine, secondary amine, p-nitrophenoxy, N-oxy-phthalimide, N-oxy-benzotriazole, chlorine, fluorine, bromine and iodine. Base used may be inorganic or organic.

[021] The compound of formula (B) is prodrug of a compound of formula (I), which is popularly known as Rivaroxaban having the structure of formula (I).



[022] When the aldehyde group of the compound of formula (B) is eliminated on exposure to an acidic or basic environment, it is converted to the active moiety, Rivaroxaban. Hence the compound of formula (B) when treated with an acid or a base in a suitable solvent departs the aldehyde group from the compound of formula (B) to obtain the compound of formula (I), i.e., Rivaroxaban:



[023] The instant invention further extends to the preparation of acid addition salt of the compound of formula (VII), in order to get the purified compound without any further purification by acid-base treatment, or solvent crystallization.

[024] The solvent used in step (a) and step (c) may be same or different, wherein the solvent is an organic solvent selected from the group consisting of aliphatic hydrocarbons, aromatic hydrocarbons, dialkylformamides, ethers, cyclic ethers, substituted cyclic ethers, alcohol, ketones, dialkylsulfoxides, dialkylacetamides, nitriles, ionic liquids, halogenated aliphatic hydrocarbons and water or mixtures thereof. In one embodiment, the solvent is neutral towards the reactants.

[025] Step (a) could be carried out at a temperature in the range of 0°C to 95°C. Usually the reaction may be carried out at temperature up to reflux temperature of the solvent.

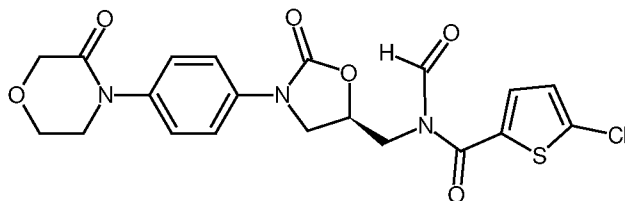
[026] The solvent used in step (b) for the preparation of the compound of formula (VI) is an organic solvent selected from the group consisting of aliphatic hydrocarbons, aromatic hydrocarbons, dialkylformamides, ethers, cyclic ethers, substituted cyclic ethers, ketones, dialkylsulfoxides, dialkylacetamides, nitriles, ionic liquids, halogenated aliphatic hydrocarbons or mixtures thereof.

[027] The solvent used in step (c) is an organic solvent selected from the group consisting of aliphatic hydrocarbons, aromatic hydrocarbons, dialkylformamides, ethers, cyclic ethers, substituted cyclic ethers, dialkylsulfoxides, dialkylacetamides, nitriles, ionic liquids, halogenated aliphatic hydrocarbons and water or mixtures thereof. Further, the compound of formula (VII) may be prepared in terms of acid addition salt by using inorganic or organic acid.

[028] In step (d) the compound of formula (VII) may be used in free base form or its acid addition salt. The solvent used in the step (d) is an organic solvent or may be mixture of water and an organic solvent. The formylating agent used in the step (d) may be formic acid, alkyl formate etc. The solvent used in the reaction may be selected from the group consisting of aromatic hydrocarbons, nitriles, aliphatic hydrocarbons, ethers. In one embodiment, the solvent used in the reaction is an aromatic hydrocarbon, preferably toluene or xylene. The base used in step (d) is selected from an organic or inorganic base.

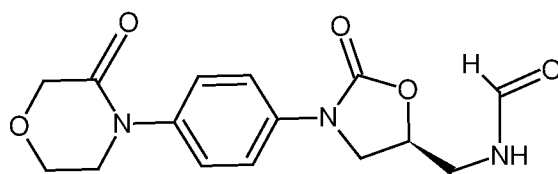
[029] In step (e), the compound of formula (A) is treated with a compound of formula (VIII) optionally in the presence of base, which may be inorganic or organic, in a solvent selected from the group consisting of aliphatic hydrocarbons, aromatic hydrocarbons, dialkylformamides, ethers, cyclic ethers, substituted cyclic ethers, dialkylsulfoxides, dialkylacetamides, nitriles, ionic liquids, esters, halogenated aliphatic hydrocarbons, ketones, cyclic amides and water or mixtures thereof to obtain Rivaroxaban precursor of the compound of formula (B). Activating agents used in the reaction of step (e) comprises CDI, DCC, HOBt, DMAP, EDCI, boric acid, boronic acid, phenyl boronic acid, and so on, and mixtures thereof.

[030] According to yet another embodiment, the present invention provides a process for preparation of a compound of formula (B):



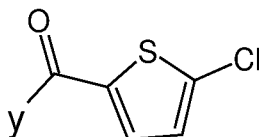
Formula (B)

comprising the step of reacting a compound of formula (A):



Formula (A)

with a compound of formula (VIII) to obtain the compound of formula (B):



Formula (VIII)

wherein Y may be alkyl sulfonyl, aryl sulfonyl, imidazole, triazole, tetrazole, alkoxy, substituted alkoxy, N-oxy-succinimide, hydroxy, primary amine, secondary amine, p-nitrophenoxy, N-oxy-phthalimide, N-oxy-benzotriazole, chlorine, fluorine, bromine and iodine. Base used may be inorganic or organic.

[031] The solvent used for the reaction may be inorganic or organic, in a solvent selected from the group consisting of aliphatic hydrocarbons, aromatic hydrocarbons, dialkylformamides, ethers, cyclic ethers, substituted cyclic ethers, dialkylsulfoxides, dialkylacetamides, nitriles, ionic liquids, esters, halogenated aliphatic hydrocarbons, ketones, cyclic amides and water or mixtures thereof to obtain Rivaroxaban precursor of a compound of formula (B). Activating agents used in the reaction comprises CDI, DCC, HOBt, DMAP, EDCI, boric acid, boronic acid, phenyl boronic acid, and so on, and mixtures thereof. The base used is selected from organic or inorganic base and optionally the compound of formula (B) may be purified or can be used as such for next reaction.

[033] In yet another embodiment of the present invention, the base used in the aforementioned step is inorganic or organic and the solvent is selected from the group consisting of aliphatic hydrocarbons, aromatic hydrocarbons, dialkylformamides, ethers, cyclic ethers, substituted cyclic ethers, dialkylsulfoxides, dialkylacetamides, nitriles, ionic liquids, esters, halogenated aliphatic hydrocarbons, ketones, cyclic amides and water or mixtures thereof to obtain Rivaroxaban of formula (I). Activating agents used in the reaction may be CDI, DCC, HOBt, DMAP, EDCI, boric acid, boronic acid, phenyl boronic acid, and so on, and mixtures thereof.

[034] As used herein, the term "hydrate" means a compound which further includes a stoichiometric or non-stoichiometric amount of water bound by non-covalent intermolecular forces. As used herein, the term "solvate" means a compound which further includes a stoichiometric or non-stoichiometric amount of solvent such as water, acetone, ethanol, methanol, dichloromethane, 2-propanol, or the like, bound by non-covalent intermolecular forces.

[035] The present invention is described in the examples given below. Further these are provided only to illustrate the invention and therefore should not be construed to limit the scope of the invention.

