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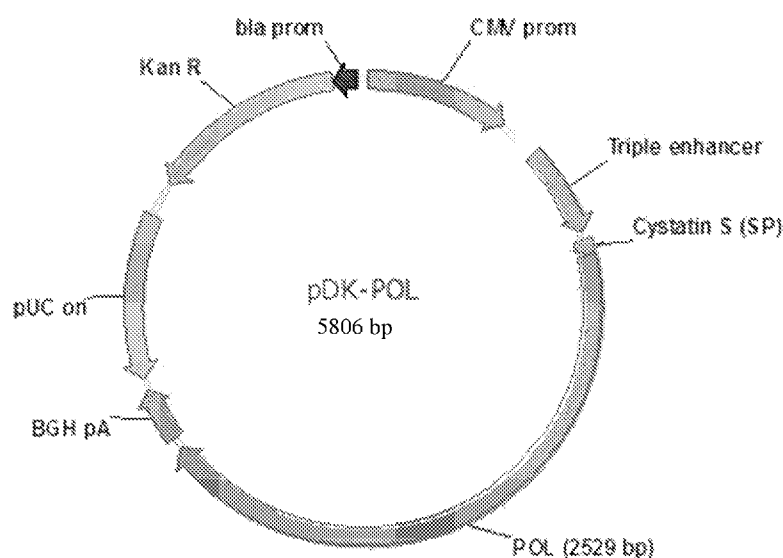


FIG. 1A

(57) Abstract: Therapeutic combinations of hepatitis B virus (HBV) vaccines and HPKI inhibitors are described. Methods of inducing an immune response against HBV or treating an HBV-induced disease, particularly in individuals having chronic HBV infection, using the disclosed therapeutic combinations of HBV vaccines and HPKI inhibitors are also described. Kits comprising the disclosed therapeutic combinations are also described.



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TITLE OF THE INVENTION

5 COMBINATION OF HEPATITIS B VIRUS (HBV) VACCINES AND
AMINOPYRIDINE DERIVATIVES AS HPK1 INHIBITORS

CROSS REFERENCE TO RELATED APPLICATION

This application claims priority to U.S. Provisional Application No. 62/862,831
filed on June 18, 2019, the disclosure of which is incorporated herein by reference in its
10 entirety.

REFERENCE TO SEQUENCE LISTING SUBMITTED ELECTRONICALLY

This application contains a sequence listing, which is submitted electronically
via EFS-Web as an ASCII formatted sequence listing with a file name
15 "065814_22WO1 Sequence Listing" and a creation date of June 4, 2020 and having a
size of 46 kb. The sequence listing submitted via EFS-Web is part of the specification
and is herein incorporated by reference in its entirety.

BACKGROUND OF THE INVENTION

20 Hepatitis B virus (HBV) is a small 3.2-kb hepatotropic DNA virus that encodes
four open reading frames and seven proteins. Approximately 240 million people have
chronic hepatitis B infection (chronic HBV), characterized by persistent virus and
subvirus particles in the blood for more than 6 months (Cohen et al. J. Viral Hepat.
(2011) 18(6), 377-83). Persistent HBV infection leads to T-cell exhaustion in
25 circulating and intrahepatic HBV-specific CD4+ and CD8+ T-cells through chronic
stimulation of HBV-specific T-cell receptors with viral peptides and circulating
antigens. As a result, T-cell polyfunctionality is decreased (i.e., decreased levels of IL-
2, tumor necrosis factor (TNF)- α , IFN- γ , and lack of proliferation).

A safe and effective prophylactic vaccine against HBV infection has been
30 available since the 1980s and is the mainstay of hepatitis B prevention (World Health
Organization, Hepatitis B: Fact sheet No. 204 [Internet] 2015 March.). The World
Health Organization recommends vaccination of all infants, and, in countries where
there is low or intermediate hepatitis B endemicity, vaccination of all children and
adolescents (<18 years of age), and of people of certain at risk population categories.

Due to vaccination, worldwide infection rates have dropped dramatically. However, prophylactic vaccines do not cure established HBV infection.

Chronic HBV is currently treated with IFN- α and nucleoside or nucleotide analogs, but there is no ultimate cure due to the persistence in infected hepatocytes of an intracellular viral replication intermediate called covalently closed circular DNA (cccDNA), which plays a fundamental role as a template for viral RNAs, and thus new virions. It is thought that induced virus-specific T-cell and B-cell responses can effectively eliminate cccDNA-carrying hepatocytes. Current therapies targeting the HBV polymerase suppress viremia, but offer limited effect on cccDNA that resides in the nucleus and related production of circulating antigen. The most rigorous form of a cure may be elimination of HBV cccDNA from the organism, which has neither been observed as a naturally occurring outcome nor as a result of any therapeutic intervention. However, loss of HBV surface antigens (HBsAg) is a clinically credible equivalent of a cure, since disease relapse can occur only in cases of severe immunosuppression, which can then be prevented by prophylactic treatment. Thus, at least from a clinical standpoint, loss of HBsAg is associated with the most stringent form of immune reconstitution against HBV.

For example, immune modulation with pegylated interferon (pegIFN)- α has proven better in comparison to nucleoside or nucleotide therapy in terms of sustained off-treatment response with a finite treatment course. Besides a direct antiviral effect, IFN- α is reported to exert epigenetic suppression of cccDNA in cell culture and humanized mice, which leads to reduction of virion productivity and transcripts (Belloni et al. J. Clin. Invest. (2012) 122(2), 529-537). However, this therapy is still fraught with side-effects and overall responses are rather low, in part because IFN- α has only poor modulatory influences on HBV-specific T-cells. In particular, cure rates are low (< 10%) and toxicity is high. Likewise, direct acting HBV antivirals, namely the HBV polymerase inhibitors entecavir and tenofovir, are effective as monotherapy in inducing viral suppression with a high genetic barrier to emergence of drug resistant mutants and consecutive prevention of liver disease progression. However, cure of chronic hepatitis B, defined by HBsAg loss or seroconversion, is rarely achieved with such HBV polymerase inhibitors. Therefore, these antivirals in theory need to be administered indefinitely to prevent reoccurrence of liver disease, similar to antiretroviral therapy for human immunodeficiency virus (HIV).

Therapeutic vaccination has the potential to eliminate HBV from chronically infected patients (Michel et al. J. Hepatol. (2011) 54(6), 1286-1296). Many strategies have been explored, but to date therapeutic vaccination has not proven successful.

BRIEF SUMMARY OF THE INVENTION

5 Accordingly, there is an unmet medical need in the treatment of hepatitis B virus (HBV), particularly chronic HBV, for a finite well-tolerated treatment with a higher cure rate. The invention satisfies this need by providing therapeutic combinations or compositions and methods for inducing an immune response against hepatitis B viruses (HBV) infection. The immunogenic compositions/combinations
10 and methods of the invention can be used to provide therapeutic immunity to a subject, such as a subject having chronic HBV infection.

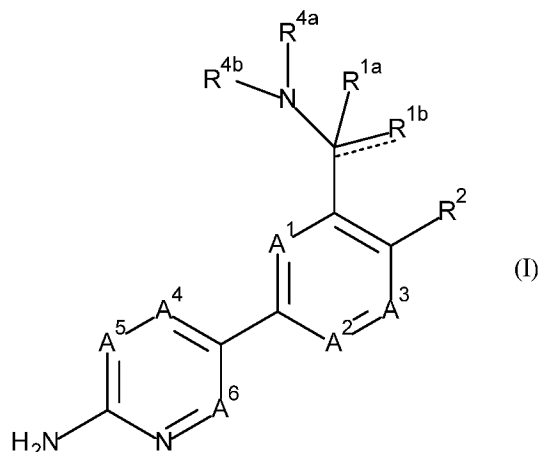
In a general aspect, the application relates to therapeutic combinations or compositions comprising one or more HBV antigens, or one or more polynucleotides encoding the HBV antigens, and an HPK1 inhibitor, for use in treating an HBV
15 infection in a subject in need thereof.

In one embodiment, the therapeutic combination comprises:

i) at least one of:

- a) a truncated HBV core antigen consisting of an amino acid sequence that is at least 95%, such as at least 95%, 96%, 97%, 98%, 99% or 100%, identical
20 to SEQ ID NO: 2,
- b) a first non-naturally occurring nucleic acid molecule comprising a first polynucleotide sequence encoding the truncated HBV core antigen;
- c) an HBV polymerase antigen having an amino acid sequence that is at least 90%, such as at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%,
25 99% or 100%, identical to SEQ ID NO: 7, wherein the HBV polymerase antigen does not have reverse transcriptase activity and RNase H activity, and
- d) a second non-naturally occurring nucleic acid molecule comprising a second polynucleotide sequence encoding the HBV polymerase antigen; and

30 ii) a compound of formula (I):



or a tautomer or a stereoisomeric form thereof, wherein:

the dotted bond towards R^{1b} is an optional bond that is optionally present when R^{1b} and R^{4b} are taken together to form a monocyclic or bicyclic aromatic heterocyclyl;

- 5 A^1 represents CH or N; A^2 represents CH; A^3 represents CH or N;
provided that only one of A^1 and A^3 represents N;
 A^4 represents CH or N; A^5 represents CR^{3a} ; A^6 represents CH;
 R^{1a} represents hydrogen;
 R^{1b} represents hydrogen or CH_3 ;
- 10 R^{4a} represents hydrogen, C_{1-4} alkyl, or C_{3-6} cycloalkyl;
 R^{4b} represents hydrogen, C_{1-4} alkyl, C_{3-6} cycloalkyl, or
a carbon linked monocyclic 4-, 5-, 6- or 7-membered non-aromatic heterocyclyl
containing 1, 2 or 3 heteroatoms each independently selected from the group
consisting of N, O, and S; wherein said S-atom is optionally substituted to form
- 15 $S(=O)$, $S(=O)_2$, or $S(=O)(=NR^x)$;
or
 R^{1b} and R^{4b} are taken together to form together with the atoms to which they are
attached a monocyclic 5-membered aromatic heterocyclyl or a monocyclic 4-, 5-, 6- or
7-membered fully saturated heterocyclyl, each containing 1 N-atom and optionally 1
- 20 or 2 additional heteroatoms each independently selected from the group consisting of
N, O, and S; wherein said optional S-atom is optionally substituted to form $S(=O)$,
 $S(=O)_2$, or $S(=O)(=NR^x)$; or
a bicyclic 6- to 12- membered aromatic or fully saturated heterocyclyl containing 1 N-
atom and optionally 1 or 2 additional heteroatoms each independently selected from

the group consisting of N, O, and S; wherein said optional S-atom is optionally substituted to form S(=O), S(=O)₂, or S(=O)(=NR^x);

wherein said monocyclic or bicyclic, aromatic or fully saturated heterocyclyl is optionally substituted on one or more of the carbon atoms with in total 1, 2 or 3

5 substituents each independently selected from the group consisting of -OH, CN, halo, R⁷, -O-R⁷,

-S(=O)₂-R⁷, -C(=O)-R⁷, -NR^{6c}R^{6d}, -C(=O)-NR^{6a}R^{6b}, and Het^c;

wherein said monocyclic or bicyclic, aromatic or fully saturated heterocyclyl is optionally substituted on the optional additional nitrogen atoms with in total 1 or 2

10 substituents each independently selected from the group consisting of R⁷, -S(=O)₂-R⁷, -C(=O)-R⁷,

-C(=O)-NR^{6a}R^{6b}, and Het^d;

provided that in case R^{1b} and R^{4b} are taken together, R^{4a} represents hydrogen; and R^{1a} represents hydrogen or R^{1a} is absent when the dotted bond towards R^{1b} is a bond;

15 or

R^{4a} and R^{4b} are taken together to form together with the N-atom to which they are attached a monocyclic 5-membered aromatic heterocyclyl or a monocyclic 4-, 5-, 6- or 7-membered fully saturated heterocyclyl, each containing 1 N-atom and optionally 1 or 2 additional heteroatoms each independently selected from the group consisting of

20 N, O, and S; wherein said optional S-atom is optionally substituted to form S(=O), S(=O)₂, or S(=O)(=NR^x); or

a bicyclic 6- to 12- membered aromatic or fully saturated heterocyclyl containing 1 N-atom and optionally 1 or 2 additional heteroatoms each independently selected from the group consisting of N, O, and S; wherein said optional S-atom is optionally

25 substituted to form S(=O), S(=O)₂, or S(=O)(=NR^x);

wherein said monocyclic or bicyclic, aromatic or fully saturated heterocyclyl is optionally substituted on one or more of the carbon atoms with in total 1, 2 or 3

substituents each independently selected from the group consisting of -OH, CN, halo, R⁷, -O-R⁷,

30 -S(=O)₂-R⁷, -C(=O)-R⁷, -NR^{6c}R^{6d}, -C(=O)-NR^{6a}R^{6b}, and Het^c;

wherein said monocyclic or bicyclic, aromatic or fully saturated heterocyclyl is optionally substituted on the nitrogen atoms with in total 1 or 2 substituents each

independently selected from the group consisting of R⁷, -S(=O)₂-R⁷, -C(=O)-R⁷, -

$C(=O)-NR^{6a}R^{6b}$, and Het^d ;

in case R^{4a} and R^{4b} are taken together, R^{1a} represents hydrogen, and R^{1b} represents hydrogen;

R^2 is selected from the group consisting of cyano; halo; $-C(=O)-NR^{8a}R^{8b}$;

- 5 $-CH_2-NR^{8c}R^{8d}$; Het^b ; $-P(=O)-(C_{1-4}alkyl)_2$; $-S(=O)_2-C_{1-4}alkyl$; $-S(=O)(=NR^x)-C_{1-4}alkyl$; $C_{1-6}alkyl$ optionally substituted with 1 or 2 substituents each independently selected from the group consisting of halo, $-OH$, cyano, and $-O-C_{1-4}alkyl$; $C_{3-6}cycloalkyl$ optionally substituted with 1 or 2 substituents each independently selected from the group consisting of halo, $-OH$, cyano and $-O-C_{1-4}alkyl$; and
- 10 $C_{3-6}cycloalkenyl$ optionally substituted with 1 or 2 substituents each independently selected from the group consisting of halo, $-OH$, cyano and $-O-C_{1-4}alkyl$; R^{3a} represents hydrogen, halo, R^7 , $-O-R^7$, cyano, $-C(=O)-NR^{6c}R^{6d}$, Het^a , or phenyl optionally substituted with 1, 2 or 3 substituents each independently selected from the group consisting of halo, cyano, $C_{1-4}alkyl$, $-O-C_{1-4}alkyl$, $C_{1-4}alkyl$ substituted with one
- 15 cyano, and $C_{1-4}alkyl$ substituted with 1, 2 or 3 halo atoms; Het^a represents a carbon linked monocyclic 5-, 6- or 7-membered aromatic heterocyclyl or carbon linked monocyclic 4-, 5-, 6- or 7-membered non-aromatic heterocyclyl, each containing 1, 2 or 3 heteroatoms each independently selected from the group consisting of N, O or S; wherein said S-atom might be substituted to form
- 20 $S(=O)$, $S(=O)_2$, or $S(=O)(=NR^x)$; or a carbon linked bicyclic 6- to 12-membered aromatic or non-aromatic heterocyclyl containing 1, 2 or 3 heteroatoms each independently selected from the group consisting of N, O and S; wherein said S-atom is optionally substituted to form $S(=O)$, $S(=O)_2$, or $S(=O)(=NR^x)$;
- 25 wherein said monocyclic or bicyclic, aromatic or non-aromatic heterocyclyl is optionally substituted on one or more of the carbon atoms with in total 1, 2 or 3 substituents each independently selected from the group consisting of $-OH$, CN, halo, R^7 , $-O-R^7$, $-S(=O)_2-R^7$, $-C(=O)-R^7$, $-NR^{6c}R^{6d}$, $-C(=O)-NR^{6a}R^{6b}$, and Het^c ;
- 30 wherein said monocyclic or bicyclic, aromatic or non-aromatic heterocyclyl is optionally substituted on the nitrogen atoms with in total 1 or 2 substituents each independently selected from the group consisting of R^7 , $-S(=O)_2-R^7$, $-C(=O)-R^7$, $-C(=O)-NR^{6a}R^{6b}$ and Het^d ;

R^{6a} , R^{6b} , R^{6c} , R^{6d} , R^{6e} , and R^{6f} are each independently selected from the group consisting of hydrogen; C₃₋₆cycloalkyl optionally substituted with one -OR⁵; and C₁₋₄alkyl optionally substituted with one -OR⁵, wherein two hydrogen atoms on the same carbon atom of said C₁₋₄alkyl are optionally taken together to form C₃.

- 5 ₆cycloalkyl;
 R^5 represents hydrogen or C₁₋₄alkyl;
 R^{8a} , R^{8c} , and R^{8d} are each independently selected from the group consisting of hydrogen;
C₁₋₄alkyl optionally substituted with one -OH or -O-C₁₋₄alkyl; and C₃₋₆cycloalkyl
10 optionally substituted with one -OH or -O-C₁₋₄alkyl;
 R^{8b} is selected from the group consisting C₁₋₄alkyl optionally substituted with one -OH or
or
-O-C₁₋₄alkyl; and C₃₋₆cycloalkyl optionally substituted with one -OH or -O-C₁₋₄alkyl;
or
15 R^{8a} and R^{8b} , or R^{8c} and R^{8d} are taken together to form together with the N-atom to
which they are attached a monocyclic fully saturated heterocyclyl containing 1 N-atom
and optionally 1 or 2 additional heteroatoms each independently selected from the
group consisting of N, O, S; wherein said optional S-atom is optionally substituted to
form S(=O), S(=O)₂, or S(=O)(=NR^x); wherein said monocyclic 4-, 5-, 6- or 7-
20 membered fully saturated heterocyclyl is optionally substituted on one or more of the
carbon atoms with in total 1, 2 or 3 substituents each independently selected from the
group consisting of -OH, CN, halo, R⁷, -O-R⁷, -S(=O)₂-R⁷, -C(=O)-R⁷, -NR^{6c}R^{6d}, and
-C(=O)-NR^{6a}R^{6b};
wherein said monocyclic fully saturated heterocyclyl is optionally substituted on the
25 nitrogen atoms with in total 1 or 2 substituents each independently selected from the
group consisting of R⁷, -S(=O)₂-R⁷, -C(=O)-R⁷, and -C(=O)-NR^{6a}R^{6b};
each Het^c independently represents a monocyclic 4-, 5-, 6- or 7-membered fully
saturated heterocyclyl containing 1, 2 or 3 heteroatoms each independently selected
from the group consisting of N, O and S; wherein said S-atom is optionally substituted
30 to form S(=O), S(=O)₂, or S(=O)(=NR^x);
each Het^d independently represents a carbon linked monocyclic 4-, 5-, 6- or 7-
membered fully saturated heterocyclyl containing 1, 2 or 3 heteroatoms each
independently selected from the group consisting of N, O and S; wherein said S-atom
is optionally substituted to form S(=O), S(=O)₂, or S(=O)(=NR^x);

Het^b represents a monocyclic 4-, 5-, 6- or 7-membered non-aromatic heterocyclyl containing 1, 2 or 3 heteroatoms each independently selected from the group consisting of N, O, and S; wherein said S-atom might be substituted to form S(=O), S(=O)₂, or S(=O)(=NR^x); or

- 5 a bicyclic 6- to 12- membered non-aromatic heterocyclyl containing 1, 2 or 3 heteroatoms each independently selected from the group consisting of N, O, and S; wherein said S-atom might be substituted to form S(=O), S(=O)₂, or S(=O)(=NR^x); wherein said monocyclic or bicyclic non-aromatic heterocyclyl might be substituted on one or more of the carbon atoms with in total 1, 2 or 3 substituents each
- 10 independently selected from the group consisting of -OH, CN, halo, R⁷, -O-R⁷, -S(=O)₂-R⁷, -C(=O)-R⁷, -NR^{6c}R^{6d}, and -C(=O)-NR^{6a}R^{6b};
- wherein said monocyclic or bicyclic non-aromatic heterocyclyl might be substituted on the nitrogen atoms with in total 1 or 2 substituents each independently selected
- 15 from the group consisting of R⁷, -S(=O)₂-R⁷, -C(=O)-R⁷, and -C(=O)-NR^{6a}R^{6b};
- each R⁷ independently represents C₃₋₆cycloalkyl optionally substituted with 1, 2 or 3 substituents each independently selected from the group consisting of halo, -OH, -O-C₁₋₄alkyl and cyano; or C₁₋₄alkyl optionally substituted with 1, 2 or 3 substituents each independently selected from the group consisting of halo, -OH, -O-C₁₋₄alkyl and
- 20 cyano; and
- each R^x independently represents hydrogen or C₁₋₄alkyl;

or a pharmaceutically acceptable addition salt, an N-oxide, or a solvate thereof.

- In one embodiment, the truncated HBV core antigen consists of the amino acid sequence of SEQ ID NO: 2 or SEQ ID NO: 4, and the HBV polymerase antigen
- 25 comprises the amino acid sequence of SEQ ID NO: 7.

In one embodiment, the therapeutic combination comprises at least one of the HBV polymerase antigen and the truncated HBV core antigen. In certain embodiments, the therapeutic combination comprises the HBV polymerase antigen and the truncated HBV core antigen.

- 30 In one embodiment, the therapeutic combination comprises at least one of the first non-naturally occurring nucleic acid molecule comprising the first polynucleotide sequence encoding the truncated HBV core antigen, and the second non-naturally occurring nucleic acid molecule comprising the second polynucleotide sequence encoding the HBV polymerase antigen. In certain embodiments, the first non-naturally

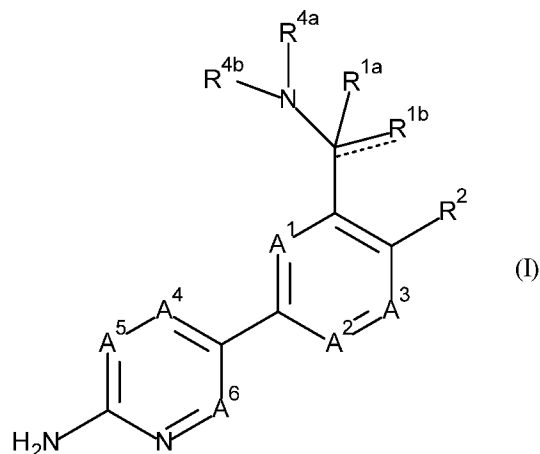
occurring nucleic acid molecule further comprises a polynucleotide sequence encoding a signal sequence operably linked to the N-terminus of the truncated HBV core antigen, and the second non-naturally occurring nucleic acid molecule further comprises a polynucleotide sequence encoding a signal sequence operably linked to the N-terminus of the HBV polymerase antigen, preferably, the signal sequence independently comprises the amino acid sequence of SEQ ID NO: 9 or SEQ ID NO: 15, more preferably, the signal sequence is encoded by the polynucleotide sequence of SEQ ID NO: 8 or SEQ ID NO: 14, respectively.

In certain embodiments, the first polynucleotide sequence comprises the polynucleotide sequence having at least 90%, such as at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%, sequence identity to SEQ ID NO: 1 or SEQ ID NO: 3.

In certain embodiments, the second polynucleotide sequence comprises a polynucleotide sequence having at least 90%, such as at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%, sequence identity to SEQ ID NO: 5 or SEQ ID NO: 6.

In an embodiment, a therapeutic combination comprises:

- a) a first non-naturally occurring nucleic acid molecule comprising a first polynucleotide sequence encoding a truncated HBV core antigen consisting of an amino acid sequence that is at least 95%, such as at least 95%, 96%, 97%, 98%, 99% or 100%, identical to SEQ ID NO: 2;
- b) a second non-naturally occurring nucleic acid molecule comprising a second polynucleotide sequence encoding an HBV polymerase antigen having an amino acid sequence that is at least 90%, such as at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%, identical to SEQ ID NO: 7, wherein the HBV polymerase antigen does not have reverse transcriptase activity and RNase H activity; and
- c) a compound of formula (I):



or a tautomer or a stereoisomeric form thereof, wherein:

the dotted bond towards R^{1b} is absent;

A^1 represents CH or N; A^2 represents CH; A^3 represents CH;

5 A^4 represents CH; A^5 represents CR^{3a} ; A^6 represents CH;

R^{1b} and R^{4b} are taken together to form together with the atoms to which they are attached a monocyclic 4-, 5-, 6- or 7-membered fully saturated heterocyclyl, each containing 1 N-atom and optionally 1 or 2 additional heteroatoms each independently selected from the group consisting of N, O, and S; wherein said optional S-atom is

10 optionally substituted to form $S(=O)$;

wherein said monocyclic fully saturated heterocyclyl is optionally substituted on one of the carbon atoms with 1 substituent selected from the group consisting of halo and R^7 ;

provided that R^{4a} represents hydrogen; and R^{1a} represents hydrogen;

15 R^2 represents Het^b ;

R^{3a} represents halo, cyano, or Het^a ;

Het^a represents a carbon linked monocyclic 5-, 6- or 7-membered aromatic heterocyclyl containing 1, 2 or 3 heteroatoms each independently selected from the group consisting of N, O and S; wherein said S-atom is optionally substituted to form

20 $S(=O)$ or $S(=O)_2$;

wherein said monocyclic aromatic heterocyclyl is optionally substituted on one of the carbon atoms with a halo substituent;

wherein said monocyclic aromatic heterocyclyl is optionally substituted on one nitrogen atom with R^7 ;

Het^b represents a monocyclic 4-, 5-, 6- or 7-membered non-aromatic heterocyclyl containing 1 oxygen atom; and each R⁷ represents C₁₋₄alkyl;

or a pharmaceutically acceptable addition salt, an N-oxide, or a solvate thereof.

5 Preferably, the therapeutic combination comprises a) a first non-naturally occurring nucleic acid molecule comprising a first polynucleotide sequence encoding an truncated HBV core antigen consisting of the amino acid sequence of SEQ ID NO: 2 or SEQ ID NO: 4; b) a second non-naturally occurring nucleic acid molecule comprising a second polynucleotide sequence encoding an HBV polymerase antigen
10 having the amino acid sequence of SEQ ID NO: 7, and (c) a compound selected from the group consisting of the exemplified compounds, particularly compounds 1 to 111 described herein, or a tautomer or stereoisomeric form, or a pharmaceutically acceptable addition salt, an N-oxide, or a solvate thereof.

Preferably, the therapeutic combination comprises a first non-naturally
15 occurring nucleic acid molecule comprising a polynucleotide sequence having at least 90%, such as at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%, sequence identity to SEQ ID NO: 1 or SEQ ID NO: 3, and a second non-naturally occurring nucleic acid molecule comprising the polynucleotide sequence having at least 90%, such as at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%,
20 98%, 99% or 100%, sequence identity to SEQ ID NO: 5 or SEQ ID NO: 6.

More preferably, the therapeutic combination comprises a) a first non-naturally occurring nucleic acid molecule comprising a first polynucleotide sequence of SEQ ID NO: 1 or SEQ ID NO: 3; b) a second non-naturally occurring nucleic acid molecule comprising a second polynucleotide sequence of SEQ ID NO: 5 or 6; and c) a
25 compound selected from the group consisting of the exemplified compounds, particularly compounds 1 to 111 described herein, or a tautomer or stereoisomeric form, or a pharmaceutically acceptable addition salt, an N-oxide, or a solvate thereof.

In an embodiment, each of the first and the second non-naturally occurring nucleic acid molecules is a DNA molecule, preferably the DNA molecule is present on
30 a plasmid or a viral vector.

In another embodiment, each of the first and the second non-naturally occurring nucleic acid molecules is an RNA molecule, preferably an mRNA or a self-replicating RNA molecule.

In some embodiments, each of the first and the second non-naturally occurring nucleic acid molecules is independently formulated with a lipid nanoparticle (LNP).

In another general aspect, the application relates to a kit comprising a therapeutic combination of the application.

5 The application also relates to a therapeutic combination or kit of the application for use in inducing an immune response against hepatitis B virus (HBV); and use of a therapeutic combination, composition or kit of the application in the manufacture of a medicament for inducing an immune response against hepatitis B virus (HBV). The use can further comprise a combination with another immunogenic
10 or therapeutic agent, preferably another HBV antigen or another HBV therapy. Preferably, the subject has chronic HBV infection.

 The application further relates to a therapeutic combination or kit of the application for use in treating an HBV-induced disease in a subject in need thereof; and use of a therapeutic combination or kit of the application in the manufacture of a
15 medicament for treating an HBV-induced disease in a subject in need thereof. The use can further comprise a combination with another therapeutic agent, preferably another anti-HBV antigen. Preferably, the subject has chronic HBV infection, and the HBV-induced disease is selected from the group consisting of advanced fibrosis, cirrhosis, and hepatocellular carcinoma (HCC).

20 The application also relates to a method of inducing an immune response against an HBV or a method of treating an HBV infection or an HBV-induced disease, comprising administering to a subject in need thereof a therapeutic combination according to embodiments of the application.

 Other aspects, features and advantages of the invention will be apparent from
25 the following disclosure, including the detailed description of the invention and its preferred embodiments and the appended claims.

30 BRIEF DESCRIPTION OF THE DRAWINGS

 The foregoing summary, as well as the following detailed description of preferred embodiments of the present application, will be better understood when read in conjunction with the appended drawings. It should be understood, however, that the application is not limited to the precise embodiments shown in the drawings.

FIG. 1A and **FIG. 1B** show schematic representations of DNA plasmids according to embodiments of the application; **FIG. 1A** shows a DNA plasmid encoding an HBV core antigen according to an embodiment of the application; **FIG. 1B** shows a DNA plasmid encoding an HBV polymerase (pol) antigen according to an embodiment of the application; the HBV core and pol antigens are expressed under control of a CMV promoter with an N-terminal cystatin S signal peptide that is cleaved from the expressed antigen upon secretion from the cell; transcriptional regulatory elements of the plasmid include an enhancer sequence located between the CMV promoter and the polynucleotide sequence encoding the HBV antigen and a bGH polyadenylation sequence located downstream of the polynucleotide sequence encoding the HBV antigen; a second expression cassette is included in the plasmid in reverse orientation including a kanamycin resistance gene under control of an Ampr (bla) promoter; an origin of replication (pUC) is also included in reverse orientation.

FIG. 2A and **FIG. 2B** show the schematic representations of the expression cassettes in adenoviral vectors according to embodiments of the application; **FIG. 2A** shows the expression cassette for a truncated HBV core antigen, which contains a CMV promoter, an intron (a fragment derived from the human ApoAI gene - GenBank accession X01038 base pairs 295 – 523, harboring the ApoAI second intron), a human immunoglobulin secretion signal, followed by a coding sequence for a truncated HBV core antigen and a SV40 polyadenylation signal; **FIG. 2B** shows the expression cassette for a fusion protein of a truncated HBV core antigen operably linked to an HBV polymerase antigen, which is otherwise identical to the expression cassette for the truncated HBV core antigen except the HBV antigen.

FIG. 3 shows ELISPOT responses of Balb/c mice immunized with different DNA plasmids expressing HBV core antigen or HBV pol antigen, as described in Example 3; peptide pools used to stimulate splenocytes isolated from the various vaccinated animal groups are indicated in gray scale; the number of responsive T-cells are indicated on the y-axis expressed as spot forming cells (SFC) per 10^6 splenocytes;

DETAILED DESCRIPTION OF THE INVENTION

Various publications, articles and patents are cited or described in the background and throughout the specification; each of these references is herein incorporated by reference in its entirety. Discussion of documents, acts, materials, devices, articles or the like which has been included in the present specification is for

the purpose of providing context for the invention. Such discussion is not an admission that any or all of these matters form part of the prior art with respect to any inventions disclosed or claimed.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood to one of ordinary skill in the art to which this invention pertains. Otherwise, certain terms used herein have the meanings as set forth in the specification. All patents, published patent applications and publications cited herein are incorporated by reference as if set forth fully herein.

It must be noted that as used herein and in the appended claims, the singular forms “a,” “an,” and “the” include plural reference unless the context clearly dictates otherwise.

Unless otherwise indicated, the term “at least” preceding a series of elements is to be understood to refer to every element in the series. Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the invention.

Throughout this specification and the claims which follow, unless the context requires otherwise, the word “comprise”, and variations such as “comprises” and “comprising”, will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps. When used herein the term “comprising” can be substituted with the term “containing” or “including” or sometimes when used herein with the term “having”.

When used herein “consisting of” excludes any element, step, or ingredient not specified in the claim element. When used herein, “consisting essentially of” does not exclude materials or steps that do not materially affect the basic and novel characteristics of the claim. Any of the aforementioned terms of “comprising”, “containing”, “including”, and “having”, whenever used herein in the context of an aspect or embodiment of the application can be replaced with the term “consisting of” or “consisting essentially of” to vary scopes of the disclosure.

As used herein, the conjunctive term “and/or” between multiple recited elements is understood as encompassing both individual and combined options. For instance, where two elements are conjoined by “and/or,” a first option refers to the applicability of the first element without the second. A second option refers to the

applicability of the second element without the first. A third option refers to the applicability of the first and second elements together. Any one of these options is understood to fall within the meaning, and therefore satisfy the requirement of the term “and/or” as used herein. Concurrent applicability of more than one of the options
5 is also understood to fall within the meaning, and therefore satisfy the requirement of the term “and/or.”

Unless otherwise stated, any numerical value, such as a concentration or a concentration range described herein, are to be understood as being modified in all instances by the term “about.” Thus, a numerical value typically includes $\pm 10\%$ of the
10 recited value. For example, a concentration of 1 mg/mL includes 0.9 mg/mL to 1.1 mg/mL. Likewise, a concentration range of 1 mg/mL to 10 mg/mL includes 0.9 mg/mL to 11 mg/mL. As used herein, the use of a numerical range expressly includes all possible subranges, all individual numerical values within that range, including integers within such ranges and fractions of the values unless the context clearly
15 indicates otherwise.

The phrases “percent (%) sequence identity” or “% identity” or “% identical to” when used with reference to an amino acid sequence describe the number of matches (“hits”) of identical amino acids of two or more aligned amino acid sequences as compared to the number of amino acid residues making up the overall length of the
20 amino acid sequences. In other terms, using an alignment, for two or more sequences the percentage of amino acid residues that are the same (e.g. 90%, 91%, 92%, 93%, 94%, 95%, 97%, 98%, 99%, or 100% identity over the full-length of the amino acid sequences) may be determined, when the sequences are compared and aligned for maximum correspondence as measured using a sequence comparison algorithm as
25 known in the art, or when manually aligned and visually inspected. The sequences which are compared to determine sequence identity may thus differ by substitution(s), addition(s) or deletion(s) of amino acids. Suitable programs for aligning protein sequences are known to the skilled person. The percentage sequence identity of protein sequences can, for example, be determined with programs such as
30 CLUSTALW, Clustal Omega, FASTA or BLAST, e.g. using the NCBI BLAST algorithm (Altschul SF, et al (1997), *Nucleic Acids Res.* 25:3389-3402).

As used herein, the terms and phrases “in combination,” “in combination with,” “co-delivery,” and “administered together with” in the context of the administration of two or more therapies or components to a subject refers to

simultaneous administration or subsequent administration of two or more therapies or components, such as two vectors, e.g., DNA plasmids, peptides, or a therapeutic combination and an adjuvant. "Simultaneous administration" can be administration of the two or more therapies or components at least within the same day. When two components are "administered together with" or "administered in combination with," they can be administered in separate compositions sequentially within a short time period, such as 24, 20, 16, 12, 8 or 4 hours, or within 1 hour, or they can be administered in a single composition at the same time. "Subsequent administration" can be administration of the two or more therapies or components in the same day or on separate days. The use of the term "in combination with" does not restrict the order in which therapies or components are administered to a subject. For example, a first therapy or component (e.g. first DNA plasmid encoding an HBV antigen) can be administered prior to (e.g., 5 minutes to one hour before), concomitantly with or simultaneously with, or subsequent to (e.g., 5 minutes to one hour after) the administration of a second therapy or component (e.g., second DNA plasmid encoding an HBV antigen), and/or a third therapy or component (e.g., HPK1 inhibitor). In some embodiments, a first therapy or component (e.g. first DNA plasmid encoding an HBV antigen), a second therapy or component (e.g., second DNA plasmid encoding an HBV antigen), and a third therapy or component (e.g., HPK1 inhibitor) are administered in the same composition. In other embodiments, a first therapy or component (e.g. first DNA plasmid encoding an HBV antigen), a second therapy or component (e.g., second DNA plasmid encoding an HBV antigen), and a third therapy or component (e.g., HPK1 inhibitor) are administered in separate compositions, such as two or three separate compositions.

As used herein, a "non-naturally occurring" nucleic acid or polypeptide, refers to a nucleic acid or polypeptide that does not occur in nature. A "non-naturally occurring" nucleic acid or polypeptide can be synthesized, treated, fabricated, and/or otherwise manipulated in a laboratory and/or manufacturing setting. In some cases, a non-naturally occurring nucleic acid or polypeptide can comprise a naturally-occurring nucleic acid or polypeptide that is treated, processed, or manipulated to exhibit properties that were not present in the naturally-occurring nucleic acid or polypeptide, prior to treatment. As used herein, a "non-naturally occurring" nucleic acid or polypeptide can be a nucleic acid or polypeptide isolated or separated from the natural source in which it was discovered, and it lacks covalent bonds to sequences with

which it was associated in the natural source. A “non-naturally occurring” nucleic acid or polypeptide can be made recombinantly or via other methods, such as chemical synthesis.

As used herein, “subject” means any animal, preferably a mammal, most preferably a human, to whom will be or has been treated by a method according to an embodiment of the application. The term “mammal” as used herein, encompasses any mammal. Examples of mammals include, but are not limited to, cows, horses, sheep, pigs, cats, dogs, mice, rats, rabbits, guinea pigs, non-human primates (NHPs) such as monkeys or apes, humans, etc., more preferably a human.

As used herein, the term “operably linked” refers to a linkage or a juxtaposition wherein the components so described are in a relationship permitting them to function in their intended manner. For example, a regulatory sequence operably linked to a nucleic acid sequence of interest is capable of directing the transcription of the nucleic acid sequence of interest, or a signal sequence operably linked to an amino acid sequence of interest is capable of secreting or translocating the amino acid sequence of interest over a membrane.

In an attempt to help the reader of the application, the description has been separated in various paragraphs or sections, or is directed to various embodiments of the application. These separations should not be considered as disconnecting the substance of a paragraph or section or embodiments from the substance of another paragraph or section or embodiments. To the contrary, one skilled in the art will understand that the description has broad application and encompasses all the combinations of the various sections, paragraphs and sentences that can be contemplated. The discussion of any embodiment is meant only to be exemplary and is not intended to suggest that the scope of the disclosure, including the claims, is limited to these examples. For example, while embodiments of HBV vectors of the application (e.g., plasmid DNA or viral vectors) described herein may contain particular components, including, but not limited to, certain promoter sequences, enhancer or regulatory sequences, signal peptides, coding sequence of an HBV antigen, polyadenylation signal sequences, etc. arranged in a particular order, those having ordinary skill in the art will appreciate that the concepts disclosed herein may equally apply to other components arranged in other orders that can be used in HBV vectors of the application. The application contemplates use of any of the applicable components in any combination having any sequence that can be used in HBV vectors

of the application, whether or not a particular combination is expressly described. The invention generally relates to a therapeutic combination comprising one or more HBV antigens and at least one HPK1 inhibitor.

Hepatitis B Virus (HBV)

5 As used herein “hepatitis B virus” or “HBV” refers to a virus of the
hepadnaviridae family. HBV is a small (e.g., 3.2 kb) hepatotropic DNA virus that
encodes four open reading frames and seven proteins. The seven proteins encoded by
HBV include small (S), medium (M), and large (L) surface antigen (HBsAg) or
envelope (Env) proteins, pre-Core protein, core protein, viral polymerase (Pol), and
10 HBx protein. HBV expresses three surface antigens, or envelope proteins, L, M, and
S, with S being the smallest and L being the largest. The extra domains in the M and
L proteins are named Pre-S2 and Pre-S1, respectively. Core protein is the subunit of
the viral nucleocapsid. Pol is needed for synthesis of viral DNA (reverse transcriptase,
RNaseH, and primer), which takes place in nucleocapsids localized to the cytoplasm
15 of infected hepatocytes. PreCore is the core protein with an N-terminal signal peptide
and is proteolytically processed at its N and C termini before secretion from infected
cells, as the so-called hepatitis B e-antigen (HBeAg). HBx protein is required for
efficient transcription of covalently closed circular DNA (cccDNA). HBx is not a viral
structural protein. All viral proteins of HBV have their own mRNA except for core
20 and polymerase, which share an mRNA. With the exception of the protein pre-Core,
none of the HBV viral proteins are subject to post-translational proteolytic processing.

 The HBV virion contains a viral envelope, nucleocapsid, and single copy of
the partially double-stranded DNA genome. The nucleocapsid comprises 120 dimers
of core protein and is covered by a capsid membrane embedded with the S, M, and L
25 viral envelope or surface antigen proteins. After entry into the cell, the virus is
uncoated and the capsid-containing relaxed circular DNA (rcDNA) with covalently
bound viral polymerase migrates to the nucleus. During that process, phosphorylation
of the core protein induces structural changes, exposing a nuclear localization signal
enabling interaction of the capsid with so-called importins. These importins mediate
30 binding of the core protein to nuclear pore complexes upon which the capsid
disassembles and polymerase/rcDNA complex is released into the nucleus. Within the
nucleus the rcDNA becomes deproteinized (removal of polymerase) and is converted
by host DNA repair machinery to a covalently closed circular DNA (cccDNA)
genome from which overlapping transcripts encode for HBeAg, HBsAg, Core protein,

viral polymerase and HBx protein. Core protein, viral polymerase, and pre-genomic RNA (pgRNA) associate in the cytoplasm and self-assemble into immature pgRNA-containing capsid particles, which further convert into mature rcDNA-capsids and function as a common intermediate that is either enveloped and secreted as infectious virus particles or transported back to the nucleus to replenish and maintain a stable cccDNA pool.

To date, HBV is divided into four serotypes (adr, adw, ayr, ayw) based on antigenic epitopes present on the envelope proteins, and into eight genotypes (A, B, C, D, E, F, G, and H) based on the sequence of the viral genome. The HBV genotypes are distributed over different geographic regions. For example, the most prevalent genotypes in Asia are genotypes B and C. Genotype D is dominant in Africa, the Middle East, and India, whereas genotype A is widespread in Northern Europe, sub-Saharan Africa, and West Africa.

HBV Antigens

As used herein, the terms “HBV antigen,” “antigenic polypeptide of HBV,” “HBV antigenic polypeptide,” “HBV antigenic protein,” “HBV immunogenic polypeptide,” and “HBV immunogen” all refer to a polypeptide capable of inducing an immune response, e.g., a humoral and/or cellular mediated response, against an HBV in a subject. The HBV antigen can be a polypeptide of HBV, a fragment or epitope thereof, or a combination of multiple HBV polypeptides, portions or derivatives thereof. An HBV antigen is capable of raising in a host a protective immune response, e.g., inducing an immune response against a viral disease or infection, and/or producing an immunity (i.e., vaccinates) in a subject against a viral disease or infection, that protects the subject against the viral disease or infection. For example, an HBV antigen can comprise a polypeptide or immunogenic fragment(s) thereof from any HBV protein, such as HBeAg, pre-core protein, HBsAg (S, M, or L proteins), core protein, viral polymerase, or HBx protein derived from any HBV genotype, e.g., genotype A, B, C, D, E, F, G, and/or H, or combination thereof.

(1) HBV Core Antigen

As used herein, each of the terms “HBV core antigen,” “HBc” and “core antigen” refers to an HBV antigen capable of inducing an immune response, e.g., a humoral and/or cellular mediated response, against an HBV core protein in a subject. Each of the terms “core,” “core polypeptide,” and “core protein” refers to the HBV viral core protein. Full-length core antigen is typically 183 amino acids in length and

includes an assembly domain (amino acids 1 to 149) and a nucleic acid binding domain (amino acids 150 to 183). The 34-residue nucleic acid binding domain is required for pre-genomic RNA encapsidation. This domain also functions as a nuclear import signal. It comprises 17 arginine residues and is highly basic, consistent with its function. HBV core protein is dimeric in solution, with the dimers self-assembling into icosahedral capsids. Each dimer of core protein has four α -helix bundles flanked by an α -helix domain on either side. Truncated HBV core proteins lacking the nucleic acid binding domain are also capable of forming capsids.

In an embodiment of the application, an HBV antigen is a truncated HBV core antigen. As used herein, a “truncated HBV core antigen,” refers to an HBV antigen that does not contain the entire length of an HBV core protein, but is capable of inducing an immune response against the HBV core protein in a subject. For example, an HBV core antigen can be modified to delete one or more amino acids of the highly positively charged (arginine rich) C-terminal nucleic acid binding domain of the core antigen, which typically contains seventeen arginine (R) residues. A truncated HBV core antigen of the application is preferably a C-terminally truncated HBV core protein which does not comprise the HBV core nuclear import signal and/or a truncated HBV core protein from which the C-terminal HBV core nuclear import signal has been deleted. In an embodiment, a truncated HBV core antigen comprises a deletion in the C-terminal nucleic acid binding domain, such as a deletion of 1 to 34 amino acid residues of the C-terminal nucleic acid binding domain, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, or 34 amino acid residues, preferably a deletion of all 34 amino acid residues. In a preferred embodiment, a truncated HBV core antigen comprises a deletion in the C-terminal nucleic acid binding domain, preferably a deletion of all 34 amino acid residues.

An HBV core antigen of the application can be a consensus sequence derived from multiple HBV genotypes (e.g., genotypes A, B, C, D, E, F, G, and H). As used herein, “consensus sequence” means an artificial sequence of amino acids based on an alignment of amino acid sequences of homologous proteins, e.g., as determined by an alignment (e.g., using Clustal Omega) of amino acid sequences of homologous proteins. It can be the calculated order of most frequent amino acid residues, found at each position in a sequence alignment, based upon sequences of HBV antigens (e.g., core, pol, etc.) from at least 100 natural HBV isolates. A consensus sequence can be

non-naturally occurring and different from the native viral sequences. Consensus sequences can be designed by aligning multiple HBV antigen sequences from different sources using a multiple sequence alignment tool, and at variable alignment positions, selecting the most frequent amino acid. Preferably, a consensus sequence of an HBV antigen is derived from HBV genotypes B, C, and D. The term “consensus antigen” is used to refer to an antigen having a consensus sequence.

An exemplary truncated HBV core antigen according to the application lacks the nucleic acid binding function, and is capable of inducing an immune response in a mammal against at least two HBV genotypes. Preferably a truncated HBV core antigen is capable of inducing a T cell response in a mammal against at least HBV genotypes B, C and D. More preferably, a truncated HBV core antigen is capable of inducing a CD8 T cell response in a human subject against at least HBV genotypes A, B, C and D.

Preferably, an HBV core antigen of the application is a consensus antigen, preferably a consensus antigen derived from HBV genotypes B, C, and D, more preferably a truncated consensus antigen derived from HBV genotypes B, C, and D. An exemplary truncated HBV core consensus antigen according to the application consists of an amino acid sequence that is at least 90% identical to SEQ ID NO: 2 or SEQ ID NO: 4, such as at least 90%, 91%, 92%, 93%, 94%, 95%, 95.5%, 96%, 96.5%, 97%, 97.5%, 98%, 98.5%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, 99.9%, or 100% identical to SEQ ID NO: 2 or SEQ ID NO: 4. SEQ ID NO: 2 and SEQ ID NO: 4 are core consensus antigens derived from HBV genotypes B, C, and D. SEQ ID NO: 2 and SEQ ID NO: 4 each contain a 34-amino acid C-terminal deletion of the highly positively charged (arginine rich) nucleic acid binding domain of the native core antigen.

In one embodiment of the application, an HBV core antigen is a truncated HBV antigen consisting of the amino acid sequence of SEQ ID NO: 2. In another embodiment, an HBV core antigen is a truncated HBV antigen consisting of the amino acid sequence of SEQ ID NO: 4. In another embodiment, an HBV core antigen further contains a signal sequence operably linked to the N-terminus of a mature HBV core antigen sequence, such as the amino acid sequence of SEQ ID NO: 2 or SEQ ID NO: 4. Preferably, the signal sequence has the amino acid sequence of SEQ ID NO: 9 or SEQ ID NO: 15.

(2) HBV Polymerase Antigen

As used herein, the term “HBV polymerase antigen,” “HBV Pol antigen” or “HBV pol antigen” refers to an HBV antigen capable of inducing an immune response, e.g., a humoral and/or cellular mediated response, against an HBV polymerase in a subject. Each of the terms “polymerase,” “polymerase polypeptide,” “Pol” and “pol” refers to the HBV viral DNA polymerase. The HBV viral DNA polymerase has four domains, including, from the N terminus to the C terminus, a terminal protein (TP) domain, which acts as a primer for minus-strand DNA synthesis; a spacer that is nonessential for the polymerase functions; a reverse transcriptase (RT) domain for transcription; and a RNase H domain.

In an embodiment of the application, an HBV antigen comprises an HBV Pol antigen, or any immunogenic fragment or combination thereof. An HBV Pol antigen can contain further modifications to improve immunogenicity of the antigen, such as by introducing mutations into the active sites of the polymerase and/or RNase domains to decrease or substantially eliminate certain enzymatic activities.

Preferably, an HBV Pol antigen of the application does not have reverse transcriptase activity and RNase H activity, and is capable of inducing an immune response in a mammal against at least two HBV genotypes. Preferably, an HBV Pol antigen is capable of inducing a T cell response in a mammal against at least HBV genotypes B, C and D. More preferably, an HBV Pol antigen is capable of inducing a CD8 T cell response in a human subject against at least HBV genotypes A, B, C and D.

Thus, in some embodiments, an HBV Pol antigen is an inactivated Pol antigen. In an embodiment, an inactivated HBV Pol antigen comprises one or more amino acid mutations in the active site of the polymerase domain. In another embodiment, an inactivated HBV Pol antigen comprises one or more amino acid mutations in the active site of the RNaseH domain. In a preferred embodiment, an inactivated HBV pol antigen comprises one or more amino acid mutations in the active site of both the polymerase domain and the RNaseH domain. For example, the “YXDD” motif in the polymerase domain of an HBV pol antigen that can be required for nucleotide/metal ion binding can be mutated, e.g., by replacing one or more of the aspartate residues (D) with asparagine residues (N), eliminating or reducing metal coordination function, thereby decreasing or substantially eliminating reverse transcriptase function. Alternatively, or in addition to mutation of the “YXDD” motif, the “DEDD” motif in the RNaseH domain of an HBV pol antigen required for Mg²⁺ coordination can be

mutated, e.g., by replacing one or more aspartate residues (D) with asparagine residues (N) and/or replacing the glutamate residue (E) with glutamine (Q), thereby decreasing or substantially eliminating RNaseH function. In a particular embodiment, an HBV pol antigen is modified by (1) mutating the aspartate residues (D) to asparagine residues (N) in the “YXDD” motif of the polymerase domain; and (2) mutating the first aspartate residue (D) to an asparagine residue (N) and the first glutamate residue (E) to a glutamine residue (Q) in the “DEDD” motif of the RNaseH domain, thereby decreasing or substantially eliminating both the reverse transcriptase and RNaseH functions of the pol antigen.

In a preferred embodiment of the application, an HBV pol antigen is a consensus antigen, preferably a consensus antigen derived from HBV genotypes B, C, and D, more preferably an inactivated consensus antigen derived from HBV genotypes B, C, and D. An exemplary HBV pol consensus antigen according to the application comprises an amino acid sequence that is at least 90% identical to SEQ ID NO: 7, such as at least 90%, 91%, 92%, 93%, 94%, 95%, 95.5%, 96%, 96.5%, 97%, 97.5%, 98%, 98.5%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, 99.9% or 100% identical to SEQ ID NO: 7, preferably at least 98% identical to SEQ ID NO: 7, such as at least 98%, 98.5%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, 99.9% or 100% identical to SEQ ID NO: 7. SEQ ID NO: 7 is a pol consensus antigen derived from HBV genotypes B, C, and D comprising four mutations located in the active sites of the polymerase and RNaseH domains. In particular, the four mutations include mutation of the aspartic acid residues (D) to asparagine residues (N) in the “YXDD” motif of the polymerase domain; and mutation of the first aspartate residue (D) to an asparagine residue (N) and mutation of the glutamate residue (E) to a glutamine residue (Q) in the “DEDD” motif of the RNaseH domain.

In a particular embodiment of the application, an HBV pol antigen comprises the amino acid sequence of SEQ ID NO: 7. In other embodiments of the application, an HBV pol antigen consists of the amino acid sequence of SEQ ID NO: 7. In a further embodiment, an HBV pol antigen further contains a signal sequence operably linked to the N-terminus of a mature HBV pol antigen sequence, such as the amino acid sequence of SEQ ID NO: 7. Preferably, the signal sequence has the amino acid sequence of SEQ ID NO: 9 or SEQ ID NO: 15.

(3) Fusion of HBV Core Antigen and HBV Polymerase Antigen

As used herein the term “fusion protein” or “fusion” refers to a single polypeptide chain having at least two polypeptide domains that are not normally present in a single, natural polypeptide.

In an embodiment of the application, an HBV antigen comprises a fusion
5 protein comprising a truncated HBV core antigen operably linked to an HBV Pol antigen, or an HBV Pol antigen operably linked to a truncated HBV core antigen, preferably via a linker.

For example, in a fusion protein containing a first polypeptide and a second heterologous polypeptide, a linker serves primarily as a spacer between the first and
10 second polypeptides. In an embodiment, a linker is made up of amino acids linked together by peptide bonds, preferably from 1 to 20 amino acids linked by peptide bonds, wherein the amino acids are selected from the 20 naturally occurring amino acids. In an embodiment, the 1 to 20 amino acids are selected from glycine, alanine, proline, asparagine, glutamine, and lysine. Preferably, a linker is made up of a majority
15 of amino acids that are sterically unhindered, such as glycine and alanine. Exemplary linkers are polyglycines, particularly (Gly)₅, (Gly)₈; poly(Gly-Ala), and polyalanines. One exemplary suitable linker as shown in the Examples below is (AlaGly)_n, wherein n is an integer of 2 to 5.

Preferably, a fusion protein of the application is capable of inducing an immune
20 response in a mammal against HBV core and HBV Pol of at least two HBV genotypes. Preferably, a fusion protein is capable of inducing a T cell response in a mammal against at least HBV genotypes B, C and D. More preferably, the fusion protein is capable of inducing a CD8 T cell response in a human subject against at least HBV genotypes A, B, C and D.

25 In an embodiment of the application, a fusion protein comprises a truncated HBV core antigen having an amino acid sequence at least 90%, such as at least 90%, 91%, 92%, 93%, 94%, 95%, 95.5%, 96%, 96.5%, 97%, 97.5%, 98%, 98.5%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, 99.9%, or 100% identical to SEQ ID NO: 2 or SEQ ID NO: 4, a linker, and an HBV Pol antigen having an amino
30 acid sequence at least 90%, such as at least 90%, 91%, 92%, 93%, 94%, 95%, 95.5%, 96%, 96.5%, 97%, 97.5%, 98%, 98.5%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, 99.9%, or 100%, identical to SEQ ID NO: 7.

In a preferred embodiment of the application, a fusion protein comprises a truncated HBV core antigen consisting of the amino acid sequence of SEQ ID NO: 2 or

SEQ ID NO: 4, a linker comprising (AlaGly)_n, wherein n is an integer of 2 to 5, and an HBV Pol antigen having the amino acid sequence of SEQ ID NO: 7. More preferably, a fusion protein according to an embodiment of the application comprises the amino acid sequence of SEQ ID NO: 16.

5 In one embodiment of the application, a fusion protein further comprises a signal sequence operably linked to the N-terminus of the fusion protein. Preferably, the signal sequence has the amino acid sequence of SEQ ID NO: 9 or SEQ ID NO: 15. In one embodiment, a fusion protein comprises the amino acid sequence of SEQ ID NO: 17.

10 Additional disclosure on HBV vaccines that can be used for the present invention are described in U.S. Patent Application No: 16/223,251, filed December 18, 2018, the contents of the application, more particularly the examples of the application, are hereby incorporated by reference in their entireties.

Polynucleotides and Vectors

15 In another general aspect, the application provides a non-naturally occurring nucleic acid molecule encoding an HBV antigen useful for an invention according to embodiments of the application, and vectors comprising the non-naturally occurring nucleic acid. A first or second non-naturally occurring nucleic acid molecule can comprise any polynucleotide sequence encoding an HBV antigen useful for the
20 application, which can be made using methods known in the art in view of the present disclosure. Preferably, a first or second polynucleotide encodes at least one of a truncated HBV core antigen and an HBV polymerase antigen of the application. A polynucleotide can be in the form of RNA or in the form of DNA obtained by recombinant techniques (e.g., cloning) or produced synthetically (e.g., chemical
25 synthesis). The DNA can be single-stranded or double-stranded, or can contain portions of both double-stranded and single-stranded sequence. The DNA can, for example, comprise genomic DNA, cDNA, or combinations thereof. The polynucleotide can also be a DNA/RNA hybrid. The polynucleotides and vectors of the application can be used for recombinant protein production, expression of the
30 protein in host cell, or the production of viral particles. Preferably, a polynucleotide is DNA.

In an embodiment of the application, a first non-naturally occurring nucleic acid molecule comprises a first polynucleotide sequence encoding a truncated HBV core antigen consisting of an amino acid sequence that is at least 90% identical to SEQ

ID NO: 2 or SEQ ID NO: 4, such as at least 90%, 91%, 92%, 93%, 94%, 95%, 95.5%, 96%, 96.5%, 97%, 97.5%, 98%, 98.5%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, 99.9% or 100% identical to SEQ ID NO: 2, preferably 98%, 99% or 100% identical to SEQ ID NO: 2 or SEQ ID NO: 4. In a particular

- 5 embodiment of the application, a first non-naturally occurring nucleic acid molecule comprises a first polynucleotide sequence encoding a truncated HBV core antigen consisting the amino acid sequence of SEQ ID NO: 2 or SEQ ID NO: 4.

Examples of polynucleotide sequences of the application encoding a truncated HBV core antigen consisting of the amino acid sequence of SEQ ID NO: 2 or SEQ ID
10 NO: 4 include, but are not limited to, a polynucleotide sequence at least 90% identical to SEQ ID NO: 1 or SEQ ID NO: 3, such as at least 90%, 91%, 92%, 93%, 94%, 95%, 95.5%, 96%, 96.5%, 97%, 97.5%, 98%, 98.5%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, 99.9% or 100% identical to SEQ ID NO: 1 or SEQ ID NO: 3, preferably 98%, 99% or 100% identical to SEQ ID NO: 1 or SEQ ID NO: 3.

- 15 Exemplary non-naturally occurring nucleic acid molecules encoding a truncated HBV core antigen have the polynucleotide sequence of SEQ ID NOs: 1 or 3.

In another embodiment, a first non-naturally occurring nucleic acid molecule further comprises a coding sequence for a signal sequence that is operably linked to the N-terminus of the HBV core antigen sequence. Preferably, the signal sequence has
20 the amino acid sequence of SEQ ID NO: 9 or SEQ ID NO: 15. More preferably, the coding sequence for a signal sequence comprises the polynucleotide sequence of SEQ ID NO: 8 or SEQ ID NO: 14.

In an embodiment of the application, a second non-naturally occurring nucleic acid molecule comprises a second polynucleotide sequence encoding an HBV
25 polymerase antigen comprising an amino acid sequence that is at least 90% identical to SEQ ID NO: 7, such as at least 90%, 91%, 92%, 93%, 94%, 95%, 95.5%, 96%, 96.5%, 97%, 97.5%, 98%, 98.5%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, 99.9% or 100% identical to SEQ ID NO: 7, preferably 100% identical to SEQ ID NO: 7. In a particular embodiment of the application, a second non-
30 naturally occurring nucleic acid molecule comprises a second polynucleotide sequence encoding an HBV polymerase antigen consisting of the amino acid sequence of SEQ ID NO: 7.

Examples of polynucleotide sequences of the application encoding an HBV Pol antigen comprising the amino acid sequence of at least 90% identical to SEQ ID

NO: 7 include, but are not limited to, a polynucleotide sequence at least 90% identical to SEQ ID NO: 5 or SEQ ID NO: 6, such as at least 90%, 91%, 92%, 93%, 94%, 95%, 95.5%, 96%, 96.5%, 97%, 97.5%, 98%, 98.5%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, 99.9% or 100% identical to SEQ ID NO: 5 or SEQ ID NO: 6, preferably 98%, 99% or 100% identical to SEQ ID NO: 5 or SEQ ID NO: 6. Exemplary non-naturally occurring nucleic acid molecules encoding an HBV pol antigen have the polynucleotide sequence of SEQ ID NOs: 5 or 6.

In another embodiment, a second non-naturally occurring nucleic acid molecule further comprises a coding sequence for a signal sequence that is operably linked to the N-terminus of the HBV pol antigen sequence, such as the amino acid sequence of SEQ ID NO: 7. Preferably, the signal sequence has the amino acid sequence of SEQ ID NO: 9 or SEQ ID NO: 15. More preferably, the coding sequence for a signal sequence comprises the polynucleotide sequence of SEQ ID NO: 8 or SEQ ID NO: 14.

In another embodiment of the application, a non-naturally occurring nucleic acid molecule encodes an HBV antigen fusion protein comprising a truncated HBV core antigen operably linked to an HBV Pol antigen, or an HBV Pol antigen operably linked to a truncated HBV core antigen. In a particular embodiment, a non-naturally occurring nucleic acid molecule of the application encodes a truncated HBV core antigen consisting of an amino acid sequence that is at least 90% identical to SEQ ID NO: 2 or SEQ ID NO: 4, such as at least 90%, 91%, 92%, 93%, 94%, 95%, 95.5%, 96%, 96.5%, 97%, 97.5%, 98%, 98.5%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, 99.9% or 100% identical to SEQ ID NO: 2 or SEQ ID NO: 4, preferably 100% identical to SEQ ID NO: 2 or SEQ ID NO: 4, more preferably 100% identical to SEQ ID NO: 2 or SEQ ID NO: 4; a linker; and an HBV polymerase antigen comprising an amino acid sequence that is at least 90% identical to SEQ ID NO: 7, such as at least 90%, 91%, 92%, 93%, 94%, 95%, 95.5%, 96%, 96.5%, 97%, 97.5%, 98%, 98.5%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, 99.9% or 100% identical to SEQ ID NO: 7, preferably 98%, 99% or 100% identical to SEQ ID NO: 7. In a particular embodiment of the application, a non-naturally occurring nucleic acid molecule encodes a fusion protein comprising a truncated HBV core antigen consisting of the amino acid sequence of SEQ ID NO: 2 or SEQ ID NO: 4, a linker comprising (AlaGly)_n, wherein n is an integer of 2 to 5; and an HBV Pol antigen comprising the amino acid sequence of SEQ ID NO: 7. In a particular

embodiment of the application, a non-naturally occurring nucleic acid molecule encodes an HBV antigen fusion protein comprising the amino acid sequence of SEQ ID NO: 16.

Examples of polynucleotide sequences of the application encoding an HBV antigen fusion protein include, but are not limited to, a polynucleotide sequence at least 90% identical to SEQ ID NO: 1 or SEQ ID NO: 3, such as at least 90%, 91%, 92%, 93%, 94%, 95%, 95.5%, 96%, 96.5%, 97%, 97.5%, 98%, 98.5%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, 99.9% or 100% identical to SEQ ID NO: 1 or SEQ ID NO: 3, preferably 98%, 99% or 100% identical to SEQ ID NO: 1 or SEQ ID NO: 3, operably linked to a linker coding sequence at least 90% identical to SEQ ID NO: 11, such as at least 90%, 91%, 92%, 93%, 94%, 95%, 95.5%, 96%, 96.5%, 97%, 97.5%, 98%, 98.5%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, 99.9% or 100% identical to SEQ ID NO: 11, preferably 98%, 99% or 100% identical to SEQ ID NO: 11, which is further operably linked a polynucleotide sequence at least 90% identical to SEQ ID NO: 5 or SEQ ID NO: 6, such as at least 90%, 91%, 92%, 93%, 94%, 95%, 95.5%, 96%, 96.5%, 97%, 97.5%, 98%, 98.5%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, 99.9% or 100% identical to SEQ ID NO: 5 or SEQ ID NO: 6, preferably 98%, 99% or 100% identical to SEQ ID NO: 5 or SEQ ID NO: 6. In particular embodiments of the application, a non-naturally occurring nucleic acid molecule encoding an HBV antigen fusion protein comprises SEQ ID NO: 1 or SEQ ID NO: 3, operably linked to SEQ ID NO: 11, which is further operably linked to SEQ ID NO: 5 or SEQ ID NO: 6.

In another embodiment, a non-naturally occurring nucleic acid molecule encoding an HBV fusion further comprises a coding sequence for a signal sequence that is operably linked to the N-terminus of the HBV fusion sequence, such as the amino acid sequence of SEQ ID NO: 16. Preferably, the signal sequence has the amino acid sequence of SEQ ID NO: 9 or SEQ ID NO: 15. More preferably, the coding sequence for a signal sequence comprises the polynucleotide sequence of SEQ ID NO: 8 or SEQ ID NO: 14. In one embodiment, the encoded fusion protein with the signal sequence comprises the amino acid sequence of SEQ ID NO: 17.

The application also relates to a vector comprising the first and/or second non-naturally occurring nucleic acid molecules. As used herein, a “vector” is a nucleic acid molecule used to carry genetic material into another cell, where it can be replicated and/or expressed. Any vector known to those skilled in the art in view of

the present disclosure can be used. Examples of vectors include, but are not limited to, plasmids, viral vectors (bacteriophage, animal viruses, and plant viruses), cosmids, and artificial chromosomes (e.g., YACs). Preferably, a vector is a DNA plasmid. A vector can be a DNA vector or an RNA vector. One of ordinary skill in the art can
5 construct a vector of the application through standard recombinant techniques in view of the present disclosure.

A vector of the application can be an expression vector. As used herein, the term “expression vector” refers to any type of genetic construct comprising a nucleic acid coding for an RNA capable of being transcribed. Expression vectors include, but
10 are not limited to, vectors for recombinant protein expression, such as a DNA plasmid or a viral vector, and vectors for delivery of nucleic acid into a subject for expression in a tissue of the subject, such as a DNA plasmid or a viral vector. It will be appreciated by those skilled in the art that the design of the expression vector can depend on such factors as the choice of the host cell to be transformed, the level of
15 expression of protein desired, etc.

Vectors of the application can contain a variety of regulatory sequences. As used herein, the term “regulatory sequence” refers to any sequence that allows, contributes or modulates the functional regulation of the nucleic acid molecule, including replication, duplication, transcription, splicing, translation, stability and/or
20 transport of the nucleic acid or one of its derivative (i.e. mRNA) into the host cell or organism. In the context of the disclosure, this term encompasses promoters, enhancers and other expression control elements (e.g., polyadenylation signals and elements that affect mRNA stability).

In some embodiments of the application, a vector is a non-viral vector.
25 Examples of non-viral vectors include, but are not limited to, DNA plasmids, bacterial artificial chromosomes, yeast artificial chromosomes, bacteriophages, etc. Examples of non-viral vectors include, but are not limited to, RNA replicon, mRNA replicon, modified mRNA replicon or self-amplifying mRNA, closed linear deoxyribonucleic acid, e.g. a linear covalently closed DNA such as linear covalently closed double
30 stranded DNA molecule. Preferably, a non-viral vector is a DNA plasmid. A “DNA plasmid”, which is used interchangeably with “DNA plasmid vector,” “plasmid DNA” or “plasmid DNA vector,” refers to a double-stranded and generally circular DNA sequence that is capable of autonomous replication in a suitable host cell. DNA plasmids used for expression of an encoded polynucleotide typically comprise an

origin of replication, a multiple cloning site, and a selectable marker, which for example, can be an antibiotic resistance gene. Examples of DNA plasmids suitable that can be used include, but are not limited to, commercially available expression vectors for use in well-known expression systems (including both prokaryotic and eukaryotic systems), such as pSE420 (Invitrogen, San Diego, Calif.), which can be used for production and/or expression of protein in *Escherichia coli*; pYES2 (Invitrogen, Thermo Fisher Scientific), which can be used for production and/or expression in *Saccharomyces cerevisiae* strains of yeast; MAXBAC® complete baculovirus expression system (Thermo Fisher Scientific), which can be used for production and/or expression in insect cells; pcDNATM or pcDNA3TM (Life Technologies, Thermo Fisher Scientific), which can be used for high level constitutive protein expression in mammalian cells; and pVAX or pVAX-1 (Life Technologies, Thermo Fisher Scientific), which can be used for high-level transient expression of a protein of interest in most mammalian cells. The backbone of any commercially available DNA plasmid can be modified to optimize protein expression in the host cell, such as to reverse the orientation of certain elements (e.g., origin of replication and/or antibiotic resistance cassette), replace a promoter endogenous to the plasmid (e.g., the promoter in the antibiotic resistance cassette), and/or replace the polynucleotide sequence encoding transcribed proteins (e.g., the coding sequence of the antibiotic resistance gene), by using routine techniques and readily available starting materials. (See e.g., Sambrook et al., *Molecular Cloning a Laboratory Manual*, Second Ed. Cold Spring Harbor Press (1989)).

Preferably, a DNA plasmid is an expression vector suitable for protein expression in mammalian host cells. Expression vectors suitable for protein expression in mammalian host cells include, but are not limited to, pcDNATM, pcDNA3TM, pVAX, pVAX-1, ADVAX, NTC8454, etc. Preferably, an expression vector is based on pVAX-1, which can be further modified to optimize protein expression in mammalian cells. pVAX-1 is commonly used plasmid in DNA vaccines, and contains a strong human intermediate early cytomegalovirus (CMV-IE) promoter followed by the bovine growth hormone (bGH)-derived polyadenylation sequence (pA). pVAX-1 further contains a pUC origin of replication and kanamycin resistance gene driven by a small prokaryotic promoter that allows for bacterial plasmid propagation.

A vector of the application can also be a viral vector. In general, viral vectors are genetically engineered viruses carrying modified viral DNA or RNA that has been rendered non-infectious, but still contains viral promoters and transgenes, thus allowing for translation of the transgene through a viral promoter. Because viral
5 vectors are frequently lacking infectious sequences, they require helper viruses or packaging lines for large-scale transfection. Examples of viral vectors that can be used include, but are not limited to, adenoviral vectors, adeno-associated virus vectors, pox virus vectors, enteric virus vectors, Venezuelan Equine Encephalitis virus vectors, Semliki Forest Virus vectors, Tobacco Mosaic Virus vectors, lentiviral vectors, etc.
10 Examples of viral vectors that can be used include, but are not limited to, arenavirus viral vectors, replication-deficient arenavirus viral vectors or replication-competent arenavirus viral vectors, bi-segmented or tri-segmented arenavirus, infectious arenavirus viral vectors, nucleic acids which comprise an arenavirus genomic segment wherein one open reading frame of the genomic segment is deleted or functionally
15 inactivated (and replaced by a nucleic acid encoding an HBV antigen as described herein), arenavirus such as lymphocytic choriomeningitis virus (LCMV), e.g., clone 13 strain or MP strain, and arenavirus such as Junin virus e.g., Candid #1 strain. The vector can also be a non-viral vector.

