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(54) Title: NOVEL REVERSE TRANSCRIPTASE INHIBITORS

(57) Abstract: Compounds that are capable of inhibiting an activity of a reverse transcriptase are disclosed. Further disclosed are pharmaceutical compositions containing these compounds, and methods of inhibiting an activity of reverse transcriptase and/or of a mutant thereof and of treating an infection caused by a retrovirus, utilizing these compounds. The disclosed compounds are either identified by computational means or are designed and newly prepared based on structural features identified, at least in part, by computational means. Thus, further disclosed is a method of identifying candidate compounds for inhibiting an activity of a wild type reverse transcriptase and/or for treating a viral infection caused by a retrovirus.



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NOVEL REVERSE TRANSCRIPTASE INHIBITORS**FIELD AND BACKGROUND OF THE INVENTION**

The present invention, in some embodiments thereof, relates to compounds
5 capable of inhibiting an activity of reverse transcriptase (RT) and to methods utilizing
same in the treatment of retroviral infections such as acquired immune deficiency
syndrome (AIDS) caused by a human immunodeficiency virus (HIV).

Retroviruses are small, single-stranded positive-sense RNA viruses. A retroviral
particle comprises two identical single-stranded positive sense RNA molecules. Their
10 genome contains, among other things, the sequence encoding the enzyme reverse
transcriptase (RT). Many molecules of reverse transcriptase are found in close
association with the genomic RNA in the mature viral particles. Upon entering a cell,
this reverse transcriptase produces a double-stranded DNA copy of the viral genome,
which is then inserted into the chromatin of a host cell. Once inserted, the viral
15 sequence is called a provirus. Retroviral integration is directly dependent upon viral
proteins. Linear viral DNA termini (the LTRs) are the immediate precursors to the
integrated proviral DNA. There is a characteristic duplication of short stretches of the
host's DNA at the site of integration.

The reverse transcriptase is therefore an essential enzyme in the retroviral life
20 cycle. After penetrating into the target cell, RT copies the viral (+) single stranded
genomic RNA into double stranded DNA. This process is catalyzed solely by RT and
depends on two fundamental activities: the DNA polymerase, which copies both RNA
and DNA into DNA, and the ribonuclease H (RNase H) that concomitantly cleaves the
viral RNA strand in the RNA/DNA heteroduplex. The resulting double-stranded DNA
25 is transported into the nucleus as part of a pre-integration complex and is subsequently
incorporated into the DNA of the cell by the viral enzyme integrase.

To date, the most widely investigated retrovirus is the human immunodeficiency
virus type-1 (HIV-1). Since the identification, two decades ago, of HIV-1 as the cause
for acquired human immunodeficiency syndrome (AIDS), a massive search for
30 molecules that block RT activities has been performed worldwide. This resulted in the
identification of two classes of anti-RT compounds: nucleoside/nucleotide RT inhibitors
(NRTIs) and the non-nucleoside RT inhibitors (NNRTIs).

NRTIs are competitive inhibitors that mimic normal nucleotides but lack the 3'-OH terminus and, hence terminate the nascent DNA chain elongation by preventing additional incorporation of nucleotides by RT.

The NNRTIs include a variety of non-competitive inhibitors that bind specifically to a hydrophobic pocket in proximity to the DNA polymerase active site of the enzyme; most of them are highly specific against HIV-1 RT with minimal effects on the closely-related HIV-2 RT. Both classes of inhibitors are currently used in therapy against HIV-1, as part of the highly active anti-retroviral therapy (HAART), which simultaneously targets the RT, the viral protease and most recently the entry step of the virus.

Currently marketed HIV-1 drugs are dominated by either nucleoside reverse transcriptase inhibitors or peptidomimetic protease inhibitors. Presently, a triple-drug therapy regimen is often used to treat AIDS patients. This "cocktail" includes a combination of a protease inhibitor called indinavir™ with two nucleoside reverse-transcriptase inhibitors, AZT™ and 3TC™. The triple drug therapy results in a decrease in measured levels of virus in both blood and lymphatic tissues. However the cocktail therapy is largely restricted by the emergence of resistant viral strains, high costs and long duration of the treatment.

Non-nucleoside reverse transcriptase inhibitors (NNRTIs) have recently gained an increasingly important role in the therapy of HIV infections. At least 30 different classes of NNRTI have been described in the literature and several NNRTIs have been evaluated in clinical trials. Dipyridodiazepinone (nevirapine), benzoxazinone (efavirenz), bis(heteroaryl) piperazine derivatives (delavirdine) and Etravirine have been approved thus far for clinical use.

However, the major drawback to the development and application of NNRTIs is the propensity for rapid emergence of drug resistant strains, both in tissue cell culture and in treated individuals, particularly those subject to monotherapy.

A significant obstacle for the use of NNRTIs is their very high specificity that reduces their efficacy against mutated variants of the virus. Given that the protein targets for therapy, especially the RT and protease, are quite flexible and can tolerate mutations and still remain active, resistance develops rapidly during treatment even when a combination of drugs is used.

Several mutant strains of HIV have been characterized, and resistance to known therapeutic agents is due to mutations in the RT gene. Some of the most commonly observed mutants clinically are: the Y181C mutant, in which a tyrosine (Y), at codon 181, has been mutated to a cysteine (C) residue, and K103N where the lysine (K) at position 103 has been replaced by asparagine (N). Other mutants, which emerge with increasing frequency during treatment with known antivirals, include the single mutants V106A, G190A, Y188C, and P236L; and the double mutants K103N/Y181C, K103N/P225H, K103N/V108I, and K103N/L100I.

As therapy and prevention of HIV infection using antivirals continues, the emergence of new resistant strains is expected to increase.

Consequently, intense efforts have been directed in recent years to find broad spectrum NNRTIs that inhibit both wild type and drug resistant variants of RT. These efforts have led to the discovery of several inhibitors, including TMC-125, GW678248 YM-215389 and R278474 (Rilpivirine) [Das et al. *J Med Chem* **2004**, 47, 2550-60; Janseen et al. *J. Med. Chem.* **2005**, 48, 1901-9; Masuda et al. *Bioorg Med Chem.* **2005**, 13, 949-61; and Romines et al. *J Med Chem.* **2006**, 49, 727-39].

Molecular modeling is an approach that could be used to narrow down a library of thousands of random molecules into a smaller list of the potentially effective inhibitors. Two major tools intensively used in this arena are virtual screening and *de novo* drug design, both being based either on known crystal structure of the specific target enzyme or on known inhibitors against the enzyme.

Virtual screening is a technique, in which each member of a large available chemical database is docked into the active site of an enzyme of interest. The compounds are then ranked according to their potential molecular interactions with the enzyme. Eventually, the top-scoring compounds can be obtained and tested for their capacity to inhibit in vitro the activity of the enzyme. Exemplary methodologies for virtual screening potential RT inhibitors are described in Herschhorn et al. [*J. Med. Chem.* **2007** 50, 2370–2384 and *Biochemistry* **2008**, 47, 490–502].

Novel inhibitors against HIV-1 RT are usually identified by screening a very large number of compounds against the recombinant RT enzyme and testing the compounds for their ability to protect susceptible cells from a productive HIV-1 infection. The latter method also requires an additional step of identifying, among the

active inhibitors, those compounds that are specifically directed against HIV-1 RT (since compounds not active against HIV-1 RT may suppress viral infectivity by other mechanisms). Such screenings and the subsequent optimization of the inhibitors by systematic chemical modifications are highly time-and resource-consuming.

5 *De novo* design of new molecules is done by first identifying functional groups that bind the active site of the target protein and then linking them into the final inhibitor molecules. The molecules are then synthesized and tested for their enzymatic inhibitory effects.

10 During the last years, the crystal structures of wild type and drug resistant variants of HIV-1 RT have been used extensively in the design of novel NNRTIs. Mao et al. [*Biochem. Pharmacol.* **2000**, 60, 1251-65] used a flexible binding site based on several structures to design PETT (phenethylthiazolylthiourea) and DABO (dihydroalkoxybenzyloxypyrimidine) derivatives with activity against drug resistant mutants of HIV-1 RT. Vinkers et al. [*J. Med. Chem.* **2003**, 46, 2765-73] have recently
15 designed several novel *de novo* NNRTIs using advanced software that includes a synthesis route for each generated molecule.

SUMMARY OF THE INVENTION

20 The present inventors have designed and successfully practiced a novel methodology for identifying compounds that inhibit reverse transcriptase. To this end, two different crystalline forms of wild type HIV-1 RT were used, and a chemical library of 46,000 compounds was screened for molecules that could bind independently to each of the tested crystalline forms. Those molecules that interacted with both RT crystalline forms were subsequently tested *in vitro* for inhibiting an activity of HIV-1 RT, and a
25 plurality of molecules were identified as potent RT inhibitors. Based on the chemical structure of a selected, highly potent compound, additional, novel compounds were designed, prepared and tested, and were also found as potent inhibitors of HIV-1 RT activity.

30 According to an aspect of some embodiments of the present invention there is provided a compound having the general Formula Ia:

Z-X-A

Formula Ia

5 or a pharmaceutically acceptable salt thereof;

wherein:

Z is selected from the group consisting of a substituted or non-substituted aryl and heteroaryl, wherein when substituted, the substituent is selected from the group consisting of halide, alkoxy, hydroxy, thioalkoxy, thioalkyl, alkyl, haloalkyl, amine, sulfonamide, sulfinyl, sulfonyl, carboxylate, cyano, nitro, amide, and OCO_2R^4 ;

X is selected from the group consisting of $\text{NR}^5\text{-C(=W)-NR}^6$ and $\text{N=CR}^7\text{-NR}^6$, whereas W is selected from the group consisting of N, O, S, =NCN , =CHNO_2 and NR^{11} ;

A is $\text{R}^8\text{-Y-R}^9$,

wherein Y is selected from the group consisting of -O- , -N- , -S- , $\text{-CR}^{10}\text{R}^{11}\text{-}$, $\text{NR}^{10}\text{-}$, -SC(=O)- , -C(=O)S- , -OC(=S)- , -C(=S)O- , -SC(=S)- , -C(=S)S- , -S(=O)- , S(=O)_2 , $\text{-NR}^{10}\text{S(=O)-}$, $\text{-S(=O)NR}^{10}\text{-}$, $\text{-S(=O)}_2\text{NR}^{10}\text{-}$, $\text{-NR}^{10}\text{S(=O)}_2\text{-}$, -C(=O)- , -C(=O)O- , -OC(=O)- , $\text{-C(=O)NR}^{11}\text{-}$, $\text{-R}^{11}\text{NC(=O)-}$, -OC(=O)O- , aryl and heterocyclic;

R^7 is selected from the group consisting of -SC(=O)R^{12} , -C(=O)SR^{12} , -SR^{12} , S(=O)R^{12} , -OC(=O)R^{12} , -C(=O)OR^{12} , $\text{-NR}^{13}\text{C(=O)R}^{12}$, $\text{-C(=O)NR}^{12}\text{R}^{13}$, -OR^{12} and $\text{-NR}^{12}\text{R}^{13}$;

R^8 and R^9 are each independently a linear or branched, saturated or unsaturated substituted or non-substituted hydrocarbon chain having 1-10 carbon atoms in its backbone, which when substituted, the substituent is selected from the group consisting of alkyl and cycloalkyl; and

R^4 , R^5 , R^6 , R^{10} , R^{11} , R^{12} and R^{13} are each independently hydrogen or alkyl;

with the proviso that:

when X is $\text{NR}^5\text{-C(=W)-NR}^6$ and W is sulfur, A is other than $(\text{CH}_2)_3\text{O}(\text{CH}_2)_2\text{CH}_3$;

when A is $(\text{CH}_2)_3\text{O}(\text{CH}_2)_2\text{CH}_3$ and X is $\text{NR}^5\text{-C(=W)-NR}^6$, W is other than sulfur;

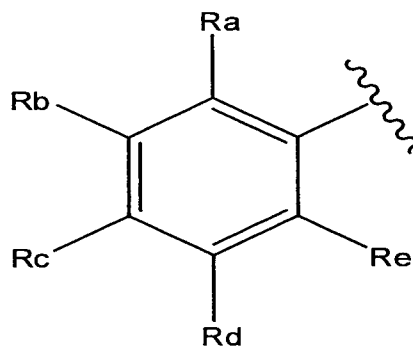
and

when X is $\text{NR}^5\text{-C(=W)-NR}^6$ and W is sulfur and A is $(\text{CH}_2)_3\text{O}(\text{CH}_2)_2\text{CH}_3$, Z is selected from the group consisting of a substituted or non-substituted heteroaryl and a substituted aryl, wherein at least one substituent on the aryl is other than alkoxy, methyl,

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and halide, the compound being capable of interacting with a hydrophobic binding site within at least one of the catalytic domains of the reverse transcriptase.

In some embodiments, Z is a substituted or non-substituted aryl having formula II:



Formula II

or a pharmaceutically acceptable salt thereof;

wherein:

10 Ra-Re are each independently selected from the group consisting of halide, alkoxy, hydroxy, thioalkoxy, thioalkyl, alkyl, haloalkyl, amine, sulfonamide, sulfinyl, sulfonyl, carboxylate, cyano, nitro, amide, and OCO_2R^4 ,

wherein at least one of the Ra-Rd is selected from the group consisting of halide, alkoxy, alkyl, carboxylate, cyano, nitro, haloalkyl and amine.

15 In some embodiments of the invention, Ra is selected from the group consisting of alkoxy, alkyl and halide.

In some embodiments of the invention, the alkoxy is methoxy.

In some embodiments of the invention, Rb is hydrogen.

20 In some embodiments of the invention, Rc is selected from the group consisting of hydrogen, halide and nitro.

In some embodiments of the invention, Rd is selected from the group consisting of hydrogen, alkyl, halide, alkoxy and haloalkyl.

In some embodiments of the invention, Rd is alkoxy.

In some embodiments of the invention, X is the $\text{NR}^5\text{-C(=W)-NR}^6$.

25 In some embodiments of the invention, W is S or O.

In some embodiments of the invention, each of R^5 and R^6 is hydrogen.

In some embodiments of the invention, Y is selected from the group consisting of -O-, -N-, -S-, -CR¹⁰R¹¹-, -NR¹⁰-, -SC(=O)-, -C(=O)S-, -OC(=S)-, -C(=S)O-, -SC(=S)-, -C(=S)S-, -S(=O)-, S(=O)₂-, -NR¹⁰S(=O)-, -S(=O)NR¹⁰-, -S(=O)₂NR¹⁰-, -NR¹⁰.S(=O)₂-, -C(=O)-, -C(=O)O-, -OC(=O)-, -C(=O)NR¹¹-, -R¹¹NC(=O)- and -OC(=O)O.

5 In some embodiments of the invention, R⁸ and R⁹ are each independently a linear, saturated, non-substituted hydrocarbon chain having 1-10 carbon atoms in its backbone.

In some embodiments of the invention, the hydrocarbon chain has 2-4 carbon atoms.

10 In some embodiments of the invention, R⁸ is a hydrocarbon chain having 2 or 3 carbon atoms.

In some embodiments of the invention, R⁹ is a hydrocarbon chain having 3 or 4 carbon atoms.

15 In some embodiments of the invention, Z has the general formula II, wherein Ra is methoxy, Rb is hydrogen, Rc is chloro, Rd is methoxy and Re is hydrogen.

In some embodiments of the invention, A is the NR⁵-C(=W)-NR⁶, W is O or S and each of R⁵ and R⁶ is hydrogen.

20 According to an aspect of some embodiments of the present invention there is provided a pharmaceutical composition comprising the compound having Formula Ia as described herein and a pharmaceutically acceptable carrier.

In some embodiments of the invention, the composition is packaged in a packaging material and identified in print, in or on the packaging material, for use in the treatment of a viral infection caused by a retrovirus.

25 According to an aspect of some embodiments of the present invention there is provided a process of synthesizing the compound having Formula IA as described herein, the process comprising:

reacting a compound having the formula Z-R¹ with a compound having the formula A-R², wherein R² is amine and R¹ is selected such that upon reacting with the amine, the X is formed.

30 According to an aspect of some embodiments of the present invention there is provided a method of inhibiting an activity of a reverse transcriptase, the method

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comprising contacting the reverse transcriptase with a compound selected from the group consisting of:

(i) a compound having the general Formula I:

5

$$Z-X-A$$

Formula I

or a pharmaceutically acceptable salt thereof;

wherein:

10 Z is selected from the group consisting of a substituted or non-substituted aryl and heteroaryl, wherein when substituted, the substituent is selected from the group consisting of halide, alkoxy, hydroxy, thioalkoxy, thioalkyl, alkyl, haloalkyl, amine, sulfonamide, sulfinyl, sulfonyl, carboxylate, cyano, nitro, amide, and OCO_2R^4 ;

X is selected from the group consisting of $NR^5-C(=W)-NR^6$ and $N=CR^7-NR^6$, whereas W is selected from the group consisting of N, O, S, $=NCN$, $=CHNO_2$ and NR^{11} ;

15

A is R^8-Y-R^9 ,

wherein Y is selected from the group consisting of $-O-$, $-N-$, $-S-$, $-CR^{10}R^{11}-$, $-NR^{10}-$, $-SC(=O)-$, $-C(=O)S-$, $-OC(=S)-$, $-C(=S)O-$, $-SC(=S)-$, $-C(=S)S-$, $-S(=O)-$, $S(=O)_2$, $-NR^{10}S(=O)-$, $-S(=O)NR^{10}-$, $-S(=O)_2NR^{10}-$, $-NR^{10}.S(=O)_2-$, $-C(=O)-$, $-C(=O)O-$, $-OC(=O)-$, $-C(=O)NR^{11}-$, $-R^{11}NC(=O)-$, $-OC(=O)O-$, aryl and heterocyclic;;

20

R^7 is selected from the group consisting of $SC(=O)R^{12}$, $C(=O)SR^{12}$, SR^{12} , $S(=O)R^{12}$, $OC(=O)R^{12}$, $C(=O)OR^{12}$, $NR^{13}C(=O)R^{12}$, $C(=O)NR^{12}R^{13}$, OR^{12} and $NR^{12}R^{13}$;

R^8 and R^9 are each independently a linear or branched, saturated or unsaturated substituted or non-substituted hydrocarbon chain having 1-10 carbon atoms in its backbone, which when substituted, the substituent is selected from the group consisting of alkyl and cycloalkyl; and

25

R^4 , R^5 , R^6 , R^{10} , R^{11} , R^{12} and R^{13} are each independently hydrogen or alkyl;

(ii) a compound having the general Formula III:

$$B^1-D-B^2$$

30

Formula III

or a pharmaceutically acceptable salt thereof;

wherein:

B¹ and B² are each independently selected from the group consisting of a substituted or unsubstituted heteroaryl and heteroalicyclic, which, when substituted, the substituent is selected from the group consisting of halide, alkoxy, hydroxy thioalkoxy, thioalkyl, alkyl, haloalkyl, amine, sulfonamide, sulfinyl, sulfonyl, carboxylate, cyano, nitro, amide, and OCO₂R¹⁵,

D is selected from a group consisting of O-, -N-, -S-, -CR¹⁶R¹⁷-, -NR¹⁶-, -SC(=O)-, -C(=O)S-, -OC(=S)-, -C(=S)O-, -SC(=S)-, -C(=S)S-, -S(=O)-, S(=O)₂-, -NR¹⁶S(=O)-, -S(=O)NR¹⁶-, -S(=O)₂NR¹⁶-, -NR¹⁶.S(=O)₂-, -C(=O)-, -C(=O)O-, -OC(=O)-, -C(=O)NR¹⁷-, -R¹⁷NC(=O)- and -OC(=O)O-, aryl and heterocyclic; and

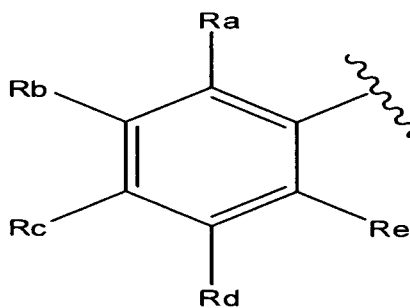
R¹⁵, R¹⁶ and R¹⁷ are each independently hydrogen or alkyl; and

(iii) a compound selected from the group consisting of Compounds **1**, **4**, **5**, **6**, **8** and **13**,

the compound being capable of interacting with a hydrophobic binding site within at least one of the catalytic domains of the reverse transcriptase.

According to some embodiments of the invention, the compound has the general Formula I.

According to some embodiments of the invention, Z is a substituted or non-substituted aryl having formula II:



Formula II

or a pharmaceutically acceptable salt thereof;

wherein:

Ra-Re are each independently selected from the group consisting of halide, alkoxy, hydroxy, thioalkoxy, thioalkyl, alkyl, haloalkyl, amine, sulfonamide, sulfinyl, sulfonyl, carboxylate, cyano, nitro, amide, and OCO₂R⁴,

provided that at least one of the Ra-Rd is selected from the group consisting of halide, alkoxy, alkyl, carboxylate, cyano, nitro, haloalkyl and amine.

According to some embodiments of the invention, Ra is selected from the group consisting of alkoxy, alkyl and halide.

5 According to some embodiments of the invention, Ra is alkoxy.

According to some embodiments of the invention, Rb is hydrogen.

According to some embodiments of the invention, Rc is selected from the group consisting of hydrogen, halide and nitro.

10 According to some embodiments of the invention, Rd is selected from the group consisting of hydrogen, alkyl, halide, alkoxy and haloalkyl.

According to some embodiments of the invention, Rd is alkoxy.

According to some embodiments of the invention, X is the $\text{NR}^5\text{-C(=W)-NR}^6$.

According to some embodiments of the invention, W is S or O.

According to some embodiments of the invention, each of R^5 and R^6 is hydrogen.

15 According to some embodiments of the invention, Y is selected from the group consisting of $-\text{O}-$, $-\text{N}-$, $-\text{S}-$, $-\text{CR}^{10}\text{R}^{11}-$, $-\text{NR}^{10}-$, $-\text{SC(=O)}-$, $-\text{C(=O)S}-$, $-\text{OC(=S)}-$, $-\text{C(=S)O}-$, $-\text{SC(=S)}-$, $-\text{C(=S)S}-$, $-\text{S(=O)}-$, S(=O)_2 , $-\text{NR}^{10}\text{S(=O)}-$, $-\text{S(=O)NR}^{10}-$, $-\text{S(=O)}_2\text{NR}^{10}-$, $-\text{NR}^{10}\text{S(=O)}_2-$, $-\text{C(=O)}-$, $-\text{C(=O)O}-$, $-\text{OC(=O)}-$, $-\text{C(=O)NR}^{11}-$, $-\text{R}^{11}\text{NC(=O)}-$, $-\text{OC(=O)O}-$, aryl and heterocyclic.

20 According to some embodiments of the invention, R^8 and R^9 are each independently a linear, saturated, non-substituted hydrocarbon chain having 1-10 carbon atoms in its backbone.

According to some embodiments of the invention, the hydrocarbon chain has 2-4 carbon atoms.

25 According to some embodiments of the invention, R^8 is a hydrocarbon chain having 2 or 3 carbon atoms.

According to some embodiments of the invention, R^9 is a hydrocarbon chain having 3 or 4 carbon atoms.

30 According to some embodiments of the invention, Z has the general formula II, wherein Ra is methoxy, Rb is hydrogen, Rc is chloro, Rd is methoxy and Re is hydrogen.

According to some embodiments of the invention, A is the $\text{NR}^5\text{-C(=W)-NR}^6$, W is O or S and each of R^5 and R^6 is hydrogen.

According to some embodiments of the invention, the compound has the general Formula III.

5 According to some embodiments of the invention, the reverse transcriptase is wild type HIV-1 reverse transcriptase or a mutant thereof and at least one heteroatom in the B^1 and B^2 is capable of interacting with Glu138 of the p51 subunit in the HIV-1 reverse transcriptase and/or Tyr318 in the p66 subunit in the HIV-1 reverse transcriptase.

10 According to some embodiments of the invention, the compound is Compound 3.
According to some embodiments of the invention, the contacting is affected *in vitro*.

According to some embodiments of the invention, the contacting is affected *in vivo*.

15 According to some embodiments of the invention, the reverse transcriptase is selected from the group consisting of a wild type reverse transcriptase and a mutant thereof.

According to some embodiments of the invention, the reverse transcriptase is an HIV-1 reverse transcriptase or a mutant thereof.

20 According to some embodiments of the invention, the reverse transcriptase is an HIV-2 reverse transcriptase or a mutant thereof.