EXAMPLE A

Abbreviations and Acronyms

LC-MS	Coupled Liquid chromatography – mass spectroscopy
HPLC	High performance liquid chromatography
LLQQ	Lower limit of quantification
SD	Standard deviation
AUC	Area under curve
DMSO	dimethyl sulphoxide
NADPH	nicotinamide adenine dinucleotide phosphate-oxida
CYP	cytochrome
BLOQ	below limit of quantification
SIF	Stimulated Intestinal fluid
SGF	Stimulated gastric fluid
CV	Concentration value
C max	Highest concentration

The following exemplary embodiment in terms of detailed study illustrates the invention but it is not restricted to these examples with procedure.

EXAMPLE 1

[036] Preparation of N-({(5S)-2-oxo-3-[4-(3-oxomorpholin-4-yl)phenyl]-1,3-oxazolidin-5-yl}methyl)formamide (aldehyde of primary amine)

In a four neck round bottom flask charged with 4-{4-[(5S)-5-(aminomethyl)-2-oxo-1,3-oxazolidin-3-yl]phenyl}morpholin-3-one free base (50 g) toluene (350 ml) and formic acid (21.63 g). Reaction mass then heated azeotropically to 110-120°C employing dean-stark apparatus for 3 to 4 h. (water removed azeotropically). Reaction mass is cooled to 25 to 30°C. Obtained solid is filtered off and washed by toluene.

Yield 96%

EXAMPLE 2

[037] Preparation of 5-chloro-N-formyl-N-({(5S)-2-oxo-3-[4-(3-oxomorpholin-4-yl)phenyl]-1,3-oxazolidin-5-yl}methyl)thiophene-2-carboxamide (compound of formula (B))

Added N-({(5S)-2-oxo-3-[4-(3-oxomorpholin-4-yl)phenyl]-1,3-oxazolidin-5-yl}methyl)formamide (1 g), dichloromethane (25 ml) in a clean dry 4 neck R.B. flask at 25 to 30°C. To this clear solution added potassium carbonate (0.89 g) and stirred at 25 to 30°C for 30 minutes. To this reaction mass, slowly added solution of 5-chlorothiophene-2-carbonyl chloride (1.0 g), and dichloromethane (5 ml). The obtained reaction mass was then stirred at 25 to 30°C for 5 to 6 h. Added water (25 ml) to reaction mass and separated organic layer. Obtained organic layer was then washed by water (25 ml X 2). Finally organic layer is dried over sodium sulfate and concentrated under reduced pressure to obtain residue. Added methanol (5 ml) to the residue and heated to reflux to get a clear solution. The obtained clear solution was gradually cooled to 15 to 20°C. The precipitated solid then filtered off and washed by chilled methanol (1 ml).

[038] ¹H-NMR (400Mz, d₆-DMSO), δ=3.74-3.77 (m, 2H), 3.84-3.87(m, 1H), 4.02-4.05(m, 2H), 4.07-4.11(m, 2H),4.12-4.15 (m, 1H), 4.34-4.41 (m, 3H), 4.94-5.00 (m, 1H), 7.00-7.01(d, 1H thiophene), 7.30-7.31 (d, 1H thiophene), 7.33-7.37 (dt, 2H aromatic), 7.55-7.58 (dt, 2H aromatic), 9.28 (s, 1H aldehyde)

Example 2 was carried out in different solvents such as acetone, toluene and ether, with the same molar ratio/parts, wherein the variations in yield are noted below:

Solvent(s)	Yield
Acetone	75 % (reaction perform as reflect in example 2)
Toluene	78 % (reaction perform as reflect in example 2)
Ether	79% (reaction perform as reflect in example 2)

EXAMPLE 3

[039] Preparation of 5-chloro-N-((5S)-2-oxo-3-[4-(3-oxomorpholin-4-yl) phenyl]-1,3-oxazolidin-5-yl)methylthiophene-2-carboxamide (Nitrile route)

To a solution of 4-{4-[(5S)-5-(aminomethyl)-2-oxo-1, 3-oxazolidin-3-yl] phenyl} morpholin-3-one hydrochloride (5.7 g) in ethanol (70 ml) added potassium carbonate (7.1 g) and the mixture was stirred 2 h at 25 to 30°C then filtered to obtain 4-{4-[(5S)-5-(aminomethyl)-2-oxo-1, 3-oxazolidin-3-yl] phenyl} morpholin-3-one (free base). In another flask charged solution of 5-chlorothiophene-2-carbonitrile (2.9 g) under nitrogen in ethanolic HCl (12 ml) and stirred for 5 h at room temperature till white precipitate was obtained. Distilled under nitrogen to avoid from moisture and obtained residue added in solution of 4-{4-[(5S)-5-(aminomethyl)-2-oxo-1, 3-oxazolidin-3-yl] phenyl} morpholin-3-one. The mixture was stirred for 16 to 18 h at reflux temperature. Aq.ethanol (5ml) was and mixture heated at reflux temperature for 10 to 12 h to obtain 5-chloro-N-((5S)-2-oxo-3-[4-(3-oxomorpholin-4-yl)phenyl]-1,3-oxazolidin-5-yl)methylthiophene-2-carboxamide (crud material) which is further purified by column chromatography.

EXAMPLE 4

[040] Preparation of N-((5S)-2-oxo-3-[4-(3-oxomorpholin-4-yl)phenyl]-1,3-oxazolidin-5-yl)methylformamide

A four neck round bottom flask was charged with 4-{4-[(5S)-5-(aminomethyl)-2-oxo-1,3-oxazolidin-3-yl]phenyl}morpholin-3-one Hydrochloride (250g), Dichloromethane (1250 ml) and ammonia (250 ml). The solution stirred for 15 min and the layers separated. Added toluene (1250ml)to the organic layer, along with water(500 ml) and formic acid (140.6g). Reaction mass was then heated azeotropically to 110-

120°C employing dean-stark apparatus for 3 to 4 h. (water removed azeotropically). Reaction mass was cooled to 25 to 30°C. Obtained solid then filtered off and washed by toluene.

Yield 80.0%

EXAMPLE 5

[041] Preparation of 5-chloro-N-formyl-N-({(5S)-2-oxo-3-[4-(3-oxomorpholin-4-yl)phenyl]-1,3-oxazolidin-5-yl}methyl)thiophene-2-carboxamide

Added N-({(5S)-2-oxo-3-[4-(3-oxomorpholin-4-yl)phenyl]-1,3-oxazolidin-5-yl}methyl)formamide (120 g), dichloromethane (2400 ml) to a clean dry 4 neck R.B. flask at 25 to 30°C. Cooled the reaction mass to 0 to 5°C. To this solution added Diisopropylethyl amine (145.7 g) dropwise manner, a solution of 5-chlorothiophene-2-carbonyl chloride (170g), and dichloromethane (240 ml) at 0 to 5°C. The obtained reaction mass was then stirred at 25 to 30°C and heated to reflux for 12hr. The reaction mass was cooled to 25 to 30°C .washed with 10 % citric acid solution (2x360ml), and separated organic layer. Obtained organic layer then washed by water (600 ml X 2) and concentrated under reduced pressure to obtain a residue. Added methanol (600 ml) to residue and stirred for 20 min. The precipitated solid was then filtered off, washed by methanol (240 ml), sucked dry and the wet cake taken into a flask methanol (600ml) added and the solution was then stirred for 30 min., solid then filtered off and washed by methanol (240 ml).