Preferably, a viral vector is an adenovirus vector, e.g., a recombinant
20 adenovirus vector. A recombinant adenovirus vector can for instance be derived from a human adenovirus (HAdV, or AdHu), or a simian adenovirus such as chimpanzee or gorilla adenovirus (ChAd, AdCh, or SAdV) or rhesus adenovirus (rhAd). Preferably, an adenovirus vector is a recombinant human adenovirus vector, for instance a recombinant human adenovirus serotype 26, or any one of recombinant human
25 adenovirus serotype 5, 4, 35, 7, 48, etc. In other embodiments, an adenovirus vector is a rhAd vector, e.g. rhAd51, rhAd52 or rhAd53. A recombinant viral vector useful for the application can be prepared using methods known in the art in view of the present disclosure. For example, in view of the degeneracy of the genetic code, several nucleic acid sequences can be designed that encode the same polypeptide. A
30 polynucleotide encoding an HBV antigen of the application can optionally be codon-optimized to ensure proper expression in the host cell (e.g., bacterial or mammalian cells). Codon-optimization is a technology widely applied in the art, and methods for obtaining codon-optimized polynucleotides will be well known to those skilled in the art in view of the present disclosure.

A vector of the application, e.g., a DNA plasmid or a viral vector (particularly an adenoviral vector), can comprise any regulatory elements to establish conventional function(s) of the vector, including but not limited to replication and expression of the HBV antigen(s) encoded by the polynucleotide sequence of the vector. Regulatory elements include, but are not limited to, a promoter, an enhancer, a polyadenylation signal, translation stop codon, a ribosome binding element, a transcription terminator, selection markers, origin of replication, etc. A vector can comprise one or more expression cassettes. An “expression cassette” is part of a vector that directs the cellular machinery to make RNA and protein. An expression cassette typically comprises three components: a promoter sequence, an open reading frame, and a 3'-untranslated region (UTR) optionally comprising a polyadenylation signal. An open reading frame (ORF) is a reading frame that contains a coding sequence of a protein of interest (e.g., HBV antigen) from a start codon to a stop codon. Regulatory elements of the expression cassette can be operably linked to a polynucleotide sequence encoding an HBV antigen of interest. As used herein, the term “operably linked” is to be taken in its broadest reasonable context, and refers to a linkage of polynucleotide elements in a functional relationship. A polynucleotide is “operably linked” when it is placed into a functional relationship with another polynucleotide. For instance, a promoter is operably linked to a coding sequence if it affects the transcription of the coding sequence. Any components suitable for use in an expression cassette described herein can be used in any combination and in any order to prepare vectors of the application.

A vector can comprise a promoter sequence, preferably within an expression cassette, to control expression of an HBV antigen of interest. The term “promoter” is used in its conventional sense, and refers to a nucleotide sequence that initiates the transcription of an operably linked nucleotide sequence. A promoter is located on the same strand near the nucleotide sequence it transcribes. Promoters can be a constitutive, inducible, or repressible. Promoters can be naturally occurring or synthetic. A promoter can be derived from sources including viral, bacterial, fungal, plants, insects, and animals. A promoter can be a homologous promoter (i.e., derived from the same genetic source as the vector) or a heterologous promoter (i.e., derived from a different vector or genetic source). For example, if the vector to be employed is a DNA plasmid, the promoter can be endogenous to the plasmid (homologous) or derived from other sources (heterologous). Preferably, the promoter is located

upstream of the polynucleotide encoding an HBV antigen within an expression cassette.

Examples of promoters that can be used include, but are not limited to, a promoter from simian virus 40 (SV40), a mouse mammary tumor virus (MMTV) promoter, a human immunodeficiency virus (HIV) promoter such as the bovine immunodeficiency virus (BIV) long terminal repeat (LTR) promoter, a Moloney virus promoter, an avian leukosis virus (ALV) promoter, a cytomegalovirus (CMV) promoter such as the CMV immediate early promoter (CMV-IE), Epstein Barr virus (EBV) promoter, or a Rous sarcoma virus (RSV) promoter. A promoter can also be a promoter from a human gene such as human actin, human myosin, human hemoglobin, human muscle creatine, or human metallothionein. A promoter can also be a tissue specific promoter, such as a muscle or skin specific promoter, natural or synthetic.

Preferably, a promoter is a strong eukaryotic promoter, preferably a cytomegalovirus immediate early (CMV-IE) promoter. A nucleotide sequence of an exemplary CMV-IE promoter is shown in SEQ ID NO: 18 or SEQ ID NO: 19.

A vector can comprise additional polynucleotide sequences that stabilize the expressed transcript, enhance nuclear export of the RNA transcript, and/or improve transcriptional-translational coupling. Examples of such sequences include polyadenylation signals and enhancer sequences. A polyadenylation signal is typically located downstream of the coding sequence for a protein of interest (e.g., an HBV antigen) within an expression cassette of the vector. Enhancer sequences are regulatory DNA sequences that, when bound by transcription factors, enhance the transcription of an associated gene. An enhancer sequence is preferably located upstream of the polynucleotide sequence encoding an HBV antigen, but downstream of a promoter sequence within an expression cassette of the vector.

Any polyadenylation signal known to those skilled in the art in view of the present disclosure can be used. For example, the polyadenylation signal can be a SV40 polyadenylation signal, LTR polyadenylation signal, bovine growth hormone (bGH) polyadenylation signal, human growth hormone (hGH) polyadenylation signal, or human β -globin polyadenylation signal. Preferably, a polyadenylation signal is a bovine growth hormone (bGH) polyadenylation signal or a SV40 polyadenylation signal. A nucleotide sequence of an exemplary bGH polyadenylation signal is shown

in SEQ ID NO: 20. A nucleotide sequence of an exemplary SV40 polyadenylation signal is shown in SEQ ID NO: 13.

Any enhancer sequence known to those skilled in the art in view of the present disclosure can be used. For example, an enhancer sequence can be human actin,
5 human myosin, human hemoglobin, human muscle creatine, or a viral enhancer, such as one from CMV, HA, RSV, or EBV. Examples of particular enhancers include, but are not limited to, Woodchuck HBV Post-transcriptional regulatory element (WPRE), intron/exon sequence derived from human apolipoprotein A1 precursor (ApoAI),
10 untranslated R-U5 domain of the human T-cell leukemia virus type 1 (HTLV-1) long terminal repeat (LTR), a splicing enhancer, a synthetic rabbit β -globin intron, or any combination thereof. Preferably, an enhancer sequence is a composite sequence of three consecutive elements of the untranslated R-U5 domain of HTLV-1 LTR, rabbit β -globin intron, and a splicing enhancer, which is referred to herein as “a triple enhancer sequence.” A nucleotide sequence of an exemplary triple enhancer sequence
15 is shown in SEQ ID NO: 10. Another exemplary enhancer sequence is an ApoAI gene fragment shown in SEQ ID NO: 12.

A vector can comprise a polynucleotide sequence encoding a signal peptide sequence. Preferably, the polynucleotide sequence encoding the signal peptide sequence is located upstream of the polynucleotide sequence encoding an HBV
20 antigen. Signal peptides typically direct localization of a protein, facilitate secretion of the protein from the cell in which it is produced, and/or improve antigen expression and cross-presentation to antigen-presenting cells. A signal peptide can be present at the N-terminus of an HBV antigen when expressed from the vector, but is cleaved off by signal peptidase, e.g., upon secretion from the cell. An expressed protein in which
25 a signal peptide has been cleaved is often referred to as the “mature protein.” Any signal peptide known in the art in view of the present disclosure can be used. For example, a signal peptide can be a cystatin S signal peptide; an immunoglobulin (Ig) secretion signal, such as the Ig heavy chain gamma signal peptide SPIgG or the Ig heavy chain epsilon signal peptide SPIgE.

30 Preferably, a signal peptide sequence is a cystatin S signal peptide. Exemplary nucleic acid and amino acid sequences of a cystatin S signal peptide are shown in SEQ ID NOs: 8 and 9, respectively. Exemplary nucleic acid and amino acid sequences of an immunoglobulin secretion signal are shown in SEQ ID NOs: 14 and 15, respectively.

A vector, such as a DNA plasmid, can also include a bacterial origin of replication and an antibiotic resistance expression cassette for selection and maintenance of the plasmid in bacterial cells, e.g., *E. coli*. Bacterial origins of replication and antibiotic resistance cassettes can be located in a vector in the same orientation as the expression cassette encoding an HBV antigen, or in the opposite (reverse) orientation. An origin of replication (ORI) is a sequence at which replication is initiated, enabling a plasmid to reproduce and survive within cells. Examples of ORIs suitable for use in the application include, but are not limited to ColE1, pMB1, pUC, pSC101, R6K, and 15A, preferably pUC. An exemplary nucleotide sequence of a pUC ORI is shown in SEQ ID NO: 21.

Expression cassettes for selection and maintenance in bacterial cells typically include a promoter sequence operably linked to an antibiotic resistance gene. Preferably, the promoter sequence operably linked to an antibiotic resistance gene differs from the promoter sequence operably linked to a polynucleotide sequence encoding a protein of interest, e.g., HBV antigen. The antibiotic resistance gene can be codon optimized, and the sequence composition of the antibiotic resistance gene is normally adjusted to bacterial, e.g., *E. coli*, codon usage. Any antibiotic resistance gene known to those skilled in the art in view of the present disclosure can be used, including, but not limited to, kanamycin resistance gene (Kanr), ampicillin resistance gene (Ampr), and tetracycline resistance gene (Tetr), as well as genes conferring resistance to chloramphenicol, bleomycin, spectinomycin, carbenicillin, etc.

Preferably, an antibiotic resistance gene in the antibiotic expression cassette of a vector is a kanamycin resistance gene (Kanr). The sequence of Kanr gene is shown in SEQ ID NO: 22. Preferably, the Kanr gene is codon optimized. An exemplary nucleic acid sequence of a codon optimized Kanr gene is shown in SEQ ID NO: 23. The Kanr can be operably linked to its native promoter, or the Kanr gene can be linked to a heterologous promoter. In a particular embodiment, the Kanr gene is operably linked to the ampicillin resistance gene (Ampr) promoter, known as the bla promoter. An exemplary nucleotide sequence of a bla promoter is shown in SEQ ID NO: 24.

In a particular embodiment of the application, a vector is a DNA plasmid comprising an expression cassette including a polynucleotide encoding at least one of an HBV antigen selected from the group consisting of an HBV pol antigen comprising an amino acid sequence at least 90%, such as 90%, 91%, 92%, 93%, 94%, 95%, 96, 97%, preferably at least 98%, such as at least 98%, 98.5%, 99%, 99.1%, 99.2%,

99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, 99.9% or 100%, identical to SEQ ID NO: 7, and a truncated HBV core antigen consisting of the amino acid sequence at least 95%, such as 95%, 96, 97%, preferably at least 98%, such as at least 98%, 98.5%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, 99.9% or 100%, identical of SEQ ID NO: 2 or SEQ ID NO: 4; an upstream sequence operably linked to the polynucleotide encoding the HBV antigen comprising, from 5' end to 3' end, a promoter sequence, preferably a CMV promoter sequence of SEQ ID NO: 18, an enhancer sequence, preferably a triple enhancer sequence of SEQ ID NO: 10, and a polynucleotide sequence encoding a signal peptide sequence, preferably a cystatin S signal peptide having the amino acid sequence of SEQ ID NO: 9; and a downstream sequence operably linked to the polynucleotide encoding the HBV antigen comprising a polyadenylation signal, preferably a bGH polyadenylation signal of SEQ ID NO: 20. Such vector further comprises an antibiotic resistance expression cassette including a polynucleotide encoding an antibiotic resistance gene, preferably a Kan^r gene, more preferably a codon optimized Kan^r gene of at least 90% identical to SEQ ID NO: 23, such as at least 90%, 91%, 92%, 93%, 94%, 95%, 95.5%, 96%, 96.5%, 97%, 97.5%, 98%, 98.5%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, 99.9% or 100% identical to SEQ ID NO: 23, preferably 100% identical to SEQ ID NO: 23, operably linked to an Ampr (bla) promoter of SEQ ID NO: 24, upstream of and operably linked to the polynucleotide encoding the antibiotic resistance gene; and an origin of replication, preferably a pUC ori of SEQ ID NO: 21. Preferably, the antibiotic resistance cassette and the origin of replication are present in the plasmid in the reverse orientation relative to the HBV antigen expression cassette.

In another particular embodiment of the application, a vector is a viral vector, preferably an adenoviral vector, more preferably an Ad26 or Ad35 vector, comprising an expression cassette including a polynucleotide encoding at least one of an HBV antigen selected from the group consisting of an HBV pol antigen comprising an amino acid sequence at least 90%, such as 90%, 91%, 92%, 93%, 94%, 95%, 96, 97%, preferably at least 98%, such as at least 98%, 98.5%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, 99.9% or 100%, identical to SEQ ID NO: 7, and a truncated HBV core antigen consisting of the amino acid sequence at least 95%, such as 95%, 96, 97%, preferably at least 98%, such as at least 98%, 98.5%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, 99.9% or 100%, identical of SEQ ID NO: 2 or SEQ ID NO: 4; an upstream sequence operably linked to

the polynucleotide encoding the HBV antigen comprising, from 5' end to 3' end, a promoter sequence, preferably a CMV promoter sequence of SEQ ID NO: 19, an enhancer sequence, preferably an ApoAI gene fragment sequence of SEQ ID NO: 12, and a polynucleotide sequence encoding a signal peptide sequence, preferably an immunoglobulin secretion signal having the amino acid sequence of SEQ ID NO: 15; and a downstream sequence operably linked to the polynucleotide encoding the HBV antigen comprising a polyadenylation signal, preferably a SV40 polyadenylation signal of SEQ ID NO: 13.

In an embodiment of the application, a vector, such as a plasmid DNA vector or a viral vector (preferably an adenoviral vector, more preferably an Ad26 or Ad35 vector), encodes an HBV Pol antigen having the amino acid sequence of SEQ ID NO: 7. Preferably, the vector comprises a coding sequence for the HBV Pol antigen that is at least 90% identical to the polynucleotide sequence of SEQ ID NO: 5 or 6, such as 90%, 91%, 92%, 93%, 94%, 95%, 95.5%, 96%, 96.5%, 97%, 97.5%, 98%, 98.5%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, 99.9% or 100% identical to SEQ ID NO: 5 or 6, preferably 100% identical to SEQ ID NO: 5 or 6.

In an embodiment of the application, a vector, such as a plasmid DNA vector or a viral vector (preferably an adenoviral vector, more preferably an Ad26 or Ad35 vector), encodes a truncated HBV core antigen consisting of the amino acid sequence of SEQ ID NO: 2 or SEQ ID NO: 4. Preferably, the vector comprises a coding sequence for the truncated HBV core antigen that is at least 90% identical to the polynucleotide sequence of SEQ ID NO: 1 or SEQ ID NO: 3, such as 90%, 91%, 92%, 93%, 94%, 95%, 95.5%, 96%, 96.5%, 97%, 97.5%, 98%, 98.5%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, 99.9% or 100% identical to SEQ ID NO: 1 or SEQ ID NO: 3, preferably 100% identical to SEQ ID NO: 1 or SEQ ID NO: 3.

In yet another embodiment of the application, a vector, such as a plasmid DNA vector or a viral vector (preferably an adenoviral vector, more preferably an Ad26 or Ad35 vector), encodes a fusion protein comprising an HBV Pol antigen having the amino acid sequence of SEQ ID NO: 7 and a truncated HBV core antigen consisting of the amino acid sequence of SEQ ID NO: 1 or SEQ ID NO: 3. Preferably, the vector comprises a coding sequence for the fusion, which contains a coding sequence for the truncated HBV core antigen at least 90% identical to SEQ ID NO: 1 or SEQ ID NO: 3, such as at least 90%, 91%, 92%, 93%, 94%, 95%, 95.5%, 96%, 96.5%, 97%, 97.5%,

98%, 98.5%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, 99.9% or 100% identical to SEQ ID NO: 1 or SEQ ID NO: 3, preferably 98%, 99% or 100% identical to SEQ ID NO: 1 or SEQ ID NO: 3, more preferably SEQ ID NO: 1 or SEQ ID NO: 3, operably linked to a coding sequence for the HBV Pol antigen at least

5 90% identical to SEQ ID NO: 5 or SEQ ID NO: 6, such as at least 90%, 91%, 92%, 93%, 94%, 95%, 95.5%, 96%, 96.5%, 97%, 97.5%, 98%, 98.5%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, 99.9% or 100% identical to SEQ ID NO: 5 or SEQ ID NO: 6, preferably 98%, 99% or 100% identical to SEQ ID NO: 5 or SEQ ID NO: 6, more preferably SEQ ID NO: 5 or SEQ ID NO: 6. Preferably, the

10 coding sequence for the truncated HBV core antigen is operably linked to the coding sequence for the HBV Pol antigen via a coding sequence for a linker at least 90% identical to SEQ ID NO: 11, such as at least 90%, 91%, 92%, 93%, 94%, 95%, 95.5%, 96%, 96.5%, 97%, 97.5%, 98%, 98.5%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, 99.9% or 100% identical to SEQ ID NO: 11, preferably 98%,

15 99% or 100% identical to SEQ ID NO: 11. In particular embodiments of the application, a vector comprises a coding sequence for the fusion having SEQ ID NO: 1 or SEQ ID NO: 3 operably linked to SEQ ID NO: 11, which is further operably linked to SEQ ID NO: 5 or SEQ ID NO: 6.

The polynucleotides and expression vectors encoding the HBV antigens of the

20 application can be made by any method known in the art in view of the present disclosure. For example, a polynucleotide encoding an HBV antigen can be introduced or “cloned” into an expression vector using standard molecular biology techniques, e.g., polymerase chain reaction (PCR), etc., which are well known to those skilled in the art.

25 **Cells, Polypeptides and Antibodies**

The application also provides cells, preferably isolated cells, comprising any of the polynucleotides and vectors described herein. The cells can, for instance, be used for recombinant protein production, or for the production of viral particles.

Embodiments of the application thus also relate to a method of making an

30 HBV antigen of the application. The method comprises transfecting a host cell with an expression vector comprising a polynucleotide encoding an HBV antigen of the application operably linked to a promoter, growing the transfected cell under conditions suitable for expression of the HBV antigen, and optionally purifying or isolating the HBV antigen expressed in the cell. The HBV antigen can be isolated or

collected from the cell by any method known in the art including affinity chromatography, size exclusion chromatography, etc. Techniques used for recombinant protein expression will be well known to one of ordinary skill in the art in view of the present disclosure. The expressed HBV antigens can also be studied
5 without purifying or isolating the expressed protein, e.g., by analyzing the supernatant of cells transfected with an expression vector encoding the HBV antigen and grown under conditions suitable for expression of the HBV antigen.

Thus, also provided are non-naturally occurring or recombinant polypeptides comprising an amino acid sequence that is at least 90% identical to the amino acid
10 sequence of SEQ ID NO: 2, SEQ ID NO: 4, or SEQ ID NO: 7. As described above and below, isolated nucleic acid molecules encoding these sequences, vectors comprising these sequences operably linked to a promoter, and compositions comprising the polypeptide, polynucleotide, or vector are also contemplated by the application.

15 In an embodiment of the application, a recombinant polypeptide comprises an amino acid sequence that is at least 90% identical to the amino acid sequence of SEQ ID NO: 2, such as 90%, 91%, 92%, 93%, 94%, 95%, 95.5%, 96%, 96.5%, 97%, 97.5%, 98%, 98.5%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, 99.9% or 100% identical to SEQ ID NO: 2. Preferably, a non-naturally
20 occurring or recombinant polypeptide consists of SEQ ID NO: 2.

In another embodiment of the application, a non-naturally occurring or recombinant polypeptide comprises an amino acid sequence that is at least 90% identical to the amino acid sequence of SEQ ID NO: 4, such as 90%, 91%, 92%, 93%, 94%, 95%, 95.5%, 96%, 96.5%, 97%, 97.5%, 98%, 98.5%, 99%, 99.1%, 99.2%,
25 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, 99.9% or 100% identical to SEQ ID NO: 4. Preferably, a non-naturally occurring or recombinant polypeptide comprises SEQ ID NO: 4.

In another embodiment of the application, a non-naturally occurring or recombinant polypeptide comprises an amino acid sequence that is at least 90% identical to the amino acid sequence of SEQ ID NO: 7, such as 90%, 91%, 92%, 93%, 94%, 95%, 95.5%, 96%, 96.5%, 97%, 97.5%, 98%, 98.5%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, 99.9% or 100% identical to SEQ ID
30 NO: 7. Preferably, a non-naturally occurring or recombinant polypeptide consists of SEQ ID NO: 7.

Also provided are antibodies or antigen binding fragments thereof that specifically bind to a non-naturally occurring polypeptide of the application. In an embodiment of the application, an antibody specific to a non-naturally HBV antigen of the application does not bind specifically to another HBV antigen. For example, an antibody of the application that binds specifically to an HBV Pol antigen having the amino acid sequence of SEQ ID NO: 7 will not bind specifically to an HBV Pol antigen not having the amino acid sequence of SEQ ID NO: 7.

As used herein, the term “antibody” includes polyclonal, monoclonal, chimeric, humanized, Fv, Fab and F(ab')₂; bifunctional hybrid (e.g., Lanzavecchia et al., Eur. J. Immunol. 17:105, 1987), single-chain (Huston et al., Proc. Natl. Acad. Sci. USA 85:5879, 1988; Bird et al., Science 242:423, 1988); and antibodies with altered constant regions (e.g., U.S. Pat. No. 5,624,821).

As used herein, an antibody that “specifically binds to” an antigen refers to an antibody that binds to the antigen with a KD of 1×10^{-7} M or less. Preferably, an antibody that “specifically binds to” an antigen binds to the antigen with a KD of 1×10^{-8} M or less, more preferably 5×10^{-9} M or less, 1×10^{-9} M or less, 5×10^{-10} M or less, or 1×10^{-10} M or less. The term “KD” refers to the dissociation constant, which is obtained from the ratio of Kd to Ka (i.e., Kd/Ka) and is expressed as a molar concentration (M). KD values for antibodies can be determined using methods in the art in view of the present disclosure. For example, the KD of an antibody can be determined by using surface plasmon resonance, such as by using a biosensor system, e.g., a Biacore® system, or by using bio-layer interferometry technology, such as a Octet RED96 system.

The smaller the value of the KD of an antibody, the higher affinity that the antibody binds to a target antigen.

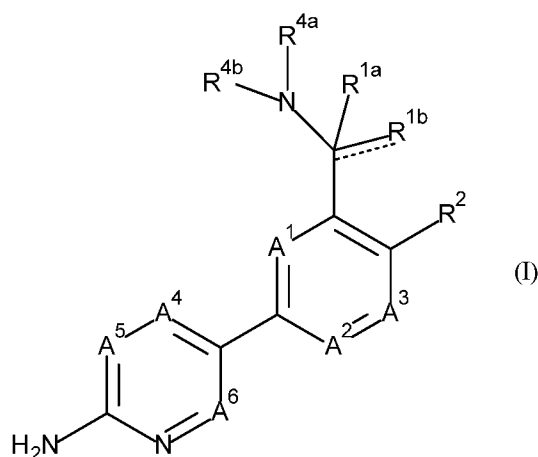
HPK1 Inhibitors

The application also relates to inhibitors of hematopoietic protein kinase 1 (HPK1). An HPK1 inhibitor is a compound that reduces HPK1 functions, such as the ability to recruit proteins to T-cell receptors (TCRs) and phosphorylate proteins, such as SLP76 and GADS. Therefore, HPK1 inhibitors can be useful in the treatment or prevention, particularly the treatment, of diseases that are susceptible to the effects of the immune system, such as cancer and viral infection. HPK1 inhibitors can enhance an immune response, increase T cell activity, and/or possess anti-tumoral properties through immune modulation. The HPK1 inhibitors described herein can be useful for

treating or preventing, in particular treating, infectious diseases, such as viral, bacterial, fungal, and parasitic infections, particularly viral infections. In some embodiments, the HPK1 inhibitors described herein can be used in the treatment of chronic infection, such as chronic viral infection, e.g., chronic HBV infection.

- 5 The HPK1 inhibitors of the application can also be combined with other agents that stimulate or enhance the immune response, such as vaccines. For example, the HPK1 inhibitors described herein can be used in compositions, therapeutic combinations, and kits comprising one or more HBV antigens, polynucleotides, and/or vectors encoding one or more HBV antigens according to the application (e.g., HBV
10 vaccines), as described in more detail below.

According to embodiments of the application, an HPK1 inhibitor is a compound of formula (I):



or a tautomer or a stereoisomeric form thereof, wherein:

- 15 the dotted bond towards R^{1b} is an optional bond that is optionally present when R^{1b} and R^{4b} are taken together to form a monocyclic or bicyclic aromatic heterocyclyl;
 A^1 represents CH or N; A^2 represents CH; A^3 represents CH or N;
 provided that only one of A^1 and A^3 represents N;
 A^4 represents CH or N; A^5 represents CR^{3a} ; A^6 represents CH;
 20 R^{1a} represents hydrogen;
 R^{1b} represents hydrogen or CH_3 ;
 R^{4a} represents hydrogen, C_{1-4} alkyl, or C_{3-6} cycloalkyl;
 R^{4b} represents hydrogen, C_{1-4} alkyl, C_{3-6} cycloalkyl, or
 a carbon linked monocyclic 4-, 5-, 6- or 7-membered non-aromatic heterocyclyl

containing 1, 2 or 3 heteroatoms each independently selected from the group consisting of N, O, and S; wherein said S-atom is optionally substituted to form S(=O), S(=O)₂, or S(=O)(=NR^x);

or

- 5 R^{1b} and R^{4b} are taken together to form together with the atoms to which they are attached a monocyclic 5-membered aromatic heterocyclyl or a monocyclic 4-, 5-, 6- or 7-membered fully saturated heterocyclyl, each containing 1 N-atom and optionally 1 or 2 additional heteroatoms each independently selected from the group consisting of N, O, and S; wherein said optional S-atom is optionally substituted to form S(=O), S(=O)₂, or S(=O)(=NR^x); or
- 10 a bicyclic 6- to 12- membered aromatic or fully saturated heterocyclyl containing 1 N-atom and optionally 1 or 2 additional heteroatoms each independently selected from the group consisting of N, O, and S; wherein said optional S-atom is optionally substituted to form S(=O), S(=O)₂, or S(=O)(=NR^x);
- 15 wherein said monocyclic or bicyclic, aromatic or fully saturated heterocyclyl is optionally substituted on one or more of the carbon atoms with in total 1, 2 or 3 substituents each independently selected from the group consisting of -OH, CN, halo, R⁷, -O-R⁷, -S(=O)₂-R⁷, -C(=O)-R⁷, -NR^{6c}R^{6d}, -C(=O)-NR^{6a}R^{6b}, and Het^c;
- 20 wherein said monocyclic or bicyclic, aromatic or fully saturated heterocyclyl is optionally substituted on the optional additional nitrogen atoms with in total 1 or 2 substituents each independently selected from the group consisting of R⁷, -S(=O)₂-R⁷, -C(=O)-R⁷, -C(=O)-NR^{6a}R^{6b}, and Het^d;
- 25 provided that in case R^{1b} and R^{4b} are taken together, R^{4a} represents hydrogen; and R^{1a} represents hydrogen or R^{1a} is absent when the dotted bond towards R^{1b} is a bond;
- or
- R^{4a} and R^{4b} are taken together to form together with the N-atom to which they are attached a monocyclic 5-membered aromatic heterocyclyl or a monocyclic 4-, 5-, 6- or
- 30 7-membered fully saturated heterocyclyl, each containing 1 N-atom and optionally 1 or 2 additional heteroatoms each independently selected from the group consisting of N, O, and S; wherein said optional S-atom is optionally substituted to form S(=O), S(=O)₂, or S(=O)(=NR^x); or

- a bicyclic 6- to 12- membered aromatic or fully saturated heterocyclyl containing 1 N-atom and optionally 1 or 2 additional heteroatoms each independently selected from the group consisting of N, O, and S; wherein said optional S-atom is optionally substituted to form S(=O), S(=O)₂, or S(=O)(=NR^x);
- 5 wherein said monocyclic or bicyclic, aromatic or fully saturated heterocyclyl is optionally substituted on one or more of the carbon atoms with in total 1, 2 or 3 substituents each independently selected from the group consisting of -OH, CN, halo, R⁷, -O-R⁷, -S(=O)₂-R⁷, -C(=O)-R⁷, -NR^{6c}R^{6d}, -C(=O)-NR^{6a}R^{6b}, and Het^c;
- 10 wherein said monocyclic or bicyclic, aromatic or fully saturated heterocyclyl is optionally substituted on the nitrogen atoms with in total 1 or 2 substituents each independently selected from the group consisting of R⁷, -S(=O)₂-R⁷, -C(=O)-R⁷, -C(=O)-NR^{6a}R^{6b}, and Het^d;
- in case R^{4a} and R^{4b} are taken together, R^{1a} represents hydrogen, and R^{1b} represents
- 15 hydrogen;
- R² is selected from the group consisting of cyano; halo; -C(=O)-NR^{8a}R^{8b}; -CH₂-NR^{8c}R^{8d}; Het^b; -P(=O)-(C₁₋₄alkyl)₂; -S(=O)₂-C₁₋₄alkyl; -S(=O)(=NR^x)-C₁₋₄alkyl; C₁₋₆alkyl optionally substituted with 1 or 2 substituents each independently selected from the group consisting of halo, -OH, cyano, and -O-C₁₋₄alkyl;
- 20 C₃₋₆cycloalkyl optionally substituted with 1 or 2 substituents each independently selected from the group consisting of halo, -OH, cyano and -O-C₁₋₄alkyl; and C₃₋₆cycloalkenyl optionally substituted with 1 or 2 substituents each independently selected from the group consisting of halo, -OH, cyano and -O-C₁₋₄alkyl;
- R^{3a} represents hydrogen, halo, R⁷, -O-R⁷, cyano, -C(=O)-NR^{6c}R^{6f}, Het^a, or phenyl
- 25 optionally substituted with 1, 2 or 3 substituents each independently selected from the group consisting of halo, cyano, C₁₋₄alkyl, -O-C₁₋₄alkyl, C₁₋₄alkyl substituted with one cyano, and C₁₋₄alkyl substituted with 1, 2 or 3 halo atoms;
- Het^a represents a carbon linked monocyclic 5-, 6- or 7-membered aromatic heterocyclyl or carbon linked monocyclic 4-, 5-, 6- or 7-membered non-aromatic
- 30 heterocyclyl, each containing 1, 2 or 3 heteroatoms each independently selected from the group consisting of N, O or S; wherein said S-atom might be substituted to form S(=O), S(=O)₂, or S(=O)(=NR^x); or a carbon linked bicyclic 6- to 12-membered aromatic or non-aromatic heterocyclyl containing 1, 2 or 3 heteroatoms each independently selected from the group

- consisting of N, O and S; wherein said S-atom is optionally substituted to form S(=O), S(=O)₂, or S(=O)(=NR^x);
- wherein said monocyclic or bicyclic, aromatic or non-aromatic heterocyclyl is optionally substituted on one or more of the carbon atoms with in total 1, 2 or 3
- 5 substituents each independently selected from the group consisting of -OH, CN, halo, R⁷, -O-R⁷, -S(=O)₂-R⁷, -C(=O)-R⁷, -NR^{6c}R^{6d}, -C(=O)-NR^{6a}R^{6b}, and Het^c;
- wherein said monocyclic or bicyclic, aromatic or non-aromatic heterocyclyl is optionally substituted on the nitrogen atoms with in total 1 or 2 substituents each
- 10 independently selected from the group consisting of R⁷, -S(=O)₂-R⁷, -C(=O)-R⁷, -C(=O)-NR^{6a}R^{6b} and Het^d;
- R^{6a}, R^{6b}, R^{6c}, R^{6d}, R^{6e}, and R^{6f} are each independently selected from the group consisting of hydrogen; C₃₋₆cycloalkyl optionally substituted with one -OR⁵; and C₁₋₄alkyl optionally substituted with one -OR⁵, wherein two hydrogen atoms on the
- 15 same carbon atom of said C₁₋₄alkyl are optionally taken together to form C₃₋₆cycloalkyl;
- R⁵ represents hydrogen or C₁₋₄alkyl;
- R^{8a}, R^{8c}, and R^{8d} are each independently selected from the group consisting of hydrogen;
- 20 C₁₋₄alkyl optionally substituted with one -OH or -O-C₁₋₄alkyl; and C₃₋₆cycloalkyl optionally substituted with one -OH or -O-C₁₋₄alkyl;
- R^{8b} is selected from the group consisting C₁₋₄alkyl optionally substituted with one -OH or
- O-C₁₋₄alkyl; and C₃₋₆cycloalkyl optionally substituted with one -OH or -O-C₁₋₄alkyl;
- 25 or
- R^{8a} and R^{8b}, or R^{8c} and R^{8d} are taken together to form together with the N-atom to which they are attached a monocyclic fully saturated heterocyclyl containing 1 N-atom and optionally 1 or 2 additional heteroatoms each independently selected from the group consisting of N, O, S; wherein said optional S-atom is optionally substituted to
- 30 form S(=O), S(=O)₂, or S(=O)(=NR^x); wherein said monocyclic 4-, 5-, 6- or 7-membered fully saturated heterocyclyl is optionally substituted on one or more of the carbon atoms with in total 1, 2 or 3 substituents each independently selected from the group consisting of -OH, CN, halo, R⁷, -O-R⁷, -S(=O)₂-R⁷, -C(=O)-R⁷, -NR^{6c}R^{6d}, and -C(=O)-NR^{6a}R^{6b};

wherein said monocyclic fully saturated heterocyclyl is optionally substituted on the nitrogen atoms with in total 1 or 2 substituents each independently selected from the group consisting of R^7 , $-S(=O)_2-R^7$, $-C(=O)-R^7$, and $-C(=O)-NR^{6a}R^{6b}$;

each Het^c independently represents a monocyclic 4-, 5-, 6- or 7-membered fully saturated heterocyclyl containing 1, 2 or 3 heteroatoms each independently selected from the group consisting of N, O and S; wherein said S-atom is optionally substituted to form $S(=O)$, $S(=O)_2$, or $S(=O)(=NR^x)$;

each Het^d independently represents a carbon linked monocyclic 4-, 5-, 6- or 7-membered fully saturated heterocyclyl containing 1, 2 or 3 heteroatoms each independently selected from the group consisting of N, O and S; wherein said S-atom is optionally substituted to form $S(=O)$, $S(=O)_2$, or $S(=O)(=NR^x)$;

Het^b represents a monocyclic 4-, 5-, 6- or 7-membered non-aromatic heterocyclyl containing 1, 2 or 3 heteroatoms each independently selected from the group consisting of N, O, and S; wherein said S-atom might be substituted to form $S(=O)$, $S(=O)_2$, or $S(=O)(=NR^x)$; or

a bicyclic 6- to 12- membered non-aromatic heterocyclyl containing 1, 2 or 3 heteroatoms each independently selected from the group consisting of N, O, and S; wherein said S-atom might be substituted to form $S(=O)$, $S(=O)_2$, or $S(=O)(=NR^x)$; wherein said monocyclic or bicyclic non-aromatic heterocyclyl might be substituted on one or more of the carbon atoms with in total 1, 2 or 3 substituents each independently selected from the group consisting of $-OH$, CN , halo, R^7 , $-O-R^7$, $-S(=O)_2-R^7$, $-C(=O)-R^7$, $-NR^{6c}R^{6d}$, and $-C(=O)-NR^{6a}R^{6b}$;

wherein said monocyclic or bicyclic non-aromatic heterocyclyl might be substituted on the nitrogen atoms with in total 1 or 2 substituents each independently selected from the group consisting of R^7 , $-S(=O)_2-R^7$, $-C(=O)-R^7$, and $-C(=O)-NR^{6a}R^{6b}$;

each R^7 independently represents C_{3-6} cycloalkyl optionally substituted with 1, 2 or 3 substituents each independently selected from the group consisting of halo, $-OH$, $-O-C_{1-4}$ alkyl and cyano; or C_{1-4} alkyl optionally substituted with 1, 2 or 3 substituents each independently selected from the group consisting of halo, $-OH$, $-O-C_{1-4}$ alkyl and cyano; and

each R^x independently represents hydrogen or C_{1-4} alkyl;

or a pharmaceutically acceptable addition salt, an N-oxide, or a solvate thereof.

The term “halo” or “halogen” as used herein represents fluoro, chloro, bromo and iodo.

The prefix “C_{x-y}” (where x and y are integers) as used herein refers to the number of carbon atoms in a given group. Thus, a C₁₋₆alkyl group contains from 1 to 6 carbon atoms, and so on.

The term “C₁₋₄alkyl” as used herein as a group or part of a group represents a straight or branched chain fully saturated hydrocarbon radical having from 1 to 4 carbon atoms, such as methyl, ethyl, *n*-propyl, isopropyl, *n*-butyl, *s*-butyl, *t*-butyl and the like.

The term “C₃₋₆cycloalkyl” as used herein as a group or part of a group defines a fully saturated, cyclic hydrocarbon radical having from 3 to 6 carbon atoms, such as cyclopropyl, cyclobutyl, cyclopentyl and cyclohexyl.

It will be clear for the skilled person that S(=O)₂ or SO₂ represents a sulfonyl moiety.

It will be clear for the skilled person that CO or C(=O) represents a carbonyl moiety.

In general, whenever the term “substituted” is used, it is meant, unless otherwise indicated or clear from the context, to indicate that one or more hydrogens, in particular from 1 to 4 hydrogens, more particularly from 1 to 3 hydrogens, preferably 1 or 2 hydrogens, more preferably 1 hydrogen, on the atom or radical indicated in the expression using ‘substituted’ are replaced with a selection from the indicated group, provided that the normal valency is not exceeded, and that the substitution results in a chemically stable compound, i.e. a compound that is sufficiently robust to survive isolation to a useful degree of purity from a reaction mixture.

Combinations of substituents and/or variables are permissible only if such combinations result in chemically stable compounds. “Stable compound” is meant to indicate a compound that is sufficiently robust to survive isolation to a useful degree of purity from a reaction mixture.

The term “optionally substituted” means that the atom or radical indicated in the expression using “optionally substituted” may or may not be substituted (this means substituted or unsubstituted respectively).

When two or more substituents are present on a moiety they may, where possible and unless otherwise indicated or clear from the context, replace hydrogens

on the same atom or they may replace hydrogen atoms on different atoms in the moiety.

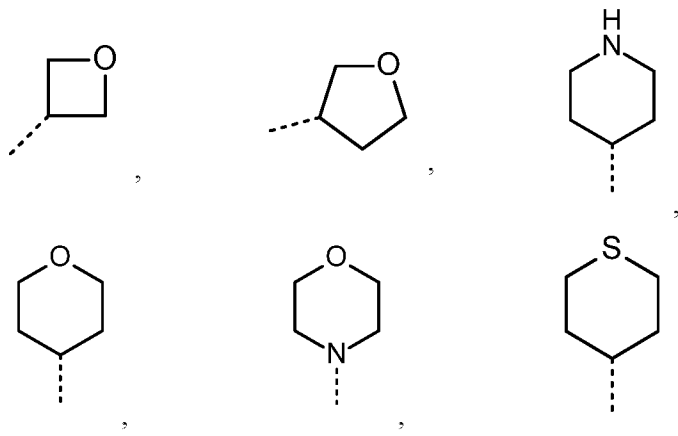
Unless otherwise is indicated or is clear from the context, a substituent on a heterocyclyl group may replace any hydrogen atom on a ring carbon atom or on a ring
5 heteroatom (e.g. a hydrogen on a nitrogen atom may be replaced by a substituent).

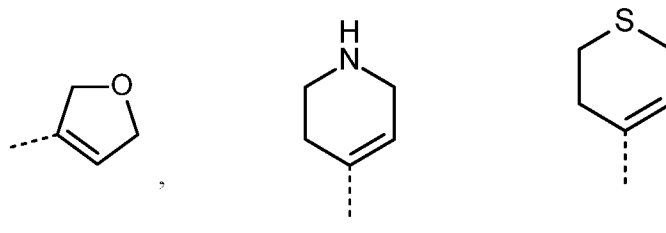
A “non-aromatic” group (e.g. a “monocyclic non-aromatic heterocyclyl”) embraces unsaturated ring systems without aromatic character, partially saturated and fully saturated carbocyclic and heterocyclic ring systems. The term “partially saturated” refers to rings wherein the ring structure(s) contain(s) at least one multiple
10 bond e.g. a C=C bond, N=C bond, etc. The term “fully saturated” refers to rings where there are no multiple bonds between ring atoms.

Unless otherwise specified or clear from the context, aromatic, non-aromatic or fully saturated heterocyclyl groups, can be attached to the remainder of the molecule of Formula (I) through any available ring carbon atom (carbon linked) or nitrogen
15 atom (nitrogen linked).

Unless otherwise specified or clear from the context, aromatic, non-aromatic or fully saturated heterocyclyl groups, can optionally be substituted, where possible, on carbon and/or nitrogen atoms according to the embodiments.

The phrase “monocyclic 4-, 5-, 6- or 7-membered non-aromatic heterocyclyl containing 1, 2 or 3 heteroatoms each independently selected from the group consisting of N, O and S” as used herein alone or as part of another group, defines a
20 monocyclic non-aromatic, cyclic hydrocarbon radical containing at least one nitrogen, oxygen or sulphur atom having from 4 to 7 ring members, as defined above. Non-limiting examples include:

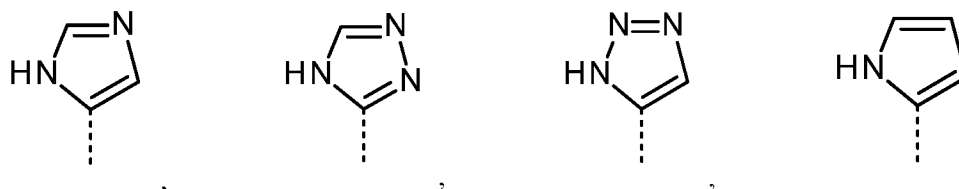




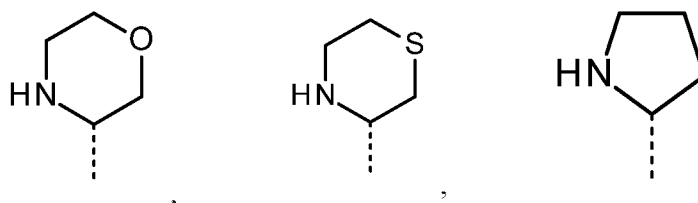
Within the context of this invention, bicyclic fully saturated heterocyclyl groups include fused, spiro and bridged saturated heterocycles. Fused bicyclic groups are two cycles that share two atoms and the bond between these atoms. Spiro bicyclic groups are two cycles that are joined at a single atom. Bridged bicyclic groups are two cycles that share more than two atoms.

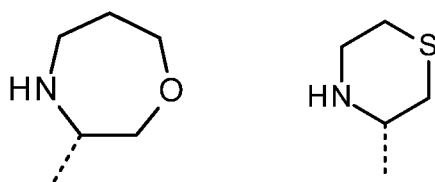
As defined in the scope of compounds of formula (I), when R^{1b} and R^{4b} are taken together, R^{4a} represents hydrogen. This means that the nitrogen atom to which R^{4a} is attached, always has a hydrogen atom and is not substituted, nor is a double bond attached to said nitrogen atom.

Non-limiting examples of R^{1b} and R^{4b} taken together to form a monocyclic 5-membered aromatic heterocyclyl containing 1 N-atom and optionally 1 or 2 additional heteroatoms each independently selected from the group consisting of N, O, and S, include, but are not limited to:

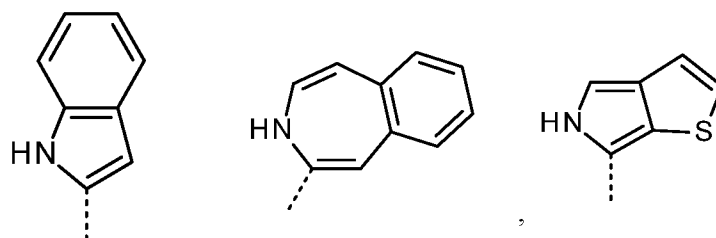


Non-limiting examples of R^{1b} and R^{4b} taken together to form a monocyclic 4-, 5-, 6- or 7-membered fully saturated heterocyclyl containing 1 N-atom and optionally 1 or 2 additional heteroatoms each independently selected from the group consisting of N, O, and S, include, but are not limited to:

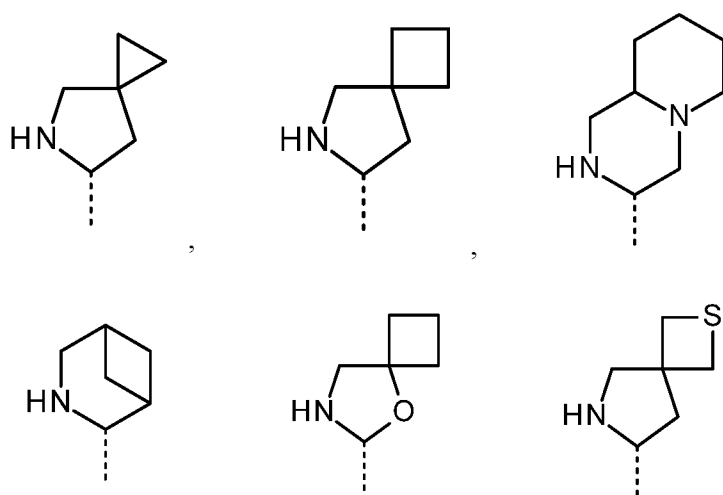




Non-limiting examples of R^{1b} and R^{4b} taken together to form a bicyclic 6- to 12-membered aromatic heterocyclyl containing 1 N-atom and optionally 1 or 2 additional heteroatoms each independently selected from the group consisting of N, O, and S, include, but are not limited to:

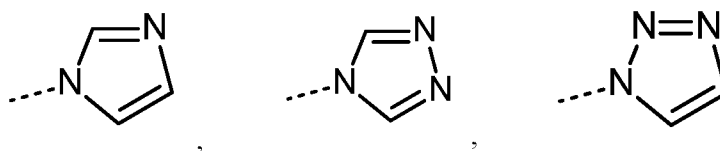


Non-limiting examples of R^{1b} and R^{4b} taken together to form a bicyclic 6- to 12-membered fully saturated heterocyclyl containing 1 N-atom and optionally 1 or 2 additional heteroatoms each independently selected from the group consisting of N, O, and S, include, but are not limited to:

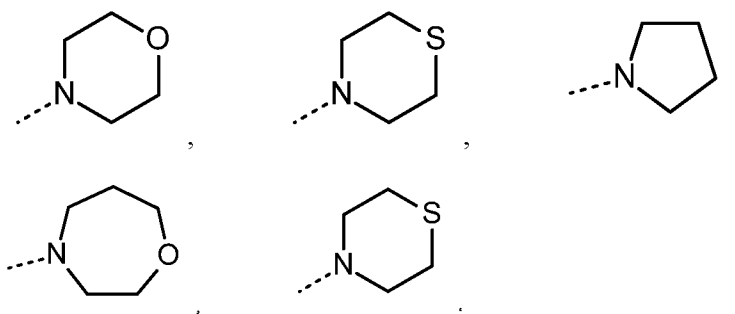


Non-limiting examples of R^{4a} and R^{4b} taken together to form together with the N-atom to which they are attached a monocyclic 5-membered aromatic heterocyclyl

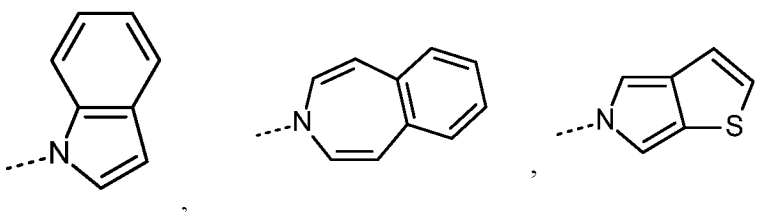
containing 1 N-atom and optionally 1 or 2 additional heteroatoms each independently selected from the group consisting of N, O, and S, include, but are not limited to:



- 5 Non-limiting examples of R^{4a} and R^{4b} taken together to form together with the N-atom to which they are attached a monocyclic 4-, 5-, 6- or 7-membered fully saturated heterocyclyl containing 1 N-atom and optionally 1 or 2 additional heteroatoms each independently selected from the group consisting of N, O, and S, include, but are not limited to:

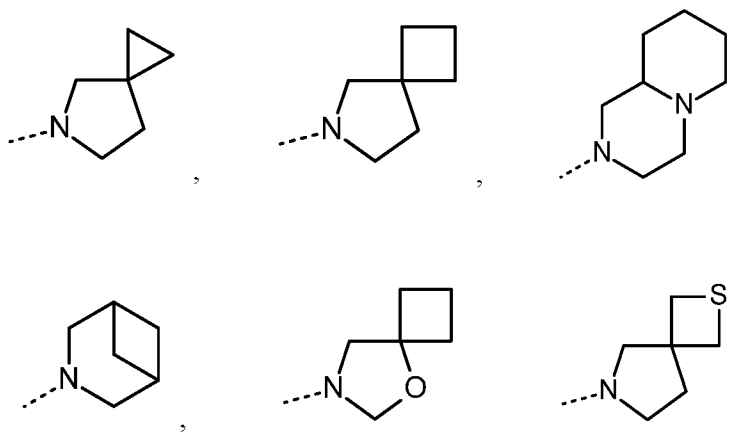


- 10 Non-limiting examples of R^{4a} and R^{4b} taken together to form together with the N-atom to which they are attached a bicyclic 6- to 12-membered aromatic heterocyclyl containing 1 N-atom and optionally 1 or 2 additional heteroatoms each independently selected from the group consisting of N, O, and S, include, but are not limited to:

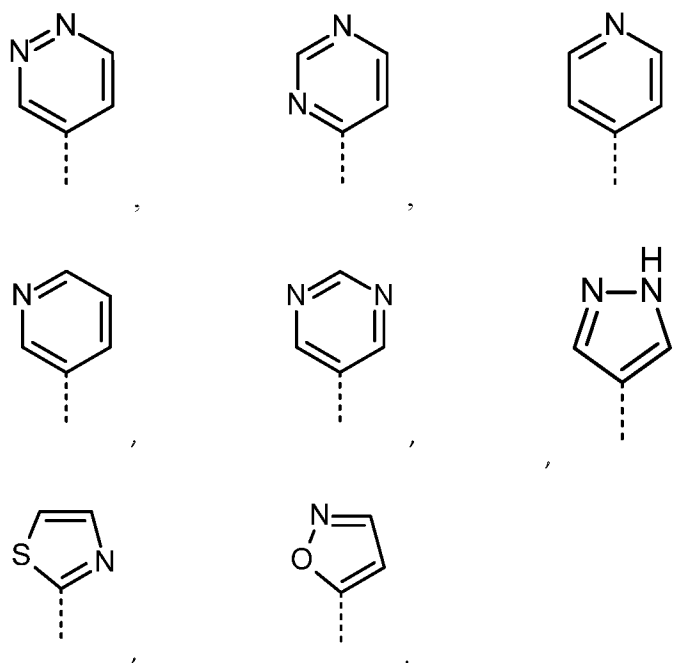


- 15 Non-limiting examples of R^{4a} and R^{4b} taken together to form together with the N-atom to which they are attached a bicyclic 6- to 12-membered fully saturated heterocyclyl containing 1 N-atom and optionally 1 or 2 additional heteroatoms each

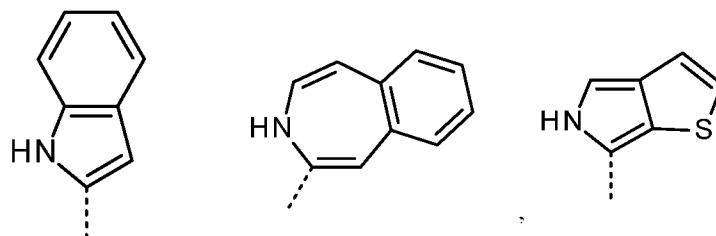
independently selected from the group consisting of N, O, and S, include, but are not limited to:



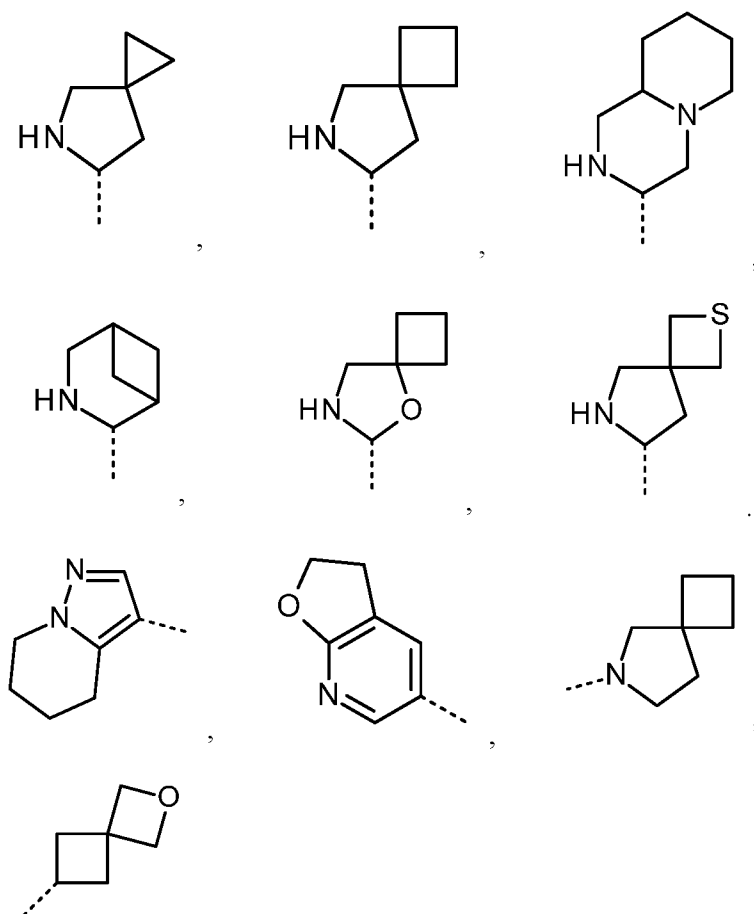
- 5 Non-limiting examples of carbon linked monocyclic 5-, 6- or 7-membered aromatic heterocyclyl containing 1, 2 or 3 heteroatoms each independently selected from the group consisting of N, O and S, include, but are not limited to:



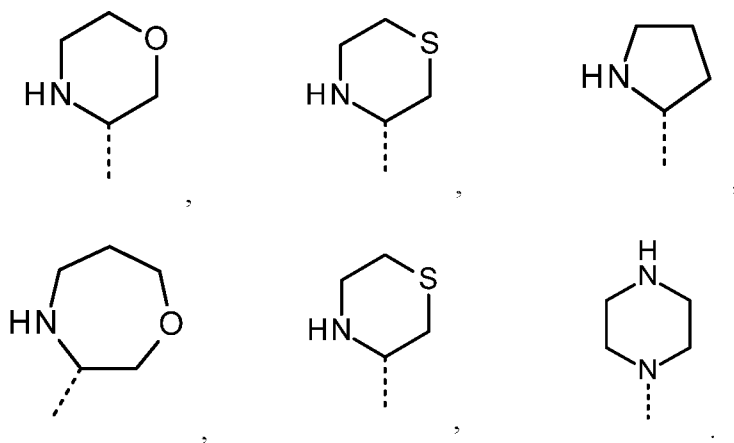
- 10 Non-limiting examples of carbon linked bicyclic 6- to 12-membered aromatic heterocyclyl containing 1, 2 or 3 heteroatoms each independently selected from the group consisting of N, O and S, include, but are not limited to:



The term “bicyclic 6- to 12-membered non-aromatic heterocyclyl containing 1, 2 or 3 heteroatoms each independently selected from the group consisting of N, O and S” as used herein alone or as part of another group, defines a bicyclic non-aromatic, cyclic hydrocarbon radical containing at least one nitrogen, oxygen or sulphur atom having from 6 to 12 ring members, as defined above. Non-limiting examples include:



Non-limiting examples of monocyclic 4-, 5-, 6- or 7-membered fully saturated heterocyclyl containing 1, 2 or 3 heteroatoms each independently selected from the group consisting of N, O, an S, include, but are not limited to:



- 5 When any variable occurs more than one time in any constituent, each definition is independent.

When any variable occurs more than one time in any formula (e.g. Formula (I)), each definition is independent.

- 10 The term “compound(s) of the application” or “compound(s) according to the application” as used herein, is meant to include the compounds of Formula (I) and tautomers, stereoisomeric forms, pharmaceutically acceptable addition salts, N-oxides, and the solvates thereof.

- 15 As used herein, any chemical formula with bonds shown only as solid lines and not as solid wedged or hashed wedged bonds, or otherwise indicated as having a particular configuration (e.g. *R*, *S*) around one or more atoms, contemplates each possible stereoisomer, or mixture of two or more stereoisomers. All stereoisomers of the compounds described herein either as a pure stereoisomer or as a mixture of two or more stereoisomers are included within the scope of the application.

- 20 The terms “stereoisomers”, “stereoisomeric forms” or “stereochemically isomeric forms” are used interchangeably.

Enantiomers are stereoisomers that are non-superimposable mirror images of each other. A 1:1 mixture of a pair of enantiomers is a racemate or racemic mixture. Atropisomers (or atropoisomers) are stereoisomers which have a particular spatial configuration, resulting from a restricted rotation about a single bond, due to large

steric hindrance. Diastereomers (or diastereoisomers) are stereoisomers that are not enantiomers, i.e. they are not related as mirror images. If a compound contains a double bond, the substituents may be in the *E* or the *Z* configuration.

Substituents on bivalent cyclic saturated or partially saturated radicals can have
5 either the *cis*- or *trans*-configuration; for example, if a compound contains a disubstituted cycloalkyl group, the substituents can be in the *cis* or *trans* configuration.

The application includes enantiomers, atropisomers, diastereomers, racemates, *E* isomers, *Z* isomers, *cis* isomers, *trans* isomers and mixtures thereof of compounds of formula (I), whenever chemically possible. The meaning of all such terms, i.e.
10 enantiomers, atropisomers, diastereomers, racemates, *E* isomers, *Z* isomers, *cis* isomers, *trans* isomers and mixtures thereof are known to the skilled person.

The absolute configuration is specified according to the Cahn-Ingold-Prelog system. The configuration at an asymmetric atom is specified by either *R* or *S*. Resolved stereoisomers whose absolute configuration is not known can be designated
15 by (+) or (-) depending on the direction in which they rotate plane polarized light. For instance, resolved enantiomers whose absolute configuration is not known can be designated by (+) or (-) depending on the direction in which they rotate plane polarized light.

When a specific stereoisomer is identified, this means that said stereoisomer is
20 substantially free, i.e. associated with less than 50%, preferably less than 20%, more preferably less than 10%, even more preferably less than 5%, in particular less than 2% and most preferably less than 1%, of the other stereoisomers. Thus, when a compound of Formula (I) is for instance specified as (*R*), this means that the compound is substantially free of the (*S*) isomer; when a compound of Formula (I) is
25 for instance specified as *E*, this means that the compound is substantially free of the *Z* isomer; when a compound of Formula (I) is for instance specified as *cis*, this means that the compound is substantially free of the *trans* isomer.

The stereochemical configuration for centers in some compounds may be designated "*R*" or "*S*" when the mixture(s) was separated; for some compounds, the
30 stereochemical configuration at indicated centers has been designated as "*R**" or "*S**" when the absolute stereochemistry is undetermined (even if the bonds are drawn stereo specifically) although the compound itself has been isolated as a single stereoisomer and is enantiomerically pure.

Some of the compounds according to Formula (I) described herein can also

exist in their tautomeric form. Such forms in so far as they may exist, although not explicitly indicated in the above Formula (I) are intended to be included within the scope of the application. It follows that a single compound may exist in both stereoisomeric and tautomeric form.

5 Pharmaceutically acceptable addition salts include acid addition salts and base addition salts. Such salts can be formed by conventional means, for example by reaction of a free acid or a free base form with one or more equivalents of an appropriate base or acid, optionally in a solvent, or in a medium in which the salt is insoluble, followed by removal of said solvent, or said medium, using standard
10 techniques (e.g. *in vacuo*, by freeze-drying or by filtration). Salts can also be prepared by exchanging a counter-ion of a compound of the application in the form of a salt with another counter-ion, for example using a suitable ion exchange resin.

The pharmaceutically acceptable addition salts as mentioned herein comprise the therapeutically active non-toxic acid and base salt forms which the compounds of
15 formula (I), N-oxides and solvates thereof, are capable of forming. Appropriate acids comprise, for example, inorganic acids such as hydrohalic acids, e.g. hydrochloric or hydrobromic acid, sulfuric, nitric, phosphoric and the like acids; or organic acids such as, for example, acetic, propanoic, hydroxyacetic, lactic, pyruvic, oxalic (i.e. ethanedioic), malonic, succinic (i.e. butanedioic acid), maleic, fumaric, malic, tartaric,
20 citric, methanesulfonic, ethanesulfonic, benzenesulfonic, p-toluenesulfonic, cyclamic, salicylic, p-aminosalicylic, pamoic and the like acids. Conversely said salt forms can be converted by treatment with an appropriate base into the free base form.

The compounds of formula (I) and solvates thereof containing an acidic proton can also be converted into their non-toxic metal or amine salt forms by treatment with
25 appropriate organic and inorganic bases. Appropriate base salt forms comprise, for example, the ammonium salts, the alkali and earth alkaline metal salts, e.g. the lithium, sodium, potassium, cesium, magnesium, calcium salts and the like, salts with organic bases, e.g. primary, secondary and tertiary aliphatic and aromatic amines such as methylamine, ethylamine, propylamine, isopropylamine, the four butylamine isomers,
30 dimethylamine, diethylamine, diethanolamine, dipropylamine, diisopropylamine, di-n-butylamine, pyrrolidine, piperidine, morpholine, trimethylamine, triethylamine, tripropylamine, quinuclidine, pyridine, quinoline and isoquinoline; the benzathine, N-methyl-D-glucamine, hydrabamine salts, and salts with amino acids such as, for example, arginine, lysine and the like. Conversely the salt form can be converted by

treatment with acid into the free acid form.

The term “solvate” comprises the solvent addition forms as well as the salts thereof, which the compounds of Formula (I) are able to form. Examples of such solvent addition forms are e.g. hydrates, alcoholates and the like.

5 The compounds of formula (I) as prepared in the processes described below can be synthesized in the form of mixtures of enantiomers, in particular racemic mixtures of enantiomers, that can be separated from one another following art-known resolution procedures. A manner of separating the enantiomeric forms of the compounds of formula (I), and pharmaceutically acceptable addition salts, N-oxides
10 and solvates thereof, involves liquid chromatography using a chiral stationary phase. Said pure stereochemically isomeric forms can also be derived from the corresponding pure stereochemically isomeric forms of the appropriate starting materials, provided that the reaction occurs stereospecifically. Preferably if a specific stereoisomer is desired, said compound would be synthesized by stereospecific methods of
15 preparation. These methods will advantageously employ enantiomerically pure starting materials.

The term “enantiomerically pure” as used herein means that the product contains at least 80% by weight of one enantiomer and 20% by weight or less of the other enantiomer. Preferably the product contains at least 90% by weight of one
20 enantiomer and 10% by weight or less of the other enantiomer. In the most preferred embodiment the term “enantiomerically pure” means that the composition contains at least 99% by weight of one enantiomer and 1% or less of the other enantiomer.

The disclosure also embraces isotopically-labeled compounds of the application which are identical to those recited herein, but for the fact that one or more
25 atoms are replaced by an atom having an atomic mass or mass number different from the atomic mass or mass number usually found in nature (or the most abundant one found in nature).

All isotopes and isotopic mixtures of any particular atom or element as specified herein are contemplated within the scope of the compounds of the
30 application, either naturally occurring or synthetically produced, either with natural abundance or in an isotopically enriched form. Exemplary isotopes that can be incorporated into compounds of the invention include isotopes of hydrogen, carbon, nitrogen, oxygen, phosphorus, sulfur, fluorine, chlorine and iodine, such as ^2H , ^3H , ^{11}C , ^{13}C , ^{14}C , ^{13}N , ^{15}O , ^{17}O , ^{18}O , ^{32}P , ^{33}P , ^{35}S , ^{18}F , ^{36}Cl , ^{122}I , ^{123}I , ^{125}I , ^{131}I , ^{75}Br , ^{76}Br ,

⁷⁷Br and ⁸²Br. Preferably, the radioactive isotope is selected from the group of ²H, ³H, ¹¹C and ¹⁸F. More preferably, the radioactive isotope is ²H. In particular, deuterated compounds are intended to be included within the scope of the application.

Certain isotopically-labeled compounds of the application (e.g., those labeled with ³H and ¹⁴C) may be useful for example in substrate tissue distribution assays. Tritiated (³H) and carbon-14 (¹⁴C) isotopes are useful for their ease of preparation and detectability. Further, substitution with heavier isotopes such as deuterium (i.e., ²H) may afford certain therapeutic advantages resulting from greater metabolic stability (e.g., increased *in vivo* half-life or reduced dosage requirements) and hence may be preferred in some circumstances. Positron emitting isotopes such as ¹⁵O, ¹³N, ¹¹C and ¹⁸F are useful for positron emission tomography (PET) studies. PET imaging in cancer finds utility in helping locate and identify tumours, stage the disease and determine suitable treatment. Human cancer cells overexpress many receptors or proteins that are potential disease-specific molecular targets. Radiolabelled tracers that bind with high affinity and specificity to such receptors or proteins on tumour cells have great potential for diagnostic imaging and targeted radionuclide therapy (Charron, Carlie L. et al. Tetrahedron Lett. 2016, 57(37), 4119-4127). Additionally, target-specific PET radiotracers can be used as biomarkers to examine and evaluate pathology, by for example, measuring target expression and treatment response (Austin R. et al. Cancer Letters (2016), doi: 10.1016/j.canlet.2016.05.008).

In some embodiments, provided is a compound of formula (I), and the tautomers and the stereoisomeric forms thereof, wherein:

the dotted bond towards R^{1b} is absent;

A¹ represents CH or N; A² represents CH; A³ represents CH;

A⁴ represents CH; A⁵ represents CR^{3a}; A⁶ represents CH;

R^{1b} and R^{4b} are taken together to form together with the atoms to which they are attached a monocyclic 4-, 5-, 6- or 7-membered fully saturated heterocyclyl, each containing 1 N-atom and optionally 1 or 2 additional heteroatoms each independently selected from the group consisting of N, O, and S; wherein said optional S-atom is

optionally substituted to form S(=O);

wherein said monocyclic fully saturated heterocyclyl is optionally substituted on one of the carbon atoms with 1 substituent selected from the group consisting of halo and R⁷;

provided that R^{4a} represents hydrogen; and R^{1a} represents hydrogen;

R^2 represents Het^b ;

R^{3a} represents halo, cyano, or Het^a ;

Het^a represents a carbon linked monocyclic 5-, 6- or 7-membered aromatic

heterocyclyl containing 1, 2 or 3 heteroatoms each independently selected from the

5 group consisting of N, O or S; wherein said S-atom is optionally substituted to form $S(=O)$ or $S(=O)_2$;

wherein said monocyclic aromatic heterocyclyl might be substituted on one of the carbon atoms with a halo substituent;

wherein said monocyclic aromatic heterocyclyl is optionally substituted on one

10 nitrogen atom with R^7 ;

Het^b represents a monocyclic 4-, 5-, 6- or 7-membered non-aromatic heterocyclyl containing 1 oxygen atom;

each R^7 represents C_{1-4} alkyl;

and the pharmaceutically acceptable addition salts, the N-oxides, and the solvates

15 thereof.

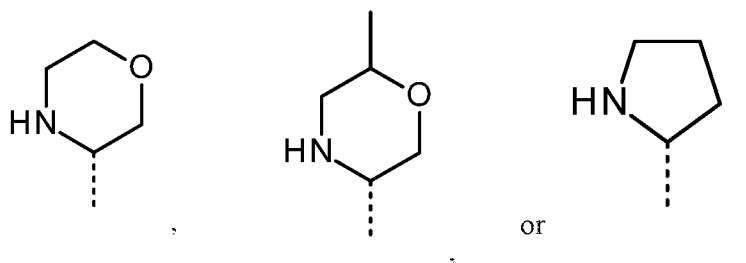
In some embodiments, provided is a compound of formula (I), and the tautomers and the stereoisomeric forms thereof, wherein:

the dotted bond towards R^{1b} is absent;

A^1 represents CH or N; A^2 represents CH; A^3 represents CH;

20 A^4 represents CH; A^5 represents CR^{3a} ; A^6 represents CH;

R^{1b} and R^{4b} are taken together to form together with the atoms to which they are attached:

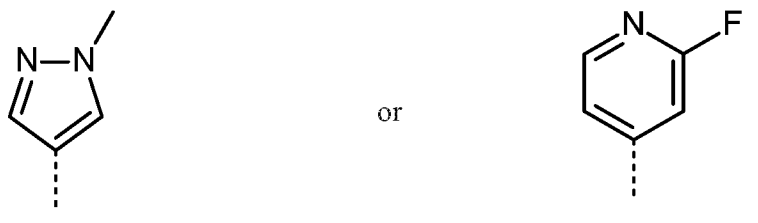


R^{4a} represents hydrogen; and R^{1a} represents hydrogen;

25 R^2 represents Het^b ;

R^{3a} represents halo, cyano, or Het^a ;

Het^a represents:



Het^b represents tetrahydropyranyl; in particular 4-tetrahydropyranyl;
and the pharmaceutically acceptable addition salts, the N-oxides, and the solvates thereof.

In some embodiments, provided is a compound of formula (I), and the
5 tautomers and the stereoisomeric forms thereof, wherein R² represents Het^b, preferably
Het^b is attached to the remainder of the molecule via a carbon atom (i.e., carbon
linked), and the pharmaceutically acceptable addition salts, the N-oxides, and the
solvates thereof.

In some embodiments, provided is a compound of formula (I), and the
10 tautomers and the stereoisomeric forms thereof, wherein Het^b represents a carbon
linked monocyclic 4-, 5-, 6- or 7-membered non-aromatic heterocyclyl containing 1, 2
or 3 heteroatoms each independently selected from the group consisting of N, O, and
S; wherein said S-atom is optionally substituted to form S(=O), S(=O)₂, or
S(=O)(=NR^x); or a carbon linked bicyclic 6- to 12- membered non-aromatic
15 heterocyclyl containing 1, 2 or 3 heteroatoms each independently selected from the
group consisting of N, O, and S; wherein said S-atom is optionally substituted to form
S(=O), S(=O)₂, or S(=O)(=NR^x); wherein said monocyclic or bicyclic non-aromatic
heterocyclyl is optionally substituted on one or more of the carbon atoms with in total
1, 2 or 3 substituents each independently selected from the group consisting of -OH,
20 CN, halo, R⁷, -O-R⁷, -S(=O)₂-R⁷, -C(=O)-R⁷, -NR^{6c}R^{6d}, and -C(=O)-NR^{6a}R^{6b};
wherein said monocyclic or bicyclic non-aromatic heterocyclyl is optionally
substituted on the nitrogen atoms with in total 1 or 2 substituents each independently
selected from the group consisting of R⁷, -S(=O)₂-R⁷, -C(=O)-R⁷, and -C(=O)-
NR^{6a}R^{6b},
25 and the pharmaceutically acceptable addition salts, the N-oxides, and the solvates
thereof.

In some embodiments, provided is a compound of formula (I), and the
tautomers and the stereoisomeric forms thereof, wherein:

R^2 represents Het^b;

Het^b represents a carbon linked monocyclic 4-, 5-, 6- or 7-membered non-aromatic heterocyclyl containing 1, 2 or 3 heteroatoms each independently selected from the group consisting of N, O, and S; wherein said S-atom is optionally substituted to form

- 5 S(=O), S(=O)₂, or S(=O)(=NR^x); or
 a carbon linked bicyclic 6- to 12- membered non-aromatic heterocyclyl containing 1, 2 or 3 heteroatoms each independently selected from the group consisting of N, O, and S; wherein said S-atom is optionally substituted to form S(=O), S(=O)₂, or S(=O)(=NR^x);
- 10 wherein said monocyclic or bicyclic non-aromatic heterocyclyl is optionally substituted on one or more of the carbon atoms with in total 1, 2 or 3 substituents each independently selected from the group consisting of -OH, CN, halo, R⁷, -O-R⁷, -S(=O)₂-R⁷, -C(=O)-R⁷, -NR^{6c}R^{6d}, and -C(=O)-NR^{6a}R^{6b}, and the pharmaceutically acceptable addition salts, the N-oxides, and the solvates
- 15 thereof.