According to some embodiments of the invention, the compound is characterized by a specific inhibition of the reverse transcriptase, as determined by the percent of residual activity of a cellular DNA polymerase in the presence of the compound divided
25 by the percent of residual activity of the reverse transcriptase in the presence of the compound (i.e. the selectivity index).

According to some embodiments of the invention, the reverse transcriptase is wild type HIV-1-reverse transcriptase and the DNA polymerase is Klenow fragment of E. coli DNA polymerase I (KF).

30 According to some embodiments of the invention, the activity of the reverse transcriptase is selected from the group consisting of RT-associated DNA-dependent DNA polymerase activity and RNA-dependent DNA polymerase activity.

According to an aspect of some embodiments of the present invention there is provided a pharmaceutical composition comprising, as an active ingredient, a compound as described herein and a pharmaceutically acceptable carrier, the composition being packaged in a packaging material and identified in print, in or on the packaging material, for use in the treatment of a viral infection caused by a retrovirus.

According to an aspect of some embodiments of the present invention there is provided a use of a compound as described herein in the preparation of a medicament for treating a viral infection caused by a retrovirus.

According to an aspect of some embodiments of the present invention there is provided a compound as described herein, being identified for use in the treatment of a viral infection caused by a retrovirus.

According to an aspect of some embodiments of the present invention there is provided a method of treating a viral infection caused by a retrovirus, the method comprising administering to a subject in need thereof a therapeutically effective amount of a compound as described herein.

According to some embodiments of the invention, the retrovirus is HIV-1.

According to some embodiments of the invention, the retrovirus is selected from the group consisting of HIV-1 and HIV-2.

According to some embodiments of the invention, any of the pharmaceutical compositions as described herein further comprises at least one agent capable of treating a viral infection caused by a retrovirus.

According to some embodiments of the invention, in any of the uses described herein the compound is utilized in combination with at least one agent capable of treating a viral infection caused by a retrovirus.

According to some embodiments of the invention, the method as described herein comprises administering to the subject a therapeutically effective amount of at least one agent capable of treating a viral infection caused by a retrovirus.

According to some embodiments of the invention, the at least one agent is selected from the group consisting of a protease inhibitor, a reverse transcriptase inhibitor, an integrase inhibitor and any combination thereof.

According to some embodiments of the invention, the at least one agent is selected from the group consisting of 3'-azido-3'-deoxythymidine (AZT), 2',3'-

dideoxyinosine (ddI), 2',3'-dideoxycytidine (ddC), d4T, 3TC, dipyrindodiazepinone (nevirapine), benzoxazinone (efavirenz) and bis(heteroaryl) piperazine derivatives (delavirdine), abacavir and any combination thereof.

According to an aspect of some embodiments of the present invention there is provided a method of identifying a candidate compound for inhibiting an activity of a reverse transcriptase, the method comprising computationally identifying a compound which is capable of specifically binding to a three-dimensional structure of an active site cavity of at least two crystalline forms of a reverse transcriptase, thereby identifying the candidate compound for inhibiting an activity of the type reverse transcriptase.

According to some embodiments of the invention, the reverse transcriptase is selected from the group consisting of a HIV-1 reverse transcriptase and a HIV-2 reverse transcriptase.

According to some embodiments of the invention, the reverse transcriptase is selected from the group consisting of a wild type reverse transcriptase and a mutant thereof.

According to some embodiments of the invention, the reverse transcriptase is a wild type HIV-1 reverse transcriptase.

According to some embodiments of the invention, the reverse transcriptase is a wild type HIV-1 reverse transcriptase and the crystalline forms of the HIV-1 reverse transcriptase have the pdb entry codes 1fk9 and 1dtq.

According to some embodiments of the invention, the computationally identifying comprises:

- (a) obtaining at least two crystalline forms of the reverse transcriptase;
- (b) detecting a location of an active site in each of the crystalline forms of the reverse transcriptase;
- (c) generating a protomol for the active site in each the crystalline forms of the reverse transcriptase;
- (d) performing a docking process of compounds from a chemical library onto the active sites using the protomol; and
- (e) identifying a compound that both spatially and chemically fits to the three-dimensional structure of the active site cavities of each crystalline form of the reverse transcriptase.

According to some embodiments of the invention, the docking process is performed by fragmenting each compound and fitting the conformation of each fragment into the protomol to yield a spatial structure that maximizes molecular similarity to the protomol.

5 According to some embodiments of the invention, the identifying is by computational means.

According to some embodiments of the invention, the method further comprises biologically assaying the candidate compound for its activity in inhibiting a catalytic activity of the reverse transcriptase.

10 According to some embodiments of the invention, the method further comprises biologically assaying the candidate compound for its activity in treating a viral infection caused by a retrovirus.

According to some embodiments of the invention, the method is being for identifying candidate compounds for treating a viral infection caused by a retrovirus.

15 Unless otherwise defined, all technical and/or scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the invention pertains. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of embodiments of the invention, exemplary methods and/or materials are described below. In case of conflict, the patent specification, including definitions, will control. In addition, the materials, methods, and
20 examples are illustrative only and are not intended to be necessarily limiting.

Implementation of the method and/or system of embodiments of the invention can involve performing or completing selected tasks manually, automatically, or a combination thereof. Moreover, according to actual instrumentation and equipment of
25 embodiments of the method and/or system of the invention, several selected tasks could be implemented by hardware, by software or by firmware or by a combination thereof using an operating system.

For example, hardware for performing selected tasks according to embodiments of the invention could be implemented as a chip or a circuit. As software, selected tasks
30 according to embodiments of the invention could be implemented as a plurality of software instructions being executed by a computer using any suitable operating system. In an exemplary embodiment of the invention, one or more tasks according to exemplary

embodiments of method and/or system as described herein are performed by a data processor, such as a computing platform for executing a plurality of instructions. Optionally, the data processor includes a volatile memory for storing instructions and/or data and/or a non-volatile storage, for example, a magnetic hard-disk and/or removable media, for storing instructions and/or data. Optionally, a network connection is provided as well. A display and/or a user input device such as a keyboard or mouse are optionally provided as well.

As used herein, the singular form “a”, “an” and “the” include plural references unless the context clearly dictates otherwise. For example, the term “a protein” or “at least one protein” may include a plurality of proteins, including mixtures thereof.

Throughout this disclosure, various aspects of this invention can be presented in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the invention. Accordingly, the description of a range should be considered to have specifically disclosed all the possible subranges as well as individual numerical values within that range. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed subranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 3, 4, 5, and 6. This applies regardless of the breadth of the range.

Whenever a numerical range is indicated herein, it is meant to include any cited numeral (fractional or integral) within the indicated range. The phrases “ranging/ranges between” a first indicate number and a second indicate number and “ranging/ranges from” a first indicate number “to” a second indicate number are used herein interchangeably and are meant to include the first and second indicated numbers and all the fractional and integral numerals therebetween.

As used herein throughout, the term “comprising” means that other steps and ingredients that do not affect the final result can be added. This term encompasses the terms “consisting of” and “consisting essentially of”.

The term “method” or “process” refers to manners, means, techniques and procedures for accomplishing a given task including, but not limited to, those manners, means, techniques and procedures either known to, or readily developed from known

manners, means, techniques and procedures by practitioners of the chemical, pharmacological, biological, biochemical and medical arts.

BRIEF DESCRIPTION OF THE DRAWINGS

5 Some embodiments of the invention are herein described, by way of example only, with reference to the accompanying drawings. With specific reference now to the drawings in detail, it is stressed that the particulars shown are by way of example and for purposes of illustrative discussion of embodiments of the invention. In this regard, the description taken with the drawings makes apparent to those skilled in the art how
10 embodiments of the invention may be practiced.

 In the drawings:

 FIGs. 1A-D present the chemical structure and inhibitory effect of Compound **20**, an exemplary reverse transcriptase inhibitor according to some embodiments of the present invention, and the inhibitory effect of Compound **3**, another exemplary reverse
15 transcriptase inhibitor according to some embodiments of the present invention. FIG. 1A presents images demonstrating the inhibition effect of various concentrations of Compound **20** on HIV-1 infectivity of B lymphocytes observed through a fluorescence microscope (left column) and plots demonstrating the HIV-1 infectivity measured quantitatively by flow cytometry (right column). FIG. 1B presents the 2D chemical
20 structure of Compound **20**. FIG. 1C presents a dose-response curve demonstrating the suppressing viral infectivity exhibited by Compound **20** and the corresponding IC_{50} value calculated on the basis of the flow cytometry results. FIG. 1D presents the toxicity and therapeutic index of Compounds **3** and **20** on B lymphocytes, wherein CC_{50} is the concentration of inhibitor at which the cell viability was reduced by 50 %
25 compared to that of the inhibitor-free control. TI is the therapeutic index (CC_{50}/IC_{50}).

 FIG. 2 presents plots demonstrating the inhibition of wild type HIV-1 RT by Compound **3** (left column) and Compound **20** (middle column), exemplary reverse transcriptase inhibitors according to some embodiments of the present invention, compared with the known NNRTI, nevirapine (right column). Wild type RT, incubated
30 with the tested compounds was assayed for DNA-dependent DNA polymerase activity (DDDP; top row), RNA-dependent DNA polymerase activity (RDDP; middle row) and RNase H activity (bottom row). The RDDP, DDDP, and RNase H activities of wild-

type HIV-1 RT were assayed in the presence of increasing concentrations of the tested compounds. The dose-response curves for each inhibitor were fitted to a four-parameter logistic equation, and the IC_{50} values as well as standard errors, sigmoidity, and correlation coefficients are reported.

5 FIGs. 3A-B present plots demonstrating the inhibition of Y181C mutant of HIV-1 RT (FIG. 3A) and a bar graph demonstrating the inhibition of HIV-2 RT (FIG. 3B) by Compounds **3** and **20**, exemplary reverse transcriptase inhibitors according to some embodiments of the present invention, and the known NNRTI, nevirapine. Y181C mutant of HIV-1 RT, incubated with Compound **3** (FIG. 3A; left column), Compound
10 **20** (FIG. 3A; middle column) and nevirapine (FIG. 3A; right column) was assayed for DNA-dependent DNA polymerase activity (DDDP). The dose response curves for each inhibitor were fitted to a four-parameter logistic equation and the IC_{50} parameters as well as standard errors, sigmoidity and correlation coefficients are reported. A bar graph comparing the inhibitory effect of Compound **3**, **20** and nevirapine, each at a
15 concentration of 140 μ M, on HIV-2 RT DNA-dependent polymerase activity (DDDP) is also shown (FIG. 3B).

FIG. 4 presents comparative plots showing of the maximal velocity of HIV-1 RT DNA-dependent DNA polymerase activity as a function of enzyme concentration. The DNA-dependent DNA polymerase activity was monitored by assessing the
20 poly(rA)_n•oligo(dT)₁₂₋₁₈-dependent incorporation of [³H]dTTP into nascent DNA in the absence (circles) or presence of 616 nM of Compound **20** (triangle). RT activity was assayed for 15 minutes at 37 °C and the extent of the [³H]dTTP incorporation was determined as described in the Examples section. The curves were fitted by linear regression analysis that resulted in high correlation coefficients (r^2) of 0.995 in the
25 absence of an inhibitor, and 0.998 in the presence of an inhibitor, indicating a strong linear relationship between V_{max} values and enzyme concentrations.

FIGs. 5A-D present kinetic analysis of the inhibition of HIV-1 RT-associated DNA-dependent DNA polymerase activity by Compound **20**, an exemplary reverse transcriptase inhibitor according to some embodiments of the present invention, with
30 respect to either the dTTP substrate (FIGs. 5A-B) or the rA•dT template (FIGs. 5C-D). FIGs. 5A and C present a double-reciprocal plot of the initial velocity of the RNA-dependent DNA polymerase activity of HIV-1 RT as a function of dTTP substrate

concentration or rA•dT template concentration, respectively. The velocity was measured with increasing concentrations of dTTP or the rA•dT template tested in the absence (filled square) or presence of 0.25 μ M (filled circle), 0.5 μ M (star), 1 μ M (filled diamond) or 2 μ M (filled triangle) of Compound **20**. FIGs. 5B and 5D present a Replot (Dixon plot) of the reciprocal maximal velocity (calculated from FIGs. 5A and 5C, respectively) *versus* various concentrations of Compound **20**. The kinetic constants K_m and K_i were calculated using linear regression analysis.

FIGs. 6A-B present a computer generated structural model of the docking of Compounds **3** and **20**, exemplary reverse transcriptase inhibitors according to some embodiments of the present invention, into the NNRTI binding site of wild-type HIV-1 RT. FIG. 4A presents the spatial structure of wild-type HIV-1 RT with its defined subdomains (left, each subdomain in the p66 subunit is depicted in a different color while the p51 is positioned behind the p66) and the location of the hydrophobic pocket along with the three aspartic acids that form the catalytic site of the DNA polymerase domain (right). FIG. 6B presents the docking of Compounds **3** (left) and **20** (right) into the HIV-1 RT structure as found in PDB entry 1dtq using Surflex. The top-scoring conformation of each molecule is shown. Amino acids in the pocket are specified in three-letter codes and different colors; Glu138 is part of the p51 HIV-1 RT subunit, and all the other amino acids are part of the p66 subunit. The two compounds are depicted in CPK (Corey, Pauling, and Koltin) colors (i.e. nitrogen blue, oxygen red sulfur yellow and carbon gray) and are displayed as a stick model. Suggested hydrogen bonds are displayed as dashed lines. All structures were displayed with discovery studio visualizer 1.6 (Accelrys Software Inc.).

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention, in some embodiments thereof, relates to compounds capable of inhibiting an activity of reverse transcriptase (RT) and to uses thereof in the treatment of retroviral infections such as acquired immune deficiency syndrome (AIDS) caused by a human immunodeficiency virus (HIV).

Before explaining at least one embodiment of the invention in detail, it is to be understood that the invention is not necessarily limited in its application to the details set

forth in the following description or exemplified by the Examples. The invention is capable of other embodiments or of being practiced or carried out in various ways.

As discussed hereinabove, the enzyme reverse transcriptase is an essential enzyme in the life cycle of retroviruses.

5 To date, the most widely investigated retrovirus is the human immunodeficiency virus type-1 (HIV-1). Since the identification, two decades ago, of HIV-1 as the cause for acquired human immunodeficiency syndrome (AIDS), a massive search for molecules that block HIV-1 RT activities has been performed worldwide.

Numerous RT inhibitors have been identified so far as agents for treating AIDS.
10 These are divided into nucleoside/nucleotide analogs RT inhibitors (NRTIs) and the non-nucleoside/nucleotide RT inhibitors (NNRTIs).

The NNRTIs include a variety of non-competitive inhibitors that bind specifically to a hydrophobic pocket in proximity to the DNA polymerase active site of the enzyme; most of them are highly specific against HIV-1 RT with minimal effects on
15 the closely-related HIV-2 RT.

As discussed hereinabove, the use of currently marketed RT inhibitors in the treatment of retroviral infections such as AIDS is restricted by the emergence of drug resistant mutants of RT.

In a search for novel NNRTIs, the present inventors have devised a novel
20 methodology for identifying such compounds.

This method is based on (i) *in silico* virtual screening of a chemical library for candidate compounds which dock onto the NNRTI binding pocket of the RT; and (ii) *in vitro* testing these candidate compounds for the selective inhibition of the RT-associated DNA polymerase activity, as further described in detail hereinbelow, so as to identify
25 candidate compounds that are indeed characterized as capable of inhibiting this RT activity.

As is known in the art, a chemical library is a collection of stored chemicals which can be used ultimately in high-throughput screening or industrial manufacture. A chemical library thus consists a series of stored chemicals, whereby for each chemical,
30 an associated information such as its chemical structure, purity, and physicochemical characteristics, is also stored.

Utilizing the above-described methodology, the present inventors have identified candidate compounds and have further tested their HIV-1 RT inhibition activity.

As presented in the Examples section that follows, compounds exhibiting selective inhibition activity towards RT-DNA polymerase function have been identified.

5 Thus, it has been shown, for example, that the compounds described herein efficiently inhibited both the HIV-1 RT RNA-dependent and DNA-dependent DNA polymerase activities (see, Tables 2 and 4 and Figure 2). Furthermore, the compounds described herein exhibited inhibition specificity toward HIV-1 RT as compared to a non-related cellular DNA polymerase (i.e. high selectivity index; see, Tables 2 and 4). It has also
10 been shown that the compounds described herein potentially inhibited the transfection of B-lymphocytes by HIV-1 pseudoviruses (see Table 2, Table 4 and Figures 1A, 1C and 1D). Taken together with their low cytotoxicity towards normal uninfected cells (see, Figure 1D) these findings demonstrate that the compounds described herein may serve as potent RT inhibitors, in the treatment of, for example, infections caused by
15 retroviruses such as HIV.

Based on the above-described findings, the present inventors have further designed and successfully prepared and practiced novel compounds. These compounds were designed based on the structural features of one of the most potent RT inhibitor that was identified by the methodology described hereinabove. Some of these
20 compounds indeed exhibited potent RT inhibition activity.

Thus, according to an aspect of some embodiments of the present invention, there is provided a method of identifying a candidate compound for inhibiting an activity of a reverse transcriptase. The method, according to this aspect of the invention, is effected by computationally-identifying a compound, which is capable of
25 specifically- binding to a three-dimensional structure of an active site cavity of at least two crystalline forms of a reverse transcriptase.

As used herein, the phrase "candidate compound" describes a compound that can potentially be utilized for an intended use, herein for inhibiting an activity of RT and/or for treating an infection caused by a retrovirus.

30 A candidate compound, according to embodiments of the invention, can act, for example, as a competitive inhibitor of RT activity, a non-competitive inhibitor of RT activity, a compound which interferes with RT binding to nucleosides, etc.

The phrase "competitive inhibitor", as used in the context of the present embodiments, describes a compound which directly binds to the same active site as a natural substrate of the enzyme RT. A competitive inhibitor typically binds the active site via reversible interactions, as in the case of a natural substrate, and thus prevents an interaction between at least some of the enzyme molecules with the natural substrate.

The phrase "non-competitive inhibitor", as used herein, describes a compound that interacts with the enzyme, herein RT, at a site other than the enzyme's active site, such that this interaction affects its interaction with its natural substrate via, for example, a conformational or chemical change in the enzyme's structure .

An "active site cavity" describes one or more amino acid(s) within the enzyme's sequence that an interaction therewith affects the interaction of the enzyme with its natural substrate, in a competitive or non-competitive manner, as defined hereinabove.

In some embodiments, the active site cavity is a hydrophobic pocket in proximity to the DNA polymerase active site of the RT. Such an active site cavity is also referred to herein interchangeably as a hydrophobic binding site within the catalytic domain of the reverse transcriptase.

As discussed hereinabove, it has been shown that most of the currently known NNRTIs bind to the hydrophobic pocket in proximity to the DNA polymerase active site of the RT, and serve as non-competitive inhibitors.

Thus, in some embodiments of the invention, the RT inhibitors described herein are non-competitive inhibitors. It is shown, for example, that an exemplary compound which was uncovered via the screening method described herein, exhibited potent non-competitive inhibition of RT activity (see, for example, Example 3 in the Examples section that follows).

As used herein, the phrase "reverse transcriptase" encompasses a wild type RT and any mutant thereof.

In some embodiments, the RT is HIV-1 RT.

In some embodiments, the method according to this aspect of the invention is effected by computationally identifying a compound which is capable of specifically binding to a three-dimensional structure of an active site cavity, as described herein, of a wild type of HIV-1 reverse transcriptase.

As demonstrated in the example section which follows, *in silico* virtual screening against two different crystalline forms of HIV-1 RT can underscore compounds with a high probability of inhibiting the infection of sensitive cells by HIV-1. A screening process which utilizes, in the docking, more than one crystalline form of the RT permits a more stringent screen that can lead to more reliable results, since it assures that subtle differences between the various RT structures will not affect the overall efficacy of the docking process.

According to some embodiments, the wild type reverse transcriptase is a HIV-1 reverse transcriptase and the crystalline forms of this HIV-1 reverse transcriptase have the pdb entry codes 1fk9 and 1dtq.

According to some embodiments, the computational identification described herein is affected by:

- (a) obtaining at least two crystalline forms of the reverse transcriptase;
- (b) detecting the location of an active site in each of the crystalline forms of the reverse transcriptase;
- (c) generating a protomol for the active site in each of the crystalline forms of the reverse transcriptase;
- (d) performing a docking process of compounds from a chemical library onto the active site using the protomol; and
- (e) identifying a compound that both spatially and chemically fits to the three-dimensional structure of the active site cavity of each crystalline form of the reverse transcriptase.

According to some embodiments, the identification described in (e) above is also effected by computational means.

When utilized for identifying inhibitors, the active site can be represented by a protomol. A protomol is an idealized representation of a ligand that makes every potential interaction with the selected active site and is an object-oriented component based framework for molecular dynamics simulations.

As noted hereinabove, in some embodiments, the active site is a hydrophobic pocket in proximity to the DNA polymerase binding site of the RT.

Thus, in some embodiments, each compound is energy-minimized to a single, optimized conformation and a docking process of the compounds onto the active site is thereafter initiated, using the generated Protomol.

In some embodiments, the docking process is performed by fragmenting each compound and fitting the conformation of each fragment into the protomol, so as to yield a spatial structure that maximizes molecular similarity to the protomol.

The phrase "three-dimensional structure" as used herein, describes the orderly geometric spatial arrangement of atoms within the protein-of-interest (i.e., tertiary structure of the protein) and is defined by its atomic coordinates. Typically, obtaining the set of atomic coordinates, which defines the three dimensional structure of the active site cavity of an enzyme (e.g., HIV-1 RT), can be effected using various approaches which are well known in the art. Examples include, but are not limited to, neutron diffraction, nuclear magnetic resonance (NMR), and X-ray crystallography. X-ray crystallography is preferred for obtaining the secondary and tertiary structure information, which requires detailed information about the arrangement of atoms within a protein.

Alternatively, a three dimensional structure of a protein-of-interest can be constructed using computer-based protein modeling techniques. In such cases, the three dimensional structure of a protein is solved by finding target sequences that are most compatible with profiles representing the structural environments of the residues in known three-dimensional protein structures (See, e.g., U.S. Pat. No. 5,436,850).

In another technique, the known three-dimensional structures of proteins in a given family are superimposed in order to define the structurally conserved regions of that protein family. This protein modeling technique also uses a known three-dimensional structure of a homologous protein to approximate the structure of a polypeptide of interest (See, e.g., U.S. Pat. Nos. 5,557,535; 5,884,230; and 5,873,052).

Regardless of the method used, structural data obtained is preferably recorded on a computer-readable medium, so as to enable data manipulation and construction of computational models. As used herein, "computer-readable medium" refers to any medium which can be read and accessed directly by a computer. Such media include, but are not limited to: magnetic storage media, such as floppy discs, hard disc storage medium, and magnetic tape; optical storage media such as optical discs or CD-ROM;

electrical storage media such as RAM and ROM; and hybrids of these categories such as magnetic/optical storage media. Selection and use of appropriate storage media is well within the capabilities of one of ordinary skill in the art.

As used herein, "recorded" refers to a process of storing information on computer readable medium.

It will be appreciated that a number of data storage devices can be used for creating a computer readable medium having recorded thereon the structural data of the present invention.

According to some embodiments of this aspect of the present invention, the coordinate data used to define the structures of the active site cavities of the RTs is retrieved from a published database (Research Collaboratory for Structural Bioinformatics site) as a complex with a known RT inhibitor (an NNRTI). Further computational manipulations can be performed so as to obtain the coordinate data of an unbound enzyme and/or to obtain the coordinate data of mutants that cannot be retrieved from an available database.

It will be appreciated that structure models of the present embodiments are preferably generated by a computing platform, which generates a graphic output of the models via a display generating device such as screen or printer. The computing platform generates graphic representations of atomic structure models *via* a processing unit which processes structure coordinate data stored in a retrievable format in the data storage device.

Suitable software applications, well known to those of skill in the art, may be used by the processing unit to process structure coordinate data so as to provide a graphic output of three-dimensional structure models generated therewith via display.

Criteria employed by software programs used in *in silico* screening processes to qualify the binding of screened chemical structures with binding pockets include gap space, hydrogen bonding, electrostatic interactions, Van der Waals forces, hydrophilicity/hydrophobicity, etc. Generally, the greater the contact area between the screened molecule and the binding region of the RT, the lower the steric hindrance, the lower the "gap space", the greater the number of hydrogen bonds, and the greater the sum total of the van der Waals forces between the screened molecule and the RT

binding region, the greater will be the capacity of the screened molecule to bind with the RT binding region.

Contact area between compounds may be directly calculated from the coordinates of the compounds in docked conformation using the MS program (Connolly ML., 1983. Science 221, 709–713).