Yield 85.0%

EXAMPLE B

Determination of the stability of the compound of formula (B) in SGF and SIF fluids

[042] The compound of formula (B), is dissolved in DMSO and then diluted with methanol: water (90:10. Stability in Buffer at Various pH buffers and SIF/SGF medium are studied:

[043] 5.7 mg of the compound of formula (B), is weighed into a 2 ml HPLC vial and dissolved in 0.250 ml DMSO. 2µl of the compound of formula (B) solution is added to 250µl of the respective buffer solution and kept at room temperature on incubator shaker for 24hr. On completion of incubation period,

the solution is centrifuged and supernatant is taken. To the supernatant, ice cold acetonitrile containing IS is added, vortexed and injected into LCMS/MS.

LC/MS/MS Method:

[044] API 4000, ESI Agilent 1100 column: Gemini Nx 100 mmx4.6 mm 5.µ; column temperature: 30°C.; eluent A: 0.1% formic acid in water, eluent B: acetonitrile; gradient: 0-2.5min 95% A, 5% B; 2.5-2.6min 5% A, 95% B; 2.6-4.2min 95% A, 5% B; flow rate: 0.8 ml/min; ESI. Q1:464.098, Q3:144.255

Decomposition of the exemplary compound in these solutions was observed at pH 7.4 and pH 7.8.

(Buffer) Solutions Employed:

[045] Prepared 0.1 mol of citric acid and 0.2 mol of di sodium hydrogen phosphate in water. Buffer pH 2.2, 4 and 7.8 are prepared using citric acid and disodium hydrogen phosphate by adjusting the pH with 0.1N HCl or 1N NaOH pH 7.4: 8.89g disodium hydrogen phosphate(Solution A) add to 1 litre of water, 1.5601g sodium di-hydrogen phosphate (solution B) are made up to 1 liter with water; Solution A (19ml) and Solution B(81ml) are mixed.

SIF/SGF: 2.38g of SIF original powder (biorelevant media) is dissolved in 1L of milliQ water. The pH of the solution is adjusted for SIF (7.4) and SGF(2.2) with 0.1N HCl. The ratios of the peak areas (F) at the respective time points in relation to the peak areas at the starting time are calculated. In this simulating SIF and SGF study samples, the simulating fluid stability of the compound of formula (B) was evaluated to see the extent the compound remaining intact at various time points at intestinal and gastric pH condition in comparison against zero minute. The peak area (F) is directly correlated to the amount of test compound which is quantified by the LC MS method.

[046] In simulating intestinal and gastric fluid, the area of the compound of formula (B) compared with zero minute area to 120min. The compound area remained the same over 120min showing stability at intestinal conditions and the similar results were observed at simulating gastric conditions.

[047] In buffer stability at pH 2.2 and 7.4

In this buffer stability of varying pH conditions of pH 2.2, 4 and 7.8, the stability of the compound of formula (B) at various pH conditions was evaluated to see the extent of the compound remaining intact at

specified time points in comparison against zero minute/or single point calibration neat aqueous standard. In these pH conditions of pH 7.4, pH 2.2, there is formation of Rivaroxaban which is monitored by LCMS. There is presence of the compound of formula (B) seen which is at pH 2.2 and 4.0 although there is a degradation and conversion to Rivaroxaban in these pH conditions.

[048] Interestingly, there is conversion of Rivaroxaban which was monitored by LCMS. This proves that the compound of formula (B) is degraded in varying pH buffer conditions and Rivaroxaban formation is observed. In buffers of pH there is a conversion to Rivaroxaban in *in vitro* conditions which is also observed to be translating in the *in vivo* conditions supported by evidence in *in vivo* rat pharmacokinetic studies in rats. Also there is evidence that conversion to Rivaroxaban is found in various *in vitro* assays like metabolic stability studies with microsomes and plasma stability studies in mouse, rat and humans.

[049] In this assay, a formation of the Rivaroxaban was found, as well as test substance (i.e., the compound of formula (B)) at various pH conditions. However, the test compound of formula (B) is stable at simulating intestinal conditions. By plotting the comparison of stability of the compound of formula (B) at various pH conditions are well illustrated in Table 1, 2 and 3.

Table 1 represents the stability comparison chart of compound of formula (B) at pH 7.8, pH 4 and pH 2.1.

	Sample Name	Analyte Peak Area (counts)	Average	SD	CV
Compound of formula (B)	pH 2.1	20975674	28315038	162.0453269	0.000572294
		35654402			
	PH 4	42841292	34879190	285.7428233	0.000819236
		26917087			
	pH 7.8	35312	38907.5	0.097829461	0.000251441
		42503			
	Standard	40131132	39849828	11.6442782	2.92204E-05
		39542018			
		39876334			

Table 2 represents the stability comparison chart of compound of formula (B) at simulating intestinal fluid.

Stability in SIF	Sample Name	Analyte Peak Area (counts)	IS Peak Area (counts)	Area Ratio	Average	SD	CV
Compound of formula (B)	Zero min	38191589	165067	231.371	226.928	6.2833509	2.7688742
		39972052	179662	222.485			
	30 min	37765238	188499	200.347	194.377	8.442855	4.3435463
		36339204	192876	188.407			
	60min	35757521	194543	183.803	182.7045	1.5535136	0.8502875
		36426366	200580	181.606			
	120min	34245401	200460	170.834	179.8485	12.748428	7.0884262
		33738135	178638	188.863			

Table 3 represents the stability comparison chart of compound of formula (B) at simulating gastric fluid.

Stability in SGF	Sample Name	Analyte Peak Area (counts)	IS Peak Area (counts)	Area Ratio	Average	SD	CV	% Parent remaining	Conclusion
Compound of formula (B)	Zero min	34733277	186737	186.001	202.267	23.003598	11.372887	100	Compound of Formula (B) is stable
		32540774	148905	218.533					
	30 min	32217743	147631	218.232	200.7875	24.670248	12.286745	99.27	
		36940283	201482	183.343					
	60min	35234526	174138	202.336	204.6365	3.2533983	1.5898426	101.17	
		35427185	171198	206.937					
	120min	36170541	185892	194.578	202.3615	11.007531	5.4395383	100.05	
		31588036	150315	210.145					

Remark (Table 1, 2 and 3)

In this assay, a formation of the Rivaroxaban was found, as well as test substance (compound of formula (B)) at various pH conditions. However, the test compound of formula (B) is stable at simulating intestinal conditions. By plotting the comparison of stability of the compound of formula (B) at various pH conditions are well illustrated in Table 1, 2 and 3.

2. In Vitro Stability in Rat, Mouse and Human Plasma (LC-MS detection)

[050] 1 mg of the compound of formula (B) is weighed into a 1.5 microfuge tube and dissolved in DMSO. The final concentration of the test compound in the assay is 5 micromolar. The compound of formula (B) was added to Rat or human plasma or mouse plasma, incubated at 37.degree. C. The 100 microlitres of aliquot at time point was removed and diluted with ice cold acetonitrile containing IS (200 .mu.L) to stop the reactions. Samples are centrifuged at 10,000 RPM for 5 minutes to precipitate proteins. Supernatants are transferred to micro centrifuge tubes and stored at -20°C for analysis of LC/MS/MS. The percent parent remaining of the test substance is calculated as ratio of peak area at each time point to peak area ratio at zero min, multiplied by 100. The compound of formula (B) is observed to be converted in to Rivaroxaban.

