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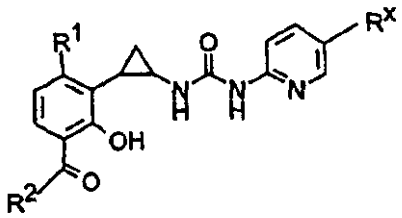

(I)

(57) Abstract

Compounds of formula (I): wherein R^x is cyano or bromo; R^1 is halo; R^2 is C_1–C_3 alkyl, and pharmaceutically acceptable salts and prodrugs thereof have activity as antiretrovirals.

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ANTIVIRALS

Technical Field

This invention relates to the field of antivirals and in particular to HIV reverse transcriptase inhibitors. The invention provides novel compounds, pharmaceutical compositions comprising these compounds and methods for the inhibition of HIV employing them.

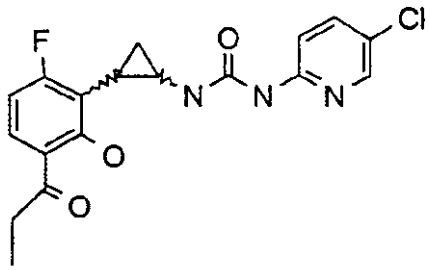
Background to the invention

Of the pharmaceuticals which have shown clinically relevant activity in the inhibition of HIV reverse transcriptase in HIV treatment, most are nucleoside analogues such as AZT, ddI, ddC and D4T. These nucleoside analogues are not as specific as is desirable and thus have to be administered at relatively high dosage levels. At these dosage levels, nucleoside analogues tend to be rather toxic, limiting their long term use.

To overcome these problems of specificity and toxicity a number of non-nucleoside inhibitors of the reverse transcriptase of HIV have been developed. For example TIBO, a reverse transcriptase from Janssen inhibits HIV at nanomolar concentrations and displays no clinically significant toxicity. Both TIBO and the non nucleotide reverse transcriptase inhibitor nevirapine proceeded rapidly to phase II clinical trials in patients. However it soon became apparent that these non-nucleoside inhibitors rapidly select out HIV mutants *in vivo* which are resistant to the usual dosages of the respective inhibitors. In the case of nevirapine for example, after only four weeks of therapy virus isolated from patient serum was 100 fold less sensitive to the drug compared with virus isolated from untreated patients (Drug Design & Discovery 1992 8 pp 255-263). A similar pattern has emerged for other non-nucleoside RT inhibitors which have entered clinical trials, Merck's L-697661 and Upjohn's delavirdine (U-87201), namely that promising in vitro activity has rapidly produced resistant HIV mutants when adminstered to patients. Notwithstanding this drawback nevirapine and delavirdine have recently been registered for clinical use, although limited to specific coadministration regimes in an attempt to retard resistance development.

International patent application no WO 95/06034 describes a series of novel urea derivatives which exhibit good in vitro activity against HIV reverse transcriptase and good inhibition of HIV replication in cell culture. However practical deployment of the compounds in WO 95/06034 is hampered by their poor pharmacokinetic performance. Additionally, as with many non-nucleoside reverse transcriptase inhibitors, the compounds presented in WO 95/06034 leave room for improvement in the key parameter of slow resistance development and a favourable pattern of activiy against HIV mutants generated by other antiviral regimes.

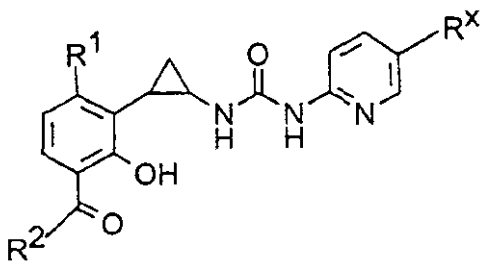
A poster of Öberg et al at the 1995 ICAR at Santa Fe disclosed inter alia a racemic compound nominally within the abovementioned WO 95/06034 and having the formula:



At the time the above depicted compound was regarded as of less interest than thiourea variants having a methoxy/acetyl bearing phenyl ring. However, we have now discovered that an alternative substitution pattern manifests an improved resistance pattern in comparison to these prior art compounds in conjunction with good pharmacokinetic performance and a prolonged time to virus resistance. The invention thus provides inhibitors which combine the superior specificity of non-nucleoside inhibitors with the clinical practicality missing from all prior art inhibitors.

Brief description of the invention

In accordance with the invention there are provided compounds of the formula I



wherein

R^1 is halo;

R^2 is C_1-C_3 alkyl;

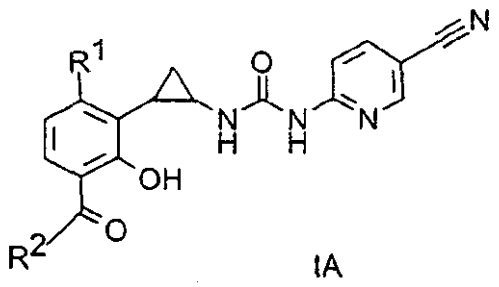
R^x is cyano or bromo;

and pharmaceutically acceptable salts and prodrugs thereof.

The invention further provides pharmaceutical compositions comprising the compounds of formula I and pharmaceutically acceptable carriers or diluents therefor. Additional aspects of the invention provide methods for the inhibition of HIV comprising administering a compound of the formula I to a subject afflicted with HIV. The invention also extends to the use of the compounds of formula I in therapy, such as in the preparation of a medicament for the treatment of HIV infections.

In treating conditions caused by HIV, the compounds of formula I are preferably administered in an amount to achieve a plasma level of around 10 to 1000 nM and more preferably 100 to 500 nM. This corresponds to a dosage rate, depending on the bioavailability of the formulation, of the order 0.01 to 10 mg/kg/day, preferably 0.1 to 2 mg/kg/day. A typical dosage rate for a normal adult will be around 0.05 to 5 g per day, preferably 0.1 to 2 g such as 500-750 mg, in one to four dosage units per day.

A preferred subset of compounds within claim 1, particularly with regard to pharmacokinetics, has the structure IA:



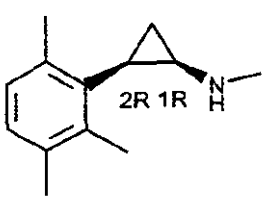
IA

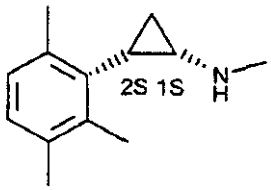
where R^1 and R^2 are as defined above, including the pharmaceutically acceptable salts and prodrugs thereof.

A further favoured subset of compounds within Formula I, particularly with regard to ease of forming prodrugs, comprise compounds wherein R^x is bromo.

Preferably R^1 is chloro and more preferably fluoro. Suitable R^2 groups include methyl, isopropyl, n-propyl and preferably ethyl.

As depicted above, the cyclopropyl ring is in the *cis* configuration, allowing two enantiomers, 1S, 2S and 1R, 2R (respectively and non-conventionally denoted 2R,1S and 2S,1R in SE 980016-7 and SE 9800113-4):

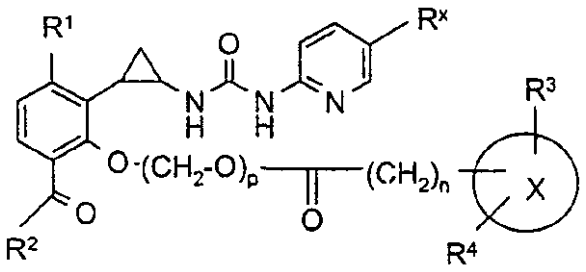




Each of these enantiomers are potent antiretrovirals, although the different enantiomers can display subtle differences in physiological properties. For instance the 1S, 2S and 1R,2R enantiomers can show a different pattern of metabolism within the P450 system. The 1S,2S enantiomer of compounds wherein R^x is cyano is particularly preferred as it appears unique in being able to avoid key components of the P450 system. Other retroviral agents such as the HIV protease inhibitor ritonavir interact extensively with the P450 system, leading to an array of undesirable

physiological responses including extensive alteration of the metabolism of other co-administered drugs. This is of particular concern with pharmaceuticals administered for a chronic infection where patients can expect to take a number of pharmaceuticals for years, if not decades.

Suitable prodrugs of the compounds of formula I include those of the formula II:



II

wherein

R^1, R^2 and R^x are as defined above,

R^3 is H, $(CH_m)_nNR^5R^6$;

R^4 is H, C_1-C_3 alkyl, $(CH_m)_nNR^5R^6$, $(CH_m)_nC(=O)R^5$, $(CH_m)_nOH$, OR^7, halo, CF_3 or CN; or

R^3 and R^4 together define a 5 or 6 membered fused ring having 0-2 hetero atoms and/or 0-2 unsaturated bonds and/or 0-2 substituents;

R^5 is H, C_1-C_3 alkyl, $C(=O)R^7$ or a peptide of 1 to 4 amino acids;

R^6 is H, C_1-C_3 alkyl; or

R^5 and R^6 together define a 5 or 6 membered ring having 0 or 1 additional hetero atom and/or 0-2 unsaturated bonds and/or 0-2 substituents;

R^7 is H, C_1-C_{12} alkyl, $(CH_m)_nNR^5R^6$;

X and its encompassing circle define a 5 or 6 membered ring having 0 to 3 unsaturated bonds and/or 0 to 3 hetero atoms selected from S, O and N;

m is independently 1 or 2;

n is independently 0, 1 or 2;

and pharmaceutically acceptable salts thereof.

Corresponding prodrugs of compounds wherein R^x is chloro form a further aspect of the invention.

The ring structure containing X, hereafter referred to the X-ring, may be saturated or have 1-3 unsaturated bonds, including rings with an aromatic character. Preferred X-rings include a cyclohexanyl or cyclohexenyl ring or more preferably a phenyl ring. Other preferred X-rings include morpholino or more preferably a pyridyl ring. Alternatively, X-ring may define a five membered ring such as pentenyl or pyrrolyl.

Suitable fused ring systems for the X-ring in the event that R^3 and R^4 join to form an optionally hetero-containing ring include napthyl, quinolyl, tetrahydroisoquinolyl, indolyl or benzimidazole ring systems. Suitable substituent rings for the X-ring in the event that R^4 and R^5 join to form a ring include morpholino and piperidino. These fused or substituent rings may be may be optionally substituted with halo, halomethyl, amino such as $(CH_m)_nNR^5R^6$, $C(=O)NR^5R^6$, hydroxy, hydroxymethyl, carboxy, carboxymethyl, C_{1-3} alkyl, C_{1-3} alkoxy and the like.

The X-ring may be spaced from the adjacent carbonyl moiety by a methylene or ethylene group which may be optionally substituted with substituents such as halo, halomethyl, amino, amino methyl, hydroxy, hydroxymethyl, carboxy, carboxymethyl, C_{1-3} alkyl, C_{1-3} alkoxy and the like. It is preferred that the X-ring is adjacent the carbonyl.

Preferably the moiety represented by the X- ring system, R^3, R^4 and, if present R^5- R^7 has a somewhat basic character. This can be achieved by selecting a suitably basic heterocycle as the X- ring, such as pyridyl or benzopyridyl. Alternatively or additionally, one or more of R^3 to R^7 may comprise a basic substituent such as a primary, secondary or tertiary amine, an amino acid etc.

Favoured R^3 and/or R^4 groups include NH_2, $N(CH_2)_2$ and NHC_1-C_3 alkyl, such as $NHCH_3$ or $NHCH_2CH_3$. Preferably R^3 is in the meta position relative to the carbonyl and its optional spacer, especially where the X-contaning ring is phenyl or R^3 is in the para position when the X-containing ring is heteroaromatic, such as pyrid-3-yl. The currently preferred value for p and/or n is zero, that is the respective groups are absent.

Preferred compounds of the invention include:

(1S, 2S)-N-[*cis*-2-(6-fluoro, 2-hydroxy, 3-propionylphenyl)-cyclopropyl]-N'-(5-cyanopyrid-2-yl)-urea,

(1S, 2S)-N-[*cis*-2-(6-fluoro, 2-hydroxy, 3-butyrylphenyl)-cyclopropyl]-N'-(5-cyanopyrid-2-yl)-urea,

(1S, 2S)-N-[*cis*-2-(6-fluoro, 2-hydroxy, 3-acetylphenyl)-cyclopropyl]-N'-(5-cyanopyrid-2-yl)-urea,

(1S, 2S)-N-[*cis*-2-(2-(3-aminophenylcarbonyloxy)-6-fluoro-3-propionylphenyl)-cyclopropyl]-N'-(5-cyanopyrid-2-yl)-urea;

(1S, 2S)-N-[*cis*-2-(2-(3-aminophenylcarbonyloxy)-6-fluoro-3-butyrylphenyl)-cyclopropyl]-N'-(5-cyanopyrid-2-yl)-urea;

(1S, 2S)-N-[*cis*-2-(2-(3-aminophenylcarbonyloxy)-6-fluoro-3-acetylphenyl)-cyclopropyl]- N'-(5-cyanopyrid-2-yl)-urea;

(1S, 2S)-N-[*cis*-2-(2-(3-ethylaminophenylcarbonyloxy)-6-fluoro-3-propionylphenyl)-cyclopropyl]-N'-(5-cyanopyrid-2-yl)-urea;

(1S, 2S)-N-[*cis*-2-(2-(3-ethylaminophenylcarbonyloxy)-6-fluoro-3-butyrylphenyl)-cyclopropyl]-N'-(5-cyanopyrid-2-yl)-urea;

(1S, 2S)-N-[*cis*-2-(2-(3-ethylaminophenylcarbonyloxy)-6-fluoro-3-acetylphenyl)-cyclopropyl]-N'-(5-cyanopyrid-2-yl)-urea;

(1S, 2S)-N-[*cis*-2-(2-(3-dimethylaminophenylcarbonyloxy)-6-fluoro-3-propionylphenyl)-cyclopropyl]- N'-(5-cyanopyrid-2-yl)-urea;

(1S, 2S)-N-[*cis*-2-(2-(3-dimethylaminophenylcarbonyloxy)- 6-fluoro-3-butyrylphenyl)-cyclopropyl]- N'-(5-cyanopyrid-2-yl)-urea;

(1S, 2S)-N-[*cis*-2-(2-(3-dimethylaminophenylcarbonyloxy)-6-fluoro-3-acetylphenyl)-cyclopropyl]- N'-(5-cyanopyrid-2-yl)-urea;

and pharmaceutically acceptable salts thereof.

Other preferred compounds include

(1S, 2S)-N-[*cis*-2-(2-(6-methylaminopyrid-3-ylcarbonyloxy)-6-fluoro-3-propionylphenyl)-cyclopropyl]- N'-(5-cyanopyrid-2-yl)-urea;

(1S, 2S)-N-[*cis*-2-(2-(6-methylaminopyrid-3-ylcarbonyloxy)- 6-fluoro-3-butyrylphenyl)-cyclopropyl]- N'-(5-cyanopyrid-2-yl)-urea;

(1S, 2S)-N-[*cis*-2-(2-(6-methylaminopyrid-3-ylcarbonyloxy)-6-fluoro-3-acetylphenyl)-cyclopropyl]- N'-(5-cyanopyrid-2-yl)-urea;

(1S, 2S)-N-[*cis*-2-(2-(6-aminopyrid-3-ylcarbonyloxy)-6-fluoro-3-propionylphenyl)-cyclopropyl]- N'-(5-cyanopyrid-2-yl)-urea;

(1S, 2S)-N-[*cis*-2-(2-(6-aminopyrid-3-ylcarbonyloxy)- 6-fluoro-3-butyrylphenyl)-cyclopropyl]- N'-(5-cyanopyrid-2-yl)-urea;

(1S, 2S)-N-[*cis*-2-(2-(6-aminopyrid-3-ylcarbonyloxy)-6-fluoro-3-acetylphenyl)-cyclopropyl]- N'-(5-cyanopyrid-2-yl)-urea;

and pharmaceutically acceptable salts thereof

Other convenient compounds of the invention include:

(1R, 2R)-N-[*cis*-2-(6-fluoro, 2-hydroxy, 3-propionylphenyl)-cyclopropyl]-N'-(5-cyanopyrid-2-yl)-urea,

(1R, 2R)-N-[*cis*-2-(6-fluoro, 2-hydroxy, 3-butyrylphenyl)-cyclopropyl]-N'-(5-cyanopyrid-2-yl)-urea,

(1R, 2R)-N-[*cis*-2-(6-fluoro, 2-hydroxy, 3-acetylphenyl)-cyclopropyl]-N'-(5-cyanopyrid-2-yl)-urea,

(1R, 2R)-N-[*cis*-2-(2-(3-aminophenylcarbonyloxy)-6-fluoro-3-propionylphenyl)-cyclopropyl]-N'-(5-cyanopyrid-2-yl)-urea;

(1R, 2R)-N-[*cis*-2-(2-(3-aminophenylcarbonyloxy)-6-fluoro-3-butyrylphenyl)-cyclopropyl]-N'-(5-cyanopyrid-2-yl)-urea;

(1R, 2R)-N-[*cis*-2-(2-(3-aminophenylcarbonyloxy)-6-fluoro-3-acetylphenyl)-cyclopropyl]- N'-(5-cyanopyrid-2-yl)-urea;

(1R, 2R)-N-[*cis*-2-(2-(3-ethylaminophenylcarbonyloxy)-6-fluoro-3-propionylphenyl)-cyclopropyl]-N'-(5-cyanopyrid-2-yl)-urea;

(1R, 2R)-N-[*cis*-2-(2-(3-ethylaminophenylcarbonyloxy)-6-fluoro-3-butyrylphenyl)-cyclopropyl]-N'-(5-cyanopyrid-2-yl)-urea;

(1R, 2R)-N-[*cis*-2-(2-(3-ethylaminophenylcarbonyloxy)-6-fluoro-3-acetylphenyl)-cyclopropyl]-N'-(5-cyanopyrid-2-yl)-urea;

(1R,2R)-N-[*cis*-2-(2-(3-dimethylaminophenylcarbonyloxy)-6-fluoro-3-propionylphenyl)-cyclopropyl]- N'-(5-cyanopyrid-2-yl)-urea;

(1R, 2R)-N-[*cis*-2-(2-(3-dimethylaminophenylcarbonyloxy)- 6-fluoro-3-butyrylphenyl)-cyclopropyl]- N'-(5-cyanopyrid-2-yl)-urea;

(1R, 2R)-N-[*cis*-2-(2-(3-dimethylaminophenylcarbonyloxy)-6-fluoro-3-acetylphenyl)-cyclopropyl]- N'-(5-cyanopyrid-2-yl)-urea;

and their pharmaceutically acceptable salts.

Other convenient compounds include;

(1R, 2R)-N-[*cis*-2-(2-(6-methylaminopyrid-3-ylcarbonyloxy)-6-fluoro-3-propionylphenyl)-cyclopropyl]- N'-(5-cyanopyrid-2-yl)-urea;

(1R, 2R)-N-[*cis*-2-(2-(6-methylaminopyrid-3-ylcarbonyloxy)- 6-fluoro-3-butyrylphenyl)-cyclopropyl]- N'-(5-cyanopyrid-2-yl)-urea;

(1R, 2R)-N-[*cis*-2-(2-(6-methylaminopyrid-3-ylcarbonyloxy)-6-fluoro-3-acetylphenyl)-cyclopropyl]- N'-(5-cyanopyrid-2-yl)-urea;

(1R, 2R)-N-[*cis*-2-(2-(6-aminopyrid-3-ylcarbonyloxy)-6-fluoro-3-propionylphenyl)-cyclopropyl]- N'-(5-cyanopyrid-2-yl)-urea;

(1R, 2R)-N-[*cis*-2-(2-(6-aminopyrid-3-ylcarbonyloxy)- 6-fluoro-3-butyrylphenyl)-cyclopropyl]- N'-(5-cyanopyrid-2-yl)-urea;

(1R, 2R)-N-[*cis*-2-(2-(6-aminopyrid-3-ylcarbonyloxy)-6-fluoro-3-acetylphenyl)-cyclopropyl]- N'-(5-cyanopyrid-2-yl)-urea;

and pharmaceutically acceptable salts thereof.

Preferred compounds of the invention include

(1S, 2S)-N-[*cis*-2-(2-(6-fluoro, 2-hydroxy, 3-propionylphenyl)-cyclopropyl]- N'-(5-bromopyrid-2-yl)-urea;

(1S, 2S)-N-[*cis*-2-(2-(3-aminophenylcarbonyloxy)- 6-fluoro-3-propionylphenyl)-cyclopropyl]- N'-(5-bromopyrid-2-yl)-urea;

(1S, 2S)-N-[*cis*-2-(2-(3-aminophenylcarbonyloxy)-6-fluoro-3-acetylphenyl)-cyclopropyl]- N'-(5-bromopyrid-2-yl)-urea;

(1S, 2S)-N-[*cis*-2-(2-(3-aminophenylcarbonyloxy)-6-fluoro-3-butyrylphenyl)-cyclopropyl]- N'-(5-bromopyrid-2-yl)-urea;

(1S, 2S)-N-[*cis*-2-(2-(3-ethylaminophenylcarbonyloxy)-6-fluoro-3-propionylphenyl)-cyclopropyl]- N'-(5-bromopyrid-2-yl)-urea;

(1S, 2S)-N-[*cis*-2-(2-(3-ethylaminophenylcarbonyloxy)- 6-fluoro-3-acetylphenyl)-cyclopropyl]- N'-(5-bromopyrid-2-yl)-urea;

(1S, 2S)-N-[*cis*-2-(2-(3-ethylaminophenylcarbonyloxy)- 6-fluoro-3-butyrylphenyl)-cyclopropyl]- N'-(5-bromopyrid-2-yl)-urea;

(1S, 2S)-N-[*cis*-2-(2-(3-dimethylaminophenylcarbonyloxy)- 6-fluoro-3-propionylphenyl)-cyclopropyl]- N'-(5-bromopyrid-2-yl)-urea;

(1S, 2S)-N-[*cis*-2-(2-(3-dimethylaminophenylcarbonyloxy)-6-fluoro-3-acetylphenyl)-cyclopropyl]- N'-(5-bromopyrid-2-yl)-urea;

(1S, 2S)-N-[*cis*-2-(2-(3-dimethylaminophenylcarbonyloxy)-6-fluoro-3-butyrylphenyl)-cyclopropyl]- N'-(5-bromopyrid-2-yl)-urea;

(1R, 2R)-N-[*cis*-2-(2-(6-fluoro, 2-hydroxy, 3-propionylphenyl)-cyclopropyl]- N'-(5-bromopyrid-2-yl)-urea;

(1R, 2R)-N-[*cis*-2-(2-(3-aminophenylcarbonyloxy)- 6-fluoro-3-propionylphenyl)-cyclopropyl]- N'-(5-bromopyrid-2-yl)-urea;

(1R, 2R)-N-[*cis*-2-(2-(3-aminophenylcarbonyloxy)-6-fluoro-3-acetylphenyl)-cyclopropyl]- N'-(5-bromopyrid-2-yl)-urea;

(1R, 2R)-N-[*cis*-2-(2-(3-aminophenylcarbonyloxy)-6-fluoro-3-butyrylphenyl)-cyclopropyl]- N'-(5-bromopyrid-2-yl)-urea;

(1R, 2R)-N-[*cis*-2-(2-(3-ethylaminophenylcarbonyloxy)-6-fluoro-3-propionylphenyl)-cyclopropyl]- N'-(5-bromopyrid-2-yl)-urea;

(1R, 2R)-N-[*cis*-2-(2-(3-ethylaminophenylcarbonyloxy)- 6-fluoro-3-acetylphenyl)-cyclopropyl]- N'-(5-bromopyrid-2-yl)-urea;

(1R, 2R)-N-[*cis*-2-(2-(3-ethylaminophenylcarbonyloxy)- 6-fluoro-3-butyrylphenyl)-cyclopropyl]- N'-(5-bromopyrid-2-yl)-urea;

(1R, 2R)-N-[*cis*-2-(2-(3-dimethylaminophenylcarbonyloxy)- 6-fluoro-3-propionylphenyl)-cyclopropyl]- N'-(5-bromopyrid-2-yl)-urea;

(1R, 2R)-N-[*cis*-2-(2-(3-dimethylaminophenylcarbonyloxy)-6-fluoro-3-acetylphenyl)-cyclopropyl]- N'-(5-bromopyrid-2-yl)-urea;

(1R, 2R)-N-[*cis*-2-(2-(3-dimethylaminophenylcarbonyloxy)-6-fluoro-3-butyrylphenyl)-cyclopropyl]- N'-(5-bromopyrid-2-yl)-urea;

and their pharmaceutically acceptable salts.

Further preferred compounds include:

(1S, 2S)-N-[*cis*-2-(2-(6-methylaminopyrid-3-ylcarbonyloxy)-6-fluoro-3-propionylphenyl)-cyclopropyl]- N'-(5-bromopyrid-2-yl)-urea;

(1S, 2S)-N-[*cis*-2-(2-(6-methylaminopyrid-3-ylcarbonyloxy)- 6-fluoro-3-butyrylphenyl)-cyclopropyl]- N'-(5-bromopyrid-2-yl)-urea;

(1S, 2S)-N-[*cis*-2-(2-(6-methylaminopyrid-3-ylcarbonyloxy)-6-fluoro-3-acetylphenyl)-cyclopropyl]- N'-(5-bromopyrid-2-yl)-urea;

(1S, 2S)-N-[*cis*-2-(2-(6-aminopyrid-3-ylcarbonyloxy)-6-fluoro-3-propionylphenyl)-cyclopropyl]- N'-(5-bromopyrid-2-yl)-urea;

(1S, 2S)-N-[*cis*-2-(2-(6-aminopyrid-3-ylcarbonyloxy)- 6-fluoro-3-butyrylphenyl)-cyclopropyl]- N'-(5-bromopyrid-2-yl)-urea;

(1S, 2S)-N-[*cis*-2-(2-(6-aminopyrid-3-ylcarbonyloxy)-6-fluoro-3-acetylphenyl)-cyclopropyl]- N'-(5-bromopyrid-2-yl)-urea;

(1R, 2R)-N-[*cis*-2-(2-(6-methylaminopyrid-3-ylcarbonyloxy)-6-fluoro-3-propionylphenyl)-cyclopropyl]- N'-(5-bromopyrid-2-yl)-urea;

(1R, 2R)-N-[*cis*-2-(2-(6-methylaminopyrid-3-ylcarbonyloxy)- 6-fluoro-3-butyrylphenyl)-cyclopropyl]- N'-(5-bromopyrid-2-yl)-urea;

(1R, 2R)-N-[*cis*-2-(2-(6-methylaminopyrid-3-ylcarbonyloxy)-6-fluoro-3-acetylphenyl)-cyclopropyl]- N'-(5-bromopyrid-2-yl)-urea;

(1R, 2R)-N-[*cis*-2-(2-(6-aminopyrid-3-ylcarbonyloxy)-6-fluoro-3-propionylphenyl)-cyclopropyl]- N'-(5-bromopyrid-2-yl)-urea;

(1R, 2R)-N-[*cis*-2-(2-(6-aminopyrid-3-ylcarbonyloxy)- 6-fluoro-3-butyrylphenyl)-cyclopropyl]- N'-(5-bromopyrid-2-yl)-urea;

(1R, 2R)-N-[*cis*-2-(2-(6-aminopyrid-3-ylcarbonyloxy)-6-fluoro-3-acetylphenyl)-cyclopropyl]- N'-(5-bromopyrid-2-yl)-urea;

and pharmaceutically acceptable salts thereof.

Appropriate pharmaceutically acceptable salts of the compounds of formula I include salts of organic carboxylic acids such as acetic, lactic, gluconic, citric, tartaric, maleic, malic, pantothenic, isethionic, oxalic, lactobionic, and succinic acids, organic

sulfonic acids such as methanesulfonic acid, ethanesulfonic acid, benzenesulfonic acid, p-chlorobenzenesulfonic acid and p-toluenesulfonic acid; and inorganic acids such as hydrochloric, hydroiodic, sulfuric, phosphoric and sulfamic acids.

In keeping with the usual practice with HIV inhibitors it is advantageous to co-administer one to three additional antivirals to provide synergistic responses and to ensure complementary resistance patterns. Such additional antivirals may include AZT, ddI, ddC, D4T, 3TC, abacavir, adefovir, adefovir dipivoxil, bis-POC-PMPA, *foscarnet, hydroxyurea, Hoechst-Bayer HBY 097, efavirenz, trovirdine, nevirapine,* delaviridine, PFA, H2G, ABT 606, DMP-450, loviride, ritonavir, saquinavir, indinavir, amprenavir (Vertex VX 478), nelfinavir and the like, typically at molar ratios reflecting their respective activities and bioavailabilities. Generally such ratio will be of the order of 25:1 to 1:25, relative to the compound of formula I.

While it is possible for the active agent to be administered alone, it is preferable to present it as part of a pharmaceutical formulation. Such a formulation will comprise the above defined active agent together with one or more acceptable carriers and optionally other therapeutic ingredients. The carrier(s) must be acceptable in the sense of being compatible with the other ingredients of the formulation and not deleterious to the recipient.

The formulations include those suitable for oral, rectal, nasal, topical (including buccal and sublingual), vaginal or parenteral (including subcutaneous, intramuscular, intravenous and intradermal) administration. The formulations may conveniently be presented in unit dosage form, e.g. tablets and sustained release capsules, and may be prepared by any methods well known in the art of pharmacy.

Such methods include the step of bringing into association the above defined active agent with the carrier. In general, the formulations are prepared by uniformly and intimately bringing into association the active agent with liquid carriers or finely divided solid carriers or both, and then if necessary shaping the product.

Formulations for oral administration in the present invention may be presented as discrete units such as capsules, cachets or tablets each containing a predetermined amount of the active agent; as a powder or granules; as a solution or a suspension of the active agent in an aqueous liquid or a non-aqueous liquid; or as an oil-in-water liquid emulsion or a water in oil liquid emulsion and as a bolus etc.

With regard to compositions for oral administration (e.g. tablets and capsules), the term suitable carrier includes vehicles such as common excipients e.g. binding agents, for example syrup, acacia, gelatin, sorbitol, tragacanth, polyvinylpyrrolidone (Povidone), methylcellulose, ethylcellulose, sodium carboxymethylcellulose, hydroxypropylmethylcellulose, sucrose and starch; fillers and carriers, for example corn starch, gelatin, lactose, sucrose, microcrystalline cellulose, kaolin, mannitol, dicalcium phosphate, sodium chloride and alginic acid; and lubricants such as magnesium stearate and other metallic stearates, stearic acid, silicone fluid, talc waxes, oils and colloidal silica. Flavouring agents such as peppermint, oil of wintergreen, cherry flavouring or the like can also be used. It may be desirable to add a colouring agent to make the dosage form readily identifiable. Tablets may also be coated by methods well known in the art.

Convenient carriers for oral dosing include liquid formulations in the form of solutions, suspensions or emulsions, optionally encapsulated or otherwise presented in unit dose form in a conventional manner. Favoured formulations include acacia/TWEEN/water, TWEEN/water, propylene glycol, vegetable oil (such as

peanut, safflower, olive and the like) with 10-20% ethanol, vegetable oil/Capmul MGM, Capmul MCM/propylene glycol, methyl cellulose/water, vegetable oil/stearoyl monoester of glycerol, vegetable oil/monounsaturated fatty acid ester of glycerol and the like.

A tablet may be made by compression or moulding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine the active agent in a free flowing form such as a powder or granules, optionally mixed with a binder, lubricant, inert diluent, preservative, surface-active or dispersing agent. Moulded tablets may be made by moulding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent. The tablets may be optionally be coated or scored and may be formulated so as to provide slow or controlled release of the active agent.

Formulations suitable for topical administration include lozenges comprising the active agent in a flavoured base, usually sucrose and acacia or tragacanth; pastilles comprising the active agent in an inert base such as gelatin and glycerin, or sucrose and acacia; and mouthwashes comprising the active agent in a suitable liquid carrier.

Formulations suitable for topical administration to the skin may be presented as ointments, creams, gels, and pastes comprising the active agent and a pharmaceutically active carrier. An exemplary topical delivery system is a transdermal patch containing the active agent. Other topical formulations include antiseptic swabs which release the active agent upon the skin prior to invasive procedures such as injection or capillary blood sampling. Such swabs neutralise HIV in the blood or serum emanating from the invasive procedure thus assisting to prevent transfer of HIV to health care workers via needle stick accidents. Such swabs

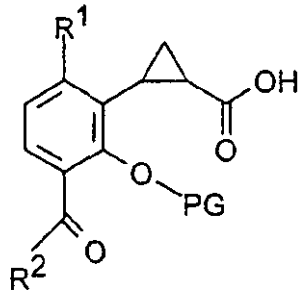
may comprise a sterile surgical gauze pad soaked in a solution of the active agent in a volatile solvent such as ethanol and single packed in a sealed sachet.

Formulations for rectal or vaginal administration may be presented as a suppository or pessary with a suitable base comprising, for example, cocoa butter or a salicylate. Other vaginal preparations can be presented as tampons, creams, gels, pastes, foams or spray formulations containing, in addition to the active agent, such carriers as are known in the art to be appropriate.

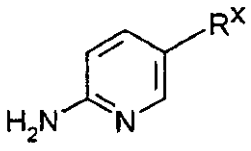
Formulations suitable for nasal administration wherein the carrier is a solid include a coarse powder having a particle size, for example, in the range 20 to 500 microns which is administered in the manner in which snuff is taken, i.e. by rapid inhalation from a container of the powder held up close to the nose. Suitable formulations wherein the carrier is a liquid for administration, for example, as a nasal spray or as nasal drops, include aqueous or oily solutions of the active agent.

Formulations suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain antioxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example sealed ampoules and vials, and may be stored in freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example water for injection, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules, and tablets of the kind previously described.

A further aspect of the invention provides methods for the preparation of the compounds of Formula I, in particular the *cis* enantiomers, comprising the Curtius rearrangement of a compound of the formula:

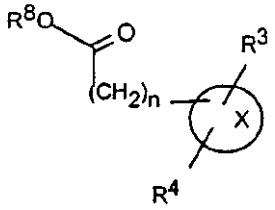


followed by coupling of a compound of the formula



and deprotection, wherein R^1, R^2 and R^x are as defined above and PG is an hydroxy-protecting group.

The methods of the invention can further comprise the step of acylating with an activated compound of the formula III:



III

where R^3, R^4, X and n are as defined above but are optionally protected, and R^8 is hydrogen or a conventional activating group. Alternatively the method of the invention may further comprise the step of alkylation with a compound of the formula IIIa:

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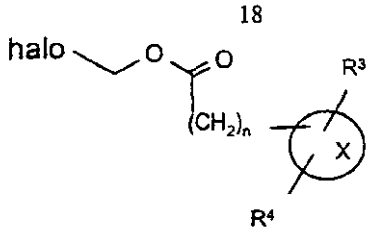
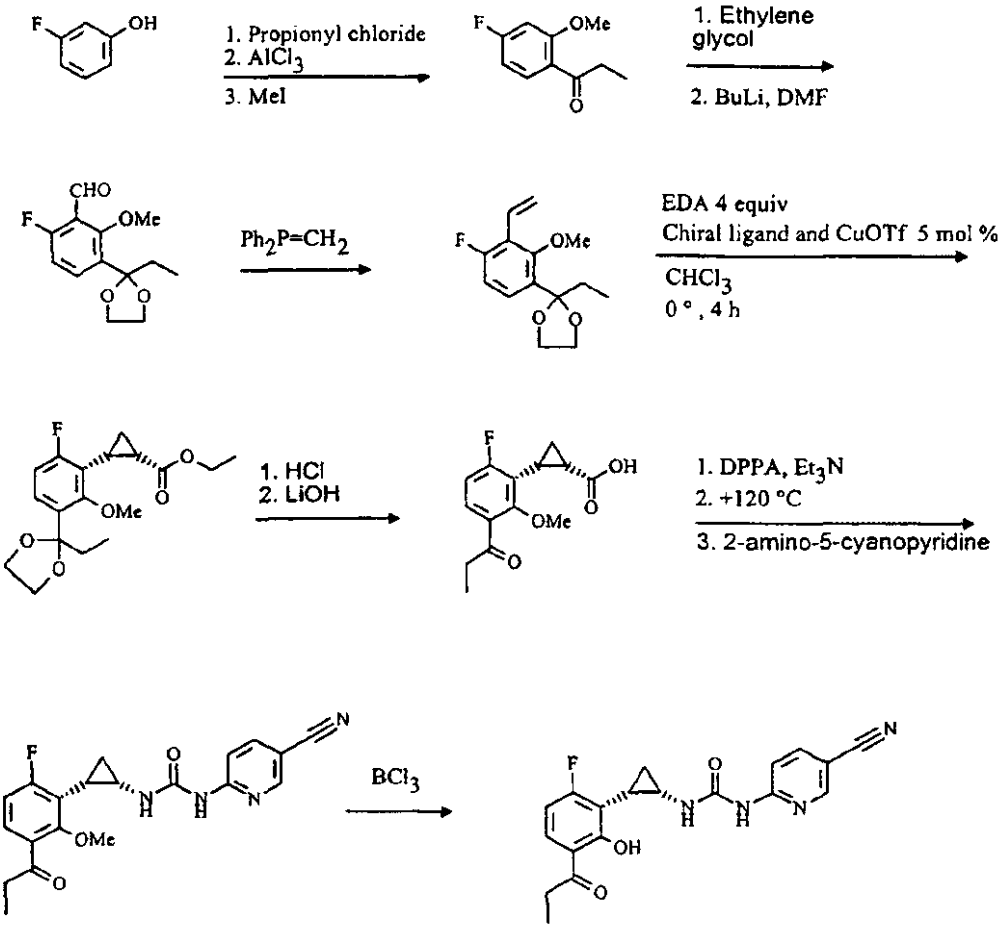


IIIa

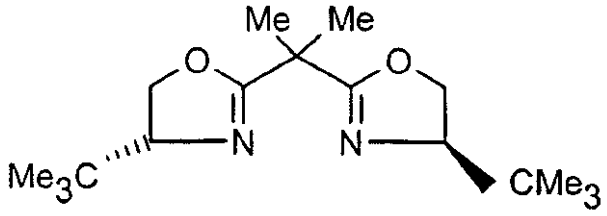
where n, R^3, R^4 and X are as defined above, but where exposed amine, hydroxy etc substituents being protected with conventional protecting groups.

Enantiomeric compounds of formula I may thus be prepared by the reaction scheme below:



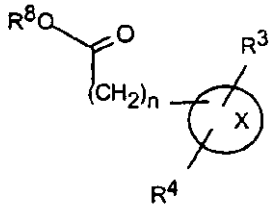
The above scheme illustrates the preparation of a (1S, 2S) compound of the invention where R^x is cyano, R^1 is F and R^2 is ethyl, but corresponding methodology is

applicable to the other R^x, R^1 and R^2 variants. The chiral ligand indicated for the fourth step may comprise, for example, a compound of the formula:



To prepare the 1R, 2R enantiomer, the mirror image chiral ligand is employed. Alternatively, the chiral ligand may be omitted in order to form the racemate.

Prodrugs of the formula II wherein p is 0 can be synthesised by acylating a compound of the formula I with with an activated compound of the formula III,



III

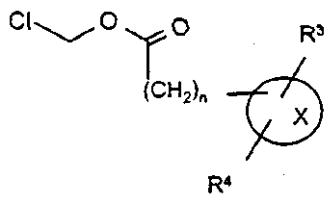
where R^3, R^4, X and n are as defined above but are optionally protected, and R^8 is hydrogen or a conventional activating group.

Activated compounds of Formula III include the acid halide, acid anhydride, activated acid ester or the acid in the presence of a coupling reagent such as dicyclohexyl-carbodiimide. Representative activated acid derivatives include the acid chloride, formic and acetic acid derived mixed anhydrides, anhydrides derived from alkoxycarbonyl halides such as isobutyloxycarbonylchloride and the like, N-hydroxysuccinamide derived esters, N-hydroxyphthalimide derived esters, N-hydroxy-5- norbornene-2,3-dicarboxamide derived esters, 2,4,5-trichlorophenol derived esters and the like. Suitable optional protecting groups for compounds of formula III, especially any constituent amines, include those groups intended to protect the N-terminus of an amino acid or peptide or to protect an amino group against undesirable reactions during synthetic procedures. Commonly used N-

protecting groups are disclosed in Greene, "Protective Groups in Organic Synthesis" (John Wiley & Sons, New York, 1981), which is hereby incorporated by reference. N-protecting groups include acyl groups such as formyl, acetyl, propionyl, pivaloyl, t-butylacetyl, 2-chloroacetyl, 2-bromoacetyl, trifluoracetyl, trichloroacetyl, phthalyl, o-nitrophenoxyacetyl, α-chlorobutyryl, benzoyl, 4-chlorobenzoyl, 4-bromobenzoyl, 4-nitrobenzoyl, and the like; sulfonyl groups such as benzenesulfonyl, p-toluenesulfonyl, and the like, carbamate forming groups such as benzyloxycarbonyl, p-chlorobenzyloxycarbonyl, p-methoxybenzyloxycarbonyl, p-nitrobenzyloxycarbonyl, 2-nitrobenzyloxycarbonyl, p-bromobenzyloxycarbonyl, 3,4-dimethoxybenzyloxycarbonyl, 4-methoxybenzyloxycarbonyl, 2-nitro-4,5-dimethoxybenzyloxycarbonyl, 3,4,5-trimethoxybenzyloxycarbonyl, 1-(p-biphenylyl)-1-methylethoxycarbonyl, α,α-dimethyl-3,5-dimethoxybenzyloxycarbonyl, benzhydryloxycarbonyl, t-butoxycarbonyl, diisopropylmethoxycarbonyl, isopropyloxycarbonyl, ethoxycarbonyl, methoxycarbonyl, allyloxycarbonyl, 2,2,2-trichloroethoxycarbonyl, phenoxycarbonyl, 4-nitrophenoxycarbonyl, fluorenyl-9-methoxycarbonyl, cyclopentyloxycarbonyl, adamantyloxycarbonyl, cyclohexyloxycarbonyl, phenylthiocarbonyl, and the like; alkyl gropus such as benzyl, triphenylmethyl, benzyloxymethyl and the like; and silyl groups such as trimethylsilyl and the like. Favoured N-protecting groups include formyl, acetyl, benzoyl, pivaloyl, t-butylacetyl, phenylsulfonyl, benzyl, t-butoxycarbonyl (BOC) and benzyloxycarbonyl (Cbz).

The acylation is carried out with conventional esterification conditions such as DMAP and DCC in a solvent such as dimethylformamide or pyridine. Optional protecting groups may be removed with conventional techniques as comprehensively discussed in Greene above, such as TFA, HCl(aq)/dioxane or hydrogenation in the presence of a catalyst to give the compound of Formula II.