In some embodiments, provided is a compound of formula (I), and the tautomers and the stereoisomeric forms thereof, wherein:

Het^b represents a carbon linked monocyclic 4-, 5-, 6- or 7-membered non-aromatic heterocyclyl containing 1, 2 or 3 heteroatoms each independently selected from the

20 group consisting of N, O, and S; wherein said S-atom is optionally substituted to form S(=O), S(=O)₂, or S(=O)(=NR^x);

wherein said monocyclic non-aromatic heterocyclyl might be substituted on the nitrogen atoms with in total 1 or 2 substituents each independently selected from the group consisting of R⁷, -S(=O)₂-R⁷, -C(=O)-R⁷, and -C(=O)-NR^{6a}R^{6b},

- 25 and the pharmaceutically acceptable addition salts, the N-oxides, and the solvates thereof.

In some embodiments, provided is a compound of formula (I) and the tautomers and the stereoisomeric forms thereof, wherein:

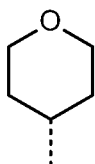
R^2 represents Het^b;

- 30 Het^b represents a carbon linked monocyclic 4-, 5-, 6- or 7-membered non-aromatic heterocyclyl containing 1, 2 or 3 heteroatoms each independently selected from the group consisting of N, O, and S; wherein said S-atom is optionally substituted to form S(=O), S(=O)₂, or S(=O)(=NR^x);

wherein said monocyclic non-aromatic heterocyclyl is optionally substituted on the nitrogen atoms with in total 1 or 2 substituents each independently selected from the group consisting of R^7 , $-S(=O)_2-R^7$, $-C(=O)-R^7$, and $-C(=O)-NR^{6a}R^{6b}$, and the pharmaceutically acceptable addition salts, the N-oxides, and the solvates thereof.

- 5 In some embodiments, provided is a compound of formula (I), and the tautomers and the stereoisomeric forms thereof, wherein:

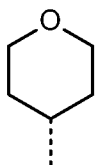
R^2 represents



, and the pharmaceutically acceptable addition salts, the N-oxides, and the solvates thereof.

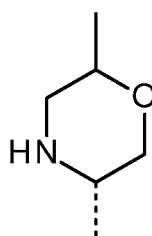
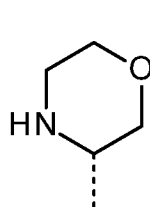
- 10 In some embodiments, provided is a compound of formula (I), and the tautomers and the stereoisomeric forms thereof, wherein:

R^2 represents

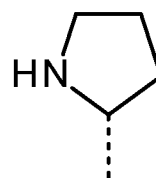


R^{1b} and R^{4b} are taken together to form together with the atoms to which they are

- 15 attached



, or



R^{4a} represents hydrogen; and

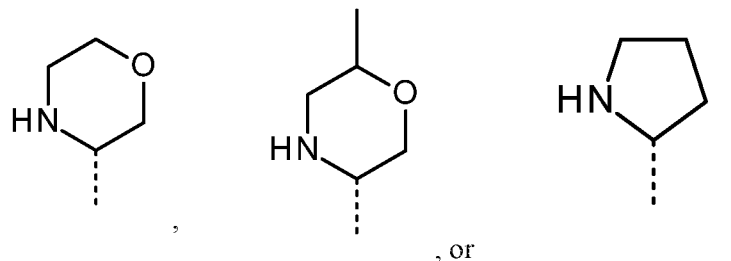
R^{1a} represents hydrogen,

and the pharmaceutically acceptable addition salts, the N-oxides, and the solvates

- 20 thereof.

In some embodiments, provided is a compound of formula (I), and the tautomers and the stereoisomeric forms thereof, wherein:

R^{1b} and R^{4b} are taken together to form together with the atoms to which they are attached



5

R^{4a} represents hydrogen; and

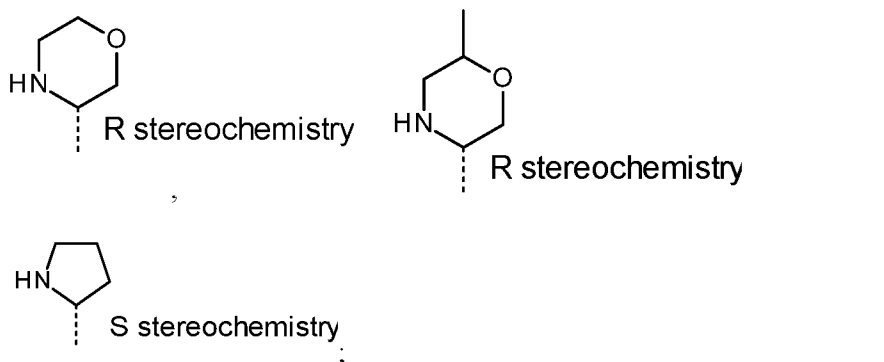
R^{1a} represents hydrogen,

and the pharmaceutically acceptable addition salts, the N-oxides, and the solvates thereof.

10

In some embodiments, provided is a compound of formula (I), and the tautomers and the stereoisomeric forms thereof, wherein:

R^{1b} and R^{4b} are taken together to form together with the atoms to which they are attached



R^{4a} represents hydrogen; and R^{1a} represents hydrogen,

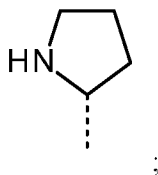
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and the pharmaceutically acceptable addition salts, the N-oxides, and the solvates thereof.

In some embodiments, provided is a compound of formula (I), and the tautomers and the stereoisomeric forms thereof, wherein:

R^{1b} and R^{4b} are taken together to form together with the atoms to which they are attached

20



R^{4a} represents hydrogen; and R^{1a} represents hydrogen, and the pharmaceutically acceptable addition salts, the N-oxides, and the solvates thereof.

- 5 In some embodiments, provided is a compound of formula (I), and the tautomers and the stereoisomeric forms thereof, wherein:

R^{1b} and R^{4b} are taken together to form together with the atoms to which they are attached



- 10 R^{4a} represents hydrogen; and R^{1a} represents hydrogen, and the pharmaceutically acceptable addition salts, the N-oxides, and the solvates thereof.

In some embodiments, provided is a compound of formula (I), and the tautomers and the stereoisomeric forms thereof, wherein:

- 15 A^1 represents CH or N; A^2 represents CH; A^3 represents CH; A^4 represents CH; A^5 represents CR^{3a} ; A^6 represents CH, and the pharmaceutically acceptable addition salts, the N-oxides, and the solvates thereof.

In some embodiments, provided is a compound of formula (I), and the tautomers and the stereoisomeric forms thereof, wherein:

- 20 A^1 represents CH or N; A^2 represents CH; A^3 represents CH; A^4 represents CH; A^5 represents CR^{3a} ; A^6 represents CH; R^2 represents Het^b; R^{3a} represents halo, cyano, or Het^a, and the pharmaceutically acceptable addition salts, the N-oxides, and the solvates thereof.

In some embodiments, provided is a compound of formula (I), and the tautomers and the stereoisomeric forms thereof, wherein:

A¹ represents CH or N; A² represents CH; A³ represents CH;

A⁴ represents CH; A⁵ represents CR^{3a}; A⁶ represents CH;

R² represents Het^b;

R^{3a} represents halo, cyano, or Het^a;

- 5 Het^a represents a carbon linked monocyclic 5-, 6- or 7-membered aromatic heterocyclyl containing 1, 2 or 3 heteroatoms each independently selected from the group consisting of N, O and S; wherein said S-atom is optionally substituted to form S(=O) or S(=O)₂;

wherein said monocyclic aromatic heterocyclyl is optionally substituted on one of the
10 carbon atoms with a halo substituent;

wherein said monocyclic aromatic heterocyclyl is optionally substituted on one nitrogen atom with R⁷;

Het^b represents a monocyclic 4-, 5-, 6- or 7-membered non-aromatic heterocyclyl containing 1 oxygen atom;

- 15 each R⁷ represents C₁₋₄alkyl,
and the pharmaceutically acceptable addition salts, the N-oxides, and the solvates thereof.

In some embodiments, provided is a compound of formula (I), and the tautomers and the stereoisomeric forms thereof, wherein:

- 20 R^{1a} represents hydrogen;

R^{1b} represents hydrogen or CH₃;

R^{4a} represents hydrogen, C₁₋₄alkyl, or C₃₋₆cycloalkyl;

R^{4b} represents hydrogen, C₁₋₄alkyl, C₃₋₆cycloalkyl, or

- a carbon linked monocyclic 4-, 5-, 6- or 7-membered non-aromatic heterocyclyl
25 containing 1, 2 or 3 heteroatoms each independently selected from the group consisting of N, O, and S; wherein said S-atom is optionally substituted to form S(=O), S(=O)₂, or S(=O)(=NR^x),

and the pharmaceutically acceptable addition salts, the N-oxides, and the solvates thereof.

- 30 In some embodiments, provided is a compound of formula (I), and the tautomers and the stereoisomeric forms thereof, wherein:

R^{1b} and R^{4b} are taken together to form together with the atoms to which they are attached a monocyclic 5-membered aromatic heterocyclyl or a monocyclic 4-, 5-, 6- or 7-membered fully saturated heterocyclyl, each containing 1 N-atom and optionally 1

or 2 additional heteroatoms each independently selected from the group consisting of N, O, and S; wherein said optional S-atom is optionally substituted to form S(=O), S(=O)₂, or S(=O)(=NR^x); or

- 5 a bicyclic 6- to 12- membered aromatic or fully saturated heterocyclyl containing 1 N-atom and optionally 1 or 2 additional heteroatoms each independently selected from the group consisting of N, O, and S; wherein said optional S-atom is optionally substituted to form S(=O), S(=O)₂, or S(=O)(=NR^x);

- wherein said monocyclic or bicyclic, aromatic or fully saturated heterocyclyl is optionally substituted on one or more of the carbon atoms with in total 1, 2 or 3 substituents each independently selected from the group consisting of -OH, CN, halo, R⁷, -O-R⁷, -S(=O)₂-R⁷, -C(=O)-R⁷, -NR^{6c}R^{6d}, -C(=O)-NR^{6a}R^{6b}, and Het^c;

- wherein said monocyclic or bicyclic, aromatic or fully saturated heterocyclyl is optionally substituted on the optional additional nitrogen atoms with in total 1 or 2 substituents each independently selected from the group consisting of R⁷, -S(=O)₂-R⁷, -C(=O)-R⁷, -C(=O)-NR^{6a}R^{6b}, and Het^d;

provided that R^{4a} represents hydrogen; and

R^{1a} represents hydrogen or R^{1a} is absent when the dotted bond towards R^{1b} is a bond, and the pharmaceutically acceptable addition salts, the N-oxides, and the solvates thereof.

- 20 In some embodiments, provided is a compound of formula (I), and the tautomers and the stereoisomeric forms thereof, wherein:

- R^{1b} and R^{4b} are taken together to form together with the atoms to which they are attached a monocyclic 5-membered aromatic heterocyclyl or a monocyclic 4-, 5-, 6- or 7-membered fully saturated heterocyclyl, each containing 1 N-atom and optionally 1 or 2 additional heteroatoms each independently selected from the group consisting of N, O, and S; wherein said optional S-atom is optionally substituted to form S(=O), S(=O)₂, or S(=O)(=NR^x);

- wherein said monocyclic aromatic or fully saturated heterocyclyl is optionally substituted on one or more of the carbon atoms with in total 1, 2 or 3 substituents each independently selected from the group consisting of -OH, CN, halo, R⁷, -O-R⁷, -S(=O)₂-R⁷, -C(=O)-R⁷, -NR^{6c}R^{6d}, -C(=O)-NR^{6a}R^{6b}, and Het^c;

- wherein said monocyclic aromatic or fully saturated heterocyclyl is optionally substituted on the optional additional nitrogen atoms with in total 1 or 2 substituents each independently selected from the group consisting of R⁷, -S(=O)₂-R⁷, -C(=O)-R⁷, -

$C(=O)-NR^{6a}R^{6b}$, and Het^d ;

provided that R^{4a} represents hydrogen; and

R^{1a} represents hydrogen or R^{1a} is absent when the dotted bond towards R^{1b} is a bond, and the pharmaceutically acceptable addition salts, the N-oxides, and the solvates

5 thereof.

In some embodiments, provided is a compound of formula (I), and the tautomers and the stereoisomeric forms thereof, wherein:

R^{1b} and R^{4b} are taken together to form together with the atoms to which they are attached a monocyclic 4-, 5-, 6- or 7-membered fully saturated heterocyclyl, each

10 containing 1 N-atom and optionally 1 or 2 additional heteroatoms each independently selected from the group consisting of N, O, and S; wherein said optional S-atom is optionally substituted to form $S(=O)$, $S(=O)_2$, or $S(=O)(=NR^x)$;

wherein said monocyclic fully saturated heterocyclyl is optionally substituted on one or more of the carbon atoms with in total 1, 2 or 3 substituents each independently

15 selected from the group consisting of $-OH$, CN , halo, R^7 , $-O-R^7$, $-S(=O)_2-R^7$, $-C(=O)-R^7$, $-NR^{6c}R^{6d}$, $-C(=O)-NR^{6a}R^{6b}$, and Het^c ;

wherein said monocyclic fully saturated heterocyclyl is optionally substituted on the optional additional nitrogen atoms with in total 1 or 2 substituents each independently selected from the group consisting of R^7 , $-S(=O)_2-R^7$, $-C(=O)-R^7$, $-C(=O)-NR^{6a}R^{6b}$,

20 and Het^d ;

provided that R^{4a} represents hydrogen; and

R^{1a} represents hydrogen or R^{1a} is absent when the dotted bond towards R^{1b} is a bond, and the pharmaceutically acceptable addition salts, the N-oxides, and the solvates thereof.

25 In some embodiments, provided is a compound of formula (I), and the tautomers and the stereoisomeric forms thereof, wherein:

R^{4a} and R^{4b} are taken together to form together with the N-atom to which they are attached a monocyclic 5-membered aromatic heterocyclyl or a monocyclic 4-, 5-, 6- or 7-membered fully saturated heterocyclyl, each containing 1 N-atom and optionally 1

30 or 2 additional heteroatoms each independently selected from the group consisting of N, O, and S; wherein said optional S-atom is optionally substituted to form $S(=O)$, $S(=O)_2$, or $S(=O)(=NR^x)$; or

a bicyclic 6- to 12- membered aromatic or fully saturated heterocyclyl containing 1 N-atom and optionally 1 or 2 additional heteroatoms each independently selected from

the group consisting of N, O, and S; wherein said optional S-atom is optionally substituted to form S(=O), S(=O)₂, or S(=O)(=NR^x);

wherein said monocyclic or bicyclic, aromatic or fully saturated heterocyclyl is optionally substituted on one or more of the carbon atoms with in total 1, 2 or 3

5 substituents each independently selected from the group consisting of -OH, CN, halo, R⁷, -O-R⁷,

-S(=O)₂-R⁷, -C(=O)-R⁷, -NR^{6c}R^{6d}, -C(=O)-NR^{6a}R^{6b}, and Het^c;

wherein said monocyclic or bicyclic, aromatic or fully saturated heterocyclyl is optionally substituted on the nitrogen atoms with in total 1 or 2 substituents each

10 independently selected from the group consisting of R⁷, -S(=O)₂-R⁷, -C(=O)-R⁷, -C(=O)-NR^{6a}R^{6b}, and Het^d;

R^{1a} represents hydrogen, and R^{1b} represents hydrogen,

and the pharmaceutically acceptable addition salts, the N-oxides, and the solvates thereof.

15 In some embodiments, provided is a compound of formula (I), and the tautomers and the stereoisomeric forms thereof, wherein:

R^{1a} represents hydrogen;

R^{1b} represents hydrogen or CH₃;

R^{4a} represents hydrogen, C₁₋₄alkyl, or C₃₋₆cycloalkyl;

20 R^{4b} represents hydrogen, C₁₋₄alkyl, C₃₋₆cycloalkyl, or

a carbon linked monocyclic 4-, 5-, 6- or 7-membered non-aromatic heterocyclyl containing 1, 2 or 3 heteroatoms each independently selected from the group consisting of N, O, and S; wherein said S-atom is optionally substituted to form S(=O), S(=O)₂, or S(=O)(=NR^x);

25 or

R^{1b} and R^{4b} are taken together to form together with the atoms to which they are attached a monocyclic 4-, 5-, 6- or 7-membered fully saturated heterocyclyl, each containing 1 N-atom and optionally 1 or 2 additional heteroatoms each independently selected from the group consisting of N, O, and S; wherein said optional S-atom is

30 optionally substituted to form S(=O), S(=O)₂, or S(=O)(=NR^x); or

a bicyclic 6- to 12- membered fully saturated heterocyclyl containing 1 N-atom and optionally 1 or 2 additional heteroatoms each independently selected from the group consisting of N, O, and S; wherein said optional S-atom is optionally substituted to form S(=O), S(=O)₂, or S(=O)(=NR^x);

- wherein said monocyclic or bicyclic, fully saturated heterocyclyl is optionally substituted on one or more of the carbon atoms with in total 1, 2 or 3 substituents each independently selected from the group consisting of -OH, CN, halo, R^7 , -O- R^7 , -S(=O)₂- R^7 , -C(=O)- R^7 , -NR^{6c}R^{6d}, -C(=O)-NR^{6a}R^{6b}, and Het^c;
- 5 wherein said monocyclic or bicyclic, fully saturated heterocyclyl is optionally substituted on the optional additional nitrogen atoms with in total 1 or 2 substituents each independently selected from the group consisting of R^7 , -S(=O)₂- R^7 , -C(=O)- R^7 , -C(=O)-NR^{6a}R^{6b}, and Het^d;
- provided that in case R^{1b} and R^{4b} are taken together, R^{4a} represents hydrogen; and
- 10 R^{1a} represents hydrogen or R^{1a} is absent when the dotted bond towards R^{1b} is a bond; or
- R^{4a} and R^{4b} are taken together to form together with the N-atom to which they are attached a monocyclic 5-, 6- or 7-membered aromatic heterocyclyl or a monocyclic 4-, 5-, 6- or 7-membered fully saturated heterocyclyl, each containing 1 N-atom and
- 15 optionally 1 or 2 additional heteroatoms each independently selected from the group consisting of N, O, and S; wherein said optional S-atom is optionally substituted to form S(=O), S(=O)₂, or S(=O)(=NR^x); or
- a bicyclic 6- to 12- membered aromatic or fully saturated heterocyclyl containing 1 N-atom and optionally 1 or 2 additional heteroatoms each independently selected from
- 20 the group consisting of N, O, and S; wherein said optional S-atom is optionally substituted to form S(=O), S(=O)₂, or S(=O)(=NR^x);
- wherein said monocyclic or bicyclic, aromatic or fully saturated heterocyclyl is optionally substituted on one or more of the carbon atoms with in total 1, 2 or 3 substituents each independently selected from the group consisting of -OH, CN, halo,
- 25 R^7 , -O- R^7 , -S(=O)₂- R^7 , -C(=O)- R^7 , -NR^{6c}R^{6d}, -C(=O)-NR^{6a}R^{6b}, and Het^c;
- wherein said monocyclic or bicyclic, aromatic or fully saturated heterocyclyl is optionally substituted on the nitrogen atoms with in total 1 or 2 substituents each independently selected from the group consisting of R^7 , -S(=O)₂- R^7 , -C(=O)- R^7 , -C(=O)-NR^{6a}R^{6b}, and Het^d;
- 30 in case R^{4a} and R^{4b} are taken together, R^{1a} represents hydrogen, and R^{1b} represents hydrogen,
- and the pharmaceutically acceptable addition salts, the N-oxides, and the solvates thereof.

In some embodiments, provided is a compound of formula (I), and the tautomers and the stereoisomeric forms thereof, wherein:

R^{1a} represents hydrogen;

R^{1b} represents hydrogen or CH_3 ;

5 R^{4a} represents hydrogen, C_{1-4} alkyl, or C_{3-6} cycloalkyl;

R^{4b} represents hydrogen, C_{1-4} alkyl, C_{3-6} cycloalkyl, or

a carbon linked monocyclic 4-, 5-, 6- or 7-membered non-aromatic heterocycl

containing 1, 2 or 3 heteroatoms each independently selected from the group

consisting of N, O, and S; wherein said S-atom is optionally substituted to form

10 $S(=O)$, $S(=O)_2$, or $S(=O)(=NR^x)$;

or

R^{1b} and R^{4b} are taken together to form together with the atoms to which they are attached a monocyclic 4-, 5-, 6- or 7-membered fully saturated heterocycl, each containing 1 N-atom and optionally 1 or 2 additional heteroatoms each independently

15 selected from the group consisting of N, O, and S; wherein said optional S-atom is optionally substituted to form $S(=O)$, $S(=O)_2$, or $S(=O)(=NR^x)$; or

a bicyclic 6- to 12- membered fully saturated heterocycl containing 1 N-atom and optionally 1 or 2 additional heteroatoms each independently selected from the group consisting of N, O, and S; wherein said optional S-atom is optionally substituted to

20 form $S(=O)$, $S(=O)_2$, or $S(=O)(=NR^x)$;

wherein said monocyclic or bicyclic, fully saturated heterocycl is optionally substituted on one or more of the carbon atoms with in total 1, 2 or 3 substituents each independently selected from the group consisting of $-OH$, CN , halo, R^7 , $-O-R^7$, $-S(=O)_2-R^7$, $-C(=O)-R^7$, $-NR^{6c}R^{6d}$, $-C(=O)-NR^{6a}R^{6b}$, and Het^c ;

25 wherein said monocyclic or bicyclic, fully saturated heterocycl might be substituted on the optional additional nitrogen atoms with in total 1 or 2 substituents each independently selected from the group consisting of R^7 , $-S(=O)_2-R^7$, $-C(=O)-R^7$, $-C(=O)-NR^{6a}R^{6b}$, and Het^d ;

provided that in case R^{1b} and R^{4b} are taken together, R^{4a} represents hydrogen; and

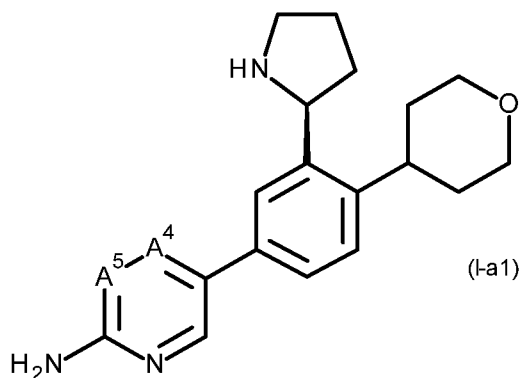
30 R^{1a} represents hydrogen or R^{1a} is absent when the dotted bond towards R^{1b} is a bond, and the pharmaceutically acceptable addition salts, the N-oxides, and the solvates thereof.

In some embodiments, provided is a compound of formula (I), and the tautomers and the stereoisomeric forms thereof, wherein:

- R^{1b} and R^{4b} are taken together to form together with the atoms to which they are attached a monocyclic 4-, 5-, 6- or 7-membered fully saturated heterocyclyl, each containing 1 N-atom and optionally 1 or 2 additional heteroatoms each independently selected from the group consisting of N, O, and S; wherein said optional S-atom is
- 5 optionally substituted to form $S(=O)$, $S(=O)_2$, or $S(=O)(=NR^x)$; or
- a bicyclic 6- to 12- membered fully saturated heterocyclyl containing 1 N-atom and optionally 1 or 2 additional heteroatoms each independently selected from the group consisting of N, O, and S; wherein said optional S-atom is optionally substituted to form $S(=O)$, $S(=O)_2$, or $S(=O)(=NR^x)$;
- 10 wherein said monocyclic or bicyclic, fully saturated heterocyclyl is optionally substituted on one or more of the carbon atoms with in total 1, 2 or 3 substituents each independently selected from the group consisting of $-OH$, CN , halo, R^7 , $-O-R^7$, $-S(=O)_2-R^7$, $-C(=O)-R^7$, $-NR^{6c}R^{6d}$, $-C(=O)-NR^{6a}R^{6b}$, and Het^c ;
- wherein said monocyclic or bicyclic, fully saturated heterocyclyl is optionally
- 15 substituted on the optional additional nitrogen atoms with in total 1 or 2 substituents each independently selected from the group consisting of R^7 , $-S(=O)_2-R^7$, $-C(=O)-R^7$, $-C(=O)-NR^{6a}R^{6b}$, and Het^d ;
- provided that R^{4a} represents hydrogen; and
- R^{1a} represents hydrogen or R^{1a} is absent when the dotted bond towards R^{1b} is a bond,
- 20 and the pharmaceutically acceptable addition salts, the N-oxides, and the solvates thereof.

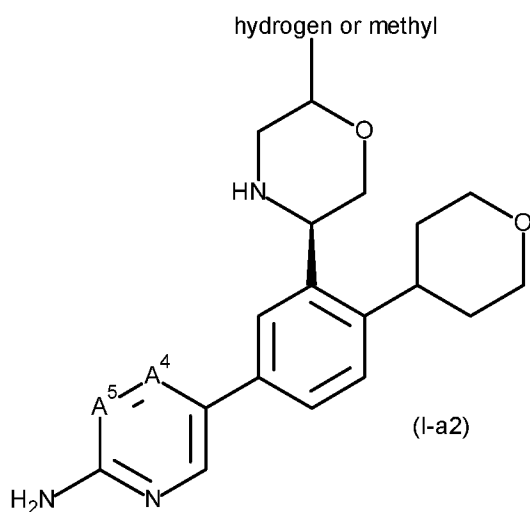
In some embodiments, provided is a compound of formula (I), and the tautomers and the stereoisomeric forms thereof, wherein Het^c represents oxetanyl, and the pharmaceutically acceptable addition salts, the N-oxides, and the solvates thereof.

- 25 In some embodiments, provided is a compound of Formula (I-a1):



, and the tautomers and the stereoisomeric forms thereof, wherein A⁴ and A⁵ are as defined in the compounds of formula (I), and the pharmaceutically acceptable addition salts, the N-oxides, and the solvates thereof.

5 In some embodiments, provided is a compound of formula (I-a2):



, and the tautomers and the stereoisomeric forms thereof, wherein A⁴ and A⁵ are as defined in the compounds of formula (I), and the pharmaceutically acceptable addition salts, the N-oxides, and the solvates thereof.

10 In particular embodiments, provided is a compound selected from the group consisting of any of the exemplified compounds, tautomers and stereoisomeric forms thereof, and any pharmaceutically acceptable addition salts, N-oxides, and solvates thereof.

All possible combinations of the above indicated embodiments are considered
15 to be embraced within the scope of the invention.

Compounds of formula (I) can be prepared according to the general preparation methods and preparation of some typical examples of the compounds of formula (I) as described below. The compounds of formula (I) are generally prepared from starting materials which are either commercially available or prepared by standard synthetic processes commonly used by those skilled in the art of organic chemistry. The following schemes are only meant to provide examples and are not limiting.

Alternatively, compounds of the application can also be prepared by analogous reaction protocols as described in the general schemes below and the specific examples, combined with standard synthetic processes commonly used by those skilled in the art.

The skilled person will realize that in the reactions described in the Schemes, although this is not always explicitly shown, it may be necessary to protect reactive functional groups (for example hydroxy, amino, or carboxy groups) where these are desired in the final product, to avoid their unwanted participation in the reactions. In general, conventional protecting groups can be used in accordance with standard practice. The protecting groups can be removed at a convenient subsequent stage using methods known from the art.

The skilled person will realize that in the reactions described in the Schemes, it may be advisable or necessary to perform the reaction under an inert atmosphere, such as for example under N₂-gas atmosphere.

It will be apparent for the skilled person that it may be necessary to cool the reaction mixture before reaction work-up (which refers to the series of manipulations required to isolate and purify the product(s) of a chemical reaction such as for example quenching, column chromatography, extraction).

The skilled person will realize that heating the reaction mixture under stirring may enhance the reaction outcome. In some reactions microwave heating may be used instead of conventional heating to shorten the overall reaction time.

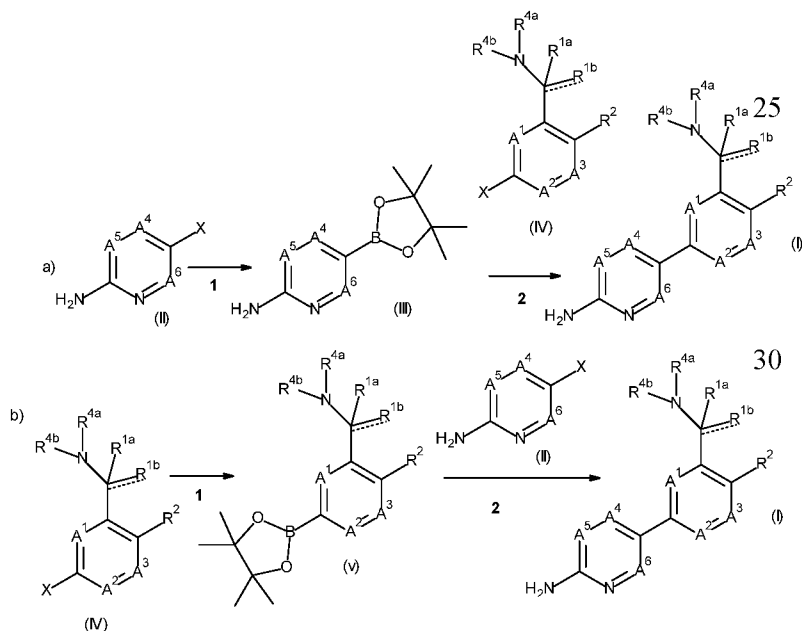
The skilled person will realize that intermediates and final compounds shown in the Schemes below may be further functionalized according to methods well-known by the person skilled in the art. The intermediates and compounds described herein can be isolated in free form or as a salt, or a solvate thereof. The intermediates and compounds described herein may be synthesized in the form of mixtures of tautomers

and stereoisomeric forms that can be separated from one another following art-known resolution procedures.

Hereinafter, “DCM” means dichloromethane; “LAH” means lithium aluminium hydride; “r.t.” means room temperature; “Boc” means *tert*-butoxycarbonyl; “MeCN” means acetonitrile; “MeOH” means methanol; “TFA” means trifluoroacetic acid; “THF” means tetrahydrofuran; “Ti(OEt)₄” means titanium ethoxide; “Pd(PPh₃)₄” means tetrakis(triphenylphosphine)palladium; “[Ir(dtbbpy)(ppy)₂]PF₆” means (4,4'-Di-*t*-butyl-2,2'-bipyridine)bis[2-(2-pyridinyl-kN)phenyl-kC]iridium(III) hexafluorophosphate; “SnAP” means tin amine protocol; “SLAP” means silicon amine protocol; “h” means hours; “PdCl₂(dppf)” means [1,1'-bis(diphenylphosphino-κP)ferrocene]dichloropalladium; “SFC” means Supercritical fluid chromatography; “LiHMDS” means Lithium bis(trimethylsilyl)amide; “SnBu₃” means tributyltin; “SiMe₃” means trimethylsilyl; “Cu(OTf)₂” means Copper (II) triflate; “PhBox” means 2,2'-Isopropylidenebis[(4*R*)-4-phenyl-2-oxazoline; “Bi(OTf)₃” means Bismuth(III) trifluoromethanesulfonate; “BF₃·2 MeOH” means boron trifluoride in methanol; “TMSOTf” means trimethylsilyltriflate; “Me-THF” means methyltetrahydrofuran; “NiCl₂” means Nickel (II) chloride.

Scheme 1

In general, compounds of Formula (I) wherein all variables are defined according to the scope of the application can be prepared according to the following reaction Scheme 1. In Scheme 1, the following definitions apply: X represents a halo. The following reaction conditions apply:

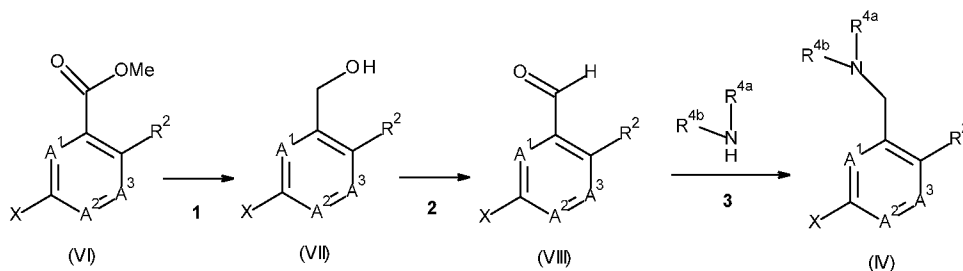


Step 1: A compound of formula (II) is converted to a compound of formula (III) (or a compound of formula (IV) is converted to a compound of formula (V)) at a suitable temperature such as for example at 80°C, in the presence of bis(pinacolatodiboron), a suitable catalyst such as for example PdCl₂(dppf), a suitable basis such as potassium acetate, in a suitable solvent such as 1,4-dioxane.

Step 2: A compound of formula (III) is reacted with a compound of formula (IV) (or a compound of formula (V) is reacted with a compound of formula (II)) at a suitable temperature such as for example at 80°C, in the presence of a suitable catalyst such as for example PdCl₂(dppf), a suitable basis such as sodium carbonate, in a suitable solvent or mixture of solvents such as 1,4-dioxane and water, thereby obtaining a compound of formula (I).

Scheme 2

Intermediates of Formula (IV) wherein R^{1a} and R^{1b} represent hydrogen, and X represent halo can be prepared according to the following reaction Scheme 2. All other variables in Scheme 2 are defined according to the scope of the application.



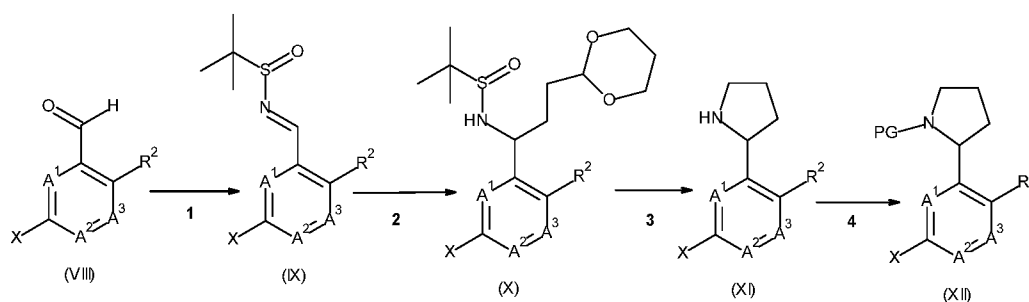
Step 1: A compound of formula (VI) is converted to a compound of formula (VII) at a suitable temperature such as for example from 0°C to rt, in the presence of a suitable reducing agent such as LAH in a suitable solvent such as for example THF.

Step 2: A compound of formula (VII) is converted to a compound of formula (VIII) at a suitable temperature such as rt, in the presence of an oxidizing agent such as for example manganese oxide or Dess-Martin periodinane, in a suitable solvent such as for example DCM.

Step 3: A compound of formula (VIII) is reacted with NHR^{4b}R^{4a} at a suitable temperature such as for example rt, in the presence of a suitable reducing agent such as for example sodium triacetoxyborohydride in a suitable solvent such as for example DCM or THF, thereby obtaining a compound of formula (I).

Scheme 3

Intermediates of Formula (IV) wherein R^{1a} represents hydrogen, R^{1b} and R^{4b} are taken together to form together with the atoms to which they attached a monocyclic 5-membered fully saturated heterocyclyl containing 1N-atom as for example intermediates of Formula (XII) can be prepared according to the following
 5 reaction Scheme 3. X represents halo, PG means protective group, and all other variables in Scheme 3 are defined according to the scope of the application.



10 Step 1: An intermediate of Formula (VIII) can react with t-Butylsulfinamide in the presence of $Ti(OEt)_4$ in a suitable solvent such as for example THF to form a compound of formula (IX).

Step 2: Reaction of a compound of formula (IX) with (1,3-dioxan-2-ylethyl) magnesium bromide in a suitable solvent, such as for example THF yields a
 15 compound of formula (X).

Step 3: A compound of formula (X) is converted to a compound of formula (XI) in the presence of a suitable acid, such as for example TFA and a reductant triethylsilane in a suitable solvent such as for example water.

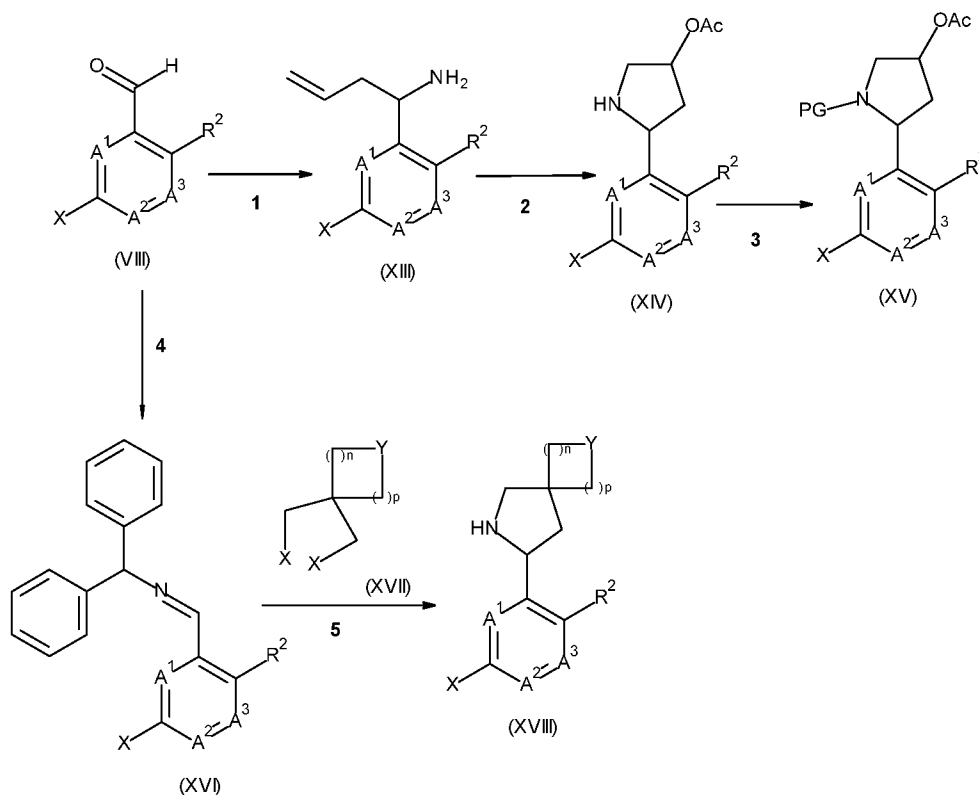
Step 4: An intermediate of Formula (XI) can be protected into a compound of
 20 Formula (XII) by reaction for example with di-tert-butyl dicarbonate in a suitable solvent such as for example DCM. The intermediates of Formula (XII) can be synthesized in the form of racemic mixtures of enantiomers which can be separated from one another using techniques such as liquid chromatography using a chiral stationary phase or SFC.

25 A skilled person will realize that similar chemistry can be used to prepare compounds wherein R^{1b} and R^{4b} are taken together to form together with the atoms to which they attached a monocyclic 4-, 6- or 7-membered fully saturated heterocyclyl containing 1N-atom.

Scheme 4

Intermediates of Formula (IV) wherein R^{1a} represents hydrogen, R^{1b} and R^{4b} are taken together to form together with the atoms to which they attached a monocyclic 5-membered fully saturated heterocyclyl containing at least 1N-atom and substituted as for example in intermediates of Formula (XV) and (XVIII), can be prepared according to the following reaction Scheme 4. X represents halo and each of n and p is 1, or 2; Y represents O or CH_2 ; and PG means protective group. All other variables in Scheme 4 are defined according to the scope of the application.

A skilled person will realize that similar chemistry can be used to prepare compounds wherein R^{1b} and R^{4b} are taken together to form together with the atoms to which they attached other sizes of fully saturated heterocyclyls containing at least 1N-atom.



Steps 1 and 2: An intermediate of Formula (VII) can be converted into a compound of formula (XIII) by reaction with Allylmagnesium chloride and LiHMDS in a suitable solvent, such as for example THF, followed by reaction with acetic anhydride in the presence of a suitable basis such as triethylamine in a suitable solvent such as DCM.

Step 3: An intermediate of Formula (XIV) can be protected into a compound of Formula (XV) by for example reaction with di-tert-butyl dicarbonate in a suitable solvent such as for example DCM.

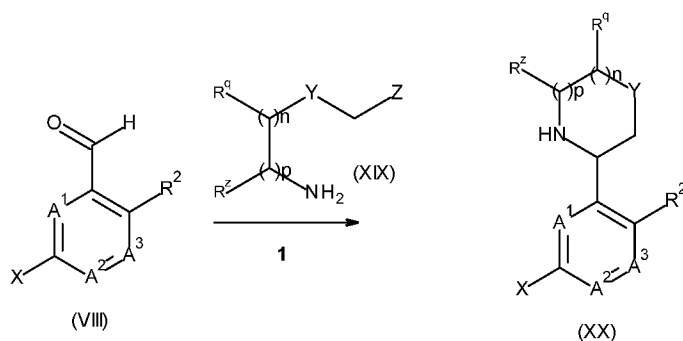
Step 4: An intermediate of Formula (VIII) can react with Benzhydrylamine
5 with magnesium sulfate in a suitable solvent such as DCM to give an intermediate of Formula (XVI)

Step 5: An intermediate of Formula (XVI) can react with a compound of Formula (XVII) in the presence of a suitable basis such as potassium tert-butoxide in a suitable solvent such as THF to yield an intermediate of formula (XVIII).

10 Intermediates of Formula (XV) can be further functionalized by a person skilled in the art. The stereoisomers of intermediates of formula (XV) and formula (XVIII) can be separated from one another using techniques such as liquid chromatography using a chiral stationary phase or SFC.

Scheme 5

15 Intermediates of Formula (IV) wherein R^{1a} represents hydrogen, R^{1b} and R^{4b} are taken together to form together with the atoms to which they attached a monocyclic 6- or 7-membered fully saturated heterocyclyl containing at least 1 N-atom and substituted as for example intermediates of Formula (XX), can be prepared according to the following reaction Scheme 5, wherein X represents halo, n and p
20 represent 1 or 2 (but only one of n and p can represent 2), Y represents O, S, or N, and Z represents $SnBu_3$ or $SiMe_3$. A skilled person will understand that R^q and R^z can be selected from the list of substituents as defined in the scope of the application and do also include bicyclic spiro moieties formed together with the N-containing heterocyclyls to which they are attached. A skilled person will understand that R^q and
25 R^z for example will not represent cyano or a directly attached amine substituent. All other variables in Scheme 5 are defined according to the scope of the application.



Step 1: An intermediate of formula (VIII) can react with a compound of formula (XIX), wherein Y represents O, S or N, Z = SnBu₃, in the presence of an oxidant, such as for example Cu(OTf)₂, a suitable ligand, such as for example Lutidine or PhBox, and a suitable solvent or solvent mixture, such as hexafluoroisopropanol (HFIP) and DCM

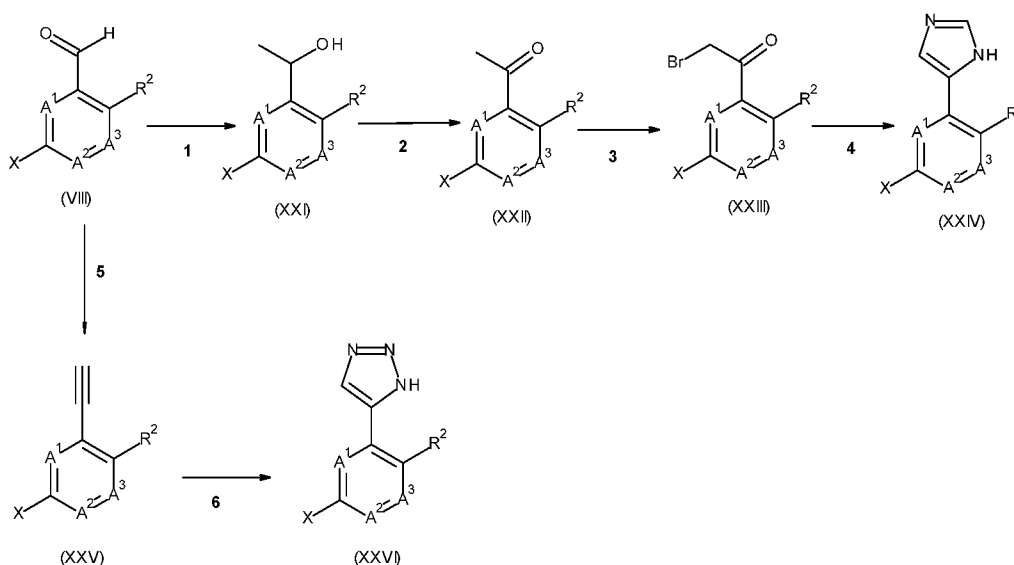
or an intermediate of Formula (VIII) can react with a compound of Formula (XIX), wherein Y = N, Z = SiMe₃, in the presence of a photocatalyst, such as for example Ir[ppy]₂(dtbbpy)PF₆, and a suitable solvent or solvent mixture, such as trifluoroethanol (TFE) and CH₃CN under blue light irradiation resulting.

Alternatively, an intermediate of formula (VIII) can react with a compound of Formula (XIX), wherein Y = S, Z = SiMe₃, in the presence of a photocatalyst, such as for example Ir[ppy]₂(dtbbpy)PF₆, a suitable acid or an acid mixture such as for example Bi(OTf)₃, Cu(OTf)₂, BF₃·2MeOH in a suitable solvent CH₃CN under blue light irradiation.

Alternatively, an intermediate of formula (VIII) can react with a compound of formula (XIX, Y = O, Z = SiMe₃) in the presence of a photocatalyst, such as for example triphenylpyrilium (TPP), a suitable acid such as for example TMSOTf, and a suitable solvent or solvent mixture, such as hexafluoroisopropanol (HFIP) and CH₃CN under blue light irradiation.

Scheme 6

Intermediates of formula (IV) wherein R^{1a} is absent when the dotted line towards R^{1b} is a bond as for example intermediates of Formula (XXIV) and (XXVI) can be prepared according to the following reaction Scheme 6. All other variables in Scheme 6 are defined according to the scope of the application.



Step 1: An intermediate of formula (VIII) can be converted into an intermediate of formula (XXI) by reaction for example with methylmagnesium bromide in a suitable solvent, such as for example THF.

Step 2: An intermediate of formula (XXI) can be converted into an intermediate of formula (XXII) by reaction for example with an oxidant such as for example manganese oxide in a suitable solvent, such as for example DCM

Step 3: An intermediate of formula (XXII) can be converted into an intermediate of formula (XXIII) by reaction for example with a bromination agent such as for example tetra-n-butylammonium tribromide in a suitable solvent, such as for example acetonitrile.

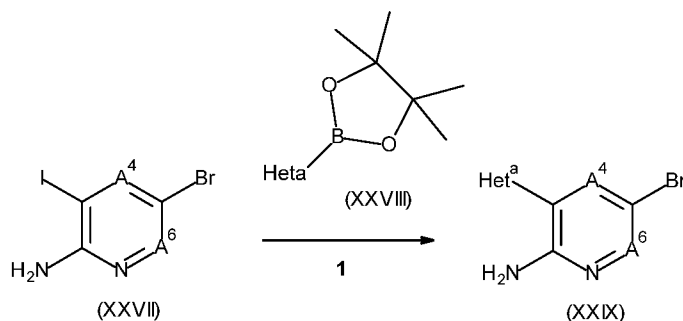
Step 4: An intermediate of formula (XXIII) can be converted into an intermediate of formula (XXIV) by reaction for example with formamide as a suitable solvent.

Step 5: An intermediate of formula (VIII) can be converted into an intermediate of formula (XXV) by reaction for example with dimethyl(1-diazo-2-oxopropyl)phosphonate in the presence of a suitable base, such as potassium carbonate in a suitable solvent, such as for example MeOH.

Step 6: An intermediate of formula (XXV) can be converted into an intermediate of formula (XXVI) by reaction for example with trimethylsilyl azide in the presence of a catalyst as for example copper iodide in a suitable solvent or mixture of solvents as for example DMF and MeOH.

Scheme 7

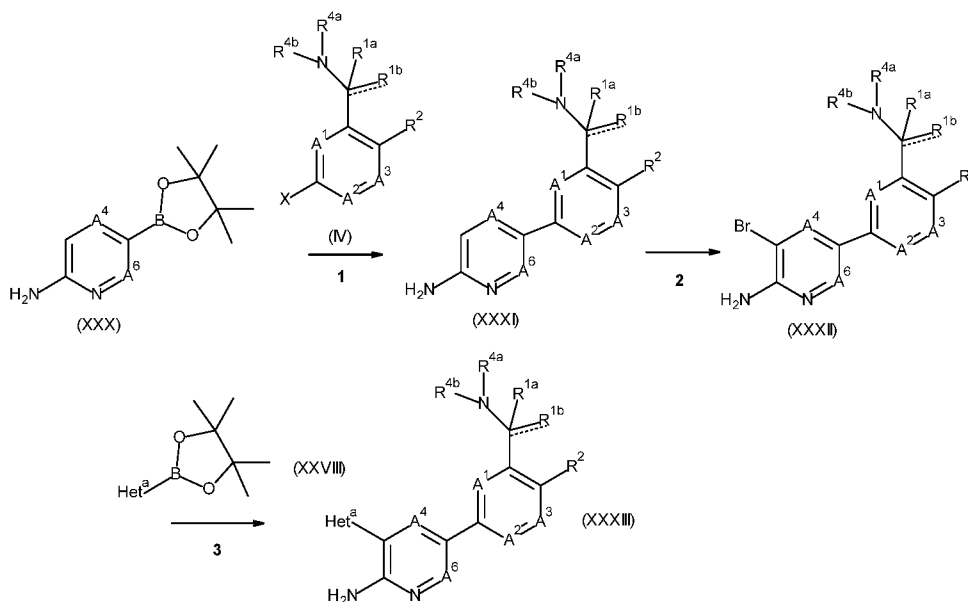
Intermediates of formula (II) wherein R^{3a} represents Het^a (or wherein R^{3a} represents an optionally substituted phenyl) and $X = Br$ can be prepared according to the following reaction Scheme 7. All other variables in Scheme 7 are defined according to the scope of the application.



Step 1: An intermediate of formula (XXVII) can react with an intermediate of formula (XXVIII) in the presence of a suitable catalyst, such as for example $Pd(PPh_3)_4$, a suitable base, such as sodium bicarbonate (Na_2CO_3), and a suitable solvent or solvent mixture, such as for example Me-THF or 1-4 dioxane and water.

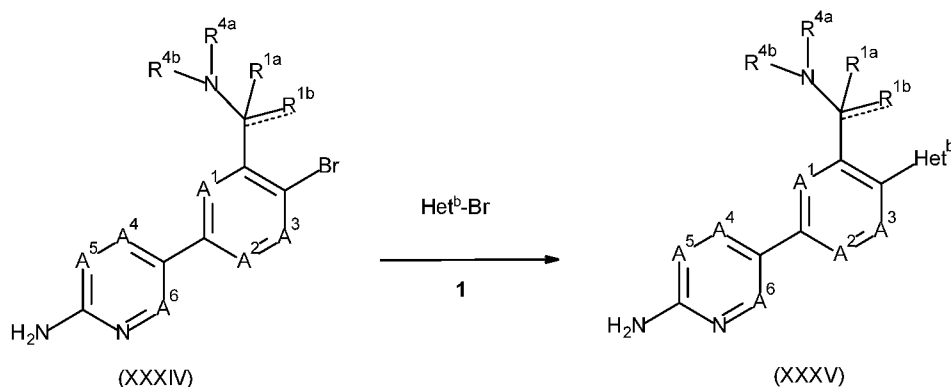
Scheme 8

An alternative to the synthesis of compounds of formula (I), wherein A^5 represents Het^a and R^2 is different from Br (compounds of Formula (XXXIII)): can be prepared according to the following reaction Scheme 8. All other variables are defined according to the scope of the application.



Scheme 9

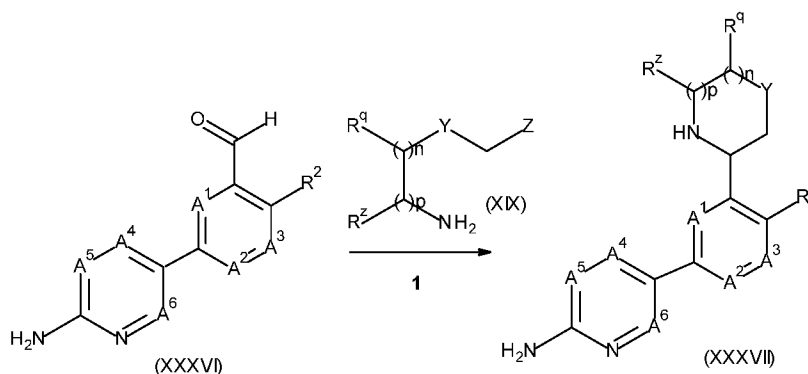
An alternative to the synthesis of compounds of Formula (I) wherein R^{3a} is different from halo, can be prepared according to the following reaction Scheme 9, as for example, compounds of Formula (XXXV) wherein R^2 represents Het^b . All other variables are defined according to the scope of the application.



- Step 1: An intermediate of formula (XXXIV) can react with Het^b bromides in the presence of a suitable photocatalyst, such as for example $\text{Ir}[\text{ppy}]_2(\text{dtbbpy})\text{PF}_6$, a suitable Nickel source, such as for example NiCl_2 , a suitable ligand, such as for example di-tert-butylbispyridine (dtbbp), a suitable reductant, such as for example tris(trimethylsilyl)silane (TTMSS), a suitable base, such as sodium bicarbonate (Na_2CO_3), and a suitable solvent, such as DME, under blue light irradiation.

Scheme 10

- An alternative to the synthesis of compounds of Formula (I), can be prepared according to the following reaction Scheme 10, as for example, compounds of Formula (XXXVII) wherein R^{1a} represents hydrogen, R^{1b} and R^{4b} are taken together to form together with the atoms to which they are attached a monocyclic 6- or 7-membered fully saturated heterocyclyl containing at least 1N-atom and substituted; n and p represent 1 or 2 (but only one of n and p can represent 2), Y represents O, S, or N, and Z represents SnBu_3 or SiMe_3 . A skilled person will understand that R^q and R^z can be selected from the list of substituents as defined in the scope of the application and do also include bicyclic spiro moieties formed together with the N-containing heterocyclyls to which they are attached. A skilled person will understand that R^q and R^z for example will not represent cyano or a directly attached amine substituent. All other variables are defined according to the scope of the application.



Step 1: An intermediate of formula (XXXVI) can react with a compound of Formula (XIX), wherein Y represents O, S or N, Z = SnBu₃, in the presence of an oxidant, such as for example Cu(OTf)₂, a suitable ligand, such as for example Lutidine or PhBox, and a suitable solvent or solvent mixture, such as hexafluoroisopropanol (HFIP) and DCM .

Alternatively, an intermediate of formula (XXXVI) can react with a compound of Formula (XIX), wherein Y = N, Z = SiMe₃, in the presence of a photocatalyst, such as for example Ir[ppy]₂(dtbbpy)PF₆, and a suitable solvent or solvent mixture, such as trifluoroethanol (TFE) and CH₃CN under blue light irradiation.

Alternatively, an intermediate of formula (XXXVI) can react with a compound of formula (XIX), wherein Y = S, Z = SiMe₃ in the presence of a photocatalyst, such as for example Ir[ppy]₂(dtbbpy)PF₆, a suitable acid or an acid mixture such as for example Bi(OTf)₃, Cu(OTf)₂, BF₃·2MeOH in a suitable solvent CH₃CN under blue light irradiation.

Alternatively, an intermediate of formula (XXXVI) can react with a compound of formula (XIX), wherein Y = O, Z = SiMe₃ in the presence of a photocatalyst, such as for example triphenylpyrilium (TPP), a suitable acid such as for example TMSOTf, and a suitable solvent or solvent mixture, such as hexafluoroisopropanol (HFIP) and CH₃CN under blue light irradiation.

In the preparation of compounds of the application according to any one of the above mentioned reaction Schemes, protection of remote functionality (e.g., primary or secondary amine) of intermediates may be necessary. The need for such protection will vary depending on the nature of the remote functionality and the conditions of the preparations methods. Suitable amino-protecting groups (NH-Pg) include t-butoxycarbonyl (Boc), acetyl, benzyl, etc. The need for such protection is readily

determined by one skilled in the art. For a general description of protecting groups and their use, see T. W. Greene and P. G. M. Wuts, *Protective Groups in Organic Synthesis*, 4th ed., Wiley, Hoboken, New Jersey, 2007.

It will be appreciated that where appropriate functional groups exist,
5 compounds of various formulae or any intermediates used in their preparation may be further derivatised by one or more standard synthetic methods employing condensation, substitution, oxidation, reduction, or cleavage reactions. Particular substitution approaches include conventional alkylation, arylation, heteroarylation, acylation, sulfonylation, halogenation, nitration, formylation and coupling procedures.

10 The compounds of formula (I) can be synthesized in the form of racemic mixtures of enantiomers which can be separated from one another following art-known resolution procedures. The racemic compounds of formula (I) containing a basic nitrogen atom can be converted into the corresponding diastereomeric salt forms by reaction with a suitable chiral acid. Diastereomeric salt forms are subsequently
15 separated, for example, by selective or fractional crystallization and the enantiomers are liberated therefrom by alkali. An alternative manner of separating the enantiomeric forms of the compounds of formula (I) involves liquid chromatography using a chiral stationary phase. Pure stereochemically isomeric forms can also be derived from the corresponding pure stereochemically isomeric forms of the
20 appropriate starting materials, provided that the reaction occurs stereospecifically.

Additional disclosure on HPK1 inhibitors that can be used in the invention are described in U.S. Provisional Application 62/823,708, filed March 26, 2019, and European Patent Application EP19167820, filed April 8, 2019, the contents of which are hereby incorporated by reference in their entirety.

25 **Compositions, Therapeutic Combinations, and Vaccines**

The application also relates to compositions, therapeutic combinations, more particularly kits, and vaccines comprising one or more HBV antigens, polynucleotides, and/or vectors encoding one or more HBV antigens according to the application. Any of the HBV antigens, polynucleotides (including RNA and DNA), and/or vectors of the
30 application described herein can be used in the compositions, therapeutic combinations or kits, and vaccines of the application.

In an embodiment of the application, a composition comprises an isolated or non-naturally occurring nucleic acid molecule (DNA or RNA) comprising polynucleotide sequence encoding a truncated HBV core antigen consisting of an

amino acid sequence that is at least 90% identical to SEQ ID NO: 2 or SEQ ID NO: 4, or an HBV polymerase antigen comprising an amino acid sequence that is at least 90% identical to SEQ ID NO: 7, a vector comprising the isolated or non-naturally occurring nucleic acid molecule, and/or an isolated or non-naturally occurring polypeptide
5 encoded by the isolated or non-naturally occurring nucleic acid molecule.

In an embodiment of the application, a composition comprises an isolated or non-naturally occurring nucleic acid molecule (DNA or RNA) comprising a polynucleotide sequence encoding an HBV Pol antigen comprising an amino acid sequence that is at least 90% identical to SEQ ID NO: 7, preferably 100% identical to
10 SEQ ID NO: 7.

In an embodiment of the application, a composition comprises an isolated or non-naturally occurring nucleic acid molecule (DNA or RNA) encoding a truncated HBV core antigen consisting of an amino acid sequence that is at least 90% identical to SEQ ID NO: 2 or SEQ ID NO: 4, preferably 100% identical to SEQ ID NO: 2 or SEQ
15 ID NO: 4.

In an embodiment of the application, a composition comprises an isolated or non-naturally occurring nucleic acid molecule (DNA or RNA) comprising a polynucleotide sequence encoding a truncated HBV core antigen consisting of an amino acid sequence that is at least 90% identical to SEQ ID NO: 2 or SEQ ID NO: 4, preferably 100% identical to SEQ ID NO: 2 or SEQ ID NO: 4; and an isolated or non-naturally occurring nucleic acid molecule (DNA or RNA) comprising a polynucleotide sequence encoding an HBV Pol antigen comprising an amino acid sequence that is at least 90% identical to SEQ ID NO: 7, preferably 100% identical to SEQ ID NO: 7.
20 The coding sequences for the truncated HBV core antigen and the HBV Pol antigen
25 can be present in the same isolated or non-naturally occurring nucleic acid molecule (DNA or RNA), or in two different isolated or non-naturally occurring nucleic acid molecules (DNA or RNA).

In an embodiment of the application, a composition comprises a vector, preferably a DNA plasmid or a viral vector (such as an adenoviral vector) comprising a
30 polynucleotide encoding a truncated HBV core antigen consisting of an amino acid sequence that is at least 90% identical to SEQ ID NO: 2 or SEQ ID NO: 4, preferably 100% identical to SEQ ID NO: 2 or SEQ ID NO: 4.

In an embodiment of the application, a composition comprises a vector, preferably a DNA plasmid or a viral vector (such as an adenoviral vector), comprising

a polynucleotide encoding an HBV Pol antigen comprising an amino acid sequence that is at least 90% identical to SEQ ID NO: 7, preferably 100% identical to SEQ ID NO: 7.

In an embodiment of the application, a composition comprises a vector,
5 preferably a DNA plasmid or a viral vector (such as an adenoviral vector), comprising a polynucleotide encoding a truncated HBV core antigen consisting of an amino acid sequence that is at least 90% identical to SEQ ID NO: 2 or SEQ ID NO: 4, preferably 100% identical to SEQ ID NO: 2 or SEQ ID NO: 4; and a vector, preferably a DNA
10 plasmid or a viral vector (such as an adenoviral vector), comprising a polynucleotide encoding an HBV Pol antigen comprising an amino acid sequence that is at least 90% identical to SEQ ID NO: 7, preferably 100% identical to SEQ ID NO: 7. The vector comprising the coding sequence for the truncated HBV core antigen and the vector comprising the coding sequence for the HBV Pol antigen can be the same vector, or two different vectors.

15 In an embodiment of the application, a composition comprises a vector, preferably a DNA plasmid or a viral vector (such as an adenoviral vector), comprising a polynucleotide encoding a fusion protein comprising a truncated HBV core antigen consisting of an amino acid sequence that is at least 90% identical to SEQ ID NO: 2 or SEQ ID NO: 4, preferably 100% identical to SEQ ID NO: 2 or SEQ ID NO: 4,
20 operably linked to an HBV Pol antigen comprising an amino acid sequence that is at least 90% identical to SEQ ID NO: 7, preferably 100% identical to SEQ ID NO: 7, or vice versa. Preferably, the fusion protein further comprises a linker that operably links the truncated HBV core antigen to the HBV Pol antigen, or vice versa. Preferably, the linker has the amino acid sequence of (AlaGly)_n, wherein n is an integer of 2 to 5.

25 In an embodiment of the application, a composition comprises an isolated or non-naturally occurring truncated HBV core antigen consisting of an amino acid sequence that is at least 90% identical to SEQ ID NO: 2 or SEQ ID NO: 4, preferably 100% identical to SEQ ID NO: 2 or SEQ ID NO: 4.

In an embodiment of the application, a composition comprises an isolated or
30 non-naturally occurring HBV Pol antigen comprising an amino acid sequence that is at least 90% identical to SEQ ID NO: 7, preferably 100% identical to SEQ ID NO: 7.

In an embodiment of the application, a composition comprises an isolated or non-naturally occurring truncated HBV core antigen consisting of an amino acid sequence that is at least 90% identical to SEQ ID NO: 2 or SEQ ID NO: 4, preferably

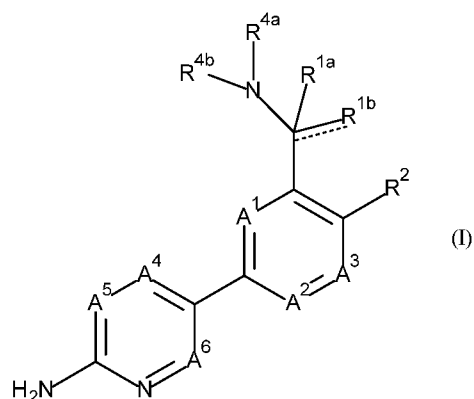
100% identical to SEQ ID NO: 2 or SEQ ID NO: 4; and an isolated or non-naturally occurring HBV Pol antigen comprising an amino acid sequence that is at least 90% identical to SEQ ID NO: 7, preferably 100% identical to SEQ ID NO: 7.

In an embodiment of the application, a composition comprises an isolated or
5 non-naturally occurring fusion protein comprising a truncated HBV core antigen consisting of an amino acid sequence that is at least 90% identical to SEQ ID NO: 2 or SEQ ID NO: 14, preferably 100% identical to SEQ ID NO: 2 or SEQ ID NO: 4, operably linked to an HBV Pol antigen comprising an amino acid sequence that is at least 90% identical to SEQ ID NO: 7, preferably 100% identical to SEQ ID NO: 7, or
10 vice versa. Preferably, the fusion protein further comprises a linker that operably links the truncated HBV core antigen to the HBV Pol antigen, or vice versa. Preferably, the linker has the amino acid sequence of (AlaGly)_n, wherein n is an integer of 2 to 5.

The application also relates to a therapeutic combination or a kit comprising polynucleotides expressing a truncated HBV core antigen and an HBV pol antigen
15 according to embodiments of the application. Any polynucleotides and/or vectors encoding HBV core and pol antigens of the application described herein can be used in the therapeutic combinations or kits of the application.

According to embodiments of the application, a therapeutic combination or kit for use in treating an HBV infection in a subject in need thereof, comprises:

- 20 i) at least one of:
- a) a truncated HBV core antigen consisting of an amino acid sequence that is at least 95% identical to SEQ ID NO: 2, and
 - b) a first non-naturally occurring nucleic acid molecule comprising a first polynucleotide sequence encoding the truncated HBV core antigen
 - 25 c) an HBV polymerase antigen having an amino acid sequence that is at least 90% identical to SEQ ID NO: 7, wherein the HBV polymerase antigen does not have reverse transcriptase activity and RNase H activity, and
 - d) a second non-naturally occurring nucleic acid molecule comprising a second polynucleotide sequence encoding the HBV polymerase antigen; and
- 30 ii) a compound of formula (I):



or a tautomer or a stereoisomeric form thereof, wherein:

the dotted bond towards R^{1b} is an optional bond that is optionally present when R^{1b} and R^{4b} are taken together to form a monocyclic or bicyclic aromatic heterocyclyl;

- 5 A^1 represents CH or N; A^2 represents CH; A^3 represents CH or N;
provided that only one of A^1 and A^3 represents N;
 A^4 represents CH or N; A^5 represents CR^{3a} ; A^6 represents CH;
 R^{1a} represents hydrogen;
 R^{1b} represents hydrogen or CH_3 ;
- 10 R^{4a} represents hydrogen, C_{1-4} alkyl, or C_{3-6} cycloalkyl;
 R^{4b} represents hydrogen, C_{1-4} alkyl, C_{3-6} cycloalkyl, or
a carbon linked monocyclic 4-, 5-, 6- or 7-membered non-aromatic heterocyclyl
containing 1, 2 or 3 heteroatoms each independently selected from the group
consisting of N, O, and S; wherein said S-atom is optionally substituted to form
- 15 $S(=O)$, $S(=O)_2$, or $S(=O)(=NR^x)$;

or

- R^{1b} and R^{4b} are taken together to form together with the atoms to which they are
attached a monocyclic 5-membered aromatic heterocyclyl or a monocyclic 4-, 5-, 6- or
- 20 7-membered fully saturated heterocyclyl, each containing 1 N-atom and optionally 1
or 2 additional heteroatoms each independently selected from the group consisting of
N, O, and S; wherein said optional S-atom is optionally substituted to form $S(=O)$,
 $S(=O)_2$, or $S(=O)(=NR^x)$; or
- a bicyclic 6- to 12- membered aromatic or fully saturated heterocyclyl containing 1 N-
- 25 atom and optionally 1 or 2 additional heteroatoms each independently selected from

the group consisting of N, O, and S; wherein said optional S-atom is optionally substituted to form S(=O), S(=O)₂, or S(=O)(=NR^x);

wherein said monocyclic or bicyclic, aromatic or fully saturated heterocyclyl is optionally substituted on one or more of the carbon atoms with in total 1, 2 or 3

5 substituents each independently selected from the group consisting of -OH, CN, halo, R⁷, -O-R⁷,

-S(=O)₂-R⁷, -C(=O)-R⁷, -NR^{6c}R^{6d}, -C(=O)-NR^{6a}R^{6b}, and Het^c;

wherein said monocyclic or bicyclic, aromatic or fully saturated heterocyclyl is optionally substituted on the optional additional nitrogen atoms with in total 1 or 2

10 substituents each independently selected from the group consisting of R⁷, -S(=O)₂-R⁷, -C(=O)-R⁷,

-C(=O)-NR^{6a}R^{6b}, and Het^d;

provided that in case R^{1b} and R^{4b} are taken together, R^{4a} represents hydrogen; and R^{1a} represents hydrogen or R^{1a} is absent when the dotted bond towards R^{1b} is a bond;

15 or

R^{4a} and R^{4b} are taken together to form together with the N-atom to which they are attached a monocyclic 5-membered aromatic heterocyclyl or a monocyclic 4-, 5-, 6- or 7-membered fully saturated heterocyclyl, each containing 1 N-atom and optionally 1 or 2 additional heteroatoms each independently selected from the group consisting of

20 N, O, and S; wherein said optional S-atom is optionally substituted to form S(=O), S(=O)₂, or S(=O)(=NR^x); or

a bicyclic 6- to 12- membered aromatic or fully saturated heterocyclyl containing 1 N-atom and optionally 1 or 2 additional heteroatoms each independently selected from

25 the group consisting of N, O, and S; wherein said optional S-atom is optionally substituted to form S(=O), S(=O)₂, or S(=O)(=NR^x);

wherein said monocyclic or bicyclic, aromatic or fully saturated heterocyclyl is optionally substituted on one or more of the carbon atoms with in total 1, 2 or 3

substituents each independently selected from the group consisting of -OH, CN, halo, R⁷, -O-R⁷,

30 -S(=O)₂-R⁷, -C(=O)-R⁷, -NR^{6c}R^{6d}, -C(=O)-NR^{6a}R^{6b}, and Het^c;

wherein said monocyclic or bicyclic, aromatic or fully saturated heterocyclyl is optionally substituted on the nitrogen atoms with in total 1 or 2 substituents each

independently selected from the group consisting of R⁷, -S(=O)₂-R⁷, -C(=O)-R⁷, -

- $C(=O)-NR^{6a}R^{6b}$, and Het^d ;
 in case R^{4a} and R^{4b} are taken together, R^{1a} represents hydrogen, and R^{1b} represents hydrogen;
- R^2 is selected from the group consisting of cyano; halo; $-C(=O)-NR^{8a}R^{8b}$;
- 5 $-CH_2-NR^{8c}R^{8d}$; Het^b ; $-P(=O)-(C_{1-4}alkyl)_2$; $-S(=O)_2-C_{1-4}alkyl$; $-S(=O)(=NR^x)-C_{1-4}alkyl$;
 $C_{1-6}alkyl$ optionally substituted with 1 or 2 substituents each independently selected from the group consisting of halo, $-OH$, cyano, and $-O-C_{1-4}alkyl$;
 $C_{3-6}cycloalkyl$ optionally substituted with 1 or 2 substituents each independently selected from the group consisting of halo, $-OH$, cyano and $-O-C_{1-4}alkyl$; and
- 10 $C_{3-6}cycloalkenyl$ optionally substituted with 1 or 2 substituents each independently selected from the group consisting of halo, $-OH$, cyano and $-O-C_{1-4}alkyl$;
 R^{3a} represents hydrogen, halo, R^7 , $-O-R^7$, cyano, $-C(=O)-NR^{6e}R^{6f}$, Het^a , or phenyl optionally substituted with 1, 2 or 3 substituents each independently selected from the group consisting of halo, cyano, $C_{1-4}alkyl$, $-O-C_{1-4}alkyl$, $C_{1-4}alkyl$ substituted with one
- 15 cyano, and $C_{1-4}alkyl$ substituted with 1, 2 or 3 halo atoms;
 Het^a represents a carbon linked monocyclic 5-, 6- or 7-membered aromatic heterocyclyl or carbon linked monocyclic 4-, 5-, 6- or 7-membered non-aromatic heterocyclyl, each containing 1, 2 or 3 heteroatoms each independently selected from the group consisting of N, O or S; wherein said S-atom might be substituted to form
- 20 $S(=O)$, $S(=O)_2$, or $S(=O)(=NR^x)$; or
 a carbon linked bicyclic 6- to 12-membered aromatic or non-aromatic heterocyclyl containing 1, 2 or 3 heteroatoms each independently selected from the group consisting of N, O and S; wherein said S-atom is optionally substituted to form $S(=O)$, $S(=O)_2$, or $S(=O)(=NR^x)$;
- 25 wherein said monocyclic or bicyclic, aromatic or non-aromatic heterocyclyl is optionally substituted on one or more of the carbon atoms with in total 1, 2 or 3 substituents each independently selected from the group consisting of $-OH$, CN, halo, R^7 , $-O-R^7$,
 $-S(=O)_2-R^7$, $-C(=O)-R^7$, $-NR^{6c}R^{6d}$, $-C(=O)-NR^{6a}R^{6b}$, and Het^c ;
- 30 wherein said monocyclic or bicyclic, aromatic or non-aromatic heterocyclyl is optionally substituted on the nitrogen atoms with in total 1 or 2 substituents each independently selected from the group consisting of R^7 , $-S(=O)_2-R^7$, $-C(=O)-R^7$, $-C(=O)-NR^{6a}R^{6b}$ and Het^d ;

R^{6a} , R^{6b} , R^{6c} , R^{6d} , R^{6e} , and R^{6f} are each independently selected from the group consisting of hydrogen; C_{3-6} cycloalkyl optionally substituted with one $-OR^5$; and C_{1-4} alkyl optionally substituted with one $-OR^5$, wherein two hydrogen atoms on the same carbon atom of said C_{1-4} alkyl are optionally taken together to form C_3 .