LC/MS/MS Method:

[051] API 4000, ESI Agilent 1100 column: Gemini Nx 100 mmx4.6 mm 5.µ; column temperature: 30°C.; eluent A: 0.1% formic acid in water, eluent B: acetonitrile; gradient: 0-2.5min 95% A, 5% B; 2.5-2.6min 5% A, 95% B; 2.6-4.2min 95% A, 5% B; flow rate: 0.8 ml/min; ESI. Q1:464.098, Q3:144.255

Table 4 represents the Plasma stability in Human, Rat and Mouse of the test compound of formula (B) and Rivaroxaban

Plasma stability in Human, Rat and Mouse of the test compound (formula-B) and Rivaroxaban (Table-4)									
Time(min)	% Parent remaining (Human)			% Parent remaining(rat)			% Parent remaining(Mouse)		
	Test Product		Rivaroxaban	Test Product			Test Product		Rivaroxaban
	Test Product (Compound formula-B)	Rivaroxaban formation*		Test Product (Compound formula-B)	Rivaroxaban formation	Rivaroxaban	Test Product (Compound formula-B)	Rivaroxaban	
0		100	100		100	100		100	100
30		95.46	100		100	82.35		100	100
60		77.84	99.68		82.43	88.4		82.18	100
Conversion	Conversion to Rivaroxaban is seen from zero min. Negligible amount of test product detected	Rivaroxaban formation in Test substance	99.99%, unchanged, stable in plasma	Conversion to Rivaroxaban is seen from zero min. Negligible amount of test product detected	Rivaroxaban formation in Test substance	88.4%, unchanged, stable in plasma	Conversion to Rivaroxaban is seen from zero min. Negligible amount of test product detected	Rivaroxaban formation in Test substance	Unchanged, stable in plasma

Remark (Table 4)

Table 4 shows the stability assay of the compound of formula (B), in plasma matrix of rat, mouse and human. The experiment was conducted to determine the stability of the compound of formula (B), as well as to see whether the conversion of the compound of formula (B) to Rivaroxaban occurs in plasma matrix of mouse, rat and human.. Rapid conversion to Rivaroxaban was observed in experiments conducted with all three species.

[052] Table 4 shows the stability assay of the compound of formula (B), in plasma matrix of rat, mouse and human. The experiment was conducted to determine the stability of the compound of formula (B), as well as to see whether the conversion of the compound of formula (B) to Rivaroxaban occurs in plasma matrix of mouse, rat and human.. Rapid conversion to Rivaroxaban was observed in experiments conducted with all three species. Negligible amount of the compound of formula (B) was observed in *in vitro* plasma stability experiment and rapid conversion to Rivaroxaban observed in *in vitro* conditions using plasma samples from the tested species

CYP Inhibition Assay

[053] The ability of substances to inhibit CYP1A2, CYP2C9, CYP2D6, CYP2C19, CYP2J2 and CYP3A4 in humans was investigated with pooled human liver microsomes as enzyme source in the presence of standard substrates (see below) which form CYP-isoform-specific metabolites. The inhibitory effects are investigated with eight different concentrations of the test compounds (0.001, 0.01, 0.1, 0.3, 1, 3, 10µM), compared with the extent of the CYP-isoform-specific metabolite formation of the standard substrates in the absence of the compound of formula (B), and the corresponding IC_{sub.50} values are calculated. A standard inhibitor which specifically inhibits a single CYP isoform serves as control of the results obtained.

Procedure:

[054] Incubation of phenacetin, diclofenac, dextromethorphan, mephenotoin, albendazole and testosterone with human liver microsomes in the presence of in each case eight different concentrations of a compound of formula (B) (as potential inhibitor) is carried out on an incubator shaker at 37 C. Standard incubation mixtures comprise NADPH and substrates in 100 mM phosphate buffer (pH 7.4) in a total volume of 200µl. Test compound are dissolved in acetonitrile. Incubated with pooled human liver microsomes at 37.degree. C. for a defined time. The reactions are stopped by adding 100µl of acetonitrile

in which a suitable internal standard is always present. Precipitated proteins are removed by centrifugation, and the supernatants analyzed by LC-MS/MS. The data represents the extrapolated IC₅₀ (μM) concentration derived from 3 μM.

CYP inhibition studies (Table 5)			
IC ₅₀ (μM) of CYP isoforms			
CYP Isoforms	Test Product(compound of formula (B))	Rivaroxaban	
1A2	1.4	18.9	
3A4	5.7	9.7	
2C9	22.4	16.7	
2C19	25.3	13.2	
2J2	6.7	No inhibition	
2D6	8.2	13.2	
Interpretation	Low drug-drug interaction (compound formula-B with other drug) when administered.		
Permeability in caco2 system			
	Papp (10 ⁻⁶ cm/sec)		Efflux ratio (B>A/A>B)
Compound	A>B	B>A	
derivative	17.41	40.3	2.31
Interpretation	Derivative showed high permeability. Classification based on Papp.		
	Derivative showed efflux of >2 and observed to be a Pgp substrate.		
(10 ⁻⁶ cm/sec) <2 = low, 2-20 = medium, >20 = high			

[055] Table 5 indicates that the CYP inhibition study using probe substrate method was carried out to determine the concentration required to inhibit different CYP isoforms. This is an essential parameter to gauge drug-drug interactions.. The compound of formula (B) showed minimal inhibition of the CYP isoforms(>1μM) that were assayed.

3. In Vitro Liver Microsomal Stability Assay

[056] Liver microsomal stability assays are conducted at 1 mg per mL liver microsome protein with an NADPH in phosphate buffer (100mM, pH 7.4). Test compounds (i.e., compounds of formula (B) of the

invention) are prepared as solutions in 20% methanol-water and added to the assay mixture (final assay concentration 1 μ M) and incubated at 37.degree. C. Aliquots (100 μ L) are taken out at times 0, 15, and 30 minutes, and diluted with ice cold acetonitrile containing IS (200 μ L) to stop the reactions. Samples are centrifuged at 10,000 RPM for 5 minutes to precipitate proteins. Supernatants are transferred to micro centrifuge tubes and stored at -20⁰C for analysis of LC/MS/MS. The percent parent remaining of the test substance is calculated as Ratio of peak area at each time point to peak area ratio at zero min, multiplied by 100. The compound of formula (B) is converted to Rivaroxaban in microsomal assay.