Compounds of the Formula II, wherein p is 1 can be prepared by reacting a compound of the formula III with iodochloromethane or mixed dichloro/iodochlor methane under conventional alkylating conditions to form a compound of the Formula IIIa:



IIIa

where n, R^3, R^4 and X are as defined above, but where exposed amine, hydroxy etc substituents being protected with conventional protecting groups. The compound of formula IIIa is then preferably converted to the corresponding iodo derivative by reaction with NaI followed by coupling to the compound of formula I, typically under basic conditions, such as an organic solvent containing sodium hydride.

Detailed Description

Aspects of the invention will now be illustrated by way of example only with reference to the following non-limiting Examples and the Drawings in which;

Fig 1 depicts rate of resistance development against time for a compound of the invention in comparison to a prior art compound, as described in Biological Example 2;

Fig 2 depicts time vs plasma levels after oral administration to rats of a compound of the invention or a prior art compound as described in Biological Example 5;

Fig 3 depicts binding kinetics to reverse transcriptase of a compound of the invention in comparison to a prior art compound, as assayed with surface plasmon resonance methodology as described in Biological Example 10.

Preparation of intermediates

Example 1

3-[1,1-(Ethylenedioxy)propyl]-6-fluoro-2-methoxybenzaldehyde

To a solution of 3-fluorophenol (22.4 g, 0.2 mol), pyridine (24 ml, 0.3 mol) and dichloromethane (200 ml) at room temperature was added 20 ml (0.225 mol) propionyl chloride over a period of 5 min. The reaction was exothermic. The solution was stirred for another 30 min. After addition of dichloromethane, the organic phase was washed with sat. $NaHCO_3$ solution and water, dried over $MgSO_4$ and concentrated *in vacuo*. 33.8 g (100%) of 3-fluoro-1-propionyloxybenzene was obtained. This compound was reacted with 33.3 g (0.25 mol) $AlCl_3$ at 150 °C for a period of 10 min. After careful quenching with water, the reaction mixture was extracted three times with ether. The ether phase was dried ($MgSO_4$) and evaporated to give 29.5 g (0.176 mol, 88%) rearranged product. This intermediate was dissolved in 200 ml of acetone and K_2CO_3 (42, 0.3 mol) and MeI (25 ml, 0.4 mol) were added. The reaction mixture was heated at 40 °C for a period of 12 h. The reaction mixture was filtered and the acetone was evaporated. The residue was dissolved in ether and the ether phase washed with a 0.5 M NaOH solution and water. Drying ($MgSO_4$) and evaporation gave 31.2 g (0.17 mol, 86 % yield for three steps) of 4-fluoro-2-methoxypropiophenone.

To a solution of 4-fluoro-2-methoxypropiophenone (31.2 g, 0.171 mol), ethylene glycol (10.5 ml, 0.188 mol) in benzene (300 ml) was added 1 g of *p*-toluenesulfonic acid. The reaction mixture was refluxed in a Dean-Stark apparatus for about 12 h. After cooling, the organic phase was washed several times with a 1M NaOH solution and dried (Na_2SO_4 and K_2CO_3). The solvent was evaporated and about 38 g of the acetal was obtained. The purity according to capillary GC was 88 % and the impurity

was basically unreacted ketone. To a solution of the acetal in THF (450 ml) at -65 °C and under nitrogen was added droppwise 128 ml (0.32 mol) of 2.5 M n-BuLi.. While keeping the temperature at about -65 °C a solution of DMF (25 ml, 0.32 mol) in THF (50 ml) was added. The reaction mixture was allowed to slowly reach room temperature and according to GC no starting material was left after about 30 min. After another 1h, the reaction mixture was quenched with sat. NH_4Cl solution and extracted three times with ether. After drying (Na_2SO_4) the residue was purified on a silica gel column (silica gel 60 from Merck, particle size 0.04-0.063 mm) eluting with EtOAc 1 and hexanes 9 to give 10 g (25 %) of the title compound.

^1H NMR ($CDCl_3$) δ 0.85 (t, 3H), 2.1 (q, 2H), 3.8-3.95 (m, 2H), 3.97 (s, 3H), 4.0-4.15 (m, 2H), 6.9 (t, 1H), 7.7-7.8 (m, 1H), 10.4 (s, 1H).

Example 2

3-[1,1-(Ethylenedioxy)propyl]-6-fluoro-2-methoxystyrene

To a suspension of methyltriphenylphosphonium bromide (14.3 g, 40 mmol) in THF (250 ml) at room temperature and under nitrogen was added 16 ml (40 mmol) of 2.5 M n-BuLi. To the almost obtained solution was then added 3-[1,1-(ethylenedioxy) -propyl]-6-fluoro-2-methoxybenzaldehyde (10 g, 39.5 mmol) in THF (30 ml). The reaction mixture was then stirred at room temperature for 2 h and poured into a mixture of hexanes and brine. The organic phase was washed two times with brine and one time with water. After evaporation of the solvent, the residue was filtered through a funnel filled with alumina (aluminium oxide 90 acc. Brockmann from Merck) and eluting with EtOAc 1 and hexanes 9 in order to remove the formed triphenylphosphonium oxide. Evaporation of the organic solvent gave a residue which was finally purified on silica gel eluting with EtOAc 1 and hexanes 9 to give

6.9 g (70 %) of the title compound with a purity of 94.5 % as determined by capillary GC.

^{1}H NMR (250 Mhz, $CDCl_3$) δ 0.85 (t, 3H), 2.1 (q, 2H), 3.8 (s, 3H), 3.8-3.95 (m, 2H), 4.0-4.1 (m, 2H), 5.55-5.65 (m, 1H), 5.95-6.05 (m, 1H), 6.7-6.85 (m, 2H), 7.3-7.4 (m, 1H).

Example 3

(1S, 2R)-*cis*-2-(6-fluoro-2-methoxy-3-propionylphenyl)cyclopropylcarboxylic acid

The ethyl ester of (1S, 2R)-*cis*-2-[3-(1,1-ethylenedioxy)ethyl-6-fluoro (2-methoxy-phenyl)cyclopropylcarboxylic acid was prepared from 3-[1,1-(ethylenedioxy) propyl]-6-fluoro-2-methoxystyrene (19.4 g, 69 mmol) and ethyl diazoacetate (29 ml, 275 mmol) using a asymmetric cyclopropanation reaction catalyzed by Cu(I)triflate (679 mg, 1.35 mmol) and the chiral ligand ([2,2'-isopropylidenbis((4R)-4-*tert*-butyl-2-oxazoline)] (794 mg, 2.7 mmol) as generally described by Evans *et al* in *J.Am.Chem.Soc.* 1991, *113*, 726-728. After silica gel chromatography, 9.4 g (40.5 %) of the ethyl ester was obtained. The enantiomeric excess was 99% as determined by HPLC on a chiral column. The ester was dissolved in 150 ml of dioxane and 30 ml of 6M HCl was added. The reaction mixture was stirred over night and partitioned between ether and brine. The solvent was evaporated to give 19 g of crude produkt. This product was dissolved in methanol (250 ml) and water (75 ml) and 6 g (250 mmol) of LiOH was added. The reaction mixture was heated to 90° C for 24 h and most of the solvent was evaporated. The remaining mixture was acidified and extracted three times with dichloromethane. Evaporation of the solvent afforded 11.2 g of the title compound.

^{1}H-NMR (250 MHz, $CDCl_3$) δ 1.15 (t, 3H), 1.59 (t, 2H), 2.10-2.17 (m, 1H), 2.22-2.32 (m, 1H), 2.91 (q, 2H), 3.80 (st, 3H), 6.82 (t, 1H), 7.44-7.50 (m, 1H), 11.30 (broad s, 1H).

Example 4

(1R,2S)-*cis*-2-(6-fluoro-2-methoxy-3-propionylphenyl)cyclopropylcarboxylic acid

This compound was prepared from 3-[1,1-ethylenedioxy)propyl]-6-fluoro-2-methoxystyrene as described for the acid in Example 3. The chiral ligand which was used was 2,2'-isopropylidenebis[(4S)-4-*tert*-butyl-2-oxazoline].

^{1}H NMR (250 Mhz, $CDCl_3$) δ 7.48 (q, 1H), 6.84 (t, 1H), 3.82 (s, 3H), 2.93 (q, 2H), 2.29 (q, 1H), 2.14 (q, 1H), 1.60 (m, 2H), 1.16 (t, 3H).

Preparation of compounds of Formula I and II

Example 5

(±)N-[*cis*-2-(2-(6-fluoro-2-hydroxy-3-propionylphenyl)-cyclopropyl]- N'-(5-cyanopyrid-2-yl)-urea

A solution of 3-[1,1-(ethylenedioxy)propyl]-6-fluoro-2-methoxystyrene (32.4 g, Example 2) and copper bromide-dimethyl sulfide complex (0.30 g) in dichloroethane (200ml) was heated to 80°C under nitrogen. Ethyl diazoacetate (54 ml) in dichloroethane (600 ml) was added during 7 h. After the addition was complete the heating was turned off. After 16 h the solvent was evaporated and the residue was purified on silica gel eluting with ethyl acetate and hexanes to give the cis-ester (6.5 g)

The cis-ester (3.7 g, 10.9 mmol) was dissolved in ethanol (20 ml) and KOH (1.8 g, 32.7 mmol) was dissolved in water (10 ml). The solutions were combined and heated to reflux for 3 h. Water (30 ml) was added and the solution was washed twice with hexanes (20 ml). The water phase was cooled in an ice bath and acidified with dilute

HCl. The solution was extracted three times with toluene. The toluene phase was dried ($MgSO_4$) and evaporated to give 1.9 g (±)-*cis*-2-[3-(1,1-ethylenedioxypropyl)-6-fluoro-2-methoxyphenyl]cyclopropylcarboxylic acid.

Triethylamine (59 µl, 0.43 mmol) and diphenylphosphoryl azide (92 µl, 0.43 mmol) was added to a solution of the acid (120 mg, 0,39 mmol) in dry toluene. The solution was stirred at room temperature for 1 h and then heated to 120°C. After 1 h 2-Amino-5-cyanopridine (51 mg, 0.43 mmol) was added. Heating was maintained for an additional 3 h. After 16 h the solvent was evaporated, the residue was dissolved in dichloromethane (30ml) , washed with dilute HCl, dried ($MgSO_4$) and evaporated to give 152 mg. This product was dissolved in dioxane and HCl (6N, 1ml) was added. After 2 h the mixture was evaporated, dissolved in dichloromethane (25ml) , washed with water (10+10 ml), dried ($MgSO_4$) and evaporated to give 117 mg. The residue was purified on silica gel eluting with ethyl acetate and hexanes to give 37 mg 2-methoxyphenyl intermediate product.

A 1M solution of boron tribromide in dichloromethane (194µl, 0.194 mmol) was added to a solution of the 2-methoxyphenyl intermediate (37 mg, 0.097 mmol) in dichloromethane at -60°C. After 10 min the cooling bath was removed and the stirring was continued for 2 h. The solution was diluted with dichloromethane, washed with dilute $NaHCO_3$ and water, dried ($MgSO_4$) and evaporated. The residue was recrystallized from MeCN giving 17 mg of the title product.

^{1}H-NMR (250 MHz, DMSO-d_6) δ 1.07-1.16 (m,4H), 1.41-1.50 (m, 1H), 1.91-2.01 (m, 1H), 3.06-3.19 (m, 3H), 6.86 (dd, 1H), 7.43 (d, 1H), 7.80-7.90 (m, 1H), 7.97-8.08 (m, 2H), 8.32 (d, 1H), 9.83 (s, 1H), 13.2 (d, 1H).

Example 6

(1R,2R)-N-(*cis*-2-(6-fluoro-2-hydroxy-3-propionylphenyl)-cyclopropyl)-N'-(5-cyanopyridy-2-yl)-urea

Triethylamine (0.85 mL, 6.1 mmol) and diphenyl phosphoryl azide (1.72 g, 6.1 mmol) was added to a solution of the acid prepared in Example 4 (1.47 g, 5.5 mmol)

in dry toluene (15 mL). The solution was stirred at room temperature under argon for 30 min and then heated to 120 °C. After 15 min a solution of 2-amino-5-cyanopyridine (0.99 g, 8.9 mmol) in DMF (3 mL) was added and heating was continued for 4 h. Toluene was evaporated, and the mixture was diluted with diethyl ether (100 mL) and ethyl acetate (50 mL) and washed with 1 M HCl, H_2O and brine. The organic layer was dried (Na_2SO_4) and concentrated. The residue was purified with silica gel flash column chromatography by eluting with ethyl acetate/n-hexane 1:10 to 1:1 to give 1.6 g (66 %) of the 2-methoxyphenyl intermediate.

A 1 M solution of boron trichloride in CH_2Cl_2 (11.0 mL, 11.0 mmol) was added to a solution of the 2-methoxyphenyl intermediate (1.40 g, 3.66 mmol) in CH_2Cl_2 (80 mL) at - 72 °C under argon. After 10 min the cold bath was removed and the stirring was continued for 1h 15 min. The solution was diluted with CH_2Cl_2 and washed with an aqueous solution of $NaHCO_3$, H_2O and brine. The organic layer was dried (Na_2SO_4) and concentrated. The precipitate from acetonitrile/H_2O 1:1 gave 0.62 g of pure title compound. The residue was concentrated and the chromatography by eluting with ethyl acetate/n-hexane 1:10 to 1:1 and ethyl acetate, and then crystallization from acetonitrile gave 0.2 g of the title product. The yield 0.82 g (61 %). The ee was 95 % as determined by HPLC on a chiral column. $[\alpha]_d^{22}$ -171.2° (c= 0.50, CH_2Cl_2)

^{1}H NMR (250 Mhz, $CDCl_3$) δ 13.35 (d, 1H), 10.02 (br s, 1H), 9.40 (br s, 1H), 8.11 (s, 1H), 7.71 (m, 2H), 7.00 (m, 1H), 6.61 (t, 1H), 3.21 (m, 1H), 3.01 (q, 2H), 2.03 (m, 1H), 1.55 (m, 1H), 1.29 (m, 4H).

Example 7

(1R, 2R)-N-[*cis*-2-(2-(3-aminophenylcarbonyloxy)- 6-fluoro-3-propionylphenyl)-cyclopropyl]- N'-(5-cyanopyrid-2-yl)-urea

To a solution of the compound described in Example 6 (1.64 g, 4.4 mmol), BOC-protected 3-aminobenzoic acid (1.6 g, 6.6 mmol) and 4-dimethylaminopyridine (269 mg, 2.2 mmol) in 20 ml of dichloromethane and 10 ml of DMF at room temperature

and under argon was added 1.36 g (6.6 mmol) of DCC. The reaction mixture was stirred for 24 hrs. The solvent was carefully evaporated and the residue purified on silica gel using hexanes/ethyl acetate 1:1 as the solvent to give 2.6 g of BOC-protected title product. This product was added to 75 ml trifluoroacetic acid at 0 °C. The mixture was then stirred at 0°C for 1 hour. The solvent was carefully removed *in vacuo*. The residue was partitioned between ethylacetate and sat. potasium carbonate. The organic phase was dried and evaporated. The residue was purified on a silica gel column using ethyl acetate/hexanes 4:1 as eluent to give 1.03 g of the free base of the title compound. This intermediate was treated with 3 ml 1M HCl in ether and 0.84 g of the titled compound was achieved. The HPLC purity was about 97 %.

^{1}H-NMR liberated amine (250 MHz, $CDCl_3$) δ 1.09 (t, 3H), 1.2-1.3 (m, 1H), 1.4-1.5 (m, 1H), 1.95-2.00 (m, 1H), 2.83 (q, 2H), 3.15-3.25 (m, 1H), 3.85 (s, 2H), 6.90 (dd, 2H), 7.09 (t, 1H), 7.20-7.27 (m, 1H), 7.44-7.46 (m, 1H), 7.56 (dd, 1H), 7.65-7.77 (m, 2H), 8.13 (d, 1H), .9.1 (broad s, 1H), 9.6 (broad s, 1H).

Example 8

(1S,2S)-N-(cis-2-(6-fluoro-2-hydroxy-3-propionoylphenyl)-cyclopropyl)-N'-(5-cyanopyrid-2-yl)-urea

Triethylamine (670µl, 4.8 mmol) and diphenyl phosphoryl azide (1.05 ml, 4.9 mmol) were added to a solution of the acid prepared in example 3 (1.2 g, 4.5 mmol) in dry toluene (10 ml) under nitrogen. The solution was stirred at room temperature for 30 min. and then heated to 120°C. After 15 min. a solution of 2-amino-5-cyanopyridine (0.80 g, 6.7 mmol) in dimethyl formamide (1.5 ml) was added and the heating was continued for 4 h. The solution was diluted with diethyl ether and washed with 1M hydrochloric acid. The organic layer was dried ($MgSO_4$) and concentrated. The residue was purified by silica gel flash chromatography (gradient starting with n-hexane:ethyl acetate 1:1, finishing with pure ethyl acetate) giving slightly unpure 2-methoxyphenyl derivative (0.93 g). Repeated chromatography, as described above, gave the pure 2-methoxyphenyl derivative. (0.70 g, 41%).

A 1M solution of boron trichloride in methylene chloride (5.5 ml, 5.5 mmol) was added to a solution of the 2-methoxyphenyl intermediate (700 mg, 1.8 mmol) in methylene chloride at -60°C. After 10 min. the cold bath was removed and the stirring continued for 2 h. The solution was diluted with methylene chloride and washed with an aqueous solution of sodium hydrogen carbonate. The organic layer was dried ($MgSO_4$) and concetrated and the residue was purified by silica gel flash chromatography (gradient, n-hexane: ethyl acetate 2:1, 1:1, 1:2, ethyl acetate:methanol (8:1) giving the title compound (500 mg, 74%).
$[\alpha]_D^{22}$ + 165.0° (C = 0.5, CH_2Cl_2).

^{1}H-NMR (DMSO-d_6) δ 1.10-1.16 (m, 4H, CH_3, CH_2-cyclopropyl), 1.45 (dd, 1H, CH_2-cyclopropyl), 1.96 (q, 1H, CH-cyclopropyl), 3.10-3.19 (m, 3H, CH-cyclopropyl, CH_2), 6.85 (t, 1H, Ar), 7.43 (d, 1H, Ar), 7.86-8.07 (m, 3H), 8.32 (s, 1H), 9.83 (s, 1H), 13.22 (s, 1H, Ar-OH).

Example 9

(1S, 2S)-N-[*cis*-2-(2-(3-aminophenylcarbonyloxy)-6-fluoro-3-propionylphenyl)-cyclopropyl]-N'-(5-cyanopyrid-2-yl)-urea

Starting from the compound described in Example 6 and using the method described in Example 7 gave the titled product as the hydrochloride salt.

^{1}H-NMR (250 MHz, DMSO-d_6) δ 0.94 (t, 3H), 0.9-1.0 (m, 1H), 1.3-1.4 (m, 1H), 1.85-1.95 (m, 1H), 2.91 (q, 2H), 3.05-3.15 (m, 1H), 7.4-7.5 (m, 2H), 7.6-7.7 (m, 1H), 7.9-8.1 (m, 5H), 8.08 (d, 1H), 9.85 (s, 1H).

Example 10

(1S, 2S)-N-(*cis*-2-(6-fluoro-2-hydroxy-3-propionylphenyl)-cyclopropyl)-N'-(5-bromopyrid-2-yl)-urea

(1S, 2R)-*cis*-2-(6-fluoro 2-methoxy-3-propionylphenyl)cyclopropylcarboxylic acid (3.0g, 11.3 mmol), triethylamine (1.58 ml, 11.3 mmol)and diphenylphosphoryl azide (2.44 ml, 11.3 ml) were dissolved in dry toluene (8 ml) at room temperature and under an atmosphere of argon. The reaction mixture was stirred at room temperature for a period of 30 min whereafter the the temperature was increased to 120 °C and kept there for another 15 min. Then, 2-amino-5-bromopyridine (2.08g, 12 mmol) was added and the reaction mixture was stirred at 120 °C for 2.5 hrs. Benzene and 1M HCl solution were added and the organic phase was evaporated. The residue was purified on silica gel using hexanes:ethyl acetate 1:1 as the eluent. The appropriate fractions were collected and 5.0 g of (1S, 2S)-N-(*cis*-2-(6-fluoro-2-methoxy-3-propionyl- phenyl) -cyclopropyl)-N'-(5-bromopyrid-2-yl)-urea was obtained. This compound was dissolved in dichloromethane (100 ml) and the solution was kept under argon and cooled to -65 °C. Boron trichloride (30 ml of a 1M solution in dichloromethane, 30 mmol) was added and the reaction mixture was allowed to reach room temperature over night. Dichloromethane and sat. sodium bicarboante were added. The organic phase was evaporated and the residue purified on silica gel using ethyl acetate: methanol 9:1 as the eluent. 1.96 g (41%) of the title compound was obtained.

Analysis: Calculated: C 51.2 , H 4.1, N 9.9. Found: C 51.5, H 3.7, N 9.5.

Mp: 198-199 °C. $[\alpha]_D^{22}$ + 149.8 ° (c= 0.50, CH_2Cl_2)

^{1}H-NMR (250 MHz, $CDCl_3$) δ 1.28 (t, 3H), 1.52-1.62 (m, 2H), 1.94-2.05 (m, 1H), 2.97-3.06 (m, 2H), 3.17-3.20 (m, 1H), 6.60 (t, 1H), 6.76 (broad s, 1H), 7.57 (dd, 1H), 7.67-7.72 (m, 1H),7.83 (broad s, 1H) 8.53 (broad s, 1H), 13.32 (d, 1H).

Example 11

(1R, 2R)-N-(*cis*-2-(6-fluoro-2-hydroxy-3-propionylphenyl)-cyclopropyl)-N'-(5-bromopyridyl-2-yl)-urea

An asymmetric cyclopropanation reaction, as described in Example 3, was performed on the compound described in Example 2 using the chiral ligand 2,2'-isopropylidinebis(4S)-4-*tert* -butyl-2-oxazoline (commercially available from Aldrich). The obtained (1R, 2S)-*cis*-2-(6-fluoro-2-methoxy-3-propionylphenyl)cyclopropylcarboxylic acid was then used in a manner analogous to Example 10 to give the title compound.

^{1}H-NMR (250 MHz, DMSO-d_6) δ 1.05-1.15 (m, 1H), 1.12 (t, 3H), 1.40-1.50 (m, 1H), 1.90 (q, 1H), 3.00-3.10 (m, 1H), 3.12 (q, 2H), 6.82 (t, 1H), 7.18 (d, 1H), 7.78 (dd, 1H), 7.88 (broad s, 1H), 7.95-8.05 (m, 1H), 9.41 (broad s, 1H), 13.20 (s, 1H).

$[\alpha]_D^{22}$ -153.8 ° (c=0.50, CH_2Cl_2)

Example 12

(1S, 2S)-N-[*cis*-2-(2-(3-aminophenylcarbonyloxy)-6-fluoro-3-propionylphenyl)-cyclopropyl]- N'-(5-bromopyrid-2-yl)-urea

To a solution of the compound of example 10 (633 mg, 1.5 mmol), BOC-protected 3-aminobenzoic acid (475 mg, 2 mmol) and 4-dimethylaminopyridine (123 mg, 1 mmol) in 20 ml of dichloromethane:DMF 1:1 at room temperature and under argon was added 415 mg (2 mmol) of DCC. The reaction mixture was stirred for 36 hrs. The solvent was carefully evaporated and the residue purified on silica gel using hexanes:ethyl acetae 1:1 as the solvent to give 811 mg of BOC-protected title product. This product was dissolved in dioxane (20 ml) and 10 ml 6M HCl was added and the mixture stirred over night. The solvent was carefully removed *in vacuo*. The residue was treated with ethanol and ether and 255 mg of the titled product was obtained as the HCl salt. The HPLC purity was about 93 %.

^{1}H-NMR (250 MHz, CD_3OD) δ 1.15 (t, 3H), 1.3-1.4 (m, 1H), 1.5-1.6 (m, 1H), 2.05-2.15 (m, 3H), 3.04 (q, 2H), 3.23-3.27 (m, 1H), 7.16 (d, 1H), 7.34 (t, 1H), 7.85-7.93 (m, 2H), 8.05 (dd, 1H, 8.19(broad d, 1H), 8.26 (broad s,1H), 8.35-8.37 (m, 1H), 8.42-8.46 (m, 1H).

Example 13

(1S, 2S)-N-[*cis*-2-(2-(3-L-alanylaminophenylcarbonyloxy)- 6-fluoro-3-propionylphenyl)-cyclopropyl]- N'-(5-bromopyrid-2-yl)-urea

The starting compound, BOC-protected 3-L-alanylaminobenzoic acid, was prepared from TCE-protected 3-aminobenzoic acid using standard chemistry, see for example Bodanszky's "The Practice of Peptide Synthesis" 2nd edition, Springer. This compound was reacted with the compound of Example 10 as described in Example 12 to give the title product as the HCl salt.

^{1}H-NMR (250 MHz, liberated amine, $CDCl_3$) δ 1.10 (t, 3H), 1.15-1.25 (m, 1H), 1.4-1.5 (m, 1H), 1.42 (d, 2H), 1.76 (broad s, 2H), 1.88-1.97 (m, 1H), 2.84 (q, 2H), 3.1-3.2 (m, 1H), 3.59-3.67 (m, 1H), 6.78 (d, 1H), 7.09 (t, 1H), 7.85-7.93 (m, 2H), 8.08 (d, 1H), 8.11 (s, 1H), 8.29 (broad s,1H), 9.05 (broad s, 1H), 9.70 (broad s, 1H).

Example 14

(1S,2S)-N-{*cis*-2-[6-fluoro-3-propionyl-2-(4-pyridylcarbonyloxy)phenyl] cyclopropyl}-N'-(5-bromopyrid-2-yl)urea

In a manner analogous to Example 12, the product of Example 10 was condensed with isonicotinic acid to give the title product as the HCl salt.

^{1}H NMR (250 MHz, CD_3OD) δ 9.26 (d,2H), 8.83 (d,2H), 8.14 (m,2H), 8.04 (dd,1H), 7.39 (t,1H), 7.10 (d,1H), 3.38 (m,1H), 3.08 (m,2H), 2.15 (m,1H), 1.62 (m,1H), 1.38 (m,1H), 1.13 (t,3H).

Example 15
(1S,2S)-N-{*cis*-2-[2-(3-dimethylaminophenylcarbonyloxy)-6-fluoro-3-propionylphenyl]cyclopropyl}-N'-(5-bromopyrid-2-yl)urea

In a manner analogous to Example 12, the product of Example 10 was condensed with 3-dimethylaminobenzoic acid to give the title product as the HCl salt.

^{1}H NMR (250 MHz, CD_3OD) δ 8.61 (s,1H), 8.45 (d,1H), 8.15-8.03 (m,4H), 7.92 (t,1H), 734 (t,1H), 7.10 (d,1H), 3.48 (s,6H), 3.28 (m,1H), 3.00 (m,2H), 2.11 (m,1H), 1.58 (m,1H), 1.38 (m,1H), 1.14 (t,3H).

Example 16
(1S,2S)-N-[*cis*-2-(2-(3-aminomethylbenzoyloxymetyloxy)-5-fluoro-3-propionylphenyl)-cyclopropyl]- N'-(5-bromopyrid-2-yl)-urea

3-t-butoxycarbonylamidomethylbenzoic acid was treated with tetrabutyl ammonium hydroxide solution (1M in MeOH) to pH 9 and evaporated. The residue was dissolved in dichloromethane and was treated with chloroiodomethane overnight. The solution was washed with water and was evaporated to obtain crude 3-t-butoxycarbonylamidomethylbenzoyloxymethylchloride. This material was reacted with the sodium salt of Example 10 (prepared with sodium hydride in DMF) with a little sodium iodide as catalyst. After 2 hours reaction the solution was quenched with acetic acid and was diluted with dichloromethane, washed with water and evaporated. The crude product was purified on silica-gel by elution with ethylacetate/hexane 1:2 and the pure material was treated with trifluoroacetic acid and evaporated to obtain the trifluoroacetate salt of the title compound as a solid.

^{1}H NMR ($CDCl_3$) δ 1.1 (t, 3H) 1.3-1.5 (m,2H) 2.2 (q, 1H) 2.9 (m, 2H) 3.2 (bs, 1H) 4.2 (s, 2H) 5.9 (q, 2H) 6.8 (d, 2H) 7.0 (t, 1H) 7.3-8.1 (m, 9H).

Example 17

(1S,2S)-N-(*cis*-2-(2-(3-amino-4-methylbenzoyloxy)-6-fluoro-3-propionylphenyl) cyclopropyl)-N'-(5-bromopyrid-2-yl)-urea

(1S,2S)-N-(*cis*-2-(6-fluoro-2-hydroxy-3-propionylphenyl)-cyclopropyl)-N'-(5-bromopyrid-2-yl)-urea from Example 10 was condensed with 3-t-butoxycarbonylamido-4-methylbenzoic acid according to the procedure in Example 12. The product was treated with trifluoroacetic acid and was evaporated to obtain a the trifluoroacetic salt of the title compound as a solid.

^{1}H NMR ($CDCl_3$) δ 1.1 (t, 3H) 1.3-1.5 (m, 2H) 1.9 (q, 1H) 2.4 (s, 3H) 2.9 (q, 2H) 3.1 (BS, 1H) 7.1 (t, 1H) 7.4 (d, 1H) 7.8 (m, 1H) 7.9 (m, 2H) 8.1 (s, 1H) 8.3 (s, 1H)

Example 18

(1S,2S)-N-(*cis*-2-(2-(3-ethylaminobenzoyloxy)-6-fluoro-3-propionylphenyl) cyclopropyl)-N'-(5-bromopyrid-2-yl)-urea

The compound of Example 10 was condensed with 3-(N-ethyl-t-butoxy carbonylamido)benzoic acid according to the procedure in Example 12 and the product was treated with trifluoroacetic acid and evaporated to obtain the trifluoroacetic salt of the title compound as a solid.

^{1}H NMR ($CDCl_3$) δ 1.1 (t, 3H) 1.3-1.6 (m,5H) 2.9 (q, 2H) 3.1 (bs, 1H) 3.5 (q, 2H) 7.1 (t, 1H) 7.2 (bs, 1H) 7.6 (t, 1H) 7.7-7.8 (m, 2H) 7.9 (d, 1H) 8.1 (s, 1H) 8.2 (d, 1H) 8.4 (s, 1H)

Example 19
(1S,2S)-N-(*cis*-2-(2-quinolo-4-yloxy-6-fluoro-3-propionylphenyl) cyclopropyl)-N'-(5-bromopyrid-2-yl)-urea

The compound of Example 10 was condensed with 4-quinolinic acid according to the procedure in Example 12 and the product was dissolved in trifluoroacetic acid and evaporated to obtain the acetic salt of the title compound as a solid.

^{1}H NMR ($CDCl_3$) δ 1.1 (t, 3H) 1.2 (m, 1H) 1.5 (m, 1H) 1.9 (m, 1H) 2.8 (q, 2H) 3.2 (bs, 1H) 6.7 (d, 1H) 7.2 (t, 1H) 7.5 (m, 1H) 7.7 (t, 1H) 7.8-8.0 (m, 2H) 8.2 (d, 1H) 8.3 (d, 1H) 8.8 (d, 1H) 9.1 (m, 2H) 9.2 (bs, 1H)

Example 20
(1S,2S)-N-(*cis*-2-(3-aminomethyl-2-methylbenzoyloxy)-fluoro-3-propionylphenyl) cyclopropyl)-N'-(5-bromopyrid-2-yl)-urea

The compound of Example 10 was condensed with 3-t-butyloxycarbonyl amido-2-methylbenzoic acid according to the procedure in Example 12. The product was treated with trifluoroacetic acid and evaporated to yield the title compound as a solid.

^{1}H NMR ($CDCl_3$) δ 1.1 (t, 3H) 1.1-1.3 (m, 2H) 1.9 (m, 1H) 2.5 (s, 3H) 2.9 (q, 2H) 3.1 (bs, 1H) 4.2 (s, 2H) 7.0-7.2 (m, 2H) 7.4 (d, 1H) 7.6-7.7 (m, 2H) 7.8-8.0 (m, 2H) 8.2 (bs, 2H)

Example 21
(1S, 2S)-N-[*cis*-2-(6-fluoro-2-(4-aminomethylphenylcarbonyloxy)-3-propionyl-phenyl)-cyclopropyl]- N'-(5-bromopyrid-2-yl)-urea

4-(*tert*-butyloxycarbonylamidomethyl)benzoic acid was prepared by adding 6.5 g of DCC to a solution of 4 g 4-cyanobenzoic acid in 200 ml MeOH. The mixture was

stirred 70 hours at room temperature, filtered to remove the precipitated dicyclohexylurea and the filtrate was concentrated in vacuo to yield 7 g a crude product. The methyl ester was dissolved in 500 ml MeOH and 9.6 g $CoCl_2 \cdot 6H_2O$ was added. The mixture was treated portionwise with $NaBH_4$. After 5 h the reaction mixture was concentrated and the precipitate was removed. The filtrate was acidified with 150 ml 1M HCl (aq.) and extracted with 2x100 ml CH_2Cl_2. The acidic water phase was treated with 100 ml 25 % NH_3 (aq.), extracted with 3x100 ml CH_2Cl_2, dried with Na_2SO_4 and concentrated to give 2.64 g brownish oil.

The oil was dissolved in 30 ml dioxane/water mixture (2:1) and treated for 20 hours with 1.5 g NaOH (s). Solvent was removed and 40 ml *t*-butanol/water mixture (1:1) added. The solution was stirred 24 hours after addition of 3.7 g di-*tert*-butyl dicarbonate, more water was then added and the mixture extracted with 2x50 ml hexane. The water phase was acidified (pH ~ 1.5 - 2.0) with $NaHSO_4$ and extracted with 3x75 ml ether. The pooled extracts were washed with 50 ml brine, dried with Na_2SO_4 and evaporated to yield the intermediate 4-(*tert*-butyloxycarbonyl-amidomethyl)benzoic acid as a white solid.

4-(*tert*-butyloxycarbonylamidomethyl)benzoic acid and (1S, 2S)-N-(*cis*-2-(6-fluoro-2-hydroxy-3-propionylphenyl)-cyclopropyl)-N'-(5-bromopyrid-2-yl)-urea from Example 10 were condensed and the BOC-protecting group removed using the method described in Example 12 to obtain the titled product as the hydrochloride salt.

^{1}H-NMR (250 MHz, $CDCl_3$) δ 0.98 (t, 3H), 1. 05-1.20 (m, 1H), 1.31-1.49 (m, 1H), 1.69-1.90 (m, 1H), 2.65 (q, 2H), 3.33-3.49 (m, 1H), 4.31 (broad s, 2H), 7.02-7.22 (m, 2H), 7.35-7.49 (m, 1H), 7.50-7.68 (m, 2H), 7.69-7.83 (m, 2H), 8.08 (d, 1H) 8.37 (broad s, 1H).

Example 22

(1S, 2SR)-N-[*cis*-2-(6-fluoro-2-(N-methylindol-5-carbonyloxy)-3-propionylphenyl)-cyclopropyl]- N'-(5-bromopyrid-2-yl)-urea

i) Preparation of N-methylindol-5-carboxylic acid

0.1 g of indol-5-carboxylic acid was mixed with 2 equivalents of methyl trifluoromethane sulfonate in 1 ml DMF at room temperature. After 5 h the solvent was evaporated and ^{1}H-NMR was recorded:

^{1}H-NMR (250 MHz, DMSO-d_6) δ 2.76 (s, 3H), 6.57 (board s, 1H), 7.46-7.50 (m, 2H), 7.75 (dd, 1H), 8.23-8.29 (m, 2H), 11.56 (broad s, 1H).

ii) Preparation of title compound.

N-methylindol-5-carboxylic acid and (1S, 2S)-N-(*cis*-2-(6-fluoro-2-hydroxy-3-propionylphenyl)-cyclopropyl)-N'-(5-bromopyrid-2-yl)-urea from Example 10 were condensed using the method described in Example 12 to obtain the title product as the hydrochloride salt.

^{1}H-NMR (250 MHz, $CDCl_3$) δ 1.08 (t, 3H), 1. 15-1.25 (m, 1H), 1.39-1.50 (m, 1H), 1.92-2.08 (m, 1H), 2.89 (q, 2H), 2.90 (s, 3H), 3.20-3.35 (m, 1H), 6.55 (broad s, 1H), 6.65 (broad d, 1H), 7.11 (t, 1H), 7.20-7.29 (m, 2H), 7.41 (dd, 1H), 7.72-7.83 (m, 2H), 7.95 (dd, 1H), 8.51 (broad s, 1H), 9.25 (broad s, 1H), 9.43 (broad s, 1H).

Example 23

(1S, 2S)-N-[*cis*-2-(6-fluoro-2-(indol-4-carbonyloxy)-3-propionylphenyl)-cyclopropyl]- N'-(5-bromopyrid-2-yl)-urea

Indol-4-carboxylic acid and (1S, 2S)-N-(*cis*-2-(6-fluoro-2-hydroxy-3-propionylphenyl)-cyclopropyl)-N'-(5-bromopyrid-2-yl)-urea of Example 10 were condensed using the method described in Example 12 to obtain the titled product as the hydrochloride salt.

^{1}H-NMR (250 MHz, $CDCl_3$) δ 1.07 (t, 3H), 1. 17-1.30 (m, 1H), 1.31-1.47 (m, 1H), 1.90-2.10 (m, 1H), 2.89 (q, 2H), 3.02-3.18 (m, 1H), 6.75 (broad d, 1H), 7.00-7.35 (m, 4H), 7.55 (dd, 1H), 7.60 (d, 1H), 7.79 (dd, 1H), 7.89 (d, 1H), 8.10 (d, 1H), 9.27 (broad d, 2H).

Example 24

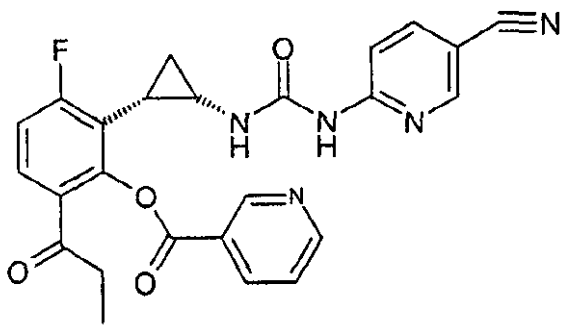
(1S, 2S)-N-[*cis*-2-(6-fluoro-2-(3-amino-4-chlorophenylcarbonyloxy)-3-propionylphenyl)-cyclopropyl]- N'-(5-bromopyrid-2-yl)-urea

3-Amino-4-chlorobenzoic acid and (1S, 2S)-N-(*cis*-2-(6-fluoro-2-hydroxy-3-propionylphenyl)-cyclopropyl)-N'-(5-bromopyrid-2-yl)-urea of Example 10 were condensed using the method described in Example 12 to obtain the title product as the hydrochloride salt.

^{1}H-NMR (250 MHz, liberated amine, $CDCl_3$) δ 1.10 (t, 3H), 1. 17-1.30 (m, 1H), 1.42-1.52 (m, 1H), 1.88-2.01 (m, 1H), 2.88 (q, 2H), 3.19-3.31 (m, 1H), 4.25 (broad s, 2H), 6.80 (broad d, 1H), 7.09 (t, 1H), 7.35 (t, 1H), 7.48-7.60 (m, 2H), 7.66 (d, 1H), 7.73-7.88 (m, 2H), 9.25 (broad s, 2H).

Example 25

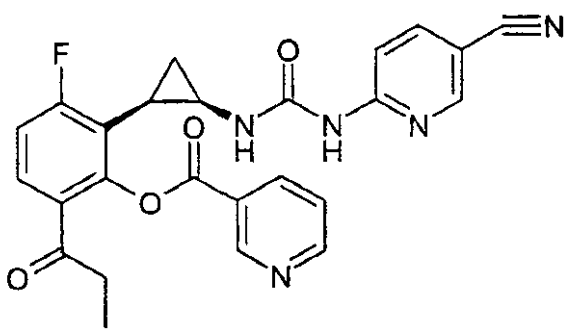
(1S,2S)-N-[*cis*-2-(6-fluoro-2-(pyrid-3-ylcarbonyloxy)-3-propionylphenyl)-cyclopropyl]- N'-(5-cyanopyrid-2-yl)-urea



A dried mixture of the compound of Example 8 (50 g, 0.68 mmol), *N,N'*-dicyclohexylcarbodiimide (0.168 g, 0.81 mmol), nicotinic acid (0.1 g, 0.81 mmol) and 4-(dimethylamino)pyridine (0.041 g, 0.34 mmol) was dissolved in CH_2Cl_2 (5 ml) and *N,N*-dimethylformamide (DMF) (2.5 ml). The mixture was then stirred at room temperature. After 20 h. the mixture was filtrated and dried in vacuum, then re-dissolved in a minimum amount of dichloromethane and filtrated. The clear solution was evaporated onto silica and purified by chromatography (ethyl acetate) to give the title compound (0.168 g, 50 %). An analytical sample was obtained by re-crystallisation from chloroform-hexane.

^{1}H NMR ($CDCl_3$): 9.89 (br s, 1H), 9.41 (m, 1H), 9.33 (br s, 1H), 8.86 (dd, 1H), 8.46 (dt, 1H), 8.18 (d, 1H), 7.80 (dd, 1H), 7.71 (dd, 1H), 7.49 (ddd, 1H), 7.13 (t, 1H) 6.92 (d, 1H) 3.18 (m, 1H), 2.88 (q, 2H), 1.99 (m, 1H), 1.52 (m, 1H), 1.25 (m, 1H), 1.13 (t, 3H).

Example 26
(1R,2R)-N-[*cis*-2-(6-fluoro-2-(pyrid-3-ylcarbonyloxy)-3-propionylphenyl)-cyclopropyl]- N'-(5-cyanopyrid-2-yl)-urea

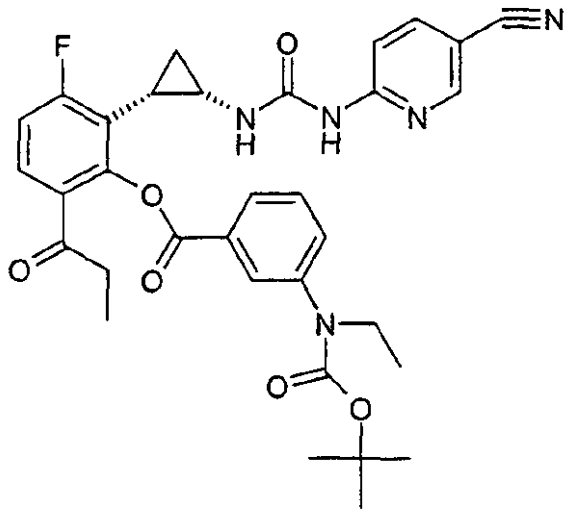


A dried mixture of the compound of Example 6 (0.1 g, 0.27 mmol), *N,N'*-dicyclohexylcarbodiimide (0.067 g, 0.33 mmol) and nicotinic acid (0.037 g, 0.3 mmol) was suspended in dichloromethane (2 ml). A minimum of DMF was added dropwise to obtain a reasonably clear solution. 4-(dimethylamino)pyridine (0.016 g, 0.14 mmol) was then added. The reaction mixture was stirred in room temperature. After 20 h the solvent was evaporated in vacuum and the crude residue was dissolved in aqueous hydrochloric acid (pH 1-2) and filtrated. The clear solution was then made slightly alkaline with sodium hydrogen carbonate and the precipitated product was filtered of. Purification by chromatography (dichloromethane-methanol, 15:1) gave the title compound 0.072 g (56 %).