Docking may be followed by energy minimization with standard molecular mechanics force fields or dynamics with programs such as CFF 1.02 force field.

As used herein, "minimization of energy" means achieving an atomic geometry of a chemical structure via systematic alteration such that any further minor perturbation of the atomic geometry would cause the total energy of the system, as measured by a molecular mechanics force-field, to increase. Minimization and molecular mechanics force fields are well understood in computational chemistry.

In some embodiments, once candidate compounds, namely, candidate RT inhibitors, are identified in the *in silico* virtual screening process, these compounds are further tested for their *in vitro* RT inhibition activity, so as to identify those compounds which may serve as potent RT inhibitors.

As demonstrated in the Example section that follows, the candidate compounds detected in the virtual screening process were further tested *in vitro* for their HIV-1 RT inhibition activity (see, Tables 2, 3 and 4). The ability of the compounds to inhibit an RT-associated DNA-dependent and RNA-dependent DNA polymerase activities and/or RNase activity was evaluated. The inhibition activity of the compounds was quantified by determining the level of inhibition of the residual activity of RT by the compounds. The residual activity was calculated by dividing the activity, detected in the presence of a specific tested compound, by the initial activity with no compound present, as detailed in the Examples section that follows.

As further demonstrated in the Example section that follows, the compounds described herein exhibit a high level of selectivity and specificity towards HIV-1 RT, as compared to other unrelated cellular DNA polymerases (see, Tables 2 and 4). This selectivity and specificity (designated as the selectivity index) was calculated by dividing the residual activity of an unrelated cellular DNA polymerases (such as Klenow fragment of *E. coli* DNA polymerase I) in the presence of each of the tested compounds, by that of HIV-1 RT in the presence of the tested compound in a similar

concentration). The results presented in Tables 2 and 3 show that there is a positive correlation between the selective inhibition of HIV-1 RT and the ability to suppress viral infectivity.

Hence according to some embodiments, the compound is characterized by a specific inhibition of a reverse transcriptase, as determined by the percent of residual activity of a cellular DNA polymerase in the presence of the compound divided by the percent of residual activity of the reverse transcriptase in the presence of said compound (also referred to herein as a selectivity index).

According to some embodiments, the selectivity index is measured using a HIV-1-reverse transcriptase and a DNA polymerase being Klenow fragment of E. coli DNA polymerase I (KF).

Therefore, according to embodiments of the invention, the method described herein further comprises biologically assaying the compound for its activity in inhibiting a catalytic activity of a reverse transcriptase, whereby compounds identified as inhibitors of RT, as described hereinabove, are further identified as capable of treating a viral infection caused by a retrovirus.

The screening method described herein can therefore be utilized for identifying compounds that can be utilized for treating a viral infection caused by a retrovirus.

In some embodiments, the retrovirus is HIV (e.g., HIV-1 or HIV-2).

Using the *in silico* screening method described herein, compounds exhibiting HIV-1 RT inhibition activity have been uncovered.

These compounds can be divided according to, for example, their structural features, into three main groups as follows:

(i) a compound having the general Formula I:



Formula I

or a pharmaceutically acceptable salt thereof;

wherein:

Z is selected from the group consisting of a substituted or non-substituted aryl and heteroaryl, wherein when substituted, the substituent is selected from the group

consisting of halide, alkoxy, hydroxy, thioalkoxy, thioalkyl, alkyl, haloalkyl, amine, sulfonamide, sulfonyl, sulfinyl, carboxylate, cyano, nitro, amide, and OCO_2R^4 ;

X is selected from the group consisting of $\text{NR}^5\text{-C(=W)-NR}^6$ and $\text{N=CR}^7\text{-NR}^6$, whereas W is selected from the group consisting of N, O, S, =NCN , =CHNO_2 and NR^{11} ;

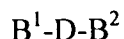
5 A is $\text{R}^8\text{-Y-R}^9$, wherein Y is selected from the group consisting of -O- , -N- , -S- , $\text{-CR}^{10}\text{R}^{11}\text{-}$, $\text{-NR}^{10}\text{-}$, -SC(=O)- , -C(=O)S- , -OC(=S)- , -C(=S)O- , -SC(=S)- , -C(=S)S- , -S(=O)- , S(=O)_2 , $\text{-NR}^{10}\text{S(=O)-}$, $\text{-S(=O)NR}^{10}\text{-}$, $\text{-S(=O)}_2\text{NR}^{10}\text{-}$, $\text{-NR}^{10}\text{S(=O)}_2\text{-}$, -C(=O)- , -C(=O)O- , -OC(=O)- , $\text{-C(=O)NR}^{11}\text{-}$, $\text{-R}^{11}\text{NC(=O)-}$, -OC(=O)O- , aryl and heterocyclic;

10 R^7 is selected from the group consisting of -SC(=O)R^{12} , -C(=O)SR^{12} , -SR^{12} , -S(=O)R^{12} , -OC(=O)R^{12} , -C(=O)OR^{12} , $\text{-NR}^{13}\text{C(=O)R}^{12}$, $\text{-C(=O)NR}^{12}\text{R}^{13}$, -OR^{12} and $\text{-NR}^{12}\text{R}^{13}$;

R^8 and R^9 are each independently a linear or branched, saturated or unsaturated substituted or non-substituted hydrocarbon chain having 1-10 carbon atoms in its backbone, which when substituted, the substituent is selected from the group consisting of alkyl and cycloalkyl; and

R^4 , R^5 , R^6 , R^{10} , R^{11} , R^{12} and R^{13} are each independently hydrogen or alkyl;

(ii) a compound having the general Formula III:



Formula III

or a pharmaceutically acceptable salt thereof;

wherein:

25 B^1 and B^2 are each independently selected from the group consisting of a substituted or unsubstituted heteroaryl and heteroalicyclic, which, when substituted, the substituent is selected from the group consisting of halide, alkoxy, hydroxy thioalkoxy, thioalkyl, alkyl, haloalkyl, amine, sulfonamide, sulfinyl, sulfonyl, carboxylate, cyano, nitro, amide, and $\text{OCO}_2\text{R}^{15}$;

30 D is selected from a group consisting of O- , -N- , -S- , $\text{-CR}^{16}\text{R}^{17}\text{-}$, $\text{-NR}^{16}\text{-}$, -SC(=O)- , -C(=O)S- , -OC(=S)- , -C(=S)O- , -SC(=S)- , -C(=S)S- , -S(=O)- , S(=O)_2 , $\text{-NR}^{16}\text{S(=O)-}$, $\text{-S(=O)NR}^{16}\text{-}$, $\text{-S(=O)}_2\text{NR}^{16}\text{-}$, $\text{-NR}^{16}\text{S(=O)}_2\text{-}$, -C(=O)- , -C(=O)O- , -OC(=O)- , $\text{-C(=O)NR}^{17}\text{-}$, $\text{-R}^{17}\text{NC(=O)-}$ and -OC(=O)O- , aryl and heterocyclic; and

R^{15} , R^{16} and R^{17} are each independently hydrogen or alkyl; and

(iii) a compound selected from the group consisting of Compounds 1, 4, 5, 6, 8 and 13, as these are presented in Tables 2 and 3 hereinbelow.

The term "amine", as used herein, describes a $-NR'R''$ group, wherein R' and R'' are each independently hydrogen, alkyl, cycloalkyl, or hydroxy, as these terms are defined hereinbelow, unless otherwise indicated.

The term "alkyl" describes a saturated aliphatic hydrocarbon including straight chain and branched chain groups. Preferably, the alkyl group has 1 to 20 carbon atoms. Whenever a numerical range; e.g., "1-20", is stated herein, it implies that the group, in this case the alkyl group, may contain 1 carbon atom, 2 carbon atoms, 3 carbon atoms, etc., up to and including 20 carbon atoms. More preferably, the alkyl is a medium size alkyl having 1 to 10 carbon atoms. Most preferably, unless otherwise indicated, the alkyl is a lower alkyl having 1 to 4 carbon atoms. The alkyl group may be substituted or unsubstituted. Unless otherwise indicated, when substituted, the substituent can be, for example, alkyl, cycloalkyl, aryl, heteroaryl, halide, trihalomethyl, hydroxyl, alkoxy, aryloxy, thiohydroxy, thioalkoxy, thioaryloxy, cyano, nitro, carbonyl, thiocarbonyl, carboxylate, carbamyl, thiocarbamyl, sulfinyl, sulfonyl, amide and amine as defined herein.

The term "cycloalkyl" describes an all-carbon monocyclic or fused ring (*i.e.*, rings which share an adjacent pair of carbon atoms) group where one or more of the rings does not have a completely conjugated pi-electron system. The cycloalkyl group may be substituted or unsubstituted. Unless otherwise indicated, when substituted, the substituent can be, for example, alkyl, cycloalkyl, aryl, heteroaryl, halide, trihalomethyl, hydroxyl, alkoxy, aryloxy, thiohydroxy, thioalkoxy, thioaryloxy, cyano, nitro, carbonyl, thiocarbonyl, carboxylate, carbamyl, thiocarbamyl, sulfinyl, sulfonyl, amide and amine as defined herein.

The term "aryl" describes an all-carbon monocyclic or fused-ring polycyclic (*i.e.*, rings which share adjacent pairs of carbon atoms) groups having a completely conjugated pi-electron system. Examples, without limitation, of aryl groups are phenyl, naphthalenyl and anthracenyl. The aryl group may be substituted or unsubstituted. Unless otherwise indicated, when substituted, the substituent can be, for example, alkyl, cycloalkyl, aryl, heteroaryl, halide, trihalomethyl, hydroxyl, alkoxy, aryloxy,

thiohydroxy, thioalkoxy, thioaryloxy, cyano, nitro, carbonyl, thiocarbonyl, carboxylate, carbamyl, thiocarbamyl, sulfinyl, sulfonyl, amide and amine as defined herein.

The term "heteroaryl" describes a monocyclic or fused ring (*i.e.*, rings which share an adjacent pair of atoms) group having in the ring(s) one or more atoms, such as, for example, nitrogen, oxygen and sulfur and, in addition, having a completely conjugated pi-electron system. Examples, without limitation, of heteroaryl groups include pyrrole, furane, thiophene, imidazole, oxazole, thiazole, pyrazole, pyridine, pyrimidine, quinoline, isoquinoline and purine. The heteroaryl group may be substituted or unsubstituted. Unless otherwise indicated, when substituted, the substituent can be, for example, alkyl, cycloalkyl, aryl, heteroaryl, halide, trihalomethyl, hydroxyl, alkoxy, aryloxy, thiohydroxy, thioalkoxy, thioaryloxy, cyano, nitro, carbonyl, thiocarbonyl, carboxylate, carbamyl, thiocarbamyl, sulfinyl, sulfonyl, amide and amine as defined herein.

A "heteroalicyclic" group describes a monocyclic or fused ring group having in the ring(s) one or more atoms such as nitrogen, oxygen and sulfur. The rings may also have one or more double bonds. However, the rings do not have a completely conjugated pi-electron system. The heteroalicyclic may be substituted or unsubstituted. Unless otherwise indicated, when substituted, the substituent can be, for example, alkyl, cycloalkyl, aryl, heteroaryl, halide, trihalomethyl, hydroxyl, alkoxy, aryloxy, thiohydroxy, thioalkoxy, thioaryloxy, cyano, nitro, carbonyl, thiocarbonyl, carboxylate, carbamyl, thiocarbamyl, sulfinyl, sulfonyl, amide and amine, as defined herein.

The term "halide", which is also referred to herein as "halo", describes fluorine, chlorine, bromine or iodine.

The term "haloalkyl" describes an alkyl group as defined hereinabove, further substituted by one or more halide(s). An example is a trihaloalkyl, such as trifluoromethyl.

The term "alkoxy" describes both an -O-alkyl and an -O-cycloalkyl group, as defined herein.

The term "aryloxy" describes both an -O-aryl and an -O-heteroaryl group, as defined herein.

The term "hydroxyl" describes a -OH group.

The term "thiohydroxy", which is also referred to herein as "thiol", describes a –SH group.

The term "thioalkoxy" describes both a -S-alkyl group, and a -S-cycloalkyl group, as defined herein.

5 The term "thioaryloxy" describes both an -S-aryl and an -S-heteroaryl group, as defined herein.

The term "sulfonamide" describes a $-S(=O)_2-NR'R''$ group and a $-NR'S(=O)_2R''$ group, with R' and R'' as defined herein.

10 The term "sulfinyl" group refers to an $-S(=O)-R'$ group, where R' is as defined herein.

The term "sulfonyl" group refers to an $-S(=O)_2-R'$ group, where R' is as defined herein.

The term "carboxylate" describes a $-C(=O)-OR'''$ or a $-OC(=O)R'''$ group, wherein R''' is hydrogen, alkyl or cycloalkyl.

15 The term "cyano", which is also referred to herein as nitrile, describes a $-C\equiv N$ group.

The term "nitro" describes an $-NO_2$ group.

The term "amide" or "amido" describes a $-C(=O)-NR'R''$ group, $-NR''C(=O)R'$ group wherein R' and R'' are as defined hereinabove.

20 The term "carbonyl" describes a $-C(=O)-R'$ group, wherein R' is as defined herein.

The term "thiocarbonyl" describes a $-C(=S)-R'$ group, where R' is as defined herein.

25 The term "carbamyl" describes an $-OC(=O)-NR'R''$ group, or an $R'OC(=O)-NR''$ -group, where R' and R'' are as defined herein.

The term "thiocarbamyl" describes an $-OC(=S)-NR'R''$ group and an $R''OC(=S)NR'-$, group where R' and R'' are as defined herein.

30 The term "hydrocarbon" describes an organic moiety that includes, as its basic skeleton, a chain of carbon atoms, substituted mainly by hydrogen atoms. The hydrocarbon can be saturated or non-saturated, be comprised of aliphatic, alicyclic or aromatic moieties, and can optionally be substituted by one or more substituents (other than hydrogen).

According to some embodiments, the compounds utilized in the context of the invention have the general formula III. An exemplary compound which belongs to this category is compound 3 (see, Table 2 hereinbelow).

As exemplified in the Examples section which follows, Compound 3 was found to be a potent HIV-RT inhibitor (see, Table 2 and Figures 1D and Figure 2). It has further been shown that Compound 3 fits into the NNRTI hydrophobic binding site in HIV-1 RT (See, Figure 6). Specifically, Molecular modeling studies have revealed that Compound 3 forms two important hydrogen bonds with Glu138 of the p51 subunit in the HIV-1 reverse transcriptase and with Tyr318 in the p66 subunit in the HIV-1 reverse transcriptase. Tyr318 is highly conserved in HIV-1 RT and interacts within a 4 Å contact distance with most NNRTIs that have been shown to bind the hydrophobic pocket.

Hence, according to some embodiments of the invention, when compounds having the general formula III are utilized in the context of the invention and the reverse transcriptase is wild type HIV-1 reverse transcriptase or a mutant thereof, at least one heteroatom in the B¹ and B² moieties in Formula III is capable of interacting with Glu138 of the p51 subunit in said HIV-1 reverse transcriptase and/or with Tyr318 in the p66 subunit in said HIV-1 reverse transcriptase.

Thus, in some embodiments, compounds having general Formula III are characterized as having a heteroaryl or heteroalicyclic moiety, in which a heteroatom thereof is capable of interacting with the above-described Glu138, and as further having an additional heteroaryl or heteroalicyclic moiety, in which a heteroatom thereof is capable of interacting with the above-described Tyr318. The heteroaryl or heteroalicyclic moieties, and the D moiety linking therebetween are therefore selected such that both these interactions can occur. In other words, the heteroaryl or heteroalicyclic moieties, and the D moiety linking therebetween, are selected such that the length, structure and flexibility of those portions of these moieties that link one heteroatom in B1 to one heteroatom in B2 allow at least one interaction between a heteroatom and the above-described Glu138 and another heteroatom and the above-described Tyr318.

In some embodiments, D is -S(=O)₂NR¹⁶- or -NR¹⁶.S(=O)₂-. R¹⁶ is preferably hydrogen.

In some embodiments, D is $-S(=O)_2NR^{16}-$, B1 is benzo[c][1,2,5]thiadiazole and B2 is 1H-pyrazole. Optionally, the 1H-pyrazole is substituted by a halide, for example, bromo.

As mentioned hereinabove, in some embodiments a compound having general
5 Formula III is Compound 3.

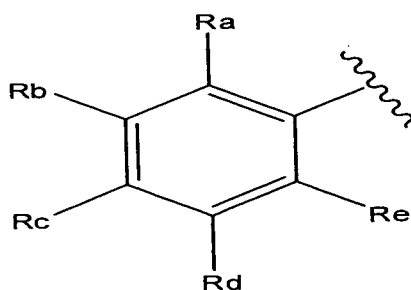
According to some embodiments, the compounds utilized in the context of the invention are selected from the group consisting of Compounds 1, 4, 5, 6, 8 and 13 (see, chemical structures in Tables 2 and 3).

As demonstrated in the Examples section which follows, these compounds
10 exhibited potent HIV-1 inhibition activity as well as a high selectivity index. Furthermore, Compounds 1, 5 and 13 were also shown to inhibit cell transfection by HIV-1 virions (see, Tables 2 and 3).

It is noted herein that most of these compound share a common structural feature, being a thiourea moiety which has at least one cyclic moiety attached, directly
15 or indirectly, to each of the nitrogen atoms therein.

As noted hereinabove, some of the most potent compounds were found to belong to the category of compounds having Formula I, as delineated hereinabove. Hence, in some embodiments, the compounds utilized in the context of the invention have general Formula I. In some embodiments, compounds having Formula I have
20 a thiourea moiety having attached to one nitrogen atom thereof an aryl moiety and to the other nitrogen atom thereof an aliphatic moiety interrupted by a heteroatom (see, Tables 2, 4 and 5). It has been suggested that the position and nature of the substituent on the aryl moiety has an effect on the potency of these compounds as RT inhibitors.

Thus, according to some embodiments, Z in formula I is a substituted or non-
25 substituted aryl having Formula II:



Formula II

wherein:

Ra-Re are each independently selected from the group consisting of halide, alkoxy, hydroxy, thioalkoxy, thioalkyl, alkyl, haloalkyl, amine, sulfonamide, sulfinyl, sulfonyl, carboxylate, cyano, nitro, amide, and OCO_2R^4 ,

whereas at least one of the Ra-Rd is selected from the group consisting of halide, alkoxy, alkyl, carboxylate, cyano, nitro, haloalkyl and amine.

In some embodiments the halide is chlorine, fluorine or bromine. In some embodiments the halide is chlorine.

In some embodiments, the alkoxy is methoxy or ethoxy, preferably methoxy.

In some embodiments, the alkyl is a methyl, propyl or tert-butyl.

In some embodiments, the haloalkyl is CF_3 .

In some embodiments the amine is a dialkylamine. In some embodiments the dialkylamine is dimethylamine.

In some embodiments, the carboxylate is $-\text{C}(=\text{O})-\text{OR}'''$ as defined hereinabove with R''' being an alkyl. In some embodiments the alkyl is methyl or ethyl.

According to some embodiments, Ra is selected from the group consisting of alkoxy, alkyl and halide. Exemplary compounds wherein Ra is an alkyl, such as methyl, include Compounds **9**, **25**, **26** and **35** (see, Tables 2 and 4). Compounds **9** and **35** exhibited potent HIV-1 RT inhibition activity with a selectivity and specificity to HIV-1 RT as compared to an unrelated cellular DNA polymerase (i.e. high selectivity index; see Table 2 and 4). Compound **9** was further tested and was able to inhibit the transfection of cells by the HIV-1 virions (see, Table 2).

Exemplary compounds wherein Ra is halide include Compounds **22**, **30**, **34** and **37** (see, Table 4). Compound **22**, in which Ra is chloride, exhibited potent HIV-1 RT inhibition activity with a high selectivity index and was also shown to inhibit the transfection of cells by the HIV-1 virions (see, Table 4).

In some embodiments Ra is alkoxy. In some embodiments the alkoxy is methoxy. An Exemplary compound in which Ra is ethoxy and which exhibited potent HIV-1 RT inhibition activity is Compound **27**. Exemplary compounds in which Ra is methoxy and which exhibited potent HIV-1 RT inhibition activity include Compounds **7**, **20**, **28**, **39**, **44**, **46**, **49**, **54**, **59**, **62**, **63**, **67**, **68**, **69**, and **70** (see, Tables 2, 4 and 5).

According to some embodiments, Rb in Formula II is hydrogen.

According to some embodiments Rc is selected from the group consisting of hydrogen, halide and nitro. Exemplary compounds wherein Rc is hydrogen include Compounds 7, 22, 25, 27, 28, 29, 32, 33, 34 and 36 (see, Tables 2 and 4). Compounds 5 7, 22, 27, 28 exhibited potent HIV-1 RT inhibition activity with a high selectivity index and inhibited the transfection of cells by the HIV-1 virions (see, Tables 2 and 4). Exemplary compounds wherein Rc is halide include Compounds 2, 9, 19, 20, 21, 24, 30, 37, 38 (see, Tables 2 and 4) and Compounds 46, 49, 54, 62, 63, 67 and 70 (see, Table 5). Compounds 2, 9, 20, 21 exhibited potent HIV-1 RT inhibition activity together with 10 a high selectivity index (see, Tables 2 and 4). Compound 20 was especially selective toward HIV-1 RT with a selectivity index of 7.4. Compound 2, 9 and 20 were also able to inhibit the transfection of cells by the HIV-1 RT virions (see, Tables 2 and 4 and Figure 1). An exemplary compound wherein Rc is nitro and which exhibited potent HIV-1 RT inhibition activity is Compound 44 (see, Table 5). Without being bound to 15 any particular theory, it has been suggested that an electron withdrawing group at position Rc may have an effect on the inhibition activity of a compound having general Formula I.

According to some embodiments, Rd is selected from the group consisting of hydrogen, alkyl, halide, alkoxy and haloalkyl. Exemplary compounds wherein Rd is 20 methyl include Compounds 19, 22, 28 and 35 (see, Table 4). Compounds 22, 28 and 35 exhibited potent HIV-1 RT inhibition activity with a high selectivity index and HIV-1 virions transfection inhibition (see, Table 4). An Exemplary compound wherein Rd is chloride and which exhibited potent HIV-1 RT inhibition activity is Compound 39. Exemplary compounds wherein Rd is hydrogen include Compounds 9, 21, 23, 25, 26, 25 27, 29, 30, 31, 34, 37 and 40 (see, Tables 2 and 4). Compounds 9, 21, 23, and 24 exhibited potent HIV-1 RT inhibition activity with a high selectivity index. Compound 27 also exhibited HIV-1 virions transfection inhibition. An exemplary compound wherein Rd is haloalkyl and which exhibited potent HIV-1 RT inhibition activity, high selectivity index and HIV-1 virions transfection inhibition is Compound 2. Exemplary 30 compounds wherein Rd is methoxy and which exhibited potent HIV-1 RT inhibition activity include Compounds 7, 20, 32 (see, Tables 2 and 4) and Compounds 44, 46, 49, 54, 59, 62, 68, 69 and 70 (see, Table 5). Without being bound to any particular theory, it

has been suggested that the presence of a methoxy group at position Rd is essential for the inhibition activity of a compound having general Formula I.

According to some embodiments, X in formula I is $\text{NR}^5\text{-C(=W)-NR}^6$, wherein R^5 and R^6 are as defined hereinabove. In some embodiments, W is S or O. Optionally, W is =CHNO_2 . In some embodiments, R^5 and R^6 are each hydrogen. Exemplary compounds in this category, in which X is NH-C(=S)-NH include Compounds **19-40** (see, Table 4) and Compounds **44, 49, 54, 59, 62, 63, 67, 68, 69** and **70** (see, Table 5). As discussed hereinabove, Compounds **7, 9, 20, 22, 27, 28** and **39** exhibited a high selectivity index and were able to inhibit transfection of cells by HIV-1 virions (see, Tables 2 and 4 and Figure 1). An exemplary compound in this category, in which X is NH-C(=O)-NH and exhibited potent HIV-1 RT inhibition is Compound **46**.

According to some embodiments, Y in formula I can be -O- , -N- , -S- , $\text{-CR}^{10}\text{R}^{11}\text{-}$, $\text{-NR}^{10}\text{-}$, -SC(=O)- , -C(=O)S- , -OC(=S)- , -C(=S)O- , -SC(=S)- , -C(=S)S- , -S(=O)- , S(=O)_2 , $\text{-NR}^{10}\text{S(=O)-}$, $\text{-S(=O)NR}^{10}\text{-}$, $\text{-S(=O)}_2\text{NR}^{10}\text{-}$, $\text{-NR}^{10}\text{.S(=O)}_2\text{-}$, -C(=O)- , -C(=O)O- , -OC(=O)- , $\text{-C(=O)NR}^{11}\text{-}$, $\text{-R}^{11}\text{NC(=O)-}$ or -OC(=O)O- , wherein R^{10} and R^{11} are as defined hereinabove.