Table 6 represents Microsomal stability in Human, Rat and Mouse of the test compound (a compound of formula (B)) and Rivaroxaban

Microsomal stability in Human, Rat and Mouse of the test compound of formula (B) and Rivaroxaban (Table 6)									
Time(m in)	% Parent remaining (Human)			% Parent remaining(rat)			% Parent remaining(Mouse)		
	Test Product		Rivaro xaban	Test Product			Test Product		Rivaroxaban
	Test Product	Rivaroxaban formation*		Test Product	Rivaroxaban formation	Rivaro xaban	Test Product	Rivaroxaban	
0	-	100	100	-	100	100	-	100	100
15	-	100	0.01	-	19.78	0.01	-	41.93	0.03
30	-	66.2	0.01	-	5.20	0.02	-	25.70	0.02
% metabol ised	Metabolised to Rivaroxaba n, seen from zero min. Negligible amount of test product detected	Rivaroxaban formation in Test substance microsomal protein. Formed Rivaroxaban is metabolised (44.8%)	99.99% metabol ised	Metabolis ed to Rivaroxa ban is seen from zero min. Negligibl e amount of test product detected	Rivaroxaban formation in Test substance in microsomal assay. The formed Rivaroxaban is metabolised (94.8%)	99.98 % metabo lised	metabolis ed to Rivaroxa ban is seen from zero min. Negligibl e amount of test product detected	Rivaroxaban formation in Test substance in microsomal assay. The formed Rivaroxaban is metabolised (74.3%)	99.98% Metabolised
Observ ation	Formation of Rivaroxaban from start of the reaction and formed Rivaroxaban is stable in human microsome		High metabol ism	Formation of Rivaroxaban from start of the reaction and formed Rivaroxaban is shown metabolism		High metabo lism	Formation of Rivaroxaban from start of the reaction and formed Rivaroxaban is shown metabolism		High metabolism

Remark (Table 6)

The findings seen in Table 6 suggest that the test compound of formula (B) is rapidly metabolized across species in rat, mouse and human microsomes. There is immediate conversion to Rivaroxaban seen in this microsomal stability experiment by LCMS. The formed Rivaroxaban was also observed to be metabolized in the microsomal experiment across species.

[057] The findings seen in Table 6 suggest that the test compound of formula (B) is rapidly metabolized across species in rat, mouse and human microsomes. There is immediate conversion to Rivaroxaban seen in this microsomal stability experiment by LCMS. The formed Rivaroxaban was also observed to be metabolized in the microsomal experiment across species.

4. Determination of Plasma Protein Binding

[058] A compound solution (1mM in DMSO) (5µL), according to the invention is added to the respective plasma matrices of rat or human or mouse (1 ml) .

Add 150 µl of phosphate buffer to receiving side of the dialysis well. Add 150µl of plasma spiked with 5µM compound of formula (B) to the sample side of the dialysis well, dialyse for 6h. Precipitate with Acetonitrile and dilute samples prior to analysis in 1.5ml polypropylene tubes. Remove 50µl from the sample side of dialysis well and add 50µl of phosphate buffer+300µl of acetonitrile containing IS. Remove 50µl from the buffer side of the dialysis well and add 50µl of respective matrix plasma +300µl of ACN. Then vortexed and centrifuged for 5min, and supernatant is taken and injected into LCMS.

[059] Different test concentration ranging from 0.1µM to 20µM are made in methanol: water (90:10). Test solutions are added to the premixed matrix containing plasma: phosphate buffer(50:50). Precipitate with 300µl of ice cold acetonitrile containing IS, vortexes and centrifuged. Supernatant is taken and injected into LCMS.

[060] Percentage of plasma protein binding was obtained via Equation (2): % Fraction unbound
=(concentration on the buffer side/concentration on the sample side)*100

Table 7

Protein Binding in Human, Rat and Mouse of the test compound of formula (B) and quantification of Rivaroxaban (Table 7)						
Time(hr)	Human		Rat		Mouse	
	Test Product		Test Product		Test Product	
	Test Product	Rivaroxaban formation	Test Product	Rivaroxaban formation	Test Product	Rivaroxaban
Free fraction (%)	NA	1.34	NA	9.39	NA	8.41
% Binding	NA	98.66	NA	96.61	NA	91.59
Observation	Not quantifiable amount of test product . Based on plasma stability, there is a conversion to Rivaroxaban and formed Rivaroxaban found to be High bound to plasma protein. Formed Rivaroxaban from test product is similar protein binding to Rivaroxaban alone.		Not quantifiable amount of test product . Based on plasma stability, there is a conversion to Rivaroxaban and formed Rivaroxaban found to be moderate-High bound to plasma protein. Formed Rivaroxaban from test product is comparatively low protein binding to Rivaroxaban alone.		Not quantifiable amount of test product . Based on plasma stability, there is a conversion to Rivaroxaban and formed Rivaroxaban found to be moderate-High bound to plasma protein. Formed Rivaroxaban from test product is similar protein binding to Rivaroxaban alone.	

Remark (Table 7)

[061] Represent protein binding in Human Rat and Mouse. The plasma protein binding assay of the compound of formula (B), was determined in plasma matrix with different species from rat, mouse and human. This is intended to see that a compound of formula (B), plasma binding as well as to see whether the conversion of the compound of formula (B), compound to Rivaroxaban in plasma matrix across species from mouse, rat to human. There was a rapid conversion seen to Rivaroxaban in experimental conducted with all three species. Negligible amount of the compound of formula (B), was observed in-vitro plasma stability experiment and rapid conversion to Rivaroxaban observed at *in vitro* conditions across species. The formed Rivaroxaban is also bound to plasma protein across species.

5. Intravenous and Oral Pharmacokinetics in Wistar Rats:

[062] On the day before administration of the substance, a catheter for obtaining blood is implanted in the jugular vein of the experimental animals (male Wistar rats, body weight 200-250 g) under Isofluran.RTM. anesthesia.

On the day of the experiment, a defined dose of the compound of formula (B) is administered as solution into the tail vein as a bolus administration and oral administration takes place as a suspension or solution. Blood samples (8-12 time points) are taken through the catheter sequentially over the course of 24 h after administration of the substance. The administration volume is 10ml/kg for oral and 1ml/kg for IV in male Wistar rats. Intravenous administration is via a formulation of 2% N-N Dimethyl acetamide/ethanol 10%/PEG400(30%)/water for IV injection (58%) and via Tween80/PEG400/sterile water in the case of oral administration.

Removal of blood is after 0.08, 0.25, 0.5, 1, 2, 3, 4, 6, 8 and 12 hours in the case of IV and, blood withdrawn after 0.25, 0.5, 1.0, 2, 3, 4, 6, 8 and 12 hours for oral administration.

[063] Plasma is obtained by centrifuging the samples in heparinized tubes. IS containing Acetonitrile is added to a defined plasma volume per time point to precipitate proteins. After centrifugation, the compound of formula (B) and, where appropriate, known cleavage products of the compound of formula (B) in the supernatant are determined quantitatively using a suitable LC/MS-MS method.

[064] The measured plasma concentrations are used to calculate pharmacokinetic parameters of the test substance and of the active ingredient, a compound (A), liberated there from, such as AUC, C.sub.max, T.sub.1/2 (half-life) and CL (clearance).

After i.v. administration of the compounds, the test substance was no longer detectable in plasma even at the first measurement point. Only the active ingredient was detectable up to the 24-hour time point too.

After oral administration of the compounds, these substances were no longer detectable in plasma even at the first measurement point. Only the active ingredient (Example 1) was detectable up to the 24-hour time point too.