^{1}H NMR ($CDCl_3$): 9.85 (br s, 1H), 9.42 (s, 1H), 9.35 (br s, 1H), 8.86 (d, 1H), 8.47 (dt, 1H), 8.18 (d, 1H), 7.81 (dd, 1H), 7.71 (dd, 1H), 7.48 (dd, 1H), 7.13 (t, 1H), 6.92 (d, 1H), 3.19 (m, 1H), 2.91 (q, 2H), 1.99 (m, 1H), 1.49 (m, 1H), 1.24 (m, 1H), 1.13 (t, 3H).

Example 27

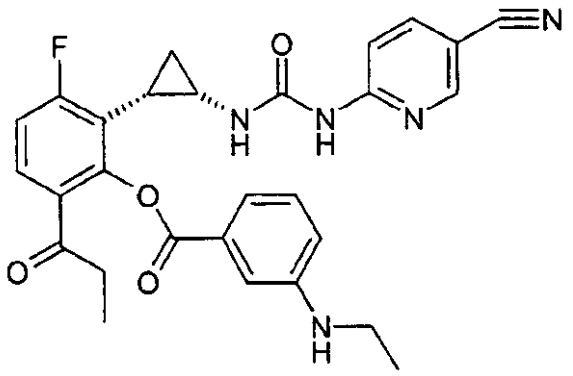
(1S,2S)-N-[*cis*-2-(2-(3-(N-ethyl,N-Boc-amino)phenylcarbonyloxy)-6-fluoro-3-propionylphenyl)-cyclopropyl]- N'-(5-cyanopyrid-2-yl)-urea



The compound of Example 8 (0.37 g, 1.0 mmol), *N,N'*-dicyclohexylcarbodiimide (0.25 g, 1.2 mmol), 4-dimethylaminopyridine (0.06 g, 0.5 mmol) and 3-(*N*-ethyl-*N*-butoxycarbonyl) aminobenzoic acid (0.320 g, 1.2 mmol) (prepared by reductive amination of 3-aminobenzoic acid, followed by protection of the amino group)were dissolved in dichloromethane (8 ml) and DMF (3 ml). The mixture was then stirred at room temperature. After 18 h. the solvent was removed in vacuum and the crude product was redissolved in dichloromethane and filtered. The clear solution was evaporated onto silica and chromatographed (ethyl acetate - hexane, 3:2) to give sufficiently pure title compound (0.24 g, 39 %).

^{1}H NMR ($CDCl_3$): 10.0 (br s, 2H), 8.20 (d, 1H), 8.06 (d, 1H), 8.03 (m, 1H), 7.77 (dd, 1H), 7.70 (dd, 1H), 7.48 (m, 2H), 7.10 (t, 1H), 6.95 (d, 1H), 3.71 (q, 2H), 3.14 (m, 1H), 2.90 (q, 2H), 1.95 (q, 1H), 1.44 (s, 10H), 1.2- 1.09 (m, 7H).

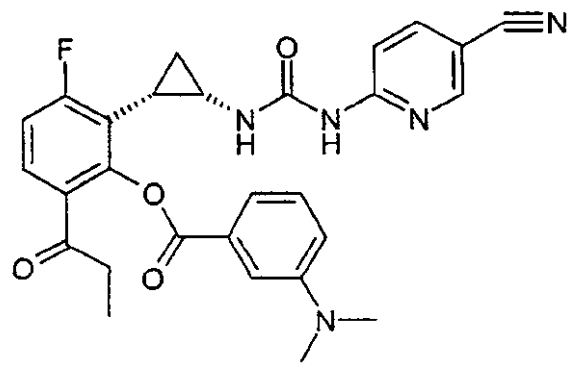
Example 28
(1S,2S)-N-[*cis*-2-(2-(3-ethylaminophenylcarbonyloxy)-6-fluoro-3-propionylphenyl)-cyclopropyl]- N'-(5-cyanopyrid-2-yl)-urea



Trifluoroacetic acid (5 ml) was added to a stirred solution of the compound of Example 27 (0.120 mg, 019 mmol) in dichloromethane (10 ml). The mixture was left at room temperature for 1-2 h. then evaporated to dryness. The crude product was purified on HPLC (prep. C-18 column, 40% water in acetonitril) to yield 0.045 g (30%) of the title compound as the trifluoroacetate salt.

^{1}H NMR ($CDCl_3$): 11.08 (br s, 2H), 9.83 (br s, 1H), 9.36 (br s, 1H), 8.23-8.08 (m, 3H), 7.82-7.54 (m, 4H), 7.13 (t, 1H), 7.02 (d, 1H), 3.42 (q, 2H), 3.20 (m, 1H), 2.83 (q, 2H), 1.94 (q, 1H), 1.46 (m, 1H), 1.34 (t, 3H), 1.24 (m, 1H), 1.06 (t, 3H).

Example 29
(1S,2S)-N-[*cis*-2-(2-(3-dimethylaminophenylcarbonyloxy)-6-fluoro-3-propionylphenyl)-cyclopropyl]- N'-(5-cyanopyrid-2-yl)-urea



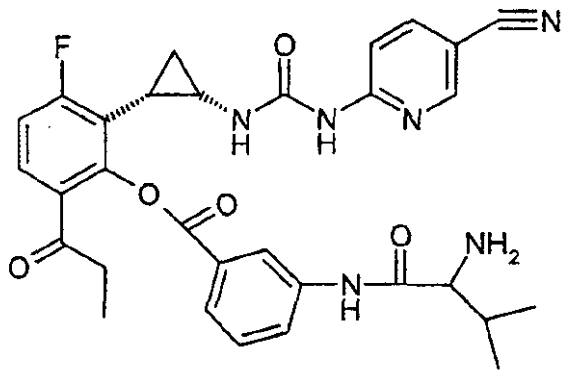
The compound of Example 8 (0.1 g, 0.27 mmol), *N,N'*-dicyclohexylcarbodiimide (0.067 g, 0.33 mmol), 4-dimethylaminopyridine (0.016 g, 0.14 mmol) and 3-

dimethylaminobenzoic acid (0.054 g, 0.39 mmol) were dissolved in dichloromethane (3 ml) and DMF (1 ml). The reaction was left at room temperature for 16 h. The solvent was then removed in vacuum and the solid redisolved in dichloromethane and filtered. Purification by chromatography (ethyl acetate - hexane, 2:1) followed by HPLC (C-18 column, 0.1 % TFA in acetonitril) yielded the title compound as the trifluoroacetate salt 0.1 g (58 %).

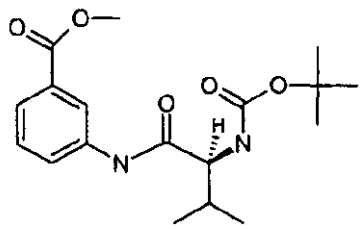
^{1}H NMR ($CDCl_3$): 8.38-8.23 (m, 3H), 7.92- 7.69 (m, 4H), 7.15 (t, 1H), 7.05 (m, 1H), 3.32 (s, 6H), 3.26 (m, 1H), 2.89 (q, 2H), 2.02 (m, 1H), 1.55-1.27 (m, 2H), 1.10 (t, 3H).

Example 30

(1S,2S)-N-[*cis*-2-(2-(3-L-valinylaminophenylcarbonyloxy)-6-fluoro-3-propionylphenyl)-cyclopropyl]- N'-(5-cyanopyrid-2-yl)-urea



a) 3-(N-Boc-L-valyl)aminomethylbenzoate

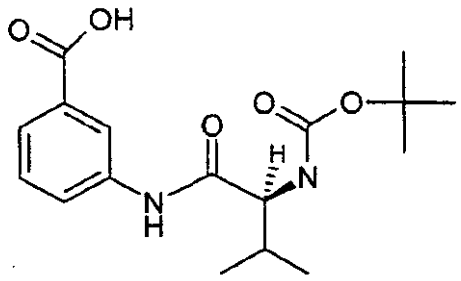


This intermediate is prepared analogously to Villaneuve & Chan, Tetrahedron Letters 1997 vol 37 6489-6492. A mixture of *N-tert*-butoxycarbonyl-L-valine (2.17 g, 10 mmol) and hexachloroacetone (1.32 g 5 mmol) in dichloromethane (20 ml) was stirred under nitrogen and cooled down to -78 degree C. Triphenylphosphine (2.6 g, 10 mmol) in dichloromethane (10 ml) was added dropwise and the mixture was stirred for 30 min. Methyl 3-aminobensoate (1.5 g, 10 mmol) in dichloromethane

(10 ml) was then added dropwise followed by triethylamine (1 g, 10 mmol) in dichloromethane. The reaction was then allowed to reach room temperature after which the solvent was evaporated under vacuum. The residue was purified by silica chromatography (hexane-ethyl acetate, 3:1) followed by recrystallization from ethyl acetate-hexane to give 0.7 g (28 %) of the pure intermediate depicted above.

^{1}H NMR ($CDCl_3$): 8.30 (br s, 1H), 8.07 (d, 1H), 7.85-7.75 (m, 2H), 7.37 (t, 1H), 5.15 (d, 1H), 4.05 (m, 1H), 3.91 (s, 3H), 2.26 (m, 1H), 1.48 (s, 9H), 1.03 (dd, 6H).

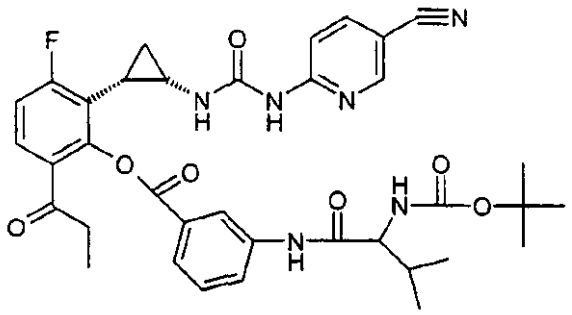
b) 3-(N-Boc-L-valyl)aminobenzoic acid



The intermediate of step a) (0.65 mg, 1.8 mmol) was suspended in methanol (6 ml) and water (2 ml). Lithium hydroxide (0.11 g, 3.9 mmol) was added and the mixture was stirred for 24 h. at room temperature. Water (10 ml) was then added and the volume reduced to half. The aqueous solution was washed with 10-20 ml of ethyl acetate then acidified with aqueous hydrochloric acid. Extraction with ethyl acetate (2 x 20 ml), drying and evaporation in vacuum yielded the pure intermediate depicted above 0.524 g (84%).

^{1}H NMR (CD_3OD): 8.23 (t, 1H), 7.84 (d, 1H), 7.76 (d, 1H), 7.42 (t, 1H), 6.70 (d, 1H), 4.00 (m, 1H), 2.08 (m, 1H), 1.45 (a, 9H), 1.00 (d, 6H).

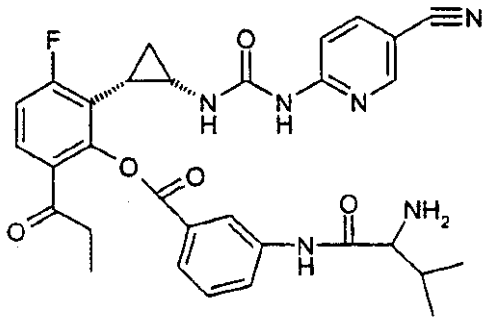
c) (1S,2S)-N-[*cis*-2-(2-(3-N-Boc-L-valinylaminophenylcarbonyloxy)-6-fluoro-3-propionylphenyl)-cyclopropyl]- N'-(5-cyanopyrid-2-yl)-urea



The compound of Example 8 (0.23 g, 0.62 mmol), *N,N'*-dicyclohexylcarbodiimide (0.153 g, 0.74 mmol), 4-dimethylaminopyridine (0.038 g, 0.3 mmol) and the intermediate of step b) (0.25 g, 0.74 mmol) were dissolved in dichloromethane (9 ml) and DMF (3 ml). The reaction was left at room temperature for 19 h. The solvent was then removed in vacuum and the solid redissolved in dichloromethane and filtered. Purification by chromatography (ethyl acetate - hexane, 1:1) gave .029 g (67%) pure N-protected title compound

^{1}H NMR (CD_3OD): 8.56 (t, 1H), 8.27 (s, 1H), 7.98-7.82 (m, 4H), 7.53 (t, 1H), 7.23 (t, 1H), 7.10 (d, 1H), 3.98 (d, 1H), 3.09 (m, 1H), 2.90 (q, 2H), 2.06-1.93 (m, 2H), 1.44 (m, 10H), 1.18-0.94 (m, 10H).

d) (1S,2S)-N-[*cis*-2-(2-(3-L-valinylaminophenylcarbonyloxy)-6-fluoro-3-propionylphenyl)-cyclopropyl]- N'-(5-cyanopyrid-2-yl)-urea



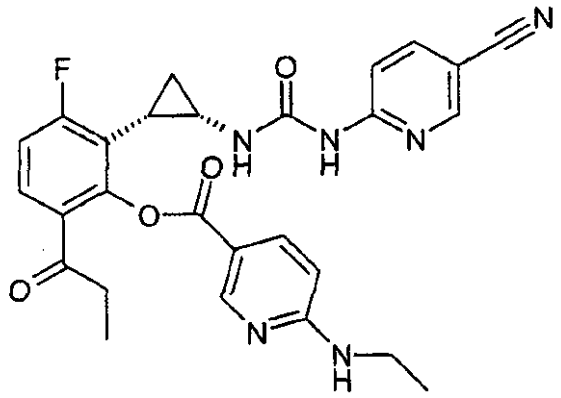
The N-protected compound of step c (0.16 g, 0.23 mmol) and thiophenol (0.054 g, 0.46 mmol) were dissolved in dichloromethane (6 ml) and cooled to 0 degree. Trifluoroacetic acid (6ml) was added and the mixture was allowed to reach room

temperature and left for 1 h. Evaporation to dryness followed by purification by chromatography (dichloromethane-methanol, 10:1.5) gave 0.150 g (90 %) of the title compound as the TFA salt.

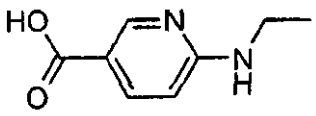
^{1}H NMR (CD_3OD) 8.60 (s, 1H), 8.25 (d, 1H), 8.0-7.85 (m, 4H), 7.53 (t, 1H), 7.21 (t, 1H), 7.09 (d, 1H), 5.0 (m, 1H), 3.12 (m, 1H), 2.96-2.87 (m, 2H), 2.20 (m, 1H), 1.97 (m, 1H), 1.46 (m, 1H), 1.09-1.03 (m, 10H).

Example 31

(1S,2S)-N-{*cis*-2-[6-fluoro-3-propionyl-2-(6-ethylaminopyrid-3-ylcarbonyloxy)phenyl] cyclopropyl}-N'-(5-cyanopyrid-2-yl)urea



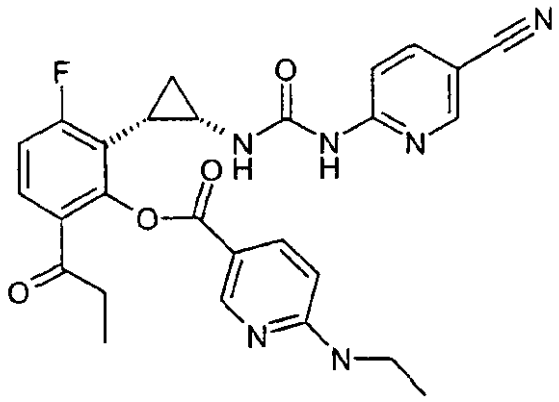
a) 6-ethylaminonicotinic acid



This intermediate is prepared from 6-chloronicotinic acid and ethylamine by the same procedure as described for Example 35 step a). 1-Butanol was substituted for ethyl acetate for the extraction. Recrystallization (MeOH-$CHCl_3$) yielded 0.53 g (50%).

^{1}H NMR (DMSO-d_6): 12.1 (br s, 1H), 8.54 (d, 1H), 7.77 (dd, 1H),7.15 (t, 1H), 6.45 (dd, 1H), 3.33 (m, 2H), 1.14 (t, 3H).

b) (1S,2S)-N-{*cis*-2-[6-fluoro-3-propionyl-2-(6-ethylaminopyrid-3-ylcarbonyloxy)phenyl] cyclopropyl}-N'-(5-cyanopyrid-2-yl)urea

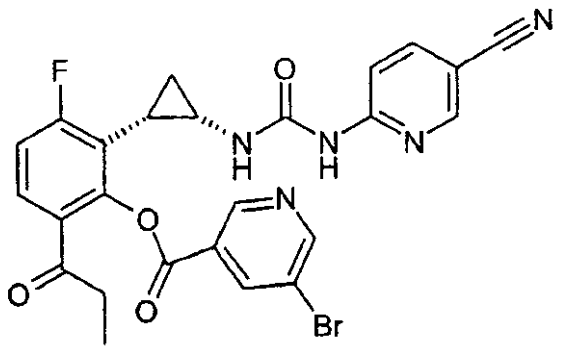


The compound of Example 8 (0.1 g, 0.27 mmol), 6-ethylaminonicotinic acid, (0.084 g, 0.54 mmol), *N,N'*-dicyclohexylcarbodiimide (0.127 g, 0.62 mmol) and 4-dimethylaminopyridine (0.016 g, 0.13 mmol) were dissolved in DMF (3 ml) and left at ambient temperature. After 19 h. the solvent was removed by vacuum and the residue suspended in dichloromethane and filterated. The solvent was removed and the crude product was purified by chromatography (ethyl acetate-hexane, 2:1) to give the title compound (0.063 g, 45 %).

^{1}H NMR ($CDCl_3$): 9.85 (br s, 1H), 9.25 (br s, 1H), 8.91 (d, 1H), 8.18- 8.02 (m, 3H), 7.76-7.67 (m, 2H), 7.65 (t, 1H), 6.96 (d, 1H), 6.37 (d, 1H), 5.40 (m, 1H), 3.37 (m, 2H), 3.19 (m, 1H), 2.8 (q, 2H), 1.98 (m, 1H), 1.49 (m, 1H), 1.28 (t, 3H), 1.15 (m, 1H), 1.10 (t, 3H).

Example 32

(1S,2S)-N-{*cis*-2-[6-fluoro-3-propionyl-2-(5-bromopyrid-3-ylcarbonyloxy)phenyl] cyclopropyl}-N'-(5-cyanopyrid-2-yl)urea

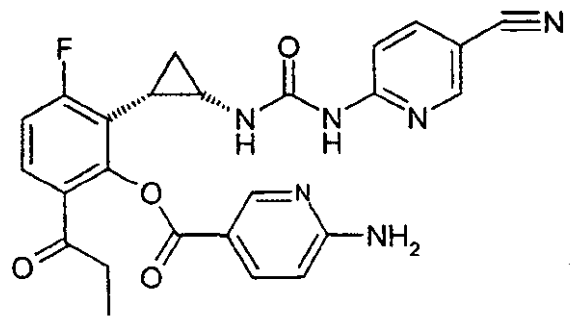


5-Bromonicotinic acid (0.065 g, 0.33 mmol), the compound of Example 8 (0.1 g, 0.27 mmol), *N,N'*-dicyclohexylcarbodiimide (0.127 g, 0.62 mmol) and 4-dimethylaminopyridine (0.016 g, 0.13 mmol) were dissolved in dichloromethane (4 ml) and left at ambient temperature. After 19 h. the mixture was filtrated and the solvent removed by vacuum. The crude product was purified by chromatography (ethyl acetate-hexane, 1:1) to give the title compound (0.040 g, 27 %).

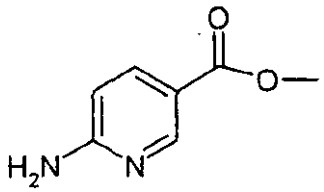
^{1}H NMR ($CDCl_3$): 9.80 (br s,1H), 9.30 (d, 1H), 9.17 (br s, 1H), 8.89 (d, 1H), 8.57 (dd, 1H), 8.57 (dd, 1H), 7.80 (dd, 1H), 7.70 (dd, 1H),7.12 (t, 1H), 6.83 (d, 1H), 3.25 (m, 1H), 2.87 (q, 2H), 2.00 (q, H), 1.50 (m, 1H), 1.24 (m, 1H), 1.12 (t, 3H).

Example 33

(1S,2S)-N-{*cis*-2-[6-fluoro-3-propionyl-2-(6-aminopyrid-3-ylcarbonyloxy)phenyl] cyclopropyl}-N'-(5-cyanopyrid-2-yl)urea



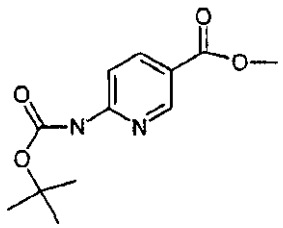
a) 6-aminonicotinic acid, methyl ester



6-Aminonicotinic acid (2 g, 22 mmol) was dissolved in methanol (10 ml) and sulphuric acid (0.5 ml). The solution was refluxed over-night and the solvent was evaporated under vacuum. The crude product was dissolved in water-EtOAc and made alkaline by aqueous sodium hydrogencarbonate. Extraction by EtoAc yielded the pure intermediate depicted above (2.3 g, 70 %).

^{1}H.NMR (DMSO-d_6): 8.51 (dd, 1H), 7.81 (dd, 1H), 6.66 (br s, 2H), 6.45 (dd, 1H), 3.77 (s, 3H).

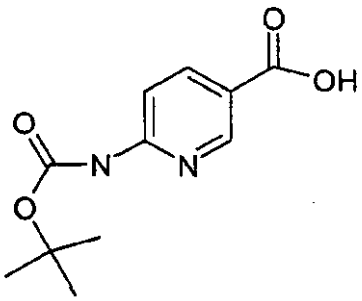
b) Methyl-6-butoxycarbonylaminonicotinate



The intermediate of step a) (0.75 g, 4.9 mmol) was dissolved in THF (5 ml). Sodium bis(trimethylsilyl)amide (5 ml, 2 M in THF) was added dropwise. After stirring at room temperature for 30 min. Di-tert-butyldicarbonate (1.1 g, 5 mmol) in THF (8 ml) was added. The reaction mixture was left over-night under nitrogen atmosphere. The solution was then evaporated under vacuum and dissolved in EtOAc (40 ml) and 0.1 M hydrochloric acid (100 ml). The layers were separated and the aqueous phase were extracted twice with EtOAc (40 ml), then made slightly alkaline with aqueous sodium hydrogencarbonate and extracted once again with EtOAc (20 ml). The organic fractions were combined, dried over sodium sulphate and purified by chromatography (EtOAc-hexane, 1:4) to give the pure intermediate depicted above (0.5 g, 40 %).

^{1}H NMR ($CDCl_3$): 8.93 (dd, 1H), 8.62 (s, 1H), 8.26 (dd, 1H), 8.06 (dd, 1H), 3.91 (s, 3H), 1.60 (s, 9H).

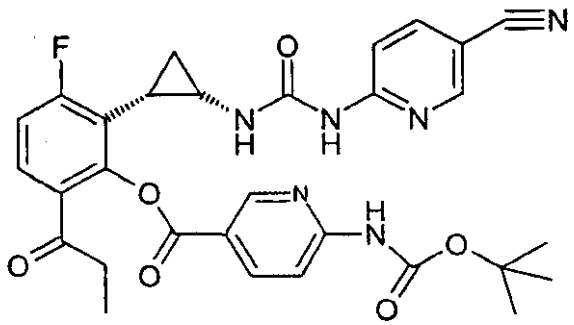
c) 6-t-butoxycarbonylaminonicotinic acid



The intermediate of step c) (0.4 g, 1.6 mmol) was suspended in methanol (4 ml) and water (1.25 ml). LiOH (0.1 g, 4 mmol) was added. The slurry was left at room temperature for 48 h. The clear solution was then concentrated under vacuum and dissolved in water and acidified with acetic acid (pH = 4-5). Extraction with EtOAc gave the pure intermediate depicted above (0.27 g, 70 %).

^{1}H.NMR (DMSO-d_6): 9.98 (s, 1H), 8.74 (d, 1H), 8.18 (d, 1H), 8.88 (d, 1H), 1.49 (s, 9H).

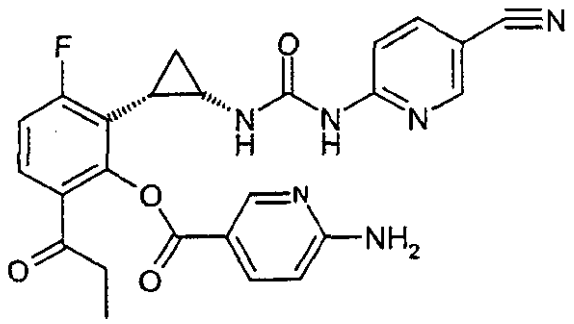
d) (1S,2S)-N-{*cis*-2-[6-fluoro-3-propionyl-2-(6-tert-butoxycarbonylamino-pyrid-3-ylcarbonyloxy)phenyl]cyclopropyl}-N'-(5-cyanopyrid-2-yl)urea



The compound of Example 8 (0.150 g, 0.41 mmol), the intermediate of step c) (0.17 g, 0.49 mmol), *N,N'*-dicyclohexylcarbodiimide (0.1 g, 0.49 mmol) and 4-dimethylaminopyridine (0.06 g, 0.49 mmol) were dissolved in DMF (2 ml). The mixture was stirred in room temperature overnight, then put in an 50 degree oil bath for 2 h. Evaporation onto silica gel and purification by chromatography yielded the N-protected title compound (0.048 g, 20 %).

^{1}H.NMR ($CDCl_3/CD_3OD$): 9.02 (s, 1H), 8.43 (dd, 1H), 8.22 (d, 1H), 8.10 (d, 1H), 7.81-7.75 (m, 2H), 7.15 (t, 1H), 7.08 (d, 1H), 3.15-3.05 (m, 1H), 2.90 (q, 2H), 1.96 (m, 1H), 1.56 (s, 9H), 1.50-1.40 (m, 1H), 1.25 –1.09 (m, 4H),

e) (1S,2S)-N-{*cis*-2-[6-fluoro-3-propionyl-2-(6-aminopyrid-3-ylcarbonyloxy)phenyl] cyclopropyl}-N'-(5-cyanopyrid-2-yl)urea

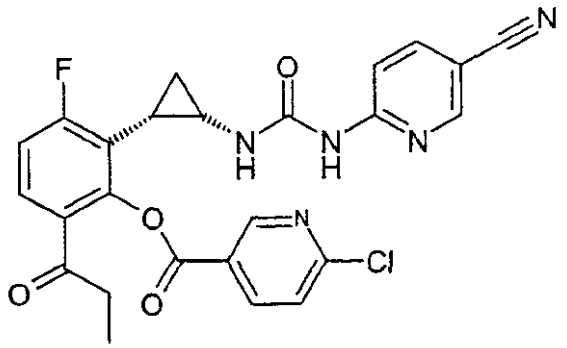


The intermediate of step d) (0.048 g, 0.08 mmol) was dissolved in dichloromethane (2 ml). Trifluoroacetic acid (1 ml) was added and the mixture was stirred for 1 h. Evaporation under vacuum yielded crude title compound. This product was dissolved in ether (2 ml) and left to stand over night. The white precipitates formed were filtrated off to give pure title compound as the trifluoracetate salt (0.032 g, 65 %).

^{1}H.NMR ($CD_3OD/CDCl_3$): 8.71 (d, 1H), 8.29 (dd, 1H), 8.16 (t, 1H), 8.82.7.74 (m, 2H), 7.20 7.10 (m, 2H), 6.96 (d, 1H), 3.25 (m, 1H), 2.86 (m, 2H), 1.96 (m, 1H), 1.52-1.43 (m, 1H), 1.24-1.19 (m, 1H), 1.09 (t, 3H).

Example 34

(1S,2S)-N-{*cis*-2-[6-fluoro-3-propionyl-2-(6-chloropyrid-3-ylcarbonyloxy)phenyl] cyclopropyl}-N'-(5-cyanopyrid-2-yl)urea

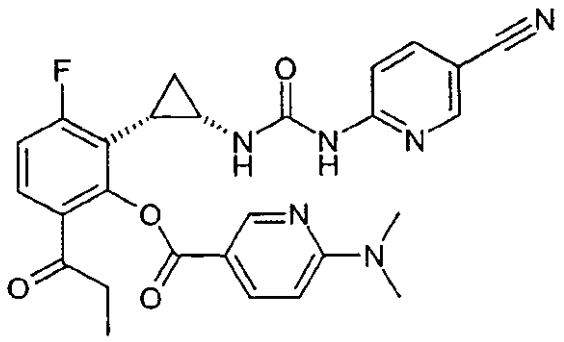


The compound of example 8 (0.15 g, 0.4 mmol), 6-chloronicotinic acid (0.076 g, 0.49 mmol), *N,N'*-dicyclohexylcarbodiimide (0.1 g, 0.49 mmol) and 4-dimethylaminopyridine (0.024 g, 0.2 mmol) were dissolved in dichloromethane (4 ml). The mixture was left over night. Evaporation under vacuum, purification by chromatography (EtOAc-hexane, 1:2) yielded the title compound (0.067 g, 32 %).

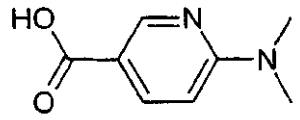
^{1}H.NMR ($CDCl_3$): 9.77 (br s, 1H), 9.18 (br d, 2H), 8.39 (dd, 1H), 8.14), 7.79 (dd, 1H), 7.71 (dd, 1H), 7.46 (d, 1H), 7.13 (t, 1H), 6.92 (d, 1H), 3.25 (m, 1H), 2.88 (q, 2H), 2.00-1.90 (m, 1H), 1.55-1.46 (m, 1H), 1.25-1.22 (m, 1H), 1.11 (t, 3H)

Example 35

(1S,2S)-N-{*cis*-2-[6-fluoro-3-propionyl-2-(6-dimethylaminopyrid-3-ylcarbonyloxy)phenyl]cyclopropyl}-N'-(5-cyanopyrid-2-yl)urea



a) 6-dimethylaminonicotinic acid



6-Chloronicotinic acid (0.5 g, 3.17 mmol) and dimethyl amine 10 ml, 40 % in water) were heated in a sealed pressure vessel at 130°C for 6h. The solvent was then removed and the residue was taken up in water and the pH was adjusted to 4-5. Extraction with dichloromethane yielded the pure intermediate depicted above (0.1 g, 20 %).

^{1}H.NMR ($CDCl_3$): 8.87 (dd, 1H), 8.04 (dd, 1H), 6.49 (dd, 1H), 3.18 (s, 6H).

b) (1S,2S)-N-{*cis*-2-[6-fluoro-3-propionyl-2-(6-dimethylaminopyrid-3-ylcarbonyloxy)phenyl]cyclopropyl}-N'-(5-cyanopyrid-2-yl)urea

The compound of Example 8 (0.13 g, 0.3 mmol), the intermediate of step a) (0.05 g, 0.3 mmol), *N,N'*-dicyclohexylcarbodiimide (0.09 g, 0.4 mmol) and 4-dimethylaminopyridine (0.02 g, 0.18 mmol) were dissolved in dichloromethane (3 ml) and DMF (1 ml). The mixture was left overnight. Evaporation under vacuum and purification by chromatography (EtOAc-hexane, 2:1) yielded the title compound (0.06 g, 39 %).

^{1}H.NMR ($CDCl_3$): 10.10 (br s, 1H), 9.29 (br s, 1H), 8.18 (d, 1H), 8.12 (dd, 1H), 7.76-7.60 (m, 2H), 7.06 (t, 1H), 6.95 (d, 1H), 6.62 (d, 1H), 3.18 (m, 7H), 2.83 (q, 2H), 2.10-1.99 (m, 1H), 1.51-1.42 (m, 1H), 1.19 (m, 1H), 1.09 (t, 3H).

Example 36

(1S, 2S)-N-[*cis*-2-(6-fluoro-2-O-3-propionylphenyl)-cyclopropyl]-N'-(5-cyanopyrid-2-yl) urea-O-4-hydroxybenzoate

a) 4-benzyloxybenzoic acid.

To a solution of 4-hydroxybenzoic acid (6.9g, 50 mmole) in 150 ml DMF was added potassium tert.-butoxide (12.34g, 110 mmole) and the mixture was stirred at room temperature for one hour. Benzyl bromide (20.5g, 120 mmole) was added and the mixture was stirred for two days at room temperature. The mixture was evaporated under reduced pressure and 100ml 1,4-dioxane and a solution of sodium hydroxide (6.0g, 150 mmole)in 50 ml water was added. The mixture was refluxed for two hours, cooled and evaporated under reduced pressure. Water was added and the mixture was acidified with acetic acid. The product was filtered, washed with cold water and dried. Yield: 10.2g = 89%.

b) 4-benzyloxybenzoyl chloride.

To a mixture of 4-benzyloxybenzoic acid (2.28g, 10 mmole) in 20 ml dried dichloromethane were added five drops of DMF and 2.5 ml thionyl chloride. The mixture was refluxed for three hours and evaporated under reduced pressure. Yield: 2.45g = 100%

c) (1S, 2H)-N-[*cis*-2-(6-fluoro-2-O-3-propionylphenyl)cyclopropyl]-N'-[2-(5-cyanopyrid-2-yl) urea O-4-benzyloxybenzoate.

To a solution of (1S, 2S)-N-[*cis*-2-(6-fluoro-2-hydroxy-3-propionylphenyl) cyclopropyl]-N'-(5-cyanopyrid-2-yl) urea (184mg, 0.5 mmole) in 3 ml DMF was added potassium tert. butoxide (78.5mg, 0.7 mmole) and the mixture was stirred for one hour at room temperature. A solution of 4-benzyloxybenzoylchloride (185mg, 0.75 mmole) in 1ml DMF was added and the mixture was stirred overnight at room temperature. 40 ml ethyl acetate were added and the organic phase was washed four times with water. The solution was dried with sodium sulfate and evaporated under reduced pressure. The product was isolated by silica gel column chromatography. Yield: 180mg = 62%.

^{1}H-NMR (DMSO δ-6) 0.92 (m, 4H) 1.31(m, 1H) 1.85 (m, 1H) 2.82 (m, 2H) 3.06 (m, 1H) 5.26 (s, 2H) 7.20 (m 2H) 7.38-8.12 (m, 11H) 8.38 (m, 1H)

d) Synthesis of (1S, 2S)-N-[*cis*-2-(6-fluoro-2-O-3-propionylphenyl) cyclopropyl]-N'-(5-cyanopyrid-2-yl)] urea-O-4-hydroxybenzoate

A solution of (1S, 2S)-N-[*cis*-2-(6-fluoro-2-O-3-propionylphenyl)cyclopropyl]-N'-(5-cyanopyrid-2-yl)urea-O-4-benzyloxybenzoate (170 mg, 0.29 mmole) in 15 ml ethyl acetate and 15 ml methanol was hydrogenated with 10% palladium on charcoal (30mg) three times at room temperature and normal pressure. The catalyst was filtered and washed with ethyl acetate and methanol and the solution was evaporated under reduced pressure. The product was isolated by silica gel column chromatography. Yield: 100 mg = 70%.

^{1}H-NMR (DMSO δ-6) 0.93 (m, 4H) 1.32 (m, 1H) 1.88 (m,1H) 2.85 (m, 2H) 3.05 (m, 1H) 6.92 (m, 2H) 7.38 (m, 2H) 8.00 (m, 4H) 8.38 (m, 1H)

Example 37

(1S, 2S)-N-[*cis*-2-(6-fluoro-2-O-3-propionylphenyl)-cyclopropyl]-N'-[2-(5-cyanopyridyl)]urea-O-methylene-4-hydroxybenzoate

a) Methyl-4-(4-methoxybenzyloxy) benzoate.

To a solution of methyl 4-hydoxybenzoate (6.85g, 45 mmole) in 80 ml DMF was added potassium tert. butoxide (5.6 g, 51 mmole) and the mixture was stirred at room temperature for one hour. 4-Methoxybenzyl chloride (8.3 g, 52 mmole) was added and the mixture was stirred overnight at room temperature. The mixture was evaporated under reduced pressure and 200 ml ethyl acetate was added. The organic phase was washed four times with water, dried with sodium sulfate and evaporated under reduced pressure. Yield: 12.3g = 100%

^{1}H-NMR ($CDCl_3$) 3.82 (s, 3H) 3.88 (s, 3H) 5.03 (s, 2H) 6.96 (m, 4H) 7.36 (d, 2H) 7.98 (d, 2H)

b) 4- (4-methoxybenzyloxy) benzoic acid

To a solution of methyl 4-(4-methoxybenzyloxy) benzoate (12.2 g, 44.8 mmole) in 50 ml 1,4-dioxane was added a solution of lithium hydroxide (2.15 g, 89,6 mmole) and the mixture was stirred overnight at 60°C. The mixture was evaporated under reduced pressure and 5% acetic acid was added. The product was filtered, washed with water and dried. Yield: 10.1g = 87%

^{1}H-NMR (DMSO δ-6) 3.74 (s, 3H) 5.08 (s, 2H) 6.92 (d, 2H) 7.06 (d, 2H) 7.36 (d, 2H) 7.90 (d, 2H)

c) Chloromethyl 4-(4-methoxybenzyloxy)benzoate

To a solution of 4-(4-methoxybenzyloxy) benzoic acid (5.16 g, 20 mmole) in 100 ml 1,4-dioxane was added a 40% solution of tetrabutylammonium hydroxide (14.27 g, 22 mmole) and the mixture was stirred 2 hours at room temperature. The mixture was evaporated under reduced pressure and co-evaporated two times with 1,4-dioxane and two times with toluene. The dried product was dissolved in 60 ml dichloromethane and iodochloromethane (35.3 g 200 mmole) was added. The solution was stirred for two days at room temperature and evaporated under reduced pressure. About 100 ml ethyl actate was added and the organic phase washed twice with water, dried with sodium sulfate and evaporated under reduced pressure. The product was isolated by silica gel column chromatography.Yield: 4.48 g = 73%

^{1}H-NMR ($CDCl_3$) 3.83 (s, 3H) 5.06 (s, 2H) 5.94 (s, 2H) 7.00 (m, 4H) 7.36 (d, 2H) 8.05 (d, 2H)

d) Iodomethyl-4-(4-methoxybenzyloxy) benzoate

To a solution of chloromethyl-4-(4-methoxybenzyloxy) benzoate (0.77g, 2.5 mmole) in 15 ml dry acetone was added sodium iodide (1.87g, 12.5 mmole) and the mixture was stirred overnight at room temperature. The mixture was evaporated under reduced pressure and extracted with ethyl actate/water. The organic phase was washed with a 5% sodium thiosulfate solution, dried with sodium sulfate and evaporated under reduced pressure. Yield 0.86g = 86%

^{1}H-NMR ($CDCl_3$) 3.84 (s, 3H) 5.05 (s, 2H) 6.14 (s, 2H) 6.98 (m, 4H) 7.36 (d, 2H) 8.00 (d, 2H)

e) (1S, 2S)-N-[*cis*-2-(6-fluoro-2-O-3-propionylphenyl (cyclopropyl] -N'-[2-(5-cyanopyridyl)urea-O-methylene-4-(4-methoxybenzyloxy) benzoate.

To a solution of (1S, 2S)-N-[*cis*-2-(6-fluoro-2-hydroxy-3-propionylphenyl) cyclopropyl]-N'-[2-(5-cyanopyridyl)]urea (368mg, 1 mmole) in 5 ml DMF was added a suspension of 60% sodium hydride in mineral oil (44mg, 1.1 mmole) and the mixture was stirred for one hour at room temperature. A solution of iodomethyl-4-(4-methoxybenzyloxy) benzoate (0.84 g, 2.1 mmole) in 2 ml THF was added and the mixture was stirred overnight at room temperature. 50 ml ethyl acetate were added and the organic phase was washed four times with water, dried with sodium sulfate and evaporated under reduced pressure. The product was isolated by silica gel column chromatography. Yield: 525 mg = 82%

^{1}H-NMR ($CDCl_3$) 0.91 (m, 3H) 1.32 (m, 1H) 1.60 (m, 1H) 2.04 (m, 1H) 2.90 (m,2H) 3.20 (m, 1H) 3.82 (s, 3H) 5.04 (s, 2H) 5.84-6.06 (m, 2H) 6.91-8.18 (m,13H)

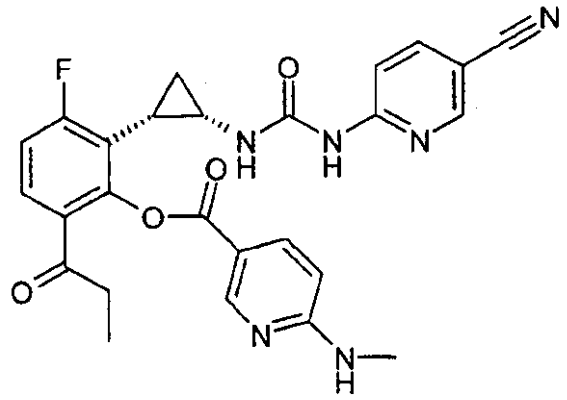
f) (1S, 2S)-N-[*cis*-2-(6-fluoro-2-O-3-propionylphenyl)cyclopropyl]-N'-[2-(5-cyanopyridyl)]urea-O-methylene-4-hydroxybenzoate

To a solution of (1S, 2S)-N-[*cis*-2-(6-fluoro-2-O-3-propionylphenyl)cyclopropyl]N'-[2-(5-cyanopyridyl)urea-O-methylene-4-(4-methoxybenzyloxy) benzoate (100 mg, 0.156 mmole) in 4 ml dichloromethane was added TFA (0.5 ml) and the solution was stirred for one hour at room temperature. The solution was evaporated under reduced pressure and the product was isolated by silica gel column chromatography.
Yield: 45mg = 55%

^{1}H-NMR (DMSO δ-6) 0.84 (m, 3H) 1.10 (m, 1H) 1.48 (m, 1H) 2.12 (m, 1H) 2.80 (m, 2H) 3.19 (m, 1H) 5.85-6.02 (m, 2H) 6.84 (m, 2H) 7.18 (m, 1H) 7.46 (m, 2H) 7.74 (m, 2H) 8.04 (m, 2H) 8.38 (m, 1H)

Example 39

(1S,2S)-N-{*cis*-2-[6-fluoro-3-propionyl-2-(6-methylaminopyrid-3-ylcarbonyloxy)phenyl] cyclopropyl}-N'-(5-cyanopyrid-2-yl)urea



This compound was prepared from 6-methylaminonicotinic acid 0.050g, 0.33 mmol) and the compound of Example 8 (0.1g, 0.27 mmol) by the same procedure as for Example 31. The crude product (containing the title compound and unreacted starting material) was purified by chromatography (ethyl acetate) to give 0.030g (22 %) of the title compound.