In some embodiments Y is -O- . Exemplary compounds in which Y is O and exhibited potent HIV-1 RT inhibition include Compounds **7, 9** and **20-40** (see, Tables 2 and 4) and Compounds **44** and **46** (see, Table 5). As discussed hereinabove, Compounds **7, 9, 20, 22, 27, 28**, and **39** exhibited inhibition of transfection of cells by HIV-1 virions (see, Tables 2 and 4 and Figure 1).

In some embodiments, Y is -S- . An Exemplary compound in which Y is S and which exhibited potent HIV-1 RT inhibition is Compound **54** (see, Table 5).

In some embodiments, Y is -C(=O)O- . An Exemplary compound in which Y is -C(=O)O- and which exhibited potent inhibition of HIV-1 RT is Compound **59** (see, Table 5).

In some embodiments Y is -OC(=O)- . Exemplary compounds in which Y is OC(=O) and which exhibited potent inhibition of HIV-1 RT include Compounds **62, 63, 67** and **68** (see, Table 5).

In some embodiments, Y is -C(=O)S- . An Exemplary compound in which Y is -C(=O)S- and which exhibited potent inhibition of HIV-1 RT is Compound **70** (see, Table 5).

According to some embodiments, R^8 and R^9 in formula I are each independently a linear, saturated, non-substituted hydrocarbon chain having 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 carbon atoms in its backbone.

In some embodiments, the hydrocarbon chain has 2, 3 or 4 carbon atoms.

5 In some embodiments R^8 is a hydrocarbon chain having 2 or 3 carbon atoms.

In some embodiments R^9 is a hydrocarbon chain having 3 or 4 carbon atoms.

Exemplary compounds wherein each of R^8 and R^9 is a hydrocarbon chain having 3 carbon atoms and which exhibited potent HIV-RT activity include Compounds **20-40** (see, Table 4) and Compounds **44, 46, 54, 59, 67, 69** and **70** (see, Table 5). An Exemplary compound wherein R^8 and R^9 are hydrocarbon chains having 2 and 4 carbon atoms, respectively, and which exhibited potent HIV-RT activity is Compound **62** (see, Table 5). An Exemplary compound wherein R^8 and R^9 are hydrocarbon chains having 3 and 4 carbon atoms, respectively, and which exhibited potent HIV-RT activity is Compound **68** (see, Table 5). An Exemplary compound wherein R^8 and R^9 are hydrocarbon chains having 2 and 3 carbon atoms, respectively, and which exhibited potent HIV-RT activity is Compound **63** (see, Table 5).

Without being bound to any particular theory, it is suggested that the total length of the aliphatic chain substituting the moiety X is Formula I, namely, the total number of carbon atoms in the hydrocarbon chains of R^8 and R^9 has an effect of the RT inhibition activity exhibited by compounds having Formula I. Thus, it is suggested that the above-described total length should be at least of 6 carbon atoms and can range, for example, from 6 to 10 carbon atoms, preferably from 6 to 8 carbon atoms.

Thus, in some embodiments the total number of carbon atoms in R^8 and R^9 together is higher than 6.

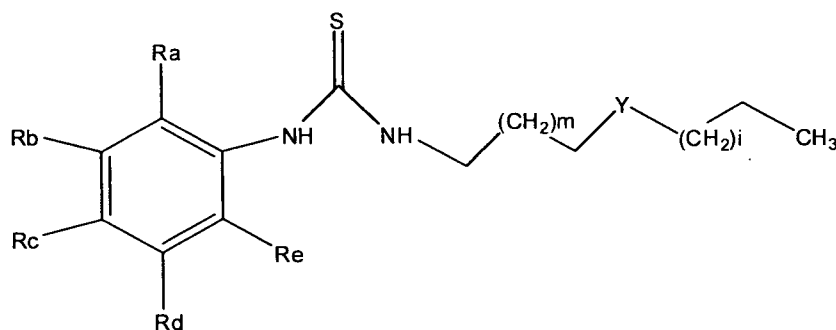
25 In some embodiments the total number of atoms in R^8 , Y and R^9 together is higher than 6. In some embodiments the total number is 7. In some embodiments the total number is 8.

Exemplary compounds which exhibited potent HIV-1 RT inhibition activity and wherein the total number of atoms in R^8 , Y and R^9 together is higher than 6 include Compounds **20-40** (see, Table 4), Compounds **63, 54, 46, and 44** (having a total number of atoms being 7), Compounds **59, 62, 67, 69** and **70** (having a total number of atoms being 8) and Compound **68** (having a total number of atoms being 9) (see, Table 5).

In some embodiments, Z has the general formula II, wherein Ra is methoxy, Rb is hydrogen, Rc is chloro, Rd is methoxy and Re is hydrogen. In a preferred embodiment, A is $\text{NR}^5\text{-C(=W)-NR}^6$, W is O or S and each of R^5 and R^6 is hydrogen. Exemplary compounds which belong to this category and exhibited potent HIV-1 RT inhibition activity include Compound **20**, and Compounds **46, 54, 59, 62, 63, 67, 68, 68** and **70**.

As exemplified in the Examples section that follows, when X is NH-C(=S)-NH and A is $(\text{CH}_2)_3\text{-O-(CH}_2)_3$, a methoxy group at position Ra is beneficial, as can be deduced from the high HIV-1 RT inhibition potency of compounds having such a structural feature (see, Compounds **7, 20, 28, 39**; Tables 2 and 4).

According to some embodiments, compounds that are suitable for use in the context of the invention can be collectively represented by the following general Formula:



wherein Y and Ra-Re are as described hereinabove, and m and i are each independently an integer from 0-5, preferably from 0-2.

While some of the compounds described herein are known compounds, uncovered as RT inhibitors in the screening method described hereinabove, some of these compounds have been newly designed and synthesized based on the structural features of one of the most potent compounds retrieved in the screening method, namely, Compound **20**.

Thus, according to embodiments of the invention, there are provided novel compounds, which can be collectively represented by general Formula Ia:

38

Z-X-A

Formula Ia

wherein:

Z is selected from the group consisting of a substituted or non-substituted aryl and heteroaryl, wherein when substituted, the substituent is selected from the group consisting of halide, alkoxy, hydroxy, thioalkoxy, thioalkyl, alkyl, haloalkyl, amine, sulfonamide, sulfinyl, sulfonyl, carboxylate, cyano, nitro, amide, and OCO_2R^4 ;

X is selected from the group consisting of $\text{NR}^5\text{-C(=W)-NR}^6$ and $\text{N=CR}^7\text{-NR}^6$, whereas W is selected from the group consisting of N, O, S, =NCN , =CHNO_2 and NR^{11} ;

A is $\text{R}^8\text{-Y-R}^9$,

wherein Y is selected from the group consisting of -O- , -N- , -S- , $\text{-CR}^{10}\text{R}^{11}\text{-}$, $\text{-NR}^{10}\text{-}$, -SC(=O)- , -C(=O)S- , -OC(=S)- , -C(=S)O- , -SC(=S)- , -C(=S)S- , -S(=O)- , S(=O)_2 , $\text{-NR}^{10}\text{S(=O)-}$, $\text{-S(=O)NR}^{10}\text{-}$, $\text{-S(=O)}_2\text{NR}^{10}\text{-}$, $\text{-NR}^{10}\text{.S(=O)}_2\text{-}$, -C(=O)- , -C(=O)O- , -OC(=O)- , $\text{-C(=O)NR}^{11}\text{-}$, $\text{-R}^{11}\text{NC(=O)-}$, -OC(=O)O- , aryl and heterocyclic;

R^7 is selected from the group consisting of -SC(=O)R^{12} , -C(=O)SR^{12} , -SR^{12} , -S(=O)R^{12} , -OC(=O)R^{12} , -C(=O)OR^{12} , $\text{-NR}^{13}\text{C(=O)R}^{12}$, $\text{-C(=O)NR}^{12}\text{R}^{13}$, -OR^{12} and $\text{-NR}^{12}\text{R}^{13}$;

R^8 and R^9 are each independently a linear or branched, saturated or unsaturated substituted or non-substituted hydrocarbon chain having 1-10 carbon atoms in its backbone, which when substituted, the substituent is selected from the group consisting of alkyl and cycloalkyl; and

R^4 , R^5 , R^6 , R^{10} , R^{11} , R^{12} and R^{13} are each independently hydrogen or alkyl;

with the proviso that:

when X is $\text{NR}^5\text{-C(=W)-NR}^6$ and W is sulfur, A is other than $(\text{CH}_2)_3\text{O}(\text{CH}_2)_2\text{CH}_3$;

when A is $(\text{CH}_2)_3\text{O}(\text{CH}_2)_2\text{CH}_3$ and X is $\text{NR}^5\text{-C(=W)-NR}^6$, W is other than sulfur;

and

when X is $\text{NR}^5\text{-C(=W)-NR}^6$ and W is sulfur and A is $(\text{CH}_2)_3\text{O}(\text{CH}_2)_2\text{CH}_3$, Z is selected from the group consisting of a substituted or non-substituted heteroaryl and a substituted aryl, wherein at least one substituent on said aryl is other than alkoxy, methyl, and halide.

The various embodiments of each of the variables in Formula Ia are as described hereinabove for Formula I.

It is noted that while the novel compounds described herein share some structural features of Compound 20, these compounds include various modifications to the chemical structure of Compound 20. These include, for example, modifications of the aliphatic side chain of the thiourea moiety; modification of the thiourea moiety; and/or modification of the substituent of the aryl ring.

Exemplary compounds having general Formula Ia are presented in Table 5 hereinbelow.

The present embodiments further encompass any pharmaceutically acceptable salt, prodrug, solvate, hydrate and, if present, purified enantiomers of each of the compounds described herein.

The phrase "pharmaceutically acceptable salt" refers to a charged species of the parent compound and its counter ion, which is typically used to modify the solubility characteristics of the parent compound and/or to reduce any significant irritation to an organism by the parent compound, while not abrogating the biological activity and properties of the administered compound.

As used herein, the term "prodrug" refers to an agent, which is converted into the active compound (the active parent drug) *in vivo*. Prodrugs are typically useful for facilitating the administration of the parent drug. They may, for instance, be bioavailable by oral administration whereas the parent drug is not. The prodrug may also have improved solubility as compared with the parent drug in pharmaceutical compositions. Prodrugs are also often used to achieve a sustained release of the active compound *in vivo*.

The term "solvate" refers to a complex of variable stoichiometry (e.g., di-, tri-, tetra-, penta-, hexa-, and so on), which is formed by a solute (the compound described herein) and a solvent, whereby the solvent does not interfere with the biological activity of the solute. Suitable solvents include, for example, ethanol, acetic acid and the like.

The term "hydrate" refers to a solvate, as defined hereinabove, where the solvent is water.

Further according to embodiments of the present invention, there are provided processes of preparing the novel compounds described herein, having Formula Ia hereinabove. These processes are described in detail in the Examples section that follows (see, Example 4).

Thus, according to an aspect of some embodiments of the invention there is provided a process of synthesizing the novel compounds, having formula Ia, as described herein. The process is effected by reacting a compound having the Formula Z-R¹ with a compound having the Formula A-R², wherein R² is amine and R¹ is selected such that upon reacting with the amine, the desired X is formed.

In some embodiments, R¹ is isothiocyanate, which, upon reaction with amine, forms a thiourea moiety.

Similarly, in some embodiments, R¹ is isocyanate, which, upon reaction with amine, forms a urea moiety.

As discussed hereinabove, the compounds described herein were selected and/or designed capable of inhibiting an activity of a reverse transcriptase.

Therefore, according to an aspect of embodiments of the present invention there is provided a method of inhibiting an activity of a reverse transcriptase, which is effected by contacting the reverse transcriptase with any of the compounds described herein.

The reverse transcriptase can be a HIV-1 RT and/or HIV-2 RT.

It is noted in this regard that it has been shown that at least Compound **20** is capable of inhibiting an activity of both HIV-1 RT and HIV-2 RT.

According to some embodiments, the contacting is effected *in vitro*. According to some embodiments the contacting is effected *in vivo*.

According to some embodiments, the activity of the reverse transcriptase is RT-associated DNA-dependent DNA polymerase activity and/or RNA-dependent DNA polymerase activity.

The phrase "DNA-dependent DNA polymerase activity", as used herein, describes the ability of a reverse transcriptase to use DNA as a template for transcription of DNA. This term is also referred to herein as "DNA polymerase activity".

The phrase "RNA-dependent DNA polymerase activity", as used herein, describes the ability of a reverse transcriptase to use RNA as a template for transcription of DNA. This term is also referred to herein as "RNA polymerase activity".

Without being bound by theory, from a mechanistic point of view, it is suggested that the compounds described herein can bind *bona fide* the NNRTI-binding hydrophobic pocket of HIV-1 RT, which is not found in other DNA polymerases. *In silico* docking experiments of the exemplary Compounds **3** and **20** onto the NNRTI-

binding hydrophobic pocket of HIV-1 RT indicated that both compounds fit well into the pocket (see, Figure 6).

As discussed hereinabove, the reverse transcriptase can be a wild type RT or a mutant thereof.

5 As demonstrated in the Examples section that follows, the compounds described herein can inhibit an activity of both a wild type HIV-1 RT and of resistant mutants thereof. For example, the exemplary Compounds **3** and **20** inhibited the RNA-dependent DNA polymerase activity of the well known drug resistant mutant HIV-1 RT Y181C (see, Figure 3A).

10 The phrase "wild type reverse transcriptase" describes an enzyme produced by an un-mutated cell.

The term "mutant" with respect to wild type RT describes an enzyme having an amino acid sequence which differs from that of the wild-type enzyme due to the genetic mutation of the virus that harbors this enzyme.

15 The term "genetic mutation" describes genetic alteration in the genome of a cell or a virus which in turn alters the amino acid sequence of the enzyme produced thereby.

As discussed hereinabove, the compounds described herein are capable of inhibiting a catalytic activity of a reverse transcriptase such as HIV-1 reverse transcriptase. The reverse transcriptase is an essential enzyme in the retroviral life cycle.

20 Therefore, according to an aspect of some embodiments of the present invention there is provided a method of treating or preventing a viral infection caused by a retrovirus (also referred to herein as a retroviral infection). This method is effected by administering to a subject in need thereof a therapeutically effective amount of any of the compounds described herein, including a combination thereof.

25 The terms "treat," "treating," and "treatment", as used herein, encompass alleviating or abrogating a disorder; or one or more of the symptoms associated with the disorder; or alleviating or eradicating the cause(s) of the disorder itself.

The terms "prevent," "preventing," and "prevention" refer to a method of delaying or precluding the onset of a disorder; and/or its attendant symptoms, barring a
30 subject from acquiring a disorder or reducing a subject's risk of acquiring a disorder.

The term "therapeutically effective amount" refers to the amount of a compound that, when administered, is sufficient to prevent development of, or alleviate to some extent, one or more of the symptoms of the disorder being treated.

As used herein, the term "subject" refers to any animal (e.g., a mammal),
5 including, but not limited to, humans, non-human primates, rodents, and the like, which is to be the recipient of a particular treatment. Typically, the terms "subject" and "patient" are used interchangeably herein in reference to a human subject.

The term "virus" describes a group of microbes which, with few exceptions, are capable of passing through fine filters that retain most bacteria, and are incapable of
10 growth or reproduction in media apart from living cells. See, e.g., Stedman's Medical Dictionary, 25th Ed., illustrated, Williams & Wilkins, Baltimore, Md., pp. 1717-1723 (1990).

The term "retrovirus", as used herein, describes a class of viruses of vertebrate animals in which the genetic material is RNA, instead of DNA. Such viruses are
15 accompanied by a polymerase enzyme known as "reverse transcriptase", which catalyzes transcription of viral single-stranded RNA into double-stranded DNA. The resultant DNA may remain in a dormant state in an infected cell for an indeterminate period of time, or become incorporated into the cells genome and actively cause the formation of new virions.

20 As used herein, the term "viral infection" or "retroviral infection" describes any disease caused by retroviruses, particularly retroviruses pathogenic to subjects such as humans.

The phrase "treating viral infection" describes a compound's ability to inhibit viral infection of cells, via, for example, cell-cell fusion or free virus infection. Such
25 infections may involve membrane fusion, as occurs in the case of enveloped viruses, or some other fusion event involving a viral structure and a cellular structure (e.g., such as the fusion of a viral pilus and bacterial membrane during bacterial conjugation).

In any of the methods and uses described herein, the reverse transcriptase can be derived from any retrovirus, including, for example, Moloney Murine Leukemia Virus
30 (M-MLV), Rous Sarcoma Virus (RSV), Avian Myeloblastosis Virus (AMV), Mouse Mammary Tumor Virus, (MMTV), Bovine Leukemia virus (BLV) Simian Immunodeficiency Virus (SIV), Human T-Cell Leukemia Virus (HTLV-1) and Human

Immunodeficiency Virus (HIV) reverse transcriptases. According to some embodiments of the present invention, the retrovirus is HIV-1.

The retrovirus designated human immunodeficiency virus (HIV), particularly the strains known as HIV type-1 (HIV-1) and type-2 (HIV-2) retroviruses, have been etiologically linked to the human immunosuppressive disease known as acquired immunodeficiency syndrome (AIDS). Therefore, according to some embodiments the present invention the method describes herein is a method of treating or preventing a viral infection caused by a retrovirus (also referred to herein as a retroviral infection), wherein the retrovirus is HIV-1 and/or HIV-2.

HIV seropositive individuals are initially asymptomatic but typically develop AIDS related complex (ARC) followed by AIDS. Affected individuals exhibit severe immunosuppression which makes them highly susceptible to debilitating and ultimately fatal opportunistic infections. Replication of HIV by a host cell requires integration of the viral genome into the host cell's DNA. Since HIV is a retrovirus, the HIV replication cycle requires transcription of the viral RNA genome into DNA by the HIV-RT.

Therefore, according to some embodiments the reverse transcriptase is a HIV-RT or a mutant thereof. Accordingly, the compounds described herein are particularly useful in the treatment (or prophylaxis) of HIV infections such as AIDS and associated conditions, such as AIDS related complex (ARC), Kaposi's sarcoma, and AIDS dementia.

Acquired immunodeficiency syndrome (AIDS) is a set of symptoms and infections resulting from the damage to the human immune system caused by the human immunodeficiency virus (HIV). This condition progressively reduces the effectiveness of the immune system and leaves individuals susceptible to opportunistic infections and tumors. HIV is transmitted through direct contact of a mucous membrane or the bloodstream with a bodily fluid containing HIV, such as blood and blood fractions, semen, vaginal fluid, preseminal fluid, and breast milk. The symptoms of AIDS are primarily the result of conditions that do not normally develop in individuals with healthy immune systems. Most of these conditions are infections caused by bacteria, viruses, fungi and parasites that are normally controlled by the elements of the immune system that HIV damages.

Subjects with AIDS also have an increased risk of developing various cancers such as Kaposi's sarcoma, cervical cancer and cancers of the immune system known as lymphomas.

AIDS related complex (ARC) is a condition in which antibody tests are positive for HIV. Patients with ARC show the mild symptoms of HIV infection, which include enlarged lymph nodes, fatigue, night sweats, weight loss, and diarrhea.

The phrase "treatment of HIV infection" refers to improvement in at least one clinical parameter associated with the HIV infection as compared to non-treated control and notably to improve in the viral load count and increase in CD4+ bearing cells. The improvement may be actual reduction in the viral load, but may also be slowing down in the rate of increase of the viral load, slowing down of the physical deterioration, and side effects associated with AIDS.

As discussed hereinabove, HIV-2 is another strain of HIV retrovirus having the ability to infect human cells. HIV-2 retrovirus is found primarily in West Africa and is similar to HIV-1 but appears less destructive to the immune system and has a slower disease progression. Most NNRTIs are highly specific against HIV-1 RT with minimal effects on the closely-related HIV-2 RT. As exemplified in the example section which follows, the compounds described herein exhibited anti-HIV-2 activity with a profound inhibition of the DNA-dependent DNA polymerase activity of the enzyme as compared to the well known NNRTI nevirapine (see Figure 3B).

Therefore, according to some embodiments, the reverse transcriptase is an HIV-2 reverse transcriptase or a mutant thereof.

In any of the methods described herein, the compounds of the present embodiments may be employed in combination with other therapeutic agents for the treatment of the above infections or conditions. Combination therapies according to embodiments of the present invention comprise the administration of at least one compound as described herein and at least one other pharmaceutically active ingredient. The compound(s) described herein and the other pharmaceutically active agents may be administered simultaneously in either the same or different pharmaceutical compositions or sequentially in any order. The amounts of the compound(s) as described herein and the other pharmaceutically active agent(s) and the relative timings of administration are selected in order to achieve the desired combined therapeutic effect.

Due to the possibility of synergistic HIV-1 replication inhibiting activities of the compounds of the invention, in some embodiments of the invention, the compounds described herein are administered to a subject together with an additional agent (e.g., a pharmaceutically active agent), as a combination therapy as described hereinabove.

5 In some embodiments, the additional agent is capable of treating the viral infection from which the subject is suffering.

Preferred combination therapies include simultaneous or sequential treatment with any of the compounds described herein and one or more of the following:

(a) reverse transcriptase inhibitors such as abacavir, adefovir, didanosine,
10 lamivudine, stavudine, zalcitabine and zidovudine;

(b) non-nucleoside reverse transcriptase inhibitors such as capavirine, delavirdine, efavirenz, etravirine (TMC-125) and nevirapine;

(c) HIV protease inhibitors such as indinavir, nelfinavir, ritonavir, and saquinavir;

(d) CCR5 antagonists such as TAK-779 or UK-427, 857;

(e) CXCR4 antagonists such as AMD-3100;

(f) integrase inhibitors, such as L-870,810 or S-1360;

(g) inhibitors of viral fusion such as T-20;

(h) investigational drugs, such as trizivir, KNI-272, amprenavir, GW-33908, FTC, PMPA, MKC-442, MSC-204, MSH-372, DMP450, PNU-140690, ABT-378, KNI-
20 764, DPC-083 and/or TMC-120;

(i) antifungal agents, such as fluconazole, itraconazole or voriconazole; and

(j) antibacterial agents, such as azithromycin.

In one embodiment, the combination therapy includes treatment with a compound as described herein and the "cocktail" therapy described hereinabove.

25 Further according to embodiments of the invention there is provided a use of any of the compounds described herein in the manufacture of a medicament for the treatment of the infections and conditions described herein (e.g., a viral infection caused by a retrovirus).

Further according to embodiments of the invention there is provided a compound
30 as described herein, which is identified for use in the treatment of a viral infection caused by a retrovirus.

As discussed hereinabove, in some embodiments, the compound is utilized in a combination therapy (co-therapy) with at least one agent capable of treating a viral infection caused by a retrovirus.

In any of the method and uses described herein, the compounds described herein
5 can be utilized either *per se* or as a part of a pharmaceutical composition, which further comprises a pharmaceutically acceptable carrier.

Hence, according to an aspect of embodiments of the present invention there is provided a pharmaceutical composition which comprises, as an active ingredient, any of the compounds described herein and a pharmaceutically acceptable carrier.

10 In some embodiments, the pharmaceutical composition is packaged in a packaging material and identified in print, in or on the packaging material, for use in the treatment of any of the conditions and infections described herein.

In some embodiments, the pharmaceutical composition is identified for inhibiting the replication of HIV-1 by inhibiting HIV-1 RT activity. In some
15 embodiments, the pharmaceutical composition is for the treatment of HIV-1 infection.

In some embodiments, the pharmaceutical composition further comprises an additional agent capable of treating a viral infection caused by a retrovirus, as described herein.

As used herein a "pharmaceutical composition" refers to a preparation of the
20 compounds presented herein, with other chemical components such as pharmaceutically acceptable and suitable carriers and excipients. The purpose of a pharmaceutical composition is to facilitate administration of a compound to an organism.

Hereinafter, the term "pharmaceutically acceptable carrier" refers to a carrier or a diluent that does not cause significant irritation to an organism and does not abrogate
25 the biological activity and properties of the administered compound. Examples, without limitations, of carriers are: propylene glycol, saline, emulsions and mixtures of organic solvents with water, as well as solid (e.g., powdered) and gaseous carriers.

Herein the term "excipient" refers to an inert substance added to a pharmaceutical composition to further facilitate administration of a compound.
30 Examples, without limitation, of excipients include calcium carbonate, calcium phosphate, various sugars and types of starch, cellulose derivatives, gelatin, vegetable oils and polyethylene glycols.