- 5 C_6 cycloalkyl;
 R^5 represents hydrogen or C_{1-4} alkyl;
 R^{8a} , R^{8c} , and R^{8d} are each independently selected from the group consisting of hydrogen;
 C_{1-4} alkyl optionally substituted with one $-OH$ or $-O-C_{1-4}$ alkyl; and C_{3-6} cycloalkyl
- 10 optionally substituted with one $-OH$ or $-O-C_{1-4}$ alkyl;
 R^{8b} is selected from the group consisting C_{1-4} alkyl optionally substituted with one $-OH$ or
 $-O-C_{1-4}$ alkyl; and C_{3-6} cycloalkyl optionally substituted with one $-OH$ or $-O-C_{1-4}$ alkyl;
or
- 15 R^{8a} and R^{8b} , or R^{8c} and R^{8d} are taken together to form together with the N-atom to
which they are attached a monocyclic fully saturated heterocyclyl containing 1 N-atom
and optionally 1 or 2 additional heteroatoms each independently selected from the
group consisting of N, O, S; wherein said optional S-atom is optionally substituted to
form $S(=O)$, $S(=O)_2$, or $S(=O)(=NR^x)$; wherein said monocyclic 4-, 5-, 6- or 7-
- 20 membered fully saturated heterocyclyl is optionally substituted on one or more of the
carbon atoms with in total 1, 2 or 3 substituents each independently selected from the
group consisting of $-OH$, CN, halo, R^7 , $-OR^7$, $-S(=O)_2-R^7$, $-C(=O)-R^7$, $-NR^{6c}R^{6d}$, and
 $-C(=O)-NR^{6a}R^{6b}$;

wherein said monocyclic fully saturated heterocyclyl is optionally substituted on the

- 25 nitrogen atoms with in total 1 or 2 substituents each independently selected from the
group consisting of R^7 , $-S(=O)_2-R^7$, $-C(=O)-R^7$, and $-C(=O)-NR^{6a}R^{6b}$;

each Het^c independently represents a monocyclic 4-, 5-, 6- or 7-membered fully
saturated heterocyclyl containing 1, 2 or 3 heteroatoms each independently selected
from the group consisting of N, O and S; wherein said S-atom is optionally substituted

- 30 to form $S(=O)$, $S(=O)_2$, or $S(=O)(=NR^x)$;

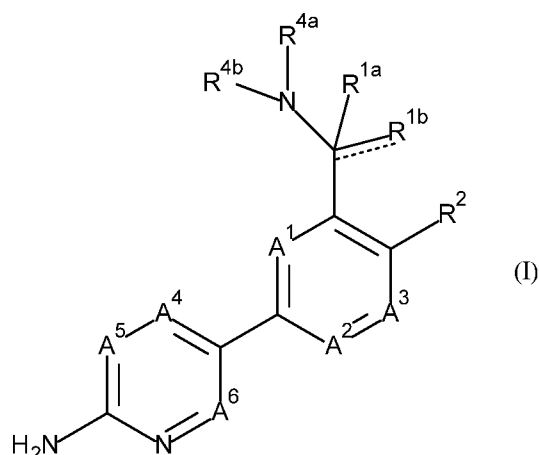
each Het^d independently represents a carbon linked monocyclic 4-, 5-, 6- or 7-
membered fully saturated heterocyclyl containing 1, 2 or 3 heteroatoms each
independently selected from the group consisting of N, O and S; wherein said S-atom
is optionally substituted to form $S(=O)$, $S(=O)_2$, or $S(=O)(=NR^x)$;

Het^b represents a monocyclic 4-, 5-, 6- or 7-membered non-aromatic heterocyclyl containing 1, 2 or 3 heteroatoms each independently selected from the group consisting of N, O, and S; wherein said S-atom might be substituted to form S(=O), S(=O)₂, or S(=O)(=NR^x); or

- 5 a bicyclic 6- to 12- membered non-aromatic heterocyclyl containing 1, 2 or 3 heteroatoms each independently selected from the group consisting of N, O, and S; wherein said S-atom might be substituted to form S(=O), S(=O)₂, or S(=O)(=NR^x); wherein said monocyclic or bicyclic non-aromatic heterocyclyl might be substituted on one or more of the carbon atoms with in total 1, 2 or 3 substituents each
- 10 independently selected from the group consisting of -OH, CN, halo, R⁷, -O-R⁷, -S(=O)₂-R⁷, -C(=O)-R⁷, -NR^{6c}R^{6d}, and -C(=O)-NR^{6a}R^{6b};
- wherein said monocyclic or bicyclic non-aromatic heterocyclyl might be substituted on the nitrogen atoms with in total 1 or 2 substituents each independently selected
- 15 from the group consisting of R⁷, -S(=O)₂-R⁷, -C(=O)-R⁷, and -C(=O)-NR^{6a}R^{6b}; each R⁷ independently represents C₃₋₆cycloalkyl optionally substituted with 1, 2 or 3 substituents each independently selected from the group consisting of halo, -OH, -O-C₁₋₄alkyl and cyano; or C₁₋₄alkyl optionally substituted with 1, 2 or 3 substituents each independently selected from the group consisting of halo, -OH, -O-C₁₋₄alkyl and
- 20 cyano; and each R^x independently represents hydrogen or C₁₋₄alkyl;
- or a pharmaceutically acceptable addition salt, an N-oxide, or a solvate thereof.

Any of the embodiments of the compounds of formula (I) described herein can be used in a therapeutic combination of the application.

- 25 In a particular embodiment of the application, a therapeutic combination or kit comprises: i) a first non-naturally occurring nucleic acid molecule comprising a first polynucleotide sequence encoding a truncated HBV core antigen consisting of an amino acid sequence that is at least 95% identical to SEQ ID NO: 2; ii) a second non-naturally occurring nucleic acid molecule comprising a second polynucleotide
- 30 sequence encoding an HBV polymerase antigen having an amino acid sequence that is at least 90% identical to SEQ ID NO: 7, wherein the HBV polymerase antigen does not have reverse transcriptase activity and RNase H activity; and iii) a compound of formula (I):



or a tautomer or a stereoisomeric form thereof, wherein:

the dotted bond towards R^{1b} is absent;

A^1 represents CH or N; A^2 represents CH; A^3 represents CH;

5 A^4 represents CH; A^5 represents CR^{3a} ; A^6 represents CH;

R^{1b} and R^{4b} are taken together to form together with the atoms to which they are attached a monocyclic 4-, 5-, 6- or 7-membered fully saturated heterocyclyl, each containing 1 N-atom and optionally 1 or 2 additional heteroatoms each independently selected from the group consisting of N, O, and S; wherein said optional S-atom is

10 optionally substituted to form $S(=O)$;

wherein said monocyclic fully saturated heterocyclyl is optionally substituted on one of the carbon atoms with 1 substituent selected from the group consisting of halo and R^7 ;

provided that R^{4a} represents hydrogen; and R^{1a} represents hydrogen;

15 R^2 represents Het^b;

R^{3a} represents halo, cyano, or Het^a;

Het^a represents a carbon linked monocyclic 5-, 6- or 7-membered aromatic heterocyclyl containing 1, 2 or 3 heteroatoms each independently selected from the group consisting of N, O and S; wherein said S-atom is optionally substituted to form

20 $S(=O)$ or $S(=O)_2$;

wherein said monocyclic aromatic heterocyclyl is optionally substituted on one of the carbon atoms with a halo substituent;

wherein said monocyclic aromatic heterocyclyl is optionally substituted on one nitrogen atom with R^7 ;

Het^b represents a monocyclic 4-, 5-, 6- or 7-membered non-aromatic heterocyclyl containing 1 oxygen atom; and
each R⁷ represents C₁₋₄alkyl,

or a pharmaceutically acceptable addition salt, an N-oxide, or a solvate thereof.

5 In certain embodiments of the application, a therapeutic combination or kit comprises a compound selected from the group consisting of the exemplified compounds, particularly compounds 1 to 111, or a tautomer or stereoisomeric form, or a pharmaceutically acceptable addition salt, an N-oxide, or a solvate thereof.

According to embodiments of the application, the polynucleotides in a vaccine
10 combination or kit can be linked or separate, such that the HBV antigens expressed from such polynucleotides are fused together or produced as separate proteins, whether expressed from the same or different polynucleotides. In an embodiment, the first and second polynucleotides are present in separate vectors, e.g., DNA plasmids or viral vectors, used in combination either in the same or separate compositions, such that the
15 expressed proteins are also separate proteins, but used in combination. In another embodiment, the HBV antigens encoded by the first and second polynucleotides can be expressed from the same vector, such that an HBV core-pol fusion antigen is produced. Optionally, the core and pol antigens can be joined or fused together by a short linker. Alternatively, the HBV antigens encoded by the first and second polynucleotides can
20 be expressed independently from a single vector using a using a ribosomal slippage site (also known as cis-hydrolase site) between the core and pol antigen coding sequences. This strategy results in a bicistronic expression vector in which individual core and pol antigens are produced from a single mRNA transcript. The core and pol antigens produced from such a bicistronic expression vector can have additional N or C-terminal
25 residues, depending upon the ordering of the coding sequences on the mRNA transcript. Examples of ribosomal slippage sites that can be used for this purpose include, but are not limited to, the FA2 slippage site from foot-and-mouth disease virus (FMDV). Another possibility is that the HBV antigens encoded by the first and second polynucleotides can be expressed independently from two separate vectors, one
30 encoding the HBV core antigen and one encoding the HBV pol antigen.

In a preferred embodiment, the first and second polynucleotides are present in separate vectors, e.g., DNA plasmids or viral vectors. Preferably, the separate vectors are present in the same composition.

According to preferred embodiments of the application, a therapeutic combination or kit comprises a first polynucleotide present in a first vector, a second polynucleotide present in a second vector. The first and second vectors can be the same or different. Preferably the vectors are DNA plasmids.

5 In a particular embodiment of the application, the first vector is a first DNA plasmid, the second vector is a second DNA plasmid. Each of the first and second DNA plasmids comprises an origin of replication, preferably pUC ORI of SEQ ID NO: 21, and an antibiotic resistance cassette, preferably comprising a codon optimized Kanr gene having a polynucleotide sequence that is at least 90% identical to SEQ ID NO: 23,
10 preferably under control of a bla promoter, for instance the bla promoter shown in SEQ ID NO: 24. Each of the first and second DNA plasmids independently further comprises at least one of a promoter sequence, enhancer sequence, and a polynucleotide sequence encoding a signal peptide sequence operably linked to the first polynucleotide sequence or the second polynucleotide sequence. Preferably, each of
15 the first and second DNA plasmids comprises an upstream sequence operably linked to the first polynucleotide or the second polynucleotide, wherein the upstream sequence comprises, from 5' end to 3' end, a promoter sequence of SEQ ID NO: 18 or 19, an enhancer sequence, and a polynucleotide sequence encoding a signal peptide sequence having the amino acid sequence of SEQ ID NO: 9 or 15. Each of the first and second
20 DNA plasmids can also comprise a polyadenylation signal located downstream of the coding sequence of the HBV antigen, such as the bGH polyadenylation signal of SEQ ID NO: 20.

In one particular embodiment of the application, the first vector is a viral vector and the second vector is a viral vector. Preferably, each of the viral vectors is an
25 adenoviral vector, more preferably an Ad26 or Ad35 vector, comprising an expression cassette including the polynucleotide encoding an HBV pol antigen or an truncated HBV core antigen of the application; an upstream sequence operably linked to the polynucleotide encoding the HBV antigen comprising, from 5' end to 3' end, a promoter sequence, preferably a CMV promoter sequence of SEQ ID NO: 19, an
30 enhancer sequence, preferably an ApoAI gene fragment sequence of SEQ ID NO: 12, and a polynucleotide sequence encoding a signal peptide sequence, preferably an immunoglobulin secretion signal having the amino acid sequence of SEQ ID NO: 15; and a downstream sequence operably linked to the polynucleotide encoding the HBV

antigen comprising a polyadenylation signal, preferably a SV40 polyadenylation signal of SEQ ID NO: 13.

In another preferred embodiment, the first and second polynucleotides are present in a single vector, e.g., DNA plasmid or viral vector. Preferably, the single
5 vector is an adenoviral vector, more preferably an Ad26 vector, comprising an expression cassette including a polynucleotide encoding an HBV pol antigen and a truncated HBV core antigen of the application, preferably encoding an HBV pol antigen and a truncated HBV core antigen of the application as a fusion protein; an upstream sequence operably linked to the polynucleotide encoding the HBV pol and
10 truncated core antigens comprising, from 5' end to 3' end, a promoter sequence, preferably a CMV promoter sequence of SEQ ID NO: 19, an enhancer sequence, preferably an ApoAI gene fragment sequence of SEQ ID NO: 12, and a polynucleotide sequence encoding a signal peptide sequence, preferably an immunoglobulin secretion signal having the amino acid sequence of SEQ ID NO: 15; and a downstream sequence
15 operably linked to the polynucleotide encoding the HBV antigen comprising a polyadenylation signal, preferably a SV40 polyadenylation signal of SEQ ID NO: 13.

When a therapeutic combination of the application comprises a first vector, such as a DNA plasmid or viral vector, and a second vector, such as a DNA plasmid or viral vector, the amount of each of the first and second vectors is not particularly
20 limited. For example, the first DNA plasmid and the second DNA plasmid can be present in a ratio of 10:1 to 1:10, by weight, such as 10:1, 9:1, 8:1, 7:1, 6:1, 5:1, 4:1, 3:1, 2:1, 1:1, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, 1:9, or 1:10, by weight. Preferably, the first and second DNA plasmids are present in a ratio of 1:1, by weight. The therapeutic combination of the application can further comprise a third vector encoding a third
25 active agent useful for treating an HBV infection.

Compositions and therapeutic combinations of the application can comprise additional polynucleotides or vectors encoding additional HBV antigens and/or additional HBV antigens or immunogenic fragments thereof, such as an HBsAg, an HBV L protein or HBV envelope protein, or a polynucleotide sequence encoding
30 thereof. However, in particular embodiments, the compositions and therapeutic combinations of the application do not comprise certain antigens.

In a particular embodiment, a composition or therapeutic combination or kit of the application does not comprise a HBsAg or a polynucleotide sequence encoding the HBsAg.

In another particular embodiment, a composition or therapeutic combination or kit of the application does not comprise an HBV L protein or a polynucleotide sequence encoding the HBV L protein.

In yet another particular embodiment of the application, a composition or
5 therapeutic combination of the application does not comprise an HBV envelope protein or a polynucleotide sequence encoding the HBV envelope protein.

Compositions and therapeutic combinations of the application can also comprise a pharmaceutically acceptable carrier. A pharmaceutically acceptable carrier is non-toxic and should not interfere with the efficacy of the active ingredient.

10 Pharmaceutically acceptable carriers can include one or more excipients such as binders, disintegrants, swelling agents, suspending agents, emulsifying agents, wetting agents, lubricants, flavorants, sweeteners, preservatives, dyes, solubilizers and coatings. Pharmaceutically acceptable carriers can include vehicles, such as lipid nanoparticles (LNPs). The precise nature of the carrier or other material can depend on
15 the route of administration, e.g., intramuscular, intradermal, subcutaneous, oral, intravenous, cutaneous, intramucosal (e.g., gut), intranasal or intraperitoneal routes. For liquid injectable preparations, for example, suspensions and solutions, suitable carriers and additives include water, glycols, oils, alcohols, preservatives, coloring agents and the like. For solid oral preparations, for example, powders, capsules,
20 caplets, gelcaps and tablets, suitable carriers and additives include starches, sugars, diluents, granulating agents, lubricants, binders, disintegrating agents and the like. For nasal sprays/inhalant mixtures, the aqueous solution/suspension can comprise water, glycols, oils, emollients, stabilizers, wetting agents, preservatives, aromatics, flavors, and the like as suitable carriers and additives.

25 Compositions and therapeutic combinations of the application can be formulated in any matter suitable for administration to a subject to facilitate administration and improve efficacy, including, but not limited to, oral (enteral) administration and parenteral injections. The parenteral injections include intravenous injection or infusion, subcutaneous injection, intradermal injection, and intramuscular
30 injection. Compositions of the application can also be formulated for other routes of administration including transmucosal, ocular, rectal, long acting implantation, sublingual administration, under the tongue, from oral mucosa bypassing the portal circulation, inhalation, or intranasal.

In a preferred embodiment of the application, compositions and therapeutic combinations of the application are formulated for parental injection, preferably subcutaneous, intradermal injection, or intramuscular injection, more preferably intramuscular injection.

5 According to embodiments of the application, compositions and therapeutic combinations for administration will typically comprise a buffered solution in a pharmaceutically acceptable carrier, e.g., an aqueous carrier such as buffered saline and the like, e.g., phosphate buffered saline (PBS). The compositions and therapeutic combinations can also contain pharmaceutically acceptable substances as required to
10 approximate physiological conditions such as pH adjusting and buffering agents. For example, a composition or therapeutic combination of the application comprising plasmid DNA can contain phosphate buffered saline (PBS) as the pharmaceutically acceptable carrier. The plasmid DNA can be present in a concentration of, e.g., 0.5 mg/mL to 5 mg/mL, such as 0.5 mg/mL, 1 mg/mL, 2 mg/mL, 3 mg/mL, 4 mg/mL, or
15 5 mg/mL, preferably at 1 mg/mL.

Compositions and therapeutic combinations of the application can be formulated as a vaccine (also referred to as an “immunogenic composition”) according to methods well known in the art. Such compositions can include adjuvants to enhance immune responses. The optimal ratios of each component in the formulation can be
20 determined by techniques well known to those skilled in the art in view of the present disclosure.

In a particular embodiment of the application, a composition or therapeutic combination is a DNA vaccine. DNA vaccines typically comprise bacterial plasmids containing a polynucleotide encoding an antigen of interest under control of a strong
25 eukaryotic promoter. Once the plasmids are delivered to the cell cytoplasm of the host, the encoded antigen is produced and processed endogenously. The resulting antigen typically induces both humoral and cell-mediated immune responses. DNA vaccines are advantageous at least because they offer improved safety, are temperature stable, can be easily adapted to express antigenic variants, and are simple to produce. Any of
30 the DNA plasmids of the application can be used to prepare such a DNA vaccine.

In other particular embodiments of the application, a composition or therapeutic combination is an RNA vaccine. RNA vaccines typically comprise at least one single-stranded RNA molecule encoding an antigen of interest, e.g., a fusion protein or HBV antigen according to the application. Once the RNA is delivered to the cell cytoplasm

of the host, the encoded antigen is produced and processed endogenously, inducing both humoral and cell-mediated immune responses, similar to a DNA vaccine. The RNA sequence can be codon optimized to improve translation efficiency. The RNA molecule can be modified by any method known in the art in view of the present
5 disclosure to enhance stability and/or translation, such by adding a polyA tail, e.g., of at least 30 adenosine residues; and/or capping the 5-end with a modified ribonucleotide, e.g., 7-methylguanosine cap, which can be incorporated during RNA synthesis or enzymatically engineered after RNA transcription. An RNA vaccine can also be self-replicating RNA vaccine developed from an alphavirus expression vector. Self-
10 replicating RNA vaccines comprise a replicase RNA molecule derived from a virus belonging to the alphavirus family with a subgenomic promoter that controls replication of the fusion protein or HBV antigen RNA followed by an artificial poly A tail located downstream of the replicase.

In certain embodiments, a further adjuvant can be included in a composition or
15 therapeutic combination of the application, or co-administered with a composition or therapeutic combination of the application. Use of another adjuvant is optional, and can further enhance immune responses when the composition is used for vaccination purposes. Other adjuvants suitable for co-administration or inclusion in compositions in accordance with the application should preferably be ones that are potentially safe,
20 well tolerated and effective in humans. An adjuvant can be a small molecule or antibody including, but not limited to, immune checkpoint inhibitors (e.g., anti-PD1, anti-TIM-3, etc.), toll-like receptor agonists (e.g., TLR7 agonists and/or TLR8 agonists), RIG-1 agonists, IL-15 superagonists (Altor Bioscience), mutant IRF3 and IRF7 genetic adjuvants, STING agonists (Aduro), FLT3L genetic adjuvant, and IL-7-
25 hyFc. For example, adjuvants can e.g., be chosen from among the following anti-HBV agents: HBV DNA polymerase inhibitors; Immunomodulators; Toll-like receptor 7 modulators; Toll-like receptor 8 modulators; Toll-like receptor 3 modulators; Interferon alpha receptor ligands; Hyaluronidase inhibitors; Modulators of IL-10; HBsAg inhibitors; Toll like receptor 9 modulators; Cyclophilin inhibitors; HBV
30 Prophylactic vaccines; HBV Therapeutic vaccines; HBV viral entry inhibitors; Antisense oligonucleotides targeting viral mRNA, more particularly anti-HBV antisense oligonucleotides; short interfering RNAs (siRNA), more particularly anti-HBV siRNA; Endonuclease modulators; Inhibitors of ribonucleotide reductase; Hepatitis B virus E antigen inhibitors; HBV antibodies targeting the surface antigens

of the hepatitis B virus; HBV antibodies; CCR2 chemokine antagonists; Thymosin agonists; Cytokines, such as IL12; Capsid Assembly Modulators, Nucleoprotein inhibitors (HBV core or capsid protein inhibitors); Nucleic Acid Polymers (NAPs); Stimulators of retinoic acid-inducible gene 1; Stimulators of NOD2; Recombinant
5 thymosin alpha-1; Hepatitis B virus replication inhibitors; PI3K inhibitors; cccDNA inhibitors; immune checkpoint inhibitors, such as PD-L1 inhibitors, PD-1 inhibitors, TIM-3 inhibitors, TIGIT inhibitors, Lag3 inhibitors, CTLA-4 inhibitors; Agonists of co-stimulatory receptors that are expressed on immune cells (more particularly T cells), such as CD27 and CD28; BTK inhibitors; Other drugs for treating HBV; IDO
10 inhibitors; Arginase inhibitors; and KDM5 inhibitors.

In certain embodiments, a therapeutic combination of the application further comprises an immune modulatory agent, such as an inhibitor of the PD-1/PD-L1 immune checkpoint axis, for example antibodies (or peptides) that bind to and/or inhibit the activity of PD-1 or the activity of PD-L1.

15 In certain embodiments, each of the first and second non-naturally occurring nucleic acid molecules is independently formulated with a lipid nanoparticle (LNP).

The application also provides methods of making compositions and therapeutic combinations of the application. A method of producing a composition or therapeutic combination comprises mixing an isolated polynucleotide encoding an HBV antigen,
20 vector, and/or polypeptide of the application with one or more pharmaceutically acceptable carriers. One of ordinary skill in the art will be familiar with conventional techniques used to prepare such compositions.

Methods of Inducing an Immune Response or Treating an HBV Infection

The application also provides methods of inducing an immune response against
25 hepatitis B virus (HBV) in a subject in need thereof, comprising administering to the subject an immunogenically effective amount of a composition or immunogenic composition of the application. Any of the compositions and therapeutic combinations of the application described herein can be used in the methods of the application.

As used herein, the term “infection” refers to the invasion of a host by a disease
30 causing agent. A disease causing agent is considered to be “infectious” when it is capable of invading a host, and replicating or propagating within the host. Examples of infectious agents include viruses, e.g., HBV and certain species of adenovirus, prions, bacteria, fungi, protozoa and the like. “HBV infection” specifically refers to invasion of a host organism, such as cells and tissues of the host organism, by HBV.

The phrase “inducing an immune response” when used with reference to the methods described herein encompasses causing a desired immune response or effect in a subject in need thereof against an infection, e.g., an HBV infection. “Inducing an immune response” also encompasses providing a therapeutic immunity for treating
5 against a pathogenic agent, e.g., HBV. As used herein, the term “therapeutic immunity” or “therapeutic immune response” means that the vaccinated subject is able to control an infection with the pathogenic agent against which the vaccination was done, for instance immunity against HBV infection conferred by vaccination with HBV vaccine. In an embodiment, “inducing an immune response” means producing an
10 immunity in a subject in need thereof, e.g., to provide a therapeutic effect against a disease, such as HBV infection. In certain embodiments, “inducing an immune response” refers to causing or improving cellular immunity, e.g., T cell response, against HBV infection. In certain embodiments, “inducing an immune response” refers to causing or improving a humoral immune response against HBV infection. In certain
15 embodiments, “inducing an immune response” refers to causing or improving a cellular and a humoral immune response against HBV infection.

As used herein, the term “protective immunity” or “protective immune response” means that the vaccinated subject is able to control an infection with the pathogenic agent against which the vaccination was done. Usually, the subject having
20 developed a “protective immune response” develops only mild to moderate clinical symptoms or no symptoms at all. Usually, a subject having a “protective immune response” or “protective immunity” against a certain agent will not die as a result of the infection with said agent.

Typically, the administration of compositions and therapeutic combinations of
25 the application will have a therapeutic aim to generate an immune response against HBV after HBV infection or development of symptoms characteristic of HBV infection, e.g., for therapeutic vaccination.

As used herein, “an immunogenically effective amount” or “immunologically effective amount” means an amount of a composition, polynucleotide, vector, or
30 antigen sufficient to induce a desired immune effect or immune response in a subject in need thereof. An immunogenically effective amount can be an amount sufficient to induce an immune response in a subject in need thereof. An immunogenically effective amount can be an amount sufficient to produce immunity in a subject in need thereof, e.g., provide a therapeutic effect against a disease such as HBV infection. An

immunogenically effective amount can vary depending upon a variety of factors, such as the physical condition of the subject, age, weight, health, etc.; the particular application, e.g., providing protective immunity or therapeutic immunity; and the particular disease, e.g., viral infection, for which immunity is desired. An

- 5 immunogenically effective amount can readily be determined by one of ordinary skill in the art in view of the present disclosure.

In particular embodiments of the application, an immunogenically effective amount refers to the amount of a composition or therapeutic combination which is sufficient to achieve one, two, three, four, or more of the following effects: (i) reduce or
10 ameliorate the severity of an HBV infection or a symptom associated therewith; (ii) reduce the duration of an HBV infection or symptom associated therewith; (iii) prevent the progression of an HBV infection or symptom associated therewith; (iv) cause regression of an HBV infection or symptom associated therewith; (v) prevent the development or onset of an HBV infection, or symptom associated therewith; (vi)
15 prevent the recurrence of an HBV infection or symptom associated therewith; (vii) reduce hospitalization of a subject having an HBV infection; (viii) reduce hospitalization length of a subject having an HBV infection; (ix) increase the survival of a subject with an HBV infection; (x) eliminate an HBV infection in a subject; (xi) inhibit or reduce HBV replication in a subject; and/or (xii) enhance or improve the
20 prophylactic or therapeutic effect(s) of another therapy.

An immunogenically effective amount can also be an amount sufficient to reduce HBsAg levels consistent with evolution to clinical seroconversion; achieve sustained HBsAg clearance associated with reduction of infected hepatocytes by a subject's immune system; induce HBV-antigen specific activated T-cell populations;
25 and/or achieve persistent loss of HBsAg within 12 months. Examples of a target index include lower HBsAg below a threshold of 500 copies of HBsAg international units (IU) and/or higher CD8 counts.

As general guidance, an immunogenically effective amount when used with reference to a DNA plasmid can range from about 0.1 mg/mL to 10 mg/mL of DNA
30 plasmid total, such as 0.1 mg/mL, 0.25 mg/mL, 0.5 mg/mL, 0.75 mg/mL, 1 mg/mL, 1.5 mg/mL, 2 mg/mL, 3 mg/mL, 4 mg/mL, 5 mg/mL, 6 mg/mL, 7 mg/mL, 8 mg/mL, 9 mg/mL, or 10 mg/mL. Preferably, an immunogenically effective amount of DNA plasmid is less than 8 mg/mL, more preferably less than 6 mg/mL, even more preferably 3-4 mg/mL. An immunogenically effective amount can be from one vector

or plasmid, or from multiple vectors or plasmids. As further general guidance, an immunogenically effective amount when used with reference to a peptide can range from about 10 µg to 1 mg per administration, such as 10, 20, 50, 100, 200, 300, 400, 500, 600, 700, 800, 9000, or 1000 µg per administration. An immunogenically effective amount can be administered in a single composition, or in multiple compositions, such as 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 compositions (e.g., tablets, capsules or injectables, or any composition adapted to intradermal delivery, e.g., to intradermal delivery using an intradermal delivery patch), wherein the administration of the multiple capsules or injections collectively provides a subject with an immunogenically effective amount. For example, when two DNA plasmids are used, an immunogenically effective amount can be 3-4 mg/mL, with 1.5-2 mg/mL of each plasmid. As yet further general guidance, an immunogenically effective amount when used with reference to an HPK1 inhibitor can range from about 0.005 mg/kg to 100 mg/kg. In particular, an effective therapeutic daily amount of an HPK1 inhibitor would be 25 mg/kg BID (twice a day) or 50 mg/kg BID. In particular, an effective therapeutic daily amount would be 50 mg/kg QD (once a day) or 100 mg/kg QD. It is also possible to administer an immunogenically effective amount to a subject, and subsequently administer another dose of an immunogenically effective amount to the same subject, in a so-called prime-boost regimen. This general concept of a prime-boost regimen is well known to the skilled person in the vaccine field. Further booster administrations can optionally be added to the regimen, as needed.

A therapeutic combination comprising two DNA plasmids, e.g., a first DNA plasmid encoding an HBV core antigen and second DNA plasmid encoding an HBV pol antigen, can be administered to a subject by mixing both plasmids and delivering the mixture to a single anatomic site. Alternatively, two separate immunizations each delivering a single expression plasmid can be performed. In such embodiments, whether both plasmids are administered in a single immunization as a mixture or in two separate immunizations, the first DNA plasmid and the second DNA plasmid can be administered in a ratio of 10:1 to 1:10, by weight, such as 10:1, 9:1, 8:1, 7:1, 6:1, 5:1, 4:1, 3:1, 2:1, 1:1, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, 1:9, or 1:10, by weight. Preferably, the first and second DNA plasmids are administered in a ratio of 1:1, by weight.

Preferably, a subject to be treated according to the methods of the application is an HBV-infected subject, in particular a subject having chronic HBV infection. Acute HBV infection is characterized by an efficient activation of the innate immune system

complemented with a subsequent broad adaptive response (e.g., HBV-specific T-cells, neutralizing antibodies), which usually results in successful suppression of replication or removal of infected hepatocytes. In contrast, such responses are impaired or diminished due to high viral and antigen load, e.g., HBV envelope proteins are
5 produced in abundance and can be released in sub-viral particles in 1,000-fold excess to infectious virus.

Chronic HBV infection is described in phases characterized by viral load, liver enzyme levels (necroinflammatory activity), HBeAg, or HBsAg load or presence of antibodies to these antigens. cccDNA levels stay relatively constant at approximately
10 10 to 50 copies per cell, even though viremia can vary considerably. The persistence of the cccDNA species leads to chronicity. More specifically, the phases of chronic HBV infection include: (i) the immune-tolerant phase characterized by high viral load and normal or minimally elevated liver enzymes; (ii) the immune activation HBeAg-
15 elevated liver enzymes are observed; (iii) the inactive HBsAg carrier phase, which is a low replicative state with low viral loads and normal liver enzyme levels in the serum that may follow HBeAg seroconversion; and (iv) the HBeAg-negative phase in which viral replication occurs periodically (reactivation) with concomitant fluctuations in liver enzyme levels, mutations in the pre-core and/or basal core promoter are common, such
20 that HBeAg is not produced by the infected cell.

As used herein, "chronic HBV infection" refers to a subject having the detectable presence of HBV for more than 6 months. A subject having a chronic HBV infection can be in any phase of chronic HBV infection. Chronic HBV infection is understood in accordance with its ordinary meaning in the field. Chronic HBV
25 infection can for example be characterized by the persistence of HBsAg for 6 months or more after acute HBV infection. For example, a chronic HBV infection referred to herein follows the definition published by the Centers for Disease Control and Prevention (CDC), according to which a chronic HBV infection can be characterized by laboratory criteria such as: (i) negative for IgM antibodies to hepatitis B core
30 antigen (IgM anti-HBc) and positive for hepatitis B surface antigen (HBsAg), hepatitis B e antigen (HBeAg), or nucleic acid test for hepatitis B virus DNA, or (ii) positive for HBsAg or nucleic acid test for HBV DNA, or positive for HBeAg two times at least 6 months apart.

Preferably, an immunogenically effective amount refers to the amount of a composition or therapeutic combination of the application which is sufficient to treat chronic HBV infection.

In some embodiments, a subject having chronic HBV infection is undergoing nucleoside analog (NUC) treatment, and is NUC-suppressed. As used herein, “NUC-suppressed” refers to a subject having an undetectable viral level of HBV and stable alanine aminotransferase (ALT) levels for at least six months. Examples of nucleoside/nucleotide analog treatment include HBV polymerase inhibitors, such as entacavir and tenofovir. Preferably, a subject having chronic HBV infection does not have advanced hepatic fibrosis or cirrhosis. Such subject would typically have a METAVIR score of less than 3 for fibrosis and a fibroscan result of less than 9 kPa. The METAVIR score is a scoring system that is commonly used to assess the extent of inflammation and fibrosis by histopathological evaluation in a liver biopsy of patients with hepatitis B. The scoring system assigns two standardized numbers: one reflecting the degree of inflammation and one reflecting the degree of fibrosis.

It is believed that elimination or reduction of chronic HBV may allow early disease interception of severe liver disease, including virus-induced cirrhosis and hepatocellular carcinoma. Thus, the methods of the application can also be used as therapy to treat HBV-induced diseases. Examples of HBV-induced diseases include, but are not limited to cirrhosis, cancer (e.g., hepatocellular carcinoma), and fibrosis, particularly advanced fibrosis characterized by a METAVIR score of 3 or higher for fibrosis. In such embodiments, an immunogenically effective amount is an amount sufficient to achieve persistent loss of HBsAg within 12 months and significant decrease in clinical disease (e.g., cirrhosis, hepatocellular carcinoma, etc.).

Methods according to embodiments of the application further comprises administering to the subject in need thereof another immunogenic agent (such as another HBV antigen or other antigen) or another anti-HBV agent (such as a nucleoside analog or other anti-HBV agent) in combination with a composition of the application. For example, another anti-HBV agent or immunogenic agent can be a small molecule or antibody including, but not limited to, immune checkpoint inhibitors (e.g., anti-PD1, anti-TIM-3, etc.), toll-like receptor agonists (e.g., TLR7 agonists and/or TLR8 agonists), RIG-1 agonists, IL-15 superagonists (Altor Bioscience), mutant IRF3 and IRF7 genetic adjuvants, STING agonists (Aduro), FLT3L genetic adjuvant, IL12 genetic adjuvant, IL-7-hyFc; CAR-T which bind HBV env (S-CAR cells); capsid

assembly modulators; cccDNA inhibitors, HBV polymerase inhibitors (e.g., entecavir and tenofovir). The one or other anti-HBV active agents can be, for example, a small molecule, an antibody or antigen binding fragment thereof, a polypeptide, protein, or nucleic acid. The one or other anti-HBV agents can e.g., be chosen from among HBV

5 DNA polymerase inhibitors; Immunomodulators; Toll-like receptor 7 modulators; Toll-like receptor 8 modulators; Toll-like receptor 3 modulators; Interferon alpha receptor ligands; Hyaluronidase inhibitors; Modulators of IL-10; HBsAg inhibitors; Toll like receptor 9 modulators; Cyclophilin inhibitors; HBV Prophylactic vaccines; HBV

10 Therapeutic vaccines; HBV viral entry inhibitors; Antisense oligonucleotides targeting viral mRNA, more particularly anti-HBV antisense oligonucleotides; short interfering RNAs (siRNA), more particularly anti-HBV siRNA; Endonuclease modulators; Inhibitors of ribonucleotide reductase; Hepatitis B virus E antigen inhibitors; HBV antibodies targeting the surface antigens of the hepatitis B virus; HBV antibodies; CCR2 chemokine antagonists; Thymosin agonists; Cytokines, such as IL12; Capsid

15 Assembly Modulators, Nucleoprotein inhibitors (HBV core or capsid protein inhibitors); Nucleic Acid Polymers (NAPs); Stimulators of retinoic acid-inducible gene 1; Stimulators of NOD2; Recombinant thymosin alpha-1; Hepatitis B virus replication inhibitors; PI3K inhibitors; cccDNA inhibitors; immune checkpoint inhibitors, such as PD-L1 inhibitors, PD-1 inhibitors, TIM-3 inhibitors, TIGIT inhibitors, Lag3 inhibitors,

20 and CTLA-4 inhibitors; Agonists of co-stimulatory receptors that are expressed on immune cells (more particularly T cells), such as CD27, CD28; BTK inhibitors; Other drugs for treating HBV; IDO inhibitors; Arginase inhibitors; and KDM5 inhibitors.

In certain embodiments, a method described herein further comprises administering to the subject in need thereof an immune modulatory agent, such as an

25 inhibitor of the PD-1/PD-L1 immune checkpoint axis, for example antibodies (or peptides) that bind to and/or inhibit the activity of PD-1 or the activity of PD-L1.

Methods of Delivery

Compositions and therapeutic combinations of the application can be administered to a subject by any method known in the art in view of the present

30 disclosure, including, but not limited to, parenteral administration (e.g., intramuscular, subcutaneous, intravenous, or intradermal injection), oral administration, transdermal administration, and nasal administration. Preferably, compositions and therapeutic combinations are administered parenterally (e.g., by intramuscular injection or intradermal injection) or transdermally.

In some embodiments of the application in which a composition or therapeutic combination comprises one or more DNA plasmids, administration can be by injection through the skin, e.g., intramuscular or intradermal injection, preferably intramuscular injection. Intramuscular injection can be combined with electroporation, i.e.,
5 application of an electric field to facilitate delivery of the DNA plasmids to cells. As used herein, the term “electroporation” refers to the use of a transmembrane electric field pulse to induce microscopic pathways (pores) in a bio-membrane. During in vivo electroporation, electrical fields of appropriate magnitude and duration are applied to cells, inducing a transient state of enhanced cell membrane permeability,
10 thus enabling the cellular uptake of molecules unable to cross cell membranes on their own. Creation of such pores by electroporation facilitates passage of biomolecules, such as plasmids, oligonucleotides, siRNAs, drugs, etc., from one side of a cellular membrane to the other. In vivo electroporation for the delivery of DNA vaccines has been shown to significantly increase plasmid uptake by host cells, while also leading
15 to mild-to-moderate inflammation at the injection site. As a result, transfection efficiency and immune response are significantly improved (e.g., up to 1,000 fold and 100 fold respectively) with intradermal or intramuscular electroporation, in comparison to conventional injection.

In a typical embodiment, electroporation is combined with intramuscular
20 injection. However, it is also possible to combine electroporation with other forms of parenteral administration, e.g., intradermal injection, subcutaneous injection, etc.

Administration of a composition, therapeutic combination or vaccine of the application via electroporation can be accomplished using electroporation devices that can be configured to deliver to a desired tissue of a mammal a pulse of energy
25 effective to cause reversible pores to form in cell membranes. The electroporation device can include an electroporation component and an electrode assembly or handle assembly. The electroporation component can include one or more of the following components of electroporation devices: controller, current waveform generator, impedance tester, waveform logger, input element, status reporting element,
30 communication port, memory component, power source, and power switch.

Electroporation can be accomplished using an in vivo electroporation device. Examples of electroporation devices and electroporation methods that can facilitate delivery of compositions and therapeutic combinations of the application, particularly those comprising DNA plasmids, include CELLECTRA® (Inovio Pharmaceuticals,

Blue Bell, PA), Elgen electroporator (Inovio Pharmaceuticals, Inc.) Tri-Grid™ delivery system (Ichor Medical Systems, Inc., San Diego, CA 92121) and those described in U.S. Patent No. 7,664,545, U.S. Patent No. 8,209,006, U.S. Patent No. 9,452,285, U.S. Patent No. 5,273,525, U.S. Patent No. 6,110,161, U.S. Patent No. 6,261,281, U.S. Patent No. 6,958,060, and U.S. Patent No. 6,939,862, U.S. Patent No. 7,328,064, U.S. Patent No. 6,041,252, U.S. Patent No. 5,873,849, U.S. Patent No. 6,278,895, U.S. Patent No. 6,319,901, U.S. Patent No. 6,912,417, U.S. Patent No. 8,187,249, U.S. Patent No. 9,364,664, U.S. Patent No. 9,802,035, U.S. Patent No. 6,117,660, and International Patent Application Publication WO2017172838, all of which are herein incorporated by reference in their entireties. Other examples of in vivo electroporation devices are described in International Patent Application entitled “Method and Apparatus for the Delivery of Hepatitis B Virus (HBV) Vaccines,” filed on the same day as this application with the Attorney Docket Number 688097-405WO, the contents of which are hereby incorporated by reference in their entireties. Also contemplated by the application for delivery of the compositions and therapeutic combinations of the application are use of a pulsed electric field, for instance as described in, e.g., U.S. Patent No. 6,697,669, which is herein incorporated by reference in its entirety.

In other embodiments of the application in which a composition or therapeutic combination comprises one or more DNA plasmids, the method of administration is transdermal. Transdermal administration can be combined with epidermal skin abrasion to facilitate delivery of the DNA plasmids to cells. For example, a dermatological patch can be used for epidermal skin abrasion. Upon removal of the dermatological patch, the composition or therapeutic combination can be deposited on the abraded skin.

Methods of delivery are not limited to the above described embodiments, and any means for intracellular delivery can be used. Other methods of intracellular delivery contemplated by the methods of the application include, but are not limited to, liposome encapsulation, lipid nanoparticles (LNPs), etc. Additionally, HPK1 inhibitors and compositions thereof as described herein can be administered systemically or topically, and are preferably administered via oral administration.

Adjuvants

In some embodiments of the application, a method of inducing an immune response against HBV further comprises administering an adjuvant. The terms

“adjuvant” and “immune stimulant” are used interchangeably herein, and are defined as one or more substances that cause stimulation of the immune system. In this context, an adjuvant is used to enhance an immune response to HBV antigens and antigenic HBV polypeptides of the application.

5 According to embodiments of the application, an adjuvant can be present in a therapeutic combination or composition of the application, or administered in a separate composition. An adjuvant can be, e.g., a small molecule or an antibody. Examples of adjuvants suitable for use in the application include, but are not limited to, immune checkpoint inhibitors (e.g., anti-PD1, anti-TIM-3, etc.), toll-like receptor
10 agonists (e.g., TLR7 and/or TLR8 agonists), RIG-1 agonists, IL-15 superagonists (Altor Bioscience), mutant IRF3 and IRF7 genetic adjuvants, STING agonists (Aduro), FLT3L genetic adjuvant, IL12 genetic adjuvant, and IL-7-hyFc. Examples of adjuvants can e.g., be chosen from among the following anti-HBV agents: HBV DNA polymerase inhibitors; Immunomodulators; Toll-like receptor 7 modulators; Toll-like
15 receptor 8 modulators; Toll-like receptor 3 modulators; Interferon alpha receptor ligands; Hyaluronidase inhibitors; Modulators of IL-10; HBsAg inhibitors; Toll like receptor 9 modulators; Cyclophilin inhibitors; HBV Prophylactic vaccines; HBV Therapeutic vaccines; HBV viral entry inhibitors; Antisense oligonucleotides targeting viral mRNA, more particularly anti-HBV antisense oligonucleotides; short interfering
20 RNAs (siRNA), more particularly anti-HBV siRNA; Endonuclease modulators; Inhibitors of ribonucleotide reductase; Hepatitis B virus E antigen inhibitors; HBV antibodies targeting the surface antigens of the hepatitis B virus; HBV antibodies; CCR2 chemokine antagonists; Thymosin agonists; Cytokines, such as IL12; Capsid Assembly Modulators, Nucleoprotein inhibitors (HBV core or capsid protein
25 inhibitors); Nucleic Acid Polymers (NAPs); Stimulators of retinoic acid-inducible gene 1; Stimulators of NOD2; Recombinant thymosin alpha-1; Hepatitis B virus replication inhibitors; PI3K inhibitors; cccDNA inhibitors; immune checkpoint inhibitors, such as PD-L1 inhibitors, PD-1 inhibitors, TIM-3 inhibitors, TIGIT inhibitors, Lag3 inhibitors, and CTLA-4 inhibitors; Agonists of co-stimulatory
30 receptors that are expressed on immune cells (more particularly T cells), such as CD27, CD28; BTK inhibitors; Other drugs for treating HBV; IDO inhibitors; Arginase inhibitors; and KDM5 inhibitors.

Compositions and therapeutic combinations of the application can also be administered in combination with at least one other anti-HBV agent. Examples of

anti-HBV agents suitable for use with the application include, but are not limited to small molecules, antibodies, and/or CAR-T therapies which bind HBV env (S-CAR cells), capsid assembly modulators, TLR agonists (e.g., TLR7 and/or TLR8 agonists), cccDNA inhibitors, HBV polymerase inhibitors (e.g., entecavir and tenofovir), and/or
 5 immune checkpoint inhibitors, etc.

The at least one anti-HBV agent can e.g., be chosen from among HBV DNA polymerase inhibitors; Immunomodulators; Toll-like receptor 7 modulators; Toll-like receptor 8 modulators; Toll-like receptor 3 modulators; Interferon alpha receptor ligands; Hyaluronidase inhibitors; Modulators of IL-10; HBsAg inhibitors; Toll like
 10 receptor 9 modulators; Cyclophilin inhibitors; HBV Prophylactic vaccines; HBV Therapeutic vaccines; HBV viral entry inhibitors; Antisense oligonucleotides targeting viral mRNA, more particularly anti-HBV antisense oligonucleotides; short interfering RNAs (siRNA), more particularly anti-HBV siRNA; Endonuclease modulators; Inhibitors of ribonucleotide reductase; Hepatitis B virus E antigen inhibitors; HBV
 15 antibodies targeting the surface antigens of the hepatitis B virus; HBV antibodies; CCR2 chemokine antagonists; Thymosin agonists; Cytokines, such as IL12; Capsid Assembly Modulators, Nucleoprotein inhibitors (HBV core or capsid protein inhibitors); Nucleic Acid Polymers (NAPs); Stimulators of retinoic acid-inducible gene 1; Stimulators of NOD2; Recombinant thymosin alpha-1; Hepatitis B virus
 20 replication inhibitors; PI3K inhibitors; cccDNA inhibitors; immune checkpoint inhibitors, such as PD-L1 inhibitors, PD-1 inhibitors, TIM-3 inhibitors, TIGIT inhibitors, Lag3 inhibitors, and CTLA-4 inhibitors; Agonists of co-stimulatory receptors that are expressed on immune cells (more particularly T cells), such as CD27, CD28; BTK inhibitors; Other drugs for treating HBV; IDO inhibitors; Arginase
 25 inhibitors; and KDM5 inhibitors. Such anti-HBV agents can be administered with the compositions and therapeutic combinations of the application simultaneously or sequentially.

Methods of Prime/Boost Immunization

Embodiments of the application also contemplate administering an
 30 immunogenically effective amount of a composition or therapeutic combination to a subject, and subsequently administering another dose of an immunogenically effective amount of a composition or therapeutic combination to the same subject, in a so-called prime-boost regimen. Thus, in an embodiment, a composition or therapeutic combination of the application is a primer vaccine used for priming an immune

response. In another embodiment, a composition or therapeutic combination of the application is a booster vaccine used for boosting an immune response. The priming and boosting vaccines of the application can be used in the methods of the application described herein. This general concept of a prime-boost regimen is well known to the skilled person in the vaccine field. Any of the compositions and therapeutic combinations of the application described herein can be used as priming and/or boosting vaccines for priming and/or boosting an immune response against HBV.

In some embodiments of the application, a composition or therapeutic combination of the application can be administered for priming immunization. The composition or therapeutic combination can be re-administered for boosting immunization. Further booster administrations of the composition or vaccine combination can optionally be added to the regimen, as needed. An adjuvant can be present in a composition of the application used for boosting immunization, present in a separate composition to be administered together with the composition or therapeutic combination of the application for the boosting immunization, or administered on its own as the boosting immunization. In those embodiments in which an adjuvant is included in the regimen, the adjuvant is preferably used for boosting immunization.

An illustrative and non-limiting example of a prime-boost regimen includes administering a single dose of an immunogenically effective amount of a composition or therapeutic combination of the application to a subject to prime the immune response; and subsequently administering another dose of an immunogenically effective amount of a composition or therapeutic combination of the application to boost the immune response, wherein the boosting immunization is first administered about two to six weeks, preferably four weeks after the priming immunization is initially administered. Optionally, about 10 to 14 weeks, preferably 12 weeks, after the priming immunization is initially administered, a further boosting immunization of the composition or therapeutic combination, or other adjuvant, is administered.

Kits

Also provided herein is a kit comprising a therapeutic combination of the application. A kit can comprise the first polynucleotide, the second polynucleotide, and the at least one HPK1 inhibitor in one or more separate compositions, or a kit can comprise the first polynucleotide, the second polynucleotide, and the HPK1 inhibitor in a single composition. A kit can further comprise one or more adjuvants or immune stimulants, and/or other anti-HBV agents.

The ability to induce or stimulate an anti-HBV immune response upon administration in an animal or human organism can be evaluated either in vitro or in vivo using a variety of assays which are standard in the art. For a general description of techniques available to evaluate the onset and activation of an immune response, see for example Coligan et al. (1992 and 1994, Current Protocols in Immunology; ed. J Wiley & Sons Inc, National Institute of Health). Measurement of cellular immunity can be performed by measurement of cytokine profiles secreted by activated effector cells including those derived from CD4+ and CD8+ T-cells (e.g. quantification of IL-10 or IFN gamma-producing cells by ELISPOT), by determination of the activation status of immune effector cells (e.g. T cell proliferation assays by a classical [3H] thymidine uptake or flow cytometry-based assays), by assaying for antigen-specific T lymphocytes in a sensitized subject (e.g. peptide-specific lysis in a cytotoxicity assay, etc.).

The ability to stimulate a cellular and/or a humoral response can be determined by antibody binding and/or competition in binding (see for example Harlow, 1989, Antibodies, Cold Spring Harbor Press). For example, titers of antibodies produced in response to administration of a composition providing an immunogen can be measured by enzyme-linked immunosorbent assay (ELISA). The immune responses can also be measured by neutralizing antibody assay, where a neutralization of a virus is defined as the loss of infectivity through reaction/inhibition/neutralization of the virus with specific antibody. The immune response can further be measured by Antibody-Dependent Cellular Phagocytosis (ADCP) Assay.

EMBODIMENTS

The invention provides also the following non-limiting embodiments.

Embodiment 1 is a therapeutic combination for use in treating a hepatitis B virus (HBV) infection in a subject in need thereof, comprising:

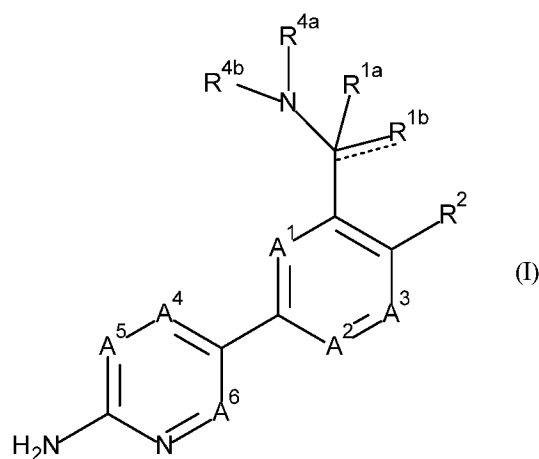
i) at least one of:

- a) a truncated HBV core antigen consisting of an amino acid sequence that is at least 95%, such as at least 95%, 96%, 97%, 98%, 99% or 100%, identical to SEQ ID NO: 2,
- b) a first non-naturally occurring nucleic acid molecule comprising a first polynucleotide sequence encoding the truncated HBV core antigen

c) an HBV polymerase antigen having an amino acid sequence that is at least 90%, such as at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%, identical to SEQ ID NO: 7, wherein the HBV polymerase antigen does not have reverse transcriptase activity and RNase H activity, and

d) a second non-naturally occurring nucleic acid molecule comprising a second polynucleotide sequence encoding the HBV polymerase antigen; and

ii) a compound of formula (I):



or a tautomer or a stereoisomeric form thereof, wherein:

the dotted bond towards R^{1b} is an optional bond that is optionally present when R^{1b} and R^{4b} are taken together to form a monocyclic or bicyclic aromatic heterocycle;

A¹ represents CH or N; A² represents CH; A³ represents CH or N;

provided that only one of A¹ and A³ represents N;

A⁴ represents CH or N; A⁵ represents CR^{3a}; A⁶ represents CH;

R^{1a} represents hydrogen;

R^{1b} represents hydrogen or CH₃;

R^{4a} represents hydrogen, C₁₋₄alkyl, or C₃₋₆cycloalkyl;

R^{4b} represents hydrogen, C₁₋₄alkyl, C₃₋₆cycloalkyl, or

a carbon linked monocyclic 4-, 5-, 6- or 7-membered non-aromatic heterocycle containing 1, 2 or 3 heteroatoms each independently selected from the group consisting of N, O, and S; wherein said S-atom is optionally substituted to form S(=O), S(=O)₂, or S(=O)(=NR^x);

or

- R^{1b} and R^{4b} are taken together to form together with the atoms to which they are attached a monocyclic 5-membered aromatic heterocyclyl or a monocyclic 4-, 5-, 6- or 7-membered fully saturated heterocyclyl, each containing 1 N-atom and optionally 1 or 2 additional heteroatoms each independently selected from the group consisting of N, O, and S; wherein said optional S-atom is optionally substituted to form S(=O), S(=O)₂, or S(=O)(=NR^x); or
- a bicyclic 6- to 12- membered aromatic or fully saturated heterocyclyl containing 1 N-atom and optionally 1 or 2 additional heteroatoms each independently selected from the group consisting of N, O, and S; wherein said optional S-atom is optionally substituted to form S(=O), S(=O)₂, or S(=O)(=NR^x);
- wherein said monocyclic or bicyclic, aromatic or fully saturated heterocyclyl is optionally substituted on one or more of the carbon atoms with in total 1, 2 or 3 substituents each independently selected from the group consisting of -OH, CN, halo, R^7 , -O- R^7 , -S(=O)₂- R^7 , -C(=O)- R^7 , -NR^{6c} R^{6d} , -C(=O)-NR^{6a} R^{6b} , and Het^c;
- wherein said monocyclic or bicyclic, aromatic or fully saturated heterocyclyl is optionally substituted on the optional additional nitrogen atoms with in total 1 or 2 substituents each independently selected from the group consisting of R^7 , -S(=O)₂- R^7 , -C(=O)- R^7 , -C(=O)-NR^{6a} R^{6b} , and Het^d;
- provided that in case R^{1b} and R^{4b} are taken together, R^{4a} represents hydrogen; and R^{1a} represents hydrogen or R^{1a} is absent when the dotted bond towards R^{1b} is a bond;

or

- R^{4a} and R^{4b} are taken together to form together with the N-atom to which they are attached a monocyclic 5-membered aromatic heterocyclyl or a monocyclic 4-, 5-, 6- or 7-membered fully saturated heterocyclyl, each containing 1 N-atom and optionally 1 or 2 additional heteroatoms each independently selected from the group consisting of N, O, and S; wherein said optional S-atom is optionally substituted to form S(=O), S(=O)₂, or S(=O)(=NR^x); or
- a bicyclic 6- to 12- membered aromatic or fully saturated heterocyclyl containing 1 N-atom and optionally 1 or 2 additional heteroatoms each independently selected from

the group consisting of N, O, and S; wherein said optional S-atom is optionally substituted to form S(=O), S(=O)₂, or S(=O)(=NR^x);

wherein said monocyclic or bicyclic, aromatic or fully saturated heterocyclyl is optionally substituted on one or more of the carbon atoms with in total 1, 2 or 3

5 substituents each independently selected from the group consisting of -OH, CN, halo, R⁷, -O-R⁷,

-S(=O)₂-R⁷, -C(=O)-R⁷, -NR^{6c}R^{6d}, -C(=O)-NR^{6a}R^{6b}, and Het^c;

wherein said monocyclic or bicyclic, aromatic or fully saturated heterocyclyl is optionally substituted on the nitrogen atoms with in total 1 or 2 substituents each

10 independently selected from the group consisting of R⁷, -S(=O)₂-R⁷, -C(=O)-R⁷, -C(=O)-NR^{6a}R^{6b}, and Het^d;

in case R^{4a} and R^{4b} are taken together, R^{1a} represents hydrogen, and R^{1b} represents hydrogen;

R² is selected from the group consisting of cyano; halo; -C(=O)-NR^{8a}R^{8b};

15 -CH₂-NR^{8c}R^{8d}; Het^b; -P(=O)-(C₁₋₄alkyl)₂; -S(=O)₂-C₁₋₄alkyl; -S(=O)(=NR^x)-C₁₋₄alkyl;

C₁₋₆alkyl optionally substituted with 1 or 2 substituents each independently selected from the group consisting of halo, -OH, cyano, and -O-C₁₋₄alkyl;

C₃₋₆cycloalkyl optionally substituted with 1 or 2 substituents each independently selected from the group consisting of halo, -OH, cyano and -O-C₁₋₄alkyl; and

20 C₃₋₆cycloalkenyl optionally substituted with 1 or 2 substituents each independently selected from the group consisting of halo, -OH, cyano and -O-C₁₋₄alkyl;

R^{3a} represents hydrogen, halo, R⁷, -O-R⁷, cyano, -C(=O)-NR^{6e}R^{6f}, Het^a, or phenyl optionally substituted with 1, 2 or 3 substituents each independently selected from the group consisting of halo, cyano, C₁₋₄alkyl, -O-C₁₋₄alkyl, C₁₋₄alkyl substituted with one

25 cyano, and C₁₋₄alkyl substituted with 1, 2 or 3 halo atoms;

Het^a represents a carbon linked monocyclic 5-, 6- or 7-membered aromatic heterocyclyl or carbon linked monocyclic 4-, 5-, 6- or 7-membered non-aromatic heterocyclyl, each containing 1, 2 or 3 heteroatoms each independently selected from the group consisting of N, O or S; wherein said S-atom might be substituted to form

30 S(=O), S(=O)₂, or S(=O)(=NR^x); or

a carbon linked bicyclic 6- to 12-membered aromatic or non-aromatic heterocyclyl containing 1, 2 or 3 heteroatoms each independently selected from the group consisting of N, O and S; wherein said S-atom is optionally substituted to form S(=O),

$S(=O)_2$, or $S(=O)(=NR^x)$;

wherein said monocyclic or bicyclic, aromatic or non-aromatic heterocyclyl is optionally substituted on one or more of the carbon atoms with in total 1, 2 or 3 substituents each independently selected from the group consisting of -OH, CN, halo,

- 5 R^7 , -O- R^7 ,
- $S(=O)_2-R^7$, -C(=O)- R^7 , -NR^{6c}R^{6d}, -C(=O)-NR^{6a}R^{6b}, and Het^c;

wherein said monocyclic or bicyclic, aromatic or non-aromatic heterocyclyl is optionally substituted on the nitrogen atoms with in total 1 or 2 substituents each independently selected from the group consisting of R^7 , - $S(=O)_2-R^7$, -C(=O)- R^7 , -

- 10 C(=O)-NR^{6a}R^{6b} and Het^d;

R^{6a} , R^{6b} , R^{6c} , R^{6d} , R^{6e} , and R^{6f} are each independently selected from the group consisting of hydrogen; C₃₋₆cycloalkyl optionally substituted with one -OR⁵; and C₁₋₄alkyl optionally substituted with one -OR⁵, wherein two hydrogen atoms on the same carbon atom of said C₁₋₄alkyl are optionally taken together to form C₃₋

- 15 ₆cycloalkyl;

R^5 represents hydrogen or C₁₋₄alkyl;

R^{8a} , R^{8c} , and R^{8d} are each independently selected from the group consisting of hydrogen;

C₁₋₄alkyl optionally substituted with one -OH or -O-C₁₋₄alkyl; and C₃₋₆cycloalkyl

- 20 optionally substituted with one -OH or -O-C₁₋₄alkyl;

R^{8b} is selected from the group consisting C₁₋₄alkyl optionally substituted with one -OH or

-O-C₁₋₄alkyl; and C₃₋₆cycloalkyl optionally substituted with one -OH or -O-C₁₋₄alkyl; or

- 25 R^{8a} and R^{8b} , or R^{8c} and R^{8d} are taken together to form together with the N-atom to which they are attached a monocyclic fully saturated heterocyclyl containing 1 N-atom and optionally 1 or 2 additional heteroatoms each independently selected from the group consisting of N, O, S; wherein said optional S-atom is optionally substituted to form S(=O), S(=O)₂, or S(=O)(=NR^x); wherein said monocyclic 4-, 5-, 6- or 7-

- 30 membered fully saturated heterocyclyl is optionally substituted on one or more of the carbon atoms with in total 1, 2 or 3 substituents each independently selected from the group consisting of -OH, CN, halo, R^7 , -O- R^7 , - $S(=O)_2-R^7$, -C(=O)- R^7 , -NR^{6c}R^{6d}, and -C(=O)-NR^{6a}R^{6b};

- wherein said monocyclic fully saturated heterocyclyl is optionally substituted on the nitrogen atoms with in total 1 or 2 substituents each independently selected from the group consisting of R^7 , $-S(=O)_2-R^7$, $-C(=O)-R^7$, and $-C(=O)-NR^{6a}R^{6b}$;
- each Het^c independently represents a monocyclic 4-, 5-, 6- or 7-membered fully saturated heterocyclyl containing 1, 2 or 3 heteroatoms each independently selected from the group consisting of N, O and S; wherein said S-atom is optionally substituted to form $S(=O)$, $S(=O)_2$, or $S(=O)(=NR^x)$;
- each Het^d independently represents a carbon linked monocyclic 4-, 5-, 6- or 7-membered fully saturated heterocyclyl containing 1, 2 or 3 heteroatoms each independently selected from the group consisting of N, O and S; wherein said S-atom is optionally substituted to form $S(=O)$, $S(=O)_2$, or $S(=O)(=NR^x)$;
- Het^b represents a monocyclic 4-, 5-, 6- or 7-membered non-aromatic heterocyclyl containing 1, 2 or 3 heteroatoms each independently selected from the group consisting of N, O, and S; wherein said S-atom might be substituted to form $S(=O)$, $S(=O)_2$, or $S(=O)(=NR^x)$; or
- a bicyclic 6- to 12- membered non-aromatic heterocyclyl containing 1, 2 or 3 heteroatoms each independently selected from the group consisting of N, O, and S; wherein said S-atom might be substituted to form $S(=O)$, $S(=O)_2$, or $S(=O)(=NR^x)$; wherein said monocyclic or bicyclic non-aromatic heterocyclyl might be substituted on one or more of the carbon atoms with in total 1, 2 or 3 substituents each independently selected from the group consisting of $-OH$, CN , halo, R^7 , $-O-R^7$, $-S(=O)_2-R^7$, $-C(=O)-R^7$, $-NR^{6c}R^{6d}$, and $-C(=O)-NR^{6a}R^{6b}$;
- wherein said monocyclic or bicyclic non-aromatic heterocyclyl might be substituted on the nitrogen atoms with in total 1 or 2 substituents each independently selected from the group consisting of R^7 , $-S(=O)_2-R^7$, $-C(=O)-R^7$, and $-C(=O)-NR^{6a}R^{6b}$;
- each R^7 independently represents C_{3-6} cycloalkyl optionally substituted with 1, 2 or 3 substituents each independently selected from the group consisting of halo, $-OH$, $-O-C_{1-4}$ alkyl and cyano; or C_{1-4} alkyl optionally substituted with 1, 2 or 3 substituents each independently selected from the group consisting of halo, $-OH$, $-O-C_{1-4}$ alkyl and cyano; and
- each R^x independently represents hydrogen or C_{1-4} alkyl;
- or a pharmaceutically acceptable addition salt, an N-oxide, or a solvate thereof.

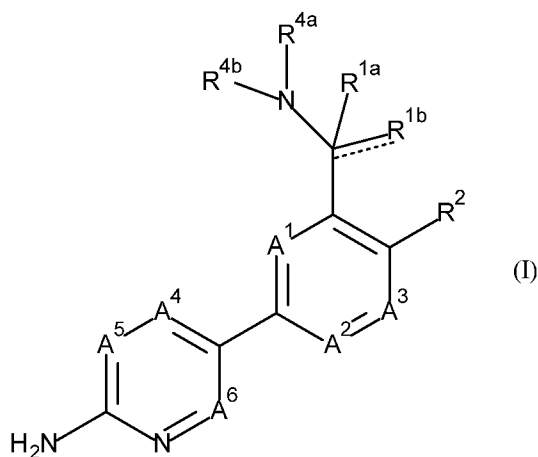
Embodiment 2 is the therapeutic combination of embodiment 1, comprising at least one of the HBV polymerase antigen and the truncated HBV core antigen.

Embodiment 3 is the therapeutic combination of embodiment 2, comprising the HBV polymerase antigen and the truncated HBV core antigen.

5 Embodiment 4 is the therapeutic combination of embodiment 1, comprising at least one of the first non-naturally occurring nucleic acid molecule comprising the first polynucleotide sequence encoding the truncated HBV core antigen, and the second non-naturally occurring nucleic acid molecule comprising the second polynucleotide sequence encoding the HBV polymerase antigen.

10 Embodiment 5 is a therapeutic combination for use in treating a hepatitis B virus (HBV) infection in a subject in need thereof, comprising

- i) a first non-naturally occurring nucleic acid molecule comprising a first polynucleotide sequence encoding a truncated HBV core antigen consisting of an amino acid sequence that is at least 95% identical to
15 SEQ ID NO: 2; and
- ii) a second non-naturally occurring nucleic acid molecule comprising a second polynucleotide sequence encoding an HBV polymerase antigen having an amino acid sequence that is at least 90% identical to SEQ ID NO: 7, wherein the HBV polymerase antigen does not have reverse
20 transcriptase activity and RNase H activity; and
- iii) a compound of formula (I):



or a tautomer or a stereoisomeric form thereof, wherein:

the dotted bond towards R^{1b} is absent;

25 A^1 represents CH or N; A^2 represents CH; A^3 represents CH;

A⁴ represents CH; A⁵ represents CR^{3a}; A⁶ represents CH;

R^{1b} and R^{4b} are taken together to form together with the atoms to which they are attached a monocyclic 4-, 5-, 6- or 7-membered fully saturated heterocyclyl, each containing 1 N-atom and optionally 1 or 2 additional heteroatoms each independently
5 selected from the group consisting of N, O, and S; wherein said optional S-atom is optionally substituted to form S(=O);

wherein said monocyclic fully saturated heterocyclyl is optionally substituted on one of the carbon atoms with 1 substituent selected from the group consisting of halo and R⁷;

10 provided that R^{4a} represents hydrogen; and R^{1a} represents hydrogen;

R² represents Het^b;

R^{3a} represents halo, cyano, or Het^a;

Het^a represents a carbon linked monocyclic 5-, 6- or 7-membered aromatic heterocyclyl containing 1, 2 or 3 heteroatoms each independently selected from the
15 group consisting of N, O and S; wherein said S-atom is optionally substituted to form S(=O) or S(=O)₂;

wherein said monocyclic aromatic heterocyclyl is optionally substituted on one of the carbon atoms with a halo substituent;

wherein said monocyclic aromatic heterocyclyl is optionally substituted on one

20 nitrogen atom with R⁷;

Het^b represents a monocyclic 4-, 5-, 6- or 7-membered non-aromatic heterocyclyl containing 1 oxygen atom; and

each R⁷ represents C₁₋₄alkyl,

or a pharmaceutically acceptable addition salt, an N-oxide, or a solvate thereof.

25 Embodiment 6 is the therapeutic combination of embodiment 4 or 5, wherein the first non-naturally occurring nucleic acid molecule further comprises a polynucleotide sequence encoding a signal sequence operably linked to the N-terminus of the truncated HBV core antigen.

Embodiment 6a is the therapeutic combination of any one of embodiments 4 to
30 6, wherein the second non-naturally occurring nucleic acid molecule further comprises a polynucleotide sequence encoding a signal sequence operably linked to the N-terminus of the HBV polymerase antigen.

Embodiment 6b is the therapeutic combination of embodiment 6 or 6a, wherein the signal sequence independently comprises the amino acid sequence of SEQ ID NO: 9 or SEQ ID NO: 15.

Embodiment 6c is the therapeutic combination of embodiment 6 or 6a, wherein
5 the signal sequence is independently encoded by the polynucleotide sequence of SEQ ID NO: 8 or SEQ ID NO: 14.

Embodiment 7 is the therapeutic combination of any one of embodiments 1-6c, wherein the HBV polymerase antigen comprises an amino acid sequence that is at least 98%, such as at least 98%, 98.5%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%,
10 99.6%, 99.7%, 99.8%, 99.9%, or 100%, identical to SEQ ID NO: 7.

Embodiment 7a is the therapeutic combination of embodiment 7, wherein the HBV polymerase antigen comprises the amino acid sequence of SEQ ID NO: 7.

Embodiment 7b is the therapeutic combination of any one of embodiments 1 to 7a, wherein the truncated HBV core antigen consists of the amino acid sequence that
15 is at least 98%, such as at least 98%, 98.5%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, 99.9%, or 100%, identical to SEQ ID NO: 2.