^{1}H.NMR ($CDCl_3$): 9.8 (br s, 1H), 9.25 (br s, 1H), 8.90 (d, 1H), 8.20 (d, 1H), 8.10 (m, 1H), 7.72 (m, 2H), 7.08 (t, 1H), 6.9 (d, 1H), 6.37 (d, 1H), 3.20 (m, 1H), 2.95 (d, 3H), 2.85 (q, 2H), 1.95 (m, 1H), 1.48 (m, 1H), 1.10 (t, 3H).

Biological Example 1

Resistance pattern

Compounds of the invention were tested for antiviral activity against a number of HIV strains, including wild type and known mutants arising from the use of other non-nucleoside reverse transcriptase inhibitors as described in the review of Schinazi at al, International Antiviral News, vol 4 no 6, pp 95-107 (1996). Results are presented in Table 1.

TABLE 1

HIV strain	Example 5	Example 6	Example 8	Prior art*
wild type	0.0012 +/- 0.0004	0.0008 +/- 0.0004	0.0007 +/- 0.0002	0.0056 +/- 0.004
wild type 50% serum	0.01 +/- 0.009	0.006 +/- 0.003	0.007 +/- 0.001	0.023 +/- 0.011
K103N	0.05 +/- 0.04	0.017 +/- 0.008	0.037 +/- 0.007	0.13 +/- 0.060
K103N 50% serum	0.38 +/- 0.31	0.17 +/- 0.07.	0.39 +/- 0.31	0.9 +/- 0.6
Y181C	0.017 +/- 0.018	0.006 +/- 0.002	0.006 +/- 0.001	0.13 +/- 0.02
Y181C 50% serum	0.10 +/- 0.06	0.08 +/- 0.05	0.08 +/- 0.06	0.13 +/- 0.07
Y188L	0.13 +/- 0.07	0.08 +/- 0.06	0.06 +/- 0.02	0.17 +/- 0.03
Y188L 50% serum	1.5 +/- 0.9	0.9 +/- 0.05	1.0 +/- 0.05	1.9 +/- 1.5
L100I, Y181C	ND	ND	0.34 +/- 0.06	1.0
L100I	ND	ND	0.009 +/- 0.001	0.026 +/- 0.009
SI	>41 600	22 500	87 000	5 900
SI 50% serum	ND	8 830	4 285	800

The assay included multiple determinations with XTT in MT-4 cells (Weislow et al, J Nat Cancer Inst 1989, vol 81 no 8, 577 et seq) including determinations in the presence of 50% human serum to indicate the contribution of protein binding. The ED_{50} is presented in µg/ml. The initial data on the calculated therapeutic index (SI) are also presented, defined as the dose producing 50% toxicity in the corresponding HIV-free cells divided by the ED_{50}. The prior art compound, from the 1995 ICAR Santa Fe is depicted above.

It will be apparent that the compounds of the invention, especially the enantiomers, have ED_{50} values which are distinctly lower than hitherto known compounds, including the values against the known problematic mutants K103N and Y181C, as well as L100I and the double mutant L100I, Y181C. Furthermore the therapeutic

indices for the enantiomers are 5 to 10 fold greater than the prior art compound. These results should be seen in the context of HIV therapy where patients can expect to take medication for many years, if not for the rest of their lives against the notoriously resistance prone virus HIV. Thus a large SI is needed to avoid cumulative toxicity, while at the same time allowing adequate dosing to maintain therapeutic pressure and prevent the spontaneous generation of multiply resistant HIV strains.

Biological Example 2

Time to resistance

2×10^4 MT4 cells per well in a microtitre plate are infected with 5-10 $TCID_{50}$ of HIV-1 $_{IIIB}$. The compounds being tested are added at concentrations around ED_{50} using 8 duplicates per concentration. After 6 days of incubation the RT activity in 10µl supernatent is measured.

The following procedure is followed at subsequent passages of the cultures once per week.: Virus produced at the concentration of test compound showing > 50% of the RT activity of untreated infected cells (SIC, Starting Inhibitory Concentration) are passaged to fresh MT4 cells. 15µl supernatent from each of the eight duplicates are transferred to cells without the test compound (control) and to cells with test compound at the same concentration, and additionally two respectively fivefold higher concentrations. (See Table 2 below)

When viral growth is permitted at the highest non-toxic concentration (5 - 40 µM), 2-4 parallel wells are collected and expanded to give material for sequence analysis and cross-wise resistance.

TABLE 2

Viral growth permitted
Virus production inhibited

				125 x SIC
			125 x SIC	***25 x SIC*** →
			25 x SIC	**5 x SIC**
		25 x SIC	***5 x SIC*** →	**No compound**
	25 x SIC	***5 x SIC*** →	**No compound**	
	5 x SIC	**SIC**		
	SIC →	**No compound**		
SIC →	**No compound**			
Pass 1	**Pass 2**	**Pass 3**	**Pass 4**	**Pass 5**

Figure 1 plots the growth of viral resistance for a compound of the invention (Example 8) against time. Also plotted is the corresponding curve for the closest Santa Fe compound, mentioned above. It will be apparent that the compounds of the invention show a significantly slower rate of resistance development.

Biological Example 3

P450 metabolism

The metabolism of compounds of the invention through the main isoforms of the human cytochrome system P450 were determined in baculovirus infected insect cells transfected with human cytochrome P450 cDNA (supersomes) Gentest Corp. Woburn USA.

The test compounds at concentrations 0.5, 5 and 50 μM were incubated in duplicate in the presence of supersomes overexpressing various cytochrome P450 isoforms,

including CYP1A2 + P450 reductase, CYP2A6 + P450 reductase, CYP2C9-Arg 144 + P450 reductase, CYP2C19 + P450 reductase, CYP2D6-Val 374 + P450 reductase and CYP3A4 + P 450 reductase. Incubates contain a fixed concentration of cytochrome P450 (eg 50 pmoles) and are conducted over 1 hour. The involvement of a given isoform in the metabolism of the test compound is determined by UV HPLC chromatographically measuring the disappearance of parent compound.

After testing the three concentrations for 7.5 minutes, the %-age remaining figures suggest that CYP3A4, 1A2, 2C19 and 2A6 are involved in the metabolism of the compound of Example 7. Similar constellations of P450 isoforms are also involved in the metabolism of the prior art Santa Fe halopyridinyl compounds.

Surprisingly, no significant p450 metabolism with any isomer was registered for the compound of Example 8, implying that the compound is stable in vivo and that the possibility of disturbance of the metabolism of coadministered drugs is correspondingly low.

Biological Example 4

Pharmacokinetics

The release of a compound of Formula I from an orally administered prodrug of Formula II was monitored in rats. The compound of Example 7 was made up in a propylene glycol vehicle and orally administered to paired fasted male Sprague Dawley rats at a dose corresponding to 0.027 mmol/kg. At the indicated time intervals, 0.2 ml blood was collected from a catheter implanted in the canis jugularis, centrifuged and frozen for later analysis. The released drug of Formula I (Example 6) was assayed by HPLC. Aliquots comprising 40-100 µl of each plasma sample are mixed with an equal volume of acetonitrile (10 seconds, Vibrofex). The sample is

centrifuged (2 min, 14000 RPM) and 30 µl of the supernantant is injected into an HPLC system, as follows.

Pre column:	RP-18, 7 µm, 15 x 3.2 mm
Column:	YMC basic, 3µm, 150 x 3 mm
Mobile phase:	60 % acetonitrile in 3 mM ammonium acetate, pH 6.4
Flow rate:	0.4 ml/min
Detection:	UV, 250 nm

Table 3

time (min)	plasma level of mother compound (µg/ml)
30	0.24, 0.35
60	0.18, 0.28
120	0.13, 0.17
240	0.07, 0.12
360	0.05, 0.07

In Table 3 it is clear that oral administration of the prodrugs of Formula II releases in vivo clinically significant amounts of the compounds of Formula I.

Biological Examples 5 - 8

i) Preparatory

The rats used in pharmacokinetic examples were male Sprague-Dawley, with a weight about 200-250 g. The rats were fasted for at least 16 hours before the experiment, but had free access to water. The day before the experiment the rats were anaesthetized using a mixture of Efrane®, laughing gas and oxygen. A catheter was

introduced into the vena jugularis. On the day of the experiment the weights of the rats were noted. The animals were shortly anaesthesized before the oral dose was given or the iv dose injected into the back of the neck. Each substance was administered to duplicate rats.

Monkeys were fasted for 12 hours prior to oral administration but had free access to water. The test compound was delivered via an infant nasogastric feeding tube. After 6 hours the monkeys received an apple.

ii) Dose preparation

Appropriate quantities of the active ingredients described in the following examples were dissolved/suspended in a solution of propylene glycol or 10 % Acacia and 1% of Tween in water for oral administration. Compounds were dissolved in DMSO for intravenous administration.

iii) Blood sampling

Blood samples (typically 0.6 ml for rats, 2 ml for monkeys) were taken before and at the indicated time intervals, as plotted, after drug administration. Monkeys were tapped from the femoral vein into EDTA-containing tubes. The blood samples were centrifuged infectious agents neutralised with 1% SDS/64°/20 min and plasma stored at -20°C.

iv) Bioanalysis

Plasma samples are prepared as follows: 40-100 µl of plasma is mixed with an equal volume of acetonitrile (10 seconds, Vibrofex). The sample is centrifuged (2 min, 14000 RPM) and 30 µl of the supernantant is injected into an HPLC system, as follows.

Pre column:	RP-18, 7 μm, 15 x 3.2 mm
Column:	YMC basic, 3μm, 150 x 3 mm
Mobile phase:	60 % acetonitrile in 3 mM ammonium acetate, pH 6.4
Flow rate:	0.4 ml/min
Detection:	UV, 250 nm

Biological Example 5

Comparison with the closest prior art compound

The in vivo stability and availability of the compounds of Formula I were compared with the closest Santa Fe compound, namely (+/-)-N-(*cis*-2-(6-fluoro-2-hydroxy-3-propionylphenyl)-cyclopropyl)-N'-(5-chloropyridyl-2-yl)-urea, whereby 0.024 mmol/kg doses of the respective compounds were administered in a DMSO vehicle. Figure 2 is a plot of plasma levels of the respective compounds (n=2 in each case) over time. It will be apparent that the respective curves follow a common pattern but that the compound of the invention has an AUC (0-4h) in excess of 1.5 times the AUC (0-4h) of the closest prior art compound. In other words the compounds of the invention provide a 50% greater in vivo exposure than the previously described derivative, although although whether this is due to a slower clearance of the compounds of the invention or a greater degree of tissue binding with the prior art compounds, etc has yet to be determined.

Biological Example 6

Bioequivalence of prodrugs and mother compound

Various compounds of Formula II (that is prodrugs of the compounds of Formula I) were administered to rats and the plasma levels of the mother compound of the invention (in this example the compound of Example 10) were monitored over time.

The vehicle was 10% acacia and 1% Tween in water or propylene glycol (asterisked).

Plasma level figures in Table 4 refer to individual animals.

TABLE 4

Compound	Dose (mmol/kg)	Time (min)	plasma level of mother compound (μg/ml)			
Example 12	0.053	30	0.2	0.3	0.06	0.11
		60	0.2	0.4	0.12	0.20
		90	0.3	0.4		
		120	0.2	0.5	0.10	0.20
		180	0.3	0.4	0.11	0.23
		240	0.3	0.4	0.08	0.24
		330			0.08	0.15
		420			0.05	0.12
Example 12	0.026	30	0.09	0.05		
		60	0.10	0.07		
		120	0.09	0.08		
		180	0.08	0.08		
		240	0.06	0.05		
		330		0.03		
		420		0.02		
Example 22	0.026	30		0.08		
		60	0.05	0.11		
		120	0.04	0.08		
		180	0.03	0.07		
		240	0.02	0.04		
		360	<0.02	<0.02		
Example 14	0.053	30	0.10	0.08		
		60	0.15	0.08		
		120	0.27	0.07		
		180	0.35	0.09		
		240	0.35	0.09		
		360	0.24	0.12		
Example 18	0.053	30	0.12	0.03		
		60	0.15	0.03		
		120	0.15	0.07		
		180	0.23	0.14		
		240	0.12	0.16		
		360	0.08	0.08		
Example 23	0.053	30	0.14	0.32		
		60	0.22	0.49		
		120	0.36	0.49		
		180	0.44	0.32		
		240	0.35	0.27		
		360	0.14	0.14		

TABLE 4 CONTINUED

Example 17	0.053	30 60 120 180 240 360	0.05 0.07 0.06 0.07 0.07 0.04	0.05 0.05 0.14 0.20 0.17 0.12		
Example 29	0.027*	30 60 120 240 360	0.258 0.268 0.128 0.051 <0.03	0.031 <0.03 <0.03 <0.03 <0.03		
Example 37	0.027*	30 60 120 240 360	0.234 0.273 0.111 0.056 0.054	0.137 0.189 0.133 0.045 0.056		

It will be apparent that the prodrugs of Formula II release in vivo clinically relevant amounts of the compounds of Formula I into the plasma. The absolute oral bioavailability (determined relative to the iv dose, as decribed in the preparatory section) was 28-33% for the compound of Example 37 and 27% for the evaluable animal with the compound of Example 27.

Biological Example 7

Bioavailability in different species

A prodrug of the invention of Formula II (Example 12) was administered at the same dose (0.026 mmol/kg) and in the same vehicle (10 % acacia and 1% Tween in water) to rats and cynomolgus monkeys. Plasma levels of the mother compound of Formula I (Example 10) was measured as a function of time.

TABLE 5

species	time (min)	plasma level of mother compound (µg/ml)	
rat	30	0.09	0.05
	60	0.10	0.07
	120	0.09	0.08
	180	0.08	0.08
	240	0.06	0.05
	330		
	420		
monkey	45	0.08	0.04
	90	0.20	0.26
	180	1.0	0.55
	240	0.72	0.54
	360	0.38	0.39
	600	0.13	0.10
	24 h	0.03	0.03

It will be apparent that the prodrugs of Formula II release in vivo clinically relevant amounts of the compounds of Formula I. Release occurs both in rodents and primates, with significantly greater plasma levels in primates.

The corresponding data for the compound of Example 28 (rat: acacia/Tween, monkey: propylene glycol) are shown in in Table 5A:

TABLE 5A

species	time (min)	plasma level of mother compound (µg/ml)	
rat	30	0.033	0.046
	60	0.039	0.084
	120	0.066	0.123
	240	0.039	0.034
	360	<0.03	<003
monkey	30	0.108	<0.03
	90	0.159	0.098
	180	0.062	0.050
	240	<0.03	0.060
	540	0.036	0.070

Biological Example 8

Antiviral activity

Compounds of Formula I were tested for HIV-1 activity against wild type HIV_{IIIB} and resistant mutants, with and without the presence of 50% human serum in the XTT-formazan assay where inhibition of cytopathogenic effects is assayed in MT4 cells. In each case the ED_{50} in μM is indicated

TABLE 6

HIV strain	Example 10	Example 10 50% serum	Example 11	Example 11 50% serum
wild type	0.01	0.06	0.009	0.05
L100I	0.05	0.33	0.09	0.95
K103N	0.38	2.4	0.09	2.0
Y181C	0.09	0.4	0.07	3.3

The compounds of formula I are thus highly active against various strains of HIV at concentrations achievable in vivo.

Biological Example 9

Antiviral activity

Compounds of the invention have also been compared to the closest prior art compound using a state of the art cell culture assay, wherein human T cell line MT4 cells are grown in RPMI 1640 medium supplemented with 10% fetal calf serum, penicillin and streptomycin seeded into 96 well microplates ($2 \bullet 10^4$ cells/well) infected with 10-20 $TCID_{50}$ per well of $HIV\text{-}1_{IIIB}$ (wild type) or mutant virus bearing RT Ile 100, Cys 181 or Asn 103 mutations. Serially diluted test compounds are added to respective wells and the culture incubated at 37°C in a CO_2 enriched atmosphere and the viability of cells is determined at day five or six with XTT vital dye. The results shown below the mean values of a number of determinations. Results are presented as ED_{50} μM.

TABLE 8

Example	wild type	wild type 50% serum	Ile100	Cys181	Asn 103
Prior art Santa Fe	0.027	nd	0.220	0.340	0.350
Example 10	0.012	0.056	0.053	0.095	0.358
Example 11	0.008	0.058	0.100	0.069	0.080
Example 8	0.003	0.019	0.021	0.019	0.086
Example 6	0.002	0.016	0.064	0.018	0.046

The compounds of the invention have significantly improved performance against wild type and especially clinically important mutations arising during treatment with NNRTIs.

Biological Example 10

Binding kinetics

The rate of association and dissociation of an NNRTI on the target enzyme can be directly assayed by surface plasmon resonance methodology, wherein reverse transcriptase is immobilized on the surface of a chip and the binding or dissociation of the putative inhibitor is monitored by observing the changes in refractive index caused by the concomitant increase or decrease in chip mass. A compound of the invention (Example 8) was compared to the closest prior art compound from Santa Fe, as depicted above. Experiments were performed on a Biacore 2000 (Biacore AB, Uppsala, Sweden), using BIAevaluation software (ver 3.0) for evaluation of data. The binding of the small analyte (NNRTI) to the much larger enzyme results in binding responses in the range of 10-20 RU. The difference in bulk refractive index between running buffer and sample makes it difficult to evaluate data obtained during the injection of sample. During the dissociation phase there is insignificant change in bulk refractive index, thus the binding of the different substances have been evaluated during this phase.

Immobilisation: The enzyme and reference protein were immobilised by direct

coupling to primary amines on a CM5 chip (Markgren et al., 1998). Antibody to Fc g (Biacore BR-1000-57) was used as reference protein and was immobilised according to instructions from the manufacturer. HIV reverse transcriptase (Unge et al., 1990) was transferred from 3 M $(NH_4)2SO_4$ to 5 mM Hepes, pH 7.6 containing 4 mM $MgCl_2$, using Nanosept Centrifugal concentrators 10K (Pall Filtron, MA, U.S.A). RT amounts corresponding to 6800-9700 RU were immobilised to the sensor chip. The sensor surface was deactivated by injection of 35 ml of 0.5 M Tris pH 7.6; 4 mM $MgCl_2$; 0.5 M KCl. The immobilisation procedure was carried out at 33° C.

Interaction with inhibitors: Stock solutions of inhibitors (1 mg/ml in DMSO) were dissolved in RT running buffer (10 mM Hepes pH 7.6; 4 mM $MgCl_2$; 0.25 mM spermine; 40 mM KCl; 0.5% Triton X-100; 3% DMSO; 0.5% fetal calf serum) to a concentration of 10 mM. Binding of substance to the RT was analysed by injection of 200 ml of the diluted substance, the flow rate was 20 ml/min and the temperature 25°C. After each injection of substance the system was washed by injection of 120 ml of 10% DMSO in RT running buffer.

The results are depicted in Fig 3. It is apparent that the compound of the invention and the prior art compound show different interaction kinetics with the compoound of the invention dissociating with the lowest rate, indicating a more efficient binding to the enzyme.

References:

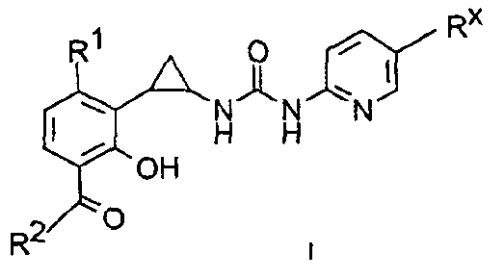
Unge T, Ahola H, Bhikhabhai R, Backbro K, Lovgren S, Fenyo EM, Honigman A, Panet A, Gronowitz JS, Strandberg B, Expression, purification, and crystallization of the HIV-1 reverse, transcriptase (RT). AIDS Res Hum Retroviruses 1990 Nov;6(11):1297-303

Markgren P-O, Hamalainen M, Danielson UH, Screening of compounds interacting with HIV-1 proteinase using optical biosensor technology. Analytical Biochemistry 1998, vol 265, in press.

Although various aspects and embodiments of the invention have been illustrated with reference to the above concrete examples, comparative examples and Figures, it will be appreciated that the invention is in no way limited to these embodiments, but extends throughout the spirit and scope of the attached claims.

CLAIMS

1. A compound of the formula I:



I

wherein

R^x is cyano or bromo;

R^1 is halo;

R^2 is C_1-C_3 alkyl,

and pharmaceutically acceptable salts and prodrugs thereof.

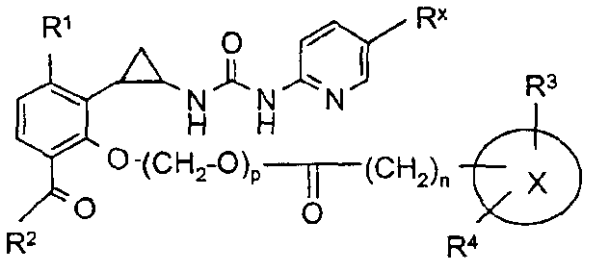
2. A compound according to claim 1, wherein R^1 is fluoro.

3. A compound according to claim 1 wherein R^2 is ethyl.

4. A compound according to claim 1, comprising at least 60 %, preferably at least 90 % 1S,2S enantiomeric form.

5. A compound according to claim 1, wherein R^x is cyano.

6. A compound according to claim 1, wherein the prodrug has the formula II:



II

wherein

R^x, R^1 and R^2 are as defined above,

R^3 is H, $(CH_m)_nNR^5R^6$;

R^4 is H, C_1-C_3 alkyl, $(CH_m)_nNR^5R^6$, $(CH_m)_nC(=O)R^5$, $(CH_m)_nOH$, OR^7, halo, CF_3 or CN; or

R^3 and R^4 together define a 5 or 6 membered fused ring having 0-2 hetero atoms and/or 0-2 unsaturated bonds and/or 0-2 substituents;

R^5 is H, C_1-C_3 alkyl, $C(=O)R^7$ or a peptide of 1 to 4 amino acids;

R^6 is H, C_1-C_3 alkyl; or

R^5 and R^6 together define a 5 or 6 membered ring having 0 or 1 additional hetero atom and/or 0-2 unsaturated bonds and/or 0-2 substituents;

R^7 is H, C_1-C_{12} alkyl, $(CH_m)_nNR^5R^6$;

X and its encompassing circle define a 5 or 6 membered ring having 0 to 3 unsaturated bonds and/or 0 to 3 hetero atoms selected from S, O and N;

m is independently 1 or 2;

n is independently 0, 1 or 2;

p is 0 or 1;

and pharmaceutically acceptable salts thereof.

7. A compound according to claim 6, wherein the X-containing ring is napthyl, pyridyl, quinolyl or phenyl.

8. A compound according to claim 7 wherein the X-containing ring is phenyl.

9. A compound according to claim 7 wherein the X-containing ring is pyrid-2-yl or preferably pyrid-3-yl.

10. A compound according to claim 6, wherein R^3 is $-NH_2$, $-NHCH_3$, $-NHCH_2CH_3$ or $-N(CH_3)_2$.

11. A compound according to claim 6 wherein R^3 is in the meta position relative to the carbonyl group especially where the X-containing ring is phenyl, or wherein R^3 is in the para position relative to the carbonyl group, especially where the X containg ring is a heterocycle.

12. A compound according to claim 6 wherein -$(CH_2)_n$- and/or -$(CH_2)_n$- are absent, that is p and/or n are 0.

13. A compound according to claim 6, wherein R^x is cyano.

14. A compound according to claim 6, wherein R^1 is fluoro.

15. A compound accoridng to claim 6, wherein R^2 is ethyl.

16. A compound according to claim 6, comprising at least 60 %, preferably at least 90 % 1S,2S enantiomeric form.

17. A compound according to claim 1 selected from (1S, 2S)-N-[*cis*-2-(6-fluoro, 2-hydroxy, 3-propionylphenyl)-cyclopropyl]-N'-(5-cyanopyrid-2-yl)-urea, (1R, 2R)-N-[*cis*-2-(6-fluoro, 2-hydroxy, 3-propionylphenyl)-cyclopropyl]-N'-(5-cyanopyrid-2-yl)-urea, (1S, 2S)-N-[*cis*-2-(6-fluoro, 2-hydroxy, 3-propionylphenyl)-cyclopropyl]-N'-(5-bromopyrid-2-yl)-urea, and (1R, 2R)-N-[*cis*-2-(6-fluoro, 2-hydroxy, 3-propionylphenyl)-cyclopropyl]-N'-(5-brompyrid-2-yl)-urea and pharmaceutically acceptable salts thereof.

18. A compound according to claim 17 denoted (1S, 2S)-N-[*cis*-2-(6-fluoro, 2-hydroxy, 3-propionylphenyl)-cyclopropyl]-N'-(5-cyanopyrid-2-yl)-urea or a pharmaceutically acceptable salt thereof.

19. A compound according to claim 1 selected from the group consisting of (1S, 2S)-N-[*cis*-2-(2-(3-aminophenylcarbonyloxy)-6-fluoro-3-propionylphenyl)-cyclopropyl]-N'-(5-cyanopyrid-2-yl)-urea,
(1S, 2S)-N-[*cis*-2-(2-(3-ethylaminophenylcarbonyloxy)-6-fluoro-3-propionylphenyl)-cyclopropyl]-N'-(5-cyanopyrid-2-yl)-urea,
(1S, 2S)-N-[*cis*-2-(2-(3-dimethylaminophenylcarbonyloxy)-6-fluoro-3-propionylphenyl)-cyclopropyl]-N'-(5-cyanopyrid-2-yl)-urea,
(1S,2S)-N-{*cis*-2-[6-fluoro-3-propionyl-2-(6-methylaminopyrid-3-ylcarbonyloxy)phenyl] cyclopropyl}-N'-(5-cyanopyrid-2-yl)urea,

(1R, 2R)-N-[*cis*-2-(2-(3-aminophenylcarbonyloxy)-6-fluoro-3-propionylphenyl)-cyclopropyl]-N'-(5-cyanopyrid-2-yl)-urea,
(1R, 2R)-N-[*cis*-2-(2-(3-ethylaminophenylcarbonyloxy)-6-fluoro-3-propionylphenyl)-cyclopropyl]-N'-(5-cyanopyrid-2-yl)-urea,
(1R, 2R)-N-[*cis*-2-(2-(3-dimethylaminophenylcarbonyloxy)-6-fluoro-3-propionylphenyl)-cyclopropyl]-N'-(5-cyanopyrid-2-yl)-urea,
(1R,2R)-N-{*cis*-2-[6-fluoro-3-propionyl-2-(6-methylaminopyrid-3-ylcarbonyloxy)phenyl] cyclopropyl}-N'-(5-cyanopyrid-2-yl)urea,

(1S, 2S)-N-[*cis*-2-(2-(3-aminophenylcarbonyloxy)-6-fluoro-3-propionylphenyl)-cyclopropyl]-N'-(5-bromopyrid-2-yl)-urea,
(1S, 2S)-N-[*cis*-2-(2-(3-ethylaminophenylcarbonyloxy)-6-fluoro-3-propionylphenyl)-cyclopropyl]-N'-(5-bromopyrid-2-yl)-urea,
(1S, 2S)-N-[*cis*-2-(2-(3-dimethylaminophenylcarbonyloxy)-6-fluoro-3-propionylphenyl)-cyclopropyl]-N'-(5-bromopyrid-2-yl)-urea,
(1S,2S)-N-{*cis*-2-[6-fluoro-3-propionyl-2-(6-methylaminopyrid-3-ylcarbonyloxy)phenyl] cyclopropyl}-N'-(5-bromopyrid-2-yl)urea,

(1R, 2R)-N-[*cis*-2-(2-(3-aminophenylcarbonyloxy)-6-fluoro-3-propionylphenyl)-cyclopropyl]-N'-(5-bromopyrid-2-yl)-urea,
(1R, 2R)-N-[*cis*-2-(2-(3-ethylaminophenylcarbonyloxy)-6-fluoro-3-propionylphenyl)-cyclopropyl]-N'-(5-bromopyrid-2-yl)-urea,
(1R, 2R)-N-[*cis*-2-(2-(3-dimethylaminophenylcarbonyloxy)-6-fluoro-3-propionylphenyl)-cyclopropyl]-N'-(5-bromopyrid-2-yl)-urea,
(1R,2R)-N-{*cis*-2-[6-fluoro-3-propionyl-2-(6-methylaminopyrid-3-ylcarbonyloxy)phenyl] cyclopropyl}-N'-(5-bromopyrid-2-yl)urea,
and pharmaceutically acceptable salts thereof.

20. A compound according to claim 19 denoted (1S,2S)-N-{*cis*-2-[6-fluoro-3-propionyl-2-(6-methylaminopyrid-3-ylcarbonyloxy)phenyl] cyclopropyl}-N'-(5-cyanopyrid-2-yl)urea and its pharmaceutically acceptable salts.

21. A compound according to claim 19 denoted (1S, 2S)-N-[*cis*-2-(2-(3-aminophenylcarbonyloxy)-6-fluoro-3-propionylphenyl)-cyclopropyl]-N'-(5-bromopyrid-2-yl)-urea and its pharmaceutically acceptable salts.

22. A pharmaceutical composition comprising a compound as defined in any one of claims 1 to 21 and a pharmaceutically acceptable carrier or diluent therefor.

23. A composition according to claim 22 further comprising one to three additional antiretroviral agents.

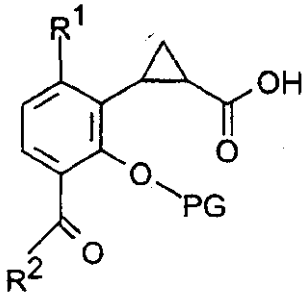
24. A composition according to claim 23 wherein the additional antiretroviral agent is selected from the group consisting of AZT, ddI, ddC, D4T, 3TC, adefovir, adefovir dipivoxil, abacavir, bis-POC-PMPA, foscarnet, hydroxyurea, efavirenz, trovirdine, nevirapine, delavirdine, PFA, H2G, ABT 606, ritonavir, saquinavir, indinavir, amprenavir (Vertex VX 478), Mitsubishi MKC-442 and nelfinavir

25 A compound according to any one of claims 1 to 21 for use in therapy

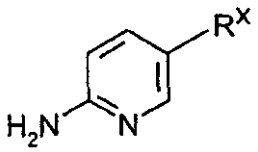
26. Use of a compound according to any one of claims 1 to 21 in the manufacture of a medicament for the treatment or prophylaxis of HIV.

27. A method for inhibiting or preventing HIV infection comprising administering an effective amount of a compound as defined in claim 1 or 6 to a subject in need thereof.

28. A method for the preparation of a compound of the Formula I comprising the comprising the Curtius rearrangement of the azide of a compound of the formula:



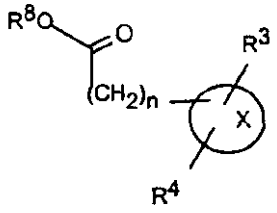
followed by coupling with a compound of the formula



and deprotection, wherein R^1, R^2 and R^x are as defined above and PG is an hydroxy-protecting group.

29. A method according to claim 28, further comprising the step of

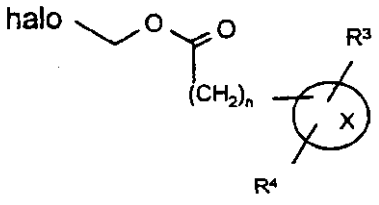
a) acylating with an activated compound of the formula III:



III

where R^3, R^4, X and n are as defined above but are optionally protected, and R^8 is hydrogen or a conventional activating group; or

b) alkylating with a compound of the formula IIIa:



IIIa

where n, R^3, R^4 and X are as defined above, but where exposed amine, hydroxy etc substituents are protected with conventional protecting groups.

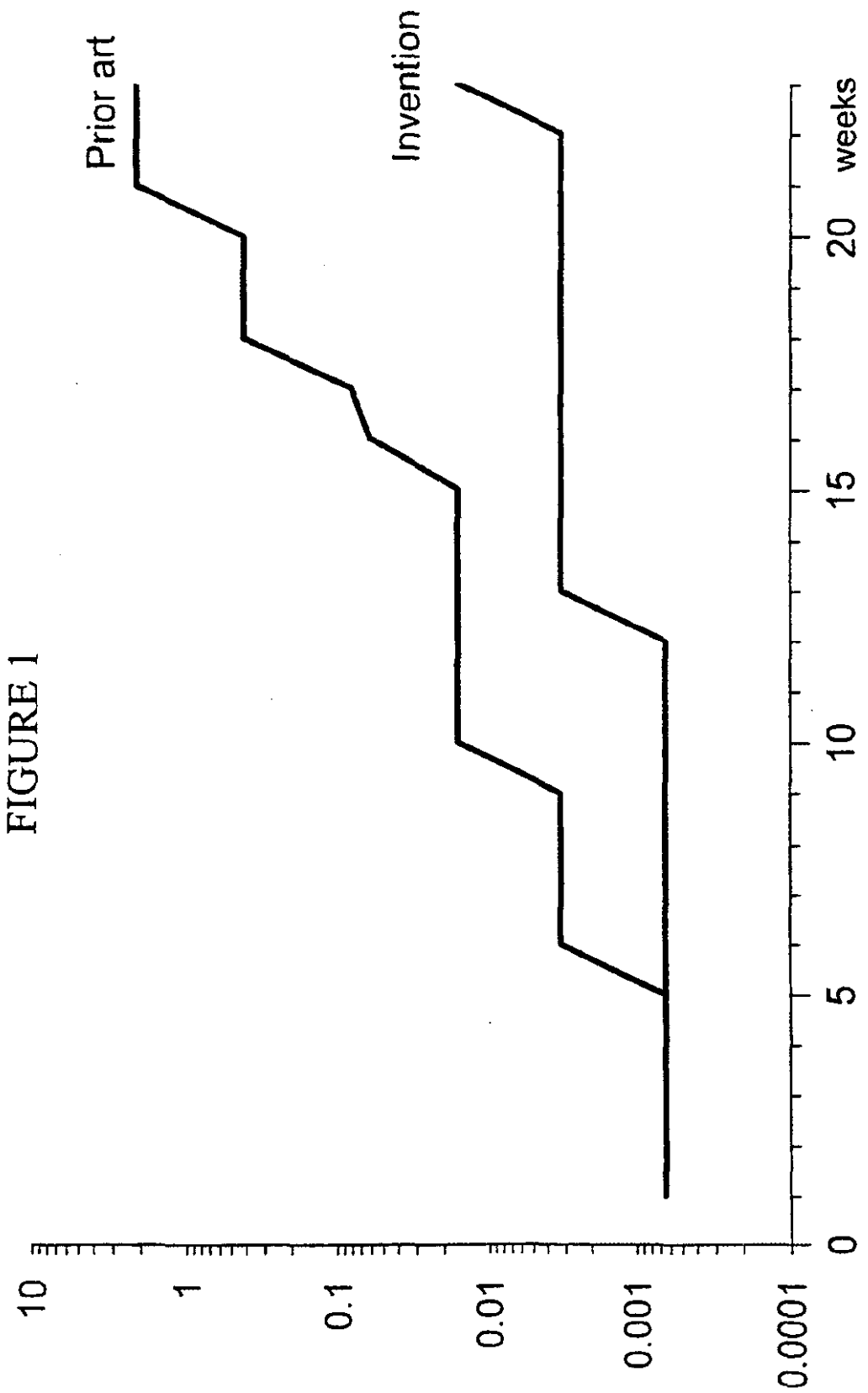
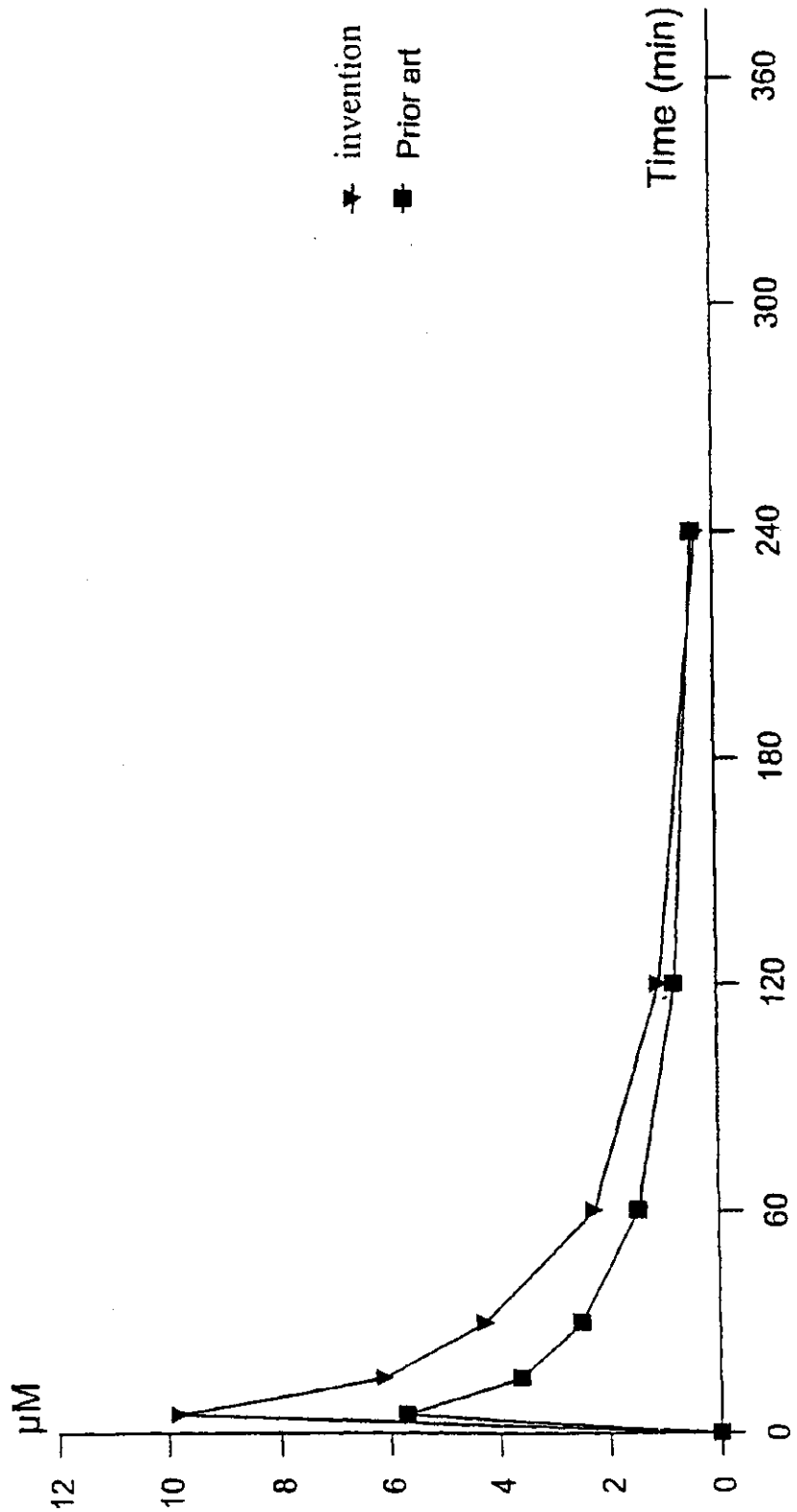


FIGURE 1

FIGURE 2


invention
Prior art
Time (min)
µM
0
60
120
180
240
300
360
0
2
4
6
8
10
12

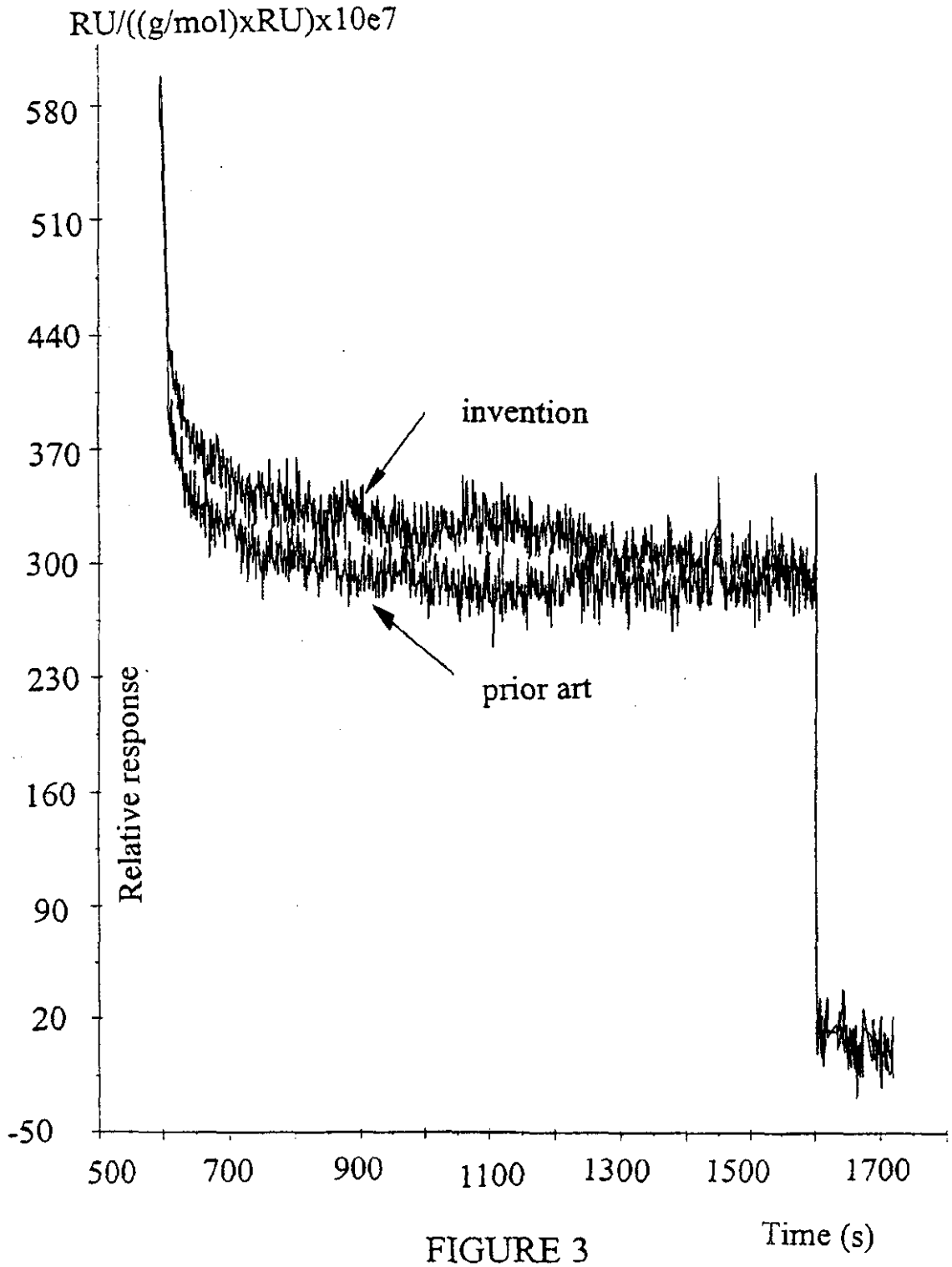

RU/((g/mol)xRU)x10e7
580
510
440
370
300
230
160
90
20
-50
Relative response
invention
prior art
500
700
900
1100
1300
1500
1700
Time (s)

FIGURE 3

HKP00210B4

[19]中华人民共和国国家知识产权局

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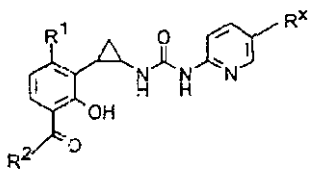
代理人 谭明胜

权利要求书6页 说明书67页 附图页数3页

[54]发明名称 抗病毒药物

[57]摘要

其中R^X为氰基或溴代,R^1为卤代,R^2为C_1-C_3烷基的式Ⅰ化合物和它们的药学上可接受的盐和前药具有抗逆转录病毒活性。



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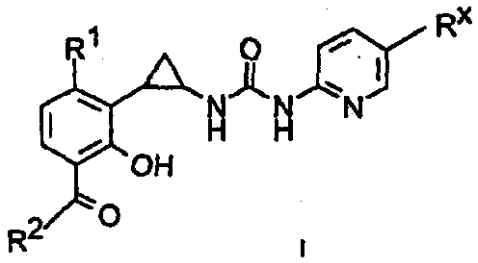
权 利 要 求 书

1. 式 I 化合物:



I

其中

R^x 为氰基或溴代;

R^1 为卤代;

R^2 为 C_1-C_3 烷基;

和它们的药学上可接受的盐和前药.