[065] Acetonitrile containing IS is added to the study samples, calibration samples and QCs, and the protein is precipitated using acetonitrile. Vortexed and centrifuged at 4000rpm and the supernatant is injected by LC-MS/MS (API 4000, AB Sciex). Chromatographic separation is carried out on an Shimadzu UFLC. The injection volume is 10µl. The separation column used is a Phenomenex Gemini NX 4.6x5µ. 100mm, adjusted to a temperature of 30.degree. C. A binary mobile phase gradient at 800 .mu.l/min is used (A: 0.1% formic acid in water, B: acetonitrile: API 4000, ESI Agilent 1100 column: Gemini Nx 100 mmx4.6 mm 5.µ; column temperature: 30°C.; eluent A: 0.1% formic acid in water, eluent B: acetonitrile; gradient: 0-2.5min 95% A, 5% B; 2.5-2.6min 5% A, 95% B; 2.6-4.2min 95% A, 5% B; flow rate: 0.8 ml/min; ESI. Q1:464.098, Q3:144.255 The temperature of the Turbo V ion source is 500.degree. C. The following MS instrument parameters are used: curtain gas 20 units, ion spray voltage 5 kV, gas 1 50 units gas 2 50 units, CAD gas 6 units. The substances are quantified by peak heights or areas using extracted ion chromatograms of specific MRM experiments.

[066] The plasma concentration/time plots determined are used to calculate the pharmacokinetic parameters such as AUC, C.sub.max, MRT (mean residence time), t.sub.1/2 (half life) and CL (clearance) employing the validated pharmacokinetic calculation programs.

6. Suspension for Intravenous Administration:

Composition:

[067] 2.2 mg of the compound according to the invention, 0.22 of ethanol (10%), 0.66ml of PEG400(30%), 1.27ml of water for injection (58%) and 0.04ml of 2% N-N-dimethyl acetamide. A single dose of 1 mg of the compound according to the invention corresponds to 1 ml of intravenous solution.

Preparation:

[068] The required quantity of the test compound is weighed in glass vial. To this, N, N dimethyl acetamide was added and vortexed. Then ethanol, PEG400 was added and vortexed. Finally, water for injection is added, mixed, vortexed and sonicated to achieve the final concentration of 1mg/ml . The final solution was clear and colorless in appearance.

7. Solution for Oral Administration:**Composition:**

[069] 8.3 of the compound of formula (B), Tween 80, PEG400 and sterile water for injection was added. The required quantity of the test compound is weighed in glass vial. To this, N, N dimethyl acetamide was added and vortexed. Then ethanol, PEG400 was added and vortexed. Finally, water for injection is added, mixed, vortexed and sonicated to achieve the final concentration of 1mg/ml . The final solution was clear and colorless in appearance

Preparation:

[070] The required quantity of the compound of formula (B) is weighed in glass vial. To this, Tween 80 was added and vortexed. Then ethanol, PEG400 was added and vortexed. Finally, water for injection is added, mixed, vortexed and sonicated to achieve the final concentration of 0.5mg/ml. The final solution was clear and colorless in appearance.

Concentration-time profile of Rivaroxaban following intravenous administration of test compound at a dose of 10 mg/kg.

Table 8

Rivaroxaban alone (Table-8)			
PK parameter	Unit	Mean IV (1 mg/kg)	Mean PO (10 mg/kg)
F	[%]	n.c.	81.99
AUC(0-t)	[ng/mL*h]	1002.09	8481.44
AUC	[ng/mL*h]	1102.73	9040.96
Δ AUC	[%]	7.11	6.01
C0(tdose)	[ng/mL]	581.14	n.c.
C(max)	[ng/mL]	n.c.	1420.27
t(max)	[h]	n.c.	1.42
t(½,z)	[h]	6.08	5.70
MRT	[h]	3.38	6.29
CL	[mL/min/kg]	25.33	18.75
V(z)	[L/kg]	10.72	9.13

Concentration-time profile of Rivaroxaban following oral administration of test compound at a dose of 10 mg/kg

Table 9

Derivative (Rivaroxaban formed on dosing of derivative)			
PK parameter	Unit	Mean IV (1 mg/kg)	Mean PO (10 mg/kg)
F	[%]	n.c.	26.95
AUC(0-t)	[ng/mL*h]	3859.31	10398.42
AUC	[ng/mL*h]	3906.91	10530.40
Δ AUC	[%]	1.51	1.49
C0(tdose)	[ng/mL]	559.12	n.c.
C(max)	[ng/mL]	n.c.	2022.31
t(max)	[h]	n.c.	1.67
t(½,z)	[h]	6.61	3.69
MRT	[h]	2.50	4.97
CL	[mL/min/kg]	4.47	17.63
V(z)	[L/kg]	2.78	5.84

[071] Tables 8 and 9 indicate that a compound of formula (B) showed an increased exposure in terms of AUC as well as increased C max as compared to Rivaroxaban. This suggests that compound of formula (B) is quickly absorbed and immediately converted to Rivaroxaban.

8. Determination of anticoagulant activity

[072] The anticoagulant action of the test substance (compound of formula (B)) and Rivaroxaban was determined in vitro using human plasma. The human plasma used for this experiment was separated from the blood collected in sodium citrate as anticoagulant. The prothrombin time (PT) was determined by using a commercial test kit (Neoplastin from Stagid) and APTT was determined using (Synthasil kit by IL). Different concentrations of test substance and Rivaroxaban used were from 0.1 to 1.0 µg/mL along with corresponding solvent as control. For determination of PT the test compound and Rivaroxaban were incubated with the plasma at 37°C for 10 minutes. Coagulation was then started by addition of thromboplastin, and time when coagulation occurred was determined. The concentration of test substance which effected a doubling of prothrombin time was determined. For determination of a PTT the test compound and Rivaroxaban were incubated with the plasma at 37°C for 10 minutes after which CaCl₂ was added. The results of assay indicated that the test compound (compound of formula (B)) has significant anticoagulant activity.

[073] In an embodiment of this invention, the compound of formula (B) can be comprised in medicament normally together with one or more inert, non-toxic, pharmaceutically suitable excipients and to the use thereof for the aforementioned purposes.

[074] The compounds can be administered to act systemically and/or locally. For this purpose, they can be administered in a suitable way and form such as, for example, by the oral, parenteral, pulmonary or nasal route, preferably orally.

[075] Suitable for oral administration are administration forms which function according to the prior art and deliver the compound according to the invention rapidly and/or in modified fashion, and which contain the compounds according to the invention in crystalline and/or amorphous and/or dissolved form, such as, for example, tablets (uncoated or coated tablets, for example having enteric coatings or coatings which are insoluble or dissolve with a delay and control the release of the compound according to the invention), tablets which disintegrate rapidly in the mouth, or films/wafers, films/lyophilizates, capsules

(for example hard or soft gelatin capsules), sugar-coated tablets, granules, pellets, powders, emulsions, suspensions, aerosols or solutions.

9. Intravenous and Oral Excretion profile in Wistar Rats:

[076] On the day before administration of the substance, a catheter for obtaining blood is implanted in the jugular vein of the experimental animals (male Wistar rats, body weight 200-250 g) under Isoflurane anesthesia.