Techniques for formulation and administration of drugs may be found in "Remington's Pharmaceutical Sciences" Mack Publishing Co., Easton, PA, latest edition, which is incorporated herein by reference.

Pharmaceutical compositions for use in accordance with the present invention
5 thus may be formulated in conventional manner using one or more pharmaceutically acceptable carriers comprising excipients and auxiliaries, which facilitate processing of the compounds into preparations which can be used pharmaceutically. Proper formulation is dependent upon the route of administration chosen. The dosage may vary depending upon the dosage form employed and the route of administration utilized.
10 The exact formulation, route of administration and dosage can be chosen by the individual physician in view of the patient's condition (see e.g., Fingl et al., 1975, in "The Pharmacological Basis of Therapeutics", Ch. 1 p.1).

The pharmaceutical composition may be formulated for administration in either one or more of routes depending on whether local or systemic treatment or
15 administration is of choice, and on the area to be treated. Administration may be done orally, by inhalation, or parenterally, for example by intravenous drip or intraperitoneal, subcutaneous, intramuscular or intravenous injection, or topically (including ophthalmically, vaginally, rectally, intranasally).

Formulations for topical administration may include but are not limited to
20 lotions, ointments, gels, creams, suppositories, drops, liquids, sprays and powders. Conventional pharmaceutical carriers, aqueous, powder or oily bases, thickeners and the like may be necessary or desirable.

Compositions for oral administration include powders or granules, suspensions or solutions in water or non-aqueous media, sachets, pills, caplets, capsules or tablets.
25 Thickeners, diluents, flavorings, dispersing aids, emulsifiers or binders may be desirable.

Formulations for parenteral administration may include, but are not limited to, sterile solutions which may also contain buffers, diluents and other suitable additives. Slow release compositions are envisaged for treatment.

30 The amount of a composition to be administered will, of course, be dependent on the subject being treated, the severity of the affliction, the manner of administration, the judgment of the prescribing physician, etc.

Compositions of the present invention may, if desired, be presented in a pack or dispenser device, such as an FDA (the U.S. Food and Drug Administration) approved kit, which may contain one or more unit dosage forms containing the active ingredient. The pack may, for example, comprise metal or plastic foil, such as, but not limited to a blister pack or a pressurized container (for inhalation). The pack or dispenser device may be accompanied by instructions for administration. The pack or dispenser may also be accompanied by a notice associated with the container in a form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals, which notice is reflective of approval by the agency of the form of the compositions for human or veterinary administration. Such notice, for example, may be of labeling approved by the U.S. Food and Drug Administration for prescription drugs or of an approved product insert. Compositions comprising the compound(s) of the invention formulated in a compatible pharmaceutical carrier may also be prepared, placed in an appropriate container, and labeled for treatment of a condition or infection as detailed hereinabove.

It is appreciated that certain features of the invention, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the invention, which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable subcombination or as suitable in any other described embodiment of the invention. Certain features described in the context of various embodiments are not to be considered essential features of those embodiments, unless the embodiment is inoperative without those elements.

Various embodiments and aspects of the present invention as delineated hereinabove and as claimed in the claims section below find experimental [support in the following examples.

EXAMPLES

Reference is now made to the following examples, which together with the above descriptions, illustrate the invention in a non limiting fashion.

EXAMPLE 1***Computer aided screening of a chemical library for non nucleoside RT inhibitors (NNRTIs)******Molecular Modeling:***

5 The RT structures 1fk9 and 1dtq were downloaded from the Research Collaboratory for Structural Bioinformatics site (www.rcsb.org/pdb) as a complex with the RT inhibitors efavirenz or Phenylethylthiazolylthiourea (PETT131A94) respectively and were saved as pdb files. 1fk9 is a structure of a wild type RT with a resolution of 2.5 Å and 1dgt is a structure of a wild type RT with a resolution of 2.8 Å.

10 All structures were modified with the Cerius 2 software as follows: first the inhibitor and water molecules were removed. Then, the structures were inspected for the correct double bonds, hydrogen atoms were added to each structure and these atoms only were energy minimized with the CFF 1.02 force field.

A Protomol was generated using the default setting of the Surflex program [see, 15 Jain, A. N. Surflex: fully automatic flexible molecular docking using a molecular similarity-based search engine. *J. Med. Chem.* **2003**, 46, 499-511]. A protomol is an idealized representation of a ligand that makes every potential interaction with the binding site. Using the three dimensional location of the removed inhibitor at the RT binding site in each structure as reference, the Protomol was defined. The Tripos 20 "Leadquest 3" chemical library, containing 46,000 compounds was downloaded from see www.tripos.com/, as a structure data (sd) file. Each compound was then energy-minimized to a single, optimized conformation using the Omega software (Openeye software) and the mmff94s force field and their 3D conformation was saved in a mol2 file format. A docking process of the compounds onto the RT binding site of 1fk9 and 25 1dtq was then initiated using the generated Protomol. The Docking was performed by fragmenting each molecule and fitting the conformation of each fragment into the Protomol to yield a spatial structure that maximizes molecular similarity to the Protomol. The 10 top conformations for each compound were retrieved according to their score as output files. These included a log output file with the scores for each 30 conformation and a structural output file with the coordinates for the suggested conformations.

Using an in-house generated Filter program, the best-fit compounds were then filtered from the log output file by using thresholds of 6 for score values, -2 for crash values and 1 for polar values. Docking conformations of these selected compounds were extracted from the structural output file with an in-house generated Extract program. Both the Filter and Extract in house programs were written in Perl and are available upon request.

The Docking procedure was repeated twice: Once for 1fk9 and once for 1dtq. Each docking was further analyzed visually with discovery studio visualizer 1.6 (Accelrys software Inc.). Compounds having the highest scores of docking onto the binding site of both RTs were purchased from Tripos Inc. For convenience, shown in Table 1 are conversions between selected Tripos compound reference numbers to the compound numbers designations used in this study.

Table 1

Compound id	Tripos number	Compound id	Tripos number
1	1515-00915	22	1526-08434
2	1526-21690	23	1526-08430
3	1554-05020	24	1526-08427
4	1503-08948	25	1526-08416
5	1526-22908	26	1526-08446
6	1503-08758	27	1526-08402
7	1526-08390	28	1526-08425
8	1519-00342	29	1526-08429
9	1526-08426	30	1526-8448
10	1515-01089	31	1526-08417
11	1539-27083	32	1526-08419
12	1527-01421	33	1526-08392
13	1526-20802	34	1526-08410

14	1515-01091	35	1526-08422
15	1526-26487	36	1526-08406
16	1526-26470	37	1526-08437
17	1515-01093	38	1526-08423
18	1526-27112	39	1526-08424
19	1526-08435	40	1526-08428
20	1526-08403	41	1526-08420
21	1526-08413		

Results:

A chemical library of 46,000 compounds was screened for optimal docking onto the binding site of two of HIV-1 RT crystal structures in order to identify novel inhibitors against HIV-1 RT. The structures of all screened compounds were energy-minimized and then docked onto the NNRTI binding pocket of the heterodimeric (p66/p51) structure of HIV-1 RT, using the Surflex software. Two RT crystal structures were used in the screening process (1fk9 and 1dtq) in order to account for subtle differences between various RT structures, thereby obtaining more reliable docking scores for the screened compounds. The docking conformation of the top ranked structures (i.e. the structures that bound with high affinity, *in silico*) to both RT structures, was further analyzed visually for determining the quality of the docking process. Consequently, 740 compounds, out of the 46,000 screened compounds in the Tripos chemical library, were selected and purchased for further experimental evaluation.

EXAMPLE 2**Activity Assays****Experimental Methods:**

Expression and purification of RTs: Recombinant, wild-type p66/p51 HIV-1 RT, derived from BH-10 clone of HIV-1, was expressed in bacteria. This enzyme, which has six histidines tag at the C-terminus of the p66 subunit, was purified on Ni²⁺

nitrilotriacetic acid agarose (Ni-NTA) column followed by cation exchange chromatography, as previously described [Sevilya et al. *Nucleic Acids Res* **2003**, 31, 1481-7].

Klenow fragment of *E. coli* DNA polymerase I (KF), which served as a control, unrelated DNA polymerase was purchased from New England Biolabs.

DNA polymerase assay: The RNA-dependent DNA polymerase activity of RT was assayed by measuring the incorporation of [^3H]dTTP into poly(rA)_n•oligo(dT)₁₂₋₁₈ template-primer as previously described [Hizi et al. *Antimicrob Agents Chemother* **1993**, 37, 1037-42]. All inhibitors were serially diluted in DMSO and 1 μl of each concentration (or 1 μl of DMSO only) was added to 79 μl of 31.25 mM Tris-HCl pH 7.5, 50 mM KCl and 8 mM MgCl₂. 10 μl of purified HIV-1 RT (14 ng) was added to each tube and the solutions were incubated on ice for 10 minutes. The enzymatic reactions were initiated by the addition of 10 μl of the substrate mix [50 $\mu\text{g}/\text{ml}$ poly(rA)_n•oligo(dT)₁₂₋₁₈, 50 μM dTTP, 150 $\mu\text{Ci}/\text{ml}$ [^3H]dTTP] followed by incubation for 30 minutes at 37 °C. The reaction was stopped by adding 50 $\mu\text{g}/\text{ml}$ herring sperm carrier DNA and 40 mM sodium pyrophosphate, followed by precipitation with ice-cold 10 % (w/v) trichloroacetic acid (TCA). The precipitates were collected on Whatman GF/C fiberglass filters, and the filters were washed with 5 % cold TCA and then with 50 % cold ethanol. The dried filters were put in a scintillation fluid and counted in a β scintillation counter. The DNA-dependent DNA polymerase activity of RT or KF was assayed in a similar manner but with activated herring sperm DNA at the final concentration of 20 $\mu\text{g}/\text{ml}$, substituting for the synthetic template•primer and with all the four dNTPs present in the reaction mixture, out of which dTTP was ^3H - labeled. The DNA-dependent DNA polymerase activity of RT and of KF was assayed under identical experimental conditions.

Measurements reported are the average of three independent experiments. The dose response curves were non-linearly fitted to the four-parameter logistic equation [DeLean et al. *Am J Physiol.* **1978**, 235, E97-102] using Origin software.

RNase H assay: The RNase H activity of RT was assayed with fluorescence resonance energy transfer technology as previously described [Parniak et al. *Anal Biochem.* **2003**, 322, 33-9]. Briefly, HIV-1 RT was incubated with a substrate of hybrid of fluorescein-RNA and DNA- 4-[[40-(dimethylamino)phenyl]azo]benzoic acid and

with (or without) a specific inhibitor. Hydrolysis of the substrate and the resulting emitting fluorescence was measured with a fluorescence spectrometer after 30 minutes of incubation at 37 °C. To improve the signal to noise ratio the assay was slightly modified to contain 6 nM RT and 1 mM DTT. All measurements were done in triplicates in a 96-well plate and IC₅₀ values were interpolated from the dose response curves.

Steady State Kinetic RNA-dependent DNA polymerase activity assays: All kinetic assays were conducted as described hereinabove for the quantitative analysis, with different concentrations of either dTTP or poly(rA)_n-oligo(dT)₁₂₋₁₈ template-primer as indicated. Reaction mixtures were incubated for 15 minutes, and the results were linearly fitted using Origin 7.5.

Cytotoxicity assay: Cytotoxicity assays were performed with the XTT substrate as previously described [Roehm et al. *J Immunol Methods*. **1991**, 142, 257-65]. Briefly, B-lymphocytes were incubated in a 96 well plate with various inhibitor concentrations at 37 °C for 72 hours. Cell viability was then assayed with XTT substrate. Absorbance was recorded at 450 nm and reference wavelength was recorded at 620 nm.

HIV pseudovirus infection: Infectious virions were prepared by transfecting HEK293T cells as previously described [Naldini et al.. *Proc. Natl. Acad. Sci. U.S.A* **1996**, 93, 11382-8] with three plasmids: (1) pCMVΔ8.2Gagpol which encodes for HIV-1 proteins (except Env), (2) pHRCMVGFP, which is transcribed to a mRNA containing both the encapsidation signal and the GFP coding sequences, and (3) pVSV-G, which supplies an envelope protein capacitated to infect wide variety of cells. 25 μM of Chloroquine was added to the medium prior to transfection. 48 hours post transfection the supernatant was collected, filtered through 0.45 μM filter, equilibrated with 50mM HEPES, pH 7.2 and stored at -80 °C. Virions were unfrozen at 37 °C and allowed to infect B lymphocytes (721.221 cells) in the presence of 5.6 μg/ml polybrene and a specific inhibitor at a tested concentration (diluted in RPMI medium + 1% DMSO). Between 48 and 72 hours post infection, the cells were collected, fixed with 1 % paraformaldehyde and analyzed by Flow cytometry (BD biosciences) or fluorescent microscopy. Values reported are from four experiments for compounds **20** and **3** and three experiments for all other compounds.

Results:***RT enzymatic activity:***

740 compounds were purchased and tested against the RNA-dependent DNA polymerase activity of HIV-1 RT, each at a final 50 µg/ml concentration (average molar concentration of 156 ± 23 µM with a range between 105 to 260 µM). The RNA-dependent DNA polymerase activity (RDDP; i.e. use of RNA as template for transcription of DNA) is unique to RTs and distinct from any other DNA polymerases, which are typically DNA-dependent DNA polymerases (DDDP; which use a DNA as a template), and therefore was tested first. Most compounds did not inhibit or inhibited to a very limited extent the RNA-dependent DNA polymerase activity of RT, while 71 compounds (9.6 %) inhibited more than 84 % of the RNA-dependent DNA polymerase activity of RT at the tested concentration (with a range between 84.1 % to 99.9%).

The level of selectivity and specificity of these 71 compounds to HIV-1 RT was tested in order to differentiate between specific inhibition and non-specific inhibition of RT due to cross reactivity with unrelated cellular DNA polymerases or interactions with other components of the enzymatic assay. Therefore, the ability of the 71 compounds to inhibit HIV-1 RT was compared to its ability to inhibit Klenow fragment of E. coli DNA polymerase I (KF), which served as a distinct and unrelated DNA polymerase. In this case, the ability of the compounds to inhibit DNA-dependent DNA polymerase activity of RT and KF was examined due to KF being a DNA polymerase devoid of any RNA-dependent DNA polymerase activity. A side-by-side, parallel comparison of the inhibition effect of each selected compound, at a similar concentration on the two enzymes under identical experimental conditions, was measured, and the specificity of each compound (designated the selectivity index) was calculated by dividing the residual KF activity by that of HIV-1 RT. Compounds with a selectivity index higher than 1 have a higher specificity towards HIV-1 RT, whereas a value lower than 1 indicates preferred specificity towards KF. Out of the total 71 compounds, only eight have demonstrated a selectivity index higher than 1.25, which was arbitrarily chosen as the threshold value for a significant anti-RT selectivity. The selectivity value for only three compounds was between 1 and 1.25 whereas all the other compounds inhibited KF more efficiently than HIV-1 RT thereby having a selectivity index below 1.

Table 2 below presents the chemical structures, selectivity and activity of the most potent and selective compounds, Compounds 1-8. These eight top molecules were filtered based on a residual RNA-dependent DNA polymerase (RDDP) activity lower than 15 % and a selectivity index higher than 1.25. Compound 9, despite showing a residual RDDP activity higher than 15 %, was extracted from the tested inhibitors based on its similarity to Compound 7 and tested as all other compounds shown.

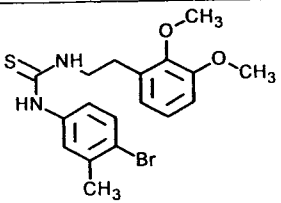
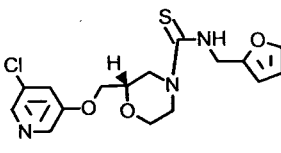
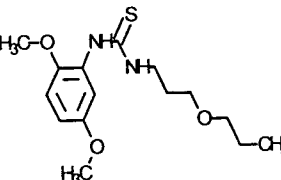
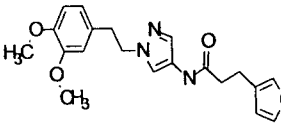
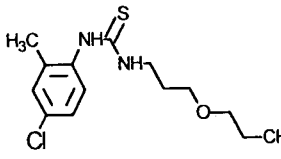
The residual activity was calculated by dividing the residual activity, detected in the presence of a final 50 µg/ml concentration of the tested compound, by the initial activity with no inhibitor present and then multiplying the outcome by 100.

Selectivity index was calculated as % residual KF-derived DNA Polymerase (DDDP) activity divided by the % residual HIV-1 RT-derived DDDP activity.

Table 2

Compound		% residual enzymatic activity			Selectivity index	IC ₅₀ PV infection [µM]
id	Structure	HIV-1 RT RDDP	HIV-1 RT DDDP	KF DDDP		
1		11.4 ± 2.17	21.7 ± 2.0	60.3 ± 3.8	2.8 ± 0.3	4.0 ± 0.6
2		0.7 ± 0.5	8.6 ± 1.8	23.8 ± 13.8	2.8 ± 1.7	1.6 ± 0.2
3		2.2 ± 0.1	13.0 ± 3.4	17.4 ± 2.1	1.6 ± 0.6	0.37 ± 0.05
4		5.6 ± 2.0	22.4 ± 7.9	43.4 ± 8.4	1.9 ± 0.8	>20

56

5		5.1 ± 1.2	15.6 ± 6.4	41.8 ± 7.3	2.7 ± 1.2	7.7 ± 1.2
6		1.1 ± 0.5	7.0 ± 2.0	16.5 ± 3.9	2.2 ± 0.8	>20
7		13.2 ± 1.5	28.7 ± 0.5	86.5 ± 1.5	3.3 ± 0.5	3.0 ± 0.3
8		10.9 ± 2.62	26.6 ± 4.8	43.5 ± 6.5	2.7 ± 0.9	>20
9		28.0 ± 4.1	52.3 ± 19.9	82.1 ± 15.0	1.5 ± 0.6	2.6 ± 0.4

Correlation between selective in vitro inhibition and protecting human cells from HIV infection:

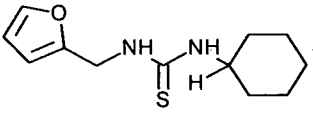
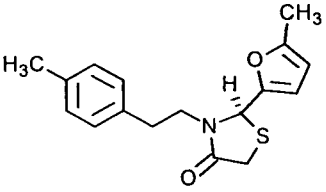
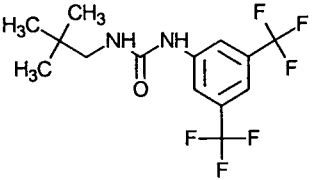
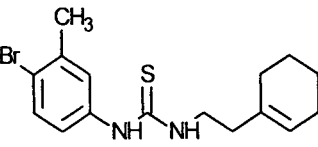
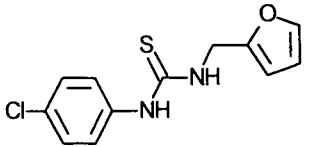
To verify that the selected 9 compounds (see, Table 2 hereinabove) indeed
 5 construct a group which specifically inhibit HIV-RT and consequently HIV infections, this group of compounds, as well as other compounds which exhibited no selectivity towards HIV-1 RT (having selectivity index < 1 and presented in Table 3 hereinbelow), were tested for their ability to protect human lymphocytes from being infected by HIV-1 pseudovirus.

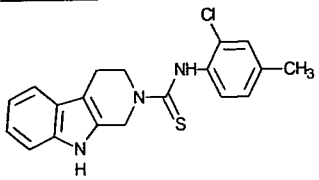
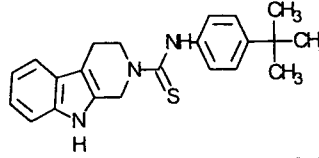
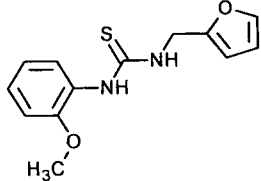
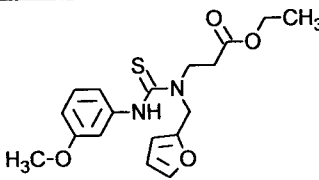
10 In this assay, HIV virions were constructed which carried: (1) viral mRNA backbone into which the coding sequence for the green fluorescent reporter gene was inserted; (2) all HIV associated proteins, which are supplied in trans during packing; and (3) vesicular stomatitis virus (VSV)-G envelope protein, enabling the virions to infect wide range of cells. Productive infection results in an expression of green

fluorescent protein (GFP) in the infected cells, which can be monitored with fluorescent microscopy and quantitatively measured by flow cytometry.

The obtained results indicated that six out of the nine anti-HIV-1 RT selective compounds tested (67 %, 62.5 % without Compound 9 that was added based on its similarity to Compound 7) inhibited HIV-1 pseudovirus infection with apparent IC_{50} values varying between about 0.37 μM and 7.7 μM (see, Table 2). In contrast, only one out of the nine non-selective tested compounds (11 %), which inhibited KF better than HIV-1 RT, suppressed the infection of lymphocytes by HIV-1 (see, Table 3, Compound 13). These results indicate a correlation between the selective inhibition of HIV-1 RT and the ability to suppress viral infectivity.

Table 3

Compound		% residual enzymatic activity			Selectivity index	IC_{50} PV infection [μM]
id	Structure	HIV-1 RT RDDP	HIV-1 RT DDDP	KF DDDP		
10		5.2 ± 2.6	10.9 ± 1.6	0.5 ± 0.3	0	> 20
11		2.0 ± 0.3	3.1 ± 2.0	2.8	0.6 ± 0.4	> 20
12		1.7 ± 0.5	7.9 ± 1.8	0	0	> 20
13		4.6 ± 0.8	9.5 ± 3.0	3.4 ± 1.1	0.36 ± 0.17	0.23 ± 0.08
14		3.4 ± 0.4	5.8 ± 0.8	0	0	> 20

15		3.7 ± 0.7	9.7 ± 2.4	0.7 ± 0.4	0.08 ± 0.04	> 20
16		5.0 ± 2.5	9.7 ± 0.5	0.6 ± 0.5	0.06 ± 0.05	> 20
17		5.9 ± 0.8	11.7 ± 0.5	0.6 ± 0.3	0.05 ± 0.03	> 20
18		8.5 ± 4.9	35.8 ± 1.1	0	0	> 20

Structure activity relationship of 1-(3-propoxypropyl)thiourea derivatives:

In the cell based HIV pseudovirus infection assay described hereinabove, the compound N-(4-bromo-1H-pyrazol-3-yl)-2,1,3-benzothiadiazole-4-sulfonamide (Compound 3) potently inhibited HIV infection with an apparent IC_{50} value of about 374 nM (see, Table 2). Among the other eight specific anti-HIV-1 RT inhibitors, two compounds, which were structurally unrelated to Compound 3, shared the 1-(3-propoxypropyl)thiourea group (Compounds 7 and 9). Both these compounds inhibited HIV-1 infection with apparent IC_{50} values of about 3 μ M (see, Table 2). Thus, compounds structurally related to Compounds 7 and 9 were also tested.

Twenty three derivatives of Compounds 7 and 9, all containing the 1-(3-propoxypropyl)thiourea group, were therefore tested for inhibiting recombinant HIV-1 RT and KF and the derivatives showing the highest selectivity index were further tested for the ability to inhibit HIV-1 infection of B lymphocytes, as detailed hereinabove. Some of these compounds were identified from the original Tripos Leadquest3 chemical library, whereas others were selected from different databases.

The results are presented in Table 4 hereinbelow and indicate that the most potent inhibitor out of these derivatives was 1-(4-chloro-2,5-dimethoxyphenyl)-3-(3-propoxypropyl)thiourea (Compound 20).

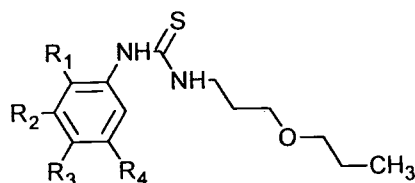
The HIV-1 infection inhibition activity of Compound **20** was evident from the reduced fluorescent intensity (due to the reduction in the transduced GFP reporter gene product) when the compound was incubated with B lymphocytes subjected to HIV-1 pseudovirus (HIV-1 PV) infection (see, FIG. 1A, left panel). The inhibition activity was dose-dependent and was confirmed by both visualizing the cells under fluorescence microscope and by measuring the fluorescent signal using flow cytometry. In the latter method, the resulting cells could be separated to uninfected and infected cells, based on their green fluorescent intensity (FIG. 1A, right panel). The average fluorescence intensity of the B lymphocytes was then plotted against the concentration of Compound **20** and fitted into a four parameter logistic equation with a corresponding IC_{50} value of approximately 168 nM (see, FIG. 1C). In order to assess whether the quantified fluorescence was due to cells infected by HIV virions, cells subjected to infection with heat-inactivated pseudovirus preparations served as control and as expected did not exhibit any level of fluorescence above background (see, FIG. 1A, lowest panel).