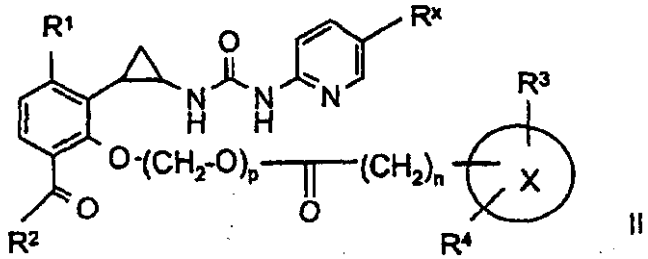
2. 权利要求 1 的化合物, 其中 R^1 为氟代.

3. 权利要求 1 的化合物, 其中 R^2 为乙基.

4. 权利要求 1 的化合物, 包括至少 60%, 优选至少 90%的 1S,2S 对映体形式.

5. 权利要求 1 的化合物, 其中 R^x 为氰基.

6. 权利要求 1 的化合物, 其中所述前药具有式 II:



II

其中

R^x 、R^1和R^2如上定义，

R^3为 H、$(CH_m)_nNR^5R^6$;

R^4为 H、C_1-C_3烷基、$(CH_m)_nNR^5R^6$、$(CH_m)_nC(=O)R^5$、$(CH_m)_nOH$、OR^7、卤代、CF_3或 CN; 或者

R^3和R^4一起定义为具有 0-2 个杂原子和/或 0-2 个不饱和键和/或 0-2 个取代基的 5 或 6 元稠环;

R^5为 H、C_1-C_3烷基、$C(=O)R^7$或 1 至 4 个氨基酸的肽;

R^6为 H、C_1-C_3烷基; 或者

R^5和R^6一起定义为具有 0 或 1 个另外的杂原子和/或 0-2 个不饱和键和/或 0-2 个取代基的 5 或 6 元环;

R^7为 H、C_1-C_{12}烷基、$(CH_m)_nNR^5R^6$;

X 和包含其的环定义为具有 0 至 3 个不饱和键和/或 0 至 3 个选自 S、O 和 N 的杂原子的 5 或 6 元环;

m 独立为 1 或 2;

n 独立为 0、1 或 2;

p 为 0 或 1;

和它们的药学上可接受的盐.

7. 权利要求 6 的化合物，其中所述含有 X 的环为萘基、吡啶基、喹啉基或苯基.

8. 权利要求 7 的化合物，其中所述含有 X 的环为苯基.

9. 权利要求 7 的化合物，其中所述含有 X 的环为吡啶-2-基或优选为吡啶-3-基.

10. 权利要求 6 的化合物，其中R^3为-NH_2、-$NHCH_3$、-$NHCH_2CH_3$或-$N(CH_3)_2$.

11. 权利要求 6 的化合物，其中R^3处于相对于所述羰基的间位，特别是其中所述含有 X 的环为苯基时，或者其中R^3处于相对于所述羰基的对位，特别是其中所述含有 X 的环为杂环时.

12. 权利要求 6 的化合物，其中-$(CH_2)_n$-和/或-$(CH_2)_n$-不存在，即 p

和/或 n 为 0.

13. 权利要求 6 的化合物，其中 R^x 为氰基.

14. 权利要求 6 的化合物，其中 R^1 为氟代.

15. 权利要求 6 的化合物，其中 R^2 为乙基.

16. 权利要求 6 的化合物，包括至少 60%，优选至少 90%的 1S,2S 对映体形式.

17. 权利要求 1 的化合物选自(1S,2S)-N-[顺式-2-(6-氟,2-羟基,3-丙酰基苯基)-环丙基]-N'-(5-氰基吡啶-2-基)-脲、(1R,2R)-N-[顺式-2-(6-氟,2-羟基,3-丙酰基苯基)-环丙基]-N'-(5-氰基吡啶-2-基)-脲、(1S,2S)-N-[顺式-2-(6-氟,2-羟基,3-丙酰基苯基)-环丙基]-N'-(5-溴吡啶-2-基)-脲和(1R,2R)-N-[顺式-2-(6-氟,2-羟基,3-丙酰基苯基)-环丙基]-N'-(5-溴吡啶-2-基)-脲和它们的药学上可接受的盐.

18. 权利要求 17 的化合物表示(1S,2S)-N-[顺式-2-(6-氟,2-羟基,3-丙酰基苯基)-环丙基]-N'-(5-氰基吡啶-2-基)-脲或它的药学上可接受的盐.

19. 权利要求 1 的化合物选自:

(1S,2S)-N-[顺式-2-(2-(3-氨基苯基羰基氧基)-6-氟-3-丙酰基苯基)-环丙基]-N'-(5-氰基吡啶-2-基)-脲,

(1S,2S)-N-[顺式-2-(2-(3-乙基氨基苯基羰基氧基)-6-氟-3-丙酰基苯基)-环丙基]-N'-(5-氰基吡啶-2-基)-脲,

(1S,2S)-N-[顺式-2-(2-(3-二甲基氨基苯基羰基氧基)-6-氟-3-丙酰基苯基)-环丙基]-N'-(5-氰基吡啶-2-基)-脲,

(1S,2S)-N-{顺式-2-[6-氟-3-丙酰基-2-(6-甲基氨基吡啶-3-基羰基氧基)苯基]-环丙基}-N'-(5-氰基吡啶-2-基)脲,

(1R,2R)-N-[顺式-2-(2-(3-氨基苯基羰基氧基)-6-氟-3-丙酰基苯基)-环丙基]-N'-(5-氰基吡啶-2-基)-脲,

(1R,2R)-N-[顺式-2-(2-(3-乙基氨基苯基羰基氧基)-6-氟-3-丙酰基苯基)-环丙基]-N'-(5-氰基吡啶-2-基)-脲,

(1R,2R)-N-[顺式-2-(2-(3-二甲基氨基苯基羰基氧基)-6-氟-3-丙酰基苯基)-环丙基]-N'-(5-氰基吡啶-2-基)-脲,

(1R,2R)-N-{顺式-2-[6-氟-3-丙酰基-2-(6-甲基氨基吡啶-3-基羰基氧基)苯基]-环丙基}-N'-(5-氰基吡啶-2-基)脲,

(1S,2S)-N-[顺式-2-(2-(3-氨基苯基羰基氧基)-6-氟-3-丙酰基苯基)-环丙基]-N'-(5-溴吡啶-2-基)-脲,

(1S,2S)-N-[顺式-2-(2-(3-乙基氨基苯基羰基氧基)-6-氟-3-丙酰基苯基)-环丙基]-N'-(5-溴吡啶-2-基)-脲,

(1S,2S)-N-[顺式-2-(2-(3-二甲基氨基苯基羰基氧基)-6-氟-3-丙酰基苯基)-环丙基]-N'-(5-溴吡啶-2-基)-脲,

(1S,2S)-N-{顺式-2-[6-氟-3-丙酰基-2-(6-甲基氨基吡啶-3-基羰基氧基)苯基]-环丙基}-N'-(5-溴吡啶-2-基)脲,

(1R,2R)-N-[顺式-2-(2-(3-氨基苯基羰基氧基)-6-氟-3-丙酰基苯基)-环丙基]-N'-(5-溴吡啶-2-基)-脲,

(1R,2R)-N-[顺式-2-(2-(3-乙基氨基苯基羰基氧基)-6-氟-3-丙酰基苯基)-环丙基]-N'-(5-溴吡啶-2-基)-脲,

(1R,2R)-N-[顺式-2-(2-(3-二甲基氨基苯基羰基氧基)-6-氟-3-丙酰基苯基)-环丙基]-N'-(5-溴吡啶-2-基)-脲,

(1R,2R)-N-{顺式-2-[6-氟-3-丙酰基-2-(6-甲基氨基吡啶-3-基羰基氧基)苯基]-环丙基}-N'-(5-溴吡啶-2-基)脲,

和它们的药学上可接受的盐.

20. 权利要求 19 的化合物表示(1S,2S)-N-{顺式-2-[6-氟-3-丙酰基-2-(6-甲基氨基吡啶-3-基羰基氧基)苯基]-环丙基}-N'-(5-氰基吡啶-2-基)脲和它的药学上可接受的盐.

21. 权利要求 19 的化合物表示(1S,2S)-N-[顺式-2-(2-(3-氨基苯基羰基氧基)-6-氟-3-丙酰基苯基)-环丙基]-N'-(5-溴吡啶-2-基)脲和它的药学上可接受的盐.

22. 药用组合物, 包括权利要求 1 至 21 中的任何一项定义的化合

物和为此药学上可接受的载体或稀释剂.

23. 权利要求 22 的组合物另外包括一种至三种另外的抗病毒药物.

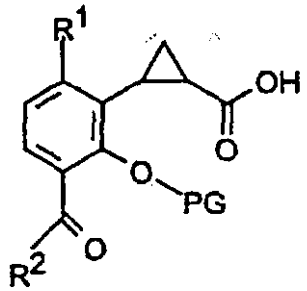
24. 权利要求 23 的组合物，其中所述另外的抗病毒药物选自 AZT、ddI、ddC、D4T、3TC、adefovir、adefovir dipivoxil、abacavir、双-POC-PMPA、膦甲酸、羟基脲、efavirenz、trovirdine、奈韦拉平、delavirdine、PFA、H2G、ABT606、ritonavir、沙奎那韦、indinavir、amprenavir(Vertex VX478)、Mitsubishi MKC-442 和 nelfinavir.

25. 权利要求 1 至 21 中任何一项的化合物用于治疗.

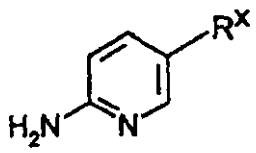
26. 权利要求 1 至 21 中任何一项的化合物用于制备用于治疗或预防 HIV 的药物.

27. 用于抑制或预防 HIV 感染的方法，包括将有效量的权利要求 1 至 6 中定义的化合物给予需要它们的患者.

28. 制备式 I 化合物的方法，包括下式化合物的叠氮化物的库尔提斯重排:



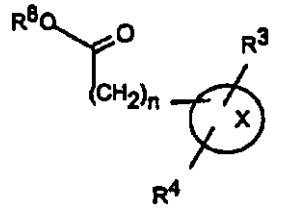
随后与下式化合物偶合和脱除保护:



其中 R^1、R^2 和 R^x 如上定义并且 PG 为羟基保护基团.

29. 权利要求 28 的方法还包括以下步骤:

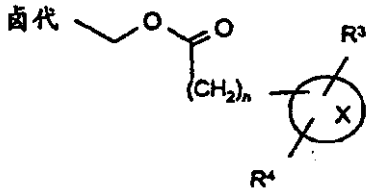
a) 以活化的式 III 化合物酰化:



III

其中 R^3、R^4、X 和 n 如上定义但任选被保护，并且 R^8 为氢或常规活化基团；或者

b) 以式 IIIa 化合物烷基化:



IIIa

其中 n、R^3、R^4 和 X 如上定义，但其中暴露的胺、羟基等取代基以常规保护基团保护.

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说　明　书

抗病毒药物

技术领域

本发明涉及抗病毒药物领域并且特别涉及HIV逆转录酶抑制剂．本发明提供新的化合物、含有这些化合物的药用组合物和使用它们用于抑制HIV的方法．

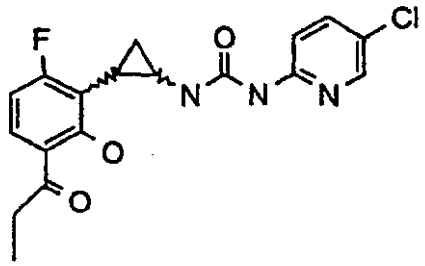
发明背景

在HIV的治疗中，在HIV逆转录酶抑制方面已经显示出临床相应活性的药物当中，大多数为核苷类似物例如AZT、ddI、ddC和D4T．这些核苷类似物并不是象所要求的那样是特异性的，因此不得不以相对高的剂量水平给药．在这些剂量水平下，核苷类似物引起相当的毒性，限制了它们的长期使用．

为克服这些特异性和毒性问题，已开发多种所述HIV逆转录酶的非核苷类抑制剂．例如来自杨森公司的逆转录酶抑制剂TIBO在纳摩尔浓度下抑制HIV且未呈现出临床显著的毒性．TIBO和所述非核苷类逆转录酶抑制剂奈韦拉平两者迅速进行患者II期临床试验．然而，不久变得明显的是这些非核苷类抑制剂体内迅速选择出其对抗通常剂量的所述各自抑制剂的HIV突变型．例如在奈韦拉平情况中，仅在治疗四周后，患者血清中分离出的病毒与未治疗患者中分离出的病毒相比较对所述药物的敏感性低100倍(Drug Design & Discovery 1992 8 第255-263页)．对已进入临床试验的其它非核苷类RT抑制剂已出现了相似的模式，即当给予患者时，有体外活性的默克公司的L-697661和普强公司的delavirdine(U-87201)已迅速产生对抗HIV的突变型．尽管限于在尝试延缓抗药性的发展中的特定联合给药方案，具有上述缺点的奈韦拉平和delavirdine最近已经登记用于临床．

国际专利申请第 WO 95/06034 号描述一系列新的脲衍生物，其呈现良好的体外抗 HIV 逆转录酶活性并在细胞培养中呈现良好的抑制 HIV 复制作用．然而，在 WO 95/06034 中所述化合物的实际应用受到它们不佳的药代动力学表现的阻碍．此外，与许多非核苷类逆转录酶抑制剂一样，在 WO 95/06034 中的化合物在缓慢抗药性发展的关键参数上和由其它的抗病毒药方案产生的抗 HIV 突变型活性的良好模式上留有改进的余地．

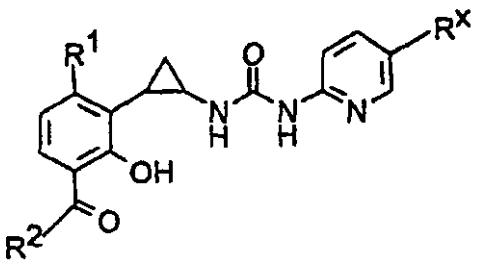
Öberg 等于 1995 年在 Santa Fe 举行的 ICAR 上用墙报特别公开了以上提及的 WO 95/06034 中并具有下式的外消旋化合物



此时，以上描述的化合物被看作比含有带甲氧基/乙酰基的苯环的硫脲的变体具有更小的意义．然而，与现有技术的具有良好的药代动力学性质和延长的病毒抗药性的时间的化合物相比较，我们现已发现另外的取代模式证实它们具有改善的抗药性模式．因此，本发明提供了将非核苷类抑制剂优越的特异性与所有现有技术抑制剂缺乏的临床实用性相结合的抑制剂．

发明简述

本发明提供式 I 化合物:



其中

R^1 为卤代;

R^2 为 C_1-C_3 烷基;

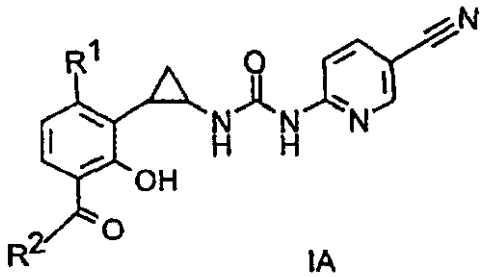
R^x 为氰基或溴代;

和它们的药学上可接受的盐和前药.

本发明另外提供包括式 I 化合物和为此药学上可接受的载体或稀释剂的药用组合物. 本发明另外的方面提供了抑制 HIV 的方法, 该方法包括将式 I 化合物给予 HIV 的患者. 本发明也扩展到治疗中式 I 化合物的用途, 例如在制备用于治疗 HIV 感染的药物中的用途.

在治疗 HIV 引起的疾病中, 所述式 I 化合物优选以达到大约 10 至 1000 nM 的血浆水平的量给药并且更优选为 100 至 500 nM. 这相应于依所述制剂的生物利用度而定的剂量比例为 0.01 至 10 mg/kg/天, 优选为 0.1 至 2 mg/kg/天. 正常成人的一般剂量比例将为每天大约 0.05 至 5 g, 优选为每天一至四个剂量单位, 0.1 至 2 g 例如 500-750 mg.

在权利要求 1 中特别关于药代动力学的优选的化合物子集具有所述结构 IA:



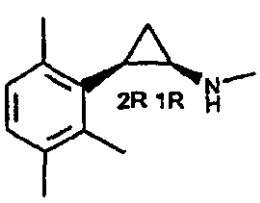
IA

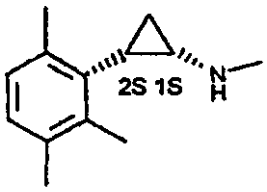
其中 R^1 和 R^2 如上定义, 包括它们的药学上可接受的盐和前药.

在式 I 中，特别易于形成前药的另一个有利的化合物子集包括其中 R^x 为溴代的化合物。

R^1 优选为氯代并且更优选为氟代。适宜的 R^2 基团包括甲基、异丙基、正丙基并且优选为乙基。

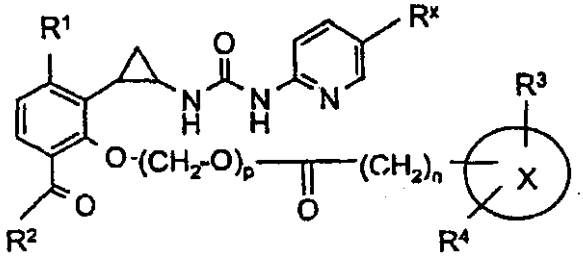
如上所述，所述环丙基环以顺式构型存在，允许存在两个对映体，1S,2S 和 1R,2R(分别和非常规指明在 SE 980016-7 和 SE 9800113-4 中为 2R,1S 和 2S,1R):





这些对映体每一个为有效的抗逆转录病毒药物，尽管所述不同的对映体在生理性质上能够表现出细微的差异。例如所述 1S,2S 和 1R,2R 对映体在所述 P450 系统中能显示出不同模式的代谢。其中 R^x 为氰基的化合物的 1S,2S 对映体为特别优选，因为其独一无二地呈现出有能力避免所述 P450 系统中的关键成分。其它的逆转录病毒药物例如所述 HIV 蛋白酶抑制剂 ritonavir 与所述 P450 系统广泛地相互作用，导致大量的不合乎需要的生理应答，其包括广泛改变其它联合给予药物的代谢。当患者期待多年(如果不是十年的话)服用多种药剂时，这与用于慢性感染所给予的药物特别相关。

式 I 化合物适宜的前药包括式 II 那些化合物:



II

其中

R^1、R^2和R^x如上定义，

R^3为 H、$(CH_m)_nNR^5R^6$;

R^4为 H、C_1-C_3烷基、$(CH_m)_nNR^5R^6$、$(CH_m)_nC(=O)R^5$、$(CH_m)_nOH$、OR^7、卤代、CF_3或 CN; 或者

R^3和R^4一起定义为具有 0-2 个杂原子和/或 0-2 个不饱和键和/或 0-2 个取代基的 5 或 6 元稠环;

R^5为 H、C_1-C_3烷基、$C(=O)R^7$或 1 至 4 个氨基酸的肽;

R^6为 H、C_1-C_3烷基; 或者

R^5和R^6一起定义为具有 0 或 1 个另外的杂原子和/或 0-2 个不饱和键和/或 0-2 个取代基的 5 或 6 元环;

R^7为 H、C_1-C_{12}烷基、$(CH_m)_nNR^5R^6$;

X 和包含其的环定义为具有 0 至 3 个不饱和键和/或 0 至 3 个选自 S、O 和 N 的杂原子的 5 或 6 元环;

m 独立为 1 或 2;

n 独立为 0、1 或 2;

和它们的药学上可接受的盐.

其中R^x为氯代的化合物的相应前药形成本发明的另一个方面.

在下文指的是 X-环的含有 X 的环结构可为饱和的或具有 1-3 个不饱和键，包括具有芳香特征的环. 优选的 X-环包括环己环或环己烯环或者更优选为苯环. 其它优选的 X-环包括吗啉代或者更优选为吡啶环. 或者，X-环可定义为五元环例如戊烯基或吡咯基.

在R^3和R^4结合形成含有任选杂原子的环的情况中，对 X-环适宜的稠合环系统包括萘基、喹啉基、四氢异喹啉基、吲哚基或苯并咪唑环系统. 在R^4和R^5结合形成环的情况中，对 X-环的适宜的取代基环包括吗啉代和哌啶子基. 这些稠合的或取代基的环可以被卤代、卤代甲基、氨基如$(CH_m)_nNR^5R^6$、$C(=O)NR^5R^6$、羟基、羟基甲基、羧基、羧基甲基、C_{1-3}烷基、C_{1-3}烷氧基等任选取代.

X-环可与相邻的羰基部分通过亚甲基或亚乙基隔开，其可以被

取代基如卤代、卤代甲基、氨基、氨基甲基、羟基、羟基甲基、羧基、羧基甲基、C_{1-3}烷基、C_{1-3}烷氧基等任选取代。优选X-环与所述羰基相邻。

由X-环系统、R^3、R^4和如果存在的R^5-R^7表示的部分优选具有一点碱性。这能通过选择适宜的碱性杂环作为所述X-环，例如吡啶基或苯并吡啶基来达到。或者，R^3至R^7中的一个或多个可包含碱性取代基如伯、仲或叔胺、氨基酸等。

有利的R^3和/或R^4基团包括NH_2、$N(CH_2)_2$和NHC_{1-3}烷基如$NHCH_3$或$NHCH_2CH_3$。R^3优选处于相对于所述羰基和其任选的间隔基团的间位，尤其是当含有X的环为苯基或者当含有X的环为杂芳香环例如吡啶-3-基时，R^3处于对位。通常优选的p和/或n的值为零，即各自的基团不存在。

本发明优选化合物包括:

(1S,2S)-N-[顺式-2-(6-氟,2-羟基,3-丙酰基苯基)-环丙基]-N'-(5-氰基吡啶-2-基)-脲;

(1S,2S)-N-[顺式-2-(6-氟,2-羟基,3-丁酰基苯基)-环丙基]-N'-(5-氰基吡啶-2-基)-脲;

(1S,2S)-N-[顺式-2-(6-氟,2-羟基,3-乙酰基苯基)-环丙基]-N'-(5-氰基吡啶-2-基)-脲;

(1S,2S)-N-[顺式-2-(2-(3-氨基苯基羰基氧基)-6-氟-3-丙酰基苯基)-环丙基]-N'-(5-氰基吡啶-2-基)-脲;

(1S,2S)-N-[顺式-2-(2-(3-氨基苯基羰基氧基)-6-氟-3-丁酰基苯基)-环丙基]-N'-(5-氰基吡啶-2-基)-脲;

(1S,2S)-N-[顺式-2-(2-(3-氨基苯基羰基氧基)-6-氟-3-乙酰基苯基)-环丙基]-N'-(5-氰基吡啶-2-基)-脲;

(1S,2S)-N-[顺式-2-(2-(3-乙基氨基苯基羰基氧基)-6-氟-3-丙酰基苯基)-环丙基]-N'-(5-氰基吡啶-2-基)-脲;

(1S,2S)-N-[顺式-2-(2-(3-乙基氨基苯基羰基氧基)-6-氟-3-丁酰基苯

基)-环丙基]-N'-(5-氰基吡啶-2-基)-脲;
(1S,2S)-N-[顺式-2-(2-(3-乙基氨基苯基羰基氧基)-6-氟-3-乙酰基苯基)-环丙基]-N'-(5-氰基吡啶-2-基)-脲;
(1S,2S)-N-[顺式-2-(2-(3-二甲基氨基苯基羰基氧基)-6-氟-3-丙酰基苯基)-环丙基]-N'-(5-氰基吡啶-2-基)-脲;
(1S,2S)-N-[顺式-2-(2-(3-二甲基氨基苯基羰基氧基)-6-氟-3-丁酰基苯基)-环丙基]-N'-(5-氰基吡啶-2-基)-脲;
(1S,2S)-N-[顺式-2-(2-(3-二甲基氨基苯基羰基氧基)-6-氟-3-乙酰基苯基)-环丙基]-N'-(5-氰基吡啶-2-基)-脲;
和它们的药学上可接受的盐.

其它优选的化合物包括:
(1S,2S)-N-[顺式-2-(2-(6-甲基氨基吡啶-3-基羰基氧基)-6-氟-3-丙酰基苯基)-环丙基]-N'-(5-氰基吡啶-2-基)-脲;
(1S,2S)-N-[顺式-2-(2-(6-甲基氨基吡啶-3-基羰基氧基)-6-氟-3-丁酰基苯基)-环丙基]-N'-(5-氰基吡啶-2-基)-脲;
(1S,2S)-N-[顺式-2-(2-(6-甲基氨基吡啶-3-基羰基氧基)-6-氟-3-乙酰基苯基)-环丙基]-N'-(5-氰基吡啶-2-基)-脲;
(1S,2S)-N-[顺式-2-(2-(6-氨基吡啶-3-基羰基氧基)-6-氟-3-丙酰基苯基)-环丙基]-N'-(5-氰基吡啶-2-基)-脲;
(1S,2S)-N-[顺式-2-(2-(6-氨基吡啶-3-基羰基氧基)-6-氟-3-丁酰基苯基)-环丙基]-N'-(5-氰基吡啶-2-基)-脲;
(1S,2S)-N-[顺式-2-(2-(6-氨基吡啶-3-基羰基氧基)-6-氟-3-乙酰基苯基)-环丙基]-N'-(5-氰基吡啶-2-基)-脲;
和它们的药学上可接受的盐.

本发明其它合适的化合物包括:
(1R,2R)-N-[顺式-2-(6-氟,2-羟基,3-丙酰基苯基)-环丙基]-N'-(5-氰基吡啶-2-基)-脲;
(1R,2R)-N-[顺式-2-(6-氟,2-羟基,3-丁酰基苯基)-环丙基]-N'-(5-氰基

吡啶-2-基)-脲;

(1R,2R)-N-[顺式-2-(6-氟,2-羟基,3-乙酰基苯基)-环丙基]-N'-(5-氰基吡啶-2-基)-脲;

(1R,2R)-N-[顺式-2-(2-(3-氨基苯基羰基氧基)-6-氟-3-丙酰基苯基)-环丙基]-N'-(5-氰基吡啶-2-基)-脲;

(1R,2R)-N-[顺式-2-(2-(3-氨基苯基羰基氧基)-6-氟-3-丁酰基苯基)-环丙基]-N'-(5-氰基吡啶-2-基)-脲;

(1R,2R)-N-[顺式-2-(2-(3-氨基苯基羰基氧基)-6-氟-3-乙酰基苯基)-环丙基]-N'-(5-氰基吡啶-2-基)-脲;

(1R,2R)-N-[顺式-2-(2-(3-乙基氨基苯基羰基氧基)-6-氟-3-丙酰基苯基)-环丙基]-N'-(5-氰基吡啶-2-基)-脲;

(1R,2R)-N-[顺式-2-(2-(3-乙基氨基苯基羰基氧基)-6-氟-3-丁酰基苯基)-环丙基]-N'-(5-氰基吡啶-2-基)-脲;

(1R,2R)-N-[顺式-2-(2-(3-乙基氨基苯基羰基氧基)-6-氟-3-乙酰基苯基)-环丙基]-N'-(5-氰基吡啶-2-基)-脲;

(1R,2R)-N-[顺式-2-(2-(3-二甲基氨基苯基羰基氧基)-6-氟-3-丙酰基苯基)-环丙基]-N'-(5-氰基吡啶-2-基)-脲;

(1R,2R)-N-[顺式-2-(2-(3-二甲基氨基苯基羰基氧基)-6-氟-3-丁酰基苯基)-环丙基]-N'-(5-氰基吡啶-2-基)-脲;

(1R,2R)-N-[顺式-2-(2-(3-二甲基氨基苯基羰基氧基)-6-氟-3-乙酰基苯基)-环丙基]-N'-(5-氰基吡啶-2-基)-脲;

和它们的药学上可接受的盐.

其它合适的化合物包括:

(1R,2R)-N-[顺式-2-(2-(6-甲基氨基吡啶-3-基羰基氧基)-6-氟-3-丙酰基苯基)-环丙基]-N'-(5-氰基吡啶-2-基)-脲;

(1R,2R)-N-[顺式-2-(2-(6-甲基氨基吡啶-3-基羰基氧基)-6-氟-3-丁酰基苯基)-环丙基]-N'-(5-氰基吡啶-2-基)-脲;

(1R,2R)-N-[顺式-2-(2-(6-甲基氨基吡啶-3-基羰基氧基)-6-氟-3-乙酰基苯基)-环丙基]-N'-(5-氰基吡啶-2-基)-脲;
(1R,2R)-N-[顺式-2-(2-(6-氨基吡啶-3-基羰基氧基)-6-氟-3-丙酰基苯基)-环丙基]-N'-(5-氰基吡啶-2-基)-脲;
(1R,2R)-N-[顺式-2-(2-(6-氨基吡啶-3-基羰基氧基)-6-氟-3-丁酰基苯基)-环丙基]-N'-(5-氰基吡啶-2-基)-脲;
(1R,2R)-N-[顺式-2-(2-(6-氨基吡啶-3-基羰基氧基)-6-氟-3-乙酰基苯基)-环丙基]-N'-(5-氰基吡啶-2-基)-脲;
和它们的药学上可接受的盐.

本发明优选的化合物包括:

(1S,2S)-N-[顺式-2-(2-(6-氟,2-羟基,3-丙酰基苯基)-环丙基]-N'-(5-溴代吡啶-2-基)-脲;
(1S,2S)-N-[顺式-2-(2-(3-氨基苯基羰基氧基)-6-氟-3-丙酰基苯基)-环丙基]-N'-(5-溴吡啶-2-基)-脲;
(1S,2S)-N-[顺式-2-(2-(3-氨基苯基羰基氧基)-6-氟-3-乙酰基苯基)-环丙基]-N'-(5-溴吡啶-2-基)-脲;
(1S,2S)-N-[顺式-2-(2-(3-氨基苯基羰基氧基)-6-氟-3-丁酰基苯基)-环丙基]-N'-(5-溴吡啶-2-基)-脲;
(1S,2S)-N-[顺式-2-(2-(3-乙基氨基苯基羰基氧基)-6-氟-3-丙酰基苯基)-环丙基]-N'-(5-溴吡啶-2-基)-脲;
(1S,2S)-N-[顺式-2-(2-(3-乙基氨基苯基羰基氧基)-6-氟-3-乙酰基苯基)-环丙基]-N'-(5-溴吡啶-2-基)-脲;
(1S,2S)-N-[顺式-2-(2-(3-乙基氨基苯基羰基氧基)-6-氟-3-丁酰基苯基)-环丙基]-N'-(5-溴吡啶-2-基)-脲;
(1S,2S)-N-[顺式-2-(2-(3-二甲基氨基苯基羰基氧基)-6-氟-3-丙酰基苯基)-环丙基]-N'-(5-溴吡啶-2-基)-脲;
(1S,2S)-N-[顺式-2-(2-(3-二甲基氨基苯基羰基氧基)-6-氟-3-乙酰基苯基)-环丙基]-N'-(5-溴吡啶-2-基)-脲;

(1S,2S)-N-[顺式-2-(2-(3-二甲基氨基苯基羰基氧基)-6-氟-3-丁酰基苯基)-环丙基]-N'-(5-溴吡啶-2-基)-脲;
(1R,2R)-N-[顺式-2-(2-(6-氟,2-羟基,3-丙酰基苯基)-环丙基]-N'-(5-溴吡啶-2-基)-脲;
(1R,2R)-N-[顺式-2-(2-(3-氨基苯基羰基氧基)-6-氟-3-丙酰基苯基)-环丙基]-N'-(5-溴吡啶-2-基)-脲;
(1R,2R)-N-[顺式-2-(2-(3-氨基苯基羰基氧基)-6-氟-3-乙酰基苯基)-环丙基]-N'-(5-溴吡啶-2-基)-脲;
(1R,2R)-N-[顺式-2-(2-(3-氨基苯基羰基氧基)-6-氟-3-丁酰基苯基)-环丙基]-N'-(5-溴吡啶-2-基)-脲;
(1R,2R)-N-[顺式-2-(2-(3-乙基氨基苯基羰基氧基)-6-氟-3-丙酰基苯基)-环丙基]-N'-(5-溴吡啶-2-基)-脲;
(1R,2R)-N-[顺式-2-(2-(3-乙基氨基苯基羰基氧基)-6-氟-3-乙酰基苯基)-环丙基]-N'-(5-溴吡啶-2-基)-脲;
(1R,2R)-N-[顺式-2-(2-(3-乙基氨基苯基羰基氧基)-6-氟-3-丁酰基苯基)-环丙基]-N'-(5-溴吡啶-2-基)-脲;
(1R,2R)-N-[顺式-2-(2-(3-二甲基氨基苯基羰基氧基)-6-氟-3-丙酰基苯基)-环丙基]-N'-(5-溴吡啶-2-基)-脲;
(1R,2R)-N-[顺式-2-(2-(3-二甲基氨基苯基羰基氧基)-6-氟-3-乙酰基苯基)-环丙基]-N'-(5-溴吡啶-2-基)-脲;
(1R,2R)-N-[顺式-2-(2-(3-二甲基氨基苯基羰基氧基)-6-氟-3-丁酰基苯基)-环丙基]-N'-(5-溴吡啶-2-基)-脲;
和它们的药学上可接受的盐.

另外优选的化合物包括:
(1S,2S)-N-[顺式-2-(2-(6-甲基氨基吡啶-3-基羰基氧基)-6-氟-3-丙酰基苯基)-环丙基]-N'-(5-溴吡啶-2-基)-脲;
(1S,2S)-N-[顺式-2-(2-(6-甲基氨基吡啶-3-基羰基氧基)-6-氟-3-丁酰基

苯基)-环丙基]-N'-(5-溴吡啶-2-基)-脲;

(1S,2S)-N-[顺式-2-(2-(6-甲基氨基吡啶-3-基羰基氧基)-6-氟-3-乙酰基苯基)-环丙基]-N'-(5-溴吡啶-2-基)-脲;

(1S,2S)-N-[顺式-2-(2-(6-氨基吡啶-3-基羰基氧基)-6-氟-3-丙酰基苯基)-环丙基]-N'-(5-溴吡啶-2-基)-脲;

(1S,2S)-N-[顺式-2-(2-(6-氨基吡啶-3-基羰基氧基)-6-氟-3-丁酰基苯基)-环丙基]-N'-(5-溴吡啶-2-基)-脲;

(1S,2S)-N-[顺式-2-(2-(6-氨基吡啶-3-基羰基氧基)-6-氟-3-乙酰基苯基)-环丙基]-N'-(5-溴吡啶-2-基)-脲;

(1R,2R)-N-[顺式-2-(2-(6-甲基氨基吡啶-3-基羰基氧基)-6-氟-3-丙酰基苯基)-环丙基]-N'-(5-溴吡啶-2-基)-脲;

(1R,2R)-N-[顺式-2-(2-(6-甲基氨基吡啶-3-基羰基氧基)-6-氟-3-丁酰基苯基)-环丙基]-N'-(5-溴吡啶-2-基)-脲;

(1R,2R)-N-[顺式-2-(2-(6-甲基氨基吡啶-3-基羰基氧基)-6-氟-3-乙酰基苯基)-环丙基]-N'-(5-溴吡啶-2-基)-脲;

(1R,2R)-N-[顺式-2-(2-(6-氨基吡啶-3-基羰基氧基)-6-氟-3-丙酰基苯基)-环丙基]-N'-(5-溴吡啶-2-基)-脲;

(1R,2R)-N-[顺式-2-(2-(6-氨基吡啶-3-基羰基氧基)-6-氟-3-丁酰基苯基)-环丙基]-N'-(5-溴吡啶-2-基)-脲;

(1R,2R)-N-[顺式-2-(2-(6-氨基吡啶-3-基羰基氧基)-6-氟-3-乙酰基苯基)-环丙基]-N'-(5-溴吡啶-2-基)-脲;

和它们的药学上可接受的盐.

式 I 化合物的适宜的药学上可接受的盐包括有机羧酸盐，例如乙酸、乳酸、葡糖酸、枸橼酸、酒石酸、马来酸、苹果酸、泛酸、羟乙磺酸、草酸、乳糖酸和琥珀酸，有机磺酸例如甲磺酸、乙磺酸、苯磺酸、对氯苯磺酸和对甲苯磺酸的盐；并且包括无机酸例如盐酸、氢碘酸、硫酸、磷酸和氨基磺酸盐.