Embodiment 7c is the therapeutic combination of embodiment 7b, wherein the truncated HBV antigen consists of the amino acid sequence of SEQ ID NO: 2 or SEQ ID NO: 4.

20 Embodiment 8 is the therapeutic combination of any one of embodiments 1-7c, wherein each of the first and second non-naturally occurring nucleic acid molecules is a DNA molecule.

Embodiment 8a is the therapeutic combination of embodiment 8, wherein the DNA molecule is present on a DNA vector.

25 Embodiment 8b is the therapeutic combination of embodiment 8a, wherein the DNA vector is selected from the group consisting of DNA plasmids, bacterial artificial chromosomes, yeast artificial chromosomes, and closed linear deoxyribonucleic acid.

Embodiment 8c is the therapeutic combination of embodiment 8, wherein the DNA molecule is present on a viral vector.

30 Embodiment 8d is the therapeutic combination of embodiment 8c, wherein the viral vector is selected from the group consisting of bacteriophages, animal viruses, and plant viruses.

Embodiment 8e is the therapeutic combination of any one of embodiments 1-7c, wherein each of the first and second non-naturally occurring nucleic acid molecules is an RNA molecule.

Embodiment 8f is the therapeutic combination of embodiment 8e, wherein the
5 RNA molecule is an RNA replicon, preferably a self-replicating RNA replicon, an mRNA replicon, a modified mRNA replicon, or self-amplifying mRNA.

Embodiment 8g is the therapeutic combination of any one of embodiments 1 to 8f, wherein each of the first and second non-naturally occurring nucleic acid molecules is independently formulated with a lipid composition, preferably a lipid
10 nanoparticle (LNP).

Embodiment 9 is the therapeutic combination of any one of embodiments 4-8g, comprising the first non-naturally occurring nucleic acid molecule and the second non-naturally occurring nucleic acid molecule in the same non-naturally occurring nucleic acid molecule.

Embodiment 10 is the therapeutic combination of any one of embodiments 4-8g, comprising the first non-naturally occurring nucleic acid molecule and the second non-naturally occurring nucleic acid molecule in two different non-naturally occurring nucleic acid molecules.

Embodiment 11 is the therapeutic combination of any one of embodiments 4-
20 10, wherein the first polynucleotide sequence comprises a polynucleotide sequence having at least 90%, such as at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%, sequence identity to SEQ ID NO: 1 or SEQ ID NO: 3.

Embodiment 11a is the therapeutic combination of embodiment 11, wherein the first polynucleotide sequence comprises a polynucleotide sequence having at least
25 98%, such as at least 98%, 98.5%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, 99.9%, or 100%, sequence identity to SEQ ID NO: 1 or SEQ ID NO: 3.

Embodiment 12 is the therapeutic combination of embodiment 11a, wherein the first polynucleotide sequence comprises the polynucleotide sequence of SEQ ID
30 NO: 1 or SEQ ID NO: 3.

Embodiment 13 is the therapeutic combination of any one of embodiments 4 to 12, wherein the second polynucleotide sequence comprises a polynucleotide sequence having at least 90%, such as at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%, sequence identity to SEQ ID NO: 5 or SEQ ID NO: 6.

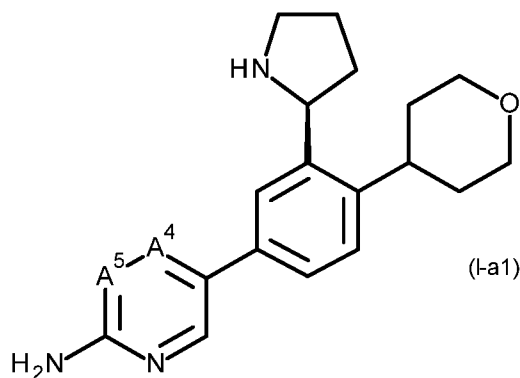
Embodiment 13a the therapeutic combination of embodiment 13, wherein the second polynucleotide sequence comprises a polynucleotide sequence having at least 98%, such as at least 98%, 98.5%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, 99.9%, or 100%, sequence identity to SEQ ID NO: 5 or SEQ ID NO:

5 6.

Embodiment 14 is the therapeutic combination of embodiment 13a, wherein the second polynucleotide sequence comprises the polynucleotide sequence of SEQ ID NO: 5 or SEQ ID NO: 6.

Embodiment 15 is the therapeutic combination of any one of embodiments 1 to 14, wherein the compound of formula (I) is selected from the group consisting of the exemplified compounds, particularly compounds 1 to 111, or a tautomer or stereisomeric form, or a pharmaceutically acceptable addition salt, an N-oxide, or solvate thereof.

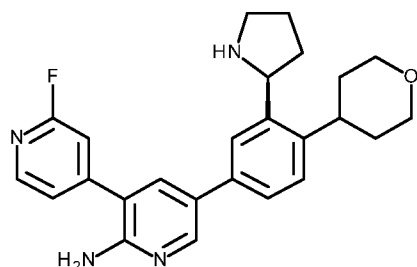
Embodiment 15a is the therapeutic combination of any one of embodiments 1 to 14, wherein the compound of formula (I) is a compound of formula (I-a1):



, or a tautomer or stereoisomeric form thereof, wherein A⁴ and A⁵ are as defined in the compounds of formula (I), or a pharmaceutically acceptable addition salt, N-oxide, or solvate thereof.

Embodiment 15b is the therapeutic combination of any one of embodiments 1 to 14, wherein the compound of formula (I) is:

20 to 14, wherein the compound of formula (I) is:



or a tautomer, stereoisomeric form,

pharmaceutically acceptable addition salt, N-oxide, or solvate thereof.

Embodiment 15c is the therapeutic combination of any one of embodiments 1 to 15b, further comprising an immune modulatory agent, preferably an inhibitor of the PD-1/PD-L1 immune checkpoint axis, more preferably an antibody or peptide that binds to and/or inhibits the activity of PD-1 or the activity of PD-L1.

Embodiment 16 is a kit comprising the therapeutic combination of any one of embodiments 1 to 15b, and instructions for using the therapeutic combination in treating a hepatitis B virus (HBV) infection in a subject in need thereof.

Embodiment 17 is a method of treating a hepatitis B virus (HBV) infection in a subject in need thereof, comprising administering to the subject the therapeutic combination of any one of embodiments 1 to 15b.

Embodiment 17a is the method of embodiment 17, wherein the treatment induces an immune response against a hepatitis B virus in a subject in need thereof, preferably the subject has chronic HBV infection.

Embodiment 17b is the method of embodiment 17 or 17a, wherein the subject has chronic HBV infection.

Embodiment 17c is the method of any one of embodiments 17 to 17b, wherein the subject is in need of a treatment of an HBV-induced disease selected from the group consisting of advanced fibrosis, cirrhosis and hepatocellular carcinoma (HCC).

Embodiment 18 is the method of any one of embodiments 17-17c, wherein the therapeutic combination is administered by injection through the skin, e.g., intramuscular or intradermal injection, preferably intramuscular injection.

Embodiment 19 is the method of embodiment 18, wherein the therapeutic combination comprises at least one of the first and second non-naturally occurring nucleic acid molecules.

Embodiment 19a is the method of embodiment 19, wherein the therapeutic combination comprises the first and second non-naturally occurring nucleic acid molecules.

Embodiment 20 is the method of embodiment 19 or 19a, wherein the non-naturally occurring nucleic acid molecules are administered to the subject by intramuscular injection in combination with electroporation.

Embodiment 21 is the method of embodiment 19 or 19a, wherein the non-naturally occurring nucleic acid molecules are administered to the subject by a lipid composition, preferably by a lipid nanoparticle.

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It will be appreciated by those skilled in the art that changes could be made to the embodiments described above without departing from the broad inventive concept thereof. It is understood, therefore, that this invention is not limited to the particular embodiments disclosed, but it is intended to cover modifications within the spirit and scope of the present invention as defined by the present description.

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EXAMPLES

Synthesis Examples

Several methods for preparing the compounds of formula (I) described herein are illustrated in the following examples. Unless otherwise noted, all starting materials were obtained from commercial suppliers and used without further purification, or alternatively can be synthesized by a skilled person by using well-known methods.

Hereinafter, "DCM" means dichloromethane; "DME" means 1,2-dimethoxyethane; "DMF-DMA" means *N,N*-dimethylformamide dimethyl acetal; "ACN" means acetonitrile; "Ac" means acetyl; "LAH" means lithium aluminium hydride; "sol." means solution; "prep." means preparative; "aq." means aqueous; "Int." Means Intermediate; "Co." means compound; "r.t." means room temperature; "r.m." means reaction mixture; "KOAc" means potassium acetate; "AcONH₄" means ammonium acetate; "BisPin" means bis(pinacolato)diboron; "DCE" means 1,2-dichloroethane; "AcOEt" or "EtOAc" means ethylacetate; "DIPE" means diisopropyl ether; "Boc" means *tert*-butoxycarbonyl; "DMA" means dimethylacetamide; "HBr" means hydrogen bromide; "MeCN" means acetonitrile; "HOBT" means 1-hydroxy-1H-benzotriazole; "TMS" means trimethylsilyl; "DIPE" means diisopropylether; "

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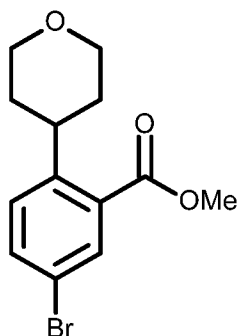
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- “DMAP” means 4-(dimethylamino)pyridine; “MeOH” means methanol; “LC” means liquid chromatography; “LCMS” means Liquid Chromatography/Mass spectrometry; “HPLC” means high-performance liquid chromatography; “NH₄Cl” means ammonium chloride; “H₂O” means water “TFA” means trifluoroacetic acid; “m.p.” means melting point; “N₂” means nitrogen; “Na₂SO₄” means sodium sulfate; “AcOH” means acetic acid; “MeOD” means deuterated methanol; “D₂” means deuterium; “RP” means reversed phase; “min” means minute(s); “EtOAc” means ethyl acetate; “Et₃N” means triethylamine; “EtOH” means ethanol; “THF” means tetrahydrofuran; “MnO₂” means manganese dioxide; “Celite®” means diatomaceous earth; “MgSO₄” means magnesium sulfate; “NH₄OH” means ammonium hydroxide; “K₂CO₃” means potassium carbonate; “DMF” means *N,N*-dimethyl formamide; “Na₂SO₃” means sodium sulfite; “NaHCO₃” means sodium bicarbonate; “Na₂CO₃” means sodium carbonate; “HCl” means hydrogen chloride “NaOH” means sodium hydroxide; “HCOOH” means formic acid; “DMSO” means dimethyl sulfoxide; “iPrOH” means 2-propanol; “iPrNH₂” means isopropylamine; “SFC” means Supercritical Fluid Chromatography; “CO₂” means carbon dioxide; “Et₃N” means triethylamine; “DIPEA” means *N,N*-diisopropylethylamine; “Pd(PPh₃)₄” means tetrakis(triphenylphosphine)palladium; “w/v” means weight/volume; “[Ir(dtbbpy)(ppy)₂]PF₆” means (4,4'-Di-*t*-butyl-2,2'-bipyridine)bis[2-(2-pyridinyl-*kN*)phenyl-*kC*]iridium(III) hexafluorophosphate; “SLAP TM” means 2-(((Trimethylsilyl)methyl)thio)ethanamine; “SLAP” means silicon amine protocol; “SLAP hydroxyridopyrazine” means (1-((trimethylsilyl)methyl)piperidin-2-yl)methanamine; “m-CPBA” means 3-chloroperbenzoic acid “PPh₃” means triphenylphosphine; “Et₂O” means diethyl ether; “Pd/C” means palladium on carbon; “Et” means ethyl; “Me” means methyl; “h” means hours; “PdCl₂(dppf)” means [1,1'-bis(diphenylphosphino-*kP*)ferrocene]dichloropalladium; and “quant.” means quantitative.

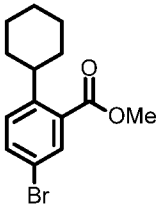
When a stereocenter is indicated with ‘RS’ this means that a racemic mixture was obtained at the indicated center, unless otherwise indicated.

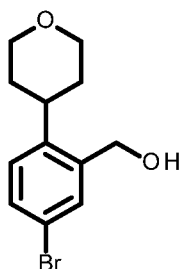
Preparation of the Intermediates and the final Compounds

**Synthesis of intermediate 1:**

To a mixture of Zn (69.9 g, 1.07 mol) in DMA (500 mL) was added ethylene dibromide (16.5 g, 88 mmol, 6.64 mL) in one portion under N₂. Then TMSCl (6.83 g, 62.9 mmol, 8.0 mL) was added slowly and the mixture was stirred for 30 min at 25 °C. A solution of 4-iodotetrahydropyran (200 g, 943 mmol) in DMA (500 mL) was added dropwise slowly (30 min) to maintain temperature below 50°C, the resulting mixture was stirred at 25 °C for 1.5 h and then added via a cannula to a solution of methyl 5-bromo-2-iodobenzoate (214.4 g, 629 mmol), Pd(dppf)Cl₂.CH₂Cl₂ (25.7 g, 31.4 mmol) and CuI (12.0 g, 62.9 mmol) in DMA (2400 mL) under N₂, the color of the mixture turned brown, then the mixture was heated and stirred at 80 °C for 14 h under N₂. Six batches were combined together. The mixture was cooled to rt and diluted with EtOAc (10 L). A saturated aqueous solution of NH₄Cl (18 L) and H₂O (10 L) were added and the mixture was stirred for 30 mins, then filtered through a pad of Celite[®]. The cake was washed with EtOAc (5 L* 2), the filtrate was extracted with EtOAc (8 L * 4), the combined organic phase was washed with brine (15 L* 2), dried with anhydrous Na₂SO₄, filtered and concentrated in vacuum to give the crude product. The crude product was purified by silica gel chromatography eluted with Petroleum ether : EtOAc =100:0-93:7 to give intermediate **1** (700 g, 61% yield) as a yellow solid.

The following intermediate was prepared via an analogous procedure :

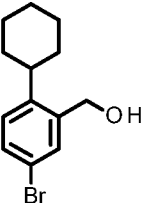
Intermediate	Structure	Quantity	Yield
Intermediate <u>2</u> Starting from iodocyclohexane		0.7 g	40%

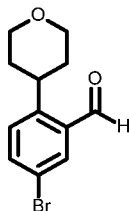


Synthesis of intermediate 3:

- To a mixture of intermediate 1 (220 g, 735 mmol) in THF (2200 mL) was
- 5 added portionwise LAH (27.9 g, 735 mmol) at 0-10 °C over 30 min. The grey suspension was stirred at 0-30 °C for 1 h. The mixture was cooled to 0 °C and H₂O (800 mL) was added dropwise and then the mixture was stirred for 30 min. The three batches were worked together. The mixture was filtered and the cake was washed with EtOAc (3 L * 3), the filtrate was extracted with EtOAc (2 L * 3), the combined
- 10 organic phase was washed with brine (2 L * 2), dried with Na₂SO₄, filtered and concentrated in vacuum. The crude product was used for next step without purification to give intermediate 3 (576 g, 87% yield) as a yellow oil.

The following intermediate was prepared via an analogous procedure :

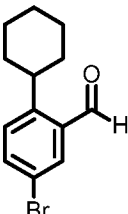
Intermediate	Structure	Quantity	Yield
Intermediate <u>4</u> Starting from intermediate 2		0.53 g	84%

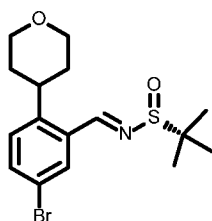


Synthesis of intermediate 5:

- To a mixture of intermediate 3 (325 g, 1.2 mol) in CH₂Cl₂ (3200 mL) was added
- 5 MnO₂ (834 g, 9.6 mol) in one portion. The black mixture was stirred at 30 °C for 34h.
- The two batches were worked together. The mixture was filtered through Celite® and the cake was washed with CH₂Cl₂ (2 L *3), the filtrate was concentrated in vacuum.
- The crude product was purified by silica gel column chromatography (Petroleum ether/Ethyl acetate=100:0-85:15) to give 560 g as a crude product which was stirred
- 10 with Petroleum ether (800 mL) at 30 °C for 4h, filtered and the cake was washed with Petroleum ether (200 mL * 2) and concentrated in high vacuum to give intermediate 5 (502 g, 1.85 mol, 77% yield) as a white solid.

The following intermediate was prepared via an analogous procedure :

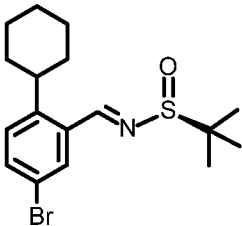
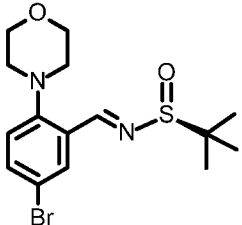
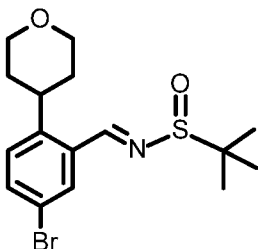
Intermediate	Structure	Quantity	Yield
Intermediate <u>6</u> Starting from intermediate 4		0.35 g	67%

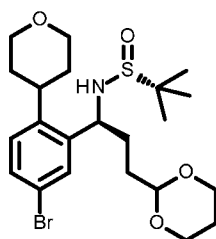


Synthesis of intermediate 7:

Titanium (IV) ethoxide (64.5 mL, 0.308 mol) was added dropwise to a solution of
 5 intermediate 5 (20.7 g, 77 mmol) and (S)-(-)-t-butylsulfinamide (18.06 g, 0.149 mol).
 The solution was stirred at rt overnight and the mixture was poured into brine and
 Ethyl acetate was added. The organic layer was separated, dried over MgSO₄, filtered
 and evaporated until dryness. The residue was purified by preparative LC (330g of
 irregular SiOH 35-40 μm GraceResolv, mobile phase: gradient from 100% DCM to
 10 DCM 95%/ MeOH 5%/NH₄OH 0.1%). The pure fractions were collected and the
 solvent evaporated until dryness to give intermediate 7 (30 g, y= quant.)

The following intermediates were prepared via an analogous procedure:

Intermediate	Structure	Quantity	Yield
Intermediate <u>8</u> Starting from intermediate 6		0.32 g	66%
Intermediate <u>9</u> Starting from 5-bromo- 2-(N-morpholino)- benzaldehyde		3.2 g	76%
Intermediate <u>10</u> Starting from intermediate 5		9.5 g	94%

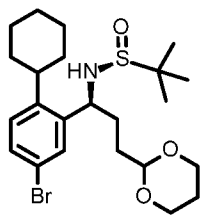
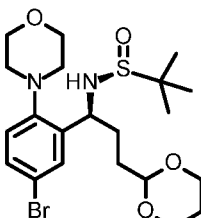


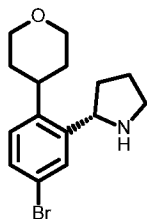
Synthesis of intermediate 11:

A solution of intermediate 7 (41 g, 0.11 mol) in THF (500 mL) was added dropwise at
5 -78°C to a solution of (1,3-dioxan-2-ylethyl)magnesium bromide (96.6 g, 0.44 mol) in
THF (550mL). The reaction mixture was stirred at -78°C for 0.5h. A saturated solution
of NH₄Cl was added and the reaction mixture was extracted with ethyl acetate, the

organic layer was separated, dried over MgSO_4 , filtered and evaporated until dryness to give intermediate **11** (60 g, y= quant.)

The following intermediates were prepared via an analogous procedure:

Intermediate	Structure	Quantity	Yield
Intermediate 12 Starting from intermediate 8		0.28 g	67%
Intermediate 13 Starting from intermediate 9		1.23 g	93%



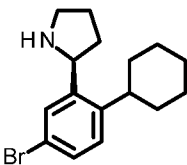
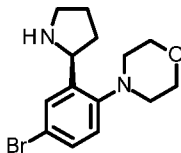
Synthesis of intermediate 14:

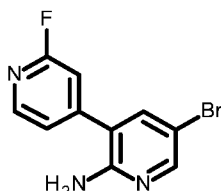
- 5 Intermediate **11** (66.4 g, 0.135 mol) was slowly added to a mixture of TFA (597 mL) and water (33 mL) to maintain temperature below 25°C. The reaction mixture was stirred at rt for 40 min. Triethylsilane (221 mL, 1.38 mol) was added and the reaction mixture vigorously stirred at rt overnight. The reaction mixture was evaporated until dryness. A purification was performed via preparative LC (stationary phase : irregular
- 10 SiOH 35-40 μM 220+330g, GraceResolv, mobile phase : from DCM 100% to DCM 80%/MeOH/20%/NH₄OH 0.1%). The pure fractions were evaporated and taken up in DCM and water, basified with K₂CO₃. The organic layer was separated, dried over

MgSO₄, filtered and evaporated to give intermediate **14** (33.2g, 79%).

[α]_D: - 47.5° (589 nm, c 0.36 w/v %, DMF, 20 °C)

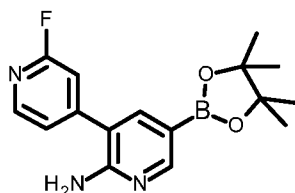
The following intermediates were prepared via an analogous procedure:

Intermediate	Structure	Quantity	Yield
Intermediate 15 Starting from intermediate 12		0.13 g	73%
Intermediate 16 Starting from intermediate 13		0.18 g	23%



5 Synthesis of intermediate 17:

A solution of 2-amino-3-iodo-5-bromopyridine (25.12 g, 84.0 mmol), 2-fluoropyridine-4-boronic acid pinacol ester (18.75 g, 84.0 mmol) and sodium carbonate (17.81 g, 168 mmol) in 1,4-dioxane (240 mL) and water (35 mL) in a sealed tube was degassed during 10 min. After addition of PdCl₂(dppf) (3.44g, 4.2 mmol), the reaction mixture was stirred at 90°C overnight. Water, potassium carbonate and ethyl acetate were added. The organic layer was separated, dried over MgSO₄, filtered and evaporated until dryness. DCM was added to the residue, the insoluble was filtered, washed with DCM and dried yielding 13.6 g (60.4%) of intermediate **17**. The filtrate was purified by preparative LC (SiOH 30μm Interchim, from 100% DCM to DCM 90%/ MeOH 10%/ NH₄OH 0.1%). The pure fractions were collected and evaporated until dryness to give intermediate **17** (6 g, 26.6%).

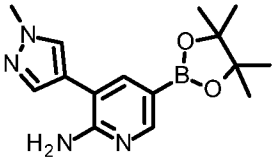
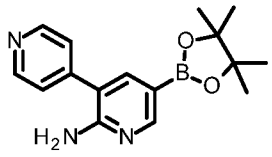


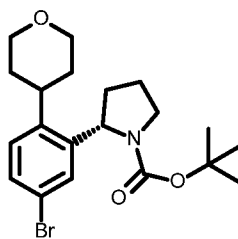
Synthesis of intermediate 18:

To a solution of intermediate **17** (13.6 g, 50.7 mmol), bis(pinacolato)diboron (14.2 g, 55.8 mmol) and potassium acetate (14.94 g, 152 mmol) in 1,4-dioxane (490 mL) was added under nitrogen atmosphere PdCl₂(dppf) (1.66 g, 2.03 mmol). The reaction mixture was heated at 80°C for 20 hours, and cooled to room temperature. Ethyl acetate was added and the mixture was filtered through a pad of celite and the filtrate was evaporated until dryness. Water and Ethyl acetate were added, the insoluble was filtered, taken up into diethylether then filtered and dried to give 9.13 g of intermediate **18** (57.1%). The organic layer was separated, dried over MgSO₄, filtered and evaporated until dryness. The residue was taken up into diethylether, stirred for 30 min then filtered and dried to give 6 g of intermediate **18** (37.7%).

The following intermediates were prepared via an analogous procedure as for intermediate 18:

Intermediate	Structure	Quantity	Yield
Intermediate 23 Starting from 5-bromo-3-(pyrimidin-5-yl)pyridine-2-amine		0.527 g	56% (80% purity)
Intermediate 24 Starting from 5-bromo-3-(pyridin-3-yl)pyridine-2-amine		0.263 g	29%

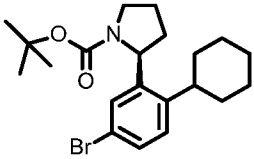
Intermediate	Structure	Quantity	Yield
Intermediate 25 Starting from 5-bromo-3-(1-methyl-1H-pyrazol-4-yl)pyridin-2-amine		0.465 g	24%
Intermediate 26 Starting from 5-bromo-[3,4'-Bipyridin]-2-amine		2.2 g	38%

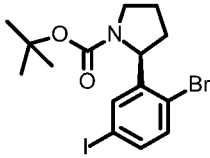


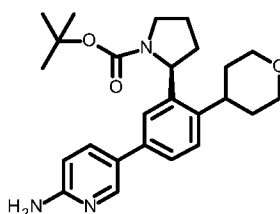
Synthesis of intermediate 19:

Di-tert-butyl dicarbonate (44.3 g, 0.203 mol) was added to a solution of intermediate **14** (21 g, 67.7 mmol) and 4-dimethylaminopyridine (1.65g 13.5 mmol) in DCM (680 mL). The reaction mixture was stirred at rt for 24h. Water and DCM were added, the organic layer was separated, dried over MgSO₄, filtered and evaporated until dryness. The residue was crystallized in diethylether, filtered and dried to give 22.2 g of intermediate **19** (80%).

The following intermediates were prepared via an analogous procedure:

Intermediate	Structure	Quantity	Yield
Intermediate 20 Starting from intermediate 15		0.2 g	Quant.

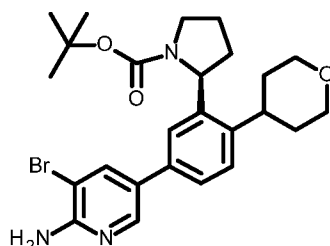
Intermediate	Structure	Quantity	Yield
Intermediate 21 Starting from Pyrrolidine,2-(2-bromo-5-iodophenyl), (2S)		27.5g	58%



Synthesis of intermediate 22:

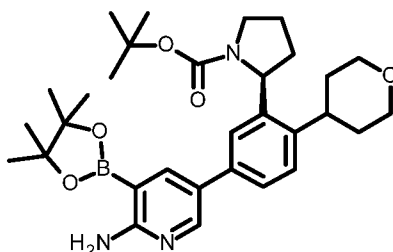
A mixture of 2-aminopyridine-5-boronic acid pinacol ester (5.92 g, 26.9 mmol), intermediate **19** (9.2g, 22.4 mmol), Pd(PPh₃)₄ (2.6 g, 2.25 mmol) and sodium carbonate (7.1 g, 67.2 mmol) in 2-methyltetrahydrofuran (250 mL) and water (25 mL) was stirred under N₂ in a sealed tube at 85°C for 15 hours. The reaction mixture was cooled down; water, potassium carbonate and ethyl acetate were added. The organic layer was separated, dried over MgSO₄, filtered and evaporated until dryness. The residue was purified by preparative LC (SiOH 30µm Interchim, from 100% DCM to DCM 90%/ MeOH 10%/ NH₄OH 0.1%). The fractions were collected and evaporated until dryness to give 11.6 g of a residue which was purified again via preparative LC (Stationary phase: irregular SiOH 40 µm 220g, Mobile phase: 60% Heptane, 5% MeOH (+5% NH₄OH), 35% AcOEt). The pure fractions were collected and evaporated until dryness to give 6 g of intermediate **22** (63%).

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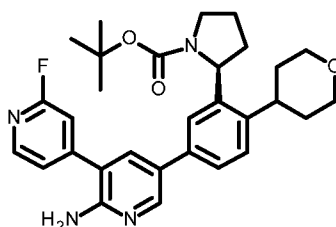
Synthesis of intermediate 27 :

Bromine (0.73 mL, 14.2 mmol) was added dropwise to a solution of intermediate **22** (6 g, 14.2 mmol) in acetic acid (90 mL) at rt. After 20 min, water and potassium carbonate were added. The aqueous phase was extracted with ethyl acetate and the organic layer was dried over MgSO_4 , filtered and evaporated until dryness. The residue was purified by preparative LC (SiOH 30 μm Interchim, from 100% DCM to DCM 90%/ MeOH 10%/ NH_4OH 0.1%). The pure fractions were collected and evaporated until dryness to give 5.8 g of intermediate **27** (81%).



Synthesis of intermediate 28:

To a solution of intermediate **27** (0.51 g, 1 mmol), bis(pinacolato)diboron (0.3 g, 1.2 mmol) and potassium acetate (0.31 g, 3.1 mmol) in 1,4-dioxane (11 mL) was added under nitrogen atmosphere $\text{PdCl}_2(\text{dppf})$ (0.084 g, 0.1 mmol) in a schlenk. The reaction mixture was stirred and heated at 110°C for 20 hours, cooled at room temperature, then DCM was added and the mixture was filtered through a cellite pad and the filtrate was evaporated until dryness and the residue was used without purification for the next step.



Synthesis of intermediate 29:

A mixture of intermediate **27** (6 g, 22.4 mmol), 2-fluoropyridine-4-boronic acid pinacol ester (3.2 g, 14.3 mmol), $\text{PdCl}_2(\text{dppf})$ (0.61 g, 0.75 mmol) and sodium carbonate (2.5 g, 23.9 mmol) in 1,4-dioxane (115 mL) and water (11.5 mL) was stirred under N_2 in a sealed tube at 90°C for 6 hours. The reaction mixture was cooled down, poured into water and potassium carbonate and the aqueous phase was extracted with ethyl acetate. The organic layer was separated, dried over MgSO_4 , filtered and

evaporated until dryness. The residue was purified by preparative LC (220 g SiOH 30µm Interchim, from 100% DCM to DCM 90%/ MeOH 10%/ NH₄OH 0.1%). The pure fractions were collected and evaporated until dryness to give 6.8 g of intermediate **29** (quant.).

5 The following intermediates were prepared via an analogous procedure:

Intermediate	Structure	Quantity	Yield
Intermediate <u>30</u> Starting from 2-Chloropyridine-4-boronic acid pinacol ester		0.75 g	71%
Intermediate <u>31</u> Starting from (4,5,6,7-Tetrahydropyrazolo[1,5-a]pyridin-3-yl)boronic acid pinacol ester		0.16 g	59%
Intermediate <u>32</u> Starting from 1-(Methylsulfonyl)pyrazole-4-boronic acid pinacol ester		0.102 g	58%
Intermediate <u>33</u> Starting from 2-(Tributylstannyl)thiazole		0.042 g	42%

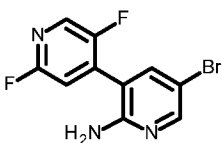
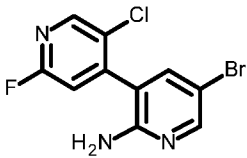
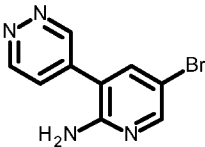
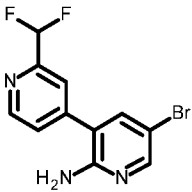
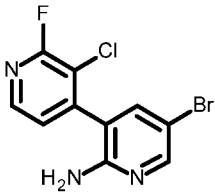
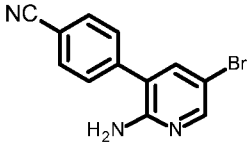
Intermediate	Structure	Quantity	Yield
Intermediate 34 Starting from 1-(2- {[tert-butyl(dimethyl) silyl]oxy}ethyl)-4-(4,4, 5,5-tetramethyl-1,3,2- dioxaborolan-2-yl)-1H- pyrazole		0.079 g	41%
Intermediate 35 Starting from 1- Isopropyl-1H-pyrazole- 4-boronic acid pinacol ester		0.058 g	36%
Intermediate 36 Starting from 1-Boc- pyrazole-4-boronic acid pinacol ester		0.086 g	59%
Intermediate 37 Starting from 3- Methoxy-4- pyridineboronic acid pinacol ester		0.073 g	99%
Intermediate 38 Starting from Pyridine- 4-boronic acid pinacol ester		0.127 g	69%

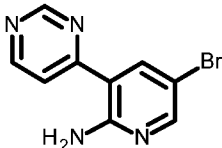
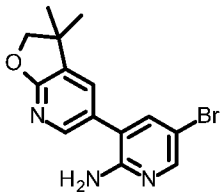
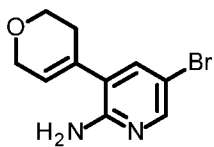
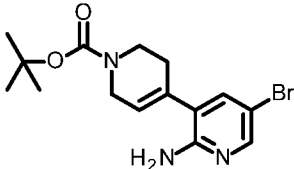
Intermediate	Structure	Quantity	Yield
Intermediate <u>39</u> Starting from 4-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)-3,6-dihydro-2H-thiopyran 1,1-dioxide		0.38 g	Quant
Intermediate <u>40</u> Starting from 6-hydroxypyridine-3-boronic acid pinacol ester		0.086 g	38%
Intermediate <u>41</u> Starting from 2-Hydroxypyridine-4-boronic acid pinacol ester		0.092g	40%
Intermediate <u>42</u> Starting from 2-Cyanopyridine-4-boronic acid pinacol ester		0.23 g	88%
Intermediate <u>43</u> Starting from 4-Fluorophenylboronic acid, pinacol ester		0.24 g	94%

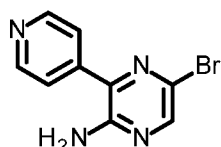
Intermediate	Structure	Quantity	Yield
Intermediate 44 Starting from 2-Trifluoromethyl-4-pyridineboronic acid pinacol ester		0.22 g	78%

The following intermediates were prepared via an analogous procedure as for intermediate 17:

Intermediate	Structure	Quantity	Yield
Intermediate 45 Starting from 2,3-difluoropyridine-4-boronic acid		0.09 g	31%
Intermediate 46 Starting from 3-fluoropyridine-4-boronic acid		0.257g	20%
Intermediate 47 Starting from (2-(2-CYANOPROPAN-2-YL)PYRIDIN-4-YL)BORONIC ACID PINACOL ESTER		0.234 g	70%
Intermediate 48 Starting from 3-chloropyridine-4-boronic acid		0.139 g	23%

Intermediate	Structure	Quantity	Yield
Intermediate <u>49</u> Starting from 2,5-difluoropyridine-4-boronic acid		0.103 g	28%
Intermediate <u>50</u> Starting from 5-chloro-2-fluoropyridine-4-yl boronic acid		0.179 g	26%
Intermediate <u>51</u> Starting from 4-(tributylstannyl)-pyridazine		0.294 g	69%
Intermediate <u>52</u>		0.04 g	31%
Intermediate <u>53</u> Starting from 3-chloro-2-fluoropyridine-4-boronic acid		0.09 g	30%
Intermediate <u>54</u> Starting from 4-Cyanophenylboronic acid		0.097 g	52%

Intermediate	Structure	Quantity	Yield
Intermediate 55 Starting from 4-(tributylstannyl)-pyrimidine		0.268 g	41%
Intermediate 56 Starting from intermediate 65		0.3g	47%
Intermediate 57 Starting from 3,6-dihydro-2H-pyran-4-boronic acid pinacol ester		0.375g	99%
Intermediate 58 Starting from N-Boc-1,2,5,6-Tetrahydropyridine-4-Boronic Acid Pinacol Ester		0.520g	83%

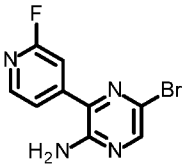


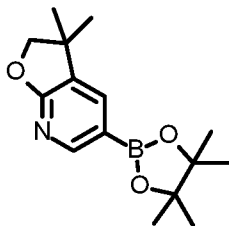
Synthesis of intermediate 59 :

A mixture of 3,5-dibromopyrazin-2-amine (1.3 g, 5.14 mmol), 4-pyridinylboronic acid (0.948 g, 7.71 mmol) and sodium carbonate (1.09 g, 10.28 mmol) in 1,4-dioxane (50 mL) and water (12.5 mL) was degazed 10 min. After addition of PdCl₂(dppf) (0.21g, 0.257 mmol) the reaction mixture was heated at 90°C for 12h. The reaction mixture

was filtered through a pad of Celite[®], water was added and the aqueous phase was extracted with DCM. The organic layer was dried over MgSO₄, filtered and concentrated. The crude mixture was purified by flash chromatography using Heptane/Ethylacetate 0% to 100% as gradient. The desired fractions were collected and concentrated to give intermediate **59** (0.5 g, 38%).

The following intermediate was prepared via an analogous procedure :

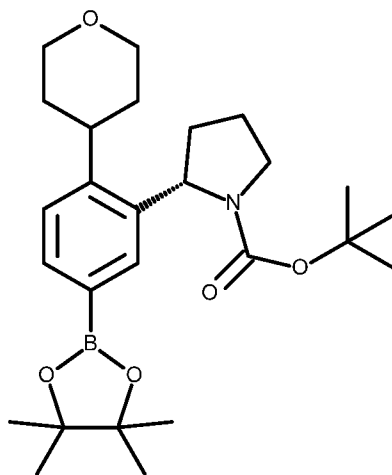
Intermediate	Structure	Quantity	Yield
Intermediate 60		0.3 g	28%



Synthesis of intermediate 61 :

- 1) A mixture of 3,3-dimethyl-2,3-dihydrofuro[2,3-b]pyridine (2.8 g, 18.77 mmol), N-bromosuccinamide (10 g, 56.3 mmol) in acetonitrile (50 mL) was heated at 60°C for 18 hours. The mixture was concentrated and diluted with ethyl acetate (100 mL). The organic layer was washed with 1M Na₂SO₃ (30 mL, twice) and 1M NaHCO₃ (30 mL, twice). The organic layer was separated, dried over MgSO₄, filtered and evaporated until dryness. The residue was purified by column chromatography over silica gel (petroleum ether/ethyl acetate : 100/0 to 4/1). The pure fractions were collected and evaporated until dryness to give 3.7 g of 5-bromo-3,3-dimethyl-2H-furo[2,3-b] pyridine (86%).
- 2) A mixture of 5-bromo-3,3-dimethyl-2H-furo[2,3-b] pyridine (0.5 g, 2.19 mmol), bis(pinacolato)diboron (0.72 g, 2.85 mmol), PdCl₂(dppf) (0.143 g, 0.175 mmol) and potassium acetate (0.645 g, 6.58 mmol) in Me-THF (7.5 mL) was stirred at 85°C under nitrogen atmosphere in a sealed tube overnight. The reaction mixture

was cooled to rt, ethyl acetate was added and the reaction mixture was filtered through decalite. The filtrate was evaporated until dryness to give intermediate **61** used without purification for the next step.

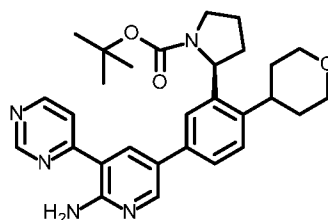


Synthesis of intermediate 62 :

- 5 A mixture of intermediate **19** (4 g, 9.7 mmol), bis(pinacolato)diboron (3 g, 11.8 mmol), PdCl₂(dppf) (0.8 g, 0.98 mmol) and potassium acetate (2.87 g, 29.2 mmol) in 1,4-dioxane (90 mL) was stirred at 90°C under nitrogen atmosphere in a schlenk flask for 15 hours. The reaction mixture was cooled to rt, water was added. The aqueous layer was extracted with ethyl acetate. The organic layer was dried over MgSO₄,
- 10 filtered and evaporated until dryness. The residue was purified by preparative LC (SiOH 35-40µm GraceResolv, from heptane/AcOEt 80/20 to 50/50). The fractions were collected and evaporated until dryness to give 5.3 g of intermediate **62** (quant.).

The following intermediate was prepared via an analogous procedure:

Intermediate	Structure	Quantity	Yield
Intermediate 63 Starting from intermediate 51		Used without purification in next step	



Synthesis of intermediate 64 :

Sodium carbonate (60 mg, 0.57 mmol) was added to a solution of intermediate **55** (71 mg, 0.28 mmol), intermediate **62** (130 mg, 0.28 mmol) in water (1 mL) and 1,4-dioxane (4 mL). The mixture was degassed with a stream of nitrogen for 5 min., then

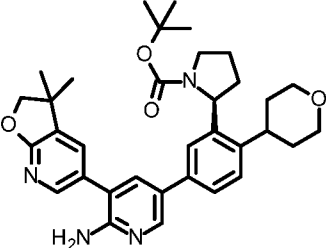
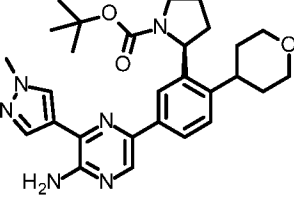
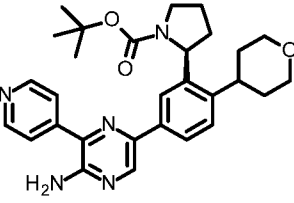
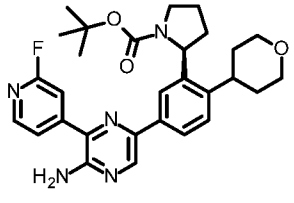
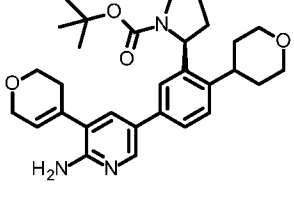
5 PdCl₂(dppf) (12 mg, 0.014 mmol) was added. The reaction mixture was stirred at 90°C overnight. The reaction mixture was cooled to rt, water (15 mL) and AcOEt (20 mL) were added. The aqueous layer was extracted with ethyl acetate (3 x 25 mL). The organic layer was dried over MgSO₄, filtered and evaporated until dryness. The residue was purified by silica gel chromatography (Heptane/EtOAc : 0-100%). The

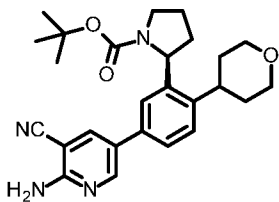
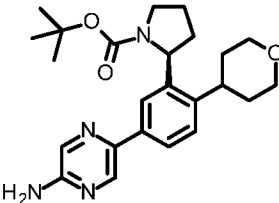
10 fractions were collected and evaporated until dryness to give 98 mg of intermediate **64** (68%).

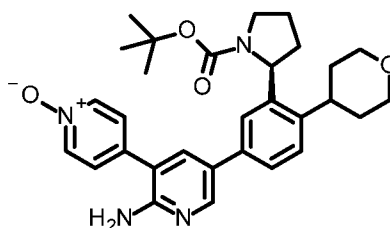
The following intermediates were prepared via an analogous procedure :

Intermediate	Structure	Quantity	Yield
Intermediate 65 Starting from Intermediate 45		0.1g	65%
Intermediate 66 Starting from Intermediate 46		0.14g	43%

Intermediate	Structure	Quantity	Yield
Intermediate 71 Starting from Intermediate 51		0.109g	51%
Intermediate 72 Starting from intermediate 52		0.04 g	73%
Intermediate 73 Starting from Intermediate 53		0.072 g	45 %
Intermediate 74 Starting from Intermediate 54		0.1 g	66 %
Intermediate 75 Starting from Intermediate 63 and 17		0.05 g	20%
Intermediate 76 Starting from 5-bromo-3-(3-methyl-1,2-oxazol-5-yl) pyridin-2-amine		0.147g	71%

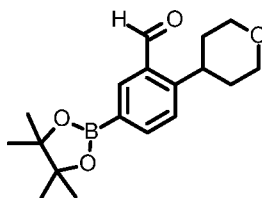
Intermediate	Structure	Quantity	Yield
Intermediate <u>77</u> Starting from Intermediate 56		0.22g	41%
Intermediate <u>78</u> Starting from 2-pyrazinamine, 5-bromo-3-(1-methyl-1H-pyrazol-4-yl)		0.111g	31%
Intermediate <u>79</u> Starting from Intermediate 59		0.054g	51%
Intermediate <u>80</u> Starting from Intermediate 60		0.107 g	65%
Intermediate <u>81</u> Starting from Intermediate 57		0.250 g	92% (70% purity)

Intermediate	Structure	Quantity	Yield
Intermediate 82 Starting from 2-amino-5-bromonicotinonitrile		1.92 g	98%
Intermediate 83 Starting from 2-Amino-5-bromopyrazine		0.63 g	86%



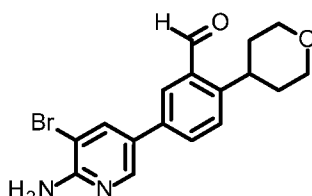
Synthesis of intermediate 84:

A mixture of intermediate **28** (0.55 g, 1 mmol), Pyridine, 4-bromo-, 1-oxide (0.174 g, 1 mmol), PdCl₂(dppf) (0.082 g, 0.1 mmol) and sodium carbonate (0.212 g, 2 mmol) in 1,4-dioxane (15 mL) and water (1.5 mL) was stirred under N₂ in a sealed tube at 90°C for 5 hours. The reaction mixture was cooled down, poured into water and potassium carbonate and the aqueous phase was extracted with ethyl acetate. The organic layer was separated, dried over MgSO₄, filtered and evaporated until dryness. The residue was purified by preparative LC (12 g SiOH 30µm Interchim, from 100% DCM to DCM 90%/ MeOH 10%/ NH₄OH 0.1%). The pure fractions were collected and evaporated until dryness to give 0.274 g of intermediate **84** (53%).



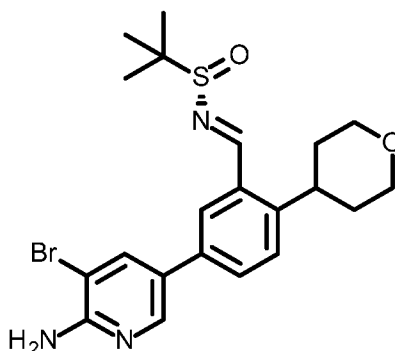
Synthesis of intermediate 85 :

PdCl₂(dppf) (5.43 g, 7.43 mmol) was added to a solution of intermediate 5 (20 g, 74.3 mmol), bis(pinacolato)diboron (22.6 g, 89.2 mmol) and potassium acetate (21.88 g, 223 mmol) under a nitrogen atmosphere in 1,4-dioxane (600 mL). The reaction mixture was stirred at 100°C for 18h. The reaction mixture was cooled to rt, diluted with AcOEt (1800 mL) and filtered. The filtrate was washed with water (400 mL, three times). The organic layer was dried over MgSO₄, filtered and evaporated until dryness to give 23.5 g of intermediate 85 (100%).



Synthesis of intermediate 86 :

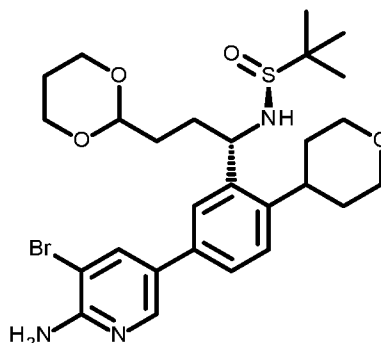
A solution of 2-amino-3-iodo-5-bromopyridine (42.92 g, 143.6 mmol), intermediate 85 (45.4 g, 143.6 mmol) and sodium carbonate (45.6 g, 430.7 mmol) in 1,4-dioxane (500 mL) and water (125 mL) was degassed during 10 min. After addition of PdCl₂(dppf) (11.72 g, 14.4 mmol), the reaction mixture was stirred at 75°C overnight. The mixture was filtered through a pad of celite and rinsed with DCM/MeOH (10/1, 500 mL). The filtrate was evaporated until dryness and purified by column chromatography over silica gel (mobile phase : DCM/MeOH 100/0 to 90/10). The pure fractions were collected and evaporated until dryness, and washed with acetonitrile (75 mL, twice) to give intermediate 86 as a brown solid (27.5 g, 51%).



Synthesis of intermediate 87 :

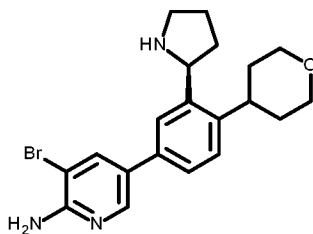
Titanium (IV) ethoxide (25.26 g, 110.7 mmol) was added dropwise to a solution of intermediate 86 (10 g, 27.7 mmol) and (S)-(-)-t-butylsulfinamide (6.71 g, 55.37

mmol). The solution was stirred at 45°C overnight and the mixture was poured into brine (400 mL) and DCM (500 mL) was added. The mixture was filtered through a pad of celite which was washed with DCM (500 mL). The organic layer was separated, dried over Na₂SO₄, filtered and evaporated until dryness. The residue was purified by column chromatography over silica gel (mobile phase : DCM/MeOH 100/0 to 90/10). The pure fractions were collected and evaporated until dryness, washed with acetonitrile (20 mL, twice) to give intermediate **87** as a pale brown solid (12 g, 93%).

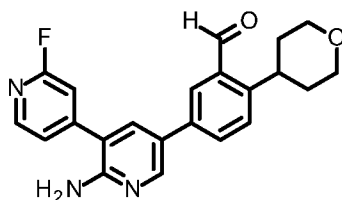


Synthesis of intermediate 88 :

To a mixture of magnesium (9.77 g, 406.96 mmol) in THF (170 mL) were added catalytic amounts of I₂ under nitrogen atmosphere. (1,3-dioxan-2-ylethyl) bromide (31.75 g, 162.78 mmol) was added dropwise. The reaction mixture was periodically cooled in a room temperature water bath to prevent over-refluxing. The mixture was stirred at rt for 1h and then cooled to -65°C. A solution of intermediate **87** (12.6 g, 27.13 mmol) in THF (80 mL) was added dropwise and the reaction mixture was stirred at -65°C for 1h and then at rt overnight. A saturated solution of NH₄Cl (120 mL) and ethyl acetate (200 mL) were added. The mixture was filtered through a pad of celite which was washed with ethyl acetate (200 mL). The organic layer was separated, dried over Na₂SO₄, filtered and evaporated until dryness. The crude compound was washed with acetonitrile (20 mL, twice) and dried to give intermediate **88** as a brown solid (12.6 g, 80%).

**Synthesis of intermediate 89 :**

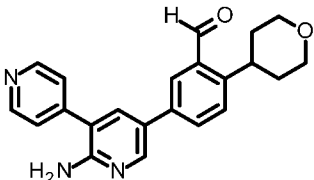
Intermediate **88** (0.35 g, 0.603 mmol) was slowly added to a mixture of TFA (2.66 mL, 34.8 mmol) and water (0.14 mL) to maintain temperature below 25°C. The reaction mixture was stirred at rt for 30 min. Triethylsilane (0.963 mL, 6.028 mmol) was added and the reaction mixture vigorously stirred at rt overnight. The reaction mixture was evaporated until dryness. A purification was performed via preparative LC (stationary phase : irregular SiOH 15-40 μ M 4 g, Interchim, mobile phase : DCM /MeOH/NH₄OH 95/5/1 to 90/10/1). The pure fractions were evaporated to give intermediate **89** (0.2 g, 82%).

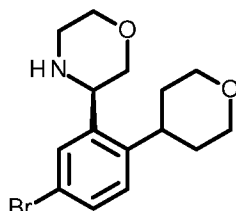
**Synthesis of intermediate 90 :**

Sodium carbonate (0.41 g, 3.88 mmol) was added to a solution of intermediate **86** (1 g, 1.94 mmol), 2-fluoropyridine-4-boronic acid pinacol ester (0.52 g, 2.33 mmol) in water (1.75 mL) and 1,4-dioxane (18.7 mL). The mixture was degassed with a stream of nitrogen for 5 min., then PdCl₂(dppf) (0.158 g, 0.194 mmol) was added. The reaction mixture was stirred at 90°C for 6 hours. The reaction mixture was cooled to rt, water and DCM were added. The aqueous layer was extracted DCM. The organic layer was dried over MgSO₄, filtered and evaporated until dryness. A purification was performed via preparative LC (stationary phase : irregular SiOH 15-40 μ M 12 g, Grace, mobile phase : DCM /MeOH/NH₄OH 100/0/0 to 90/10/1). The pure fractions were evaporated to give intermediate **90** (0.66 g, 90%).

The following intermediate was prepared via an analogous procedure:

Intermediate	Structure	Quantity	Yield

Intermediate 91			
Starting from intermediate 58 and [3,4'-Bipyridin]-2-amine, 5-bromo		0.1 g	49%

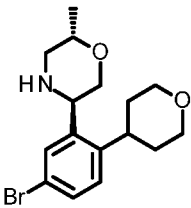
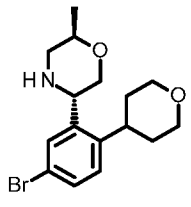
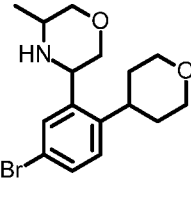
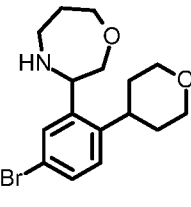


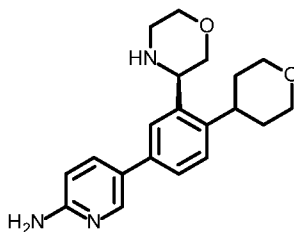
Synthesis of intermediate 92 :

- Intermediate **5** (7.39 g, 27.46 mmol) and molecular sieves 4 A (3.6g) were added to [(2-aminoethoxy)methyl]tributyl stannane (10 g, 27.46 mmol) in DCM (95 mL). The reaction mixture was stirred at rt for 6h, filtered over Celite[®] and the filtrate was evaporated until dryness to give the imine. (R,R)-2,2'-Isopropylidenebis(4-phenyl-2-oxazoline) (1.84 g, 5.5 mmol) was added in one portion to a suspension of copper (II) triflate in hexafluoroisopropanol (50 mL). To this mixture was added the imine in hexafluoroisopropanol (50 mL). The reaction mixture was stirred at rt overnight. 1N NaOH was added, the mixture was stirred and extracted with DCM, the organic layer was separated, dried over MgSO₄, filtered and evaporated till dryness. A purification was performed via preparative LC (Stationary phase: SiOH 35-40μm, 330g interchim, Mobile phase: 100% DCM to 50/50 DCM/AcOEt). The fractions were collected and evaporated until dryness (8.5 g, 95%). Both enantiomers were separated via chiral SFC (Stationary phase: CHIRALPAK AD-H 5μm 250*30mm, Mobile phase: 70% CO₂, 30% iPrOH(0.3% iPrNH₂)) yielding 1.86 g of enantiomer A (20.8%, [α]_D: + 7.05° (589 nm, c 0.19 w/v %, DMF, 20 °C) and 4.63 g of enantiomer B (intermediate **92**, 51.7 %, [α]_D: - 76.67° (589 nm, c 0.27 w/v %, DMF, 20 °C).

The following intermediates were prepared via an analogous procedure :

Intermediate	Structure	Quantity	Yield

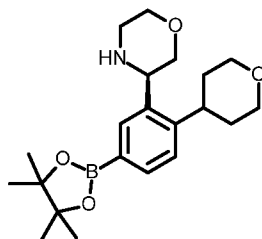
Intermediate	Structure	Quantity	Yield
Intermediate 93a Starting from intermediate 5 and {[(1-aminopropan-2-yl) oxy]methyl}tributylstannane	 $[\alpha]_d: -73.4^\circ$ (589 nm, c 0.29 w/v %, DMF, 20 °C)	2.6 g	26%
Intermediate 93b Starting from intermediate 5 and {[(1-aminopropan-2-yl) oxy]methyl}tributylstannane	 $[\alpha]_d: +71.3^\circ$ (589 nm, c 0.24 w/v %, DMF, 20 °C)	2.8 g	28%
Intermediate 94 Starting from intermediate 5 and [(2-aminopropoxy) methyl]tributylstannane		0.8 g	63%
Intermediate 95 Starting from intermediate 5 and 3-[(Tributylstannyl) methoxy]-1-propanamine		0.54 g	35 %

**Synthesis of intermediate 96 :**

A mixture of 2-Aminopyridine-5-boronic acid pinacol ester (1.2 g, 5.45 mmol), intermediate **92** (1.44 g, 4.41 mmol), Pd(PPh₃)₄ (0.51 g, 0.44 mmol), Na₂CO₃ (1.4 g, 13.2 mmol) in Me-THF (50 mL) and water (5 mL) was heated under N₂ in a sealed tube at 85°C for 15 hours. H₂O, K₂CO₃ and AcOEt were added; the organic layer was extracted, dried over MgSO₄, filtered and evaporated until dryness. The residue was purified by preparative LC (SiOH 30µm Interchim, gradient from 100% DCM to 90% DCM 10% CH₃OH 0.1% NH₄OH). The pure fractions were collected and evaporated until dryness, yielding intermediate **96** (1.24 g, 83%).

10 The following intermediate was prepared via an analogous procedure :

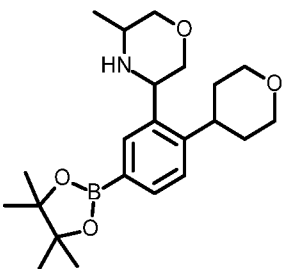
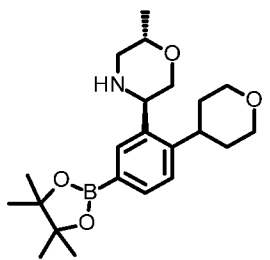
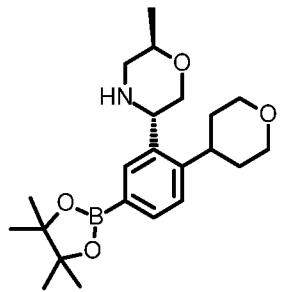
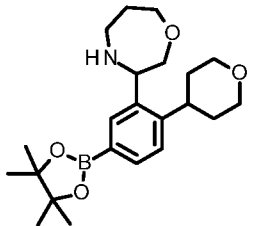
Intermediate	Structure	Quantity	Yield
Intermediate 97 Starting from intermediate 93a		2.3 g	87%

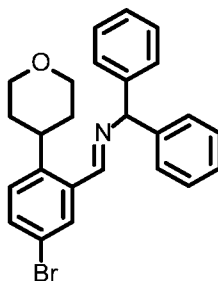
**Synthesis of intermediate 98 :**

A mixture of intermediate **92** (0.28 g, 0.86 mmol), bis(pinacolato)diboron (0.261 g, 1 mmol), PdCl₂(dppf) (0.045 g, 0.05 mmol), potassium acetate (0.252 mg, 2.6 mmol) in 1,4-dioxane (8 mL) was stirred at 120°C using one single mode microwave (Biotage

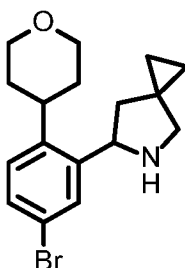
Initiator EXP 60) with a power output ranging from 0 to 400 W for 45min. The reaction mixture was filtered over Celite®, H₂O was added to the filtrate, the filtrate was extracted with ethyl acetate, the organic layer was separated, dried over MgSO₄, filtered and evaporated. Compound **98** was used as it for next step.

5 The following intermediates were prepared via an analogous procedure :

Intermediate	Structure	Quantity
Intermediate 99 Starting from intermediate 94		Used without purification in next step
Intermediate 100a Starting from intermediate 93a		Used without purification in next step
Intermediate 100b Starting from intermediate 93b		Used without purification in next step
Intermediate 101 Starting from intermediate 95		Used without purification in next step

**Synthesis of intermediate 102 :**

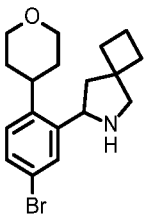
Magnesium sulfate (3.94 g, 32.7 mmol) and intermediate 5 (4 g, 14.86 mmol) were added successively to a stirred solution of Benhydramine (2.64 mL, 14.86 mmol) in DCM (52 mL). The resulting mixture was stirred at rt overnight, filtered. The filtrate
5 was evaporated until dryness, dried under vacuum and used directly in the next step without further purification.

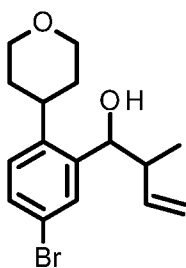
**Synthesis of intermediate 103:**

Intermediate 102 (1.079 g, 2.48 mmol) was dissolved in THF (17 mL) under nitrogen atmosphere and cooled to -78 °C. A 1M solution of Potassium tert-butoxide in dry
10 THF (334.6 mg, 2.98 mmol) was added dropwise (changing of color to violet). After 30 min 1,1-bis(iodomethyl)cyclopropane (2.4 g, 7.45 mmol) was added rapidly. The mixture was stirred for 1h at -78 °C, then warmed to room temperature (after 5 min changing of color to grey) and stirred overnight. Water and DCM were added, the phases were separated and the aqueous phase extracted with DCM (3 x 100 mL). The
15 combined organic extracts were dried over MgSO₄, filtered, the solvent removed under reduced pressure and the residue obtained dried under high vacuum. This residue was dissolved in acetone (10 mL), 3M HCl (3.56 mL, 10.67 mmol) was added and the mixture stirred at room temperature overnight. The mixture was basified with sat. Na₂CO₃ aqueous solution and extracted with DCM. The layers were separated and
20 the aqueous phase extracted with DCM (5 x 50 mL). The combined organic extracts were dried over MgSO₄, filtered and the solvent removed under reduced pressure. The crude mixture was purified by silicagel column chromatography (DCM/MeOH : 0-

15%). The pure fractions were collected and evaporated until dryness to give intermediate **103** (425 mg, 51%).

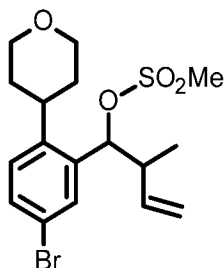
The following intermediate was prepared via an analogous procedure:

Intermediate	Structure	Quantity	Yield
Intermediate 104 Starting from intermediate 102 and 1,1-Bis(iodomethyl) cyclobutane		0.128 g	15%

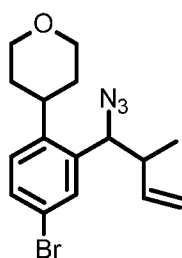


5 Synthesis of intermediate 105:

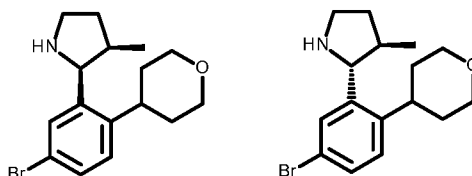
Zinc dust (1.21 g, 18.58 mmol) and crotyl bromide (0.9 mL, 7.43 mmol) were added to a solution of intermediate **5** (1g, 3.42 mmol) in THF (75 mL). A saturated aqueous solution of NH_4Cl (37.5 mL) was added dropwise to the previous mixture and the reaction stirred at room temperature for 50 min. The mixture was filtered through a pad of celite. The filtrate was acidified with 2N HCl and extracted with EtOAc (3 x 50 mL). The combined organic extracts were washed with brine, dried over MgSO_4 , filtered and concentrated. The crude mixture was purified by silicagel column chromatography (Heptane:EtOAc; 0-40%). The pure fractions were collected and evaporated until dryness to give intermediate **105** (1.16 g, 94%).

**Synthesis of intermediate 106:**

Triethylamine (0.944 mL, 6.8 mmol) was added at 0 °C to a solution of intermediate **105** (1.16 g, 3.57 mmol) in DCM (35 mL) under argon atmosphere. Then methane sulfonyl chloride (0.414 mL, 5.35 mmol) was added slowly to the previous solution and the mixture warmed to room temperature and stirred for 20 h. The mixture was diluted with water and the layers separated and the aqueous phase was extracted with DCM (3 x 40 mL). The combined organic extracts were dried over MgSO₄, filtered and the solvent removed under reduced pressure. The crude intermediate **106** obtained was dried under high vacuum and used directly in the next step without further purification.

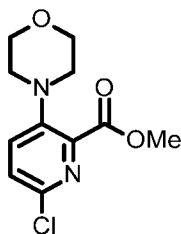
**Synthesis of intermediate 107:**

Sodium azide (0.616 g, 9.48 mmol) was added carefully to a solution of intermediate **106** (1.53 g, 3.79 mmol) in dry DMF (35 mL) and the mixture stirred at 45 °C overnight. The mixture was diluted with water (200 mL) and extracted with EtOAc (3 x 40 mL). The combined organic extracts were washed with brine (25 mL), dried over MgSO₄, filtered and the solvent removed under reduced pressure. The crude mixture was purified by silicagel column chromatography (Heptane:EtOAc; 0-10%). The pure fractions were collected and evaporated until dryness to give intermediate **107** (0.929 g, 67%).

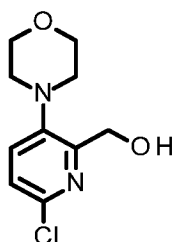


Synthesis of intermediate 108 and 109:

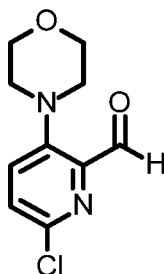
A solution of Borane dimethyl sulphide complex (0.550 mL, 5.8 mmol) in 2.9 mL of dry THF (2 M solution) was added dropwise at 0 °C to a solution of cyclohexene (1.18 mL, 11.6 mmol) in 1.7 mL of dry THF. The resulting white suspension was stirred at 0 °C for 1h, then cooled to -15 °C and a solution of intermediate **107** (0.677 g, 1.93 mmol) in dry THF (5.4 mL) was added dropwise to it. The reaction was allowed to warm to room temperature, stirred at this temperature for 3 h. The reaction was quenched with MeOH at 0 °C and evaporated to dryness. The crude mixture was dissolved in EtOAc and washed with 1M HCl aqueous solution. The layers were separated and the aqueous phase extracted with EtOAc (10 x 20 mL). The combined organic extracts were dried over MgSO₄, filtered and the solvent removed under reduced pressure. The crude mixture was purified by silicagel column chromatography (DCM:MeOH; 0-15%). All fractions containing product were combined and concentrated to afford a mixture of intermediates **108** and **109** (0.421 g, 67%) which was separated by reverse phase using a GILSON Semi-Preparative System, operated by Trilution software, equipped with a Phenomenex Gemini C18 100A column (100 mm long x 30 mm I.D.; 5 µm particles) at 25 °C, with a flow rate of 20 or 40 mL/min. A gradient elution (method ISO15-AF): [start: 85% H₂O (0.1% HCOOH) - 15% ACN-MeOH; finish: 85% H₂O (0.1% HCOOH) - 15% ACN-MeOH]. The desired fractions were collected and partially concentrated to remove the organic solvents. Then the fractions were basified with saturated Na₂CO₃ aqueous solution and extracted with EtOAc. The combined organic extracts were dried over MgSO₄, filtered, the solvent removed under reduced pressure and dried under high vacuum to afford intermediate **108** (0.079 g, 12.6%) and intermediate **109** (0.180 g, 28.7%).

**Synthesis of intermediate 110:**

To a solution of Methyl 6-chloro-3-fluoropicolinate (1.64 g, 8.65 mmol) in DMSO (10 mL) were added morpholine (0.908 mL, 10.38 mmol) and N,N-diisopropylethylamine (4.52 mL, 25.9 mmol). The reaction mixture was stirred at rt over the weekend. The mixture was diluted with AcOEt. The organic phase was washed with water and the aqueous phase extracted with AcOEt. The combined organic extracts were dried over MgSO₄, filtered and the solvent removed under reduced pressure. The crude mixture was purified by silicagel column chromatography (Heptane:EtOAc; 0-20%). The pure fractions were collected and evaporated until dryness to give intermediate **110** (1.82 g, 82%).

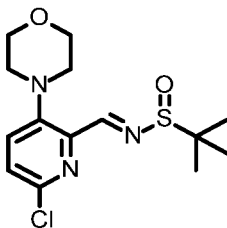
**Synthesis of intermediate 111:**

To a mixture of intermediate **110** (1.7g, 6.62 mmol) and calcium chloride (1.47 g, 13.25 mmol) in ethanol (17 mL) was added sodium borohydride (0.751 g, 19.9 mmol) at 0 °C. The reaction mixture was stirred for 2 h at 0 °C and 4 h at rt. Then water was added to the reaction mixture, and it was extracted with DCM. The organic layer was dried over anhydrous magnesium sulfate and filtered. The crude mixture was purified by flash column chromatography eluting with a mixture of Heptane/AcOEt (40%) to give intermediate **111** (1.37 g, 90%).

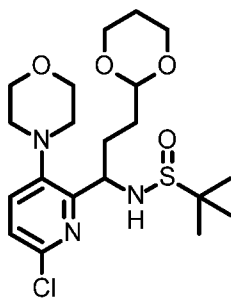
**Synthesis of intermediate 112:**

To a solution of intermediate **111** (1.37 g, 5.99 mmol) in dry DCM (30 mL) was added Dess-Martin Periodinane (3.81g, 8.99 mmol). The reaction mixture was stirred at rt for 3 h. The reaction was quenched with $\text{Na}_2\text{S}_2\text{O}_3$ and Na_2CO_3 was added until basic pH.

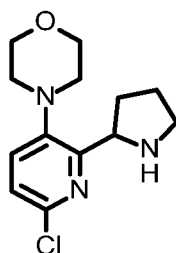
- 5 Then it was extracted with DCM, the organic phase was dried over MgSO_4 , filtered and the solvent was concentrated in vacuo. The crude mixture was purified by flash column chromatography eluting with a mixture of Heptane/AcOEt (50%) to yield intermediate **112** (1.34 g, 99%).

**Synthesis of intermediate 113:**

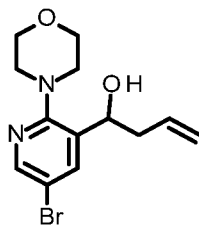
- 10 Titanium (IV) ethoxide (4.96 mL, 23.65 mmol) was added dropwise under nitrogen atmosphere to a solution of intermediate **112** (1.34 g, 5.91 mmol) and 2-methyl-2-propanesulfonamide in dry THF (17 mL). The mixture was stirred at room temperature for 16 h. The reaction mixture was diluted with DCM and brine. The resulting precipitate was removed by filtration through celite and washed with DCM. The layers
- 15 were separated and the aqueous phase extracted with DCM. The combined organic extracts were dried over MgSO_4 , filtered and the solvent removed under reduced pressure. The crude mixture was purified by flash column chromatography eluting with a mixture of Heptane/AcOEt (50%) to afford intermediate **113** (1.3 g, 67%).

**Synthesis of intermediate 114:**

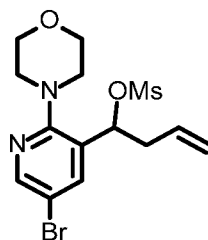
(1,3-dioxan-2-ylethyl)magnesium bromide (28.7 mL, 14.37 mmol) was added dropwise at -78 °C to a solution of intermediate **113** (1.58 g, 4.79 mmol) in dry THF (49 mL). The mixture was stirred at -78 °C for 1 h, quenched with a saturated NH₄Cl aqueous solution and slowly warmed to room temperature. The mixture was diluted with EtOAc and the aqueous phase was extracted with EtOAc. The combined organic extracts were dried over MgSO₄, filtered and the solvent removed under reduced pressure. The crude mixture was purified by flash column chromatography over silica gel, eluting with a mixture of Heptane /AcOEt (60%) to yield intermediate **114** (1.82 g, 85%).

**Synthesis of intermediate 115:**

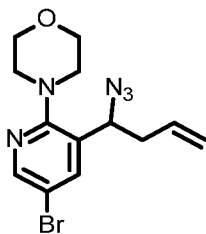
Intermediate **114** (2 g, 4.48 mmol) was slowly added to a mixture of TFA (19.8 mL, 259 mmol) and water (1.3 mL) to maintain temperature below 25°C. The reaction mixture was stirred at rt for 30 min. Triethylsilane (7.16 mL, 44.8 mmol) was added and the reaction mixture vigorously stirred at rt overnight. The reaction mixture was evaporated until dryness. The crude mixture was purified by silicagel column chromatography, gradient in DCM/DCM:MeOH (9:1) (50%) to yield intermediate **115** (1.09 g, 88%).