As presented in Table 4, Compound **20** also exhibited a high selectivity toward HIV-1 RT (selectivity index of 7.4). Furthermore, the cytotoxicity of this compound toward B lymphocytes was examined. The cytotoxicity assays were performed following a 72 hours incubation of the cells with the inhibitor at 37 °C, under the identical experimental conditions used for assaying its HIV transfection inhibitory effect. The concentration of inhibitor at which the percent of viable cells was reduced by 50 % (compared to the percent of viable cells without incubation with the inhibitor, referred to as CC_{50} value) was calculated to be about 58 μ M, and the resulting CC_{50}/IC_{50} (therapeutic index, TI) value for Compound **20** was calculated to be about 345.

Compound **3**, for comparison, was less effective than Compound **20** in inhibiting HIV-1 pseudovirus infection of B lymphocytes (with an apparent IC_{50} value of about 374 nM), but was found to be less cytotoxic, with a calculated therapeutic index above 446 (see, FIG. 1D). Nevirapine, an NNRTI currently used for HIV-1 therapy, was used as a reference and inhibited HIV transfection of B lymphocytes with an IC_{50} of 14 ± 3 nM.

60

Table 4



Compound					% residual DDDP activity		Selectivity index	IC ₅₀ PV infection [μM]
id	R1	R2	R3	R4	HIV-1 RT	KF		
19	H	H	Br	CH ₃	24.8 ± 6.3	14.4 ± 3.4	0.6 ± 0.2	
20	OCH ₃	H	Cl	OCH ₃	8.4 ± 2.2	62.2 ± 14.4	7.4 ± 2.6	0.17 ± 0.02
21	H	H	Br	H	27.8 ± 3.2	48.4 ± 18.5	1.7 ± 0.7	
22	Cl	H	H	CH ₃	17.3 ± 0.8	76.5 ± 13.8	4.4 ± 0.8	0.67 ± 0.08
23	H	H	(CH ₂) ₃ CH ₃	H	31.4 ± 13.7	74.2 ± 3.2	2.4 ± 1.0	
24	H	H	Cl	CF ₃	2.2 ± 1.7	4.4 ± 2.8	0.5 ± 0.5	
25	CH ₃	Cl	H	H	28.9 ± 12.9	10.8 ± 5.2	0.4 ± 0.2	
26	CH ₃	H	(CH ₂) ₃ CH ₃	H	27.5 ± 7.7	4.5 ± 1.2	0.2 ± 0.1	
27	OCH ₂ CH ₃	H	H	H	16.2 ± 0.2	87.1 ± 10.1	5.4 ± 0.6	0.43 ± 0.07
28	OCH ₃	H	H	CH ₃	16.7 ± 0.2	71.7 ± 6.4	4.3 ± 0.4	0.89 ± 0.11
29	H	H	COOCH ₃	H	13.6 ± 3.8	2.6 ± 1.7	0.2 ± 0.1	
30	Br	H	F	H	39.4 ± 10.3	47.6 ± 11.0	1.2 ± 0.4	
31	H	H	C(CH ₃) ₃	H	34.3 ± 19.9	13.8 ± 1.8	0.4 ± 0.2	
32	H	H	H	OCH ₃	30.5 ± 3.5	98.7 ± 9.4	3.2 ± 0.5	
33	H	H	H	CN	69.0 ± 1.5	75.2 ± 27.3	1.1 ± 0.4	
34	Cl	H	H	H	49.2 ± 4.8	59.3 ± 19.3	1.2 ± 0.4	
35	CH ₃	H	CH ₃	CH ₃	21.9 ± 0.7	64.4 ± 3.3	2.9 ± 0.2	
36	H	H	H	CO ₂ CH ₂ CH ₃	47.1 ± 11.8	56.6 ± 14.4	1.2 ± 0.4	
37	Cl	H	Cl	H	53.4 ± 7.8	13.2 ± 6.4	0.2 ± 0.1	
38	H	H	Br	Cl	5.5 ± 0.4	1.0 ± 0.7	0.2 ± 0.1	
39	OCH ₃	H	H	Cl	15.7 ± 0.8	50.8 ± 12.3	3.2 ± 0.8	0.32 ± 0.11
40	H	H	N(CH ₃) ₂	H	55.0 ± 7.0	11.1 ± 1.4	0.2 ± 0.0	
41	F	H	H	F	82.3 ± 12.3	47.5 ± 13.4	0.6 ± 0.2	

The effects of compounds 3 and 20 on the activity of wild-Type HIV-1 RT, drug resistant mutants of HIV-1 RT and Wild-Type HIV-2 RT :

The IC₅₀ of the observed *in vitro* RT inhibition by Compounds 3 and 20, was also evaluated.

5 As shown in FIG. 2, Compound 3 inhibited the RNA-dependent and DNA-dependent DNA polymerase activities of HIV-1 RT with apparent IC₅₀ values of about 2.8 µM and 3.3 µM, respectively. Compound 20 inhibited these two DNA polymerase activities with IC₅₀ values of about 0.51 µM and 0.94 µM, respectively. For reference, the inhibition activity of Nevirapine was also tested. Nevirapine exhibited IC₅₀ values
10 of approximately 1.7 µM and 0.63 µM, for the RNA-dependent and DNA-dependent DNA polymerase activities, respectively. As expected, neither compounds (including nevirapine) inhibited significantly the RNase H activity of RT, as similar to most NNRTIs.

Y181C mutant of HIV-1 RT, is known to be highly resistant to nevirapine.
15 However, it was found that the Y181C mutant of HIV-1 RT was inhibited effectively by compounds 3 and 20 (with apparent IC₅₀ values of 20 µM and 70 µM, respectively), whereby for nevirapine an apparent IC₅₀ of 100 µM was observed (see, FIG. 3A).

As shown in FIG. 3B, Compounds 3 and 20 also inhibit the activity of HIV-2 RT at relatively high concentrations (e.g., 140 µM). This feature is unique to these
20 compounds and is not shared by most classic NNRTIs, such as nevirapine.

EXAMPLE 3

Kinetics of RT inhibition by Compound 20

Reversibility of HIV-1 RT Inhibition by Compound 20:

25 The mode by which Compound 20 inhibited RT was further evaluated. To test the reversibility of the binding between HIV-1 RT and Compound 20, the RNA-dependent DNA polymerase activity of RT was assayed at increasing concentrations of the enzyme in the absence or presence of 616 nM inhibitor. As shown in FIG. 4, Plotting HIV-1 RT concentrations against their apparent V_{max} values for the reactions
30 with and without Compound 20 showed intersecting rather than parallel lines. In other words, the RT activity in the presence of Compound 20 was proportionally increased as a function of RT concentration with 59 ± 6 % residual activity at all tested RT

concentrations. This result excludes the possibility that a fraction of the RT was irreversibly eliminated by the inhibitor and hence suggests a reversible binding between RT and Compound 20.

Mode of Inhibition of the RDDP Activity of HIV-1 RT by Compound 20:

5 To gain a better insight into the mechanism by which HIV-1 RT is inhibited by Compound 20, steady state kinetic studies were performed by assaying the RNA-dependent DNA polymerase activity of wild-type HIV-1 RT in the presence of increasing concentrations of each substrate that was used (either dTTP or rA·dT) and a specific concentration of Compound 20. These assays were repeated with a range of
10 Compound 20 concentrations, and the results were analyzed with double-reciprocal (Lineweaver-Burk) plots. The results are presented in FIGs. 5A-D and demonstrate that RT inhibition with respect to both substrates showed a classical noncompetitive behavior with no significant change in the apparent K_m values in the presence of Compound 20.

15 In the kinetic study with respect to the dTTP substrate (see, FIGs. 5A and 5B), the control calculated value for the reaction without inhibitor was 4.3 μM dTTP, whereas the calculated K_m values in the presence of 0.25, 0.5, 1, and 2 μM Compound 20 were 4.5, 4.8, 4.3, and 4.2 μM , respectively (average of $4.5 \pm 0.25 \mu\text{M}$). In accordance with this mode of inhibition, the k_{cat} values ($V_{max}/[RT]$) were decreased by
20 Compound 20 from approximately 0.38 s^{-1} in the absence of inhibitor to 0.28, 0.25, 0.20, and 0.13 s^{-1} in the presence of 0.25, 0.5, 1, and 2 μM Compound 20, respectively.

In the second kinetic study with respect to the rA·dT substrate, a similar pattern was observed, with a K_m value of 0.100 $\mu\text{g/ml}$ for the control reaction and K_m values of $0.096 \pm 0.001 \mu\text{g/ml}$ for 0.25, 0.5, 1 and 2 μM Compound 20 (see, FIGs. 5C and 5D).
25 In this case, the k_{cat} values could not be calculated in the standard units of inverse seconds, due to the heterogeneous length of the commercial substrate [poly(rA)_n·oligo(dT)₁₂₋₁₈].

Further analysis of both kinetic studies via replotting the $1/V_{max}$ values against the inhibitor concentrations (Dixon plot) showed a linear trend with a high correlation
30 coefficient (r^2) of 0.99. The K_i values calculated from these plots yielded a value of 1.1 μM with respect to the dTTP substrate and 1.8 μM with respect to the rA·dT substrate (see, FIGs. 5B and 5D). On the basis of these data, it is likely that neither the dTTP

substrate nor the rA·dT substrate competed with the inhibitor for binding to the enzyme and each of the molecules could bind RT independently.

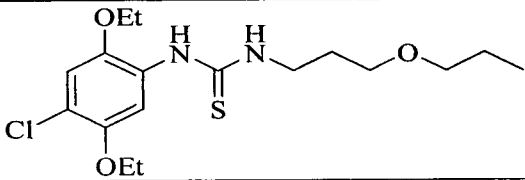
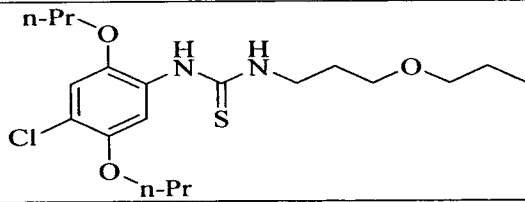
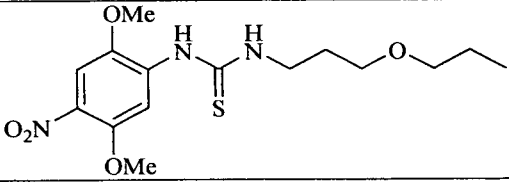
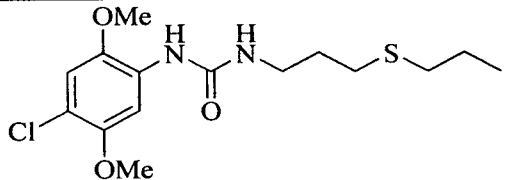
EXAMPLE 4

RT Inhibition by derivatives of Compound 20

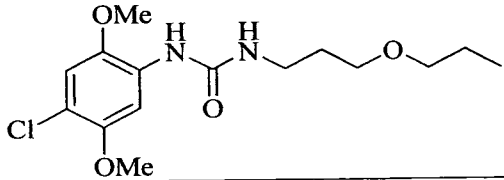
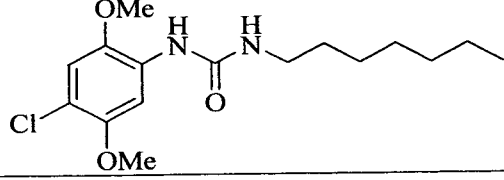
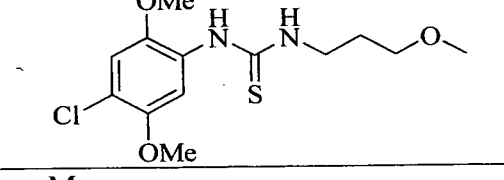
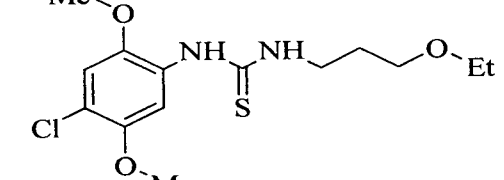
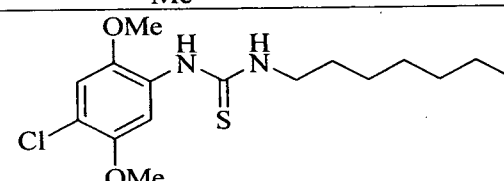
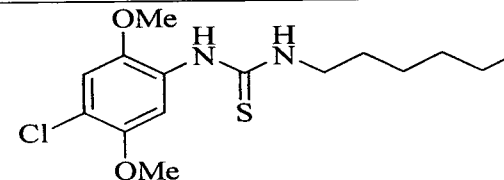
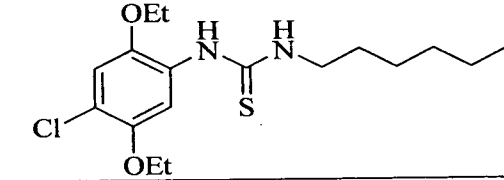
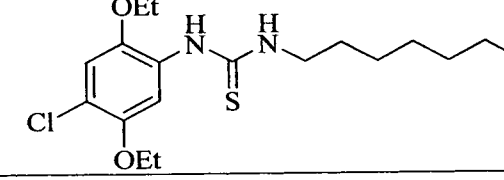
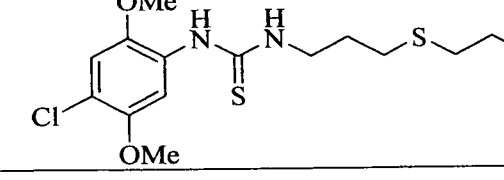
In view of the highly potent parameters of the activity of Compound 20, derivatives of Compound 20 were designed, synthesized and tested for their RT inhibition activity.

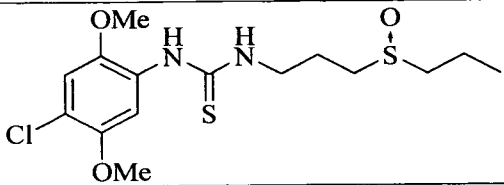
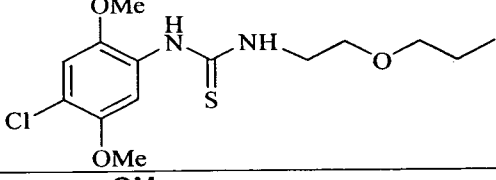
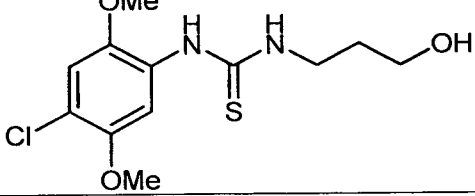
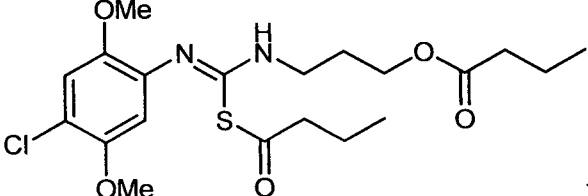
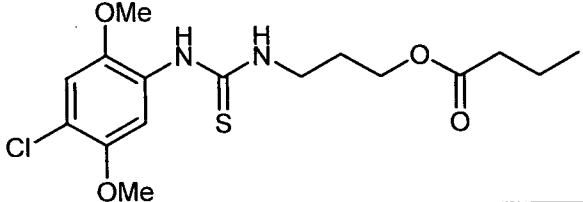
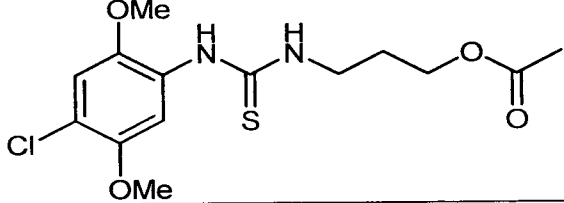
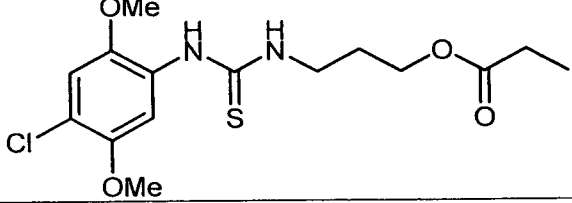
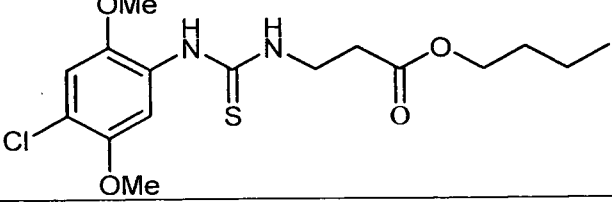
Table 5 presents the chemical structures of all the compounds synthesized to this effect, and the corresponding RT inhibition thereof. The extent of RT inhibition was determined by the extent of reduction in residual HIV-1 PV infection in the presence of the inhibitor, at the indicated concentration, according to the procedure described in Example 2 hereinabove.

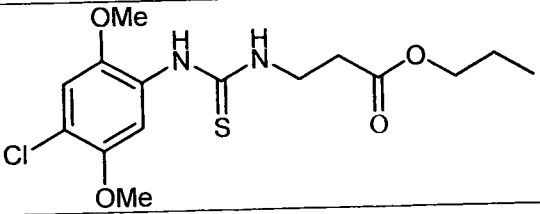
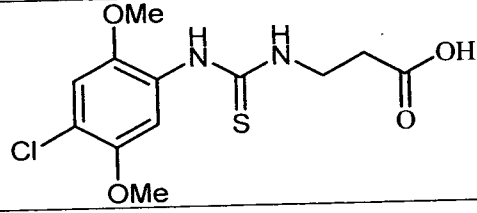
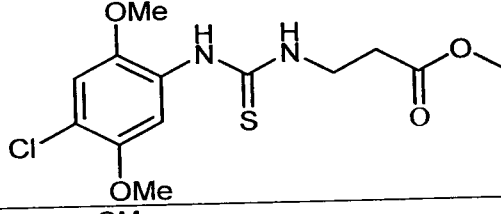
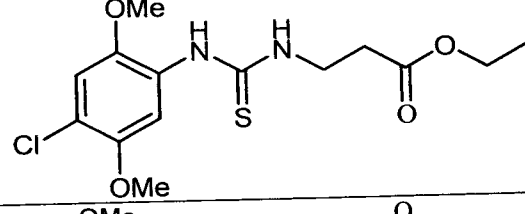
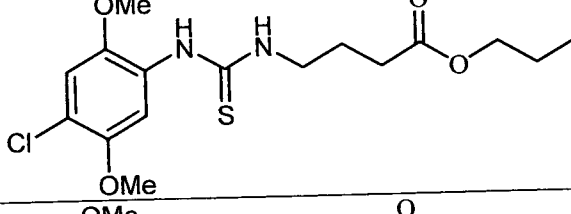
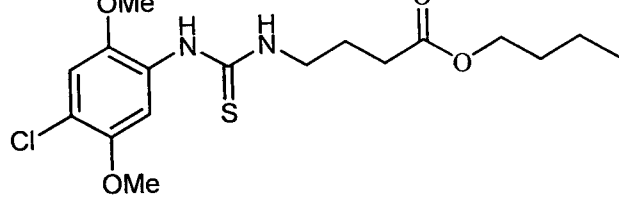
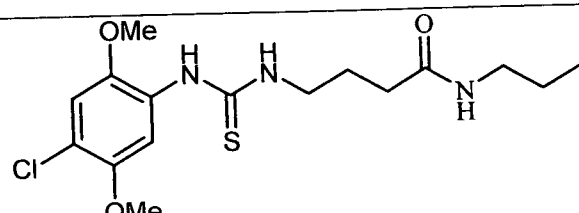
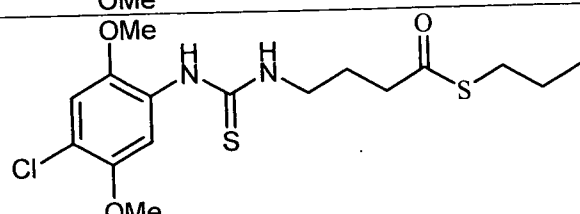
Table 5

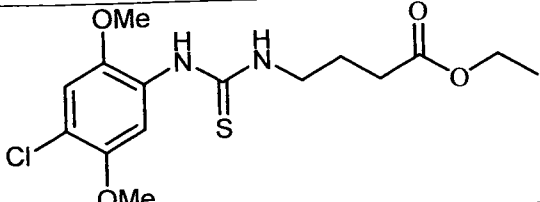
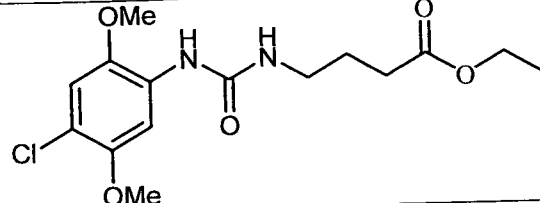
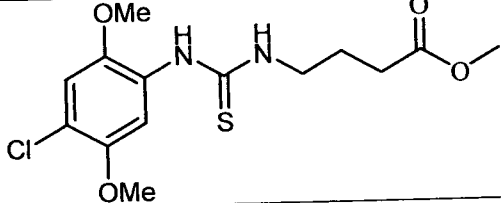
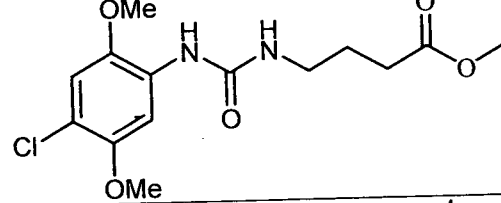
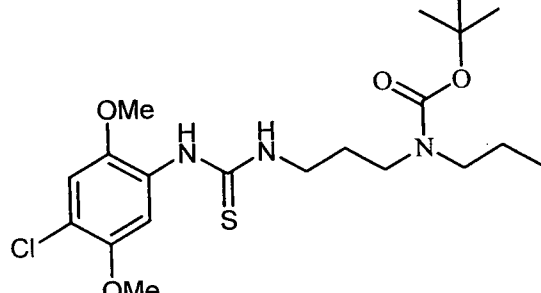
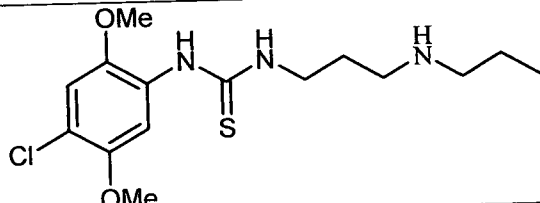
Comp No.	Structure	Inhibitor final concentration [μM]	Residual HIV-1 PV infection (%)
42		1.0	86.17
43		1.0	86.40
44		5.0	36.19
45		2.5	82.42

64

46		3.0	37.25
47		3.0	82.52
48		3.0	92.63
49		5.0	56.29
50		3	69.21
51		3.0	100.82
52		1.0	103.44
53		1.0	81.69
54		2.5	26.08

65			
55		5.0	91.52
56		5.0	75.77
57		2.5	80.26
58		2.5	70.04
59		2.5	49.85
60		2.5	73.65
61		5	73.97
62		5	39.82

66			
63	 <chem>CCOC(=O)CCNC(=S)Nc1cc(OC)c(Cl)cc1OC</chem>	5	46.63
64	 <chem>OC(=O)CCNC(=S)Nc1cc(OC)c(Cl)cc1OC</chem>	5	95.14
65	 <chem>COC(=O)CCNC(=S)Nc1cc(OC)c(Cl)cc1OC</chem>	5	88
66	 <chem>CCOC(=O)CCNC(=S)Nc1cc(OC)c(Cl)cc1OC</chem>	5	76.49
67	 <chem>CCCC(=O)OCCNC(=S)Nc1cc(OC)c(Cl)cc1OC</chem>	5	16.28
68	 <chem>CCCCC(=O)OCCNC(=S)Nc1cc(OC)c(Cl)cc1OC</chem>	5	19.73
69	 <chem>CCCC(=O)NCCNC(=S)Nc1cc(OC)c(Cl)cc1OC</chem>	5	54.34
70	 <chem>CCCC(=O)SCCNC(=S)Nc1cc(OC)c(Cl)cc1OC</chem>	5	7.01

67			
71			
72			
73			
74			
75		5	88.62
76		5	67.13

The following describes the preparation of the compounds presented in Table 5 above.