在保持采用 HIV 抑制剂通常实践中，共同给予一至三种另外的

抗病毒药物以提供协同应答和确保互补抗性模式是有利的．这样另外的抗病毒药可包括 AZT、ddI、ddC、D4T、3TC、abacavir、adefovir、adefovir dipivoxil、双-POC-PMPA、膦甲酸、羟基脲、赫斯特-拜耳 HBY097、efavirenz、trovirdine、奈韦拉平、delaviridine、PFA、H2G、ABT606、DMP-450、loviride、ritonavir、沙奎那韦、indinavir、amprenavir (Vertex VX478)、nelfinavir 等，一般的以反应它们各自的活性和生物利用度的摩尔比例给药．一般这样的比例相对于式 I 化合物为 25:1 至 1:25．

尽管对于所述活性剂而言可单独给药，但是其优选作为药用制剂的一部分存在．这样的制剂将包括与一种或多种可接受的载体一起的以上定义的活性剂并且任选包括其它的治疗成分．所述载体必须在与所述制剂的其它成分相容性方面是可接受的并且对接受者无害．

所述制剂包括那些适宜于口服、直肠、鼻、局部(包括颊和舌下)、阴道或非肠道(包括皮下、肌内、静脉和透皮)给药．所述制剂可便利地以单位剂型例如片剂和缓释胶囊剂形式存在，并且可通过任何药学领域熟知的方法进行制备．

这样的方法包括使以上定义的活性剂与所述载体混合在一起的步骤．所述制剂一般通过使活性剂与液体载体或粉末的固体载体或两者皆有之均匀地和紧密地混合在一起，然后如果必要，使所述产物成形来制备．

在本发明中用于口服的制剂可作为独立的单位如每一种含有预先确定量的所述活性剂的胶囊剂、扁囊剂或片剂，作为散剂或颗粒剂，作为所述活性剂在水溶性或非水溶性液体中的溶液剂或混悬剂或者作为水包油液体乳剂或油包水液体乳剂和作为大药丸(bolus)等呈现．

关于口服给药的组合物(例如片剂和胶囊剂)，术语适宜的载体包括媒介物例如普通的赋形剂，例如粘合剂如糖浆、阿拉伯胶、明胶、

山梨糖醇、黄蓍胶、聚乙烯吡咯烷酮(povidone)、甲基纤维素、乙基纤维素、羧甲基纤维素钠、羟基丙基甲基纤维素、蔗糖和淀粉；填充剂和载体如玉米淀粉、明胶、乳糖、蔗糖、微晶纤维素、高岭土、甘露糖醇、磷酸二钙、氯化钠和藻酸；和润滑剂如硬脂酸镁和其它的金属硬脂酸盐、硬脂酸、硅酮液、滑石粉、蜡类、油类和胶态二氧化硅．也能够使用矫味剂例如薄荷、冬青油、樱桃香精等．可要求加入着色剂以使所述剂型易于辨别．也可以本领域熟知的方法包衣片剂．

用于口服给药的合适的载体包括以溶液剂、混悬剂或乳剂形式存在的，任选以常规方法包囊化的或以单位剂型存在的液体制剂．有利的制剂包括阿拉伯胶/吐温/水、吐温/水、丙二醇、具有10-20%乙醇的植物油(如花生油、红花油、橄榄油等)、植物油/Capmul MGM、Capmul MCM/丙二醇、甲基纤维素/水、植物油/硬脂酰基单甘油酯、植物油/单不饱和脂肪酸甘油酯等．

片剂可通过任选与一种或多种辅助成分一起压制或模压来制备．通过在适宜的机械中，压制与粘合剂、润滑剂、惰性稀释剂、防腐剂、表面活性剂或分散剂任选混合的，以自由流动的形式如粉末或颗粒存在的所述活性剂可制备压制片剂．通过在适宜的机械中，模压用惰性液体稀释剂湿润的粉末状化合物的混合物可制备模压片剂．所述片剂可任选被包衣或刻痕并且配制以便提供缓慢或控制释放的所述活性剂．

适宜用于局部给药的制剂包括含有在矫味的基质通常为蔗糖和阿拉伯胶或黄蓍胶中的所述活性剂的糖锭剂、在惰性基质例如明胶和甘油或蔗糖和阿拉伯胶中的所述活性剂的锭剂和含有在适宜的液体载体中的所述活性剂的漱口剂．

适宜用于皮肤局部给药的制剂包括可作为含有活性剂和药学上活性载体的软膏剂、霜剂、凝胶剂和糊剂呈现．举例说明的局部传递系统为含有所述活性剂的透皮贴剂．其它的局部制剂包括防腐药

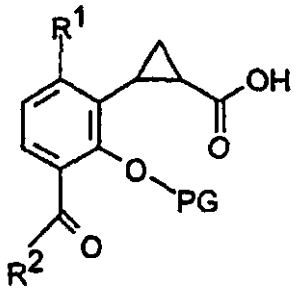
签，其在例如注射器或毛细管采血样的侵袭性过程之前释放所述活性剂到皮肤上．这样的药签中和来自所述侵袭性过程流出的血液或血清中的 HIV，由此帮助防止 HIV 经针头意外转移至健康的护理工作者．这样的药签可包括浸泡在挥发性溶剂例如乙醇中的所述活性剂溶液中的灭菌手术纱布垫并且单个包装在密封的香囊中．

用于直肠或阴道给药的制剂可作为具有包括例如可可豆脂或水杨酸盐的适宜的基质的栓剂和阴道栓．其它的阴道制剂能够作为棉塞剂、霜剂、凝胶剂、糊剂、泡沫剂或喷雾剂呈现，它们含有除了所述活性剂以外的作为在本领域是已知的适当的载体．

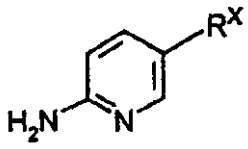
适宜用于鼻给药的其中所述载体为固体的制剂包括具有粒子体积例如在所述范围 20 至 500 微米的粗粉末，该粉末以其中服用鼻吸药的方式给药，即通过从举至贴近鼻子的所述粉末容器迅速吸入给药．适宜用于例如作为鼻喷雾剂或者作为鼻滴剂给药的其中所述载体为液体的制剂包括所述活性剂的水或油溶液．

适宜用于非肠道给药的制剂包括可含有抗氧化剂、缓冲液、杀菌剂和使所述制剂与所打算的接受者的血液等渗的溶质的水和非水灭菌注射溶液剂和其可包括悬浮剂和增稠剂的水和非水灭菌混悬剂．所述制剂可以单位-剂量或多-剂量容器例如密封安瓿和小瓶呈现，并且可在冻干(冷冻干燥)条件下贮存，使用前仅需要立即加入所述灭菌液体载体例如注射用水．可由先前描述的那种灭菌散剂、颗粒剂和片剂制备临时的注射溶液和悬浮液剂．

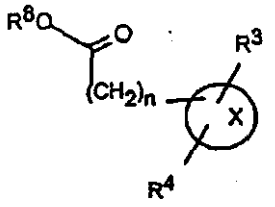
本发明另一方面提供用于制备式 I 化合物尤其是所述顺式异构体的方法，该方法包括下式化合物的库尔提斯重排:



随后通过使下式化合物偶合和脱除保护，其中 R^1、R^2 和 R^x 如上定义并且 PG 为羟基保护基团:

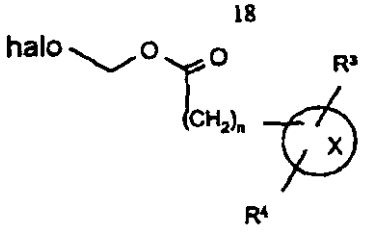


本发明所述方法另外包括用活化的式 III 化合物酰化的步骤:



III

其中 R^3、R^4、X 和 n 如上定义但任选被保护，并且 R^8 为氢或常规活化基团. 或者本发明所述方法可另外包括用式 IIIa 化合物烷基化的步骤:

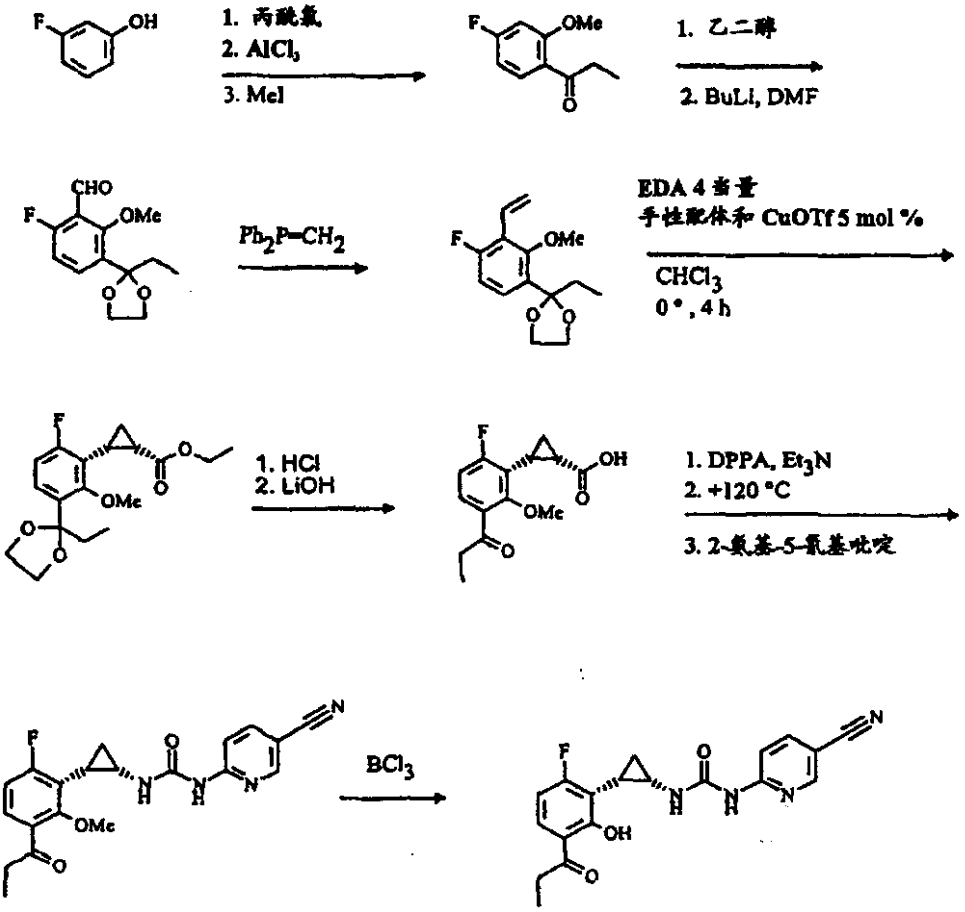


IIIa

其中 n、R^3、R^4 和 X 如上定义，但暴露的胺、羟基等取代基用常规

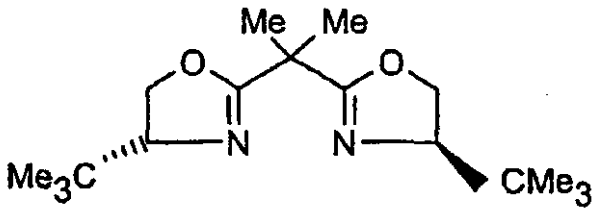
保护基团保护.

因此，式I对映体化合物可通过以下反应流程制备:



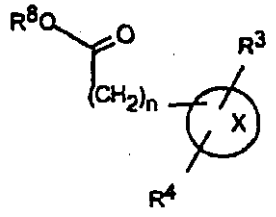
以上流程阐明其中R^x为氰基、R^1为F和R^2为乙基的本发明(1S,2S)化合物的制备，但是相应的方法适合于其它的R^x、R^1和R^2的变体.

适用于第四步骤的所述手性配体可包括例如下式化合物:



为制备 1R,2R 对映体，使用镜像手性配体．或者为形成外消旋体而省去所述手性配体．

其中 p 为 0 的式 II 的前药能够通过用活化的式 III 化合物酰化式 I 化合物来合成，



III

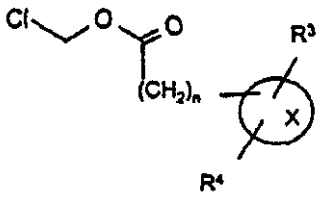
其中 R^3、R^4、X 和 n 如上定义但是任选被保护，并且 R^8 为氢或常规活化基团．

活化的式 III 化合物包括在偶合剂例如二环己基-碳二亚胺存在下的酰卤、酸酐、活化酸的酯或者所述酸．代表性的活化酸衍生物包括酰氯、甲酸和乙酸衍生的混合酸酐、衍生于烷氧基羰基卤例如异丁氧基羰基氯等的酸酐、N-羟基琥珀酰胺衍生的酯、N-羟基苯邻二甲酰亚胺衍生的酯、N-羟基-5-降冰片烯-2,3-二甲酰胺衍生的酯、2,4,5-三氯苯酚衍生的酯等．用于式 III 化合物特别是任何取代基的胺的适宜的任选保护基团包括那些打算保护氨基酸或肽的 N-末端或者打算保护氨基基团以在合成过程中对抗不合乎需要的反应的基团．常用的 N 保护基团在 Greene 的“在有机合成中的保护基团”(John Wiley & Sons, New York, 1981)中公开，其通过引用结合到本文中．N-保护基团包括酰基基团例如甲酰基、乙酰基、丙酰基、三甲基乙酰基、叔丁基乙酰基、2-氯乙酰基、2-溴乙酰基、三氟乙酰基、三氯乙酰基、邻苯二甲酰基、邻硝基苯氧基乙酰基、α-氯丁酰基、苯甲酰基、4-氯苯甲酰基、4-溴苯甲酰基、4-硝基苯甲酰基等；磺酰基例如苯磺酰基、对甲苯磺酰基等，氨基甲酸酯形成基团例如苄氧基羰基、对氯苄氧基羰基、对甲氧基苄氧基羰基、对硝基苄氧基羰基、2-硝基苄氧基羰基、对溴苄氧基羰基、3,4-二甲氧基苄氧基羰基、4-甲氧基苄氧

基羰基、2-硝基-4,5-二甲氧基苄氧基羰基、3,4,5-三甲氧基苄氧基羰基、1-(对联苯基)-1-甲基乙氧基羰基、α,α-二甲基-3,5-二甲氧基苄氧基羰基、二苯甲氧基羰基、叔丁氧基羰基、二异丙基甲氧基羰基、异丙氧基羰基、乙氧基羰基、甲氧基羰基、烯丙氧基羰基、2,2,2-三氯乙氧基羰基、苯氧基羰基、4-硝基苯氧基羰基、芴基-9-甲氧基羰基、环戊氧基羰基、金刚烷氧基羰基、环己氧基羰基、苯基硫代羰基等；烷基例如苄基、三苯基甲基、苄氧基甲基等；和甲硅烷基例如三甲基甲硅烷基等. 有利的 N-保护基团包括甲酰基、乙酰基、苯甲酰基、三甲基乙酰基、叔丁基乙酰基、苯磺酰基、苄基、叔丁氧基羰基(BOC)和苄氧基羰基(Cbz).

所述酰化反应用常规酯化条件例如 DMAP 和 DCC 在溶剂例如二甲基甲酰胺或吡啶中进行. 任选保护基团可以用如在以上 Greene 中综合讨论的常规技术除去，例如 TFA、HCl(aq)/二氧六环或在催化剂存在下氢化，得到式 II 化合物.

其中 p 为 1 的式 II 化合物能够通过如下方法制备：在常规烷基化条件下，使式 III 化合物与碘氯甲烷或者混合的二氯甲烷/碘氯甲烷反应，形成式 IIIa 化合物:



IIIa

其中 n、R^3、R^4和 X 如上定义，但是暴露的胺、羟基等取代基用常规保护基团保护. 然后，一般在碱性条件下，例如含有氢化钠的有机溶剂中，式 IIIa 化合物通过与 NaI 反应优选转化为相应的碘代衍生物随后与式 I 化合物偶合.

详细描述

本发明的这些方面现在将通过实施例阐明，仅参照以下非限制性实施例和附图，其中；

图 1 描述如同在生物实施例 2 中介绍的本发明化合物与现有技术化合物相比较的抗药性发展速率与时间的关系.

图 2 描述如同在生物实施例 5 中介绍的本发明化合物或者现有技术化合物口服给予大鼠后的时间与血浆水平的关系.

图 3 描述以如同在生物实施例 10 中介绍的如表面细胞质基因组共振方法测定的本发明化合物与现有技术化合物相比较的逆转录酶的结合动力学.

中间体的制备

实施例 1

3-[1,1-(亚乙二氧基)丙基]-6-氟-2-甲氧基苯甲醛

在室温下，于 5 分钟内，向 3-氟苯酚(22.4 g, 0.2 mol)、吡啶(24 ml, 0.3 mol)和二氯甲烷(200 ml)的溶液中加入 20 ml(0.225 mol)丙酰氯. 该反应放热. 将所述溶液搅拌另外 30 分钟. 加入二氯甲烷后，以饱和 $NaHCO_3$ 溶液和水洗涤所述有机相，经 $MgSO_4$ 干燥并真空浓缩. 得到 33.8 g(100%)3-氟-1-丙酰氧基苯. 在 150℃下，使该化合物与 33.3 g(0.25 mol)$AlCl_3$ 反应 10 分钟. 在小心以水骤冷后，所述反应混合物以乙醚提取三次. 干燥($MgSO_4$)所述醚相并蒸发，得到 29.5 g(0.176 mol, 88%)重排产物. 将该中间体溶于 200 ml 丙酮和 K_2CO_3(42, 0.3 mol)中并加入 MeI(25 ml, 0.4 mol). 在 40℃下，将所述反应混合物加热 12 小时. 过滤反应混合物并蒸发丙酮. 将残余物溶于乙醚并以 0.5 M NaOH 溶液和水洗涤所述醚相. 干燥($MgSO_4$)并蒸发，得到 31.2 g(0.17 mol, 三步收率 86%)的 4-氟-2-甲氧基苯基乙基酮.

向 4-氟-2-甲氧基苯基乙基酮(31.2 g, 0.171 mol)、乙二醇(10.5 ml, 0.188 mol)在苯(300 ml)的溶液中加入 1 g 对甲苯磺酸. 在迪安-斯达克

装置中将所述反应混合物回流大约 12 小时. 在冷却后, 以 1 M NaOH 溶液洗涤所述有机相几次并干燥(Na_2SO_4 和 K_2CO_3). 蒸发溶剂并得到大约 38 g 缩醛. 根据毛细管 GC 其纯度为 88%和杂质基本上为未反应的酮. 在-65℃和在氮气氛下, 向所述缩醛在 THF(450 ml)的溶液中滴加入 128 ml(0.32 mol)的 2.5 M 正丁基锂. 在保持所述温度在大约-65℃的同时, 加入 DMF(25 ml, 0.32 mol)在 THF(50 ml)中的溶液. 使所述反应混合物缓慢达到室温并根据 GC 在大约 30 分钟后未留下起始原料. 在另一个 1 小时后, 所述反应混合物以饱和 NH_4Cl 溶液骤冷并以乙醚提取三次. 干燥(Na_2SO_4)后在硅胶柱(来自默克硅胶 60, 粒度 0.04-0.063 mm)上纯化所述残余物, 以 EtOAc 1 和己烷 9 洗脱, 得到 10 g (25%)标题化合物.

^{1}H NMR($CDCl_3$) δ 0.85 (t, 3H), 2.1 (q, 2H), 3.8-3.95 (m, 2H), 3.97 (s, 3H), 4.0-4.15 (m, 2H), 6.9 (t, 1H), 7.7-7.8 (m, 1H), 10.4 (s, 1H).

实施例 2

3-[1,1-(亚乙二氧基)丙基]-6-氟-2-甲氧基苯乙烯

在室温下和在氮气氛中, 向甲基三苯基溴化鏻(14.3 g, 40 mmol)在 THF(250 ml)的悬浮液中加入 16 ml(40 mmol)的 2.5 M 正丁基锂. 然后向得到的溶液中加入在 THF(30 ml)中的 3-[1,1-(亚乙二氧基)丙基]-6-氟-2-甲氧基苯甲醛(10 g, 39.5 mmol). 然后在室温下将所述反应混合物搅拌 2 小时并倾入到己烷和盐水的混合物中. 所述有机相以盐水洗涤两次和以水洗涤一次. 蒸发溶剂后, 通过以氧化铝(来自默克的氧化铝 90 acc. Brockmann)填充的漏斗过滤所述残余物并以 EtOAc 1 和己烷 9 洗脱, 以便于除去所形成的三苯基氧化鏻. 蒸发有机溶剂得到残余物, 最后将其在硅胶柱上纯化, 以 EtOAc 1 和己烷 9 洗脱, 得到 6.9 g (70%)如同通过毛细管 GC 测定的具有 94.5%纯度的标题化合物.

^{1}H NMR(250 MHz, $CDCl_3$) δ 0.85 (t, 3H), 2.1 (q, 2H), 3.8 (s, 3H), 3.8-

3.95 (m, 2H), 4.0-4.1 (m, 2H), 5.55-5.65 (m, 1H), 5.95-6.05 (m, 1H), 6.7-6.85 (m, 2H), 7.3-7.4 (m, 1H).

实施例 3

(1S,2R)-顺式-2-(6-氟-2-甲氧基-3-丙酰基苯基)环丙基羧酸

如同 Evans 等在 *J. Am. Chem. Soc.* 1991, *113*, 726-728 中通常描述的那样，使用通过三氟甲磺酸铜(I)(679 mg, 1.35 mmol)和手性配体([2,2'-亚异丙基双((4R)-4-叔丁基-2-噁唑啉)](794 mg, 2.7 mmol)催化的不对称环丙烷化反应，由 3-[1,1-(亚乙二氧基)丙基]-6-氟-2-甲氧基苯乙烯(19.4 g, 69 mmol)和重氮基乙酸乙酯(29 ml, 275 mmol)制备(1S,2R)-顺式-2-[3-(1,1-亚乙二氧基)乙基-6-氟(2-甲氧基-苯基)环丙基羧酸乙酯．在硅胶层析法后，得到 9.4 g(40.5%)所述乙酯．通过在手性柱上的 HPLC 测定，对映体过量为 99%．将所述酯溶于 150 ml 二氧六环中并且加入 30 ml 的 6 M HCl．将所述反应混合物搅拌过夜并在乙醚和盐水之间分配．蒸发溶剂，得到 19 g 粗品．将所述产物溶于甲醇(250 ml)和水(75 ml)中并加入 6 g(250 mmol)的 LiOH．将所述反应混合物加热至 90℃反应 24 小时并且蒸发大部分溶剂．将残留的混合物酸化并以二氯甲烷提取三次．蒸发溶剂，得到 11.2 g 的标题化合物．

^{1}H NMR(250 MHz, $CDCl_3$) δ 1.15 (t, 3H), 1.59 (t, 2H), 2.10-2.17 (m, 1H), 2.22-2.32 (m, 1H), 2.91 (q, 2H), 3.80 (st, 3H), 6.82 (t, 1H), 7.44-7.50 (m, 1H), 11.30 (宽 s, 1H).

实施例 4

(1R,2S)-顺式-2-(6-氟-2-甲氧基-3-丙酰基苯基)环丙基羧酸

如同对实施例 3 中所述酸描述的那样，由 3-[1,1-(亚乙二氧基)丙基]-6-氟-2-甲氧基苯乙烯制备该化合物．所使用的手性配体为 2,2'-亚异丙基双((4S)-4-叔丁基-2-噁唑啉).

^{1}H NMR(250 Mhz, $CDCl_3$) δ 7.48 (q, 1H), 6.84 (t, 1H), 3.82 (s, 3H), 2.93 (q, 2H), 2.29 (q, 1H), 2.14 (q, 1H), 1.60 (m, 2H), 1.16 (t, 3H).

制备式 I 和 II 化合物

实施例 5

(±)N-[顺式-2-(2-(6-氟-2-羟基-3-丙酰基苯基)-环丙基]-N'-(5-氰基吡啶-2-基)-脲

在氮气氛下，将 3-[1,1-(亚乙二氧基)丙基]-6-氟-2-甲氧基苯乙烯(32.4 g, 实施例 2)和溴化铜-二甲硫复合物(0.30 g)在二氯乙烷(200 ml)中的溶液加热至 80℃. 在 7 小时内加入在二氯乙烷(600 ml)中的重氮基乙酸乙酯(54 ml). 加入完成后，结束加热. 在 16 小时后，蒸发溶剂并在硅胶上纯化所述残余物，以乙酸乙酯和己烷洗脱，得到顺式-酯(6.5 g).

将所述顺式-酯(3.7 g, 10.9 mmol)溶于乙醇(20 ml)中并将 KOH (1.8 g, 32.7 mmol)溶于水(10 ml)中. 合并所述溶液并加热至回流 3 小时. 加入水(30 ml)并以己烷(20 ml)洗涤所述溶液两次. 在冰浴上冷却水相并以稀 HCl 酸化. 以甲苯提取所述溶液三次. 干燥($MgSO_4$)甲苯相并蒸发，得到 1.9 g 的(±)-顺式-2-[3-(1,1-亚乙二氧基丙基)-6-氟-2-甲氧基苯基]环丙基羧酸.

将三乙胺(59 μl, 0.43 mmol)和二苯基磷酰基叠氮化物(92 μl, 0.43 mmol)加入到所述酸(120 mg, 0.39 mmol)在干燥甲苯的溶液中. 在室温下，将所述溶液搅拌 1 小时，然后加热至 120℃. 在 1 小时后，加入 2-氨基-5-氰基吡啶(51 mg, 0.43 mmol). 维持加热另外 3 小时. 在 16 小时后，蒸发溶剂，所述残余物溶于二氯甲烷(30 ml)中并以稀 HCl 洗涤，干燥($MgSO_4$)并蒸发，得到 152 mg. 将所述产物溶于二氧六环中并加入 HCl(6 N, 1 ml). 在 2 小时后，蒸发所述混合物，使其溶于二氯甲烷(25 ml)中，以水(10+10 ml)洗涤，干燥($MgSO_4$)并蒸发，得到 117 mg. 所述残余物在硅胶上纯化，以乙酸乙酯和己烷洗脱，得

到 37 mg 的 2-甲氧基苯基中间体产物.

在-60℃下，将三溴化硼在二氯甲烷中的 1 M 溶液(194 μl, 0.194 mmol)加入到所述 2-甲氧基苯基中间体(37 mg, 0.097 mmol)在二氯甲烷中的溶液中. 在 10 分钟后，撤除冷却浴并继续搅拌 2 小时. 以二氯甲烷稀释所述溶液，以稀 $NaHCO_3$ 和水洗涤，干燥($MgSO_4$)并蒸发. 所述残余物由 MeCN 重结晶，得到 17 mg 标题化合物.

^{1}H NMR(250 MHz, DMSO-d_6) δ 1.07-1.16 (m, 4H), 1.41-1.50 (m, 1H), 1.91-2.01 (m, 1H), 3.06-3.19 (m, 3H), 6.86 (dd, 1H), 7.43 (d, 1H), 7.80-7.90 (m, 1H), 7.97-8.08 (m, 2H), 8.32 (d, 1H), 9.83 (s, 1H), 13.2 (d, 1H).

实施例 6

(1R,2R)-N-(顺式-2-(6-氟-2-羟基-3-丙酰基苯基)-环丙基)-N'-(5-氰基吡啶-2-基)-脲

将三乙胺(0.85 ml, 6.1 mmol)和二苯基磷酰基叠氮化物(1.72 g, 6.1 mmol)加入到在实施例 4 中制备的所述酸(1.47 g, 5.5 mmol)在干燥甲苯(15 mL)中的溶液中. 在室温下，于氩气氛中将所述溶液搅拌 30 分钟，然后加热至 120℃. 在 15 分钟后，加入 2-氨基-5-氰基吡啶(0.99 g, 8.9 mmol)在 DMF(3 mL)中的溶液. 继续加热 4 小时. 蒸发甲苯，所述混合物以乙醚(100 mL)和乙酸乙酯(50 mL)稀释并以 1 M HCl、H_2O 和盐水洗涤. 干燥(Na_2SO_4)所述有机层并浓缩. 所述残余物以硅胶快速柱层析法纯化，以乙酸乙酯/正己烷 1:10 至 1:1 洗脱，得到 1.6 g (66%) 所述 2-甲氧基苯基中间体.

在-72℃下，于氩气氛中将三氯化硼在 CH_2Cl_2 中的 1 M 溶液(11.0 mL, 11.0 mmol)加入到所述 2-甲氧基苯基中间体(1.40 g, 3.66 mmol)在 CH_2Cl_2(80 mL)中的溶液中. 在 10 分钟后，撤除冷却浴并继续搅拌 1 小时 15 分钟. 所述溶液以 CH_2Cl_2 稀释并以 $NaHCO_3$ 水溶液、H_2O 和盐水洗涤. 干燥($NaSO_4$)有机层并浓缩. 来自乙腈/H_2O 1:1 的沉淀给出 0.62 g 纯的标题化合物. 将其残余物浓缩并通过以乙酸乙酯/正己

烷 1:10 至 1:1 和乙酸乙酯洗脱的层析法，然后从乙腈结晶，给出 0.2 g 标题产物．其产量为 0.82 g(61%)．如同通过在手性柱上的 HPLC 测定的那样，其 ee 为 95%．$[\alpha]_d^{22}$ –171.2° (c=0.50, CH_2Cl_2)．

^{1}H NMR(250 Mhz, $CDCl_3$) δ 13.35 (d, 1H), 10.02 (br s, 1H), 9.40 (br s, 1H), 8.11 (s, 1H), 7.71 (m, 2H), 7.00 (m, 1H), 6.61 (t, 1H), 3.21 (m, 1H), 3.01 (q, 2H), 2.03 (m, 1H), 1.55 (m, 1H), 1.29 (m, 4H).

实施例 7

(1R,2R)-N-[顺式-2-(2-(3-氨基苯基羰基氧基)-6-氟-3-丙酰基苯基)环丙基]-N'-(5-氰基吡啶-2-基)-脲

在室温下，于氩气氛中向在实施例 6 中描述的化合物(1.64 g, 4.4 mmol)、BOC-保护的 3-氨基苯甲酸(1.6 g, 6.6 mmol)和 4-二甲基氨基吡啶(269 mg, 2.2 mmol)在 20 ml 二氯甲烷和 10 ml 的 DMF 中的溶液中加入 1.36 g(6.6 mmol)DCC．将所述反应混合物搅拌 24 小时．小心蒸发溶剂并使用作为溶剂的己烷/乙酸乙酯 1:1，在硅胶上纯化残余物，得到 2.6 g 的 BOC-保护的标题产物．在 0℃下，将所述产物加入到 75 ml 三氟乙酸中．然后在 0℃下将所述混合物搅拌 1 小时．小心真空除去溶剂．在乙酸乙酯和饱和碳酸钾之间分配所述残余物．干燥所述有机相并蒸发．使用作为洗脱剂的乙酸乙酯/己烷 4:1，在硅胶柱上纯化残余物，得到 1.03 g 为游离碱的标题化合物．以在乙醚中的 3 ml 的 1 M HCl 处理该中间体并得到标题化合物 0.84 g．HPLC 纯度为大约 97%．

^{1}H NMR 释出的胺(250 MHz, $CDCl_3$) δ 1.09 (t, 3H), 1.2-1.3 (m, 1H), 1.4-1.5 (m, 1H), 1.95-2.00 (m, 1H), 2.83 (q, 2H), 3.15-3.25 (m, 1H), 3.85 (s, 2H), 6.90 (dd, 2H), 7.09 (t, 1H), 7.20-7.27 (m, 1H), 7.44-7.46 (m, 1H), 7.56 (dd, 1H), 7.65-7.77 (m, 2H), 8.13 (d, 1H), 9.1 (宽 s, 1H), 9.6 (宽 s, 1H).

实施例 8

(1S,2S)-N-(顺式-2-(6-氟-2-羟基-3-丙酰基苯基)-环丙基)-N'-(5-氰基吡啶-2-基)-脲

在氮气氛下,将三乙胺(670 μl, 4.8 mmol)和二苯基磷酰基叠氮化物(1.05 ml, 4.9 mmol)加入到在实施例 3 中制备的所述酸(1.2 g, 4.5 mmol)在干燥甲苯(10 ml)中的溶液中. 在室温下, 将所述溶液搅拌 30 分钟, 然后加热至 120℃. 在 15 分钟后, 加入 2-氨基-5-氰基吡啶(0.80 g, 6.7 mmol)在二甲基甲酰胺(1.5 ml)中的溶液并继续加热 4 小时. 所述溶液以乙醚稀释并以 1 M 盐酸洗涤. 干燥($MgSO_4$)所述有机层并浓缩. 残余物经硅胶快速层析法纯化(梯度洗脱, 开始以正己烷/乙酸乙酯 1:1, 以纯的乙酸乙酯完成), 得到稍微不纯的 2-甲氧基苯基衍生物(0.93 g). 如同以上描述的那样, 重复层析法, 得到所述纯的 2-甲氧基苯基衍生物(0.70 g, 41%).

在-60℃下, 将三氯化硼在二氯甲烷中的 1 M 溶液(5.5 ml, 5.5 mmol)加入到所述 2-甲氧基苯基中间体(700 mg, 1.8 mmol)在二氯甲烷中的溶液中. 在 10 分钟后, 撤除冷却浴并继续搅拌 2 小时. 所述溶液以二氯甲烷稀释并以碳酸氢钠水溶液洗涤. 干燥($MgSO_4$)所述有机层并浓缩, 将残余物通过硅胶快速层析法(梯度, 正己烷:乙酸乙酯 2:1、1:1、1:2, 乙酸乙酯:甲醇(8:1)纯化, 得到标题化合物(500 mg, 74%).

$[\alpha]_D^{22}$ +165.0° (C=0.5, CH_2Cl_2).

^1H NMR(DMSO-d_6) δ 1.10-1.16 (m, 4H, CH_3, CH_2-环丙基), 1.45 (dd, 1H, CH_2-环丙基), 1.96 (q, 1H, CH-环丙基), 3.10-3.19 (m, 3H, CH-环丙基, CH_2), 6.85 (t, 1H, Ar), 7.43 (d, 1H, Ar), 7.86-8.07 (m, 3H), 8.32 (s, 1H), 9.83 (s, 1H), 13.22 (s, 1H, Ar-OH).

实施例 9

(1S,2S)-N-[顺式-2-(2-(3-氨基苯基羰基氧基)-6-氟-3-丙酰基苯基)-环丙基]-N'-(5-氰基吡啶-2-基)-脲

从在实施例 6 中描述的化合物开始，并使用在实施例 7 中描述的方法，得到作为盐酸盐的标题产物.

^{1}H NMR(250 MHz, DMSO-d_6) δ 0.94 (t, 3H), 0.9-1.0 (m, 1H), 1.3-1.4 (m, 1H), 1.85-1.95 (m, 1H), 2.91 (q, 2H), 3.05-3.15 (m, 1H), 7.4-7.5 (m, 2H), 7.6-7.7 (m, 1H), 7.9-8.1 (m, 5H), 8.08 (d, 1H), 9.85 (s, 1H).

实施例 10

(1S,2S)-N-(顺式-2-(6-氟-2-羟基-3-丙酰基苯基)-环丙基)-N'-(5-溴吡啶-2-基)-脲

在室温下，于氩气氛中将(1S,2R)-顺式-2-(6-氟-2-甲氧基-3-丙酰基苯基)环丙基羧酸(3.0 g, 11.3 mmol)、三乙胺(1.58 ml, 11.3 mmol)和二苯基磷酰基叠氮化物(2.44 ml, 11.3 mmol)溶于干燥甲苯(8 ml)中. 在室温下，将所述反应混合物搅拌 30 分钟，之后将温度升至 120℃并维持另外 15 分钟. 然后，加入 2-氨基-5-溴吡啶(2.08 g, 12 mmol)并在 120℃下将所述反应混合物搅拌 2.5 小时. 加入苯和 1 M HCl 溶液并将所述有机相蒸发. 使用己烷/乙酸乙酯 1:1 作为洗脱剂，在硅胶上纯化残余物. 收集适当的部分并得到 5.0g(1S,2S)-N-(顺式-2-(6-氟-2-甲氧基-3-丙酰基苯基)-环丙基)-N'-(5-溴吡啶-2-基)-脲. 将所述化合物溶于二氯甲烷(100 ml)中，将所述溶液保持在氩气氛下并冷却至-65℃. 加入三氯化硼(30 ml 在二氯甲烷中的 1 M 溶液, 30 mmol)并使所述反应混合物达到室温过夜. 加入二氯甲烷和饱和碳酸氢钠. 蒸发有机相并使用乙酸乙酯:甲醇 9:1 作为洗脱液，在硅胶上纯化残余物. 得到 1.96 g(41%)标题化合物.

分析: 计算值: C 51.2, H 4.1, N 9.9. 实测值: C 51.5, H 3.7, N 9.5.

Mp: 198-199℃. $[\alpha]_D^{22}$ +149.8° (c=0.50, CH_2Cl_2).

^{1}H NMR(250 MHz, $CDCl_3$) δ 1.28 (t, 3H), 1.52-1.62 (m, 2H), 1.94-2.05 (m, 1H), 2.97-3.06 (m, 2H), 3.17-3.20 (m, 1H), 6.60 (t, 1H), 6.76 (宽 s, 1H), 7.57 (dd, 1H), 7.67-7.72 (m, 1H), 7.83 (宽 s, 1H), 8.53 (宽 s, 1H), 13.32 (d, 1H).

实施例 11

(1R,2R)-N-(顺式-2-(6-氟-2-羟基-3-丙酰基苯基)-环丙基)-N'-(5-溴吡啶-2-基)-脲

使用手性配体 2,2'-亚异丙基双(4S)-4-叔丁基-2-噁唑啉(从 Aldrich 得到), 如同在实施例 3 中描述的那样, 对在实施例 2 中描述的化合物实施不对称环丙烷化反应. 然后把得到的(1R,2S)-顺式-2-(6-氟-2-甲氧基-3-丙酰基苯基)环丙基羧酸以类似于实施例 10 的方法使用, 得到标题化合物.

^{1}H NMR(250 MHz, DMSO-d_6) δ 1.05-1.15 (m, 1H), 1.12 (t, 3H), 1.40-1.50 (m, 1H), 1.90 (q, 1H), 3.00-3.10 (m, 1H), 3.12 (q, 2H), 6.82 (t, 1H), 7.18 (d, 1H), 7.78 (dd, 1H), 7.88 (宽 s, 1H), 7.95-8.05 (m, 1H), 9.41 (宽 s, 1H), 13.20 (s, 1H).

$[\alpha]_D^{22}$ -153.8° (c=0.50, CH_2Cl_2).

实施例 12

(1S,2S)-N-[顺式-2-(2-(3-氨基苯基羰基氧基)-6-氟-3-丙酰基苯基)-环丙基]-N'-(5-溴吡啶-2-基)-脲

在室温下, 于氩气氛中向实施例 10 的化合物(633 mg, 1.5 mmol)、BOC-保护的 3-氨基苯甲酸(475 mg, 2 mmol)和 4-二甲基氨基吡啶(123 mg, 1 mmol)在 20 ml 二氯甲烷:DMF 1:1 中的溶液中加入 415 mg (2 mmol)的 DCC. 将所述反应混合物搅拌 36 小时. 小心蒸发溶剂并使用作为溶剂的己烷:乙酸乙酯 1:1, 在硅胶上纯化残余物, 得到 811 mg 的 BOC-保护的标题产物. 将该产物溶于二氧六环(20 ml)中并加入 10

ml 的 6M HCl，并将所述混合物搅拌过夜．小心真空除去溶剂．以乙醇和乙醚处理残余物，得到255 mg作为HCl盐的标题产物．所述HPLC纯度为大约93%．

^{1}H-NMR(250 MHz, CD_3OD) δ 1.15 (t, 3H), 1.3-1.4 (m, 1H), 1.5-1.6 (m, 1H), 2.05-2.15 (m, 3H), 3.04 (q, 2H), 3.23-3.27 (m, 1H), 7.16 (d, 1H), 7.34 (t, 1H), 7.85-7.93 (m, 2H), 8.05 (dd, 1H), 8.19 (宽 d, 1H), 8.26 (宽 s, 1H), 8.35-8.37 (m, 1H), 8.42-8.46 (m, 1H).

实施例 13

(1S,2S)-N-[顺式-2-(2-(3-L-丙氨酰氨基苯基羰基氧基)-6-氟-3-丙酰基苯基)-环丙基]-N'-(5-溴吡啶-2-基)-脲

参见例如 Bodanszky 的“肽合成的实践”第二版，Springer，使用标准化学方法从TCE-保护的3-氨基苯甲酸制备起始化合物BOC-保护的3-L-丙氨酰氨基苯甲酸．如同在实施例12中描述的那样，使该化合物与实施例10的化合物反应，得到作为HCl盐的标题产物．

^{1}H-NMR(250 MHz, 释出的胺, $CDCl_3$) δ 1.10 (t, 3H), 1.15-1.25 (m, 1H), 1.4-1.5 (m, 1H), 1.42 (d, 2H), 1.76 (宽 s, 2H), 1.88-1.97 (m, 1H), 2.84 (q, 2H), 3.1-3.2 (m, 1H), 3.59-3.67 (m, 1H), 6.78 (d, 1H), 7.09 (t, 1H), 7.85-7.93 (m, 2H), 8.08 (d, 1H), 8.11 (s, 1H), 8.29 (宽 s, 1H), 9.05 (宽 s, 1H), 9.70 (宽 s, 1H).

实施例 14

(1S,2S)-N-{顺式-2-[6-氟-3-丙酰基-2-(4-吡啶基羰基氧基)苯基]环丙基}-N'-(5-溴吡啶-2-基)脲

以类似实施例12的方法，使实施例10的产物与异烟酸缩合，得到作为HCl盐的标题产物．

^{1}H-NMR(250 MHz, CD_3OD) δ 9.26 (d, 2H), 8.83 (d, 2H), 8.14 (m, 2H), 8.04 (dd, 1H), 7.39 (t, 1H), 7.10 (d, 1H), 3.38 (m, 1H), 3.08 (m, 2H), 2.15

(m, 1H), 1.62 (m, 1H), 1.38 (m, 1H), 1.13 (t, 3H).

实施例 15

(1S,2S)-N-{顺式-2-[2-(3-二甲基氨基苯基羰基氧基)-6-氟-3-丙酰基苯基]环丙基}-N'-(5-溴吡啶-2-基)脲

以类似于实施例 12 的方法，使实施例 10 的产物与 3-二甲基氨基苯甲酸缩合，得到作为 HCl 盐的标题产物。

^{1}H-NMR(250 MHz, CD_3OD) δ 8.61 (s, 1H), 8.45 (d, 1H), 8.15-8.03 (m, 4H), 7.92 (t, 1H), 7.34 (t, 1H), 7.10 (d, 1H), 3.48 (s, 6H), 3.28 (m, 1H), 3.00 (m, 2H), 2.11 (m, 1H), 1.58 (m, 1H), 1.38 (m, 1H), 1.14 (t, 3H).

实施例 16

(1S,2S)-N-[顺式-2-(2-(3-氨基甲基苯甲酰氧基甲氧基)-5-氟-3-丙酰基苯基)-环丙基]-N'-(5-溴吡啶-2-基)-脲

以四丁基氢氧化铵溶液(1 M 在 MeOH 中)处理 3-叔丁氧基羰基酰氨基甲基苯甲酸至 pH 9 并蒸发。将残余物溶于二氯甲烷中并以氯碘甲烷处理过夜。以水洗涤所述溶液并蒸发，得到粗品 3-叔丁氧基羰基酰氨基甲基苯甲酰氧基甲基氯。用少量碘化钠作为催化剂，该物料与实施例 10 的钠盐(在 DMF 中用氢化钠制备)反应。在 2 小时反应后，以乙酸骤冷所述溶液并以二氯甲烷稀释，以水洗涤并蒸发。经以乙酸乙酯/己烷 1:2 洗脱，在硅胶上纯化所述粗品产物，并以三氟乙酸处理所述纯的物料并蒸发，得到作为固体的标题化合物的三氟乙酸盐。

^{1}H-NMR($CDCl_3$) δ 1.1 (t, 3H), 1.3-1.5 (m, 2H), 2.2 (q, 1H), 2.9 (m, 2H), 3.2 (bs, 1H), 4.2 (s, 2H), 5.9 (q, 2H), 6.8 (d, 2H), 7.0 (t, 1H), 7.3-8.1 (m, 9H).

实施例 17

(1S,2S)-N-(顺式-2-(2-(3-氨基-4-甲基苯甲酰氧基)-6-氟-3-丙酰基苯基)环丙基)-N'-(5-溴吡啶-2-基)-脲

按照在实施例 12 中方法，使来自实施例 10 的(1S,2S)-N-(顺式-2-(6-氟-2-羟基-3-丙酰基苯基)-环丙基)-N'-(5-溴吡啶-2-基)-脲与 3-叔丁氧基羰基酰氨基-4-甲基苯甲酸缩合．将其产物以三氟乙酸处理并蒸发，得到作为固体的标题化合物的三氟乙酸盐．

^{1}H-NMR($CDCl_3$) δ 1.1 (t, 3H), 1.3-1.5 (m, 2H), 1.9 (q, 1H),2.4 (s, 3H), 2.9 (q, 2H), 3.1 (BS, 1H), 7.1 (t, 1H), 7.4 (d, 1H), 7.8 (m, 1H), 7.9 (m, 2H), 8.1 (s, 1H), 8.3 (s, 1H).

实施例 18

(1S,2S)-N-(顺式-2-(2-(3-乙基氨基苯甲酰氧基)-6-氟-3-丙酰基苯基)环丙基)-N'-(5-溴吡啶-2-基)-脲

按照在实施例 12 中方法，将实施例 10 的化合物与 3-(N-乙基-叔丁氧基羰基酰氨基)苯甲酸缩合，并将其产物以三氟乙酸处理并蒸发，得到作为固体的标题化合物的三氟乙酸盐．

^{1}H NMR($CDCl_3$) δ 1.1 (t, 3H), 1.3-1.6 (m, 5H), 2.9 (q, 2H), 3.1 (bs, 1H), 3.5 (q, 2H), 7.1 (t, 1H), 7.2 (bs, 1H), 7.6 (t, 1H), 7.7-7.8 (m, 2H), 7.9 (d, 1H), 8.1 (s, 1H), 8.2 (d, 1H), 8.4 (s, 1H).

实施例 19

(1S,2S)-N-(顺式-2-(2-quinolo-4-基氧基-6-氟-3-丙酰基苯基)环丙基)-N'-(5-溴吡啶-2-基)-脲

按照实施例 12 中方法，将实施例 10 的化合物与 4-喹啉酸缩合，并将其产物溶于三氟乙酸中并蒸发，得到作为固体的标题化合物的乙酸盐．

^{1}H NMR($CDCl_3$) δ 1.1 (t, 3H), 1.2 (m, 1H), 1.5 (m, 1H), 1.9 (m, 1H), 2.8

(q, 2H), 3.2 (bs, 1H), 6.7 (d, 1H), 7.2 (t, 1H), 7.5 (m, 1H), 7.7 (t, 1H), 7.8-8.0 (m, 2H), 8.2 (d, 1H), 8.3 (d, 1H), 8.8 (d, 1H), 9.1 (m, 2H), 9.2 (bs,1H).