[077] On the day of the experiment, a defined dose of the compound of formula (B) is administered as solution into the tail vein as a bolus administration and oral administration takes place as a suspension or solution. Urine and faeces are taken collected from metabolic cages over the course of 144 h after administration of the substance. The administration volume is 10ml/kg for oral and 1ml/kg for IV in male Wistar rats. Intravenous administration is via a formulation of 2% N-N Dimethyl acetamide/ethanol 10%/PEG400(30%)/water for IV injection (58%) and via Tween80/PEG400/sterile water in the case of oral administration. Urine and faeces collection is 0-4,4- 8,8- 24, 24-48,48-72,72-96,96-120,120-144 in the case of IV and for oral administration.

[078] Urine and faeces was processed and IS containing Acetonitrile is added to a defined urine/faeces and precipitated. After centrifugation, the compound of formula (B) and, where appropriate, known cleavage products of the compound of formula (B) in the supernatant are determined quantitatively using a suitable LC/MS-MS method.

[079] The measured urine and faeces concentrations are used to calculate parameters of the test substance and of the active ingredient compound (A) liberated there from, such as AUC and C.sub.max.

[080] After i.v. administration of the compounds, the test substance was no longer detectable in urine and faeces even at the first measurement point. Only the active ingredient was detectable up to in both urine and faeces.

[081] After oral administration of the compounds, these substances were no longer detectable in urine and faeces even at the first measurement point. Only the active ingredient (Example 1) was detectable in urine as well as in faeces.

[082] Acetonitrile containing IS is added to the study samples, calibration samples and QCs, and the protein is precipitated using acetonitrile. Vortexed and centrifuged and the supernatant is injected by LC-MS/MS (API 4000, AB Sciex). Chromatographic separation is carried out on an Shimadzu UFLC. The injection volume is 10µl. The separation column used is a Phenomenex Gemini NX 4.6x5µ. 100mm, adjusted to a temperature of 30.degree. C. A binary mobile phase gradient at 800 .mu.l/min is used (A: 0.1% formic acid in water , B: acetonitrile: API 4000, ESI Agilent 1100 column: Gemini Nx 100 mmx4.6 mm 5.µ; column temperature: 30°C.; eluent A: 0.1% formic acid in water, eluent B: acetonitrile; gradient: 0-2.5min 95% A, 5% B; 2.5-2.6min 5% A, 95% B; 2.6-4.2min 95% A, 5% B; flow rate: 0.8 ml/min; ESI. Q1:464.098, Q3:144.255.

The temperature of the Turbo V ion source is 500.degree. C. The following MS instrument parameters are used: curtain gas 20 units, ion spray voltage 5 kV, gas 1 50 units gas 2 50 units, CAD gas 6 units. The substances are quantified by peak heights or areas using extracted ion chromatograms of specific MRM experiments.

10. Suspension for Intravenous Administration:

[083] Composition:

2.2 mg of the compound according to the invention, 0.22 of ethanol (10%), 0.66ml of PEG400(30%), 1.27ml of water for injection (58%) and 0.04ml of 2% N-N-dimethyl acetamide. A single dose of 1 mg of the compound according to the invention corresponds to 1 ml of intravenous solution.

[084] Preparation:

The required quantity of the test compound is weighed in glass vial. To this, N, N dimethyl acetamide was added and vortexed. Then ethanol, PEG400 was added and vortexed. Finally, water for injection is added, mixed, vortexed and sonicated to achieve the final concentration of 1mg/ml . The final solution was clear and colorless in appearance.

11. Solution for Oral Administration:

Composition:

[085] compound of formula (B), Tween 80, PEG400 and sterile water for injection was added. The required quantity of the test compound is weighed in glass vial. To this, N, N dimethyl acetamide was

added and vortexed. Then ethanol, PEG400 was added and vortexed. Finally, water for injection is added, mixed, vortexed and sonicated to achieve the final concentration of 1mg/ml . The final solution was clear and colorless in appearance.

Preparation:

[086] The required quantity of the compound of formula (B) is weighed in glass vial. To this, Tween 80 was added and vortexed. Then ethanol, PEG400 was added and vortexed. Finally, water for injection is added, mixed, vortexed and sonicated. The final solution was clear and colorless in appearance.

Concentration-time profile of Rivaroxaban following intravenous administration of test compound (compound of formula (B)) at a dose of 1 mg/kg and oral administration of test compound at a dose of 10 mg/kg.

Table 10 (A)

Group	Animal weights (g)	Dose (mg/kg)	Dose volume (mL/kg)	Dose conc. (mg/mL)	Dosing route	No. of animals	Sample time (h) for urine/faeces
1	250-300	1	1	1	i.v.	3	0-4,4- 8,8- 24, 24-48,48-72,72-96,96-120,120-144
2	250-300	10	10	1	p.o.	3	0-4,4- 8,8- 24, 24-48,48-72,72-96,96-120,120-144

Table 10 (B)

	Rivaroxaban (IV 1 mg/Kg)		Rivaroxaban (PO 10 mg/Kg)		Derivative (IV 1 mg/Kg)		Derivative (PO 10 mg/Kg)	
Time [h]	Mean (ng)	Mean (ng/G)	Mean (ng)	Mean (ng/G)	Mean (ng)	Mean (ng/G)	Mean (ng)	Mean (ng/G)
	Urine	Faeces	Urine	Faeces	Urine	Faeces	Urine	Faeces
4	559.4	442.8	2381.3	5458.8	345.5	3122.7	1200.6	9675.3
8	28.9	1442.0	1770.9	19583.2	447.0	911.6	1828.5	11184.7
24	169.4	1328.8	1838.1	236961.7	451.5	614.6	3296.0	6274.2
48	333.6	342.8	464.5	35049.4	24.6	164.5	1166.5	4881.3
72	539.5	110.7	236.1	6655.8	20.0	7.4	130.8	360.4
96	41.9	32.6	22.9	2291.2	7.1	3.3	92.5	5.0
120	BLQ	40.3	48.4	63.9	BLQ	5.2	30.6	45.0
144	BLQ	16.8	30.4	57.4	4.2	BLQ	29.7	70.7

Table 10 (C)

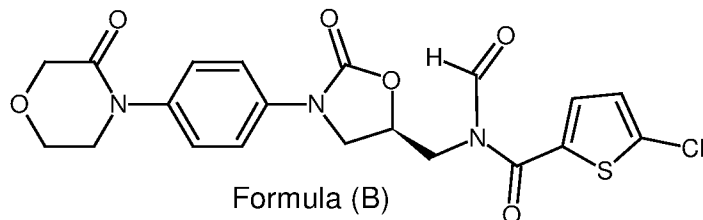
Summary Results				
	oral(10mg/kg)		IV(1mg/kg)	
	Rivaroxaban	derivative	Rivaroxaban	derivative
urine(ng)	6720.4	7744.6	1425.9	1152.8
faeces(ng/g)	408546.9	32503.0	2937.9	4974.9
plasma-AUC	9041.0	10530.4	1102.7	3906.9
Mean Urine AUC	76389.7	121079.3	20998.3	55548.0
Mean Faeces AUC	8189000.0	354379.0	12537.0	30631.7
Tmax	1.4	1.7	581.2	559.1

[087] Tables 10 (A), 10 (B) and 10 (C), indicate that, Plasma exposure is higher in Test product (compound of formula (B)) than the Rivaroxaban. The Urine excretion profile of Rivaroxaban and test compound (compound of formula (B)) showed similar excretion profile but the unabsorbed Rivaroxaban is lesser on oral administration of Test product (compound of formula (B)).