Syntheses of compounds 41-76 – general procedure:

¹H and ¹³C NMR spectra 200, 300 and 600 MHz were obtained on Bruker AC-200 and AM-300 spectrometers, respectively. Chemical shifts are expressed in ppm downfield from Me₄Si used as internal standard. The values are given in δ scale.

Mass spectra were obtained on a Varian Mat 731 spectrometer.

HRMS were obtained on a AutoSpec Premier (Waters UK) spectrometer in CI, CH₄.

LRMS were obtained on a QToF micro (Waters UK) spectrometer in ESI.

To a solution of a substituted phenylisothiocyanate or a substituted phenylisocyanate, the appropriate amine is added and the mixture is stirred at room temperature for the indicated time. In cases where the amine is present as a hydrochloride salt thereof, an equimolar amount of triethylamine is added. The solvent is thereafter evaporated, and the obtained residue is purified by flash chromatography, so as to obtain the desired compound.

The following describes the synthesis particulars of each of the compounds:

1-(4-Chloro-2,5-diethoxyphenyl)-3-(3-propoxypropyl)thiourea (Compound 42): Compound 42 was obtained as a colorless oil in 81 % yield by reacting 1-chloro-2,5-diethoxy-4-isothiocyanatobenzene and 3-propoxypropan-1-amine hydrochloride (reaction solvent EtOH; chromatography eluent hexane:CH₂Cl₂ 2.5:1). Herein "d", "t", etc. indicate multiplets with second order characteristics.

¹H NMR (300 M Hz, acetone-*d*₆): δ = 0.84 (t, *J* = 7. Hz, 3H, H-12), 1.35 (t, *J* = 7. Hz, 3H, OCH₂CH₃), 1.39 (t, *J* = 7.2 Hz, 3H), 1.46 (sex., *J* = 7 Hz, 2H, H-11), 1.86 (quint, *J* = 6.3 Hz, 2H, H-8), 3.30 (t, *J* = 6.6 Hz, 2H, H-9), 3.48 (t, *J* = 6.0 Hz, 2H, H-10), 3.67 (q, *J* = 6.6 Hz, 2H, H-7), 4.07 (q, *J* = 7.0 Hz, 2H, ArOCH₂), 4.08 (q, *J* = 7.0 Hz, 2H, ArOCH₂), 7.04 (s, 1H, H-3), 7.50 (bt, 1H, NH), 7.85 (bs, 1H, H-6), 8.25 (bs, 1H, NH).

¹³C NMR (200 M Hz, acetone-*d*₆): δ = 10.8 (C-12), 15.0 (OCH₂CH₃), 23.4, 28.6, 29.6 (CH₂, CH₂, CH₂), 43.5 (C-7, broad), 65.7 (OCH₂, OCH₂), 69.5 (OCH₂), 73.0 (OCH₂), 111.9 (C-6), 115.4 (C-3), 118.6 (C-4) 128.5 (C-1), 145.9 (C-2), 148.8 (C-5), 181.7 (CS).

MS (CI+): *m/z* (%) = 357.153 (MH⁺, 100), 329.109 (MH⁺-EtOH, 29.8).

HRMS: calcd. for $C_{17}H_{28}N_2O_3^{35}SCl$ 357.1509; found 357.1528, calcd. for $C_{17}H_{28}N_2O_3^{37}SCl$ 377.1480; found 377.1497.

1-(4-Chloro-2,5-dipropoxyphenyl)-3-(3-propoxypropyl)thiourea (Compound 43): Compound 43 was obtained as a colorless oil in 81 % yield by reacting 1-chloro-4-isothiocyanato-2,5-dipropoxybenzene and 3-propoxypropan-1-amine hydrochloride (reaction solvent EtOH, chromatography eluent CH_2Cl_2 :hexane 1:1 until all isothiocyanate was washed, then pure CH_2Cl_2). Herein "d", "t", etc. indicate multiplets with second order characteristics.

1H NMR (300 M Hz, acetone- d_6): δ = 0.67 (t, J = 7.35 Hz, 3H, H-12), 0.97 (t, J = 7.5 Hz, 3H, $ArO(CH_2)_2CH_3$), 1.00 (t, J = 7.5 Hz, 3H, $ArO(CH_2)_2CH_3$), 1.20 (sex., J = 6.3 Hz, 2H, H-11), 1.76 (m, 6H, $ArOCH_2CH_2$, $ArOCH_2CH_2$, H-8), 3.15 (t, J = 6.6 Hz, 2H, H-10), 3.68 (t, J = 6.6 Hz, 2H, H-10), 3.45 (t, J = 5.4 Hz, 2H, H-9), 3.67 (bq, J = 5.4 Hz, 2H, H-7), 3.84 (t, J = 6.6 Hz, 2H, $ArOCH_2$), 3.86 (t, J = 6.6 Hz, 2H, $ArOCH_2$), 6.90 (s, 1H, H-3), 7.11 (bt, 1H, NH), 7.55 (bs, 1H, H-6).

^{13}C NMR (300 M Hz, $CDCl_3$): δ = 10.3 (C-12), 10.5 ($ArO(CH_2)_2CH_3$, $ArO(CH_2)_2CH_3$), 22.3 (C-11), 22.5 ($ArOCH_2CH_2$, $ArOCH_2CH_2$), 28.4 (C-8), 45.4 (C-7, broad), 70.3 (CH_3 , broad), 71.2 (CH_2), 73.0 (C-9), 110.8 (C-6), 115 (C-3), 120.2 (C-4), 125.0 (C-1), 145.6 (C-2), 148.6 (C-5), 180.0 (CS).

MS (CI⁺): m/z (%) = 403.18 (MH^+ , 42.5).

HRMS: calcd. for $C_{19}H_{32}N_2O_3SCl$ 403.1822; found 403.1801.

1-(2,5-Dimethoxy-4-nitrophenyl)-3-(3-propoxypropyl)thiourea (Compound 44): Compound 44 was obtained as a yellow solid in 69 % yield by reacting 1-isothiocyanato-2,5-dimethoxy-4-nitrobenzene and 3-propoxypropan-1-amine hydrochloride (reaction solvent EtOH, chromatography eluent CH_2Cl_2). Herein "d", "t", etc. indicate multiplets with second order characteristics.

m.p. = 116-119 °C.

1H NMR (300 M Hz, acetone- d_6 + CD_3OD): δ = 0.85 (t, J = 7.5 Hz, 3H, H-12), 1.51 (sex., J = 7.5 Hz, 2H, H-11), 1.84 (quint, J = 6.6 Hz, 2H, H-8), 3.33 (t, J = 6.6 Hz, 2H), 3.47 (t, J = 6.3 Hz, 2H), 3.61 (t., J = 6.9 Hz, 2H), 3.85 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 7.50 (s, 1H, H-6), 8.93 (s, 1H, H-3).

^{13}C NMR (300 M Hz, acetone- d_6): δ = 10.8 (C-12), 23.7 (CH_2), 28.6 (CH_2), 42.7 (C-7), 57.0 (OCH_3), 57.4 (OCH_3), 69.3 (C-10), 73.5 (C-9), 107.2 (bs, CH

aromatic), 108.5 (CH aromatic) 133.4, 136.5 (C-1, C-4), 143.4 (C-2), 149.5 (C-5), 181.34 (CS). MS (CI+) m/z (%) = 358.146 (MH^+ , 10).

HRMS: calcd. for $C_{15}H_{24}N_3O_5S$ 358.1437; found 35.1457.

1-(4-Chloro-2,5-dimethoxyphenyl)-3-(3-(propylthio)propyl)urea (Compound

5 **45)**: Compound **45** was obtained as a colorless oil in quantitative yield by reacting 1-chloro-4-isocyanato-2,5-dimethoxybenzene and 3-(propylthio)propan-1-amine (reaction solvent $CHCl_3$). Herein "d", "t", etc. indicate multiplets with second order characteristics.

1H NMR (300 M Hz, $CDCl_3$): δ = 0.94 (t, J = 7.5 Hz, 3H, H-12), 1.58 (sex, J = 7.2 Hz, 2H, H-11), 1.80 (quint, J = 6.9 Hz, 2H, H-8), 2.44, 2.50 (bt, 2H, bt, 2H, H-10, H-9), 3.36 (t, J = 6.6 Hz, 2H, H-7), 3.75 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 6.83 (s, 1H, H-3), 7.20 (bs, 1H, NH), 7.97 (s, 1H, H-6).

^{13}C NMR (300 M Hz, $CDCl_3$): δ = 13.5 (C-12), 22.9 (CH_2), 29.4 (CH_2), 29.7 (CH_2), 34.2 (CH_2), 39.4 (C-7), 56.4 (OCH_3), 56.6 (OCH_3), 104.4 (C-6), 112.4 (C-3) 15 114.0 (C-4), 128.2 (C-1), 141.8 (C-2), 149.2 (C-5), 155.59 (CO).

MS (CI+): m/z (%) = 347.123 (MH^+ , 27.9).

HRMS: calcd. for $C_{15}H_{23}N_2O_3S^{35}Cl$ 347.1196; found 347.1227, calcd. for $C_{15}H_{23}N_2O_3S^{37}Cl$ 348.1088; found 348.1137.

1-(4-Chloro-2,5-dimethoxyphenyl)-3-(3-propoxypropyl)urea (Compound 46):

20 Compound **46** was obtained as a white solid oil in 73 % yield by reacting 1-chloro-4-isocyanato-2,5-dimethoxybenzene 3-propoxypropan-1-amine hydrochloride (reaction solvent $CHCl_3$, chromatography eluent CH_2Cl_2). Herein "d", "t", etc. indicate multiplets with second order characteristics.

m.p. = 90-92 °C

25 1H NMR (200 M Hz, acetone- d_6): δ = 0.87 (t, J = 7 Hz, 3H, H-12) 1.50 (sex., J = 6.4 Hz, 2H, H-11), 1.72 (quint, J = 7.4 Hz, 2H, H-8), 3.29 (q, J = 5.6 Hz, 2H, H-7), 3.32 (t., J = 5.6 Hz, 2H, H-10), 3.45 (t., J = 6.1 Hz, 2H, H-9), 3.79 (s, 6H, H-10), 3.43 (t, J = 6.6 Hz, 2H, H-9), 3.66 (bq, J = 6.6 Hz, 2H, H-7), 3.83 (s, 3H, OCH_3 , OCH_3), 6.52 (bt, 1H, NH), 6.92 (s, 1H, H-3), 7.74 (bs, 1H, NH), 8.24 (s, 1H, H-6).

30 ^{13}C NMR (300 M Hz, acetone- d_6): δ = 10.9 (C-12), 23.6 (CH_2), 31.1 (CH_2), 37.7 (C-7), 56.7 (OCH_3), 56.9 (OCH_3), 68.7 (C-10), 73.0 (C-9), 109.6 (C-6), 113.1 (C-3, C-4) 130.6 (C-1), 142.5 (C-2), 149.9 (C-5), 156.1 (CO).

MS (CI⁺): m/z (%) = 331.14 (MH⁺, 94.1), 330.14 (M⁺, 83.0).

HRMS: calcd. for C₁₅H₂₃N₂O₄Cl 330.1346; found 330.1361.

1-(4-Chloro-2,5-dimethoxyphenyl)-3-heptylurea (Compound 47): Compound 47 was obtained as a white solid in 62 % yield by reacting 1-chloro-4-isocyanato-2,5-dimethoxybenzene and heptylamine (reaction solvent CHCl₃, chromatography eluent CH₂Cl₂).

m.p. = 87-89 °C.

¹H NMR (200 M Hz, acetone-*d*₆): δ = 0.88 (m, 3H, H-13), 1.31 (m, 8H, H-9, H-10, H-11, H-12), 1.52 (m, 2H, H-8), 3.23 (q, J = 6.7 Hz, 2H, H-7), 3.808 (s, 3H, OCH₃), 3.816 (s, 3H, OCH₃), 6.46 (bt, J = 6.7 Hz, 1H, NH), 6.94 (s, 1H, H-3), 7.70 (bs, 1H, NH), 8.27 (s, 1H, H-6).

¹³C NMR (200 M Hz, acetone-*d*₆): δ = 14.3 (C-13), 23.3 (CH₂), 27.6 (CH₂), 29.796 (CH₂), 27.969 (CH₂), 32.6 (CH₂), 40.4 (C-7), 56.7 (OCH₃), 56.9 (OCH₃), 104.5 (C-6), 113.02 (C-4), 113.11 (C-3), 130.7 (C-1), 142.5 (C-2), 150.0 (C-5), 155.9 (CO).

MS (CI⁺): m/z (%) = 329.16 (MH⁺, 49.3), 328.16 (M⁺, 39.7).

HRMS: calcd. for C₁₆H₂₅N₂O₃Cl 328.1554; found 328.1567.

1-(4-Chloro-2,5-dimethoxyphenyl)-3-(3-methoxypropyl)thiourea (Compound 48): Compound 48 was obtained as a colorless oil by reacting 1-chloro-4-isothiocyanto-2,5-dimethoxybenzene and 3-methoxypropan-1-amine (reaction solvent CHCl₃).

¹H NMR (300 M Hz, acetone-*d*₆): δ = 1.85 (quint, J = 6.6 Hz, 2H, H-8), 3.21 (s, 3H, H-10), 3.43 (t, J = 6.6 Hz, 2H, H-9), 3.66 (bq, J = 6.6 Hz, 2H, H-7), 3.83 (s, 3H, OCH₃), 3.84 (s, 3H, OCH₃), 7.06 (s, 1H, H-3), 7.51 (bt, J = 6.6 Hz, 1H, NH), 7.89 (bs, 1H, H-6), 8.34 (bs, 1H, NH).

¹³C NMR (300 M Hz, acetone-*d*₆): δ = 29.5 (C-8), 43.4 (C-7), 56.7 (OCH₃), 58.5 (C-10), 71.5 (C-9), 110.86 (C-6), 114. (C-3), 118.2 (C-4), 127.8 (C-1), 146.8 (C-2), 149.4 (C-5), 181.7 (CS).

MS (CI⁺): m/z (%) = 319.08 (MH⁺, 13.6).

HRMS: calcd. for C₁₃H₂₀N₂O₃S 319.0883; found 319.0837.

1-(4-Chloro-2,5-dimethoxyphenyl)-3-(3-ethoxypropyl)thiourea (Compound 49): Compound 49 was obtained as a colorless oil by reacting 1-chloro-4-isothiocyanto-2,5-dimethoxybenzene and 3-ethoxypropan-1-amine (reaction solvent

CHCl₃). The NMR data assignment was aided by several two-dimensional spectra including COSY, HMQC and HMBC analysis. Herein "d", "t", etc. indicate multiplets with second order characteristics.

¹H NMR (600 M Hz, acetone-*d*₆): δ = 1.04 (t, *J* = 7.0 Hz, 3H, H-11), 1.86 (quint, *J* = 6.0 Hz, 2H, H-8), 3.39 (q, *J* = 7.0 Hz, 2H, H-10), 3.48 (t, *J* = 6.0 Hz, 2H, H-9), 3.67 (t, 2H, H-7), 3.84 (s, 6H, C₂OCH₃), 3.85 (s, 3H, C₅OCH₃), 7.09 (s, 1H, H-3), 7.65 (bt, 1H, NH), 7.91 (bs, 1H, H-6), 8.47 (bs, 1H, NH).

¹³C NMR (600 M Hz, acetone-*d*₆): δ = 15.3 (C-11), 29.8 (C-8), 43.2 (C-7), 56.6 (OCH₃, OCH₃), 66.4 (C-10), 69.2 (C-9), 110.1 (C-6), 113.9 (C-3), 117.7 (C-4), 127.3 (C-1), 146.3 (C-2), 149.1 (C-5), 181.1 (CS).

MS (CI⁺): *m/z* (%) = 333.108 (MH⁺, 87.8), 301.077 (MH⁺-MeOH, 100).

HRMS: calcd. for C₁₄H₂₂N₂O₃SCl 333.1040; found 333.1081.

1-(4-Chloro-2,5-dimethoxyphenyl)-3-heptylthiourea (Compound 50):

Compound **50** was obtained as a white solid in quantitative yield by reacting 1-chloro-4-isothiocyanato-2,5-dimethoxybenzene and heptylamine (reaction solvent CHCl₃, chromatography eluent CH₂Cl₂).

m.p. = 70-72 °C.

¹H NMR (200 M Hz, acetone-*d*₆): δ = 0.84 (m, 3H, H-13), 0.84 (m, 8H, H-9, H-10, H-11, H-12), 1.61 (bt, *J* = 6.8, 2H, H-8), 3.57 (q, *J* = 6.8 Hz, 2H, H-7), 3.81 (s, 6H, OCH₃, OCH₃), 7.02 (s, 1H, H-3), 7.74 (bt, *J* = 6.8 Hz, 1H, NH), 8.02 (s, 1H, H-6), 8.47 (bs, 1H, NH).

¹³C NMR (200 M Hz, acetone-*d*₆): δ = 13.1 (C-13), 21.9 (CH₂), 26.3 (CH₂), 28.3 (CH₂), 28.5 (CH₂), 31.2 (CH₂), 43.9 (C-7), 55.63 (OCH₃), 109.0 (C-6), 112.7 (C-3), 117. (C-4), 127.1 (C-1), 144.9 (C-2), 148.0 (C-5), 180.3 (CS).

MS (CI⁺): *m/z* (%) = 345.193 (MH⁺, 100).

HRMS: calcd. for C₁₆H₂₆N₂O₃S³⁵Cl 345.1404; found 345.1387, calcd. for C₁₆H₂₆N₂O₃S³⁷Cl 347.1359; found 347.1369.

1-(4-Chloro-2,5-dimethoxyphenyl)-3-hexylthiourea (Compound 51):

Compound **51** was obtained as a white solid in 26 % yield by reacting 1-chloro-4-isothiocyanato-2,5-dimethoxybenzene and hexylamine (reaction solvent CHCl₃, chromatography eluent Hex:CH₂Cl₂).

m.p. = 84-86 °C.

^{13}C NMR (200 M Hz, acetone- d_6): δ = 14.2 (C-12), 23.2 (CH_2), 27.3 (CH_2), 29.5 (CH_2), 32.3 (CH_2), 45.2 (C-7), 56.9 (OCH_3 , OCH_3), 110.4 (C-6), 114.1 (C-3) 117.6 (C-4), 128.3 (C-1), 146.3 (C-2), 149.4 (C-5), 181.7 (CS).

1-(4-Chloro-2,5-diethoxyphenyl)-3-hexylthiourea (Compound 52): Compound 52 was obtained as a white solid in 51 % yield by reacting 1-chloro-2,5-diethoxy-4-isothiocyanatobenzene and hexylamine (reaction solvent CHCl_3 , chromatography eluent Hex: CH_2Cl_2 1:1).

m.p. = 82-84 °C.

^1H NMR (300 M Hz, acetone- d_6): δ = 0.88 (m, 3H, H-12), 1.31 (m, 6H, H-9, H-10, H-11), 1.41 (t, J = 7 Hz, OCH_2CH_3), 1.44 (t, J = 7 Hz, OCH_2CH_3), 1.60 (bsex, 2H, H-11), 3.51 (bt, 2H, H-7), 4.02 (q, 2H, J = 7 Hz, OCH_2), 4.05 (q, 2H, J = 7 Hz, OCH_2), 6.24 (bs, 1H, NH), 6.97 (s, 1H, H-3), 7.66 (bs, 1H, H-6).

^{13}C NMR (300 M Hz, acetone- d_6): δ = 14.0 (C-12), 14.8 (OCH_2CH_3), 22.5 (CH_2), 26.7 (CH_2), 28.1 (CH_2), 31.4 (CH_2), 45.4 (C-7), 65.4 (OCH_2), 65.7 (OCH_2), 110.7 (C-6), 115.5 (C-3), 120.3 (C-4), 125.2 (C-1), 145.3 (C-2), 148.5 (C-5), 180.1 (CS).

MS (CI+): m/z (%) = 159.15 (MH^+ , 2.1).

HRMS: calcd. for $\text{C}_{17}\text{H}_{28}\text{N}_2\text{O}_2\text{ClS}$ 359.1560; found 359.1530.

1-(4-Chloro-2,5-diethoxyphenyl)-3-heptylthiourea (Compound 53): Compound 53 was obtained as a white solid in 55 % yield by reacting 1-chloro-2,5-diethoxy-4-isothiocyanatobenzene and heptylamine (reaction solvent EtOH, chromatography eluent CH_2Cl_2 :Hex 1.5:1).

m.p. = 76-78 °C.

^1H NMR (300 M Hz, acetone- d_6): δ = 0.89 (m, 3H, H-12), 1.30 (m, 8H, H-8, H-9, H-10, H-11), 1.40 (t, J = 7 Hz, OCH_2CH_3), 1.44 (t, J = 7 Hz, OCH_2CH_3), 1.60 (bsex, 2H, H-12), 3.57 (bt, 2H, H-7), 4.02 (q, 2H, J = 7 Hz, OCH_2), 4.05 (q, 2H, J = 7 Hz, OCH_2), 6.28 (bs, 1H, NH), 6.96 (s, 1H, H-3), 7.71 (bs, 1H, H-6).

^{13}C NMR (300 M Hz, acetone- d_6): δ = 14.0 (C-12), 14.75 (OCH_2CH_3), 14.78 (OCH_2CH_3), 22.5 (CH_2), 26.9 (CH_2), 28.9 (CH_2), 29.6 (CH_2), 31.7 (CH_2), 45.3 (C-7), 65.4 (OCH_2), 65.6 (OCH_2), 110.7 (C-6), 115.5 (C-3), At the concentration of the sample, the quaternary (C-4) was not observed 125.3 (C-1), 145.3 (C-2), 148.5 (C-5), 180.1 (CS).

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MS (CI⁺): m/z (%) = 373.17(MH⁺, 100).

HRMS: calcd. for C₁₈H₃₀N₂O₂SCl 373.1717; found 373.1695.

1-(4-Chloro-2,5-dimethoxyphenyl)-3-(3-(propylthio)propyl)thiourea

(Compound 54): Compound 54 was obtained as a colorless oil in quantitative yield by reacting 1-chloro-4-isothiocyanato-2,5-dimethoxybenzene and 3-(propylthio)propan-1-amine (reaction solvent CHCl₃). Herein "d", "t", etc. indicate multiplets with second order characteristics.

¹H NMR (200 M Hz, acetone-*d*₆): δ = 1.06 (t, *J* = 7.4 Hz, 3H, H-12), 1.66 (sex, *J* = 7.2 Hz, 2H, H-11), 2.01 (quint, *J* = 7.2 Hz, 2H, H-8), 2.59 (t, *J* = 7.4 Hz, 2H, H-10), 2.67 (t, *J* = 7.0 Hz, 2H, H-9), 3.80 (q, *J* = 5.6 Hz, 2H, H-7), 3.93 (s, 3H, OCH₃), 3.94 (s, 3H, OCH₃), 7.41 (s, 1H, H-3), 7.72 (bt, 1H, NH), 8.09 (s, 1H, H-6), 8.53 (bs, 1H, NH).

¹³C NMR (200 M Hz, acetone-*d*₆): δ = 13.6 (C-12), 23.5 (CH₂), 29.6 (CH₂), 29.7 (CH₂), 30.6 (CH₂), 40.3 (C-7), 56.9 (OCH₃, OCH₃), 110.7 (C-6), 114.2 (C-3), 118.1 (C-4), 128.0 (C-1), 146.6 (C-2), 149.4 (C-5), 181.9 (CS).

MS (CI⁺): m/z (%) = 363.10 (MH⁺, 11.6).

HRMS: calcd. for C₁₅H₂₄N₂O₂S₂Cl 363.0968; found 363.0992.

1-(4-Chloro-2,5-dimethoxyphenyl)-3-(3-(propylsulfinyl)propyl)thiourea

(Compound 55): Compound 55 was obtained by reacting 1-chloro-4-isothiocyanato-2,5-dimethoxybenzene and 3-(propylsulfinyl)propan-1-amine (reaction solvent CHCl₃), the product could not be purified by flash chromatography. Herein "d", "t", etc. indicate multiplets with second order characteristics.

¹H NMR (300 M Hz, CDCl₃): δ = 0.94 (t, *J* = 7.5 Hz, 3H, H-12), 1.58 (sex, *J* = 7.2 Hz, 2H, H-11), 1.80 (quint, *J* = 6.9 Hz, 2H, H-8), 2.44, 2.50 (bt, 2H, bt, 2H, H-10, H-9), 3.36 (t, *J* = 6.6 Hz, 2H, H-7), 3.75 (s, 3H, OCH₃), 3.84 (s, 3H, OCH₃), 6.83 (s, 1H, H-3), 7.20 (bs, 1H, NH), 7.97 (s, 1H, H-6).