实施例 20

(1S,2S)-N-(顺式-2-(3-氨基甲基-2-甲基苯甲酰氧基)-氟-3-丙酰基苯基)环丙基)-N'-(5-溴吡啶-2-基)-脲

按照实施例 12 中方法，将实施例 10 的化合物与 3-叔丁氧基羰基酰氨基-2-甲基苯甲酸缩合．将其产物以三氟乙酸处理并蒸发，得到作为固体的标题化合物．

^{1}H NMR($CDCl_3$) δ 1.1 (t, 3H), 1.1-1.3 (m, 2H), 1.9 (m, 1H), 2.5 (s, 3H), 2.9 (q, 2H), 3.1 (bs, 1H), 4.2 (s, 2H), 7.0-7.2 (m, 2H), 7.4 (d, 1H), 7.6-7.7 (m, 2H), 7.8-8.0 (m, 2H), 8.2 (bs, 2H).

实施例 21

(1S,2S)-N-[顺式-2-(6-氟-2-(4-氨基甲基苯基羰基氧基)-3-丙酰基苯基)-环丙基]-N'-(5-溴吡啶-2-基)-脲

通过向 4g4-氰基苯甲酸在 200 ml MeOH 中的溶液中加入 6.5 g DCC 制备 4-(叔丁氧基羰基酰氨基甲基)苯甲酸．将所述混合物在室温下搅拌 70 小时，过滤除去沉淀出的二环己基脲并在真空下浓缩滤液，得到 7 g 粗产物．将所述甲基酯溶于 500 ml MeOH 中并加入 9.6 g $CoCl_2$ $6H_2O$．将所述混合物以 $NaBH_4$ 分次处理．5 小时后把所述反应混合物浓缩并除去沉淀．滤液以 150 ml 的 1M HCl(aq.)酸化并以 2 × 100 ml 的 CH_2Cl_2 提取．该酸性的水相以 100 ml 的 25% NH_3(aq.)处理，以 3 × 100 ml CH_2Cl_2 提取，以 Na_2SO_4 干燥并浓缩，得到 2.64 g 棕色的油．

将该油溶于 30 ml 的二氧六环/水混合物(2:1)中并以 1.5g NaOH(s) 处理 20 小时．除去溶剂并加入 40 ml 叔丁醇/水混合物(1:1)．加入 3.7g

二碳酸二叔丁基酯后将该溶液搅拌 24 小时，然后加入更多的水并用 2 × 50 ml 己烷提取该混合物．将所述水相以 $NaHSO_4$ 酸化(pH~1.5-2.0)并以 3 × 75 ml 乙醚提取．将该合并的提取液以 50 ml 盐水洗涤，以 Na_2SO_4 干燥并蒸发，得到作为白色固体的中间体 4-(叔丁氧基羰基酰氨基甲基)苯甲酸．

将 4-(叔丁氧基羰基酰氨基甲基)苯甲酸和来自实施例 10 的 (1S,2S)-N-(顺式-2-(6-氟-2-羟基-3-丙酰基苯基)-环丙基)-N'-(5-溴吡啶-2-基)-脲缩合，并使用实施例 12 中描述的方法除去 BOC-保护基团，得到作为盐酸盐的标题产物．

^{1}H-NMR(250 MHz, $CDCl_3$) δ 0.98 (t, 3H), 1.05-1.20 (m, 1H), 1.31-1.49 (m, 1H), 1.69-1.90 (m, 1H), 2.65 (q, 2H), 3.33-3.49 (m, 1H), 4.31 (宽 s, 2H), 7.02-7.22 (m, 2H), 7.35-7.49 (m, 1H), 7.50-7.68 (m, 2H), 7.69-7.83 (m, 2H), 8.08 (d, 1H), 8.37 (宽 s, 1H).

实施例 22

(1S,2SR)-N-[顺式-2-(6-氟-2-(N-甲基吲哚-5-羰基氧基)-3-丙酰基苯基)-环丙基]-N'-(5-溴吡啶-2-基)-脲

i) N-甲基吲哚-5-羧酸的制备

在室温下，将 0.1 g 吲哚-5-羧酸与 2 当量的三氟甲磺酸甲酯在 1 ml DMF 中混合．5 小时后蒸发溶剂并记录 ^{1}H-NMR:

^{1}H-NMR(250 MHz, DMSO-d_6) δ 2.76 (s, 3H), 6.57 (宽 s, 1H), 7.46-7.50 (m, 2H), 7.75 (dd, 1H), 8.23-8.29 (m, 2H), 11.56 (宽 s, 1H).

ii) 制备标题化合物

使用实施例 12 中描述的方法，将 N-甲基吲哚-5-羧酸和来自实施例 10 的(1S,2S)-N-(顺式-2-(6-氟-2-羟基-3-丙酰基苯基)-环丙基)-N'-(5-溴吡啶-2-基)-脲缩合，得到作为盐酸盐的标题产物．

^{1}H-NMR(250 MHz, $CDCl_3$) δ 1.08 (t, 3H), 1.15-1.25 (m, 1H), 1.39-1.50 (m, 1H), 1.92-2.08 (m, 1H), 2.89 (q, 2H), 2.90 (s, 3H), 3.20-3.35 (m, 1H),

6.55 (宽 s, 1H), 6.65 (宽 d, 1H), 7.11 (t, 1H), 7.20-7.29 (m, 2H), 7.41 (dd, 1H), 7.72-7.83 (m, 2H), 7.95 (dd, 1H), 8.51 (宽 s, 1H), 9.25 (宽 s, 1H), 9.43 (宽 s, 1H).

实施例 23

(1S,2S)-N-[顺式-2-(6-氟-2-(吲哚-4-羰基氧基)-3-丙酰基苯基)-环丙基]-N'-(5-溴吡啶-2-基)-脲

使用实施例 12 中描述的方法，将吲哚-4-羧酸和来自实施例 10 的(1S,2S)-N-(顺式-2-(6-氟-2-羟基-3-丙酰基苯基)-环丙基)-N'-(5-溴吡啶-2-基)-脲缩合，得到作为盐酸盐的标题产物.

^{1}H-NMR(250 MHz, $CDCl_3$) δ 1.07 (t, 3H), 1.17-1.30 (m, 1H), 1.31-1.47 (m, 1H), 1.90-2.10 (m, 1H), 2.89 (q, 2H), 3.02-3.18 (m, 1H), 6.75 (宽 d, 1H), 7.00-7.35 (m, 4H), 7.55 (dd, 1H), 7.60 (d, 1H), 7.79 (dd, 1H), 7.89 (d, 1H), 8.10 (d, 1H), 9.27(宽 d, 2H).

实施例 24

(1S,2S)-N-[顺式-2-(6-氟-2-(3-氨基-4-氯苯基羰基氧基)-3-丙酰基苯基)-环丙基]-N'-(5-溴吡啶-2-基)-脲

使用实施例 12 中描述的方法，将 3-氨基-4-氯苯甲酸和来自实施例 10 的(1S,2S)-N-(顺式-2-(6-氟-2-羟基-3-丙酰基苯基)-环丙基)-N'-(5-溴吡啶-2-基)-脲缩合，得到作为盐酸盐的标题产物.

^{1}H-NMR(250 MHz, 释出的胺, $CDCl_3$) δ 1.10 (t, 3H), 1.17-1.30 (m, 1H), 1.42-1.52 (m, 1H), 1.88-2.01 (m, 1H), 2.88 (q, 2H), 3.19-3.31 (m, 1H), 4.25 (宽 s, 2H), 6.80 (宽 d, 1H), 7.09 (t, 1H), 7.35 (t, 1H), 7.48-7.60 (m, 2H), 7.66 (d, 1H), 7.73-7.88 (m, 2H), 9.25 (宽 s, 2H).

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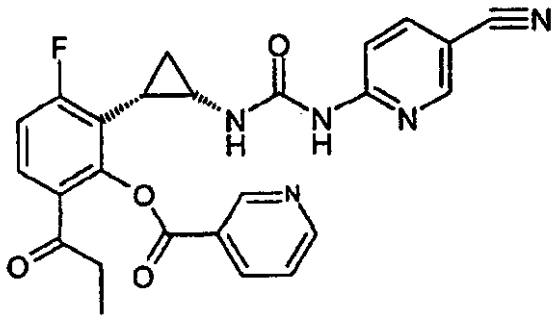
实施例 25

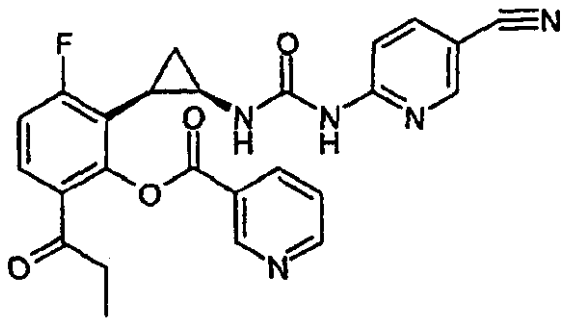
(1S,2S)-N-[顺式-2-(6-氟-2-(吡啶-3-基羰基氧基)-3-丙酰基苯基)-环丙基]-N'-(5-氰基吡啶-2-基)-脲



将实施例 8 的化合物(50 g, 0.68 mmol)、*N,N'*-二环己基碳二亚胺(0.168 g, 0.81 mmol)、烟酸(0.1 g, 0.81 mmol)和 4-(二甲基氨基)吡啶(0.041 g, 0.34 mmol)的干燥混合物溶于 CH_2Cl_2(5 ml)和 *N,N*-二甲基甲酰胺(DMF)(2.5 ml)中．然后在室温下搅拌该混合物．20 小时后，过滤所述混合物并真空干燥，然后再溶解在最小量的二氯甲烷中并过滤．将该澄清的溶液在硅胶上蒸发并通过层析法(乙酸乙酯)纯化，得到标题化合物(0.168 g, 50%)．通过从氯仿-己烷重结晶得到分析样品．^{1}H NMR($CDCl_3$): 9.89 (br s, 1H), 9.41 (m, 1H), 9.33 (br s, 1H), 8.86 (dd, 1H), 8.46 (dt, 1H), 8.18 (d, 1H), 7.80 (dd, 1H), 7.71 (dd, 1H), 7.49 (ddd, 1H), 7.13 (t, 1H), 6.92 (d, 1H), 3.18 (m, 1H), 2.88 (q, 2H), 1.99 (m, 1H), 1.52 (m, 1H), 1.25 (m, 1H), 1.13 (t, 3H).

实施例 26

(1R,2R)-N-[顺式-2-(6-氟-2-(吡啶-3-基羰基氧基)-3-丙酰基苯基)-环丙基]-N'-(5-氰基吡啶-2-基)-脲

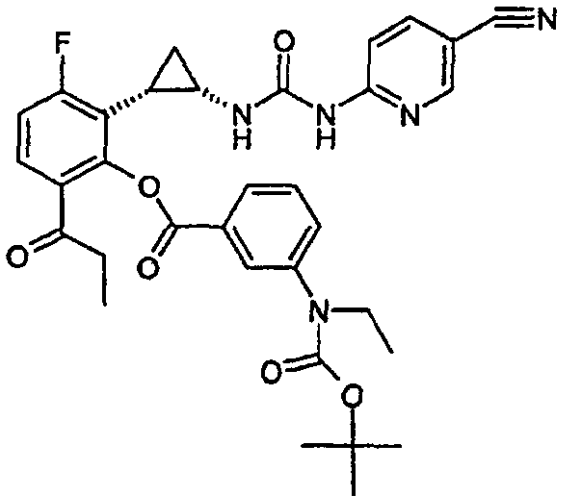


将实施例 6 的化合物(0.1 g, 0.27 mmol)、*N,N'*-二环己基碳二亚胺(0.067 g, 0.33 mmol)和烟酸(0.037 g, 0.3 mmol)的干燥混合物悬浮于二氯甲烷(2 ml)中. 滴加最少的 DMF 以得到相当澄清的溶液. 然后加入 4-(二甲基氨基)吡啶(0.016 g, 0.14 mmol). 在室温下, 搅拌该反应混合物. 20 小时后, 真空蒸发溶剂并将粗品残余物溶于盐酸水溶液(pH 1-2)中并过滤. 然后以碳酸氢钠使所述澄清溶液呈稍微碱性并过滤所沉淀出的产物. 通过层析法(二氯甲烷-甲醇, 15:1)纯化, 得到标题化合物 0.072 g(56%).

^{1}H NMR($CDCl_3$): 9.85 (br s, 1H), 9.42 (s, 1H), 9.35 (br s, 1H), 8.86 (d, 1H), 8.47 (dt, 1H), 8.18 (d, 1H), 7.81 (dd, 1H), 7.71 (dd, 1H), 7.48 (dd, 1H), 7.13 (t, 1H), 6.92 (d, 1H), 3.19 (m, 1H), 2.91 (q, 2H), 1.99 (m, 1H), 1.49 (m, 1H), 1.24 (m, 1H), 1.13 (t, 3H).

实施例 27

(1S,2S)-N-[顺式-2-(2-(3-(N-乙基,N-Boc-氨基)苯基羰基氧基)-6-氟-3-丙酰基苯基)-环丙基]-N'-(5-氰基吡啶-2-基)-脲

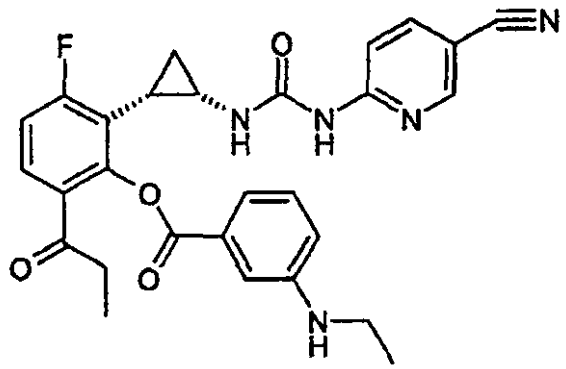


将实施例 8 的化合物(0.37 g, 1.0 mmol)、*N,N'*-二环己基碳二亚胺(0.25 g, 1.2 mmol)、4-二甲基氨基吡啶(0.06 g, 0.5 mmol)和 3-(N-乙基-N-丁氧基羰基)氨基苯甲酸(0.320 g, 1.2 mmol)(通过 3-氨基苯甲酸的还原性胺化,随后保护所述氨基来制备)溶于二氯甲烷(8 ml)和DMF(3 ml)中. 然后在室温下将所述混合物搅拌. 在 18 小时后, 真空除去溶剂并将粗品产物再溶于二氯甲烷中且过滤. 所述澄清溶液在硅胶上蒸发并通过层析法(乙酸乙酯-己烷, 3:2)纯化, 得到足够纯的标题化合物(0.24 g, 39%).

^{1}H NMR($CDCl_3$): 10.0 (br s, 2H), 8.20 (d, 1H), 8.06 (d, 1H), 8.03 (m, 1H), 7.77 (dd, 1H), 7.70 (dd, 1H), 7.48 (m, 2H), 7.10 (t, 1H), 6.95 (d, 1H), 3.71 (q, 2H), 3.14 (m, 1H), 2.90 (q, 2H), 1.95 (q, 1H), 1.44 (s, 10H), 1.2-1.09 (m, 7H).

实施例 28

(1S,2S)-N-[顺式-2-(2-(3-乙基氨基苯基羰基氧基)-6-氟-3-丙酰基苯基)-环丙基]-N'-(5-氰基吡啶-2-基)-脲

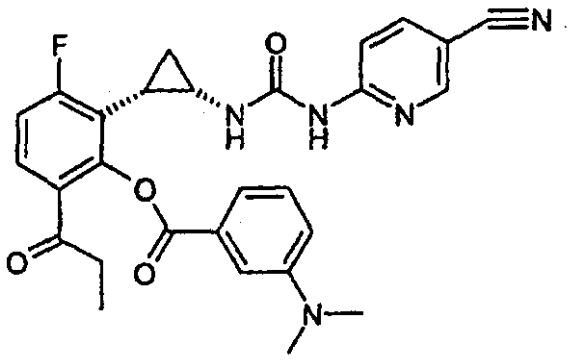


将三氟乙酸(5 ml)加入到实施例 27 的化合物(0.120 mg, 0.19 mmol)在二氯甲烷(10 ml)中搅拌着的溶液中. 在室温下, 将所述混合物放置 1-2 小时. 然后蒸发至干. 在 HPLC(制备型 C-18 柱, 40%水在乙腈中)上纯化所述粗品产物, 得到 0.045 g(30%)作为三氟乙酸盐的标题化合物.

^{1}H NMR($CDCl_3$): 11.08 (br s, 2H), 9.83 (br s, 1H), 9.36 (br s, 1H), 8.23-8.08 (m, 3H), 7.82-7.54 (m, 4H), 7.13 (t, 1H), 7.02 (d, 1H), 3.42 (q, 2H), 3.20 (m, 1H), 2.83 (q, 2H), 1.94 (q, 1H), 1.46 (m, 1H), 1.34 (t, 3H), 1.24 (m, 1H), 1.06 (t, 3H).

实施例 29

(1S,2S)-N-[顺式-2-(2-(3-二甲基氨基苯基羰基氧基)-6-氟-3-丙酰基苯基)-环丙基]-N'-(5-氰基吡啶-2-基)-脲

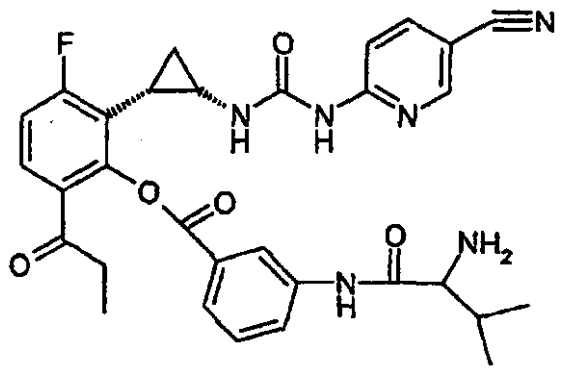


将实施例 8 的化合物(0.1 g, 0.27 mmol)、*N,N'*-二环己基碳二亚胺(0.067 g, 0.33 mmol)、4-二甲基氨基吡啶(0.016 g, 0.14 mmol)和 3-二甲基氨基苯甲酸(0.054 g, 0.39 mmol)溶于二氯甲烷(3 ml)和 DMF (1 ml)中．在室温下将所述反应物放置 16 小时．然后真空除去溶剂并将固体再溶于二氯甲烷中且过滤．通过层析法(乙酸乙酯-己烷, 2:1)随后经 HPLC(C-18 柱, 0.1% TFA 在乙腈中)纯化，得到作为三氟乙酸盐的标题化合物 0.1 g(58%).

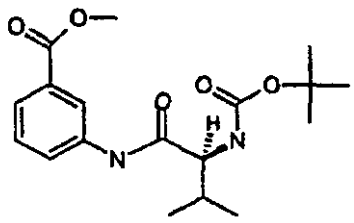
^{1}H NMR($CDCl_3$): 8.38-8.23 (m, 3H), 7.92-7.69 (m, 4H), 7.15 (t, 1H), 7.05 (m, 1H), 3.32 (s, 6H), 3.26 (m, 1H), 2.89 (q, 2H), 2.02 (m, 1H), 1.55-1.27 (m, 2H), 1.10 (t, 3H).

实施例 30

(1S,2S)-N-[顺式-2-(2-(3-L-缬氨酰氨基苯基羰基氧基)-6-氟-3-丙酰基苯基)-环丙基]-N'-(5-氰基吡啶-2-基)-脲



a) 3-(N-Boc-L-缬氨酰)氨基甲基苯甲酸酯

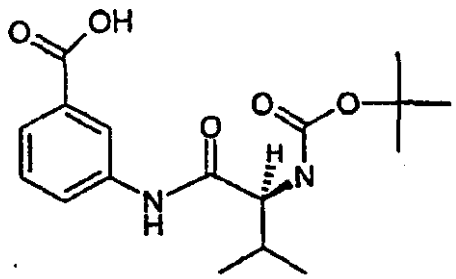


类似 Villaneuve 和 Chan 的方法，四面体快报, 1997, 第 37 卷 第 6489-6492 页制备该中间体. 在氮气氛下，将 *N*-叔丁氧基羰基-L-缬氨酸(2.17 g, 10 mmol)和六氯丙酮(1.32 g, 5 mmol)在二氯甲烷(20 ml)中的混合物搅拌并冷却至-78℃. 滴加入在二氯甲烷(10 ml)中的三苯基膦(2.6 g, 10 mmol)并把所述混合物搅拌 30 分钟. 然后滴加入在二氯甲烷(10 ml)中的 3-氨基苯甲酸甲酯(1.5 g, 10 mmol)，随后加入在二氯甲烷中的三乙胺(1 g, 10 mmol). 然后使所述反应物达到室温，之后真空下蒸发溶剂. 经硅胶层析法(己烷-乙酸乙酯, 3:1)纯化残余物，随后从乙酸乙酯-己烷中重结晶，得到 0.7 g(28%)以上描述的纯的中间体.

^{1}H NMR($CDCl_3$): 8.30 (br s, 1H), 8.07 (d, 1H), 7.85-7.75 (m, 2H), 7.37 (t,

1H), 5.15 (d, 1H), 4.05 (m, 1H), 3.91 (s, 3H), 2.26 (m, 1H), 1.48 (s, 9H), 1.03 (dd, 6H).

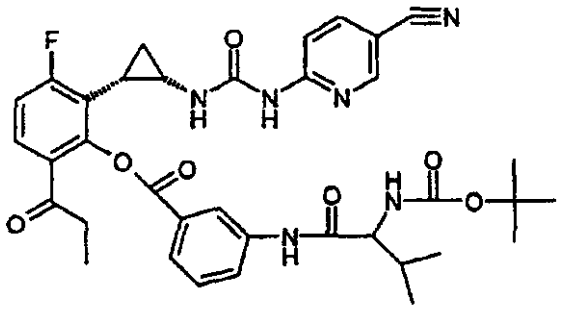
b) 3-(N-Boc-L-缬氨酰)氨基苯甲酸



将步骤 a)中间体(0.65 mg, 1.8 mmol)悬浮于甲醇(6 ml)和水(2 ml)中．加入氢氧化锂(0.11 g, 3.9 mmol)并在室温下将所述混合物搅拌 24 小时．然后加入水(10 ml)并把其体积减少至一半．以 10-20 ml 乙酸乙酯洗涤所述水溶液，然后以盐酸水溶液酸化．以乙酸乙酯(2 × 20 ml)提取，干燥并真空蒸发，得到以上描述的纯的中间体 0.524 g(84%).

^{1}H NMR(CD_3OD): 8.23 (t, 1H), 7.84 (d, 1H), 7.76 (d, 1H), 7.42 (t, 1H), 6.70 (d, 1H), 4.00 (m, 1H), 2.08 (m, 1H), 1.45 (a, 9H), 1.00 (d, 6H).

c) (1S,2S)-N-[顺式-2-(2-(3-N-Boc-L-缬氨酰氨基苯基羰基氧基)-6-氟-3-丙酰基苯基)-环丙基]-N'-(5-氰基吡啶-2-基)-脲

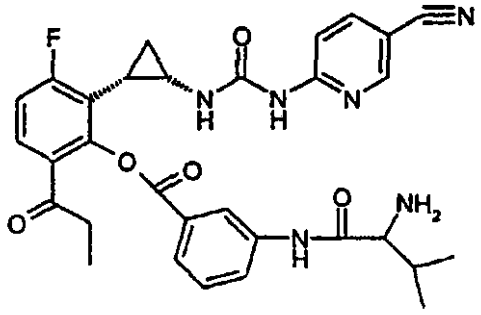


将实施例 8 的化合物(0.23 g, 0.62 mmol)、*N,N'*-二环己基碳二亚胺(0.153 g, 0.74 mmol)、4-二甲基氨基吡啶(0.038 g, 0.3 mmol)和步骤 b)中间体(0.25 g, 0.74 mmol)溶于二氯甲烷(9 ml)和 DMF (3 ml)中．在

室温下，将所述反应物放置 19 小时．真空除去溶剂并将固体重新溶于二氯甲烷中且过滤．通过层析法(乙酸乙酯-己烷, 1:1)纯化，得到 0.029 g(67%)纯的 N-保护标题化合物．

^{1}H NMR(CD_3OD): 8.56 (t, 1H), 8.27 (s, 1H), 7.98-7.82 (m, 4H), 7.53 (t, 1H), 7.23 (t, 1H), 7.10 (d, 1H), 3.98 (d, 1H), 3.09 (m, 1H), 2.90 (q, 2H), 2.06-1.93 (m, 2H), 1.44 (m, 10H), 1.18-0.94 (m, 10H).

d) (1S,2S)-N-[顺式-2-(2-(3-L-缬氨酰氨基苯基羰基氧基)-6-氟-3-丙酰基苯基)-环丙基]-N'-(5-氰基吡啶-2-基)-脲

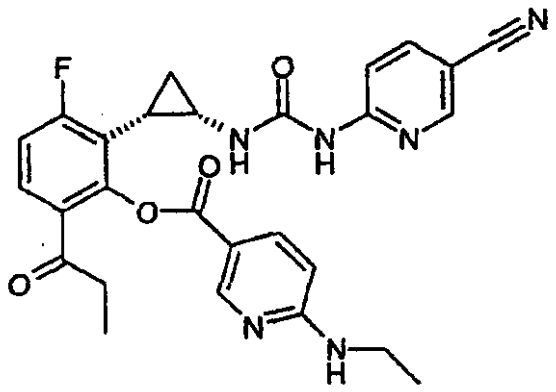


将 N-保护的步骤 c)的化合物(0.16 g, 0.23 mmol)和苯硫酚(0.054 g, 0.46 mmol)溶于二氯甲烷(6 ml)中并冷却至 0℃．加入三氟乙酸(6 ml)并使所述混合物达到室温且放置 1 小时．蒸发至干，随后经层析法(二氯甲烷-甲醇, 10:1.5)纯化，得到 0.150 g(90%)作为 TFA 盐的标题化合物．

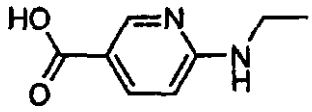
^{1}H NMR(CD_3OD): 8.60 (s, 1H), 8.25 (d, 1H), 8.0-7.85 (m, 4H), 7.53 (t, 1H), 7.21 (t, 1H), 7.09 (d, 1H), 5.0 (m, 1H), 3.12 (m, 1H), 2.96-2.87 (m, 2H), 2.20 (m, 1H), 1.97 (m, 1H), 1.46 (m, 1H), 1.09-1.03 (m, 10H).

实施例 31

(1S,2S)-N-{顺式-2-[6-氟-3-丙酰基-2-(6-乙基氨基吡啶-3-基羰基氧基)苯基]环丙基}-N'-(5-氰基吡啶-2-基)脲



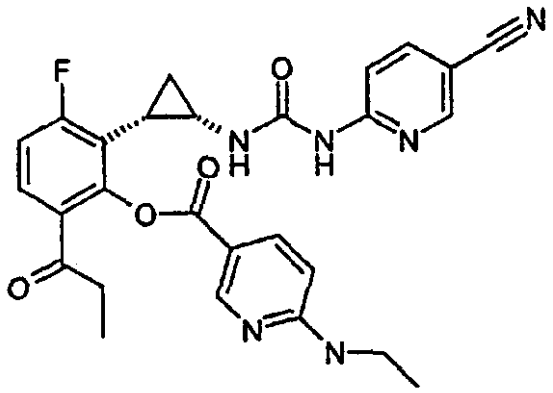
a) 6-乙基氨基烟酸



通过与实施例 35 步骤 a)中描述的相同的方法，从 6-氯烟酸和乙基胺制备该中间体，以 1-丁醇替代乙酸乙酯用于提取，重结晶 ($MeOH-CHCl_3$)得到 0.53 g(50%)。

^1H NMR(DMSO-d_6): 12.1 (br s, 1H), 8.54 (d, 1H), 7.77 (dd, 1H), 7.15 (t, 1H), 6.45 (dd, 1H), 3.33 (m, 2H), 1.14 (t, 3H).

b) (1S,2S)-N-{顺式-2-[6-氟-3-丙酰基-2-(6-乙基氨基吡啶-3-基羰基氧基)苯基]环丙基}-N'-(5-氰基吡啶-2-基)脲

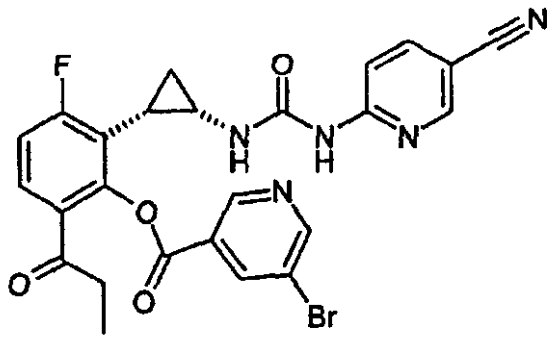


将实施例 8 的化合物(0.1 g, 0.27 mmol)、6-乙基氨基烟酸(0.084 g, 0.54 mmol)、*N,N'*-二环己基碳二亚胺(0.127 g, 0.62 mmol)和 4-二甲基氨基吡啶(0.016 g, 0.13 mmol)溶于 DMF(3 ml)中并在环境温度下放置. 在 19 小时后, 真空除去溶剂并把残余物悬浮于二氯甲烷中且过滤. 除去溶剂并经层析法(乙酸乙酯-己烷, 2:1)纯化所述粗品产物, 得到标题化合物(0.063 g, 45%).

^{1}H NMR($CDCl_3$): 9.85 (br s, 1H), 9.25 (br s, 1H), 8.91 (d, 1H), 8.18-8.02 (m, 3H), 7.76-7.67 (m, 2H), 7.65 (t, 1H), 6.96 (d, 1H), 6.37 (d, 1H), 5.40 (m, 1H), 3.37 (m, 2H), 3.19 (m, 1H), 2.8 (q, 2H), 1.98 (m, 1H), 1.49 (m, 1H), 1.28 (t, 3H), 1.15 (m, 1H), 1.10 (t, 3H).

实施例 32

(1S,2S)-N-{顺式-2-[6-氟-3-丙酰基-2-(5-溴吡啶-3-基羰基氧基)苯基]环丙基}-N'-(5-氰基吡啶-2-基)脲

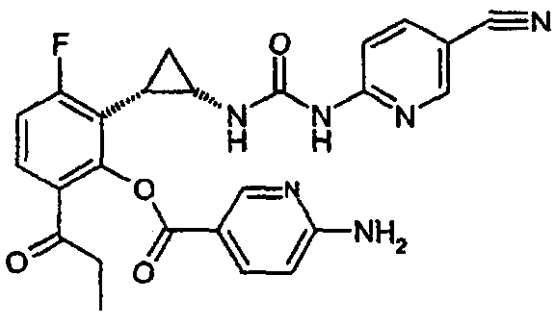


将 5-溴烟酸(0.065 g, 0.33 mmol)、实施例 8 的化合物(0.1 g, 0.27 mmol)、*N,N'*-二环己基碳二亚胺(0.127 g, 0.62 mmol)和 4-二甲基氨基吡啶(0.016 g, 0.13 mmol)溶于二氯甲烷(4 ml)中并在环境温度下放置. 在 19 小时后, 将所述混合物过滤并真空除去溶剂. 经层析法(乙酸乙酯-己烷, 1:1)纯化所述粗品产物, 得到标题化合物 (0.040 g, 27%).

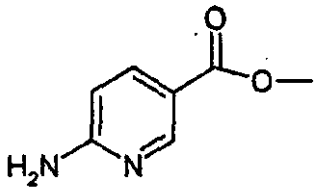
^{1}H NMR($CDCl_3$): 9.80 (br s, 1H), 9.30 (d, 1H), 9.17 (br s, 1H), 8.89 (d, 1H), 8.57 (dd, 1H), 8.57 (dd, 1H), 7.80 (dd, 1H), 7.70 (dd, 1H), 7.12 (t, 1H), 6.83 (d, 1H), 3.25 (m, 1H), 2.87 (q, 2H), 2.00 (q, H), 1.50 (m, 1H), 1.24 (m, 1H), 1.12 (t, 3H).

实施例 33

(1S,2S)-N-{顺式-2-[6-氟-3-丙酰基-2-(6-氨基吡啶-3-基羰基氧基)苯基]环丙基}-N'-(5-氰基吡啶-2-基)脲



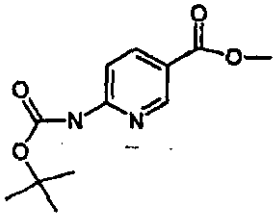
a) 6-氨基烟酸甲酯



将 6-氨基烟酸(2 g, 22 mmol)溶于甲醇(10 ml)和硫酸(0.5 ml)中. 将所述溶液回流过夜并真空蒸发溶剂. 将其粗品产物溶于水-EtOAc 中并经碳酸氢钠水溶液使之碱化. 通过 EtoAc 提取, 得到以上描述的纯的中间体(2.3 g, 70%).

^{1}H NMR(DMSO-d_6): 8.51 (dd, 1H), 7.81 (dd, 1H), 6.66 (br s, 2H), 6.45 (dd, 1H), 3.77 (s, 3H).

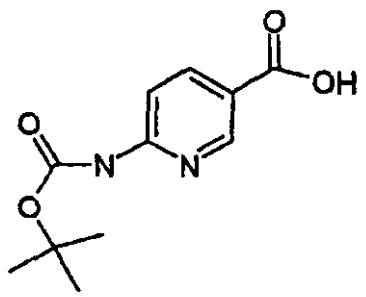
b) 6-丁氧基羰基氨基烟酸甲酯



将步骤 a)中间体(0.75 g, 4.9 mmol)溶于 THF (5 ml)中. 滴加入双

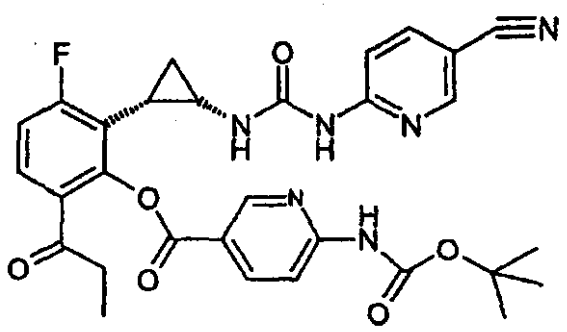
(三甲基甲硅烷基)氨化钠(5 ml, 2 M 在 THF 中). 在室温下搅拌 30 分钟. 加入在 THF(8 ml)中的二碳酸二叔丁基酯(1.1 g, 5 mmol). 在氮气氛下, 将所述反应混合物放置过夜. 然后将所述溶液真空蒸发并使之溶于 EtOAc(40 ml)和 0.1 M 盐酸(100 ml)中. 分层并以 EtOAc (40 ml)提取所述水相两次, 然后以碳酸氢钠水溶液将其稍微碱化并以 EtOAc (20 ml)再提取一次. 合并所述有机部分, 经硫酸钠干燥并经层析法(EtOAc-己烷, 1:4)纯化, 得到以上描述的纯的中间体(0.5 g, 40%).
^{1}H NMR($CDCl_3$): 8.93 (dd, 1H), 8.62 (s, 1H), 8.26 (dd, 1H), 8.06 (dd, 1H), 3.91 (s, 3H), 1.60 (s, 9H).

c) 6-叔丁氧基羰基氨基烟酸



将步骤 c)中间体(0.4 g, 1.6 mmol)悬浮于甲醇(4 ml)和水(1.25 ml)中. 加入 LiOH(0.1 g, 4 mmol). 在室温下, 将所述浆化物放置 48 小时. 然后将澄清的溶液真空浓缩并溶于水中, 以乙酸酸化(pH=4-5). 以 EtOAc 提取, 得到以上描述的纯的中间体(0.27 g, 70%).
^{1}H NMR(DMSO-d_6): 9.98 (s, 1H), 8.74 (d, 1H), 8.18 (d, 1H), 8.88 (d, 1H), 1.49 (s, 9H).

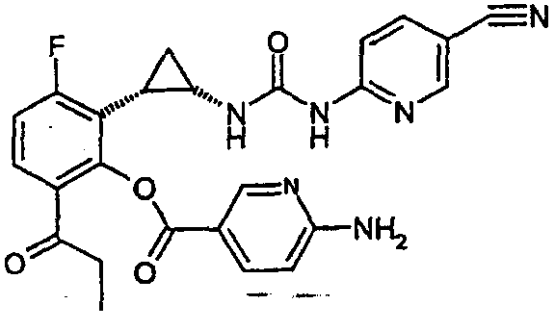
d) (1S,2S)-N-{顺式-2-[6-氟-3-丙酰基-2-(6-叔丁氧基羰基氨基-吡啶-3-基羰基氧基)苯基]环丙基}-N'-(5-氰基吡啶-2-基)脲



将实施例 8 的化合物(0.150 g, 0.41 mmol)、步骤 c)中间体(0.17 g, 0.49 mmol)、*N,N'*-二环己基碳二亚胺(0.1 g, 0.49 mmol)和 4-二甲基氨基吡啶(0.06 g, 0.49 mmol)溶于 DMF(2 ml)中．在室温下，将所述混合物搅拌过夜，然后放入 50℃油浴中 2 小时．在硅胶上蒸发并经层析法纯化，得到 N-保护的标题化合物(0.048 g, 20%).

^{1}H NMR($CDCl_3/CD_3OD$): 9.02 (s, 1H), 8.43 (dd, 1H), 8.22 (d, 1H), 8.10 (d, 1H), 7.81-7.75 (m, 2H), 7.15 (t, 1H), 7.08 (d, 1H), 3.15-3.05 (m, 1H), 2.90 (q, 2H), 1.96 (m, 1H), 1.56 (s, 9H), 1.50-1.40 (m, 1H), 1.25-1.09 (m, 4H).

e) (1S,2S)-N-{顺式-2-[6-氟-3-丙酰基-2-(6-氨基吡啶-3-基羰基氧基)苯基]环丙基}-N'-(5-氰基吡啶-2-基)脲



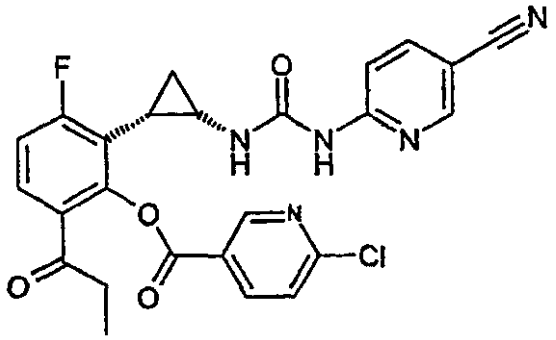
将步骤 d)中间体(0.048 g, 0.08 mmol)溶于二氯甲烷(2 ml)中．加

入三氟乙酸(1 ml)并把所述混合物搅拌 1 小时. 真空蒸发, 得到粗品标题化合物. 将所述产物溶于乙醚(2 ml)中并放置过夜. 滤出形成的白色沉淀, 得到作为三氟乙酸盐的纯的标题化合物(0.032 g, 65%).

^{1}H NMR($CD_3OD/CDCl_3$): 8.71 (d, 1H), 8.29 (dd, 1H),8.16 (t, 1H), 8.82.7.74 (m, 2H), 7.20 7.10 (m, 2H), 6.96 (d, 1H), 3.25 (m, 1H), 2.86 (m, 2H), 1.96 (m, 1H), 1.52-1.43 (m, 1H), 1.24-1.19 (m, 1H), 1.09 (t, 3H).

实施例 34

(1S,2S)-N-{顺式-2-[6-氟-3-丙酰基-2-(6-氯吡啶-3-基羰基氧基)苯基]环丙基}-N'-(5-氰基吡啶-2-基)脲

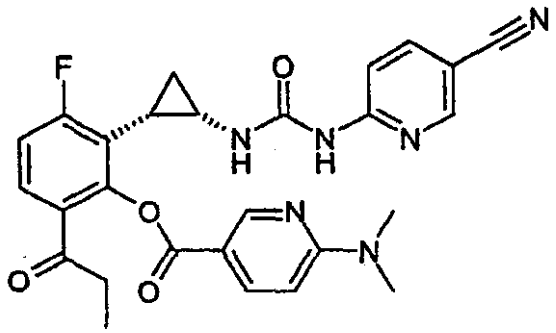


将实施例 8 的化合物(0.15 g, 0.4 mmol)、6-氯烟酸(0.076 g, 0.49 mmol)、*N,N'*-二环己基碳二亚胺(0.1 g, 0.49 mmol)和 4-二甲基氨基吡啶(0.024 g, 0.2 mmol)溶于二氯甲烷(4 ml)中. 将所述混合物放置过夜, 真空蒸发, 经层析法(EtOAc-己烷, 1:2)纯化, 得到标题化合物(0.067 g, 32%).

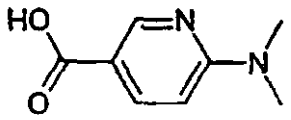
^{1}H NMR($CDCl_3$): 9.77 (br s, 1H), 9.18 (br d, 2H), 8.39 (dd, 1H), 8.14, 7.79 (dd, 1H), 7.71 (dd, 1H), 7.46 (d, 1H), 7.13 (t, 1H), 6.92 (d, 1H), 3.25 (m, 1H), 2.88 (q, 2H), 2.00-1.90 (m, 1H), 1.55-1.46 (m, 1H), 1.25-1.22 (m, 1H), 1.11 (t, 3H).

实施例 35

(1S,2S)-N-{顺式-2-[6-氟-3-丙酰基-2-(6-二甲基氨基吡啶-3-基羰基氧基)苯基]环丙基}-N'-(5-氰基吡啶-2-基)脲



a) 6-二甲基氨基烟酸



在 130℃下，在密封压力容器中将 6-氯烟酸(0.5 g, 3.17 mmol)和二甲基胺(10 ml, 40%在水中)加热 6 小时．然后除去溶剂并以水提取残余物，并把其 pH 调至 4-5．以二氯甲烷提取，得到以上描述的纯的中间体(0.1 g, 20%).

^{1}H NMR($CDCl_3$): 8.87 (dd, 1H), 8.04 (dd, 1H), 6.49 (dd, 1H), 3.18 (s, 6H).

b) (1S,2S)-N-{顺式-2-[6-氟-3-丙酰基-2-(6-二甲基氨基吡啶-3-基羰基氧基)苯基]环丙基}-N'-(5-氰基吡啶-2-基)脲

将实施例 8 的化合物(0.13 g, 0.3 mmol)、步骤 a)中间体(0.05 g, 0.3 mmol)、*N,N'*-二环己基碳二亚胺(0.09 g, 0.4 mmol)和 4-二甲基氨基吡啶(0.02 g, 0.18 mmol)溶于二氯甲烷(3ml)和 DMF(1ml)中．将所述混合物放置过夜，真空蒸发并经层析法(EtOAc-己烷, 2:1)纯化，得到标题化合物(0.06 g, 39%).

^{1}H NMR($CDCl_3$): 10.10 (br s, 1H), 9.29 (br s, 1H), 8.18 (d, 1H), 8.12 (dd, 1H), 7.76-7.60 (m, 2H), 7.06 (t, 1H), 6.95 (d, 1H), 6.62 (d, 1H), 3.18 (m, 7H), 2.83 (q, 2H), 2.10-1.99 (m, 1H), 1.51-1.42 (m, 1H), 1.19 (m, 1H), 1.09 (t, 3H).