**Synthesis of intermediate 116:**

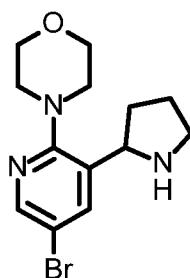
Zinc dust (1.27 g, 19.4 mmol) and allyl bromide (0.67 mL, 7.75 mmol) were added to a solution of 5-bromo-2-(morpholin-4-yl)pyridine-3-carbaldehyde (1.05 g, 3.9 mmol) in THF (75 mL). NH₄Cl (sat. aq. Solution, 39 mL) was added dropwise to the previous mixture and the reaction stirred at room temperature for 1 h. The mixture was filtered through a pad of celite. The filtrate was acidified with 2N HCl and extracted with EtOAc (3 x 50 mL). The combined organic extracts were washed with brine, dried over MgSO₄, filtered and concentrated. The crude mixture was purified by silicagel column chromatography (Heptane:EtOAc; 0-40%) to yield intermediate **116** (1.09 g, 89%).

**Synthesis of intermediate 117:**

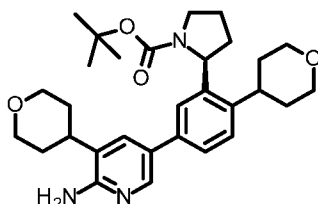
Triethylamine (0.922 mL, 6.6 mmol) was added at 0 °C to a solution of intermediate **116** (1.09 g, 3.48 mmol) in DCM (35 mL) under argon atmosphere. Then methanesulfonyl chloride (0.404 mL, 5.2 mmol) was added slowly to the previous solution and the mixture warmed to room temperature and stirred for 2 h. The mixture was diluted with water and the layers separated and the aqueous phase was extracted with DCM (3 x 40 mL). The combined organic extracts were dried over MgSO₄, filtered and the solvent removed under reduced pressure. The yellow oil obtained was dried under high vacuum and used directly in the next step without further purification.

**Synthesis of intermediate 118:**

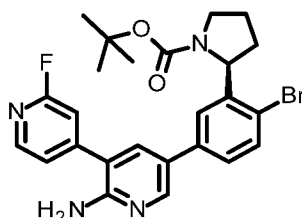
Sodium azide (0.429 g, 6.6 mmol) was added carefully to a solution of intermediate **117** (1.29 g, 3.3 mmol) in dry DMF (25 mL) and the mixture stirred at 60°C for 5 hours. The mixture was diluted with water (100 mL) and extracted with EtOAc (3 x 30 mL). The combined organic extracts were washed with brine (25 mL), dried over MgSO₄, filtered and the solvent removed under reduced pressure. The crude mixture was purified by silicagel column chromatography (Heptane:EtOAc; 0-10%) yielding intermediate **118** (0.72 g, 64%).

**Synthesis of intermediate 119:**

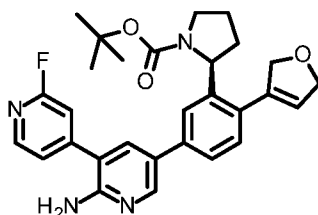
10 A solution of Borane dimethyl sulfide complex (0.6 mL, 6.1 mmol) in dry THF (2M solution, 3 mL) was added dropwise at 0 °C to a solution of cyclohexene (1.2 mL, 12.2 mmol) in dry THF (2 mL). The resulting white suspension was stirred at 0 °C for 1h, then cooled to -15 °C and a solution of intermediate **118** (0.691 g, 2.04 mmol) in dry THF (6 mL) was added dropwise. The reaction was allowed to warm to room
15 temperature and stirred overnight at this temperature. The reaction was quenched with MeOH at 0 °C, the mixture was evaporated to dryness and the crude mixture was purified by silicagel column chromatography (DCM:MeOH; 0-15%) yielding intermediate **119** (0.47 g, 71%).

**Synthesis of intermediate 120:**

To a solution of intermediate **81** (0.335 g, 0.53 mmol, 80% purity) in methanol (20 mL) was added Pd/C 10% under nitrogen atmosphere. The resulting reaction mixture was stirred at room temperature under hydrogen atmosphere for 3 hours. After completion of the reaction, the catalyst was filtered off on Celite[®] and washed with methanol. The filtrate was concentrated under reduced pressure to yield intermediate **120** (0.232 g, 60%, 70% purity).

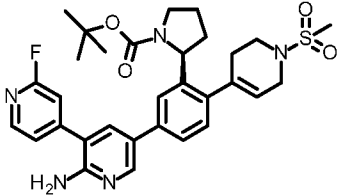
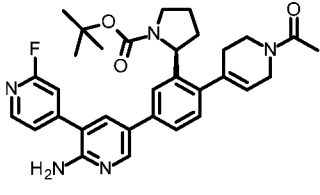
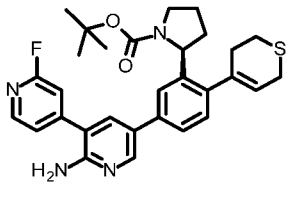
**Synthesis of intermediate 121:**

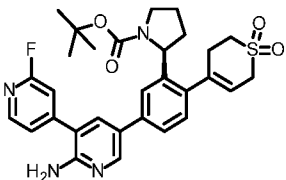
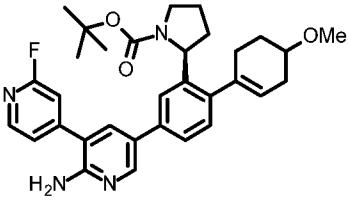
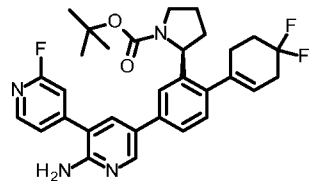
A mixture of intermediate **18** (13.45 g, 42.7 mmol), intermediate **21** (19.3 g, 42.7 mmol), PdCl₂(dppf) (3.49 g, 4.27 mmol) and sodium carbonate (13.6 g, 128.1 mmol) in 1,4-dioxane (386 mL) and water (64 mL) under N₂ was heated at 90°C for 3 hours. The reaction mixture was cooled to rt, diluted with ethyl acetate (1000 mL) and filtered. The filtrate was washed with water (3*300 mL), the aqueous layer was extracted with ethyl acetate. The organic layer was dried over MgSO₄, filtered and evaporated until dryness. The residue was purified by chromatography over silica gel (petroleum ether/ethyl acetate : from 100/0 to /100). The fractions were collected and evaporated until dryness to give intermediate **121** (15.7 g, 70%).

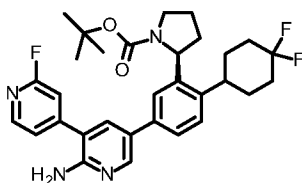
**Synthesis of intermediate 122:**

A mixture of intermediate **121** (0.846 g, 1.59 mmol), 2,5-dihydrofuran-3-ylboronic acid pinacol ester (2.43 g, 3.18 mmol), PdCl₂(dppf) (0.130 g, 0.159 mmol) and sodium

- carbonate (0.506 g, 4.77 mmol) in 1,4-dioxane (42 mL) and water (8 mL) under N₂ was heated at 95°C for 18 hours. The reaction mixture was cooled to rt, diluted with ethyl acetate (200 mL) and filtered. The filtrate was washed with water (3*50 mL), the aqueous layer was extracted with ethyl acetate. The organic layer was dried over
- 5 MgSO₄, filtered and evaporated until dryness. The residue was purified by chromatography over silica gel (petroleum ether/ethyl acetate : from 100/0 to /100) followed by preparative HPLC (Phenomenex Gemini 150*25 mm*10μm; water (0.05% ammonia hydroxide v/v) and MeCN : from 55/45 to 25/75). The fractions were collected and evaporated until dryness to give intermediate **122** (0.39 g, 47%).
- 10 The following intermediates were prepared via an analogous procedure :

Intermediate	Structure	Quantity	Yield
Intermediate 123 Starting from intermediates 121 and 1-Methanesulfonyl-4-(tetramethyl-1,3,2-dioxaborolan-2-yl)-1,2,3,6-tetrahydropyridine		0.75 g	98%
Intermediate 124 Starting from intermediates 121 and 1-Acetyl-5,6-dihydro-2H-pyridine-4-boronic acid, pinacol ester		0.56 g	78%
Intermediate 125 Starting from intermediates 121 and 3,6-Dihydro-2H-thiopyran-4-ylboronic acid pinacol ester		0.53 g	75%

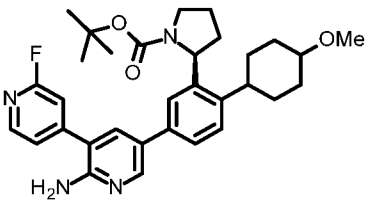
Intermediate	Structure	Quantity	Yield
Intermediate <u>126</u> Starting from intermediates 121 and 1,1-Dioxo-1,2,3,6-tetrahydro-2H-thiopyran-4-yl-boronic acid pinacol ester		0.28 g	Quant.
Intermediate <u>127</u> Starting from intermediates 121 and 4-Methoxycyclohexene-1-boronic acid pinacol ester		0.45 g	Quant.
Intermediate <u>128</u> Starting from intermediates 121 and 4,4-Difluoro-1-cyclohexene-1-boronic acid pinacol ester		0.22 g	67%

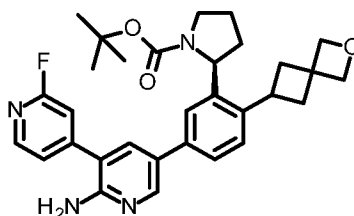


Synthesis of intermediate 129:

A mixture of intermediate 128 (0.15 g, 0.272 mmol), Pd/C 10% (0.15g, 0.272 mmol) in MeOH (3 mL) was hydrogenated under 5 bars pressure at 80°C overnight. The reaction mixture was filtered over celite, washed with DCM/MeOH and the filtrate was evaporated. The residue was purified by preparative LC (12 g SiOH 40 µm irregular, mobile phase : DCM/MeOH/NH₄OH 98/2/0 to 90/10/0.1) The pure fractions were collected and evaporated until dryness yielding 0.017 g of intermediate 129 (11%).

The following intermediate was prepared via an analogous procedure :

Intermediate	Structure	Quantity	Yield
Intermediate 130 Starting from intermediate 127		0.045g	10%

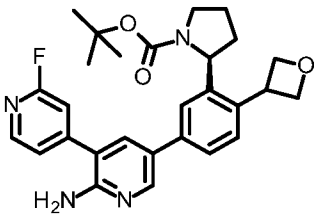
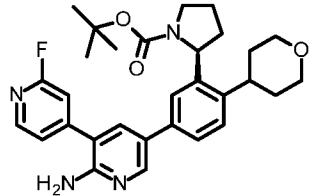


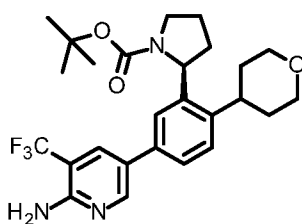
Synthesis of intermediate 131:

To a solution of intermediate **121** (0.2 g, 0.39 mmol), tris(trimethylsilyl)silane (0.145 g, 0.584 mmol), [Ir(dtbbpy)(ppy)₂](PF₆) (0.035 g, 0.039 mmol), sodium carbonate (0.124 g, 1.17 mmol), 6-Bromo-2-oxaspiro[3.3]heptane (0.138 g, 0.779 mmol) in DME (3.5 mL) was added a solution of Nickel (II) chloride ethylene glycol dimethyl ether complex (4.3 mg, 0.02 mmol) and 4'-di-tert-butyl-2, 2'-bipyridine (5.3 mg, 0.02 mmol) in DME (1.5 mL). The reaction mixture was degassed with nitrogen and irradiated under blue light in a Hepatochem device for 20 hours (Hepatochem photoreactor – irradiated with a Cree lamp 18W). Water and ethyl acetate were added, the organic layer was separated, dried over MgSO₄, filtered and evaporated until dryness. The residue was purified by preparative LC (irregular SiOH 40µm 12g, from 100% DCM to DCM 95%/ MeOH 5%/ NH₄OH 0.1%). The fractions were collected and evaporated until dryness to give intermediate **131** (0.094 g, 45%).

The following intermediates were prepared via an analogous procedure:

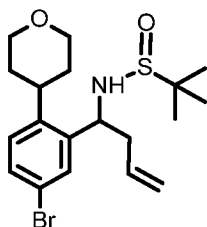
Intermediate	Structure	Quantity	Yield
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Intermediate	Structure	Quantity	Yield
Intermediate 132 Starting from intermediate 121		0.2 g	70%
Intermediate 132b Starting from intermediate 121		0.053 g	26%

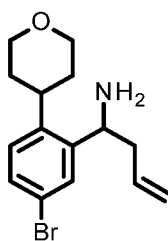


Synthesis of intermediate 133:

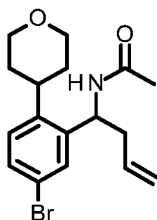
A mixture of intermediate **83** (0.25 g, 0.589 mmol), ammonium persulfate (0.205 g, 0.898 mmol), (Ir[dF(CF₃)ppy]₂(dtbpy))PF₆ (10 mg, 0.009 mmol) and sodium trifluoromethanesulfinate (0.14 g, 0.897 mmol) in DMSO (12.5 mL) was irradiated with a Kessil lamp for 5 hours. The solution was poured into H₂O then extracted with DCM. The organic layer was dried over MgSO₄, filtered and evaporated until dryness. The residue was purified by preparative LC (12g of SiOH 40μm Buchi, gradient from 100% DCM to 90% DCM 10% CH₃OH 0.1% NH₄OH). The fractions were collected and evaporated until dryness. Another purification was performed by preparative LC (12g of SiOH 15μm Buchi, gradient from 100% DCM to 90% DCM 10% MeOH 0.1% NH₄OH). The fractions were collected and evaporated until dryness yielding intermediate **133** (0.064 g, 22%).

**Synthesis of intermediate 134:**

Allylmagnesium chloride (12 mL, 20.5 mmol) was added dropwise to a solution of intermediate **10** (1.9 g, 5.1 mmol) in THF (55 mL) at -70°C under N₂. The reaction mixture was stirred for 1 hour, poured into H₂O and NH₄Cl. The aqueous phase was
 5 extracted with AcOEt. The organic layer was dried over MgSO₄, filtered and evaporated until dryness yielding intermediate **134** (2.07 g, 98%).

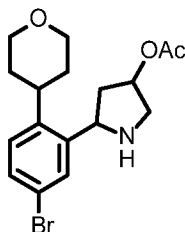
**Synthesis of intermediate 135:**

HCl 3M (35 mL) was added slowly to a solution of intermediate **134** (2.07 g, 5 mmol) in THF (18 mL) at 5°C. The reaction mixture was stirred for 20 hours at room
 10 temperature. Water was added and the aqueous phase was basified by addition of potassium carbonate, then extracted with AcOEt. The organic layer was dried over MgSO₄, filtered and evaporated until dryness yielding intermediate **135** (1.6 g, Quant.).

**Synthesis of intermediate 136:**

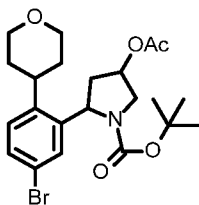
A mixture of intermediate **135** (1.6 g, 5.2 mmol) and Et₃N (2.9 mL, 20.9 mmol) in DCM (25 mL) was cooled at 5°C; Acetic anhydride (1.45 mL, 15.5 mmol) was added and the reaction mixture was stirred for 3 hours. Water and potassium carbonate were added and the organic layer was extracted, dried over MgSO₄, filtered and evaporated until dryness. The residue was purified by preparative LC (12g of SiOH 15μm
 15

Interchim, gradient from 100% DCM to 90% DCM 10% MeOH 0.1% NH₄OH). The fractions were collected and evaporated until dryness yielding intermediate **136** (1.55 g, 85%).



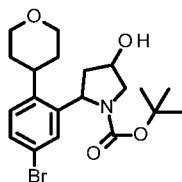
Synthesis of intermediate 137:

- 5 Iodine (10.8 g, 42.6 mmol) was added to a solution of intermediate **136** (5 g, 14.2 mmol) in THF (40 mL) and water (10 mL) at room temperature. The reaction mixture was heated at 50°C for 1 hour, and then poured into a saturated solution of NaHCO₃. The aqueous phase was extracted with AcOEt. The organic layer was washed with a saturated solution of Na₂S₂O₃, dried over MgSO₄, filtered and evaporated until
- 10 dryness. The residue was purified by preparative LC (80g of SiOH 35-40μm GraceResolv, gradient from 100% DCM to 90% DCM 10% CH₃OH 0.1% NH₄OH). The fractions were collected and evaporated until dryness yielding intermediate **137** (2.21 g, 42%).



Synthesis of intermediate 138:

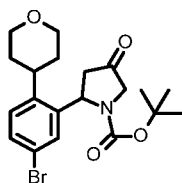
- 15 Di-tert-butyl dicarbonate (3.56 g, 16.3 mmol) was added to a solution of intermediate **137** (2 g, 5.43 mmol), 4-Dimethylaminopyridine (0.133 g, 1.09 mmol) in DCM (60 mL) at room temperature. The reaction mixture was stirred for 2 days, poured into water. The organic layer was extracted, dried over MgSO₄, filtered and evaporated until dryness. The residue was purified by preparative LC (12g of SiOH 35-40μm
- 20 GraceResolv, gradient from 100% DCM to 95% DCM 5% CH₃OH). The fractions were collected and evaporated until dryness yielding intermediate **138** (2.19 g, 86%).

**Synthesis of intermediate 139:**

NaOH 1M (5 mL, 5 mmol) was slowly added to a mixture of intermediate **138** (2.1 g, 4.5 mmol) in MeOH (25 mL) at 5°C. The reaction mixture was stirred for 2 hours.

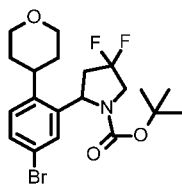
MeOH was evaporated at room temperature and the residue was taken up with water

- 5 then neutralized with HCl 1N. The insoluble was filtered and dried yielding intermediate **139** (1.92 g, Quantitatif).

**Synthesis of intermediate 140:**

DMSO (0.5 mL, 7 mmol) was added dropwise to oxalyl chloride (1.76 mL, 3.52 mmol) in DCM (5 mL) at -78°C, the solution was stirred for 10 min then intermediate

- 10 **139** (0.5 g, 1.17 mmol) in DCM (5 mL) was added dropwise and the reaction was stirred at -50°C for 30 min. After dropwise addition of Et₃N (1.5 mL, 10.8 mmol), the reaction mixture was allowed to warm to room temperature over 30 min. The mixture was poured into NH₄Cl and H₂O. The organic layer was extracted, dried over MgSO₄, filtered and evaporated until dryness. The residue was purified by preparative LC (24
- 15 g of SiOH 35-40µm GraceResolv, gradient from 100% DCM to 95% DCM 5% CH₃OH). The fractions were collected and evaporated until dryness yielding intermediate **140** (0.312 g, 63%).

**Synthesis of intermediate 141:**

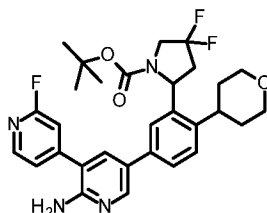
Diethylaminosulfur trifluoride (0.31 mL, 2.19 mmol) was added dropwise to a

- 20 solution of intermediate **140** (0.31 g, 0.73 mmol) in DCM (6 mL) at -70°C under N₂.

The mixture was stirred for 15 hours at room temperature, cooled at -70°C then

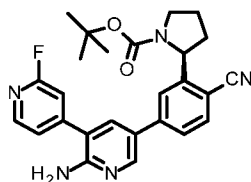
diethylaminosulfur trifluoride (0.31 mL, 2.19 mmol) was added dropwise. The

reaction mixture was stirred for 2.5 hours at room temperature. Water was added and the aqueous phase was basified potassium carbonate. The organic layer was extracted, dried over MgSO_4 , filtered and evaporated until dryness. The residue was purified by preparative LC (12 g of SiOH 35-40 μm GraceResolv, gradient from 100% DCM to 95% DCM 5% CH_3OH). The fractions were collected and evaporated until dryness yielding intermediate **141** (0.3 g, 92%).



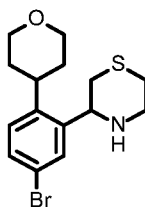
Synthesis of intermediate 142:

A mixture of intermediate **18** (0.233 g, 0.74 mmol), intermediate **141** (0.3 g, 0.67 mmol), $\text{PdCl}_2(\text{dppf})$ (0.071 g, 0.087 mmol) and sodium carbonate (0.143 g, 1.34 mmol) in 1,4-dioxane (10 mL) and water (1 mL) under N_2 was heated at 90°C for 8 hours. Water and potassium carbonate were added. The aqueous layer was extracted with ethyl acetate. The organic layer was dried over MgSO_4 , filtered and evaporated until dryness. The residue was purified by preparative LC (24 g of SiOH 35-40 μm GraceResolv, gradient from 100% DCM to 90% DCM 10% CH_3OH 0.1% NH_4OH). The fractions were collected and evaporated until dryness yielding intermediate **142** (0.225 g, 92%).



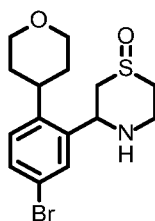
Synthesis of intermediate 143:

A mixture of intermediate **121** (300 mg, 0.58 mmol), Zinc cyanide (316 mg, 2.7 mmol), $\text{Pd}(\text{PPh}_3)_4$ (67.5 mg, 0.06 mmol) in DMF (2.9 mL) was stirred in a Biotage apparatus at 140°C for 45min. Water and AcOEt were added, the organic layer was separated, dried over MgSO_4 , filtered and evaporated. A purification was performed via preparative LC (Stationary phase: irregular SiOH 40 μm 25 g, Mobile phase: 98/2 to 90% DCM 10% CH_3OH 0.1% NH_4OH) yielding intermediate **143** (0.2 g, 74%).



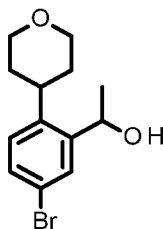
Synthesis of intermediate 144:

Intermediate **5** (0.5 g, 1.86 mmol) and molecular sieves 4A (0.1 g) were added to SLAP TM (0.38 g, 2.32 mmol) in DCM (8 mL). The reaction mixture was stirred at rt 5 h, filtered over celite®, the filtrate was evaporated until dryness to afford the imine which was added to a solution of [Ir(dtbpy)(ppy)₂](PF₆)₃ (17 mg, 0.0186 mmol), Copper (II) trifluoromethanesulfonate (0.67 g, 1.86 mmol) and Bismuth(III) trifluoromethane sulfonate (0.612 g, 0.93 mmol) in MeCN (10 mL). The reaction mixture was stirred at rt for 16 h under blue light (34W - Hepatochem photoreactor – irradiated with a Kessil lamp). The reaction mixture was poured into H₂O and K₂CO₃ and extracted with AcOEt. The organic layer was dried over MgSO₄, filtered and evaporated until dryness. The residue was purified by preparative LC (Stationary phase: irregular SiOH 15-40µm 40g GraceResolv, gradient from 100% heptane to 50% heptane 50% AcOEt). The fractions were collected and evaporated until dryness yielding intermediate **144** (0.4 g, 63%).

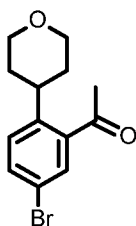


Synthesis of intermediate 145:

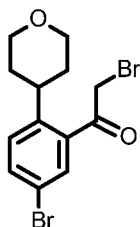
m-CPBA (0.221 g, 0.85 mmol) was added portion wise to intermediate **144** (0.291 g, 0.85 mmol) in DCM (12 mL) at 0°C. The reaction mixture was stirred at room temperature for 15 hours. H₂O and K₂CO₃ were added and the mixture was stirred for 1 hour. The organic layer was extracted, dried over MgSO₄, filtered and evaporated until dryness yielding intermediate **145** (0.3 g, 98%).

**Synthesis of intermediate 146:**

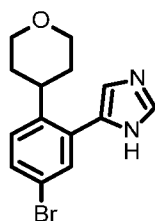
Methylmagnesium bromide (1.4 M in THF, 3.8 mL, 5.35 mmol) was added at 0 °C to a solution of intermediate **5** (1.2 g, 4.46 mmol) in dry THF (8 mL) under nitrogen atmosphere. The reaction was stirred at 0 °C for 30 min and then warmed to room temperature for 1.5 h. The reaction was quenched with a saturated NH₄Cl aqueous solution. The aqueous phase was extracted with EtOAc (5 x 15 mL). The combined organic extracts were dried over MgSO₄, filtered and the solvent removed under reduced pressure. The crude mixture was purified by silica gel column chromatography gradient n-heptane/Ethyl Acetate from 100/0 to 65/35. The desired fractions were collected and evaporated in vacuo to afford intermediate **146** (1.18 g, 93%).

**Synthesis of intermediate 147:**

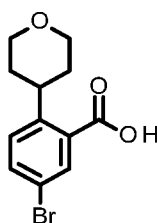
Manganese oxide (8.45 g, 86.6 mmol) was added to a solution of intermediate **146** (1.18 g, 4.13 mmol) in DCM (20 mL). The resulting suspension was stirred at room temperature overnight. The mixture was filtered through a pad of celite and was washed with DCM and MeOH. The solvents were evaporated under reduced pressure and the crude mixture was purified by flash silicagel column chromatography (Heptane: EtOAc; 0- 50%). The fractions were collected and concentrated to afford intermediate **147** (0.51 g, 44%).

**Synthesis of intermediate 148:**

Tetra-n-butylammonium tribromide (1.6 g, 3.31 mmol) was added to a solution of intermediate **147** (0.94 g, 3.31 mmol) in acetonitrile (10 mL). The reaction was stirred at rt for 2h. The solvent was concentrated under reduced pressure, and the residue was re-dissolved in EtOAc. The solution was washed with saturated aqueous NaHCO₃, dried over MgSO₄, and concentrated under reduced pressure. The crude mixture was purified by silicagel column chromatography (Heptane: EtOAc; 0-35%). All fractions were combined and concentrated to afford intermediate **148** (1.14 g, 95%).

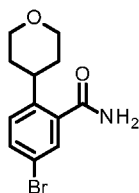
**Synthesis of intermediate 149:**

Intermediate **148** (0.6 g, 1.66 mmol) was dissolved in formamide (3 mL) and the reaction was stirred for 11 h at 165 °C. The mixture obtained was diluted with EtOAc, washed with saturated NaHCO₃ and the layers were separated. The aqueous phase was extracted with DCM (3 x 20 mL), the combined organic extracts dried over MgSO₄, filtered and concentrated under reduced pressure. The crude mixture was purified by silicagel column chromatography with Heptane/EtOAc (gradient: 0-70% EtOAc). All fractions were combined and concentrated to afford intermediate **149** (0.250 g, 49%).

**Synthesis of intermediate 150:**

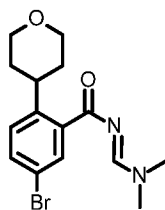
To a solution of intermediate **5** (0.6 g, 2.23 mmol) in DMF (20 mL) was added oxone (0.75g, 2.45 mmol). The reaction mixture was stirred at rt for 48 hours. 1N HCl was

used to dissolve the salts and the aqueous phase was extracted with ethyl acetate. The organic layer was washed with 1N HCl (x 3) and brine, dried over Na₂SO₄ and the solvent was reduced under pressure. The crude mixture was purified by flash chromatography using Heptane/Ethyl acetate 0% to 100% as gradient. The desired fractions were collected and concentrated yielding intermediate **150** (0.46 g, 66%).



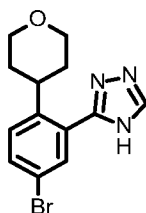
Synthesis of intermediate 151:

To a solution of intermediate **150** (0.46 g, 1.48 mmol) in dry THF (20 mL) was added portion wise 1,1-carbonylimidazole (0.36g, 2.22 mmol). The mixture was stirred at rt for 2 h. An ammonium hydroxide solution (0.91 g/mL, 11.4 mL, 74 mmol) was added and the reaction was stirred at rt overnight. The reaction mixture was diluted with ethyl acetate (50 ml) and washed with water. The phases were separated and the aqueous layer was extracted with Ethyl acetate (5x30 mL). The organic layer was dried over MgSO₄, filtered and evaporated until dryness. The residue was purified by flash chromatography using Heptane/Ethyl acetate 0% to 100% as gradient. The desired fractions were collected and concentrated to afford intermediate **151** (0.349 g, 82%).

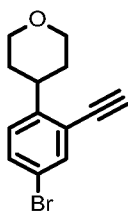


Synthesis of intermediate 152:

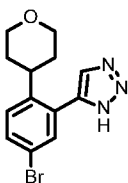
A solution of intermediate **151** (0.27 g, 0.95 mmol) in DMF-DMA (3.4 mL, 25.6 mmol) was stirred and heated at 110°C overnight. The reaction mixture was concentrated and used directly in the next reaction.

**Synthesis of intermediate 153:**

To a solution of intermediate **152** (0.32 g, 0.95 mmol) in AcOH (1.68 mL) was added hydrazine monohydrate (0.101 mL, 2.08 mmol). The reaction mixture was stirred and heated at 120°C for 1 hour. The reaction mixture was concentrated and was triturated
5 with diethyl ether, filtered and the solid was dried under high vacuum yielding intermediate **150** (0.3 g, Quant.).

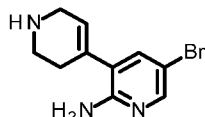
**Synthesis of intermediate 154:**

Dimethyl(1-diazo-2-oxopropyl)phosphonate (0.4 mL, 2.68 mmol) was added to a mixture of intermediate **5** (0.6g, 2.23 mmol) and potassium carbonate (0.62 g, 4.46
10 mmol) in methanol (30 mL). The mixture was stirred at r.t. for 4 h. Methanol was evaporated in vacuo. Half saturated aqueous solution of NaHCO₃ was added and the aqueous phase was extracted with dichloromethane (x4). The combined organic extracts were dried over MgSO₄, filtered and concentrated. The residue was purified by flash column chromatography (Heptane/EtOAc gradient from 100:0 to 80:20)
15 yielding intermediate **154** (0.45 g, 76%).

**Synthesis of intermediate 155:**

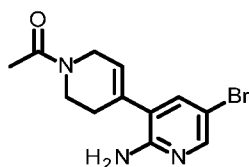
Intermediate **154** (0.25 g, 0.943 mmol) and copper iodide (9 mg, 0.047 mmol) were added in a vial which was sealed with a screw-cap septum. It was backfilled with nitrogen (3 times), then trimethylsilyl azide (0.19 mL, 1.41 mmol) was added via
20 syringe, followed by addition of DMF/MeOH (4/1) (2 mL). The mixture was stirred at

80°C for 10 h. The solvents were evaporated and the mixture was diluted with water and AcOEt. The layers were separated and the aqueous phase extracted with AcOEt. The combined organic extracts were dried over MgSO_4 , filtered and concentrated. The residue was purified by flash column chromatography (Heptane/ AcOEt gradient from 100:0 to 85:15) yielding intermediate **155** (0.15 g, 52%).



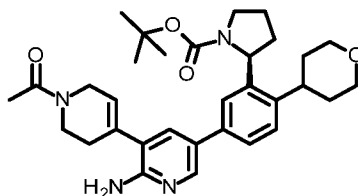
Synthesis of intermediate 156:

A mixture of intermediate **58** (0.52 g) and a hydrogen chloride solution (4 M in dioxane, 8 mL) in dioxane was stirred at rt overnight. The solvents were evaporated in vacuo and the crude mixture was used in the next step without further purification.



Synthesis of intermediate 157:

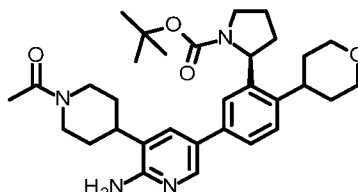
Triethylamine (0.51 mL, 3.67 mmol) was added to a mixture of intermediate **156** (0.427 g, 1.47 mmol), acetic anhydride (0.15 mL, 1.62 mmol) in DCM (15 mL) at room temperature. The mixture was stirred overnight. The solvents were evaporated and the crude mixture was purified by flash column chromatography over silica gel, gradient: DCM/DCM-MeOH (9:1) from 100/0 to 0/100% yielding intermediate **157** (0.41 g, 90%).



Synthesis of intermediate 158:

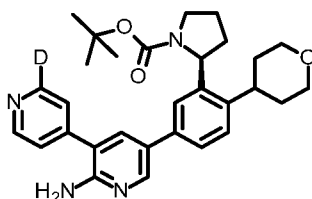
Sodium carbonate (139 mg, 1.31 mmol) was added to a solution of intermediate **157** (0.243 g, 0.66 mmol), intermediate **62** (0.3 g, 0.66 mmol) in water (2 mL) and 1,4-dioxane (12 mL). The mixture was degassed with a stream of nitrogen for 5 min., then $\text{PdCl}_2(\text{dppf})$ (27 mg, 0.033 mmol) was added. The reaction mixture was stirred at 90°C overnight. The reaction mixture was cooled to rt, water and AcOEt were added. The aqueous layer was extracted with ethyl acetate. The organic layer was dried over

MgSO₄, filtered and evaporated until dryness. The residue was purified by silicagel chromatography (DCM/DCM: MeOH (4:1) from 100/0 to 0/100. The fractions were collected and evaporated until dryness to give 0.252 g of intermediate **158** (53%, purity 75%).



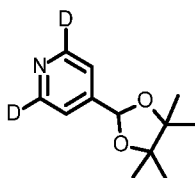
5 **Synthesis of intermediate 159:**

To a solution of intermediate **158** (0.252 g, 0.35 mmol, 75% purity) in methanol (15 mL) was added Pd/C 10% under nitrogen atmosphere. The resulting reaction mixture was stirred at room temperature under hydrogen atmosphere for 3 hours. After completion of the reaction, the catalyst was filtered off on celite and washed with methanol. The filtrate was concentrated under reduced pressure to get intermediate **159** (0.205 g, 97%, 90% purity).



Synthesis of intermediate 160:

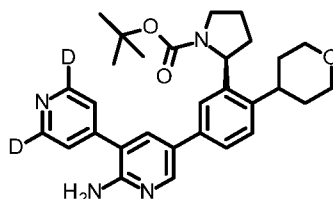
A mixture of intermediate **30** (0.1 g, 0.187 mmol), Pd/C (10 mg, 0.094 mmol) in MeOD (10 mL) was deuterated with D₂ (1atm) at room temperature in a round bottom flask and stirred for 20 hours. The mixture was filtered through a pad of Celite® and the filtrate was evaporated until dryness. The residue was purified by preparative LC (12 g of SiOH 30µm Interchim, gradient from 100% DCM to 90% DCM 10% CH₃OH 0.1% NH₄OH). The fractions were collected and evaporated until dryness yielding intermediate **160** (0.067 g, 71%).



20 **Synthesis of intermediate 161:**

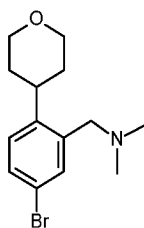
A mixture of 2,6-Dichloropyridine-4-boronic acid, pinacol ester (1 g, 3.65 mmol),

Pd/C (0.194 g, 1.82 mmol) in MeOD (20 mL) was deuterated with D₂ (1atm) at room temperature in a round bottom flask and stirred for 20 hours. The mixture was filtered through a pad of Celite[®] and the filtrate was evaporated until dryness used directly in the next step.



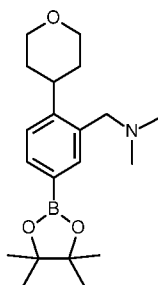
5 **Synthesis of intermediate 162:**

A mixture of intermediate **27** (0.5 g, 1 mmol), intermediate **161** (0.516 g, 2.5 mmol), sodium carbonate (0.21 g, 2 mmol), PdCl₂(dppf) (0.04 g, 0.05 mmol) in 1,4-dioxane (10 mL) and water (1 mL) was heated at 90°C under N₂ for 3 hours in a sealed tube. The reaction mixture was poured into water and potassium carbonate and extracted with ethyl acetate. The organic layer was dried over MgSO₄, filtered and evaporated until dryness. The residue was purified by preparative LC (12 g of SiOH 35-40μm GraceResolv, gradient from 100% DCM to 88% DCM 12% CH₃OH 0.1% NH₄OH) yielding intermediate **162** (0.204 g, 41%).



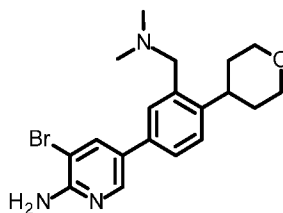
Synthesis of intermediate 163:

15 To a solution of Benzenemethanamine, 5-amino-*N,N*-dimethyl-2-(tetrahydro-2*H*-pyran-4-yl) (6.82 g, 26.75 mmol) in HBr (48% in water, 2 mL) cooled in an ice bath, was added dropwise sodium nitrite (2.03 g, 29.42 mmol) in water. The mixture was stirred for 30 min at 0-4°C. Then this mixture was added dropwise to a solution of copper bromide (2.12 g, 14.71 mmol) in HBr (48% in water, 5.5 mL) cooled in an ice bath. The reaction mixture was stirred at 100°C for 2 hours, then poured into aqueous NaOH (10 mol/L, 200mL) and was extracted with ethyl acetate (100mL*5). The organic layer was dried over MgSO₄, filtered and evaporated until dryness. The residue was purified by column chromatography over silica gel (eluent: petrol/ethyl acetate=100:0-2:1) yielding intermediate **163** (6.02 g, 76.6%) as a white solid.



Synthesis of intermediate 164:

- A mixture of intermediate **163** (1.5 g, 5.1 mmol), bis(pinacolato)diboron (1.5 g, 6.1 mmol), PdCl₂(dppf) (0.267 g, 0.3 mmol) and potassium acetate (1.5 g, 15.3 mmol) in 1,4-dioxane (20 mL) was stirred at 120°C using one single mode microwave (Biotage Initiator EXP 60) with a power output ranging from 0 to 400 W for 45min. The reaction mixture was cooled to rt, filtered over celite and water was added. The organic layer was dried over MgSO₄, filtered and evaporated until dryness. The residue was crystallized in DIPE/pentane, the precipitate was filtered off (catalyst derivatives) and the filtrate was evaporated and used as it for next step.

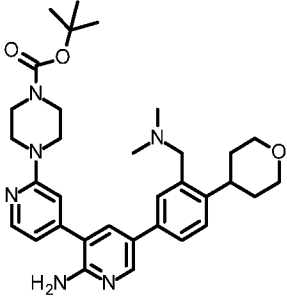


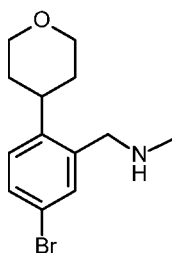
Synthesis of intermediate 165:

- In a sealed tube, a mixture of intermediate **164** (1.75 g, 5.1 mmol), 3-Bromo-5-iodopyridin-2-amine (1.5 g, 5.1 mmol), sodium carbonate (1.6 g, 15.2 mmol), PdCl₂(dppf) (0.415 g, 0.51 mmol) in 1,4-dioxane (22.6 mL) and water (5.3 mL) was stirred at 90°C for 16h. The mixture was poured into H₂O and potassium carbonate and extracted with AcOEt. The organic layer was dried over MgSO₄, filtered and evaporated until dryness. The residue was purified by preparative LC (Stationary phase: irregular 15-40µm 80g Merck, Mobile phase: 0.4% NH₄OH, 96% DCM, 4% MeOH) yielding intermediate **165** (0.685 g, 35%).

- Intermediate 166 was prepared starting from intermediate 165 and following an analogous reaction protocol as was used for intermediate 158.

Intermediate	Structure	Quantity	Yield
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Intermediate 166 Starting from intermediate 165 and 2-(4-tert-butoxycarbonylpiperazin-1-yl)pyridine-4-boronic acid, pinacol ester		0.128 g	87%
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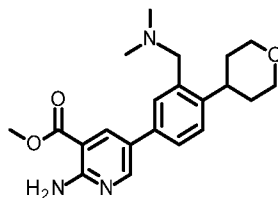


Synthesis of intermediate 167:

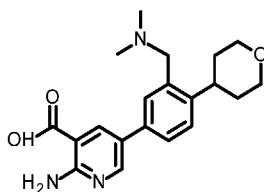
A mixture of intermediate **5** (0.4 g, 1.49 mmol) and methylamine (aqueous solution, 0.129 mL, 1.49 mmol) in MeOH (5 mL) and acetic acid (five drops) was stirred at room temperature for 15 min. Sodiumtriacetoxyborohydride (0.945 g, 4.46 mmol) was added to the resulting mixture and the mixture was stirred at room temperature for 2h.

1 eq of methylamine (0.130 ml) and 1.5 eq of sodiumtriacetoxyborohydride (470 mg) were added to the reaction mixture which was stirred at room temperature overnight.

The mixture was evaporated under vacuo and diluted with water, extracted with dichloromethane three times. The combined organic phases were dried over MgSO_4 , filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (dichloromethane/(dichloromethane/methanol 9:1) gradient 100:0 to 0:100). The desired fractions were collected and the solvent removed under reduced pressure to afford 0.15 g of intermediate **167** (0.15 g, 35%).

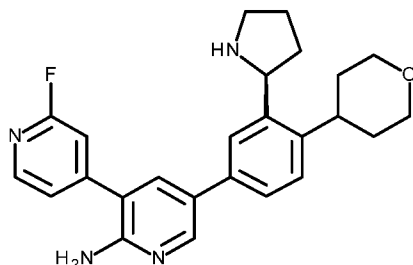
**Synthesis of intermediate 168:**

Sodium carbonate (2.36 g, 22.24 mmol) was added to a solution of intermediate **163** (3.35 g, 11.12 mmol) and methyl 2-amino-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)nicotinate (4.02 g, 14.5 mmol) in 1,4-dioxane (60 mL) and water (12 mL). The mixture was degassed with a stream of nitrogen for 5 min, then PdCl₂(dppf) (0.455 g, 0.56 mmol) was added. The reaction mixture was stirred at 90 °C overnight. The reaction was diluted with water (20 mL). The layers were separated and the aqueous phase extracted with DCM (3 x 20 mL). The combined organic extracts were dried over MgSO₄, filtered and the solvent removed under reduced pressure. The crude mixture was purified by silicagel column chromatography (Heptane/ EtOAc : 0-40%). The desired fractions were collected and the solvent removed under reduced pressure to afford intermediate **168** (3.3 g, 79%).

**Synthesis of intermediate 169:**

Lithium hydroxide (1.09 g, 17.9 mmol) was added to a solution of intermediate **168** in methanol (12 mL) and water (3 mL). The reaction mixture was stirred at 55 °C for 14h. Methanol was evaporated under reduced pressure and the residue obtained was diluted with EtOAc and water. The organic layer was discarded, and the aqueous layer was acidified with HCl 6N and extracted with EtOAc (10 x 20 mL). The organic layer and aqueous layer were filtrated under reduce pressure to obtain a brown solid washed with DCM and dried to yield intermediate **169** (1.05 g, 65%).

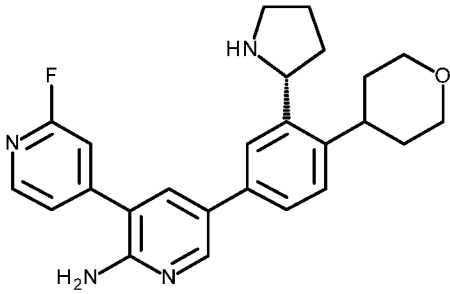
Synthesis of final compounds**Preparation of Co. 1 (1st approach)**

**Synthesis of Co.1:**

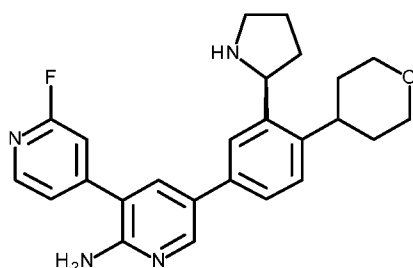
A mixture of intermediate **18** (21.7 g, 67.4 mmol), intermediate **14** (16.04 g, 51.7 mmol), PdCl₂(dppf) (6.78 g, 8.3 mmol) and sodium carbonate (11 g, 103.4 mmol) in 1,4-dioxane (800 mL) and water (80 mL) under N₂ was heated at 80°C for 4 hours.

- 5 The reaction mixture was cooled to rt, water and potassium carbonate were added. The aqueous layer was extracted with ethyl acetate. The organic layer was dried over MgSO₄, filtered and evaporated until dryness. The residue was purified by preparative LC (SiOH 30-40µm GraceResolv, from 100% DCM to DCM 80%/ MeOH 20%/ NH₄OH 1%). The fractions were collected and evaporated until dryness to give **Co.1**
- 10 which was further purified in multiple batches via preparative LC (Stationary phase: irregular SiOH 40µm 300 g, mobile phase: DCM 96%/ MeOH 4%/ NH₄OH 0.5%). The pure fractions were collected and evaporated until dryness to give **Co.1** (15.3 g, 71%). **Co.1** was taken up in acetonitrile, heated at 50°C 15 min, cooled down. The compound was filtered off and dried yielding 11.5 g of **Co.1** (53%) as an off-white
- 15 powder. [α]_D: - 42.22° (589 nm, c 0.27 w/v %, DMF, 20 °C); m.p. = 218°C (DSC).

The following compound was prepared via an analogous procedure:

Compound	Structure	Quantity	Yield
Co. 1b	 $[\alpha]_D^{20} +20.3^\circ$ (589 nm, c 0.093, w/v %, MeOH, 23 °C)	0.085 g	54%

Preparation of Co. 1 (2nd approach)



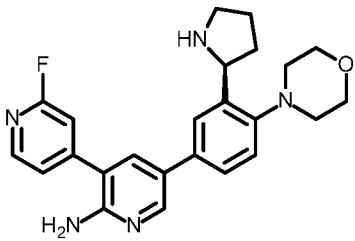
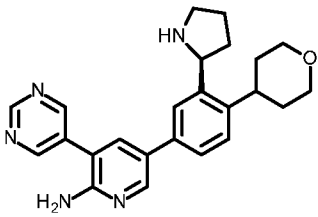
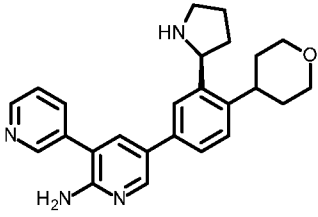
Synthesis of Co.1:

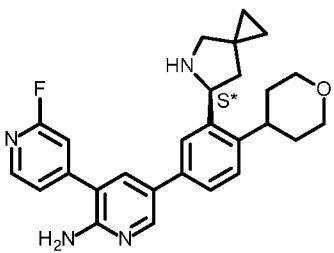
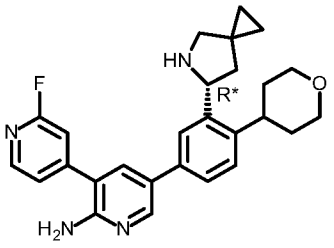
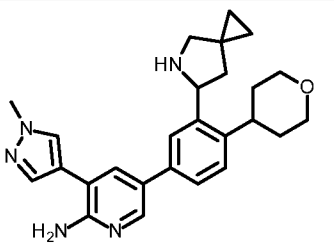
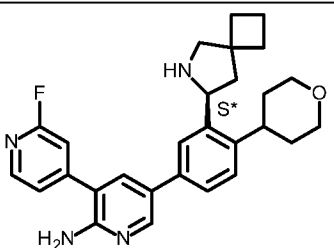
- Trifluoroacetic acid (13 mL, 0.17 mol) was added dropwise to a solution of
- 5 intermediate **29** (3.1 g, 6 mmol) in DCM (80 mL). The mixture was stirred at rt for 15h, poured into water, basified with potassium carbonate. The aqueous phase was extracted with ethyl acetate. The organic layer was separated, dried over MgSO₄, filtered and evaporated until dryness. The residue was purified by preparative LC (40 g SiOH 30μm Interchim, from 100% DCM to DCM 80%/ MeOH 20%/ NH₄OH 1%).
- 10 Two fractions were obtained: F1 = 2 g and F2 = 0.5 g. F1 was crystallized in acetonitrile, filtered, washed with diethylether and dried to give F3. The filtrate and F2 were combined and purified by preparative LC (40 g SiOH 30μm Interchim, from 100% DCM to DCM 80%/ MeOH 20%/ NH₄OH 1%). The fractions were collected and evaporated until dryness to give F4 = 0.75 g. F4 and F3 were combined, taken up
- 15 in DCM and a solution of potassium carbonate 10%. The organic layer was separated, dried over MgSO₄, filtered and evaporated until dryness. The compound was

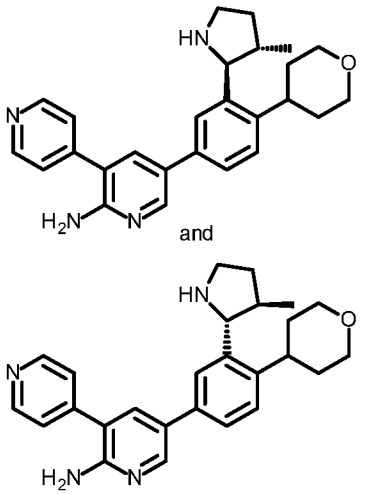
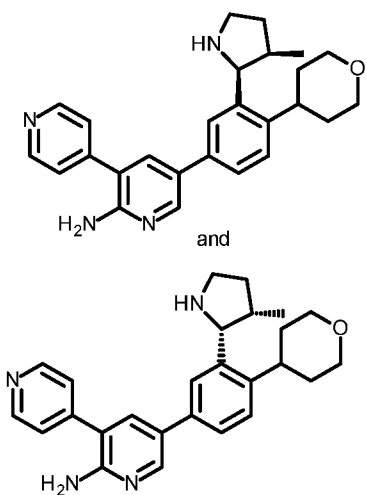
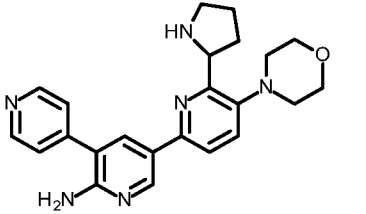
crystallized in acetonitrile, filtered and dried to give 1.54 g of **Co. 1** (62%). $[\alpha]_d$: -35.95° (589 nm, c 0.194 w/v %, DMF, 20 °C); m.p. = 218°C (DSC).

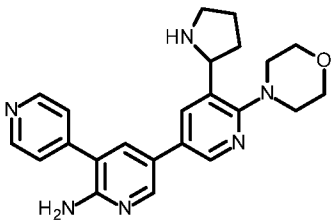
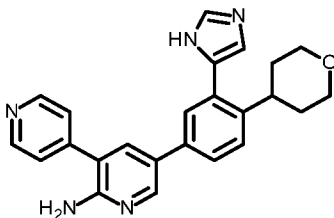
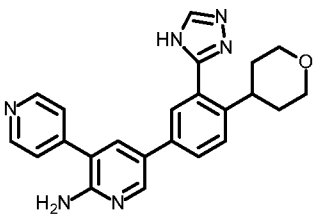
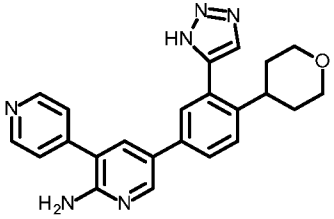
The compounds listed in the table below have been prepared by an analogous reaction protocol as was used for the synthesis of **Co. 1** (1st approach).

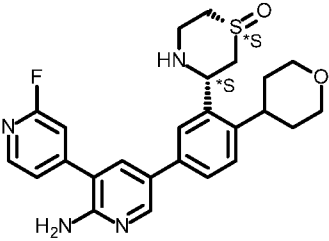
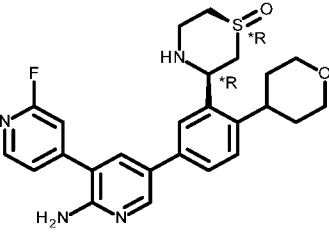
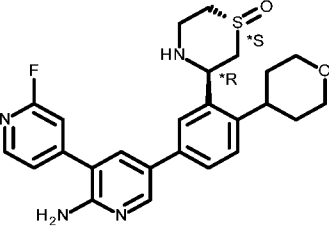
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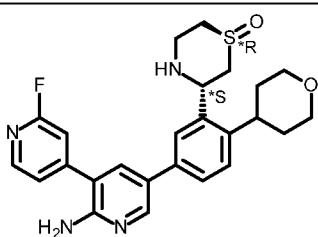
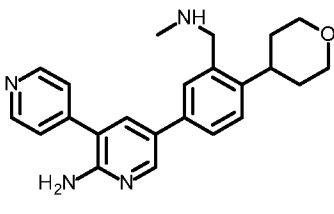
Compound	Structure	Quantity	Yield
Co. 2 Starting from Intermediate 16	 $[\alpha]_d$: -26.5° (589 nm, c 0.43 w/v %, MeOH, 23 °C); m.p. > 300°C (Mettler Toledo MP50)	0.197 g	95%
Co.3 Starting from Intermediate 23	 $[\alpha]_d$: -34° (589 nm, c 0.11 w/v %, MeOH, 23 °C); m.p. = 245°C (Mettler Toledo MP50)	0.105 g	80%
Co.4 Starting from Intermediate 24	 $[\alpha]_d$: -11.9° (589 nm, c 0.186 w/v %, MeOH, 23 °C); m.p. = 257°C (Mettler Toledo MP50)	0.12 g	92%

Compound	Structure	Quantity	Yield
Co.5a Starting from Intermediate 103	 $[\alpha]_d: -7.42^\circ$ (589 nm, c 0.31 w/v %, DMF, 20 °C)	0.064 g	35%
Co.5b Starting from Intermediate 103	 $[\alpha]_d: +7.2^\circ$ (589 nm, c 0.25 w/v %, DMF, 20 °C)	0.055 g	30%
Co.6 Starting from Intermediate 103 & 25	 m.p. = 196.5°C (Mettler Toledo MP50)	0.088 g	32%
Co.7a Starting from Intermediate 104 & 18	 $[\alpha]_d: +7.78^\circ$ (589 nm, c 0.18	0.024 g	28%

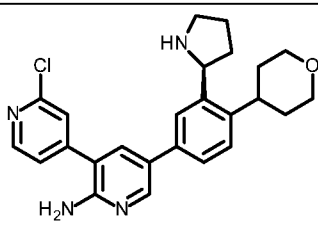
Compound	Structure	Quantity	Yield
Co.9 Starting from Intermediate 104 & 109	Racemic mixture of 2 trans forms  m.p. = 218.3°C (Mettler Toledo MP50)	0.080 g	36%
Co.10 Starting from Intermediate 104 & 108	Racemic mixture of 2 cis forms 	0.014 g	14%
Co.11 Starting from Intermediate 104 & 115		0.163 g	43%

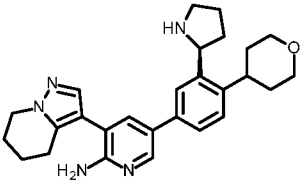
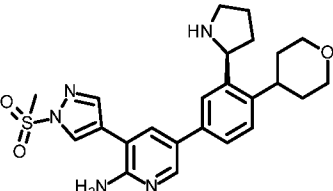
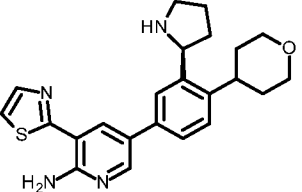
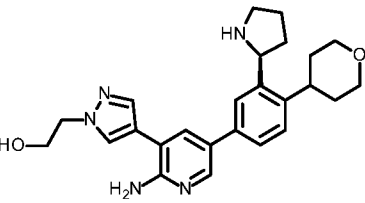
Compound	Structure	Quantity	Yield
	m.p. = 245°C (Mettler Toledo MP50)		
Co.12 Starting from Intermediate 104 & 119	 m.p. = 196°C (Mettler Toledo MP50)	0.21 g	36%
Co.13 Starting from Intermediate 149 and 104	 m.p. = 257°C (Mettler Toledo MP50)	0.07 g	21%
Co.14 Starting from Intermediate 153 and 104	 m.p. = 263°C (Mettler Toledo MP50)	0.14 g	54%
Co.15 Starting from Intermediate 155 and 104		0.03 g	16%

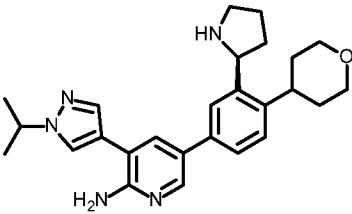
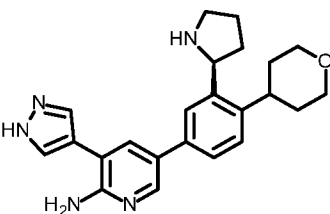
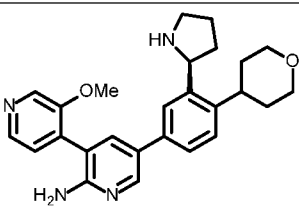
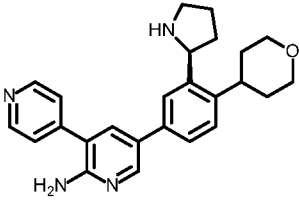
Compound	Structure	Quantity	Yield
	m.p. > 300°C (Mettler Toledo MP50)		
Co. 15a Starting from intermediates 145 and 18	 $[\alpha]_d: +29.6^\circ$ (589 nm, c 0.22, w/v %, DMF, 20 °C)	0.039 g	10 %
Co. 15b Starting from intermediates 145 and 18	 $[\alpha]_d: -9^\circ$ (589 nm, c 0.2, w/v %, DMF, 20 °C)	0.037 g	9.5%
Co. 15c Starting from intermediates 145 and 18	 $[\alpha]_d: -4.4^\circ$ (589 nm, c 0.23, w/v %, DMF, 20 °C)	0.026 g	6.7%

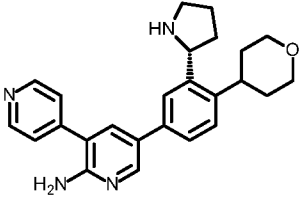
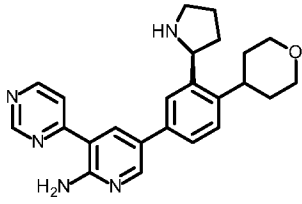
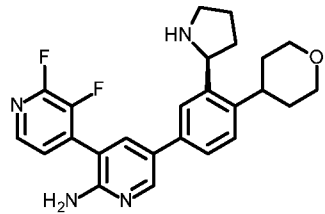
Compound	Structure	Quantity	Yield
Co. 15d Starting from intermediates 145 and 18	 $[\alpha]_d$: +9° (589 nm, c 0.19, w/v %, DMF, 20 °C)	0.028 g	7.2%
Co.16 Starting from intermediate 167 and 104	 m.p. = 265°C (Mettler Toledo MP50)	0.065 g	32%

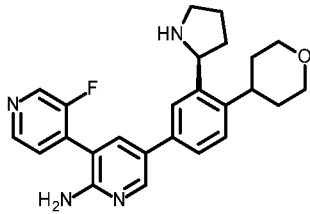
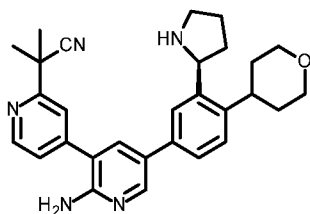
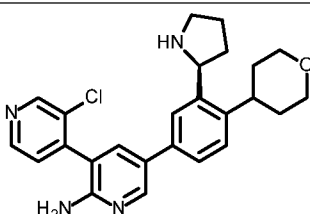
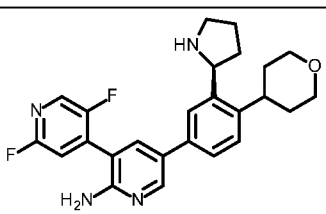
The compounds listed in the table below have been prepared by an analogous reaction protocol as was used for the synthesis of Co. 1 (2nd approach).

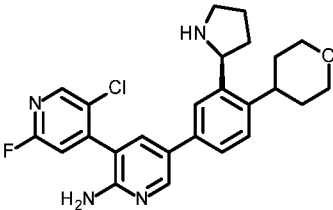
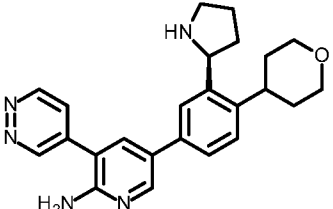
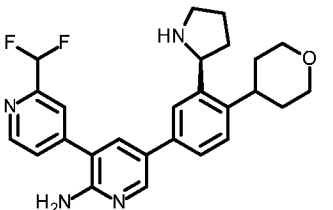
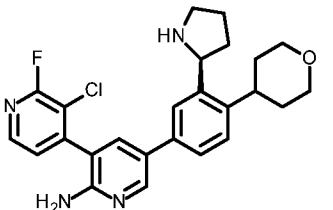
Compound	Structure	Quantity	Yield
Co. 17 Starting from Intermediate 30	 $[\alpha]_d$: - 36.4° (589 nm, c 0.25 w/v %, DMF, 20 °C)	0.241 g	70%

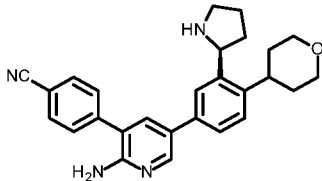
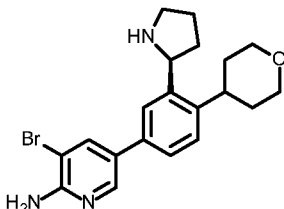
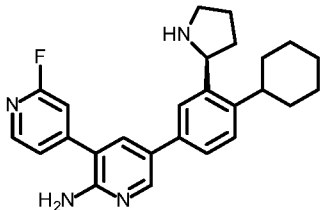
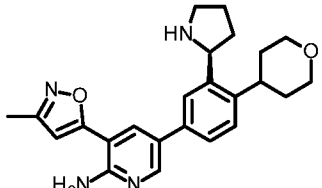
Compound	Structure	Quantity	Yield
Co. 18 Starting from Intermediate 31	 $[\alpha]_d: -45^\circ$ (589 nm, c 0.24 w/v %, DMF, 20 °C)	0.052 g	40%
Co. 19 Starting from Intermediate 32	 $[\alpha]_d: -55^\circ$ (589 nm, c 0.18 w/v %, DMF, 20 °C)	0.019 g	23%
Co. 20 Starting from Intermediate 33	 $[\alpha]_d: -39.6^\circ$ (589 nm, c 0.23 w/v %, DMF, 20 °C)	0.026 g	77%
Co. 21 Starting from Intermediate 34	 $[\alpha]_d: -41.8^\circ$ (589 nm, c 0.22 w/v %, DMF, 20 °C)	0.024 g	45%

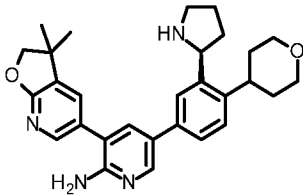
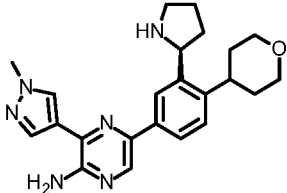
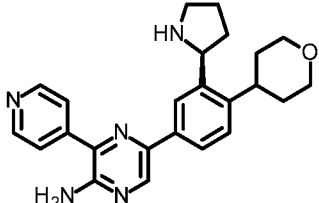
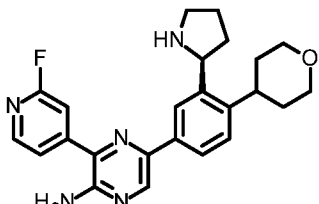
Compound	Structure	Quantity	Yield
Co. 22 Starting from intermediate 35	 $[\alpha]_d$: - 40.9° (589 nm, c 0.22 w/v %, DMF, 20 °C); m.p. = 191.7 °C (DSC)	0.026 g	55%
Co. 23 Starting from Intermediate 36	 $[\alpha]_d$: - 46.5° (589 nm, c 0.2 w/v %, DMF, 20 °C); m.p. = 223.4° C (DSC)	0.026 g	38%
Co. 24 Starting from intermediate 37	 $[\alpha]_d$: - 36.1° (589 nm, c 0.31 w/v %, DMF, 20 °C); m.p. = 217.5° C (DSC)	0.025 g	42%
Co. 25 Starting from Intermediate 38	 $[\alpha]_d$: - 44.4° (589 nm, c 0.25 w/v	0.057 g	56%

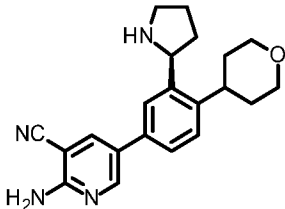
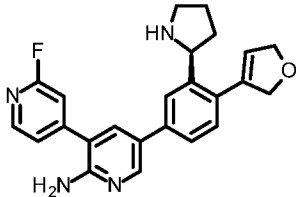
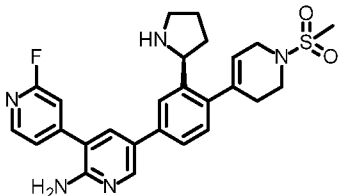
Compound	Structure	Quantity	Yield
	%, DMF, 20 °C); m.p. = 238.1° C (DSC)		
Co. 25b	 [α]_D: + 36° (589 nm, c 0.28 w/v %, DMF, 20 °C)	0.033 g	26%
Co. 26 Starting from Intermediate 64	 [α]_D: - 21.7° (589 nm, c 0.12 w/v %, MeOH, 23 °C); m.p. = 201° C (Mettler Toledo MP50)	0.065 g	85%
Co. 27 Starting from Intermediate 65	 [α]_D: -94.8° (589 nm, c 0.25 w/v %, DMF, 20 °C); m.p. > 300° C (Mettler Toledo MP50)	0.068 g	83%

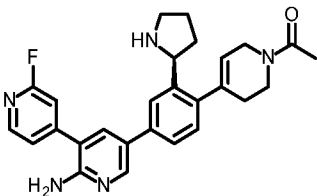
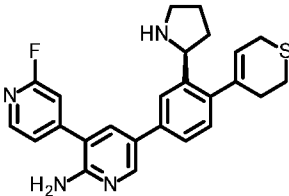
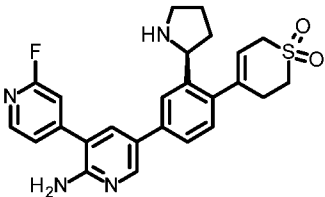
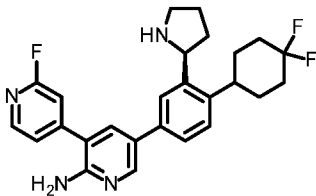
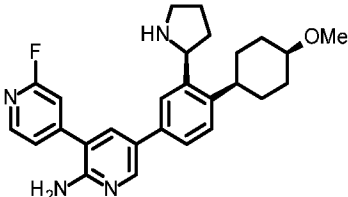
Compound	Structure	Quantity	Yield
Co. 28 Starting from Intermediate 66	 $[\alpha]_D^{25}$: - 29.6° (589 nm, c 0.36 w/v %, MeOH, 23 °C); m.p. = 230° C (Mettler Toledo MP50)	0.052 g	46 %
Co. 29 Starting from Intermediate 67	 $[\alpha]_D^{25}$: - 18.2° (589 nm, c 0.39 w/v %, MeOH, 23 °C); m.p. = 228° C (Mettler Toledo MP50)	0.11 g	68%
Co. 30 Starting from Intermediate 68	 $[\alpha]_D^{25}$: - 23.9° (589 nm, c 0.27 w/v %, MeOH, 23 °C); m.p. > 300° C (Mettler Toledo MP50)	0.088 g	71%
Co. 31 Starting from Intermediate 69	 $[\alpha]_D^{25}$: - 25.5° (589 nm, c 0.093	0.085 g	68%

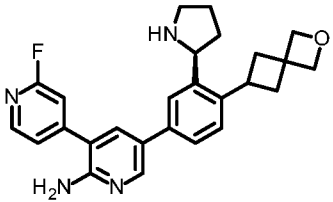
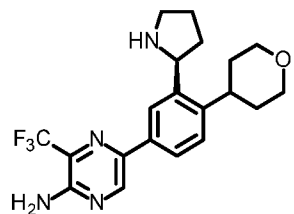
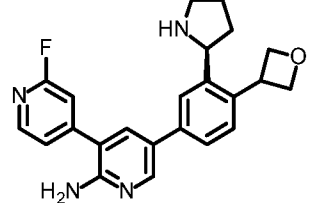
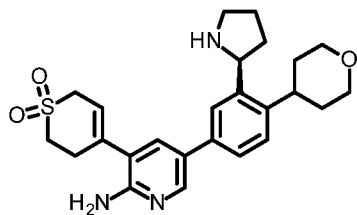
Compound	Structure	Quantity	Yield
	w/v %, MeOH, 23 °C); m.p. = 225° C (Mettler Toledo MP50)		
Co. 32 Starting from Intermediate 70	 $[\alpha]_D^{25}$: - 28.1° (589 nm, c 0.73 w/v %, MeOH, 23 °C); m.p. = 221.6° C (Mettler Toledo MP50)	0.093 g	49%
Co. 33 Starting from Intermediate 71	 $[\alpha]_D^{25}$: - 23.1° (589 nm, c 0.73 w/v %, MeOH, 23 °C); m.p. = 240° C (Mettler Toledo MP50)	0.071 g	39%
Co. 34 Starting from Intermediate 72		0.007 g	21%
Co. 35 Starting from Intermediate 73	 $[\alpha]_D^{25}$: - 16.6° (589 nm, c 0.53 w/v	0.049 g	82%

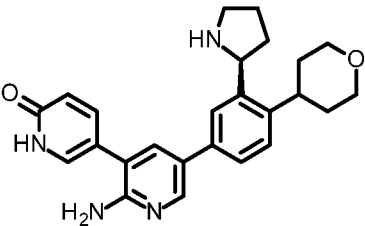
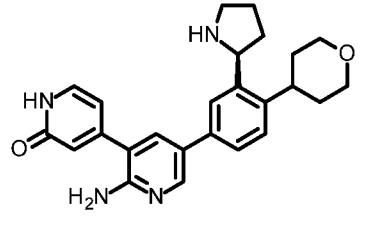
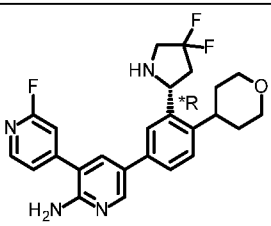
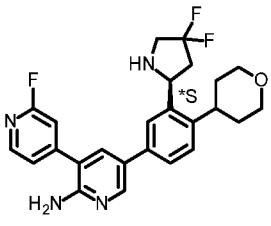
Compound	Structure	Quantity	Yield
	%, MeOH, 23 °C); m.p. = 218° C (Mettler Toledo MP50)		
Co. 36 Starting from Intermediate 74	 [α] _d : - 22.6° (589 nm, c 0.1 w/v %, MeOH, 23 °C); m.p. = 231.6° C (Mettler Toledo MP50)	0.049 g	82%
Co. 37 Starting from Intermediate 27	 [α] _d : - 42.1° (589 nm, c 0.24 w/v %, DMF, 20 °C); m.p. = 173° C (Mettler Toledo MP50)	0.052 g	65%
Co. 38 Starting from Intermediate 75	 0.015 g	37%	
Co.39 Starting from Intermediate 76	 [α] _d : - 18.7° (589 nm, c 0.13 w/v %, MeOH, 23 °C); m.p. =	0.075 g	64%

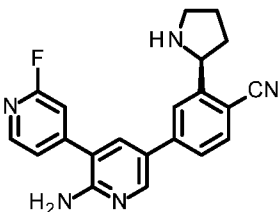
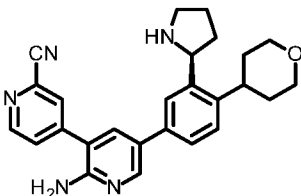
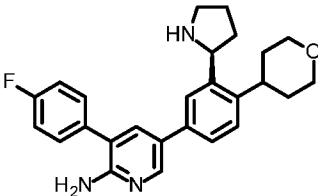
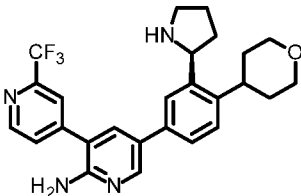
Compound	Structure	Quantity	Yield
	184.8° C (Mettler Toledo MP50)		
Co. 40 Starting from Intermediate 77		0.04 g	22%
Co. 41 Starting from Intermediate 78	 $[\alpha]_D^{25}$: - 33.9° (589 nm, c 0.1 w/v %, MeOH, 23 °C); m.p. = 216.5° C (Mettler Toledo MP50)	0.1 g	49%
Co. 42 Starting from Intermediate 79	 $[\alpha]_D^{25}$: - 37.47° (589 nm, c 0.13 w/v %, MeOH, 23 °C); m.p. = 262° C (Mettler Toledo MP50)	0.03 g	68%
Co. 43 Starting from Intermediate 80	 $[\alpha]_D^{25}$: - 24.8° (589 nm, c 0.08 w/v %, MeOH, 23 °C); m.p. = 217°	0.063 g	80%

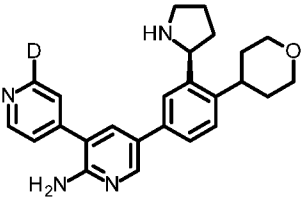
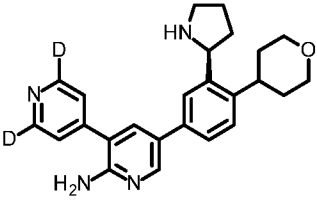
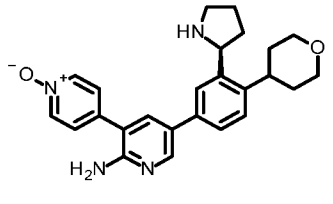
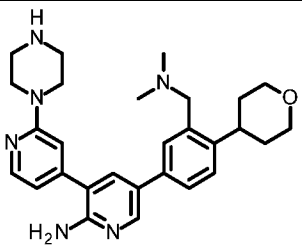
Compound	Structure	Quantity	Yield
	C (Mettler Toledo MP50)		
Co. 44 Starting from Intermediate 82	 $[\alpha]_d$: - 56.8° (589 nm, c 0.19 w/v %, DMF, 20 °C); m.p. = 206°C (DSC)	0.404 g	27%
Co. 45 Starting from intermediate 122	 $[\alpha]_d$: - 50.4° (589 nm, c 0.28 w/v %, DMF, 20 °C); m.p. = 206.7°C (DSC)	0.25 g	95%
Co. 46 Starting from intermediate 123	 m.p. = 224°C (DSC)	0.33 g	53%

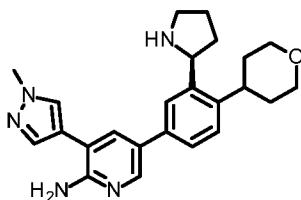
Compound	Structure	Quantity	Yield
Co. 47 Starting from intermediate 124		0.1 g	22%
Co. 48 Starting from intermediate 125 m.p. = 208°C (DSC)		0.41 g	97%
Co. 49 Starting from intermediate 126		0.05 g	29%
Co. 50 Starting from intermediate 129 m.p. = 219°C (DSC)		0.036 g	38%
Co. 51 Starting from intermediate 130		0.018 g	49%

Compound	Structure	Quantity	Yield
Co. 52 Starting from intermediate 131	 $[\alpha]_d$: - 26.2° (589 nm, c 0.29 w/v %, DMF, 20 °C); m.p. = 222°C (DSC)	0.016 g	26%
Co. 53 Starting from intermediate 133	 $[\alpha]_d$: - 44.55° (589 nm, c 0.11 w/v %, DMF, 20 °C)	0.019 g	40%
Co. 54 Starting from intermediate 132	 $[\alpha]_d$: - 90° (589 nm, c 0.42 w/v %, DMF, 20 °C)	0.02 g	13%
Co. 55 Starting from intermediate 39	 $[\alpha]_d$: - 33.7° (589 nm, c 0.27 w/v %, DMF, 20 °C)	0.045 g	20%

Compound	Structure	Quantity	Yield
Co. 56 Starting from intermediate 40		0.019 g	20%
Co. 57 Starting from intermediate 41	 $[\alpha]_d: -30.5^\circ$ (589 nm, c 0.22 w/v %, DMF, 20 °C)	0.039 g	40%
Co. 58 Starting from intermediate 142	 $[\alpha]_d: +26.8^\circ$ (589 nm, c 0.22 w/v %, DMF, 20 °C); m.p. = 216°C (DSC)	0.039 g	21%
Co. 59 Starting from intermediate 142	 $[\alpha]_d: -23.9^\circ$ (589 nm, c 0.26 w/v %, DMF, 20 °C); m.p. = 215°C (DSC)	0.045 g	24%

Compound	Structure	Quantity	Yield
Co. 60 Starting from intermediate 143		0.076 g	49%
Co. 61 Starting from intermediate 42	 $[\alpha]_d$: - 29.3° (589 nm, c 0.15, w/v %, DMF, 20 °C)	0.072 g	39%
Co. 62 Starting from intermediate 43	 $[\alpha]_d$: - 35.83° (589 nm, c 0.26 w/v %, DMF, 20 °C); m.p. = 216°C (DSC)	0.102 g	52%
Co. 63 Starting from intermediate 44	 $[\alpha]_d$: - 40° (589 nm, c 0.1, w/v %, DMF, 20 °C); m.p. = 213°C (DSC)	0.084 g	46%

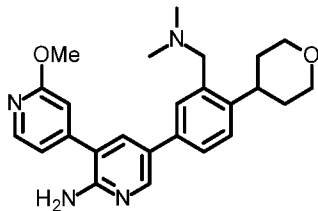
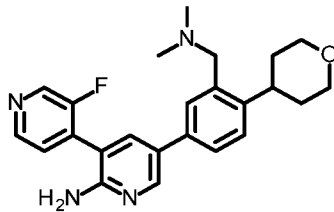
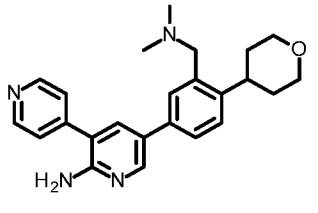
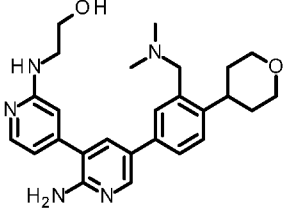
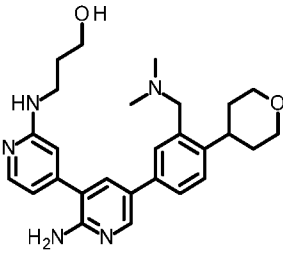
Compound	Structure	Quantity	Yield
Co. 64 Starting from intermediate 160		0.04 g	33 %
Co. 65 Starting from intermediate 162		0.106 g	65%
Co. 66 Starting from intermediate 84	 $[\alpha]_D$: -32.2° (589 nm, c 0.27, w/v %, DMF, 20 °C); m.p. > 260°C (Kofler)	0.110 g	48%
Co.67 Starting from intermediate 166	 m.p. = 185°C (Kofler)	0.05 g	48%

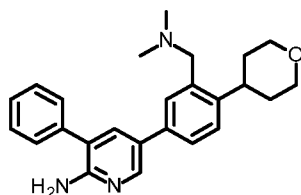
**Synthesis of Co.68 :**

Sodium carbonate (119 mg, 1.12 mmol) was added to a solution of intermediate **89** (200 mg, 0.497 mmol), 1-Methyl-1H-pyrazole-4-boronic acid pinacol ester (124 mg, 0.6 mmol) in water (1.16 mL) and 1,4-dioxane (5.8 mL). The mixture was degassed with a stream of nitrogen for 5 min., then PdCl₂(dppf) (34 mg, 0.041 mmol) was added. The reaction mixture was stirred at 90°C overnight in a sealed tube. The reaction mixture was cooled to rt, water and AcOEt were added. The aqueous layer was extracted with ethyl acetate. The organic layer was dried over MgSO₄, filtered and evaporated until dryness. The residue was purified by preparative LC (12 g SiOH 15-40 µm Interchim, mobile phase: DCM/MeOH/NH₄OH 97/3/0 to 90/10/0.1). The pure fractions were collected and evaporated until dryness to give 80 mg of **Co.68** (39%) which was taken up in Et₂O. The precipitate was filtered off and dried yielding 53 mg of **Co.68** (26%).