[088] The compound of formula (B), showed lesser amount of Rivaroxaban present in faeces as compared to Rivaroxaban alone. Which indicate that an advantageous in having higher exposure in plasma and lower excretion in faeces as compared to Rivaroxaban.

We Claim:

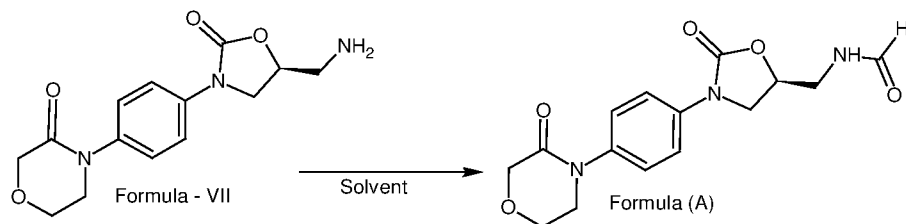
- 1) A compound of formula (B):



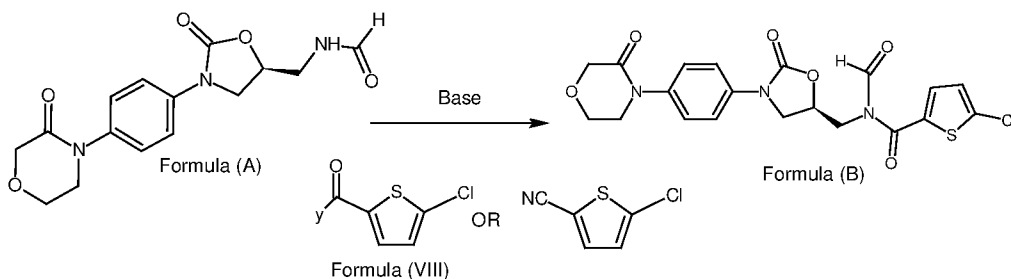
or a pharmaceutically acceptable salt or solvate or hydrate form.

- 2) A process for the preparation of a compound of formula (B), as claimed in claim 1, by the method comprising:

- a) treating a compound of formula (VII) with formic acid in a suitable solvent(s) to obtain an intermediate of formula (A), *N*-((5*S*)-2-oxo-3-[4-(3-oxomorpholin-4-yl)phenyl]-1,3-oxazolidin-5-yl)methyl)formamide:



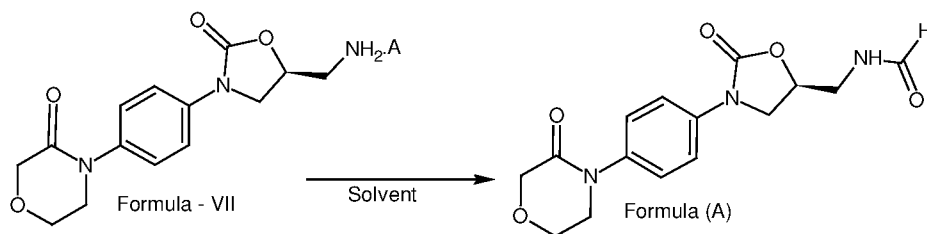
- b) treating the compound of formula (A) with a compound of formula (VIII) or 5-chlorothiophene-2-carbonitrile, in a suitable solvent(s) selected from methylene dichloride, acetone, toluene and ether, or mixture thereof, in presence of a base to obtain the compound of formula (B):



wherein Y is alkyl sulfonyl, aryl sulfonyl, imidazole, triazole, tetrazole, alkoxy, substituted alkoxy, N-oxy-succinimide, hydroxy, primary amine, secondary amine, p-nitrophenoxy, N-oxy-phthalimide, N-oxy-benzotriazole, chlorine, fluorine, bromine and iodine.

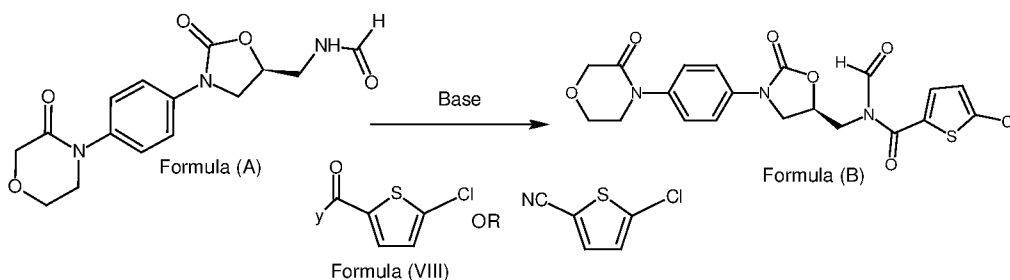
3) A process for the preparation of a compound of formula (B), as claimed in claim 1, by the method comprising:

- a) treating an acid addition salt of a compound of formula (VII) with a base in a suitable solvent(s) to obtain a base of the compound of formula (VII), which is further treated with formic acid in to obtain the novel intermediate formula (A), *N*-({(5*S*)-2-oxo-3-[4-(3-oxomorpholin-4-yl)phenyl]-1,3-oxazolidin-5-yl}methyl)formamide:



wherein A = is acid addition salt;

- b) treating the compound of formula (A) with a compound of formula (VIII) or 5-chlorothiophene-2-carbonitrile, in a suitable solvent(s) selected from methylene dichloride, acetone, toluene and ether, or mixture thereof, in presence of a base to obtain the compound of formula (B):



wherein Y is alkyl sulfonyl, aryl sulfonyl, imidazole, triazole, tetrazole, alkoxy, substituted alkoxy, N-oxy-succinimide, hydroxy, primary amine, secondary amine, p-nitrophenoxy, N-oxy-phthalimide, N-oxy-benzotriazole, chlorine, fluorine, bromine and iodine.

- 4) The process according to claim 2 or 3, wherein the compound of formula (B) is purified by a combination of one or more of the process of washing, sedimentation, filtration, drying and/or distillation.
- 5) A pharmaceutical composition comprising a compound of formula (B), as claimed in claim 1, and one or more pharmaceutically acceptable excipients.
- 6) Use of a compound of formula (B) as claimed in claim 1, or a pharmaceutical composition as claimed in claim 5, in the manufacture of a medicament for treatment and/or prevention of thromboembolic disorders in humans and animals.
- 7) Use of a compound of formula (B) as claimed in claim 1, or a pharmaceutical composition as claimed in claim 5, in the manufacture of an anti-coagulant.
- 8) A method for the treatment and/or prevention of thromboembolic disorders in a human or animal comprising administering to the human or animal a compound of formula (B) as claimed in claim 1, or a pharmaceutical composition as claimed in claim 5.
- 9) A method of coagulating blood in a human or animal comprising administering to the human or animal a compound of formula (B) as claimed in claim 1, or a pharmaceutical composition as claimed in claim 5.
- 10) An anti-coagulant comprising a compound of formula (B) as claimed in claim 1, or a pharmaceutical composition as claimed in claim 5.