实施例 36

(1S,2S)-N-[顺式-2-(6-氟-2-O-3-丙酰基苯基)-环丙基]-N'-(5-氰基吡啶-2-基)脲-O-4-羟基苯甲酸酯

a) 4-苄氧基苯甲酸

向 4-羟基苯甲酸(6.9 g, 50 mmole)在 150 ml 的 DMF 中的溶液中加入叔丁醇钾(12.34 g, 110 mmole), 并在室温下将所述混合物搅拌一小时. 加入苄基溴(20.5 g, 120 mmole)并在室温下将所述混合物搅拌两天. 减压下蒸发所述混合物并加入 100 ml 的 1,4-二氧六环和氢氧化钠(6.0 g, 150 mmole)在 50 ml 水中的溶液. 将所述混合物回流两小时, 冷却并减压下蒸发. 加入水并以乙酸使所述混合物酸化. 将所述产物过滤, 以冷水洗涤并干燥. 产量: 10.2 g=89%.

b) 4-苄氧基苯甲酰氯

向 4-苄氧基苯甲酸(2.28 g, 10 mmole)在 20 ml 干燥二氯甲烷中的混合物中加入 5 滴 DMF 和 2.5 ml 亚硫酰氯. 将所述混合物回流三小时并减压下蒸发. 产量: 2.45 g=100%.

c) (1S,2H)-N-[顺式-2-(6-氟-2-O-3-丙酰基苯基)环丙基]-N'-[2-(5-氰基吡啶-2-基)脲-O-4-苄氧基苯甲酸酯

向(1S,2S)-N-[顺式-2-(6-氟-2-羟基-3-丙酰基苯基)环丙基]-N'-(5-氰基吡啶-2-基)脲(184 mg, 0.5 mmole)在 3 ml 的 DMF 中的溶液中加入叔丁醇钾(78.5 mg, 0.7 mmole), 并在室温下将所述混合物搅拌一小时. 加入 4-苄氧基苯甲酰氯(185 mg, 0.75 mmole)在 1 ml 的 DMF 中的溶液并在室温下把所述混合物搅拌过夜. 加入 40 ml 乙酸乙酯并以水将所述有机相洗涤四次. 以硫酸钠干燥所述溶液并减压下蒸发.

产物经硅胶柱层析法分离. 产量: 180 mg=62%.

^{1}H-NMR(DMSO δ -6): 0.92 (m, 4H), 1.31 (m, 1H), 1.85 (m, 1H), 2.82 (m, 2H), 3.06 (m, 1H), 5.26 (s, 2H), 7.20 (m, 2H), 7.38-8.12 (m, 11H), 8.38 (m, 1H).

d) (1S,2S)-N-[顺式-2-(6-氟-2-O-3-丙酰基苯基)环丙基]-N'-(5-氰基吡啶-2-基)]脲-O-4-羟基苯甲酸酯的合成

在室温和正常压力下, 将(1S,2S)-N-[顺式-2-(6-氟-2-O-3-丙酰基苯基)环丙基]-N'-(5-氰基吡啶-2-基)脲-O-4-苄氧基苯甲酸酯(170 mg, 0.29 mmole)在 15 ml 乙酸乙酯和 15 ml 甲醇中的溶液用 10%披钯炭(30 mg)氢化三次. 过滤催化剂并以乙酸乙酯和甲醇洗涤, 并减压下将所述溶液蒸发. 产物经硅胶柱层析法分离. 产量: 100 mg=70%.

^{1}H-NMR(DMSO δ -6): 0.93 (m, 4H), 1.32 (m, 1H), 1.88 (m, 1H), 2.85 (m, 2H), 3.05 (m, 1H), 6.92 (m, 2H), 7.38 (m, 2H), 8.00 (m, 4H), 8.38 (m, 1H).

实施例 37

(1S,2S)-N-[顺式-2-(6-氟-2-O-3-丙酰基苯基)-环丙基]-N'-[2-(5-氰基吡啶基)]脲-O-亚甲基-4-羟基苯甲酸酯

a) 4-(4-甲氧基苄氧基)苯甲酸甲酯

向 4-羟基苯甲酸甲酯(6.85 g, 45 mmole)在 80 ml DMF 中的溶液中加入叔丁醇钾(5.6 g, 51 mmole)并在室温下将所述混合物搅拌一小时. 加入 4-甲氧基苄基氯(8.3 g, 52 mmole)并在室温下将所述混合物搅拌过夜. 减压下将所述混合物蒸发并加入 200 ml 乙酸乙酯. 以水将所述有机相洗涤四次, 以硫酸钠干燥并减压下蒸发. 产量: 12.3 g =100%.

^{1}H-NMR($CDCl_3$): 3.82 (s, 3H),3.88 (s, 3H), 5.03 (s, 2H), 6.96 (m, 4H), 7.36 (d, 2H), 7.98 (d, 2H).

b) 4-(4-甲氧基苄氧基)苯甲酸

向 4-(4-甲氧基苄氧基)苯甲酸甲酯(12.2 g, 44.8 mmole)在 50 ml 的 1,4-二氧六环中的溶液中加入氢氧化锂(2.15 g, 89.6 mmole)溶液并在 60℃下把所述混合物搅拌过夜. 减压下蒸发所述混合物并加入 5%乙酸. 将所述产物过滤, 以水洗涤并干燥. 产量: 10.1 g = 87%.

^{1}H-NMR(DMSO δ -6): 3.74 (s, 3H), 5.08 (s, 2H), 6.92 (d, 2H), 7.06 (d, 2H), 7.36 (d, 2H), 7.90 (d, 2H).

c) 4-(4-甲氧基苄氧基)苯甲酸氯甲酯

向 4-(4-甲氧基苄氧基)苯甲酸(5.16 g, 20 mmole)在 100 ml 的 1,4-二氧六环中的溶液中加入 40%氢氧化四丁基铵(14.27 g, 22 mmole)溶液并在室温下将所述混合物搅拌 2 小时. 减压下将所述混合物蒸发并与 1,4-二氧六环共同蒸发两次且与甲苯共同蒸发两次. 将所述干燥的产物溶于 60 ml 二氯甲烷中并加入碘氯甲烷(35.3 g, 200 mmole). 在室温下, 将所述溶液搅拌两天并减压下蒸发. 加入大约 100 ml 乙酸乙酯并以水洗涤所述有机相两次. 以硫酸钠干燥并减压下蒸发. 经硅胶柱层析法纯化其产物. 产量: 4.48 g = 73%.

^{1}H-NMR($CDCl_3$): 3.83 (s, 3H), 5.06 (s, 2H), 5.94 (s, 2H), 7.00 (m, 4H), 7.36 (d, 2H), 8.05 (d, 2H).

d) 4-(4-甲氧基苄氧基)苯甲酸碘甲酯

向 4-(4-甲氧基苄氧基)苯甲酸氯甲酯(0.77 g, 2.5 mmole)在 15 ml 干燥丙酮中的溶液中加入碘化钠(1.87 g, 12.5 mmole)并在室温下将所述混合物搅拌过夜. 减压下将所述混合物蒸发并以乙酸乙酯/水提取. 以 5%硫代硫酸钠溶液洗涤所述有机相, 以硫酸钠干燥并减压下蒸发. 产量: 0.86 g = 86%.

^{1}H-NMR($CDCl_3$): 3.84 (s, 3H), 5.05 (s, 2H), 6.14 (s, 2H), 6.98 (m, 4H), 7.36 (d, 2H), 8.00 (d, 2H).

e) (1S,2S)-N-[顺式-2-(6-氟-2-O-3-丙酰基苯基)环丙基]-N'-[2-(5-氰基吡啶基)脲-O-亚甲基-4-(4-甲氧基苄氧基)苯甲酸酯

向(1S,2S)-N-[顺式-2-(6-氟-2-羟基-3-丙酰基苯基)环丙基]-N'-[2-(5-氰基吡啶基)脲(368 mg, 1 mmole)在 5 ml 的 DMF 中的溶液中加入 60%氢化钠在矿物油中的悬浮液(44 mg, 1.1 mmole)并在室温下将所述混合物搅拌一小时. 加入 4-(4-甲氧基苄氧基)苯甲酸碘甲酯(0.84 g, 2.1 mmole)在 2 ml 的 THF 中的溶液并在室温下将所述混合物搅拌过夜. 加入 50 ml 乙酸乙酯并以水把所述有机相洗涤四次, 以硫酸钠干燥并减压下蒸发. 经硅胶柱层析法分离所述产物. 产量: 525 mg = 82%.

^{1}H-NMR($CDCl_3$): 0.91 (m, 3H), 1.32 (m, 1H), 1.60 (m, 1H), 2.04 (m, 1H), 2.90 (m, 2H), 3.20 (m, 1H), 3.82 (s, 3H), 5.04 (s, 2H), 5.84-6.06 (m, 2H), 6.91-8.18 (m, 13H).

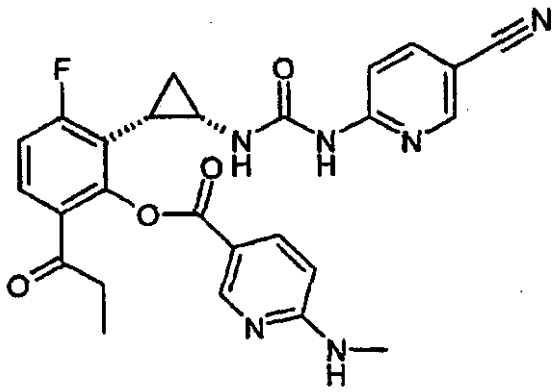
f) (1S,2S)-N-[顺式-2-(6-氟-2-O-3-丙酰基苯基)环丙基]-N'-[2-(5-氰基吡啶基)]脲-O-亚甲基-4-羟基苯甲酸酯

向(1S,2S)-N-[顺式-2-(6-氟-2-O-3-丙酰基苯基)环丙基]-N'-[2-(5-氰基吡啶基)脲-O-亚甲基-4-(4-甲氧基苄氧基)苯甲酸酯(100 mg, 0.156 mmole)在 4 ml 二氯甲烷中的溶液中加入 TFA(0.5 ml)并在室温下将所述溶液搅拌一小时. 减压下将所述溶液蒸发并经硅胶柱层析法分离产物. 产量: 45 mg = 55%.

^{1}H-NMR(DMSO δ -6): 0.84 (m, 3H), 1.10 (m, 1H), 1.48 (m, 1H), 2.12 (m, 1H), 2.80 (m, 2H), 3.19 (m, 1H), 5.85-6.02 (m, 2H), 6.84 (m, 2H), 7.18 (m, 1H), 7.46 (m, 2H), 7.74 (m, 2H), 8.04 (m, 2H), 8.38 (m, 1H).

实施例 39

(1S,2S)-N-{顺式-2-[6-氟-3-丙酰基-2-(6-甲基氨基吡啶-3-基羰基氧基)苯基]环丙基}-N'-(5-氰基吡啶-2-基)脲



通过以实施例 31 相同的方法，由 6-甲基氨基烟酸(0.050 g, 0.33 mmol)和实施例 8 的化合物(0.1 g, 0.27 mmol)制备该化合物．经层析法(乙酸乙酯)纯化所述粗品产物(含有标题化合物和未反应的原料)，得到 0.030 g(22%)标题化合物．

^{1}H.NMR($CDCl_3$): 9.8 (br s, 1H), 9.25 (br s, 1H), 8.90 (d, 1H), 8.20 (d, 1H), 8.10 (m, 1H), 7.72 (m, 2H), 7.08 (t, 1H), 6.9 (d, 1H), 6.37 (d, 1H), 3.20 (m, 1H), 2.95 (d, 3H), 2.85 (q, 2H), 1.95 (m, 1H), 1.48 (m, 1H), 1.10 (t, 3H).

生物实施例 1

抗性模式

检测本发明化合物用于抗多种 HIV 株的抗病毒活性，所述的多种 HIV 株包括野生型和使用从在 Schinazi 等的综述中, International Antiviral News, 第 4 卷，第 6 期, 95-107 页(1996)描述的其它非核苷类逆转录酶抑制剂所出现的已知突变型．结果呈现在表 1 中．

表 1

HIV 株	实施例 5	实施例 6	实施例 8	现有技术*
野生型	0.0012 +/-0.0004	0.0008 +/-0.0004	0.0007 +/-0.0002	0.0056 +/-0.004
野生型 50%血清	0.01 +/-0.009	0.006 +/-0.003	0.007 +/-0.001	0.023 +/-0.011
K103N	0.05 +/-0.04	0.017 +/-0.008	0.037 +/-0.007	0.13 +/-0.060
K103N 50%血清	0.38 +/-0.31	0.17 +/-0.07	0.39 +/-0.31	0.9 +/-0.6
Y181C	0.017 +/-0.018	0.006 +/-0.002	0.006 +/-0.001	0.13 +/-0.02
Y181C 50%血清	0.10 +/-0.06	0.08 +/-0.05	0.08 +/-0.06	0.13 +/-0.07
Y188L	0.13 +/-0.07	0.08 +/-0.06	0.06 +/-0.02	0.17 +/-0.03
Y188L 50%血清	1.5 +/-0.9	0.9 +/-0.05	1.0 +/-0.05	1.9 +/-1.5
L100I,Y181C	ND	ND	0.34 +/-0.06	1.0
L100I	ND	ND	0.009 +/-0.001	0.026 +/-0.009
SI	>41 600	22 500	87 000	5 900
SI 50%血清	ND	8 830	4 285	800

所述测试包括在 MT-4 细胞中以 XTT 诱导的多种测定(Weislow 等, J Nat Cancer Inst 1989, 第 81 卷 第 8 期, 577 页等), 这包括在 50% 人血清存在下表明有助于蛋白结合的作用的测定. ED_{50} 以 μ g/ml 呈现. 基于计算的治疗指数(SI)的原始数据也被呈现, 其定义在相应于无 HIV 细胞产生 50%毒性的剂量除以所述 ED_{50}. 来自所述 1995 ICAR

Santa Fe 的现有技术的化合物如上描述.

显而易见地是本发明化合物特别是所述对映体具有比迄今已知的化合物明显更低的 ED_{50} 值，包括抗已知未定的突变型 K103N 和 Y181C 以及 L100I 和双重突变型 L100I、Y181C 的值. 此外，所述对映体的治疗指数比现有技术的化合物高 5 至 10 倍. 在 HIV 疗法的内容中将观察到这些结果，即使患者生命的其余部分不能显著抗众所周知的原病毒 HIV，那么患者也能够期待长期服药治疗. 因此，需要大的 SI 以避免蓄积毒性，同时适当给药以维持治疗压力并且防止自发产生对 HIV 株的抗性.

生物实施例 2

时间与抗性

在微量滴定板上，以 5-10 $TCID_{50}$ 的 $HIV\text{-}1_{IIIB}$ 感染每孔 2×10^4 MT4 细胞. 使用每浓度 8 对，以大约 ED_{50} 的浓度加入受试化合物. 孵育 6 天后测量在 10 μl 上清液中的 RT 活性.

在随后每周一次培养物传代之后，进行以下过程：使在显示>50%的未处理感染细胞的 RT 活性的受试化合物的浓度(SIC，初始抑制浓度)下产生的病毒传代给新鲜的 MT4 细胞. 将来自所述八对试验的每一个试验的 15 μl 上清液转移至无受试化合物的细胞(对照组)中和含有相同浓度受试化合物的细胞中，并且另两种分别具有五倍高的浓度. (参见以下表 2).

当允许病毒在最高非毒性浓度(5-40 μM)下生长时，收集 2-4 平行孔并且扩展给出物料用于结果的分析和交叉方式抗性.

表 2

允许病毒生长

所抑制的病毒产生

				125 × SIC
			125 × SIC	***25 × SIC*** →
			25 × SIC	***5 × SIC***
		25 × SIC	***5 × SIC*** →	***无化合物***
	25 × SIC	***5 × SIC*** →	***无化合物***	
	5 × SIC	SIC		
	SIC →	***无化合物***		
SIC →	***无化合物***			
传代(pass)1	**传代 2**	**传代 3**	**传代 4**	**传代 5**

图 1 描绘了对于本发明化合物(实施例 8)病毒抗性生长对时间的曲线. 也描绘了以上提到的最近的 Santa Fe 化合物的相应曲线. 显而易见的是本发明化合物显示显著低的抗药性发展的速率.

生物实施例 3

P450 代谢

在以人细胞色素 P450 cDNA(超体(supersomes))Gentest 公司 Woburn USA 转染的杆状病毒感染的昆虫细胞中，测定本发明化合物经过人细胞色素系统 P450 的主要同种型的代谢.

在过度表达多种细胞色素 P450 同种型的超体的存在下，在 0.5、5 和 50 μ M 的浓度下，一式两份孵育所述受试化合物，该同种型包括 CYP1A2 + P450 还原酶、CYP2A6 + P450 还原酶、CYP2C9-Arg 144 + P450 还原酶、CYP2C19 + P450 还原酶、CYP2D6-Val 374 + P450 还原酶和 CYP3A4 + P450 还原酶. 孵育液含有固定浓度(例如 50 皮摩尔)的细胞色素 P450 并且进行 1 小时以上. 通过 UV HPLC 色谱测量母体化合物的消失来测定在所述受试化合物代谢中所涉及的给定

同种型.

将所述三种浓度测试 7.5 分钟后，所述寿命保留百分比图提示 CYP3A4、1A2、2C19 和 2A6 参与实施例 7 化合物的代谢. 相似构象的 P450 同种型也参与现有技术的 Santa Fe 卤代吡啶基化合物的代谢.

令人惊奇地是对实施例 8 化合物，未记录到明显的具有任何异构体的 P450 代谢，暗示所述化合物在体内稳定并且联合给予药物的代谢干扰的可能性是相应低的.

生物实施例 4

药物代谢动力学

监控来自口服给予式 II 的前药的式 I 化合物在大鼠中的释放. 以相应于 0.027 mmol/kg 的剂量，将实施例 7 化合物溶解在丙二醇媒介中并且口服给予成对禁食的雄性 Sprague-Dawley 大鼠. 在指明的时间间隔，从植入 canis 颈静脉的导管采集 0.2 ml 的血，离心并冷冻用于以后的分析. 释放的式 I 药物(实施例 6)经 HPLC 测试. 将含有 40-100 μl 的每个血浆样品的等分试样与等体积乙腈混合(10 秒, Vibrofex). 将所述样品离心(2 分钟, 14000 RPM)并把 30 μl 的上清液注射进入如下的 HPLC 系统.

预柱:	RP-18, 7 μM, 15×3.2 mm
柱:	YMC 基质，3 μM, 150×3 mm
流动相:	60%乙腈在 3 mM 乙酸铵中，pH 6.4
流速:	0.4 ml/min
检测:	UV, 250 nm

表 3

时间 (min)	母体化合物的血浆水平(μg/ml)
30	0.24, 0.35
60	0.18, 0.28
120	0.13, 0.17
240	0.07, 0.12
360	0.05, 0.07

在表 3 中，很清楚，口服给予式 II 的前药体内释放临床上显著量的式 I 化合物.

生物实施例 5-8

i) 预备

在药代动力学实施例中使用的大鼠为体重大约 200-250 g 的雄性 Sprague-Dawley 大鼠. 在实验之前，所述大鼠至少禁食 16 小时，但是自由接近水. 在所述实验的前一天，使用的 Efrane®、笑气和氧的混合物麻醉所述大鼠. 将导管插入大鼠颈静脉. 在实验那天，记录大鼠的体重. 在口服给予药物或静脉给药注射进入到所述颈的背部之前，短暂麻醉所述动物. 每种物质给予两只大鼠.

在口服给药之前，猴子禁食 12 小时，但是自由接近水. 借助婴儿鼻胃喂饲管给予所述受试化合物. 在 6 小时后，所述猴子得到苹果.

ii) 剂量制备

把在以下实施例中描述的适当量的所述活性成分溶解/悬浮在丙二醇溶液中或 10%阿拉伯胶和 1%吐温在水的溶液中，以用于口服给药. 将化合物溶于 DMSO 中以用于静脉给药.

iii) 血样采集

在药物给予前，采集血样(大鼠一般为 0.6 ml, 猴子为 2 ml)并在在药物给予后于指定的时间间隔内(如图所示)，如同前者取得血样. 从股静脉轻叩使猴血进入含有 EDTA 的试管中. 将血样离心，以 1% SDS/64*/20 分钟中和感染的药物并将血浆于-20℃下贮存.

iv) 生物分析

如下制备血浆样品：将 40-100μl 的血浆与等体积乙腈混合(10 秒, Vibrofex)．将所述样品离心(2 分钟, 14000 RPM)并把 30μl 的上清液注射进入如下的 HPLC 系统．

预柱:	RP-18, 7 μM, 15×3.2 mm
柱:	YMC 基质，3 μM, 150×3 mm
流动相:	60%乙腈在 3 mM 乙酸铵中，pH 6.4
流速:	0.4 ml/min
检测:	UV, 250 nm

生物实施例 5

与最接近的现有技术化合物的比较

将式 I 化合物的体内稳定性和有效性与最接近的 Santa Fe 化合物即(+/-)-N-(顺式-2-(6-氟-2-羟基-3-丙酰基苯基)-环丙基)-N'-(5-氯吡啶-2-基)-脲相比较，从而给予在 DMSO 媒介中 0.024 mmol/kg 剂量的所述各化合物．图 2 为整个时间内所述各化合物的血浆水平的曲线图(在每种情况下 n=2)．显而易见的是所述各曲线按照常规模式，但是本发明化合物具有超过最接近的现有技术化合物的 AUC(0-4 小时)1.5 倍的 AUC(0-4 小时)．换言之，本发明化合物比先前描述的衍生物提供大于 50%的体内接触，无论是否由于本发明化合物比现有技术化合物更缓慢的清除率还是更高程度的组织结合也已经被测定．

生物实施例 6

前药与母体化合物的生物等效性

将多种式 II 化合物(即式 I 化合物的前药)给予大鼠并全程监控本发明母体化合物(在这个实施例中为实施例 10 化合物)的血浆水平．所述溶媒为在水或丙二醇中 10%阿拉伯胶和 1%吐温(加星号)．在表 4 中的血浆水平数据指的是个体动物．

表 4

化合物	剂量 (mmol/kg)	时间 (min)	母体化合物的血浆水平 (μg/ml)			
实施例 12	0.053	30	0.2	0.3	0.06	0.11
		60	0.2	0.4	0.12	0.20
		90	0.3	0.4		
		120	0.2	0.5	0.10	0.20
		180	0.3	0.4	0.11	0.23
		240	0.3	0.4	0.08	0.24
		330			0.08	0.15
		420			0.05	0.12
实施例 12	0.026	30	0.09	0.05		
		60	0.10	0.07		
		120	0.09	0.08		
		180	0.08	0.08		
		240	0.06	0.05		
		330		0.03		
		420		0.02		
实施例 22	0.026	30		0.08		
		60	0.05	0.11		
		120	0.04	0.08		
		180	0.03	0.07		
		240	0.02	0.04		
		360	<0.02	<0.02		
实施例 14	0.053	30	0.10	0.08		
		60	0.15	0.08		
		120	0.27	0.07		
		180	0.35	0.09		
		240	0.35	0.09		
		360	0.24	0.12		
实施例 18	0.053	30	0.12	0.03		
		60	0.15	0.03		
		120	0.15	0.07		
		180	0.23	0.14		
		240	0.12	0.16		
		360	0.08	0.08		

实施例 23	0.053	30	0.14	0.32		
		60	0.22	0.49		
		120	0.36	0.49		
		180	0.44	0.32		
		240	0.35	0.27		
		360	0.14	0.14		
实施例 17	0.053	30	0.05	0.05		
		60	0.07	0.05		
		120	0.06	0.14		
		180	0.07	0.20		
		240	0.07	0.17		
		360	0.04	0.12		
实施例 29	0.027*	30	0.258	0.031		
		60	0.268	<0.03		
		120	0.128	<0.03		
		240	0.051	<0.03		
		360	<0.03	<0.03		
实施例 37	0.027*	30	0.234	0.137		
		60	0.273	0.189		
		120	0.111	0.133		
		240	0.056	0.045		
		360	0.054	0.056		

显而易见地是式 II 的前药体内将临床相应量的式 I 化合物释放进入血液. 所述绝对口服生物利用度(相对于所述 iv 剂量进行测定, 如同在制备部分描述的那样)对实施例 37 的化合物为 28-33%和对服用实施例 27 的化合物的评价动物为 27%.

生物实施例 7

不同物种中的生物利用度

将本发明式 II 的前药(实施例 12)以相同剂量(0.026 mmol/kg)和相同的溶媒(在水中 10%阿拉伯胶和 1%吐温)给予大鼠和短尾猴. 式 I 母体化合物(实施例 10)的血浆水平作为时间的函数来测量.

表 5

物种	时间 (min)	母体化合物的血浆水平 (μg/ml)	
大鼠	30	0.09	0.05
	60	0.10	0.07
	120	0.09	0.08
	180	0.08	0.08
	240	0.06	0.05
	330		
	420		
猴子	45	0.08	0.04
	90	0.20	0.26
	180	1.0	0.55
	240	0.72	0.54
	360	0.38	0.39
	600	0.13	0.10
	24 小时	0.03	0.03

显而易见地是式 II 的前药体内释放临床相应量的式 I 化合物. 释放既发生在啮齿动物也发生在灵长目动物，在灵长目动物中存在显著更高的血浆水平.

实施例 28 的化合物的相应数据(大鼠：阿拉伯胶/吐温，猴子：丙二醇)显示在表 5A 中:

表 5A

物种	时间 (min)	母体化合物的血浆水平 (μg/ml)	
大鼠	30	0.033	0.046
	60	0.039	0.084
	120	0.066	0.123
	240	0.039	0.034
	360	<0.03	<0.03
猴子	30	0.108	<0.03
	90	0.159	0.098
	180	0.062	0.050
	240	<0.03	0.060
	540	0.036	0.070

生物实施例 8

抗病毒活性

在所述 XTT 甲臜测试中，在有和无 50%人血清存在下，测试式 I 化合物用于抗野生型 HIV_{IIIB} 和抗性突变型的 HIV-1 活性，其中在 MT4 细胞中测试对致细胞病变效应的抑制作用，在每一种情况下 ED_{50} 以μM 表明。

表 6

HIV 株	实施例 10	实施例 10 50%血清	实施例 11	实施例 11 50%血清
野生型	0.01	0.06	0.009	0.05
L100I	0.05	0.33	0.09	0.95
K103N	0.38	2.4	0.09	2.0
Y181C	0.09	0.4	0.07	3.3

在体内可达到的浓度下，式 I 化合物对抗多种 HIV 株具有高度活性。

生物实施例 9

抗病毒活性

使用现有技术细胞培养测试也对本发明化合物与所述最接近的现有技术化合物进行了比较，其中人 T 细胞系 MT4 细胞在接种进入 96 孔微量滴定板($2 \cdot 10^4$ 细胞/孔)的补充 10%胎牛血清、青霉素和链霉素的 RPMI 1640 培养基中生长，以每孔的 $HIV\text{-}1_{IIIB}$(野生型)或者载荷有 RT Ile 100、Cys 181 或 Asn 103 突变的突变型病毒 10-20 $TCID_{50}$ 感染．将被连续稀释的受试化合物加入到各自的孔中并将所述培养物在 37℃下于富含 CO_2 的气氛中孵育，并且在第五天或第六天用 XTT 活体染料测定细胞生存力．所述结果显示以下多种测定的平均值．结果表示为 ED_{50} μM.

表 8

实施例	野生型	野生型 50%血清	Ile100	Cys181	Asn103
现有技术 Santa Fe	0.027	nd	0.220	0.340	0.350
实施例 10	0.012	0.056	0.053	0.095	0.358
实施例 11	0.008	0.058	0.100	0.069	0.080
实施例 8	0.003	0.019	0.021	0.019	0.086
实施例 6	0.002	0.016	0.064	0.018	0.046

本发明化合物已显著改善抗野生型并且特别是抗临床重要的、在以 NNRTIs 治疗期间出现的突变型的性能.

生物实施例 10

结合动力学

NNRTI 在所述靶酶上的结合与解离率能够直接通过表面细胞质基因组共振方法进行测试，其中将逆转录酶固定在嵌片的表面并且通过观察由伴随嵌片的质量增加或减少引起的折射率的变化来监控

假定的抑制剂的结合或者解离．如同以上描述的那样，将本发明化合物(实施例 8)与来自 Santa Fe 的最接近的现有技术的化合物进行比较．实验在 Biacore 2000(Biacore AB, Uppsala, 瑞典)上进行，使用 BIA 评价软件(3.0 版)用于评价数据．所述小的分析物(NNRTI)对大得多的酶的结合导致在 10-20 RU 的范围内结合应答．在流动的缓冲液和样品之间的总体折射率的差异使得评价在注射样品期间得到的数据是困难的．在所述解离相期间，在总体折射率方面存在不明显的变化，因此在这个相中已评价了不同物质的结合．

固定：通过直接偶合于伯胺，所述酶和对照蛋白固定在 CM5 嵌片上(Markgren 等, 1998)．对 Fc g(Biacore BR-1000-57)的抗体被用作对照蛋白并且按照制造商的说明书固定．使用 Nanosept 离心浓缩器 10K(Pall Filtron, MA, U.S.A)，将 HIV 逆转录酶(Unge 等, 1990)从 3 M 的$(NH_4)_2SO_4$转移至含有 4 mM $MgCl_2$ 的 5 mM Hepes, pH 7.6 中．将相应于 6800-9700 RU 量的 RT 固定于所述传感器嵌片上．通过注射 35 ml 的 0.5 M Tris pH 7.6、4 mM $MgCl_2$、0.5 M KCl 钝化所述传感器表面．所述固定方法在 33℃下进行．

与抑制剂的相互作用：将抑制剂的贮存液(1 mg/ml 在 DMSO 中)溶于 RT 流动的缓冲液(10 mM Hepes pH 7.6、4 mM $MgCl_2$、0.25 mM 精胺、40 mM KCl、0.5% Triton X-100、3% DMSO、0.5%胎牛血清)中以达到 10 mM 的浓度．通过注射 200 ml 的所述稀释物质来分析物质对 RT 的结合，流速为 20 ml/min 和温度为 25℃．在每一次注射物质后，通过注射在 RT 的流动的缓冲液中的 120 ml 的 10%的 DMSO 洗涤所述系统．

所述结果在图 3 中得到描绘．显而易见地是本发明化合物和现有技术的化合物显示不同的相互作用动力学，本发明化合物具有最低的解离率，这表明其更有效地与所述酶结合．

参考文献:

Unge T, Ahola H, Bhikhabhai R, Backbro K, Lovgren S, Fenyo EM,

Honigman A, Panet A, Gronowitz JS, Strandberg B, HIV-1 逆转录酶(RT)的表达、纯化和结晶. AIDS Res Hum Retroviruses 1990 年 11 月; 6(11):1297-303.

Markgren P-O, Hamalainen M, Danielson UH, 使用光学生物传感器技术筛选与 HIV-1 蛋白酶相互作用的化合物. Analytical Biochemistry 1998, 第 265 卷、(在印刷中).

尽管参照以上具体的实施例、比较实施例和附图已阐明了本发明的多个方面和实施方案，易于理解的是本发明不以任何方式局限于这些实施方案，而是扩展到整个所附的权利要求书的精神和范围内.

图 1

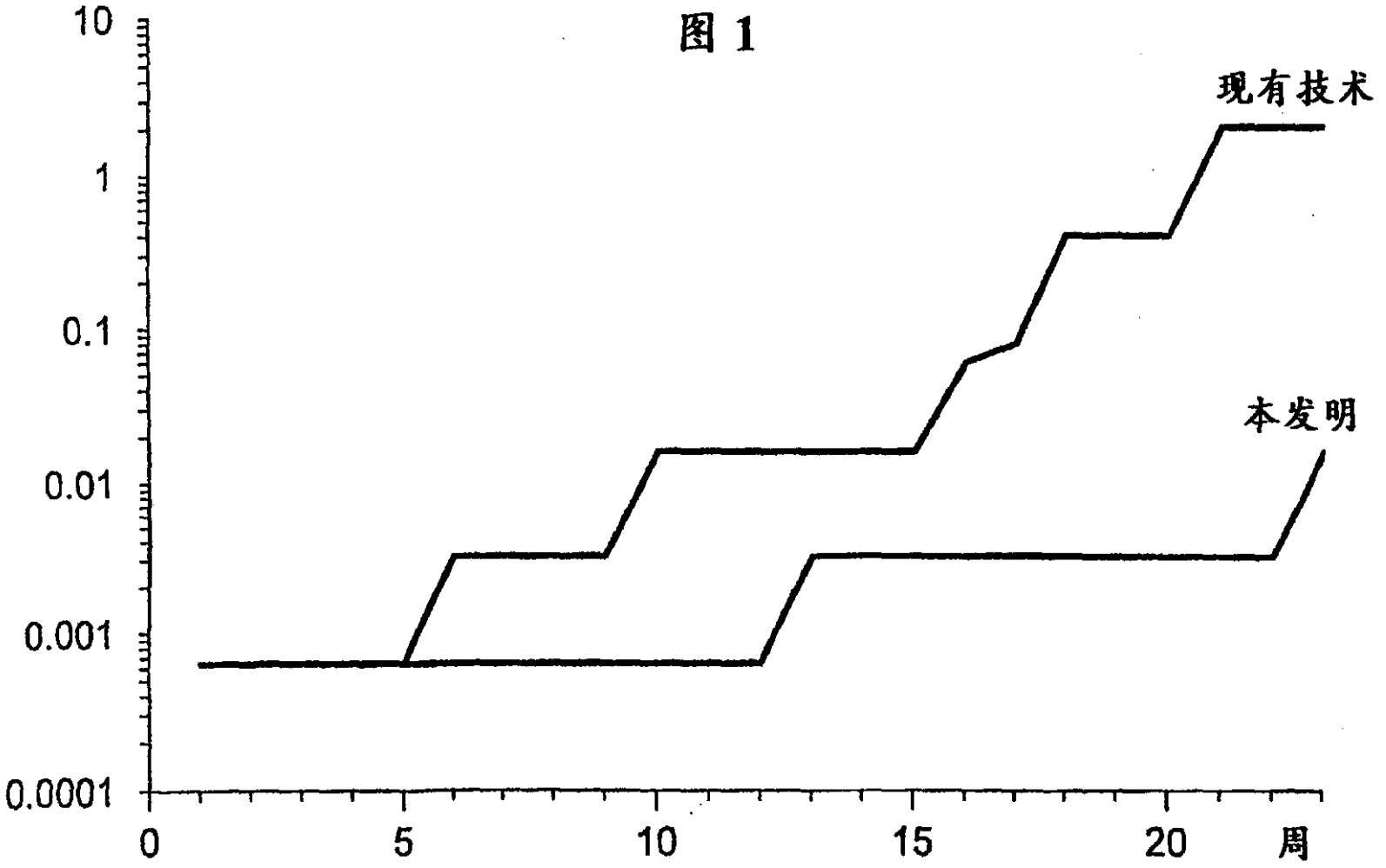
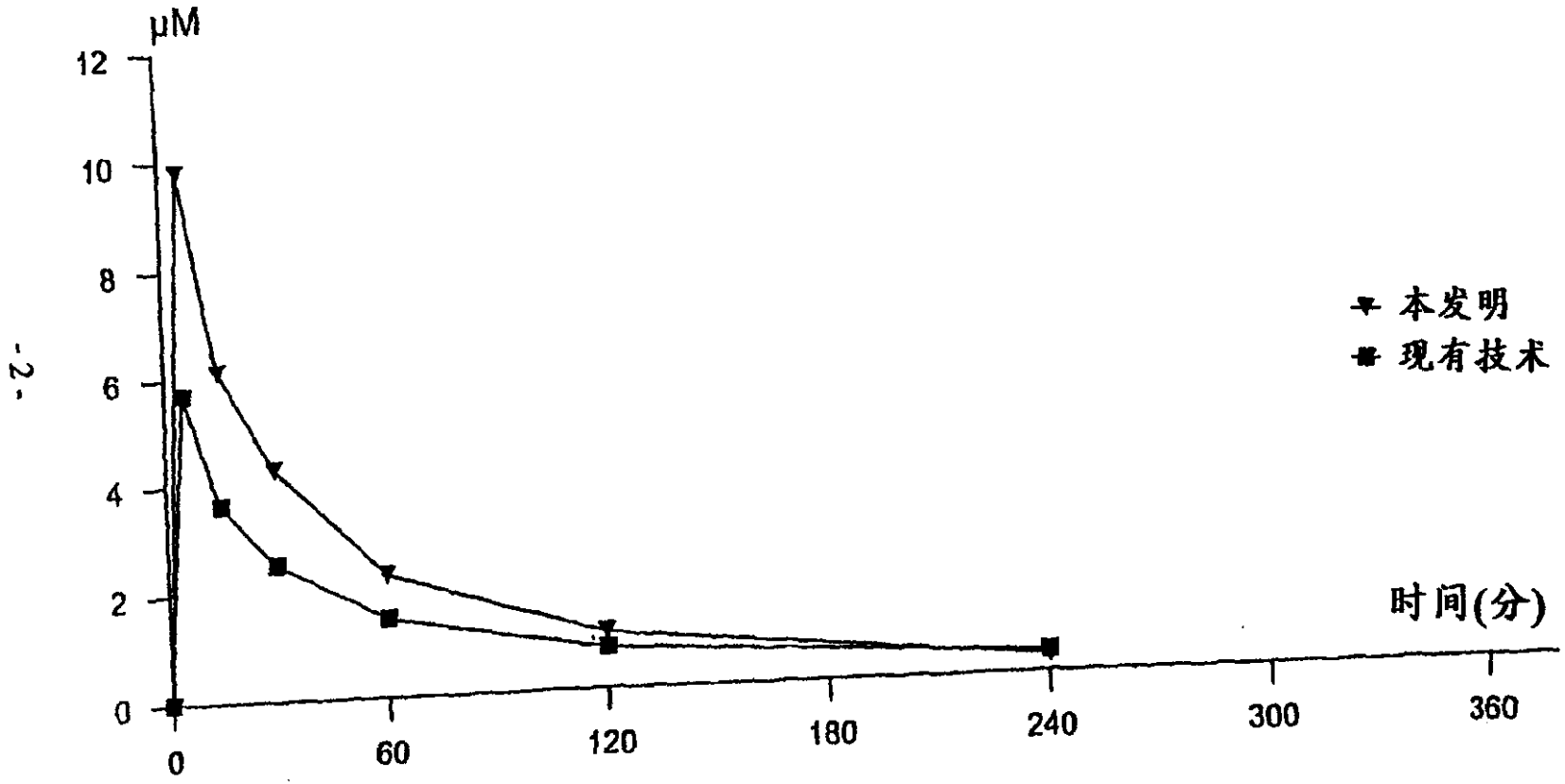


图 2



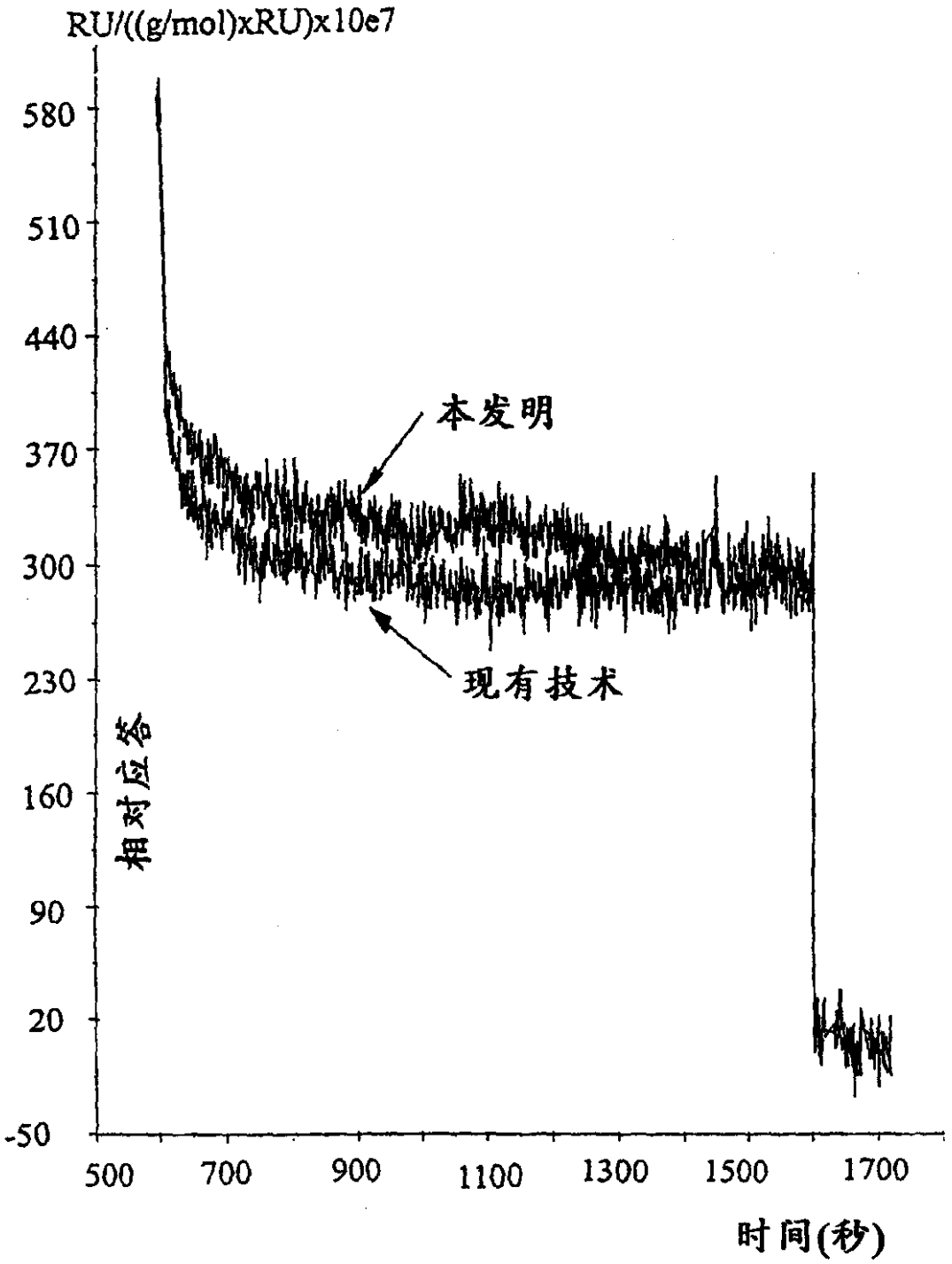

RU/((g/mol)xRU)x10e7
580
510
440
370
300
230
160
90
20
-50
本发明
现有技术
相对应答
500
700
900
1100
1300
1500
1700
时间(秒)

图 3