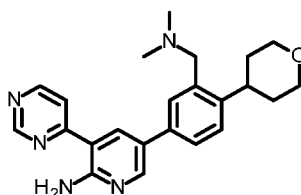
The compounds listed in the table below have been prepared by an analogous reaction protocol:

Compound	Structure	Quantity	Yield
Co. 69	 m.p. = 236°C (Kofler)	0.023 g	22%
Co. 70	 m.p. = 225°C	0.057 g	55%

Compound	Structure	Quantity	Yield
Co. 71 Starting from Intermediate 165	 m.p. = 222°C (Kofler)	0.056 g	52%
Co.72 Starting from Intermediate 165	 m.p. = 234°C (Kofler)	0.063 g	60%
Co.73 Starting from Intermediate 165	 m.p. = 227°C (DSC)	0.064 g	80%
Co.74 Starting from intermediate 165 and 2- (4-(4,4,5,5-tetramethyl- 1,3,2-dioxaborolan-2-yl) pyridin-2-ylamino) ethanol		0.039 g	34%
Co.75 Starting from intermediate 165 and 3- (4-(4,4,5,5-tetramethyl- 1,3,2-dioxaborolan-2-yl) pyridin-2-ylamino) propan-1-ol	 m.p. = 170°C (Kofler)	0.071g	60%

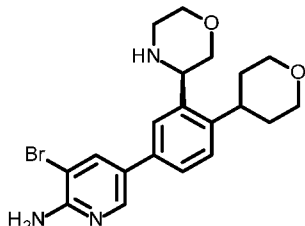
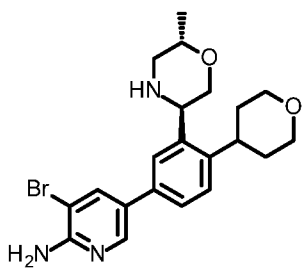
**Synthesis of Co.76:**

A mixture of intermediate **164** (0.119 g, 0.345 mmol), 5-bromo-3-phenylpyridine-2-amine (0.08 g, 0.321 mmol), PdCl₂(dppf) (0.028 g, 0.0345 mmol) and sodium carbonate (0.11 g, 1.034 mmol) in 1,4-dioxane (1.5 mL) and water (0.4 mL) in a sealed tube was stirred at 120°C using one single mode microwave (Biotage Initiator EXP 60) with a power output ranging from 0 to 400 W for 30 min. The reaction mixture was poured into water and potassium carbonate, extracted with AcOEt. The organic layer was dried over MgSO₄, filtered and evaporated until dryness. The residue was purified by preparative LC (24 g of SiOH 30µm Interchim, gradient from 100% DCM to 90% DCM 10% CH₃OH 0.1% NH₄OH). The fractions were collected and evaporated until dryness and the residue was crystallized in DIPE. The precipitate was filtered off and dried yielding **Co.76** (0.028g, 21%). m.p. = 173°C (Kofler).

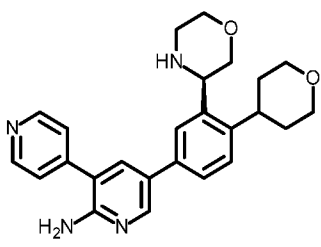
**Synthesis of Co.77:**

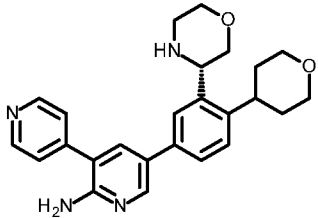
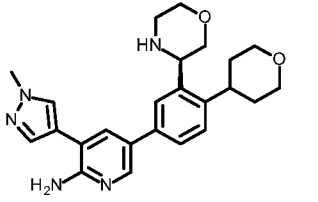
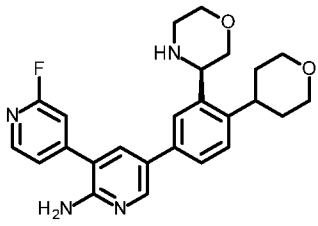
A mixture of intermediate **165** (0.100 g, 0.26 mmol), 4-(tributylstannyl)pyrimidine (0.114 g, 0.307 mmol), Copper iodide (11.7 mg, 0.062 mmol), Pd(PPh₃)₄ (0.021g, 0.018 mmol) and lithium chloride (0.016 g, 0.384 mmol) in 1,4-dioxane (5 mL) was stirred at 130°C in a Biotage Microwave for 20 minutes. The reaction mixture was poured into water, extracted with AcOEt. The organic layer was dried over MgSO₄, filtered and evaporated until dryness. The residue was purified by preparative LC (40g of SiOH, 95% DCM 5% CH₃OH 0.1% NH₄OH). The fractions were collected and evaporated until dryness yielding **Co.77** (0.051g, 51%).

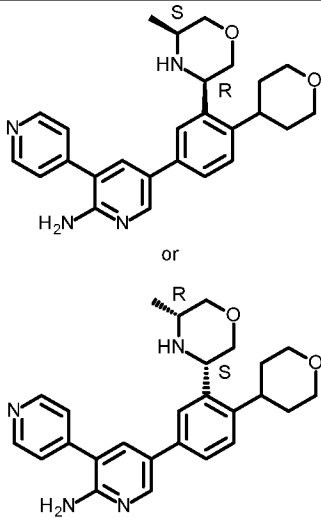
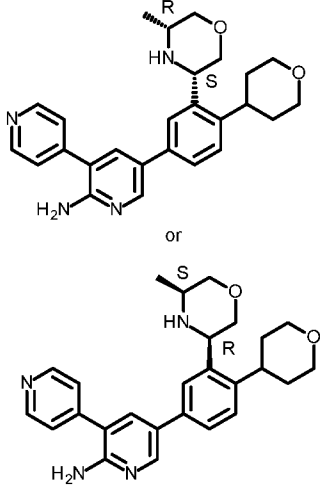
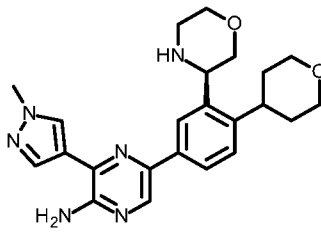
The compounds listed in the table below have been prepared by an analogous reaction protocol as described for the preparation of intermediate 27:

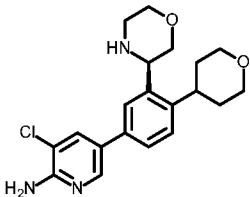
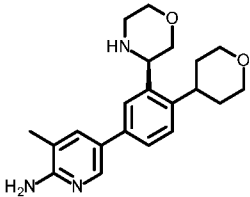
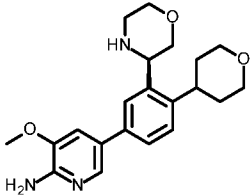
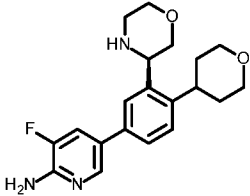
Compound	Structure	Quantity	Yield
Co. 78 Starting from Intermediate 96	 $[\alpha]_d$: - 77.8° (589 nm, c 0.27 w/v %, DMF, 20 °C); m.p. = 208°C (DSC)	0.5 g	56%
Co. 79 Starting from Intermediate 97		1.85 g	66%

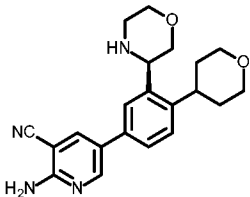
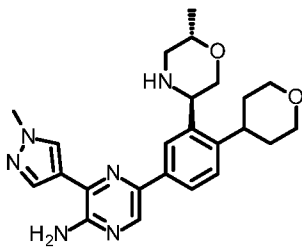
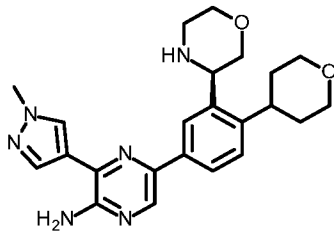
The compounds listed in the table below have been prepared by an analogous reaction protocol as for preparation of intermediate 64:

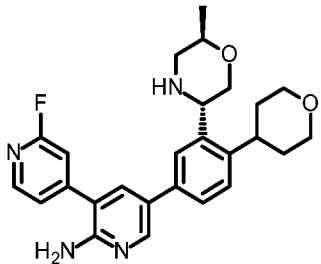
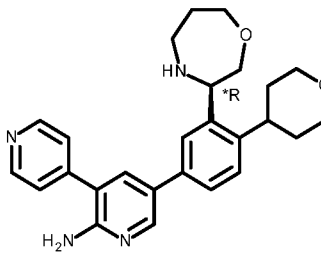
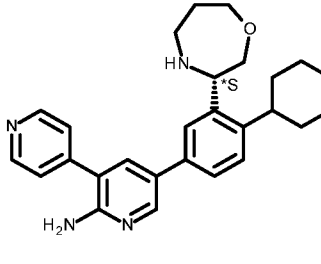
Compound	Structure	Quantity	Yield
Co. 80a Starting from Intermediate 98 and 5-bromo-[3,4'-bipyridin]-2-amine	 $[\alpha]_d$: - 28.4° (589 nm, c 0.26 w/v %, DMF, 20 °C)	0.07 g	24%

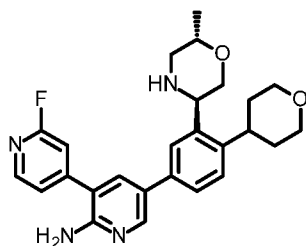
Compound	Structure	Quantity	Yield
Co. 80b	 $[\alpha]_d$: +48° (589 nm, c 0.11 w/v %, DMF, 20 °C)	0.022 g	17%
Co. 81 Starting from Intermediate 98 and 5- bromo-3-(1-methyl- 1H-pyrazol-4-yl) pyridin-2-amine	 $[\alpha]_d$: - 70° (589 nm, c 0.21 w/v %, DMF, 20 °C)	0.025 g	10%
Co. 82 Starting from Intermediate 98 and 17	 $[\alpha]_d$: - 73.25° (589 nm, c 0.24 w/v %, DMF, 20 °C)	0.072 g	36%

Compound	Structure	Quantity	Yield
Co. 83a Starting from Intermediate 99 and 5- bromo-[3,4'-bipyridin]- 2-amine	 $[\alpha]_d$: - 84.9° (589 nm, c 0.35 w/v %, DMF, 20 °C)	0.04 g	5%
Co. 83b	 $[\alpha]_d$: +82.2° (589 nm, c 0.35 w/v %, DMF, 20 °C)	0.036 g	4%
Co. 84 Starting from Intermediate 98 and 2- pyrazinamine, 5- bromo-3-(1-methyl- 1H-pyrazol-4-yl)	 $[\alpha]_d$: - 79.6° (589 nm, c 0.26 w/v %, DMF, 20 °C) m.p. =	0.06 g	23%

Compound	Structure	Quantity	Yield
	243°C (DSC)		
Co. 85 Starting from Intermediate 98 and 2- pyridinamine, 5- bromo-3-chloro	 $[\alpha]_d$: - 82.4° (589 nm, c 0.25 w/v %, DMF, 20 °C) m.p. = 213°C (DSC)	0.148 g	52%
Co. 86 Starting from Intermediate 98 and 2- Amino-5-bromo-3- methylpyridine	 $[\alpha]_d$: - 22.3° (589 nm, c 0.22 w/v %, DMF, 20 °C) m.p. = 266°C (DSC)	0.069 g	32%
Co. 87 Starting from Intermediate 98 and 5- bromo-3- methoxypyridin-2- amine	 $[\alpha]_d$: - 78.9° (589 nm, c 0.19 w/v %, DMF, 20 °C) m.p. = 239°C (DSC)	0.053 g	23%
Co. 88 Starting from Intermediate 98 and 5- bromo-3-fluoropyridin-	 $[\alpha]_d$: - 83.5° (589 nm, c 0.2	0.089 g	41%

Compound	Structure	Quantity	Yield
2-amine	w/v %, DMF, 20 °C) m.p. = 225°C (DSC)		
Co. 89 Starting from Intermediate 98 and 2-Amino-5-bromonicotinonitrile	 $[\alpha]_d$: - 108.3° (589 nm, c 0.24 w/v %, DMF, 20 °C) m.p. = 258°C (DSC)	0.086 g	39%
Co. 90 Starting from Intermediate 100a and 5-bromo-3-(1-methyl-1H-pyrazol-4-yl)pyrazin-2-amine	 $[\alpha]_d$: - 45.6° (589 nm, c 0.16 w/v %, DMF, 20 °C) m.p. = 246°C (DSC)	0.195 g	61%
Co. 91 Starting from Intermediate 98 and 5-bromo-3-(1-methyl-1H-pyrazol-4-yl)pyrazin-2-amine	 $[\alpha]_d$: - 73.2° (589 nm, c 0.25 w/v %, DMF, 20 °C) m.p. = 242°C (DSC)	0.76 g	46%

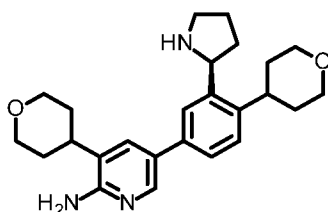
Compound	Structure	Quantity	Yield
Co.92 Starting from Intermediate 100b and 17	 $[\alpha]_d: +60.6^\circ$ (589 nm, c 0.17 w/v %, DMF, 20 °C) m.p. = 209°C (DSC)	0.052 g	19%
Co.93a Starting from Intermediate 101 and 5-bromo-[3,4'- bipyridin]-2-amine		0.02 g	3.5 %
Co.93b Starting from Intermediate 101 and 5-bromo-[3,4'- bipyridin]-2-amine		0.028 g	5 %



Synthesis of Co.94 :

Sodium carbonate (0.402 g, 3.79 mmol) was added to a solution of **Co.79** (0.82 g, 1.90 mmol), 2-Fluoropyridine-4-boronic acid pinacol ester (0.508 g, 2.28 mmol) in water

(1.8 mL) and 1,4-dioxane (18 mL). The mixture was degassed with a stream of nitrogen for 5 min., then PdCl₂(dppf) (0.085 g, 0.104 mmol) was added. The reaction mixture was stirred at 90°C for 6 hours in a sealed tube. The reaction mixture was cooled to rt, poured into water and K₂CO₃. The aqueous layer was extracted with ethyl acetate. The organic layer was dried over MgSO₄, filtered and evaporated until dryness. The residue was purified by preparative LC (40 g SiOH 15 µm Interchim, mobile phase : DCM/MeOH/NH₄OH 100/0/0 to 90/10/0.1). The pure fractions were collected and evaporated until dryness. The compound was taken up in diisopropylether, stirred, filtered and dried yielding **Co.94** (0.735 g, 85%). [α]_D: - 82.7° (589 nm, c 0.22 w/v %, DMF, 20 °C).

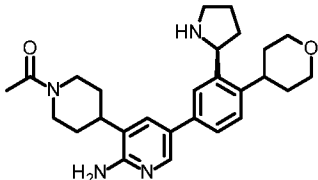


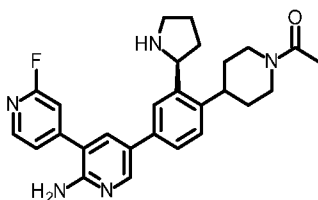
Synthesis of Co.95 :

To a solution of intermediate **120** (0.240 g, 0.331 mmol) in DCM (5 mL) was added trifluoroacetic acid (2.54 mL) dropwise under an ice bath. The resulting reaction mixture was stirred at room temperature for 3 hours. The mixture was evaporated in vacuo and the crude mixture was neutralized with NaHCO₃ until basic-pH. The solvents were evaporated in vacuo and the crude was purified by reverse phase: Column: Brand Phenomenex; Type Gemini; I.D. (mm) 100 x 21.2; Particle size 5µm (C18). Method: From 81 % of H₂O (25mM NH₄HCO₃)- 19 % (ACN: MeOH 1:1) until 45 % of H₂O (25mM NH₄HCO₃)- 55 % (ACN: MeOH 1:1). The desired fractions were collected and the solvents were evaporated in vacuo. The solid was washed with acetonitrile and evaporated under high vacuum three times at 60°C until a white solid appeared to give pure **Co.95** (0.062g, 45%). [α]_D: - 19.3° (589 nm, c 0.13 w/v %, methanol, 23°C). m.p. = 223° C (Mettler Toledo MP50)

The compound listed in the table below was prepared by an analogous reaction protocol:

Compound	Structure	Quantity	Yield
Co. 96			

Compound	Structure	Quantity	Yield
Starting from intermediate 159	 [α]_d: - 44.6° (589 nm, c 0.12 w/v %, MeOH, 23 °C) m.p. = 237° C (Mettler Toledo MP50)	0.115 g	75%

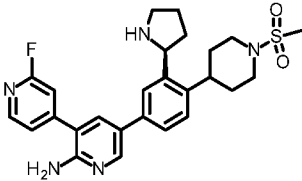
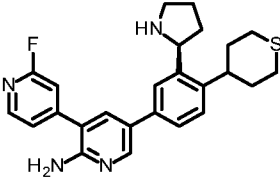
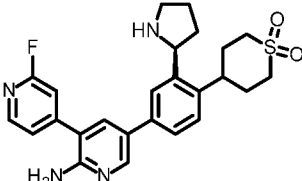
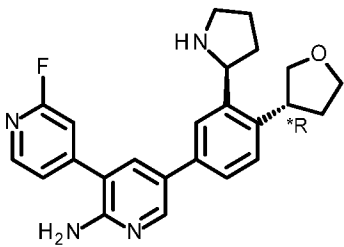
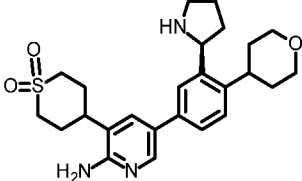


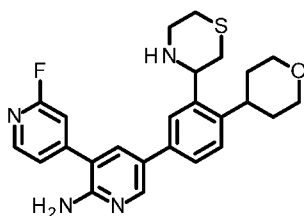
Synthesis of Co.97:

- A mixture of **Co.47** (0.324 g, 0.709 mmol), Pd/C 10% (0.3g, 0.282 mmol) in THF (10 mL) was hydrogenated under 10 bars pressure at 55°C overnight. The reaction mixture
- 5 was filtered over celite, washed with DCM and the filtrate was evaporated. The residue was purified by preparative LC (12 g SiOH 40 µm irregular, mobile phase: DCM/MeOH/NH₄OH 95/5/1 to 90/10/1). The pure fractions were collected and evaporated until dryness. The compound was taken up in diisopropylether, stirred, filtered and dried yielding **Co.97** (0.08 g, 25%). [α]_d: - 30.71° (589 nm, c 0.28 w/v %, 10 DMF, 20 °C).

The compounds listed in the table below have been prepared by an analogous reaction protocol:

Compound	Structure	Quantity	Yield
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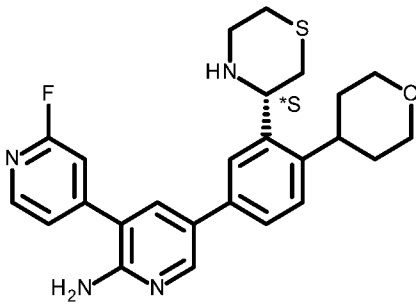
Compound	Structure	Quantity	Yield
Co. 98 Starting from Co.46	 $[\alpha]_d: -16.8^\circ$ (589 nm, c 0.28 w/v %, DMF, 20 °C)	0.1 g	35%
Co. 99 Starting from Co.48	 m.p. = 222°C (DSC)	0.097 g	27 %
Co. 100 Starting from Co. 49		0.15 g	45 %
Co. 101 Starting from Co. 45		0.05 g	20%
Co. 102 Starting from Co. 55	 $[\alpha]_d: -33.7^\circ$ (589 nm, c 0.27 w/v %, DMF, 20 °C)	0.024g	28%

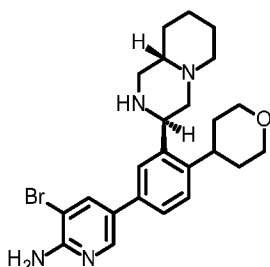


Synthesis of Co.103 :

- Intermediate **90** (1.45 g, 3.84 mmol) and molecular sieves 4A (0.2 g) were added to SLAP TM (0.78 g, 4.8 mmol) in DCM (16 mL). The reaction mixture was stirred at rt 5 h, and filtered over celite®. The filtrate was evaporated until dryness to afford the imine which was added to a solution of [Ir(dtbbpy)(ppy)₂](PF₆) (35 mg, 0.0384 mmol), Copper (II) trifluoromethanesulfonate (1.39 g, 3.85 mmol) and Bismuth(III) trifluoromethane sulfonate (1.26 g, 1.93 mmol) in MeCN (80 mL). The reaction mixture was stirred at rt for 16 h under blue light (34W - Hepatochem photoreactor – irradiated with a Kessil lamp). The reaction mixture was poured into H₂O and K₂CO₃ and extracted with DCM. The organic layer was dried over MgSO₄, filtered and evaporated until dryness. The residue was purified by preparative LC twice (Stationary phase: irregular SiOH 15-40µm 80 and 40g GraceResolv, gradient from DCM/MeOH/NH₄OH 98/2/0 to 95/5/0.1). The fractions were collected and evaporated until dryness yielding **Co.103** (1.09 g, 63%).
- The compounds listed in the table below have been prepared by an analogous reaction protocol as was described for preparation of Co.103:

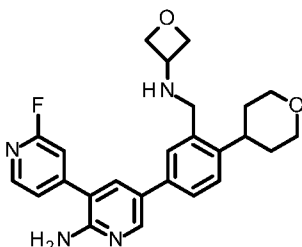
Compound	Structure
Co. 103a	[α]_D: - 40.8° (589 nm, c 0.26 w/v %, DMF, 20 °C)

Compound	Structure
Co. 103b	 [α]_D: +77.7° (589 nm, c 0.26 w/v %, DMF, 20 °C)



Synthesis of Co.104 :

A mixture of intermediate **86** (100 mg, 0.28 mmol), SLAP hydropyridopyrazine (55 mg, 0.28 mmol), molecular sieves (30 mg) in DCM (7 mL) was stirred at rt for 5h. The imine was filtered off over celite, and the filtrate was evaporated. Acetonitrile (4 mL), and trifluoromethanol (1 mL) and DCM (3 mL) were added and then the photocatalyst [Ir(dtbbpy)(ppy)₂PF₆] (2.5mg). The reaction mixture was stirred at rt for 16h under blue light (34W - Hepatochem photoreactor – irradiated with a Kessil lamp). Water and AcOEt were added, the aqueous phase was extracted, the organic layer was separated, dried over MgSO₄, filtered and evaporated. A first purification was performed via preparative LC (Stationary phase: irregular SiOH 40 μm 4g, Mobile phase: 98/2 to 90/10/1 DCM/MeOH/NH₄OH) followed by a second one via reverse phase (Stationary phase: YMC-actus Triart C18 10μm 30*150mm, Mobile phase: Gradient from 65% NH₄HCO₃ 0.2% , 35% ACN to 25% NH₄HCO₃ 0.2% , 75% ACN) yielding **Co. 104** (15 mg, 11%).



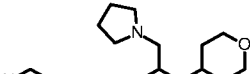
Synthesis of Co.105 :

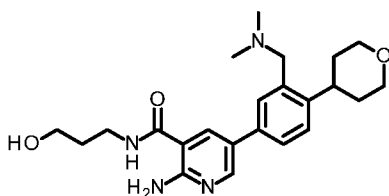
Sodium triacetoxyborohydride (0.168 g, 0.8 mmol) was added portionwise to a mixture of intermediate **90** (0.150 g, 0.4 mmol), 3-Oxetanamine (0.090 g, 1.2 mmol) in DCM (3mL) at room temperature. The reaction mixture was stirred for 15 hours.

- 5 Water was added and the mixture was basified with potassium carbonate. The organic layer was extracted, dried over MgSO_4 , filtered and evaporated until dryness. The residue was purified by preparative LC (12 g of SiOH 15 μm Interchim, gradient from 100% DCM to 90% DCM 10% CH_3OH 0.1% NH_4OH). The fractions were collected and evaporated until dryness and the compound was crystallized in diisopropylether,
- 10 filtered and dried to give **Co.105** (0.085 g, 49%).

The compounds listed in the table below have been prepared by an analogous reaction protocol:

Compound	Structure	Quantity	Yield
Co. 106 Starting from intermediate 91	 m.p. = 245°C (DSC)	0.023 g	13%
Co. 107 Starting from intermediate 91		0.02 g	11%

Compound	Structure	Quantity	Yield
Co. 108 Starting from intermediate 91		0.026 g	26%

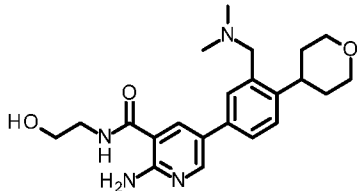
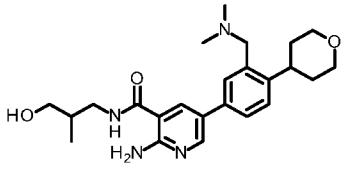


Synthesis of Co.109 :

Aminopropanol (0.045 mL, 0.59 mmol) was added to a stirring solution of intermediate **169** (0.15 g, 0.422 mmol), DIPEA (0.147 mL, 0.84 mmol), 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (0.121 g, 0.63 mmol), HOBt (0.086 g, 0.63 mmol) in DCM (3.2 mL) and DMSO (1.5 mL). The reaction mixture was stirred at rt overnight. To complete the reaction, 1.5 eq of 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride, 1.5 eq of HOBt, 1.4 eq of aminopropanol, 2 eq of DIPEA were added. After 14h at rt, the reaction mixture was diluted with water (5 mL) and DCM (20 mL). The layers were separated and the aqueous phase extracted with DCM (3 x 5 mL). The organic layer was dried over MgSO₄, filtered and evaporated until dryness. The residue was purified by flash chromatography (silica, gradient: 0-100% DCM-DCM/MeOH (4/1)). The desired fractions were collected and evaporated yielding Co. **109** (0.092 g, 52%). m.p. = 203°C

The compounds listed in the table below have been prepared by an analogous reaction protocol:

Compound	Structure	Quantity	Yield
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Compound	Structure	Quantity	Yield
Co. 110 Starting from intermediate 169	 m.p. = 272°C (MP50 Mettler Toledo)	0.060 g	35%
Co. 111 Starting from intermediate 169	 m.p. = 228°C (MP50 Mettler Toledo)	0.075g	41%

Analytical Part

OPTICAL ROTATION (OR)

- Optical rotations were measured at 20°C or 23°C on a Perkin Elmer 341 digital polarimeter at $\lambda = 589$ nm (i.e., sodium D line), using a 0.2 mL cell ($l = 1$ dm), and are given as $[\alpha]_D$ (concentration in g/100 mL solvent).

LCMS (Liquid Chromatography/Mass spectrometry)

LCMS General procedure

- The High Performance Liquid Chromatography (HPLC) measurement was performed using a LC pump, a diode-array (DAD) or a UV detector and a column as specified in the respective methods. If necessary, additional detectors were included (see table of methods below).
- Flow from the column was brought to the Mass Spectrometer (MS) which was configured with an atmospheric pressure ion source. It is within the knowledge of the skilled person to set the tune parameters (e.g. scanning range, dwell time, etc.) in order to obtain ions allowing the identification of the compound's nominal monoisotopic molecular weight (MW). Data acquisition was performed with appropriate software.
- Compounds are described by their experimental retention times (R_t) and ions. If not specified differently in the table of data, the reported molecular ion corresponds to the

[M+H]⁺ (protonated molecule) and/or [M-H]⁻ (deprotonated molecule). In case the compound was not directly ionizable the type of adduct is specified (i.e. [M+NH₄]⁺, [M+HCOO]⁻, etc.). For molecules with multiple isotopic patterns (Br, Cl, etc.), the reported value is the one obtained for the lowest isotope mass. All results were obtained with experimental uncertainties that are commonly associated with the method used.

Hereinafter, “MSD” means Mass Selective Detector, “DAD” Diode Array Detector.

10 Table: LCMS Method codes (Flow expressed in mL/min; column temperature (T) in °C; Run time in minutes).

Method code	Instrument	Column	Mobile phase	gradient	Flow	Run time
					Column T	
1	Agilent: 1100-DAD and MSD	YMC: Pack ODS-AQ (3μm, 4.6x50mm)	A: HCOOH 0.1% in water, B: CH ₃ CN	95% A to 5% A in 4.8min, held for 1min, back to 95% A in 0.2min.	2.6	6
					35	
					60	
2	Agilent 1290 Infinity DAD TOF-LC/MS G6224A	YMC-pack ODS-AQ C18 (50 x 4.6 mm, 3 μm)	A: 0.1% HCOOH in H ₂ O B: CH ₃ CN	ISET 2V1.0 Emulated Agilent Pump G1312A V1.0 From 94.51% A to 5% A in 4.8 min, held for 1.0 min, to 95% A in 0.2 min	2.6	6
					35	
3	Waters: Acquity UPLC® - DAD and Quattro	Waters: BEH C18 (1.7μm, 2.1x100mm)	A: 95% CH ₃ COON H ₄ 7mM / 5%CH ₃ CN, B: CH ₃ CN	84.2% A for 0.49min, to 10.5% A in 2.18min, held for 1.94min, back to	0.343	6.2
					40	

	Micro TM			84.2% A in 0.73min, held for 0.73min.		
4	Waters: Acquity [®] H-Class - DAD and SQD2 TM	Waters: BEH C18 (1.7 μ m, 2.1x100mm)	A: 95% CH ₃ COONH ₄ 7mM / 5%CH ₃ CN, B: CH ₃ CN	84.2% A to 10.5% A in 2.18 min, held for 1.96 min, back to 84.2% A in 0.73 min, held for 0.73 min.	0.343	6.1
					40	
5	Waters: Acquity [®] UPLC - DAD and Quattro TM	Waters HSS [®] T3 (1.8 μ m, 2.1x100mm)	A: CH ₃ COONH ₄ 7mM 95%/CH ₃ CN 5%, B: CH ₃ CN	99%A/1%B for 0.5min, to 15%A in 3.5min, held for 2min, back to 99% A/1%B in 0.5min, held for 1.5min.	0.35	8
					40	
					40	

Melting Points

For a number of compounds, melting points (m.p.) were determined with a DSC 1 STAR[®] System from Mettler Toledo (method 1) or a Mettler Toledo MP50 (method 2) or a Kofler bench (method 3). Melting points were measured with a temperature gradient of 10°C/minute up to 350 °C. Melting points are given by peak values.

The results of the analytical measurements are shown in Table 3.

Table 3: Retention time (R_t) in min., $[M+H]^+$ peak (protonated molecule), LCMS method and m.p. (melting point in °C) (n.d. means not determined).

Co. No.	R_t	$[M+H]^+$	LC MS Meth od	m.p. (°C)	m.p. metho d
1	2.03	419.3	3	218	1
2	2.02	420.3	3	>300	2

Co. No.	R_t	$[M+H]^+$	LC MS Meth od	m.p. (°C)	m.p. metho d
6	1.53	430.2	1	196	2
9	1.44	415.2	1	218	2

Co. No.	R _t	[M+H] ⁺	LC MS Method	m.p. (°C)	m.p. method
10	1.42	415.2	1	n.d.	
11	1.79	403.3	3	245	2
12	1.59	403.3	3	196	2
13	1.20	398.1	1	257	2
14	1.46	399.1	1	263	2
15	1.60	399.1	1	>300	2
16	1.35	375.1	1	265	2
17	2.10	435.2	3	n.d.	
18	2.09	444.2	3	n.d.	
19	2.08	468.2	3	n.d.	
20	2.34	407.2	3	n.d.	
21	1.76	434.2	3	n.d.	
22	2.16	432.2	3	192	1
23	1.78	390.1	3	223	1
24	1.92	431.2	3	218	1
25	1.90	401.2	3	238	1
26	1.90	402.3	3	201	2
27	1.82	437.2	1	>300	2
28	n.d.	419.2	1	230	2
29	1.79	468.2	1	228	2
31	1.79	437.2	1	225	2
32	1.86	453.1	1	222	2
33	1.37	402.2	1	240	2
34	2.17	451.2	3	n.d.	
35	1.88	453.1	1	218	2
36	1.74	425.1	1	232	2
37	2.16	404.0	3	173	1

Co. No.	R _t	[M+H] ⁺	LC MS Method	m.p. (°C)	m.p. method
38	2.84	417.2	3	n.d.	
39	2.07	405.3	3	185	
40	2.13	471.6	4	n.d.	
41	1.82	405.2	1	217	2
43	2.03	420	1	217	2
44	1.90	349.2	3	206	1
45	2.05	403.1	3	207	1
46	2.11	494.2	3	224	1
47	1.96	458.2	3	227	1
48	2.37	433.1	3	208	1
49	1.94	465.1	3	n.d.	
50	2.49	453.2	3	219	1
51	2.34	447.3	3	n.d.	
52	2.07	472.2 [M+CH ₃ CN] ⁺	3	222	1
53	2.21	393.1	3	219	1
54	1.86	391	3	n.d.	
55	1.67	454.5	4	n.d.	
56	1.44	417.5	4	n.d.	
57	1.47	417.5	4	n.d.	
58	2.57	455.5	4	216	1
59	2.55	455.5	4	215	1
60	1.85	360.1	3	n.d.	
61	2.06	426.3	3	n.d.	
62	2.33	418.2	3	216	1
63	2.27	469.3	3	213	1
64	1.91	402.2	3	n.d.	

Co. No.	R _t	[M+H] ⁺	LC MS Meth od	m.p. (°C)	m.p. metho d
65	1.78	403	4	n.d.	
66	2.56	417.4	5	>260	3
67	1.79	473.6	4	185	3
68	1.73	404.6	4	n.d.	
69	2.02	403.6	4	236	3
70	2.19	403.1	3	225	3
71	2.45	419.1	3	222	3
72	2.04	407.5	4	234	3
73	2.04	389.1	3	227	1
74	1.81	448.6	4	n.d.	
75	2.00	462.2	3	170	3
76	2.67	388	3	173	3
77	2.12	390.1	3	n.d.	
78	2.32	418.1	3	208	1
79	2.45	474.9 [M+CH ₃ CN] ⁺	3	176	1
81	2.05	420.1	3	n.d.	
82	2.30	435.2	3	n.d.	
84	1.98	421.2	3	243	1
85	2.25	374	3	213	1
86	2.05	354	3	266	1
87	2.03	370	3	239	1
88	2.08	358	3	225	1
89	2.10	406.0 [M+CH ₃ CN] ⁺	3	258	1
90	2.11	435.2	3	246	1
92	2.46	449.2	3	209	1

Co. No.	R _t	[M+H] ⁺	LC MS Meth od	m.p. (°C)	m.p. metho d
94	2.46	449.2	3	205	1
95	1.91	408.4	3	223	2
96	1.37	449.4	1	237	2
98	2.11	496.4	3	n.d.	
99	2.41	435.1	3	222	1
100	1.94	467.1	3	n.d.	
101	1.99	405.1	3	n.d.	
102	1.80	456.2	3	n.d.	
105	2.25	435.2	3	n.d.	
106	2.91	415.2	3	245	1
107	2.75-2.79	451.2	3	n.d.	
108	2.06	415.2	3	n.d.	
109	1.16	413.3	2	203	2
110	1.05	399.2	2	272	2
111	1.26	427.3	2	228	2
103a	2.55	451.3	3	n.d.	
103b	2.55	451.3	3	n.d.	
15a	1.97	508.3 [M+CH ₃ CN] ⁺	3	n.d.	
15b	1.97	467.2	3	n.d.	
15c	2.02	467.2	3	n.d.	
15d	2.02	467.2	3	n.d.	
1b	1.63	419	1	n.d.	
25b	1.89	401.2	3	n.d.	
5a	2.32	445.2	3	n.d.	
5b	2.32	445.1	3	n.d.	

Co. No.	R _t	[M+H] ⁺	LC MS Method	m.p. (°C)	m.p. method
7a	2.47	459.2	3	n.d.	
7b	2.46	459.2	3	n.d.	
80a	2.05	417.2	3	n.d.	
80b	2.05	417.3	3	n.d.	
83a	2.17	431.4	3	n.d.	
83b	2.20	431.3	3	n.d.	

Co. No.	R _t	[M+H] ⁺	LC MS Method	m.p. (°C)	m.p. method
8a	2.09	427.2	3	n.d.	
8b	2.08	427.2	3	n.d.	
93a	1.88	431.6	4	n.d.	
93b	1.89	431.6	4	n.d.	

NMR

Some NMR experiments were carried out using a Bruker Avance 500 spectrometer equipped with a Bruker 5mm BBFO probe head with z gradients and operating at 500 MHz for the proton and 125 MHz for carbon. Chemical shifts (δ) are reported in parts per million (ppm). J values are expressed in Hz.

Some NMR experiments were carried out using a Bruker Avance III 400 spectrometer at ambient temperature (298.6 K), using internal deuterium lock and equipped with reverse double-resonance (^1H , ^{13}C , SEI) probe head with z gradients and operating at 400 MHz for the proton. Chemical shifts (δ) are reported in parts per million (ppm). J values are expressed in Hz.

Table 4: ^1H NMR results

Co. No.	^1H NMR result
1	^1H NMR (500 MHz, DMSO- d_6) δ 8.28-8.37 (m, 2H), 7.76 (d, $J=1.98$ Hz, 1H), 7.68 (d, $J=2.44$ Hz, 1H), 7.52 (td, $J=1.56, 5.11$ Hz, 1H), 7.44 (dd, $J=2.06, 8.01$ Hz, 1H), 7.35 (s, 1H), 7.29 (d, $J=8.09$ Hz, 1H), 6.08 (s, 2H), 4.32 (t, $J=7.78$ Hz, 1H), 3.91-4.02 (m, 2H), 3.43-3.54 (m, 2H), 3.33 (br s, 2H), 3.05-3.19 (m, 2H), 2.88 (td, $J=7.67, 9.54$ Hz, 1H), 2.71 (br s, 1H), 2.10-2.20 (m, 1H), 1.66-1.88 (m, 4H), 1.51-1.65 (m, 2H), 1.34-1.49 (m, 1H)
25	^1H NMR (400 MHz, DMSO- d_6) δ ppm 8.63 - 8.68 (m, 2H), 8.36 (d, $J=2.3$ Hz, 1H), 7.77 (d, $J=1.8$ Hz, 1H), 7.70 (d, $J=2.3$ Hz, 1H), 7.52 - 7.59 (m, 3H), 7.36 (d, $J=8.2$ Hz, 1H), 6.00 (s, 2H), 4.62 (t, $J=8.2$ Hz, 1 H), 3.96 (br d, $J=11.1$ Hz, 2H), 3.45 - 3.55 (m, 2H), 3.20 - 3.30 (m, 1 H), 3.07-3.18 (m, 2H), 2.19 - 2.30 (m, 1H), 1.85 - 2.03 (m, 2H), 1.61 - 1.82 (m, 4H), 1.56 (br d, $J=12.7$ Hz, 1H)
41	^1H NMR (500 MHz, DMSO- d_6) δ ppm 8.37 (s, 1H), 8.32 (s, 1H), 8.12 (s, 1H), 8.04 (s, 1H), 7.80 (br d, $J=7.9$ Hz, 1H), 7.32 (br d, $J=8.2$ Hz, 1H), 6.12 (br s, 2H), 4.39 (br t, $J=7.6$ Hz, 1H), 3.88 - 4.02 (m, 5H), 3.49 (br t, $J=11.3$ Hz, 2H), 3.09 - 3.21 (m, 2H), 2.89 - 3.02 (m, 1H), 2.11 - 2.23 (m, 1H), 1.68 - 1.96 (m, 4H), 1.54 - 1.68 (m, 2H), 1.43 - 1.54 (m, 1H)

Co. No.	¹ H NMR result
44	¹ H NMR (500 MHz, DMSO- <i>d</i> ₆) δ ppm 8.50 (d, <i>J</i> =2.5 Hz, 1H), 8.14 (d, <i>J</i> =2.5 Hz, 1H), 7.73 (d, <i>J</i> =1.9 Hz, 1H), 7.42 (dd, <i>J</i> =7.9, 1.9 Hz, 1H), 7.30 (d, <i>J</i> =8.2 Hz, 1H), 6.92 (s, 2H), 4.33 (t, <i>J</i> =7.7 Hz, 1H), 3.89 - 4.00 (m, 2H), 3.43 - 3.53 (m, 2H), 3.06 - 3.20 (m, 2H), 2.83 - 2.94 (m, 1H), 2.70 (br s, 1H), 2.08 - 2.22 (m, 1H), 1.66 - 1.90 (m, 4H), 1.50 - 1.65 (m, 2H), 1.44 (dq, <i>J</i> =11.7, 8.4 Hz, 1H)
73	¹ H NMR (500 MHz, DMSO- <i>d</i> ₆) δ ppm 8.65 (d, <i>J</i> =5.7 Hz, 2H), 8.32 (d, <i>J</i> =2.2 Hz, 1H), 7.66 (d, <i>J</i> =1.9 Hz, 1H), 7.57 (d, <i>J</i> =5.7 Hz, 2H), 7.52 (dd, <i>J</i> =7.9, 1.3 Hz, 1H), 7.47 (s, 1H), 7.33 (d, <i>J</i> =8.2 Hz, 1H), 5.92 (s, 2H), 3.96 (br dd, <i>J</i> =11.0, 3.5 Hz, 2H), 3.38 - 3.52 (m, 4H), 3.12 - 3.26 (m, 1H), 2.17 (s, 6H), 1.70 (qd, <i>J</i> =12.3, 3.8 Hz, 2H), 1.58 - 1.64 (m, 2H)
78	¹ H NMR (500 MHz, DMSO- <i>d</i> ₆) δ ppm 8.25 (d, <i>J</i> =1.9 Hz, 1H), 7.99 (d, <i>J</i> =1.9 Hz, 1H), 7.75 (d, <i>J</i> =1.9 Hz, 1H), 7.46 (dd, <i>J</i> =8.2, 1.9 Hz, 1H), 7.32 (d, <i>J</i> =8.2 Hz, 1H), 6.28 (s, 2H), 4.12 (dd, <i>J</i> =9.9, 2.7 Hz, 1H), 3.96 (td, <i>J</i> =10.7, 3.8 Hz, 2H), 3.76 (br d, <i>J</i> =9.5 Hz, 1H), 3.65 (dd, <i>J</i> =10.9, 2.7 Hz, 1H), 3.46 - 3.56 (m, 3H), 3.25 (d, <i>J</i> =10.4 Hz, 1H), 3.10 - 3.19 (m, 1H), 2.97 (td, <i>J</i> =12, 2.8 Hz, 1H), 2.91 (d, <i>J</i> =11 Hz, 1H), 2.77 (br s, 1H), 1.79 (qd, <i>J</i> =12.3, 4.3 Hz, 1H), 1.60 - 1.73 (m, 2H), 1.56 (br d, <i>J</i> =12.6 Hz, 1H)
84	¹ H NMR (500 MHz, DMSO- <i>d</i> ₆) δ ppm 8.37 (s, 1H), 8.32 (s, 1H), 8.14 (d, <i>J</i> =1.6 Hz, 1H), 8.04 (s, 1H), 7.85 (dd, <i>J</i> =8.0, 1.7 Hz, 1H), 7.35 (d, <i>J</i> =8.2 Hz, 1H), 6.15 (s, 2H), 4.14 (br dd, <i>J</i> =9.8, 2.2 Hz, 1H), 3.89 - 4.02 (m, 5H), 3.78 (br d, <i>J</i> =10.7 Hz, 1H), 3.67 (dd, <i>J</i> =10.7, 2.5 Hz, 1H), 3.46 - 3.59 (m, 3H), 3.25-3.35 (m, 1H, partially obscured by solvent peak), 3.15 - 3.23 (m, 1H), 2.88 - 3.02 (m, 2H), 2.72 (br s, 1H), 1.81 (qd, <i>J</i> =12.3, 4.3 Hz, 1H), 1.62 - 1.74 (m, 2H), 1.58 (br d, <i>J</i> =12.9 Hz, 1H)
94	¹ H NMR (400 MHz, DMSO- <i>d</i> ₆) δ ppm 8.28 - 8.34 (m, 2H), 7.78 (d, <i>J</i> =1.7 Hz, 1H), 7.69 (d, <i>J</i> =2.3 Hz, 1H), 7.52 (br d, <i>J</i> =6.4 Hz, 2H), 7.31 - 7.37 (m, 2H), 6.11 (s, 2H), 4.10 (br d, <i>J</i> =8.7 Hz, 1H), 3.91 - 4.01 (m, 2H), 3.68 (br dd, <i>J</i> =11.0, 2.4 Hz, 1H), 3.56 - 3.64 (m, 1H), 3.51 (br t, <i>J</i> =11.1 Hz, 2H), 3.26 - 3.26 (m, 1H, partially obscured by solvent peak), 3.07 - 3.21 (m, 1H), 2.98 (br d, <i>J</i> =11.2 Hz, 1H), 2.56 - 2.65 (m, 1H), 1.60 - 1.85 (m, 3H), 1.55 (br d, <i>J</i> =12.6 Hz, 1H), 1.09 (d, <i>J</i> =6.2 Hz, 3H)

Experimental Examples

Example 1. HBV core plasmid & HBV pol plasmid

A schematic representation of the pDK-pol and pDK-core vectors is shown in Fig. 1A and 1B, respectively. An HBV core or pol antigen optimized expression cassette containing a CMV promoter (SEQ ID NO: 18), a splicing enhancer (triple composite sequence) (SEQ ID NO: 10), Cystatin S precursor signal peptide SPCS (NP_0018901.1) (SEQ ID NO: 9), and pol (SEQ ID NO: 5) or core (SEQ ID NO: 2) gene was introduced into a pDK plasmid backbone, using standard molecular biology techniques.

The plasmids were tested *in vitro* for core and pol antigen expression by Western blot analysis using core and pol specific antibodies, and were shown to provide consistent expression profile for cellular and secreted core and pol antigens (data not shown).

Example 2. Generation of Adenoviral Vectors Expressing a Fusion of Truncated HBV Core Antigen with HBV Pol Antigen

The creation of an adenovirus vector has been designed as a fusion protein expressed from a single open reading frame. Additional configurations for the expression of the two proteins, e.g. using two separate expression cassettes, or using a 2A-like sequence to separate the two sequences, can also be envisaged.

Design of expression cassettes for adenoviral vectors

The expression cassettes (diagrammed in FIG. 2A and FIG. 2B) are comprised of the CMV promoter (SEQ ID NO: 19), an intron (SEQ ID NO: 12) (a fragment derived from the human ApoAI gene - GenBank accession X01038 base pairs 295 – 523, harboring the ApoAI second intron), followed by the optimized coding sequence – either core alone or the core and polymerase fusion protein preceded by a human immunoglobulin secretion signal coding sequence (SEQ ID NO: 14), and followed by the SV40 polyadenylation signal (SEQ ID NO: 13).

A secretion signal was included because of past experience showing improvement in the manufacturability of some adenoviral vectors harboring secreted transgenes, without influencing the elicited T-cell response (mouse experiments).

The last two residues of the Core protein (VV) and the first two residues of the Polymerase protein (MP) if fused results in a junction sequence (VVMP) that is present

on the human dopamine receptor protein (D3 isoform), along with flanking homologies.

The interjection of an AGAG linker between the core and the polymerase sequences eliminates this homology and returned no further hits in a Blast of the human proteome.

Example 3. *In Vivo* Immunogenicity Study of DNA Vaccine in Mice

An immunotherapeutic DNA vaccine containing DNA plasmids encoding an HBV core antigen or HBV polymerase antigen was tested in mice. The purpose of the study was designed to detect T-cell responses induced by the vaccine after intramuscular delivery via electroporation into BALB/c mice. Initial immunogenicity studies focused on determining the cellular immune responses that would be elicited by the introduced HBV antigens.

In particular, the plasmids tested included a pDK-Pol plasmid and pDK-Core plasmid, as shown in FIGS. 1A and 1B, respectively, and as described above in Example 1. The pDK-Pol plasmid encoded a polymerase antigen having the amino acid sequence of SEQ ID NO: 7, and the pDK-Core plasmid encoding a Core antigen having the amino acid sequence of SEQ ID NO: 2. First, T-cell responses induced by each plasmid individually were tested. The DNA plasmid (pDNA) vaccine was intramuscularly delivered via electroporation to Balb/c mice using a commercially available TriGridTM delivery system-intramuscular (TDS-IM) adapted for application in the mouse model in cranialis tibialis. See International Patent Application Publication WO2017172838, and U.S. Patent Application No. 62/607,430, entitled "Method and Apparatus for the Delivery of Hepatitis B Virus (HBV) Vaccines," filed on December 19, 2017 for additional description on methods and devices for intramuscular delivery of DNA to mice by electroporation, the disclosures of which are hereby incorporated by reference in their entireties. In particular, the TDS-IM array of a TDS-IM v1.0 device having an electrode array with a 2.5 mm spacing between the electrodes and an electrode diameter of 0.030 inch was inserted percutaneously into the selected muscle, with a conductive length of 3.2 mm and an effective penetration depth of 3.2 mm, and with the major axis of the diamond configuration of the electrodes oriented in parallel with the muscle fibers. Following electrode insertion, the injection was initiated to distribute DNA (e.g., 0.020 ml) in the muscle. Following completion of the IM injection, a 250 V/cm electrical field (applied voltage of 59.4 -65.6 V, applied current

limits of less than 4 A, 0.16 A/sec) was locally applied for a total duration of about 400 ms at a 10% duty cycle (i.e., voltage is actively applied for a total of about 40 ms of the about 400 ms duration) with 6 total pulses. Once the electroporation procedure was completed, the TriGrid™ array was removed and the animals were recovered. High-dose (20 µg) administration to BALB/c mice was performed as summarized in Table 1. Six mice were administered plasmid DNA encoding the HBV core antigen (pDK-core; Group 1), six mice were administered plasmid DNA encoding the HBV pol antigen (pDK-pol; Group 2), and two mice received empty vector as the negative control. Animals received two DNA immunizations two weeks apart and splenocytes were collected one week after the last immunization.

Table 1: Mouse immunization experimental design of the pilot study.

Group	N	pDNA	Unilateral Admin Site (alternate sides)	Dose	Vol	Admin Days	Endpoint (spleen harvest) Day
1	6	Core	CT + EP	20 µg	20 µL	0, 14	21
2	6	Pol	CT + EP	20 µg	20 µL	0, 14	21
3	2	Empty Vector (neg control)	CT + EP	20 µg	20 µL	0, 14	21

CT, cranialis tibialis muscle; EP, electroporation.

Antigen-specific responses were analyzed and quantified by IFN-γ enzyme-linked immunospot (ELISPOT). In this assay, isolated splenocytes of immunized animals were incubated overnight with peptide pools covering the Core protein, the Pol protein, or the small peptide leader and junction sequence (2 µg/ml of each peptide). These pools consisted of 15 mer peptides that overlap by 11 residues matching the Genotypes BCD consensus sequence of the Core and Pol vaccine vectors. The large 94 kDa HBV Pol protein was split in the middle into two peptide pools. Antigen-specific T cells were stimulated with the homologous peptide pools and IFN-γ-positive T cells were assessed using the ELISPOT assay. IFN-γ release by a single antigen-specific T cell was visualized by appropriate antibodies and subsequent chromogenic detection as a colored spot on the microplate referred to as spot-forming cell (SFC).

Substantial T-cell responses against HBV Core were achieved in mice immunized with the DNA vaccine plasmid pDK-Core (Group 1) reaching 1,000 SFCs per 10^6 cells (FIG. 3). Pol T-cell responses towards the Pol 1 peptide pool were strong (~1,000 SFCs per 10^6 cells). The weak Pol-2-directed anti-Pol cellular responses were likely due to the limited MHC diversity in mice, a phenomenon called T-cell immunodominance defined as unequal recognition of different epitopes from one antigen. A confirmatory study was performed confirming the results obtained in this study (data not shown).

The above results demonstrate that vaccination with a DNA plasmid vaccine encoding HBV antigens induces cellular immune responses against the administered HBV antigens in mice. Similar results were also obtained with non-human primates (data not shown).

Example 4: Enzymatic Assays

HPK1 Kinase Assay

A recombinant fusion protein consisting of full-length human Hpk1 with an N-terminal Glutathione S-transferase (GST) tag was produced in a baculovirus/insect cell expression system. GST-Hpk1 protein was purified from cell lysates by glutathione sepharose affinity chromatography. Kinase inhibition was determined using the ADP Glo™ Kinase Assay Kit (Promega, V9101). Test compounds were prepared by serial dilution in dimethyl sulfoxide (DMSO) and 0.1 µl of each dilution was spotted onto a 384-well white Proxiplate (Perkin Elmer, 6008289). 8 µl of kinase assay buffer (40 mM Tris pH 7.5, 2 mM dithiothreitol (DTT), 0.05% Bovine Serum Albumin, 5 mM MgCl₂) containing Hpk1 protein was transferred to each well and incubated for 30-45 minutes at room temperature. The enzymatic reaction was started by adding 2 µl of kinase assay buffer containing 75 µM ATP. After 120 minutes, the reaction was stopped by adding 3 µl of ADP Glo™ reagent (Promega, V9101) and incubating 45 minutes at room temperature. After adding 6 µl Kinase Detection Reagent (Promega, V9101) and incubating for at least 20 minutes at room temperature, the plate was transferred to an EnVision Plate Reader (Perkin Elmer) for luminescence detection and IC₅₀ values were determined. The results are shown below in Table 2.

SLP76 phosphorylation assay

HPK1 inhibition in cells was determined by detection of SLP76 phosphorylation at S376 in lysates from HEK293 cells engineered to express HPK1 and SLP76. Serial dilutions of test compounds were prepared in cell culture medium and 20 μ l was transferred into each well of a 96-well plate containing HEK293 cells in 180 μ l of culture medium. After incubation for 4 hours at 37°C/5% CO₂, the culture medium was removed, plates were put on ice and cells washed with cold Minimal Essential Medium before lysing in 90 μ l of cold Mammalian Protein Extraction Reagent (Thermo Scientific, 78501) for 30 minutes on ice. The cell lysates were transferred to another 96-well plate, centrifuged 10 minutes at 3000 rcf (4°C) and 30 μ l transferred to a 96-well High-bind plate (MSD, L15XB-3). After coating overnight at 4°C, the cell lysate was removed, wells were washed with base buffer (TBS, 0.2% Tween-20), and blocking solution (base buffer containing 3% MSD blocker A) was added to each well for 1 hour at room temperature. The blocking solution was removed, the wells were washed with base buffer, and detection antibody (SLP76 pS376) was added in 25 μ l base buffer containing 1% MSD blocker A to each well. After a 2h incubation at room temperature, the detection antibody was removed, wells were washed four times with base buffer, 25 μ l SULFO-TAG labeled goat anti-rabbit secondary antibody (MSD, R32AB-1) in base buffer containing 1% MSD blocker A was added to each well and left for 1 hour at room temperature. Wells were washed 4 times with base buffer, 150 μ l Read Buffer T was added to each well, and the plates were read in a Mesoscale plate reader (MSD). IC₅₀ values were determined by curve-fitting the electrochemiluminescent signals obtained from the lysates of HEK293 cells expressing HPK1 and SLP76 treated with test compounds at various concentrations minus the signal obtained from HEK293 cells expressing SLP76 only. The results are shown below in Table 2.

Table 2: Data of the HPK1 kinase assay and the SLP76 phosphorylation assay. Co. No. means Compound Number.

Co. No.	Hpk1 biochemical (pIC ₅₀)	pSLP76 (HEK293) (pIC ₅₀)
1	8.12	6.42
2	7.38	6.06
3	7.27	5.25
4	7.37	5.43
5a	7.99	6.11
5b	6.04	n.d.
7a	7.92	6.87
7b	5.71	5.14
8a	8.2	6.46
8b	6.87	5.16
9	7.37	5.67
10	8.18	6.55
11	7.45	5.3
12	6.95	5.13
13	6.73	5.05
14	6.08	<5
15	6.24	<5
15a	5.03	<5
15b	6.7	<5
15c	6.2	<5
15d	5.16	<5
17	7.8	5.8
18	7.75	5.81
19	8.37	6.01
20	8.5	6.77
21	7.73	5.16
22	8.56	6.47
23	8.99	6.52
24	7.88	6.05
25	8.01	6.37
25b	6.02	<5
26	8.69	6.61
27	8.24	6.62
28	7.99	6.5
29	7.42	5.22
30	7.55	6.31
31	7.69	5.98
32	7.55	5.73
33	7.84	5.64
34	7.76	5.74
35	8.09	6.17
36	8.1	6.16

Co. No.	Hpk1 biochemical (pIC ₅₀)	pSLP76 (HEK293) (pIC ₅₀)
37	8.14	6.19
38	8.12	5.89
39	8.63	6.56
40	8.05	5.96
41	8.34	6.21
42	7.61	5.7
43	7.67	<5
44	8.03	6.47
45	7.38	5.95
46	7.45	5.99
47	7.61	5.87
48	7.6	5.88
49	7.62	5.6
50	7.7	5.61
51	7.74	6.26
52	7.41	6.07
53	7.8	6.01
54	6.34	<5
55	7.02	<5
56	7.45	<5
57	7.58	<5
58	5.06	<5
59	7.11	5.25
60	6.6	<5
61	7.46	5.4
62	7.5	5.97
63	7.23	5.27
64	7.77	6.42
65	7.57	5.92
66	7.07	<5
67	7.33	5.78
68	8.2	5.85
69	6.5	n.d.
70	6.94	5.36
71	7.38	5.69
72	7.39	5.64
73	7.37	5.81
74	7.25	5.73
75	7.38	5.64
76	7.46	5.71
77	7.85	5.59
78	8.09	6.04

Co. No.	Hpk1 biochemical (pIC ₅₀)	pSLP76 (HEK293) (pIC ₅₀)
79	7.59	6.1
80a	7.84	6.07
80b	5.43	<5
81	7.81	6.09
82	8.06	6.9
83a	7.35	5.87
83b	5.24	n.d.
84	7.6	6.32
85	7.42	5.94
86	6.92	5.4
87	7.32	5.96
88	6.95	5.38
89	7.67	6.07
90	7.45	5.91
92	5.71	<5
93a	6.79	5.34
93b	7.73	5.91
94	7.89	6.24
95	7.36	5.65
96	6.84	5.1
98	7.58	6.14
99	7.67	6.19
100	7.71	5.73
101	8.43	6.16
102	6.53	<5
103a	8.11	5.71
103b	6.22	<5
104	7.23	5.1
105	6.42	<5
106	6.75	5.13
107	6.08	n.d.
108	6.93	5.12
110	7.05	5.33
111	6.96	5.23

Example 5: Impact of Anti-PD-1 on HBV Vaccine-Induced Immune Response in AAV/HBV Infected Mice

To assess whether blocking HPK-1 impacts HBV-vaccine induced cell-mediated immunity (CMI) in AAV/HBV infected mice, adult C57BL/6 mice are infected with AAV/HBV for 28 days. Afterwards, 1 group of 8 animals is vaccinated by DNA

electroporation (2.5µg of each plasmid) at day 28 (d28) and day 49 (d49), and group 2 is treated with compound **Co.1** QD 100mpk. Group 3 is vaccinated by DNA electroporation (2.5µg of each plasmid) at d28 and d49 and treated with compound **Co.1** QD 100mpk. Group 4 is a control group and is only receiving PBS. At day 56 (d56) mice are sacrificed. Splenocytes are isolated as well as intrahepatic lymphocytes (IHL), and proliferative capacity of CD8 T-cells is measured. During the whole treatment, serum is taken to evaluate viral parameter and ALT levels every week.

Materials and methods

Animals:

C57BL/6 mice are infected with rAAV8-1.3HBV (Fiveplus Medical Institute, China) via the tail vein at MOI 10¹¹. Vaccination is performed when HBV chronicity is reached 28 days after infection.

Plasmid DNA

The DNA vaccine was a combination of two separate DNA plasmids encoding the Core (pDF-Core) and Polymerase (Pol) proteins (pDF-Pol) of HBV, respectively. The pDF-Core and pDF-Pol have the same sequences as the pDK-Core and pDK-Pol, respectively, as described in Example 1 and as shown in Figure 1A and Figure 1B. 2.5µg of each plasmid is electroporated with trigrid system i.m.

Study design

Adult C57BL/6 mice are infected with AAV/HBV for 28 days. Afterwards, 1 group of 8 animals is vaccinated by DNA electroporation (2.5µg of each plasmid) at d28 and d49. Group 2 is treated with compound **Co. 1** QD 100mpk from day 28 until d56. Group 3 is vaccinated by DNA electroporation at d28 and d49 and is treated with compound **Co. 1** QD 100mpk from day 28 until d56. The control group is treated with PBS only.

Isolation of splenocytes and IHL

Spleens are isolated from mice, tissue disruption is performed using GentleMACS Dissociater (Miltenyi Biotec), and cells are counted and used in the designated assays. The intrahepatic lymphocytes are obtained by perfusing the liver with PBS, and tissue disruption is performed using GentleMACS Dissociater, followed by a 33.75% (v/v) Percoll® / PBS2%FCS gradient. Centrifugation at 700xg (12 minutes,

room temperature, max acceleration and brake at 1) separates the hepatocytes from the lymphocytes. These last ones are aspirated carefully and used in designated assay.

Immunophenotyping

5 Splenocytes and IHL are plated 100.000 cells per well. Next, they are stained with viability dye. Afterwards, cells are fixed and permeabilized to perform intracellular staining. Cells are stained with the following markers (anti-CD3, anti-CD8, anti-CD4 and ki67 which is a marker for proliferative T-cells). After 1 hour, cells are washed twice and resuspended in 100µl stain buffer, and read-out is done on FACS Fortessa.

10 It is understood that the examples and embodiments described herein are for illustrative purposes only, and that changes could be made to the embodiments described above without departing from the broad inventive concept thereof. It is understood, therefore, that this invention is not limited to the particular embodiments disclosed, but it is intended to cover modifications within the spirit and scope of the invention as defined
15 by the appended claims.

CLAIMS

It is claimed:

1. A therapeutic combination for use in treating a hepatitis B virus (HBV) infection in a subject in need thereof, comprising:

5

i) at least one of:

a) a truncated HBV core antigen consisting of an amino acid sequence that is at least 95% identical to SEQ ID NO: 2,

b) a first non-naturally occurring nucleic acid molecule comprising a first polynucleotide sequence encoding the truncated HBV core antigen,

10

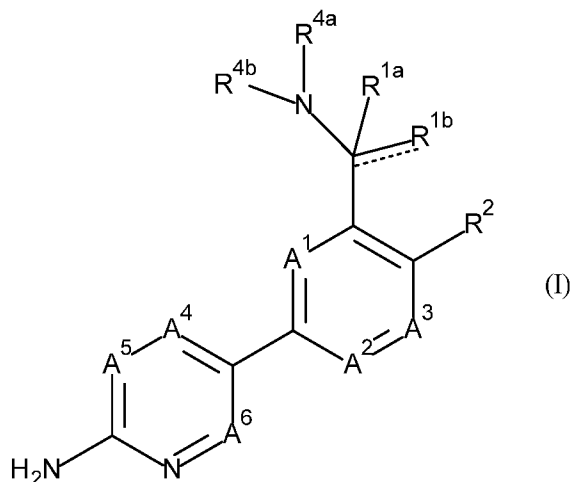
c) an HBV polymerase antigen having an amino acid sequence that is at least 90% identical to SEQ ID NO: 7, wherein the HBV polymerase antigen does not have reverse transcriptase activity and RNase H activity, and

d) a second non-naturally occurring nucleic acid molecule comprising a second polynucleotide sequence encoding the HBV polymerase antigen;

15

and

ii) a compound of formula (I):



or a tautomer or a stereoisomeric form thereof, wherein:

the dotted bond towards R^{1b} is an optional bond that is optionally present when R^{1b} and R^{4b} are taken together to form a monocyclic or bicyclic aromatic heterocyclyl;

20

A^1 represents CH or N; A^2 represents CH; A^3 represents CH or N;

provided that only one of A^1 and A^3 represents N;

A^4 represents CH or N; A^5 represents CR^{3a} ; A^6 represents CH;

R^{1a} represents hydrogen;

R^{1b} represents hydrogen or CH_3 ;

R^{4a} represents hydrogen, C_{1-4} alkyl, or C_{3-6} cycloalkyl;

5 R^{4b} represents hydrogen, C_{1-4} alkyl, C_{3-6} cycloalkyl, or
a carbon linked monocyclic 4-, 5-, 6- or 7-membered non-aromatic heterocycl
containing 1, 2 or 3 heteroatoms each independently selected from the group consisting
of N, O, and S; wherein said S-atom is optionally substituted to form $S(=O)$, $S(=O)_2$, or
 $S(=O)(=NR^x)$;

10 or

R^{1b} and R^{4b} are taken together to form together with the atoms to which they are attached
a monocyclic 5-membered aromatic heterocycl or a monocyclic 4-, 5-, 6- or 7-
membered fully saturated heterocycl, each containing 1 N-atom and optionally 1 or 2
additional heteroatoms each independently selected from the group consisting of N, O,
15 and S; wherein said optional S-atom is optionally substituted to form $S(=O)$, $S(=O)_2$, or
 $S(=O)(=NR^x)$; or

a bicyclic 6- to 12- membered aromatic or fully saturated heterocycl containing 1 N-
atom and optionally 1 or 2 additional heteroatoms each independently selected from the
group consisting of N, O, and S; wherein said optional S-atom is optionally substituted to
20 form $S(=O)$, $S(=O)_2$, or $S(=O)(=NR^x)$;

wherein said monocyclic or bicyclic, aromatic or fully saturated heterocycl is optionally
substituted on one or more of the carbon atoms with in total 1, 2 or 3 substituents each
independently selected from the group consisting of $-OH$, CN , halo, R^7 , $-OR^7$,
 $-S(=O)_2-R^7$, $-C(=O)-R^7$, $-NR^{6c}R^{6d}$, $-C(=O)-NR^{6a}R^{6b}$, and Het^c ;

25 wherein said monocyclic or bicyclic, aromatic or fully saturated heterocycl is optionally
substituted on the optional additional nitrogen atoms with in total 1 or 2 substituents each
independently selected from the group consisting of R^7 , $-S(=O)_2-R^7$, $-C(=O)-R^7$,
 $-C(=O)-NR^{6a}R^{6b}$, and Het^d ;

provided that in case R^{1b} and R^{4b} are taken together, R^{4a} represents hydrogen; and

30 R^{1a} represents hydrogen or R^{1a} is absent when the dotted bond towards R^{1b} is a bond;

or

R^{4a} and R^{4b} are taken together to form together with the N-atom to which they are attached a monocyclic 5-membered aromatic heterocyclyl or a monocyclic 4-, 5-, 6- or 7-membered fully saturated heterocyclyl, each containing 1 N-atom and optionally 1 or 2 additional heteroatoms each independently selected from the group consisting of N, O, and S; wherein said optional S-atom is optionally substituted to form $S(=O)$, $S(=O)_2$, or $S(=O)(=NR^x)$; or

a bicyclic 6- to 12- membered aromatic or fully saturated heterocyclyl containing 1 N-atom and optionally 1 or 2 additional heteroatoms each independently selected from the group consisting of N, O, and S; wherein said optional S-atom is optionally substituted to form $S(=O)$, $S(=O)_2$, or $S(=O)(=NR^x)$;

wherein said monocyclic or bicyclic, aromatic or fully saturated heterocyclyl is optionally substituted on one or more of the carbon atoms with in total 1, 2 or 3 substituents each independently selected from the group consisting of -OH, CN, halo, R^7 , -O- R^7 ,

- $S(=O)_2-R^7$, -C(=O)- R^7 , -NR^{6c}R^{6d}, -C(=O)-NR^{6a}R^{6b}, and Het^c;

wherein said monocyclic or bicyclic, aromatic or fully saturated heterocyclyl is optionally substituted on the nitrogen atoms with in total 1 or 2 substituents each independently selected from the group consisting of R^7 , - $S(=O)_2-R^7$, -C(=O)- R^7 , -C(=O)-NR^{6a}R^{6b}, and Het^d;

in case R^{4a} and R^{4b} are taken together, R^{1a} represents hydrogen, and R^{1b} represents hydrogen;

R^2 is selected from the group consisting of cyano; halo; -C(=O)-NR^{8a}R^{8b}; -CH₂-NR^{8c}R^{8d}; Het^b; -P(=O)-(C₁₋₄alkyl)₂; - $S(=O)_2$ -C₁₋₄alkyl; - $S(=O)(=NR^x)$ -C₁₋₄alkyl; C₁₋₆alkyl optionally substituted with 1 or 2 substituents each independently selected from the group consisting of halo, -OH, cyano, and -O-C₁₋₄alkyl;

C₃₋₆cycloalkyl optionally substituted with 1 or 2 substituents each independently selected from the group consisting of halo, -OH, cyano and -O-C₁₋₄alkyl; and

C₃₋₆cycloalkenyl optionally substituted with 1 or 2 substituents each independently selected from the group consisting of halo, -OH, cyano and -O-C₁₋₄alkyl;

R^{3a} represents hydrogen, halo, R^7 , -O- R^7 , cyano, -C(=O)-NR^{6e}R^{6f}, Het^a, or phenyl optionally substituted with 1, 2 or 3 substituents each independently selected from the

group consisting of halo, cyano, C₁₋₄alkyl, -O-C₁₋₄alkyl, C₁₋₄alkyl substituted with one cyano, and C₁₋₄alkyl substituted with 1, 2 or 3 halo atoms;

Het^a represents a carbon linked monocyclic 5-, 6- or 7-membered aromatic heterocyclyl or carbon linked monocyclic 4-, 5-, 6- or 7-membered non-aromatic heterocyclyl, each
 5 containing 1, 2 or 3 heteroatoms each independently selected from the group consisting of N, O or S; wherein said S-atom might be substituted to form S(=O), S(=O)₂, or S(=O)(=NR^x); or

a carbon linked bicyclic 6- to 12-membered aromatic or non-aromatic heterocyclyl containing 1, 2 or 3 heteroatoms each independently selected from the group consisting
 10 of N, O and S; wherein said S-atom is optionally substituted to form S(=O), S(=O)₂, or S(=O)(=NR^x);

wherein said monocyclic or bicyclic, aromatic or non-aromatic heterocyclyl is optionally substituted on one or more of the carbon atoms with in total 1, 2 or 3 substituents each independently selected from the group consisting of -OH, CN, halo, R⁷, -O-R⁷,

15 -S(=O)₂-R⁷, -C(=O)-R⁷, -NR^{6c}R^{6d}, -C(=O)-NR^{6a}R^{6b}, and Het^c;

wherein said monocyclic or bicyclic, aromatic or non-aromatic heterocyclyl is optionally substituted on the nitrogen atoms with in total 1 or 2 substituents each independently selected from the group consisting of R⁷, -S(=O)₂-R⁷, -C(=O)-R⁷, -C(=O)-NR^{6a}R^{6b} and Het^d,

20 R^{6a}, R^{6b}, R^{6c}, R^{6d}, R^{6e}, and R^{6f} are each independently selected from the group consisting of hydrogen; C₃₋₆cycloalkyl optionally substituted with one -OR⁵; and

C₁₋₄alkyl optionally substituted with one -OR⁵, wherein two hydrogen atoms on the same carbon atom of said C₁₋₄alkyl are optionally taken together to form C₃₋₆cycloalkyl;

R⁵ represents hydrogen or C₁₋₄alkyl;

25 R^{8a}, R^{8c}, and R^{8d} are each independently selected from the group consisting of hydrogen;

C₁₋₄alkyl optionally substituted with one -OH or -O-C₁₋₄alkyl; and C₃₋₆cycloalkyl optionally substituted with one -OH or -O-C₁₋₄alkyl;

R^{8b} is selected from the group consisting C₁₋₄alkyl optionally substituted with one -OH or -O-C₁₋₄alkyl; and C₃₋₆cycloalkyl optionally substituted with one -OH or -O-C₁₋₄alkyl;

30 or

R^{8a} and R^{8b}, or R^{8c} and R^{8d} are taken together to form together with the N-atom to which

they are attached a monocyclic fully saturated heterocyclyl containing 1 N-atom and optionally 1 or 2 additional heteroatoms each independently selected from the group consisting of N, O, S; wherein said optional S-atom is optionally substituted to form S(=O), S(=O)₂, or S(=O)(=NR^x); wherein said monocyclic 4-, 5-, 6- or 7-membered fully saturated heterocyclyl is optionally substituted on one or more of the carbon atoms with in total 1, 2 or 3 substituents each independently selected from the group consisting of -OH, CN, halo, R⁷, -O-R⁷, -S(=O)₂-R⁷, -C(=O)-R⁷, -NR^{6c}R^{6d}, and -C(=O)-NR^{6a}R^{6b};

wherein said monocyclic fully saturated heterocyclyl is optionally substituted on the nitrogen atoms with in total 1 or 2 substituents each independently selected from the group consisting of R⁷, -S(=O)₂-R⁷, -C(=O)-R⁷, and -C(=O)-NR^{6a}R^{6b};

each Het^c independently represents a monocyclic 4-, 5-, 6- or 7-membered fully saturated heterocyclyl containing 1, 2 or 3 heteroatoms each independently selected from the group consisting of N, O and S; wherein said S-atom is optionally substituted to form S(=O), S(=O)₂, or S(=O)(=NR^x);

each Het^d independently represents a carbon linked monocyclic 4-, 5-, 6- or 7-membered fully saturated heterocyclyl containing 1, 2 or 3 heteroatoms each independently selected from the group consisting of N, O and S; wherein said S-atom is optionally substituted to form S(=O), S(=O)₂, or S(=O)(=NR^x);

Het^b represents a monocyclic 4-, 5-, 6- or 7-membered non-aromatic heterocyclyl containing 1, 2 or 3 heteroatoms each independently selected from the group consisting of N, O, and S; wherein said S-atom might be substituted to form S(=O), S(=O)₂, or S(=O)(=NR^x); or

a bicyclic 6- to 12- membered non-aromatic heterocyclyl containing 1, 2 or 3 heteroatoms each independently selected from the group consisting of N, O, and S; wherein said S-atom might be substituted to form S(=O), S(=O)₂, or S(=O)(=NR^x);

wherein said monocyclic or bicyclic non-aromatic heterocyclyl might be substituted on one or more of the carbon atoms with in total 1, 2 or 3 substituents each independently selected from the group consisting of -OH, CN, halo, R⁷, -O-R⁷, -S(=O)₂-R⁷, -C(=O)-R⁷, -NR^{6c}R^{6d}, and -C(=O)-NR^{6a}R^{6b};

wherein said monocyclic or bicyclic non-aromatic heterocyclyl might be substituted on the nitrogen atoms with in total 1 or 2 substituents each independently selected from the group consisting of R^7 , $-S(=O)_2-R^7$, $-C(=O)-R^7$, and $-C(=O)-NR^{6a}R^{6b}$;

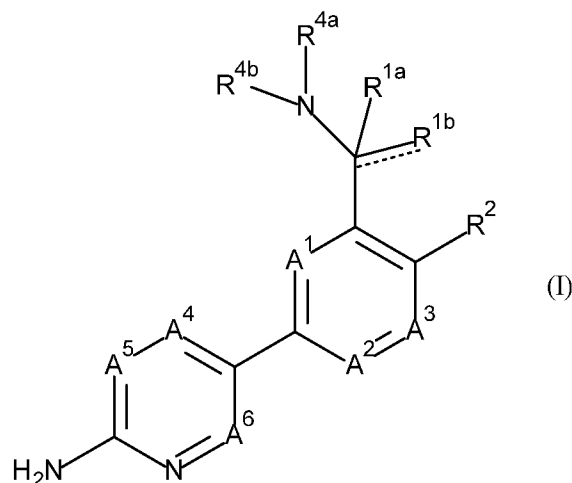
each R^7 independently represents C_{3-6} cycloalkyl optionally substituted with 1, 2 or 3

5 substituents each independently selected from the group consisting of halo, -OH, -O- C_{1-4} alkyl and cyano; or C_{1-4} alkyl optionally substituted with 1, 2 or 3 substituents each independently selected from the group consisting of halo, -OH, -O- C_{1-4} alkyl and cyano; and

each R^x independently represents hydrogen or C_{1-4} alkyl;

10 or a pharmaceutically acceptable addition salt, an N-oxide, or a solvate thereof.

2. The therapeutic combination of claim 1, comprising at least one of the HBV polymerase antigen and the truncated HBV core antigen.
3. The therapeutic combination of claim 2, comprising the HBV polymerase antigen and the truncated HBV core antigen.
- 15 4. The therapeutic combination of claim 1, comprising at least one of the first non-naturally occurring nucleic acid molecule comprising the first polynucleotide sequence encoding the truncated HBV core antigen and the second non-naturally occurring nucleic acid molecule comprising the second polynucleotide sequence encoding the HBV polymerase antigen.
- 20 5. A therapeutic combination for use in treating a hepatitis B virus (HBV) infection in a subject in need thereof, comprising
 - i) a first non-naturally occurring nucleic acid molecule comprising a first polynucleotide sequence encoding a truncated HBV core antigen consisting of an amino acid sequence that is at least 95% identical to SEQ
 - 25 ID NO: 2; and
 - ii) a second non-naturally occurring nucleic acid molecule comprising a second polynucleotide sequence encoding an HBV polymerase antigen having an amino acid sequence that is at least 90% identical to SEQ ID NO: 7, wherein the HBV polymerase antigen does not have reverse
 - 30 transcriptase activity and RNase H activity; and
 - iii) a compound of formula (I):



or a tautomer or a stereoisomeric form thereof, wherein:

the dotted bond towards R^{1b} is absent;

A^1 represents CH or N; A^2 represents CH; A^3 represents CH;

5 A^4 represents CH; A^5 represents CR^{3a} ; A^6 represents CH;

R^{1b} and R^{4b} are taken together to form together with the atoms to which they are attached a monocyclic 4-, 5-, 6- or 7-membered fully saturated heterocyclyl, each containing 1 N-atom and optionally 1 or 2 additional heteroatoms each independently selected from the group consisting of N, O, and S; wherein said optional S-atom is optionally substituted to form S(=O);

wherein said monocyclic fully saturated heterocyclyl is optionally substituted on one of the carbon atoms with 1 substituent selected from the group consisting of halo and R^7 ; provided that R^{4a} represents hydrogen; and R^{1a} represents hydrogen;

R^2 represents Het^b;

15 R^{3a} represents halo, cyano, or Het^a;

Het^a represents a carbon linked monocyclic 5-, 6- or 7-membered aromatic heterocyclyl containing 1, 2 or 3 heteroatoms each independently selected from the group consisting of N, O and S; wherein said S-atom is optionally substituted to form S(=O) or S(=O)₂; wherein said monocyclic aromatic heterocyclyl is optionally substituted on one of the

20 carbon atoms with a halo substituent;

wherein said monocyclic aromatic heterocyclyl is optionally substituted on one nitrogen atom with R^7 ;

Het^b represents a monocyclic 4-, 5-, 6- or 7-membered non-aromatic heterocyclyl containing 1 oxygen atom; and

each R⁷ represents C₁₋₄alkyl,

or a pharmaceutically acceptable addition salt, an N-oxide, or a solvate thereof.

5

6. The therapeutic combination of claim 4 or 5, wherein the first non-naturally occurring nucleic acid molecule further comprises a polynucleotide sequence encoding a signal sequence operably linked to the N-terminus of the truncated HBV core antigen, and the second non-naturally occurring nucleic acid molecule further comprises a polynucleotide sequence encoding a signal sequence operably linked to the N-terminus of the HBV polymerase antigen, preferably, the signal sequence independently comprises the amino acid sequence of SEQ ID NO: 9 or SEQ ID NO: 15, preferably the signal sequence is independently encoded by the polynucleotide sequence of SEQ ID NO: 8 or SEQ ID NO: 14.

10

7. The therapeutic combination of any one of claims 1-6, wherein
a) the truncated HBV core antigen consists of the amino acid sequence of SEQ ID NO: 2 or SEQ ID NO: 4; and
b) the HBV polymerase antigen comprises the amino acid sequence of SEQ ID NO: 7.

15

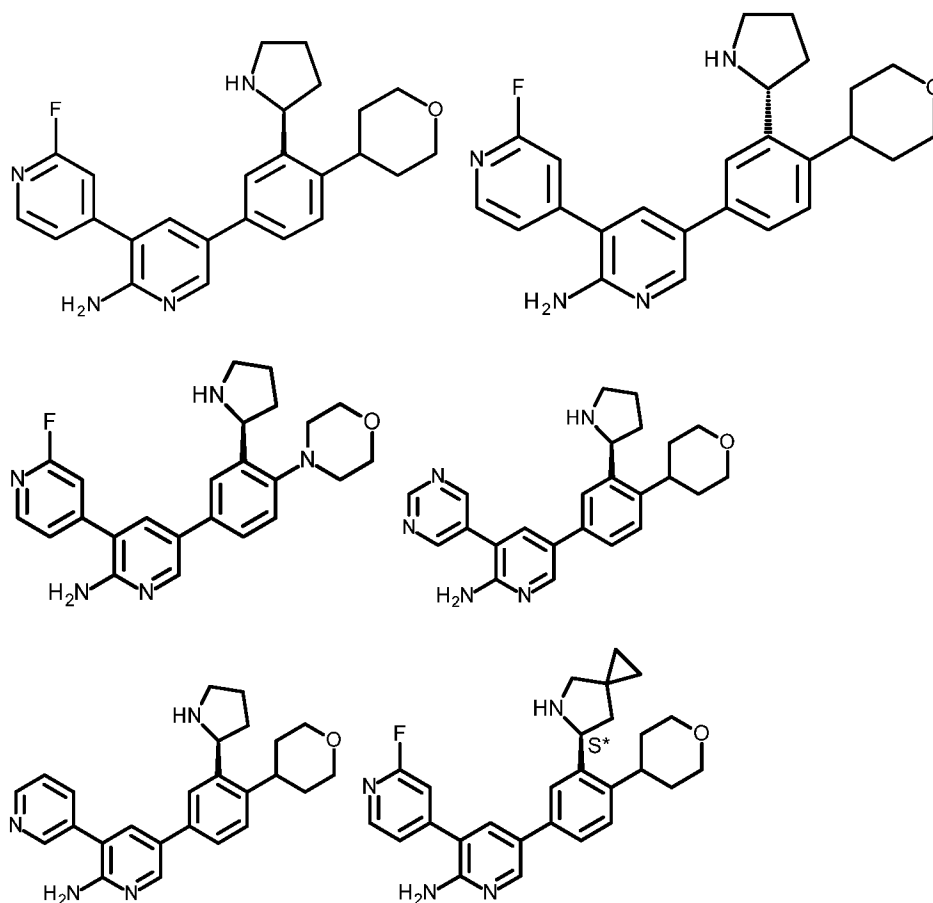
8. The therapeutic combination of any one of claims 1-7, wherein each of the first, and second non-naturally occurring nucleic acid molecules is a DNA molecule, preferably the DNA molecule is present on a plasmid or a viral vector.
9. The therapeutic combination of any one of claims 4 to 8, comprising the first non-naturally occurring nucleic acid molecule and the second non-naturally occurring nucleic acid molecule in the same non-naturally nucleic acid molecule.

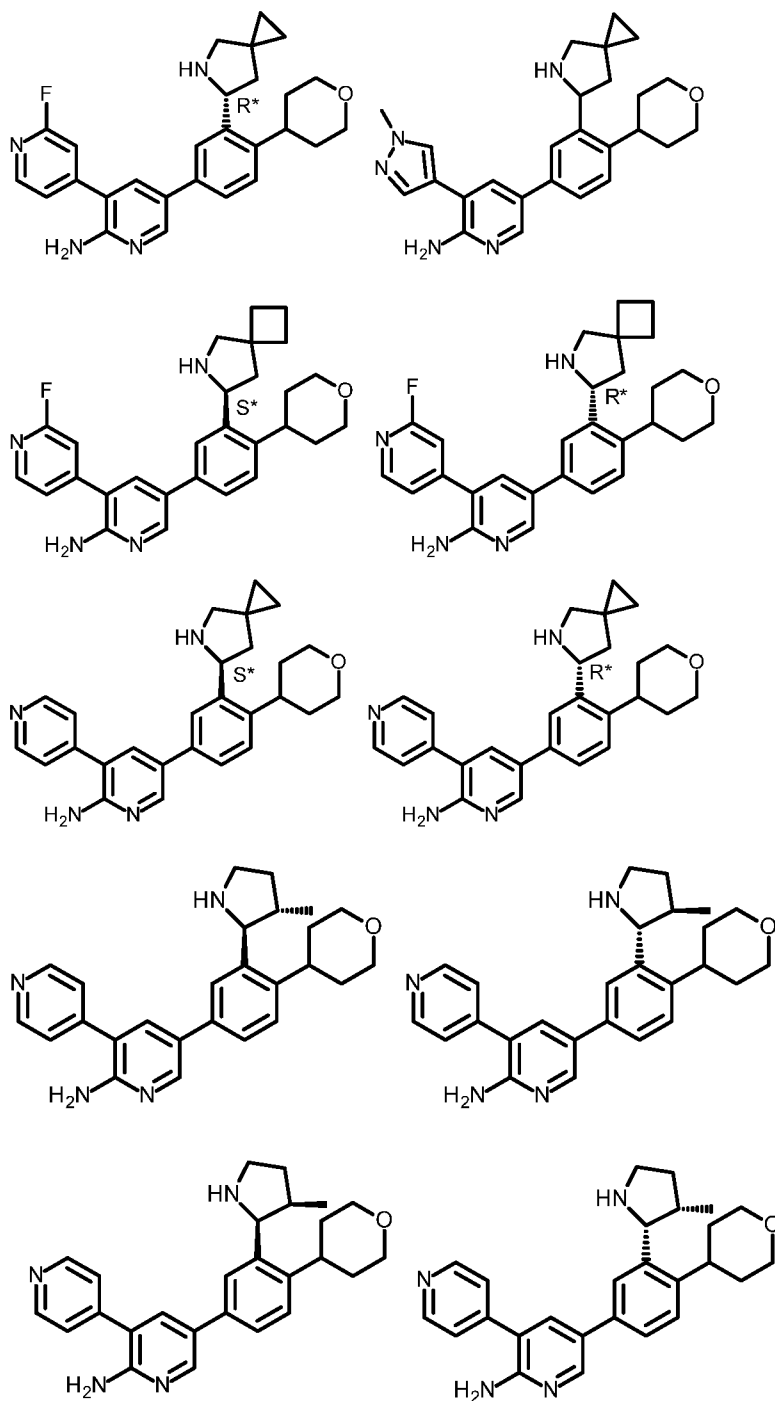
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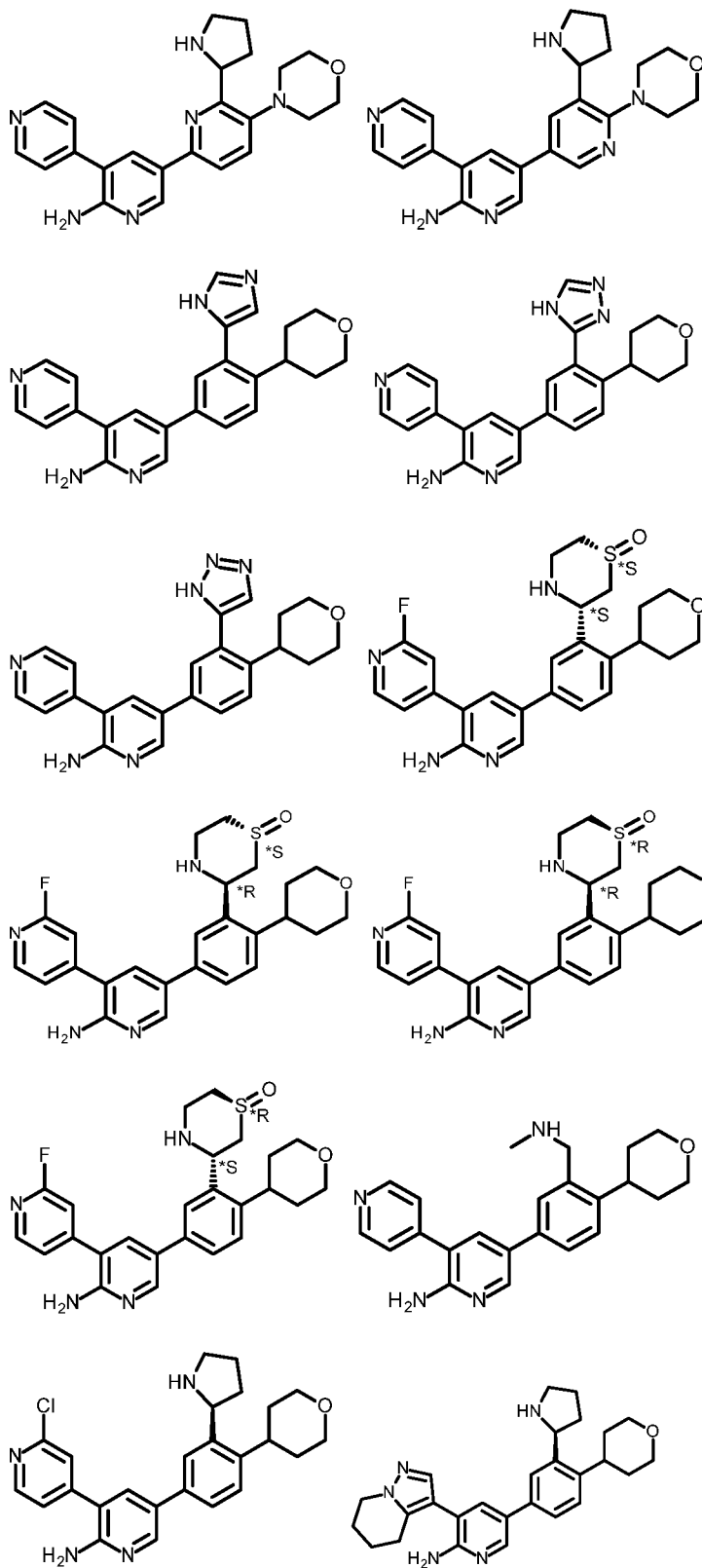
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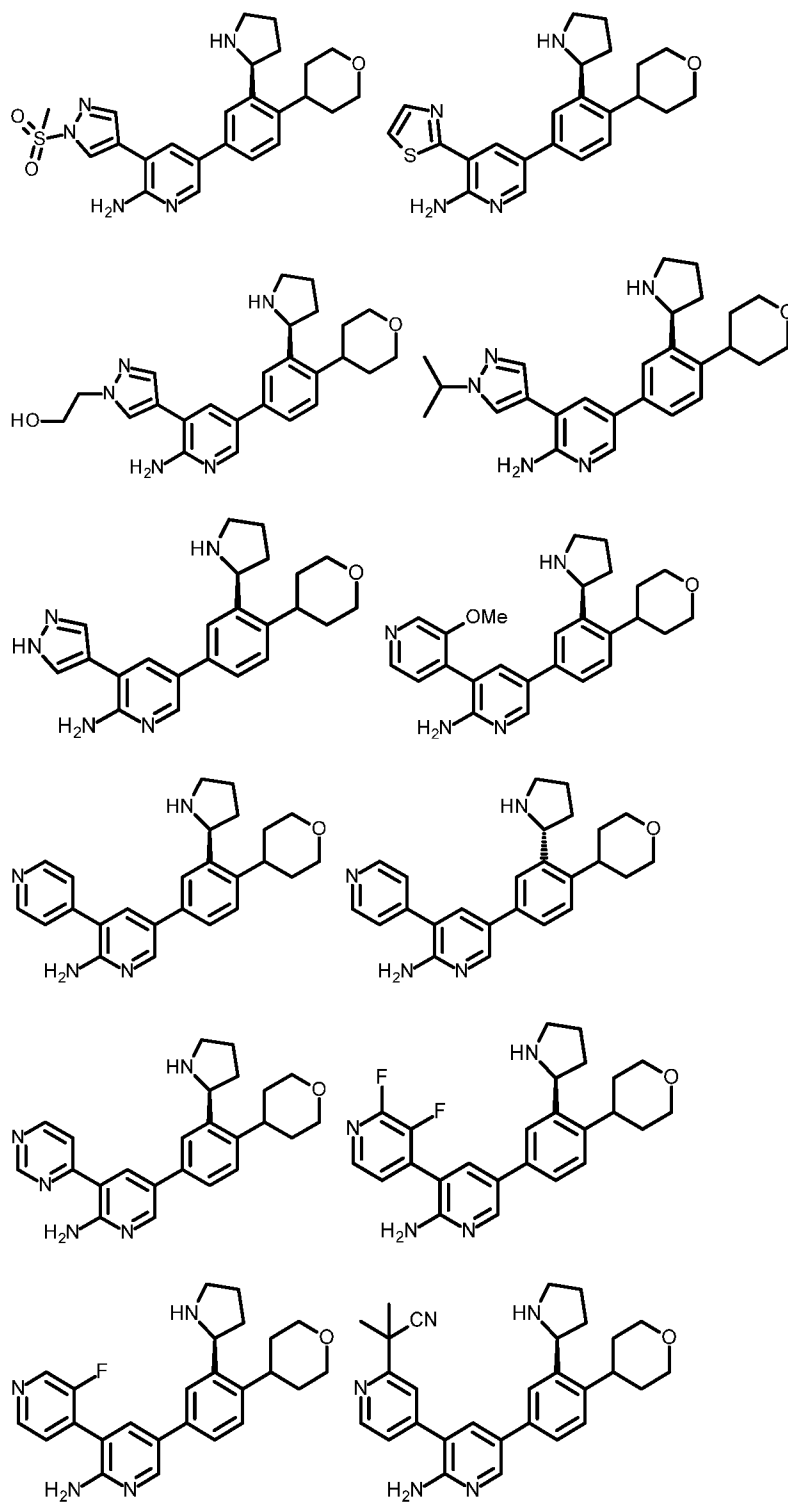
10. The therapeutic combination of any one of claims 4 to 8, comprising the first non-naturally occurring nucleic acid molecule and the second non-naturally occurring nucleic acid molecule in two different non-naturally occurring nucleic acid molecules.

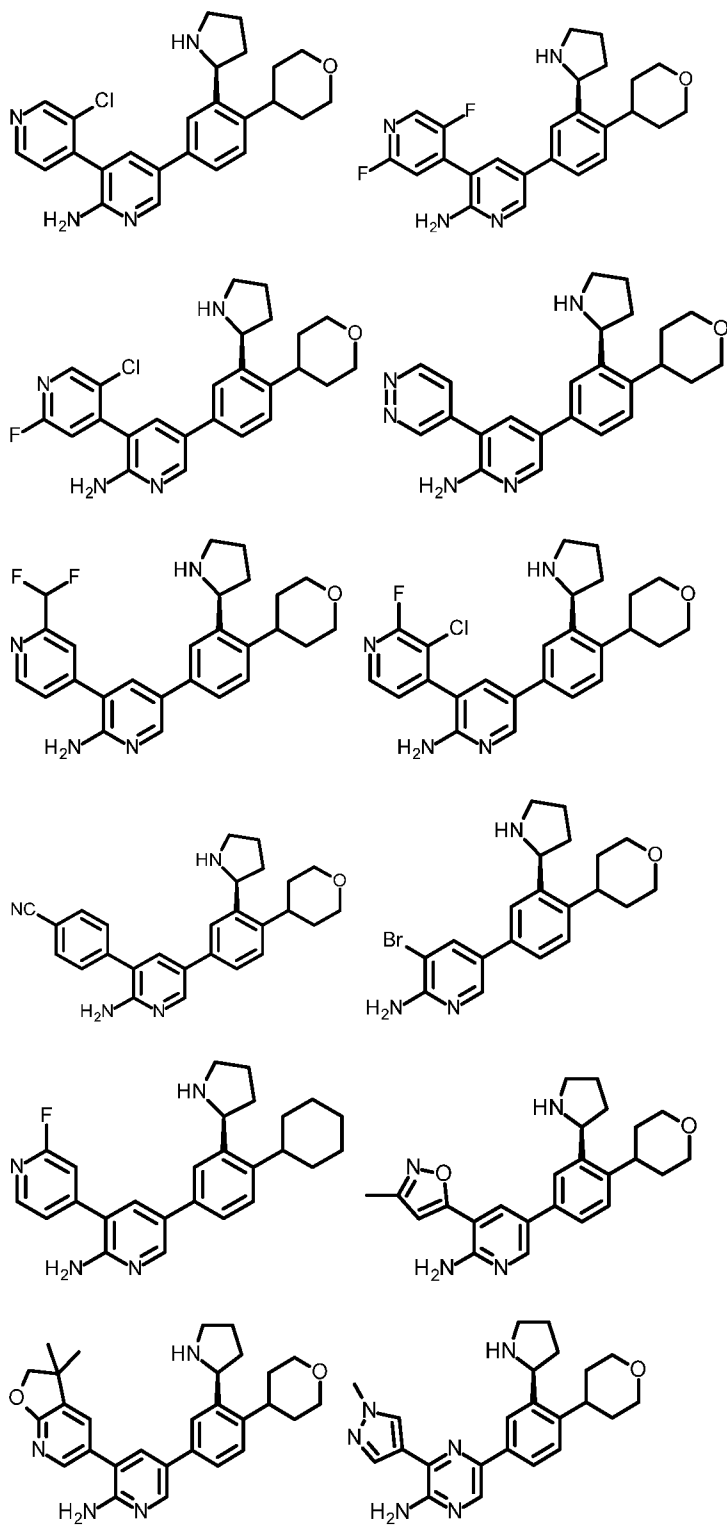
11. The therapeutic combination of any one of claims 4 to 10, wherein the first polynucleotide sequence comprises a polynucleotide sequence having at least 90% sequence identity to SEQ ID NO: 1 or SEQ ID NO: 3.
12. The therapeutic combination of claim 11, wherein the first polynucleotide sequence comprises the polynucleotide sequence of SEQ ID NO: 1 or SEQ ID NO: 3.
13. The therapeutic combination of any one of claims 4 to 12, wherein the second polynucleotide sequence comprises a polynucleotide sequence having at least 90% sequence identity to SEQ ID NO: 5 or SEQ ID NO: 6.
14. The therapeutic combination of claim 13, wherein the second polynucleotide sequence comprises the polynucleotide sequence of SEQ ID NO: 5 or SEQ ID NO: 6.
15. The therapeutic combination of any one of claims 1-14, wherein the compound is selected from the group consisting of:

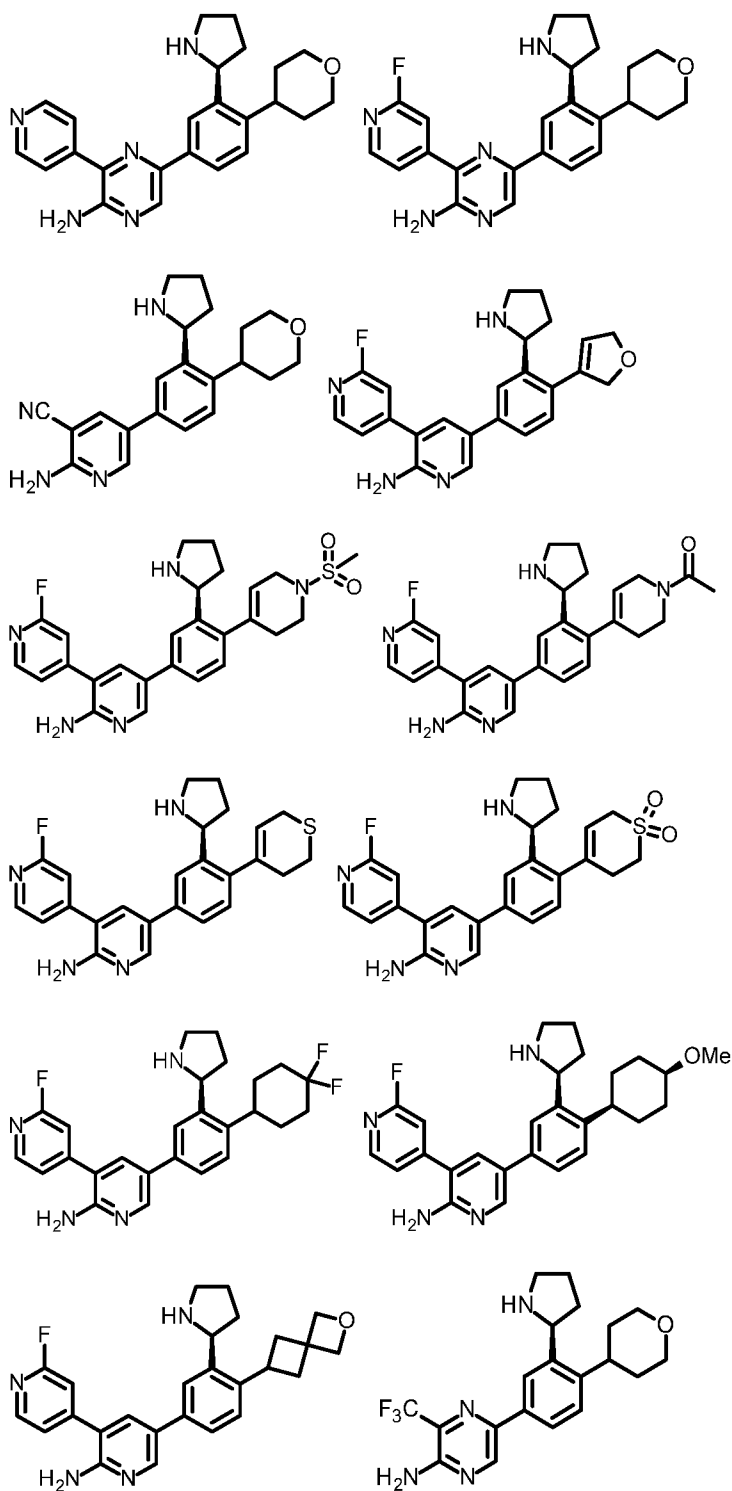


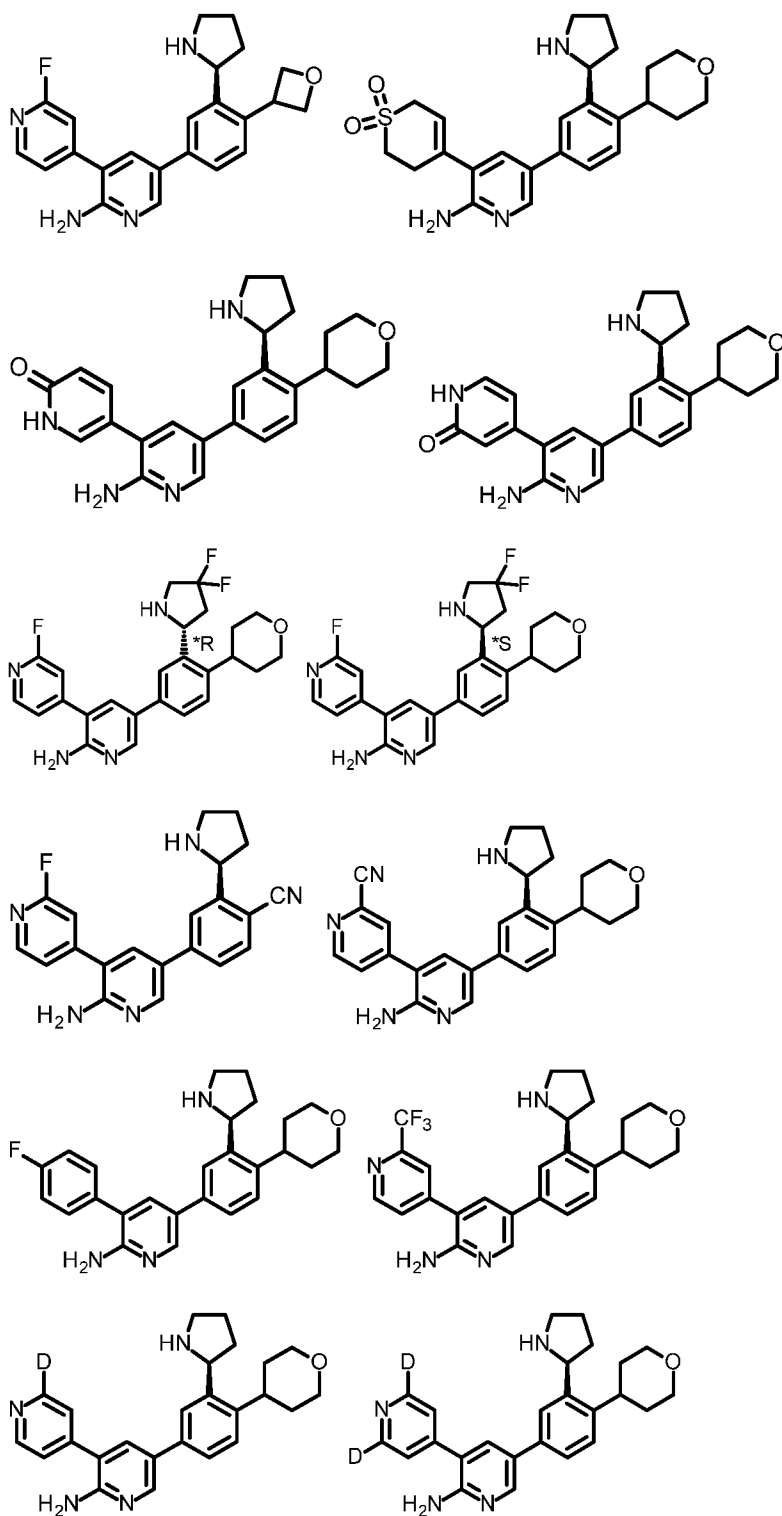


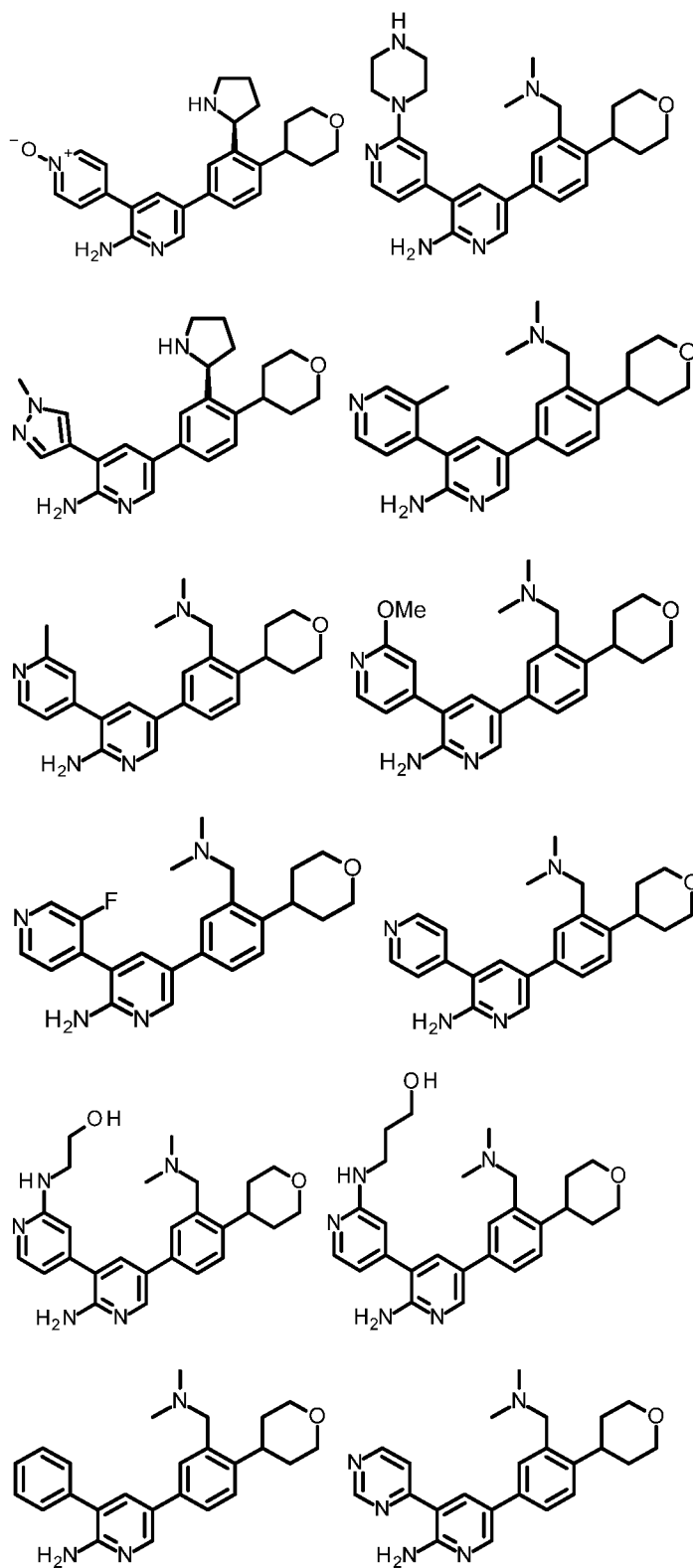


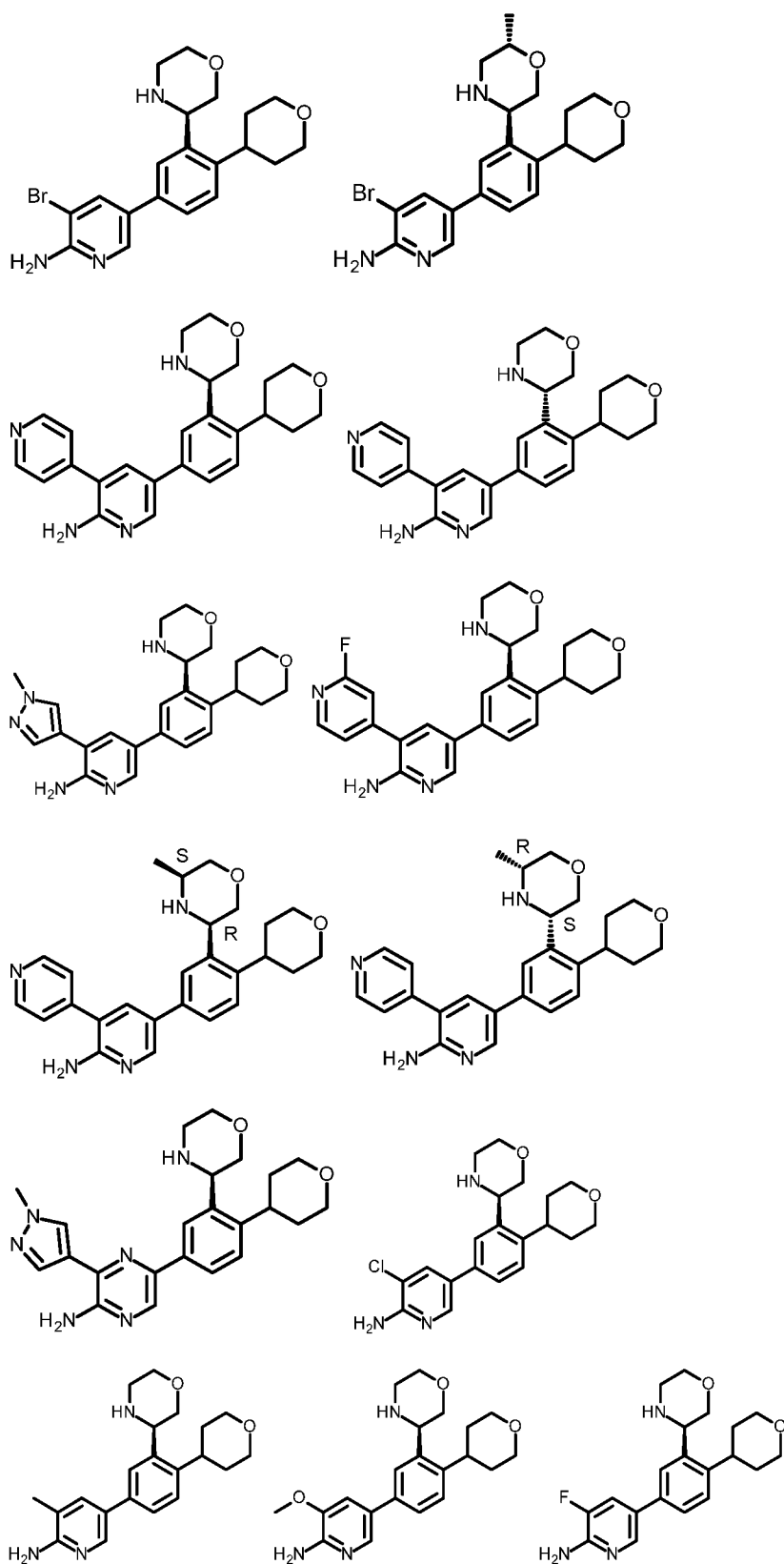


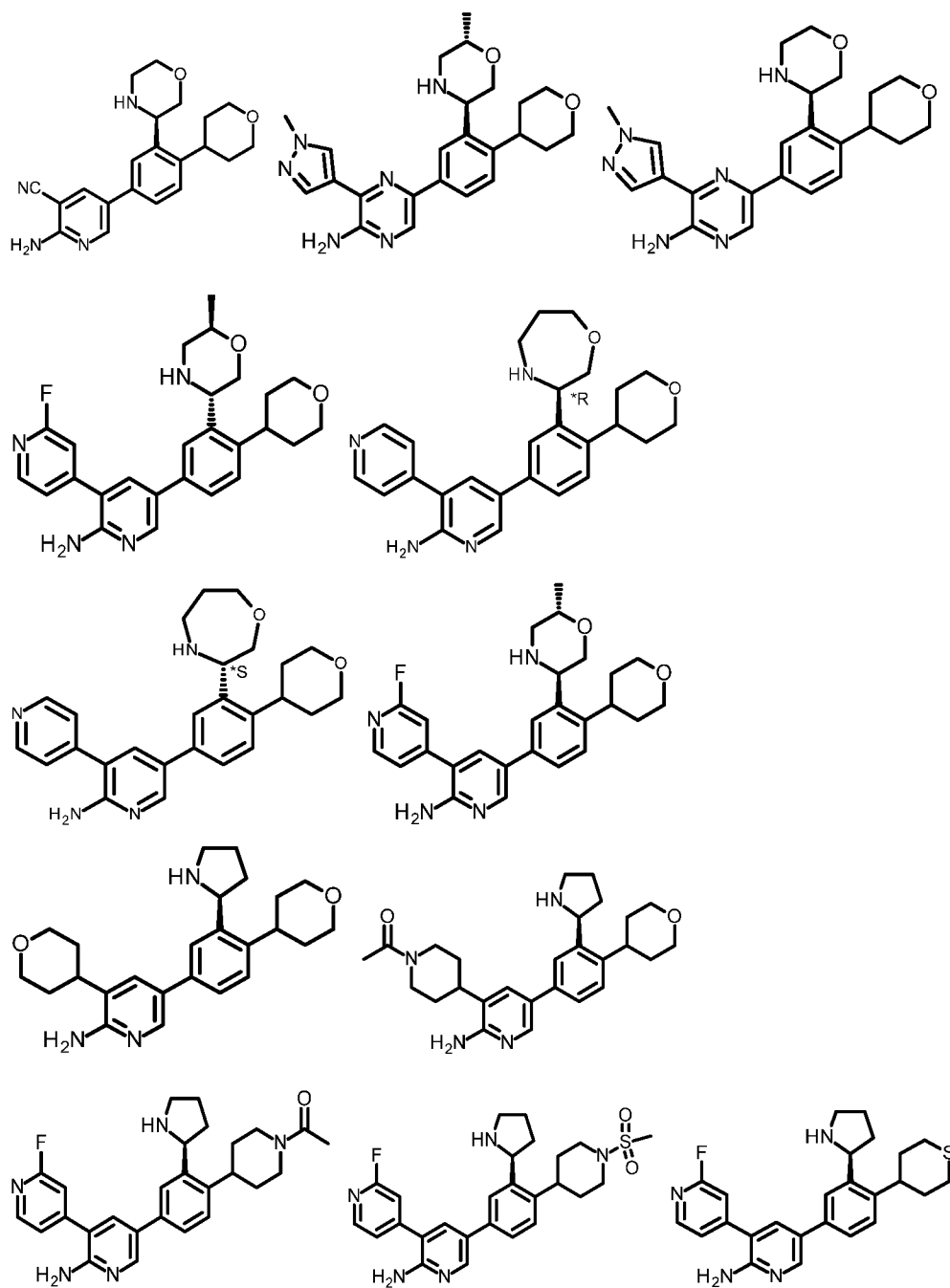


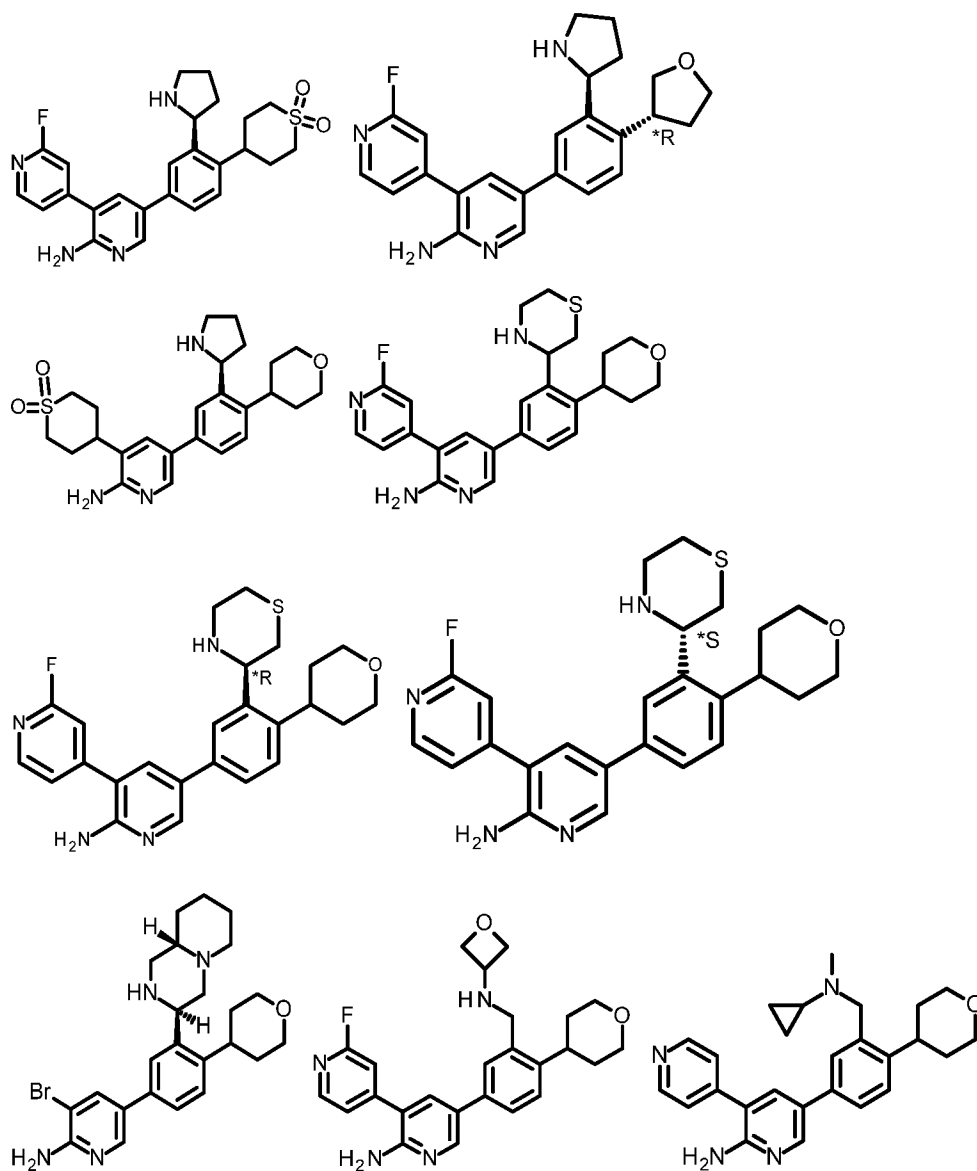


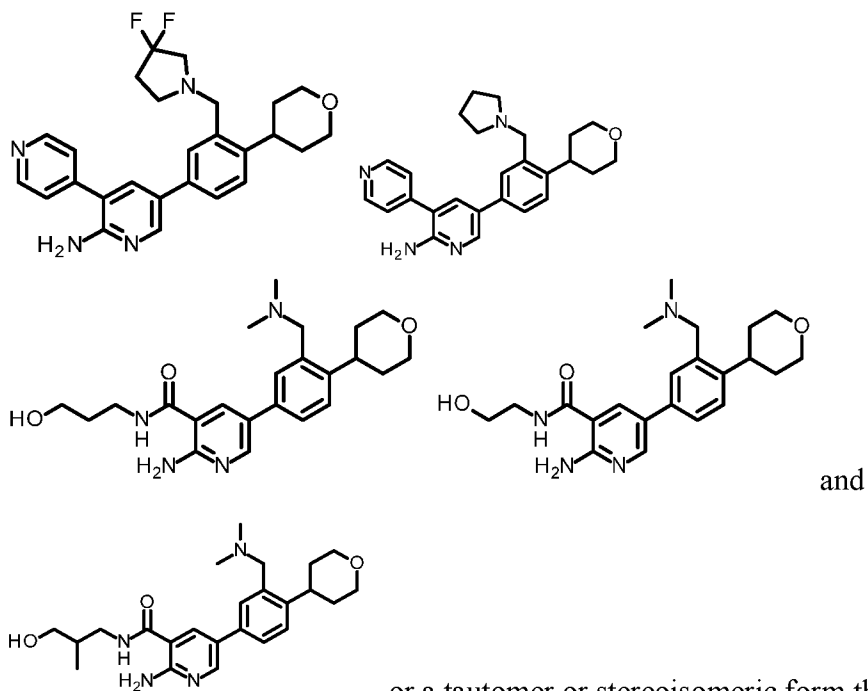












- 5 16. A kit comprising the therapeutic combination of any one of claims 1-15, and instructions for using the therapeutic combination in treating a hepatitis B virus (HBV) infection in a subject in need thereof.
17. The therapeutic combination of any one of claims 1 to 15 for use in treating a hepatitis B virus (HBV) infection in a subject in need thereof.

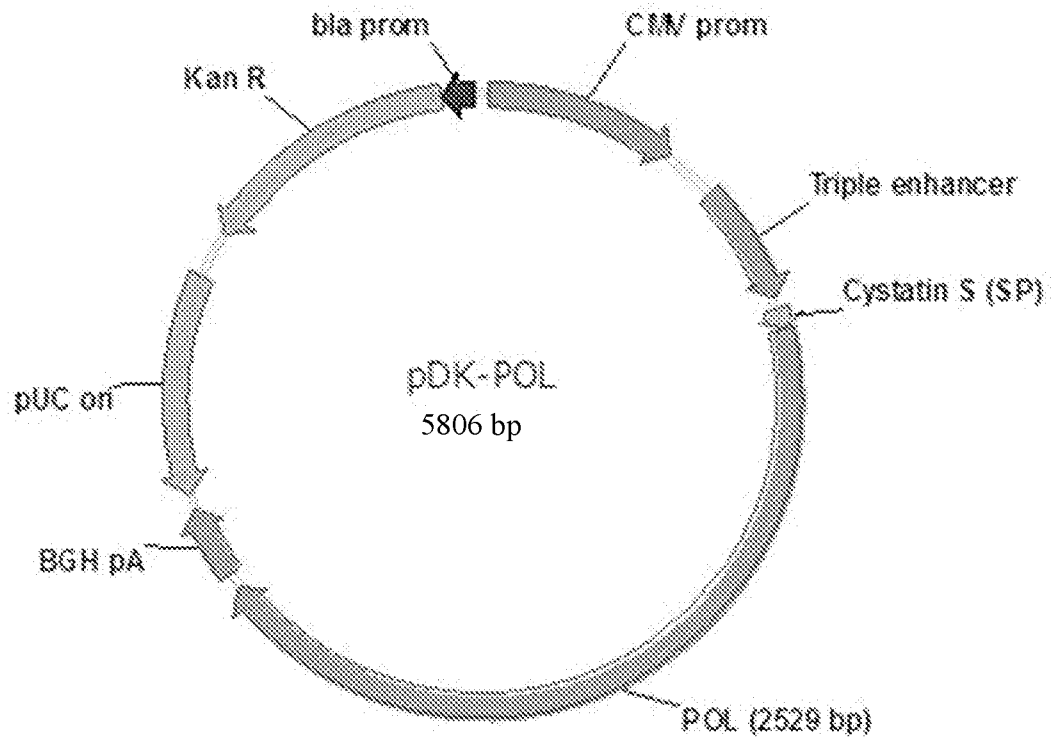
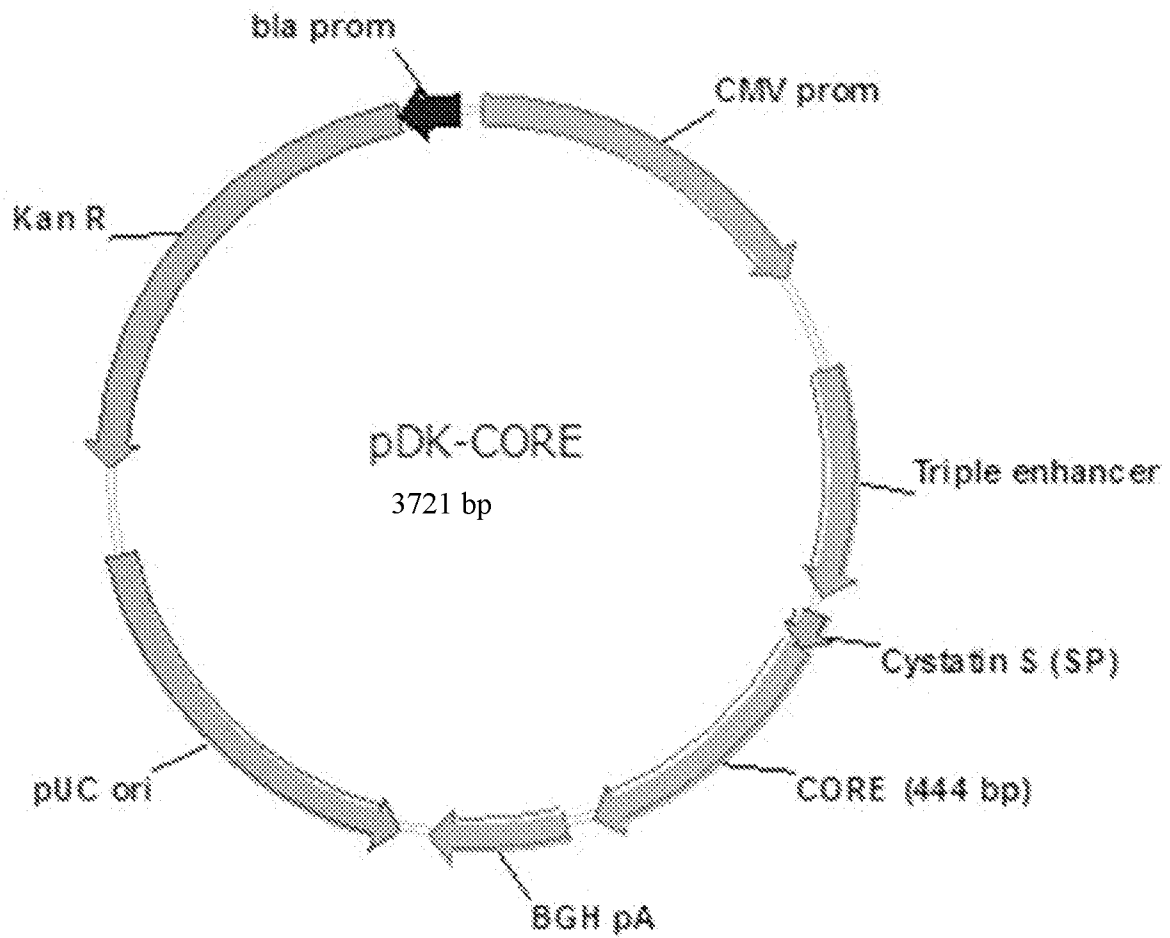
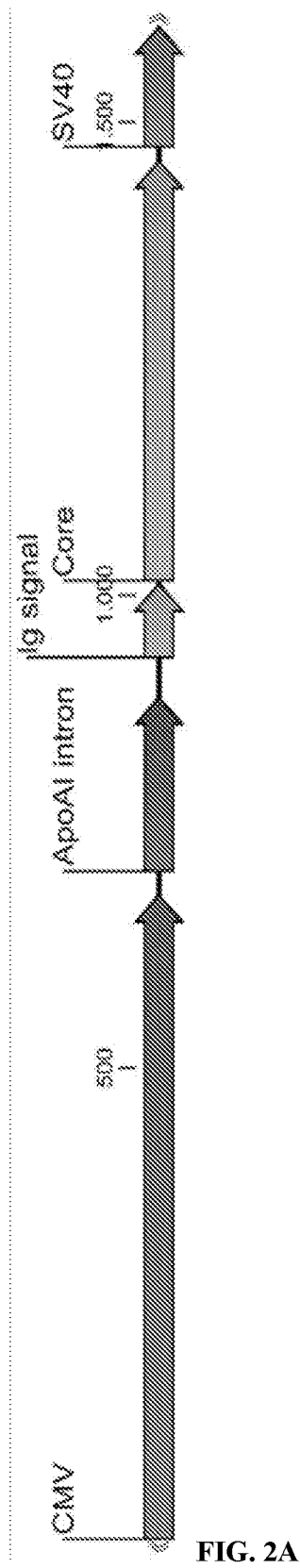


FIG. 1A

**FIG. 1B**



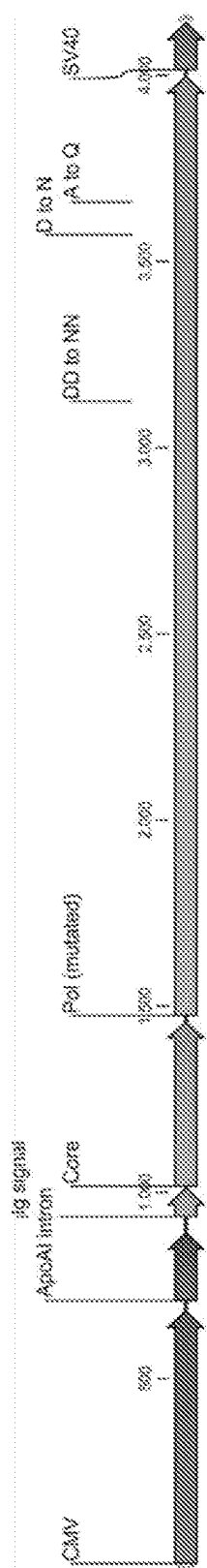


FIG. 2B

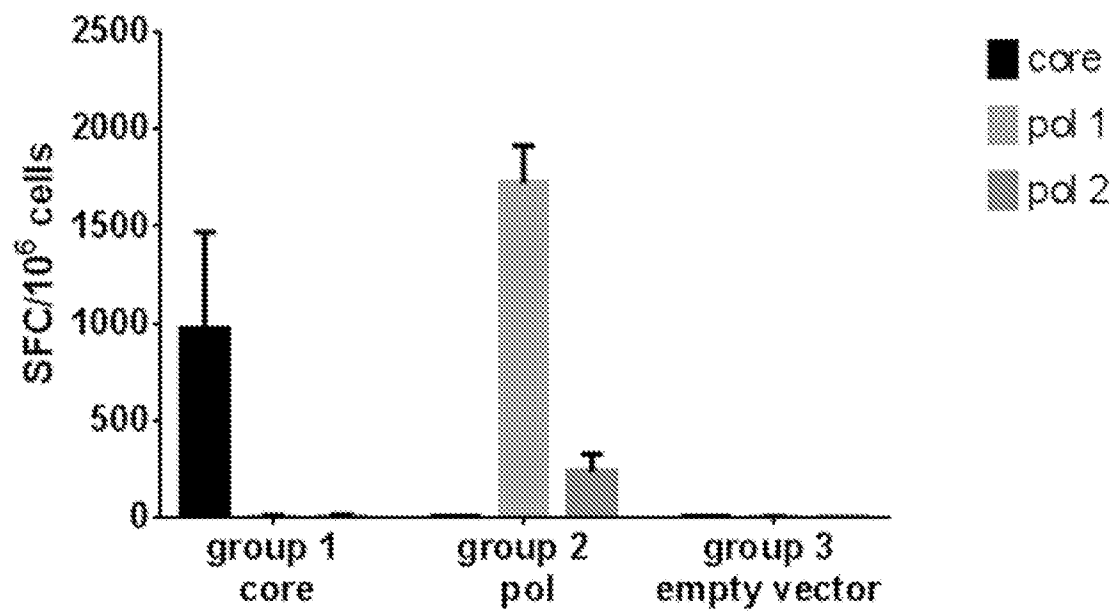


FIG. 3

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2020/055718

A. CLASSIFICATION OF SUBJECT MATTER

INV. A61K31/4433 A61K31/4439 A61K31/444 A61K31/501 A61K31/506
A61K39/12 A61K39/39 A61P31/20 A61K45/06 C07D401/14
C07D403/14 C07D405/14 C07D413/14 C07D417/14 C07D498/14

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K A61P C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, CHEM ABS Data, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2016/020538 A1 (TRANSGENE SA [FR]; PASTEUR INSTITUT [FR]; INST NAT SANTE RECH MED [FR]) 11 February 2016 (2016-02-11) claims 1-2 page 28, line 1 - page 29, line 9 page 43, line 20 - page 44, line 21 examples; sequence 6 -----	1-17
A	WO 2019/016071 A1 (BAYER PHARMA AG [DE]; BAYER AG [DE]; DEUTSCHES KREBSFORSCH [DE]) 24 January 2019 (2019-01-24) claims 1-19 MAP4K1 = HPK1; page 61, line 31 - page 62, line 12 ----- -/--	1-17



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

29 September 2020

Date of mailing of the international search report

12/10/2020

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INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2020/055718

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2018/167147 A1 (HOFFMANN LA ROCHE [CH]; GENENTECH INC [US]) 20 September 2018 (2018-09-20) pages 1-2; table B1 claims 1-49	1-17
A	----- US 2019/076401 A1 (VECHORKIN OLEG [US] ET AL) 14 March 2019 (2019-03-14) Formula IIa6-IIa9; claim 29 examples	1-17
A	----- JAMES W. LEAHY ET AL: "Discovery of a Novel Series of Potent and Orally Bioavailable Phosphoinositide 3-Kinase [gamma] Inhibitors", JOURNAL OF MEDICINAL CHEMISTRY, vol. 55, no. 11, 14 June 2012 (2012-06-14) , pages 5467-5482, XP055085698, ISSN: 0022-2623, DOI: 10.1021/jm300403a abstract figure 1 tables -----	1-17

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/IB2020/055718

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		EP 3655406 A1	27-05-2020
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		UY 37813 A	28-02-2019
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		WO 2019051199 A1	14-03-2019