¹³C NMR (300 M Hz, CDCl₃): δ = 13.5 (C-12), 22.9 (CH₂), 29.4 (CH₂), 29.7 (CH₂), 34.2 (CH₂), 39.4 (C-7), 56.4 (OCH₃), 56.6 (OCH₃), 104.4 (C-6), 112.4 (C-3), 114.0 (C-4), 128.2 (C-1), 141.8 (C-2), 149.2 (C-5), 155.59 (CO).

1-(4-Chloro-2,5-dimethoxyphenyl)-3-(2-propoxyethyl)thiourea (Compound

56): Compound 56 was obtained as a white solid in quantitative yield by reacting 1-chloro-4-isothiocyanato-2,5-dimethoxybenzene and 2-propoxyethanamine (reaction

solvent CHCl_3 , recrystallized from hexane- CH_2Cl_2). Herein "d", "t", etc. indicate multiplets with second order characteristics.

m.p. = 95-97 °C

^1H NMR (200 M Hz, acetone- d_6): δ = 0.80 (t, J = 7.35 Hz, 3H, CH_2CH_3), 1.53 (sex., J = 7 Hz, 2H, CH_2CH_3), 3.39 (t, J = 5.8 Hz, 2H, $\text{CH}_2\text{CH}_2\text{CH}_3$), 3.59 (t, J = 5.2 Hz, 2H, NCH_2CH_2), 3.77 (td, J = 5 Hz, 1.6Hz 2H, NCH_2), 3.83 (s, 6H, OCH_3), 7.06 (s, 1H, H-3), 7.55 (bs, 1H, NH), 8.095, 8.126 (s, 1H, H-6, s, 1H, H-6), 8.52 (bs, 1H, NH).

^{13}C NMR (200 M Hz, acetone- d_6): δ = 10.8 (CH_2CH_3), 23.5 (CH_2CH_3), 45.11, 45.24 (NCH_2), 57.0 (OCH_3), 69.4 (OCH_2), 73.1 (OCH_2), 110.3, 110.45 (C-6), 114.1 (C-3), 117.7 (C-4) 128.3 (C-1), 146.3.9 (C-2), 149.4 (C-5), 181.991, 182.100 (CS). MS (CI⁺): m/z (%) = 333.103 (MH^+ , 98.33), 301.073 (MH^+ -MeOH, 98.40). HRMS: calcd. for $\text{C}_{14}\text{H}_{22}\text{N}_2\text{O}_3\text{S}^{35}\text{Cl}$ 333.1040; found 333.1032, calcd. for $\text{C}_{14}\text{H}_{22}\text{N}_2\text{O}_3\text{S}^{37}\text{Cl}$ 335.1010; found 335.1018.

1-(4-Chloro-2,5-dimethoxyphenyl)-3-(3-hydroxypropyl)thiourea (Compound 57): Compound 57 was obtained by reacting 1-chloro-2,5-dimethoxy-4-isothiocyanatobenzene and 3-amino-1-propanol. The residue was purified by silica gel chromatography eluted with $\text{CH}_2\text{Cl}_2/\text{EtOAc}$ (4:1) to obtain Compound 57 in 90 % yield.

m.p. = 100 °C.

^1H NMR (200 MHz, acetone- d_6): δ = 8.39 (bs, 1H, H-N9), 7.795 (bs, 1H, H-C5), 7.58 (bt, 1H, H-N11), 7.06 (s, 1H, H-C2), 3.84 (s, 3H, H-C8), 3.83 (s, 3H, H-C7), 3.72 (q, 2H, J = 6.4 Hz, H-C12), 3.63 (t, 2H, J = 6 Hz, H-C14), 2.99 (bs, 1H, H-O15), 1.77 (quintet, 2H, J = 6.4 Hz, H-C13).

^{13}C NMR (300 MHz, acetone- d_6): δ = 181.71 (q, C10), 149.44 (q, C4), 146.91 (q, C1), 127.5 (q, C6), 118 (q, C3), 114.3 (CH, C2), 110.94 (CH, C5), 60.05 (CH_2 , C14), 56.92 (CH_3 , C8), 56.88 (CH_3 , C7), 42.93 (CH_2 , C12), 32.55 (CH_2 , C13).

MS (CI⁺): m/z (%) = 305.075 (MH^+ , 7.85), 230.006 ($[\text{MH}^+ - \text{H}_2\text{NCH}_2\text{CH}_2\text{CH}_2\text{OH}]$, 100), 230.006 ($[\text{MH}^+ - \text{H}_3\text{NCH}_2\text{CH}_2\text{CH}_2\text{OH}]$, 56.34).

HRMS: calcd. for $\text{C}_{12}\text{H}_{18}\text{O}_3\text{N}_2\text{SCl}$ (MH^+ , Cl^+) 305.0725 found 305.075, calcd. for $\text{C}_9\text{H}_9\text{NO}_2\text{SCl}$ (MH^+ , Cl^+) 230.0043 found 230.0061.

Butyric(Z)-N-(3-(butyryloxy)propyl)-N-(4-chloro-2,5-dimethoxyphenyl) carbamimidic thioanhydride (Compound 58): To a solution of Compound 57 (0.19

mmol) in CH_2Cl_2 (20 ml) at 0 °C, under N_2 , 4-dimethylaminopyridine (DMAP) (0.19 mmol) and Et_3N (0.19 mmol) were added. The resulting mixture was stirred for a few minutes and a butyryl chloride (0.19 mmol) in CH_2Cl_2 (10 ml) was added dropwise. The solution was brought to room temperature and stirred over night. The mixture was quenched with diluted water and extracted with CH_2Cl_2 . The organic phase was washed with 1N HCl, saturated NaHCO_3 solution and brine, dried over MgSO_4 and evaporated. The residue was purified by chromatography (hexane/EtOAc 6:1) to obtain Compound **58** as a colorless oil in 29 % yield.

^1H NMR (600 MHz, acetone- d_6): δ = 11.58 (bt, 1H, $H\text{-N10}$), 7.19 (s, 1H, $H\text{-C5}$), 7.1 (s, 1H, $H\text{-C2}$), 4.18 (t, 2H, J = 6.6 Hz, $H\text{-C13}$), 3.84 (s, 3H, $H\text{-C8}$), 3.83 (s, 3H, $H\text{-C7}$), 3.76 (td, 2H, J = 6.6 Hz, J = 1.8 Hz, $H\text{-C11}$), 2.33 (t, 2H, J = 7.2 Hz, $H\text{-C15}$), 2.09-2.03 (m, 4H, $H\text{-C12}$, $H\text{-C19}$), 1.63 (sextet, 2H, J = 7.2 Hz, $H\text{-C16}$), 1.53 (sextet, 2H, J = 7.2 Hz, $H\text{-C20}$), 0.93 (t, 3H, J = 7.2 Hz, $H\text{-C17}$), 0.81 (t, 3H, J = 7.2 Hz, $H\text{-C21}$).

^{13}C NMR (300 MHz, acetone- d_6): δ = 184.57 (q, C9), 178.8 (q, C18), 173.6 (q, C14), 150.7 (q, C4), 150.13 (q, C1), 130.3 (q, C6), 123.5 (q, C1), 116.97 (CH, C2), 115.12 (CH, C5), 62.8 (CH₂, C13), 57.17 (CH₃, C8), 57.03 (CH₃, C7), 44.28 (CH₂, C11), 36.41 (CH₂, C15), 30.35 (CH₂, C19), 27.53 (CH₂, C12), 19.04 (CH₂, C16), 18.4 (CH₂, C20), 13.91 (CH₃, C17), 13.66 (CH₃, C21).

MS (CI⁺): m/z (%) = 447.147 ($\text{MH}^+(\text{Cl}^{37})$, 28.39), 446.149 ($\text{M}^+(\text{Cl}^{37})$, 33.69), 445.146 (MH^+ , 69.65), 444.146 (M^+ , 40.55), 187.029 ($[\text{MH}^+ + \text{H}^+ - \text{C}(\text{SC}(\text{O})(\text{CH}_2)_2\text{CH}_3)(\text{HN}(\text{CH}_2)_3\text{OC}(\text{O})(\text{CH}_2)_2\text{CH}_3]$, 100), 172.01 ($[\text{M}^+ - \text{NC}(\text{SC}(\text{O})(\text{CH}_2)_2\text{CH}_3)(\text{HN}(\text{CH}_2)_3\text{OC}(\text{O})(\text{CH}_2)_2\text{CH}_3]$, 55.57), 129.092 ($[\text{MH}^+ - \text{C}_6\text{H}_2\text{Cl}(\text{OMe})_2\text{N}=\text{C}(\text{NH}_2)(\text{SC}(\text{O})(\text{CH}_2)_2\text{CH}_3]$, 49.81), 128.098 ($[\text{M}^+ - \text{C}_6\text{H}_2\text{Cl}(\text{OMe})_2\text{N}=\text{C}(\text{NH}_2)(\text{SC}(\text{O})(\text{CH}_2)_2\text{CH}_3]$, 81.82).

HRMS: calcd. for $\text{C}_{20}\text{H}_{29}\text{N}_2\text{O}_5\text{SCl}^{35}$ (MH^+ , Cl^+) 444.1486 found 444.1455, calcd. for $\text{C}_{20}\text{H}_{30}\text{N}_2\text{O}_5\text{SCl}^{35}$ (MH^+ , Cl^+) 445.1564 found 445.1517, calcd. for $\text{C}_{20}\text{H}_{29}\text{N}_2\text{O}_5\text{SCl}^{37}$ 446.1456 found 446.1493, calcd. for (MH^+ , Cl^+) $\text{C}_{20}\text{H}_{30}\text{N}_2\text{O}_5\text{SCl}^{37}$ 447.1534 found 447.1475.

3-(3-(4-Chloro-2,5-dimethoxyphenyl)thioureido)propyl butyrate (Compound 59): Compound **59** was obtained by reacting 1-chloro-2,5-dimethoxy-4-isothiocyantobenzene and 3-aminopropyl butyrate and was purified by

chromatography (hexane/EtOAc 2:1) to provide the desired Compound **59** as a colorless oil.

¹H NMR (300 MHz, acetone-*d*₆): δ = 8.38 (bs, 1H, *H*-N9), 7.91 (bs, 1H, *H*-C5), 7.53 (bt, 1H, *H*-N11), 7.06 (s, 1H, *H*-C2), 4.13 (t, 2H, *J* = 6.6 Hz, *H*-C14), 3.84 (s, 3H, *H*-C8), 3.83 (s, 3H, *H*-C7), 3.7 (q, 2H, *J* = 6.6 Hz, *H*-C12), 2.25 (t, 2H, *J* = 7.2 Hz, *H*-C16), 1.96 (quintet, 2H, *J* = 6.6 Hz, *H*-C13), 1.59 (sextet, 2H, *J* = 7.2 Hz, *H*-C17), 0.91 (t, 3H, *J* = 7.2 Hz, *H*-C18).

¹³C NMR (300 MHz, acetone-*d*₆): δ = 181.94 (q, C10), 173.65 (q, C15), 149.46 (q, C4), 146.76 (q, C1), 127.73 (q, C6), 118.26 (q, C3), 114.27 (CH, C2), 110.78 (CH, C5), 62.45 (CH₂, C14), 56.95 (CH₃, C7+C8), 42.12 (CH₂, C12), 36.4 (CH₂, C16), 28.998 (CH₂, C13), 18.997 (CH₂, C17), 13.88 (CH₃, C18).

MS (CI⁺): *m/z* (%) = 375.111 (MH⁺, 27.16), 230 ([MH⁺-H₂N(CH₂)₃OCO(CH₂)₂CH₃], 100), 188.048 ([MH(CI³⁷)⁺-C(S)NH(CH₂)₃OC(O)CH₂CH₂CH₃], 29.68), 187.04 ([MH⁺-C(S)NH(CH₂)₃OC(O)CH₂CH₂CH₃], 35.27), 129.084 ([M⁺-C₆H₂(Cl)(OMe)₂(NHC(S)NH), 69.93).

HRMS: calcd. for C₁₆H₂₄N₂O₄SCl³⁵ (MH⁺, Cl⁺) 375.1145 found 375.1109.

3-(3-(4-Chloro-2,5-dimethoxyphenyl)thioureido)propyl acetate (Compound 60): Compound **60** was obtained by reacting 1-chloro-2,5-dimethoxy-4-isothiocyanatobenzene and 3-aminopropyl acetate and was purified by chromatography (hexane/EtOAc 2:1), to provide the desired Compound **60**.

m.p. = 74-76 °C.

¹H NMR (200 MHz, acetone-*d*₆): δ = 8.41 (bs, 1H, *H*-N9), 7.87 (bs, 1H, *H*-C5), 7.5 (bt, 1H, *H*-N11), 7.06 (s, 1H, *H*-C2), 4.1 (t, 2H, *J* = 6.4 Hz, *H*-C14), 3.83 (s, 3H, *H*-C8), 3.82 (s, 3H, *H*-C7), 3.69 (q, 2H, *J* = 6.4 Hz, *H*-C12), 1.98 (s, 3H, *H*-C16), 1.96 (quintet, 2H, *J* = 6.6 Hz, *H*-C13).

¹³C NMR (200 MHz, acetone-*d*₆): δ = 181.78 (q, C10), 171.12 (q, C15), 149.34 (q, C4), 146.76 (q, C1), 127.5 (q, C6), 118.29 (q, C3), 114.19 (CH, C2), 110.78 (CH, C5), 62.57 (CH₂, C14), 56.89 (CH₃, C7+C8), 42.00 (CH₂, C12), 28.83 (CH₂, C13), 20.78 (CH₃, C16).

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MS (CI⁺): m/z (%) = 347.084 (MH⁺, 46.09), 230.005 ([MH⁺-H₂N(CH₂)₃OCOCH₃], 92.21), 187.04 ([MH⁺-C(S)NH(CH₂)₃OC(O)CH₃], 56.08), 101.053 ([M⁺-C₆H₂Cl(OMe)₂(NHC(S)NH)], 100).

HRMS: calcd. for C₁₄H₂₀N₂O₄SCl³⁵ (MH⁺, Cl⁺) 347.0832 found 347.0844.

5 **3-(3-(4-Chloro-2,5-dimethoxyphenyl)thioureido)propyl propionate (Compound 61):** Compound 61 was obtained by reacting 1-chloro-2,5-dimethoxy-4-isothiocyanatobenzene and 3-aminopropyl propionate and was purified by silica gel chromatography eluted with hexane/EtOAc (3:1) to obtain Compound 61 in 48 % yield as a colorless oil.

10 ¹H NMR (300 MHz, acetone-*d*₆): δ = 8.38 (bs, 1H, *H*-N9), 7.91 (bs, 1H, *H*-C5), 7.53 (bt, 1H, *J* = 4.8 Hz, *H*-N11), 7.06 (s, 1H, *H*-C2), 4.12 (t, 2H, *J* = 6.3 Hz, *H*-C14), 3.84 (s, 3H, *H*-C8), 3.83 (s, 3H, *H*-C7), 3.7 (q, 2H, *J* = 6.6 Hz, *H*-C12), 2.29 (q, 2H, *J* = 7.5 Hz, *H*-C16), 1.94 (quintet, 2H, *J* = 6.6 Hz, *H*-C13), 1.06 (t, 3H, *J* = 7.5 Hz, *H*-C17).

15 ¹³C NMR (300 MHz, acetone-*d*₆): δ = 181.96 (q, C10), 174.48 (q, C15), 149.46 (q, C4), 146.78 (q, C1), 127.73 (q, C6), 118.27 (q, C3), 114.27 (CH, C2), 110.81 (CH, C5), 62.51 (CH₂, C14), 56.95 (CH₃, C7+C8), 42.09 (CH₂, C12), 28.97 (CH₂, C16), 27.77 (CH₂, C13), 9.38 (CH₃, C17).

20 MS (CI⁺): m/z (%) = 361.104 (MH⁺, 50.52), 360.098 (M⁺, 29.47), 329.077 ([MH⁺-MeOH], 53.54), 287.062 ([MH⁺-HOOCCH₂CH₃], 38.19), 230.007 ([MH⁺-H₂N(CH₂)₃OCOCH₂CH₃], 75.8), 229.01 ([MH⁺-H₃N(CH₂)₃OCOCH₂CH₃], 99.99), 187.049 ([MH⁺-C(S)NH(CH₂)₃OCOCH₂CH₃], 55.11).

HRMS: calcd. for C₁₅H₂₂N₂O₄SCl³⁵ (MH⁺, Cl⁺) 361.0989 found 361.1035, calcd. for C₁₄H₁₈N₂O₃SCl³⁵ 329.0727 found 329.0770.

25 **Butyl 3-(amino-N-(4-chloro-2,5-dimethoxyphenyl) methanethioamino) propanoate (Compound 62):** Compound 62 was obtained by reacting 1-chloro-2,5-dimethoxy-4-isothiocyanatobenzene and butyl 3-aminopropanoate and was purified by silica gel chromatography eluted with hexane/EtOAc (4:1) to obtain Compound 62 in 39 % yield as a colorless oil.

30 ¹H NMR (300 MHz, CDCl₃): δ = 7.81 (bs, 1H, *H*-N9), 7.13 (bs, 1H, *H*-C5), 7.07 (bt, 1H, *H*-N11), 6.92 (s, 1H, *H*-C2), 3.99 (t, 2H, *J* = 6.6 Hz, *H*-C15), 3.88-3.82 (m, 5H, *H*-C8, *H*-C12), 3.73 (s, 3H, *H*-C7), 2.65 (t, 2H, *J* = 5.7 Hz, *H*-C13), 1.52 (quintet, 2H, *J* = 6.6 Hz, *H*-C16), 1.29 (sextet, 2H, *J* = 7.2 Hz, *H*-C17), 0.86 (t, 3H, *J* = 7.2 Hz, *H*-C18).

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^{13}C NMR (300 MHz, CDCl_3): δ = 179.9 (q, C10), 173.08 (q, C14), 149.08 (q, C4), 146.02 (q, C1), 124.46 (q, C6), 119.7 (q, C3), 114.16 (CH, C2), 109.28 (CH, C5), 64.72 (CH_2 , C15), 56.73 (CH_3 , C8), 56.39 (CH_3 , C7), 40.59 (CH_2 , C12), 33.12 (CH_2 , C13), 30.5 (CH_2 , C16), 19.05 (CH_2 , C17), 13.65 (CH_3 , C18).

MS (CI⁺): m/z (%) = 377.11 ($\text{MH}^+(\text{Cl}^{37})$, 25.84), 375.113 ($\text{MH}^+(\text{Cl}^{35})$, 64.91), 374.107 (M^+ , 23.41), 229.995 ($[\text{MH}^+ - \text{H}_2\text{N}(\text{CH}_2)_2\text{COO}(\text{CH}_2)_3\text{CH}_3]$, 100), 228.989 ($[\text{MH}^+ - \text{H}_3\text{N}(\text{CH}_2)_2\text{COO}(\text{CH}_2)_3\text{CH}_3]$, 80.44), 187.04 ($[\text{MH}^+ - \text{C}(\text{S})\text{NH}(\text{CH}_2)_2\text{COO}(\text{CH}_2)_3\text{CH}_3]$, 52.73).

HRMS: calcd. for $\text{C}_{16}\text{H}_{24}\text{N}_2\text{O}_4\text{SCl}^{35}$ (MH^+ , Cl^+) 375.1145 found 375.1127, calcd. for $\text{C}_{16}\text{H}_{24}\text{N}_2\text{O}_4\text{SCl}^{37}$ (MH^+ , Cl^+) 377.1116 found 377.1104, calcd. for $\text{C}_{16}\text{H}_{23}\text{N}_2\text{O}_4\text{SCl}^{35}$ (M , Cl^+) 374.1067 found 374.1071.

Propyl 3-(amino-N-(4-chloro-2,5-dimethoxyphenyl)methanethioamino) propanoate (Compound 63): Compound 63 was obtained by reacting 1-chloro-2,5-dimethoxy-4-isothiocyanatobenzene and propyl 3-aminopropanoate, was purified by column chromatography (hexane/EtOAc 3:1) and was isolated as a yellowish oil in 30 % yield.

^1H NMR (300 MHz, acetone- d_6): δ = 8.47 (bs, 1H, $H\text{-N9}$), 7.93 (bs, 1H, $H\text{-C5}$), 7.6 (bt, 1H, J = 4.2 Hz, $H\text{-N11}$), 7.07 (s, 1H, $H\text{-C2}$), 4.01 (t, 2H, J = 6.6 Hz, $H\text{-C15}$), 3.87 (q, 2H, J = 6.6 Hz, $H\text{-C12}$), 3.85 (s, 3H, $H\text{-C8}$), 3.82 (s, 3H, $H\text{-C7}$), 2.71 (t, 2H, J = 6.6 Hz, $H\text{-C13}$), 1.62 (sextet, 2H, J = 7.5 Hz, $H\text{-C16}$), 0.91 (t, 3H, J = 7.5 Hz, $H\text{-C17}$).

^{13}C NMR (300 MHz, acetone- d_6): δ = 181.83 (q, C10), 172.67 (q, C14), 149.45 (q, C4), 146.59 (q, C1), 127.76 (q, C6), 118.17 (q, C3), 114.25 (CH, C2), 110.61 (CH, C5), 66.48 (CH_2 , C15), 56.95 (CH_3 , C8+C7), 40.88 (CH_2 , C12), 33.96 (CH_2 , C13), 22.62 (CH_2 , C16), 10.60 (CH_3 , C17).

MS (CI⁺): m/z (%) = 363.096 ($\text{MH}^+(\text{Cl}^{37})$, 37.54), 361.098 ($\text{MH}^+(\text{Cl}^{35})$, 99.99), 360.092 (M , 45.07), 329.073 ($[\text{MH}^+ - \text{MeOH}]$, 53.75), 301.041 ($[\text{MH}^+ - \text{HO}(\text{CH}_2)_2\text{CH}_3]$, 12.65), 230.0 ($[\text{MH}^+ - \text{H}_2\text{N}(\text{CH}_2)_2\text{COO}(\text{CH}_2)_2\text{CH}_3]$, 44.97), 187.037 ($[\text{MH}^+ - \text{C}(\text{S})\text{NH}(\text{CH}_2)_2\text{COO}(\text{CH}_2)_2\text{CH}_3]$, 41.76).

HRMS: calcd. for $\text{C}_{15}\text{H}_{22}\text{N}_2\text{O}_4\text{SCl}^{35}$ (MH^+ , Cl^+) 361.0989 found 361.0983, calcd. for $\text{C}_{15}\text{H}_{22}\text{N}_2\text{O}_4\text{SCl}^{37}$ (MH^+ , Cl^+) 363.0959 found 363.0964 calcd. for $\text{C}_{15}\text{H}_{21}\text{N}_2\text{O}_4\text{SCl}^{35}$ (M , Cl^+) 360.0911 found 360.0918.

3-(Amino-N-(4-chloro-2,5-dimethoxyphenyl)methanethioamino)propanoic acid (Compound 64): Compound **64** was obtained by reacting 1-chloro-2,5-dimethoxy-4-isothiocyantobenzene and 3-aminopropanoic acid, was purified by column chromatography (CH₂Cl₂/MeOH 20:1) and was isolated as a white solid in 2 % yield.

5 m.p. = 157-159 °C.

¹H NMR (200 MHz, acetone-*d*₆): δ = 8.49 (bs, 1H, *H*-N9), 7.91 (bs, 1H, *H*-C5), 7.6 (bt, 1H, *H*-N11), 7.06 (s, 1H, *H*-C2), 3.86 (q, 2H, *J* = 6.4 Hz, *H*-C12), 3.84 (s, 3H, *H*-C8), 3.82 (s, 3H, *H*-C7), 2.71 (t, 2H, *J* = 6.4 Hz, *H*-C13).

¹³C NMR (300 MHz, acetone-*d*₆): δ = 181.65 (q, C10), 173.65 (q, C14), 149.44
10 (q, C4), 146.43 (q, C1), 127.9 (q, C6), 118.1 (q, C3), 114.2 (CH, C2), 110.38 (CH, C5), 56.92 (CH₃, C7+C8), 40.72 (CH₂, C12), 33.52 (CH₂, C13).

MS (CI⁺): *m/z* (%) = 347.083 ([M⁺+C₂H₅], 5.63), 232.01 ([MH⁺(Cl³⁷)-
H₂N(CH₂)₂COOH], 35.86), 230.011 ([MH⁺- H₂N(CH₂)₂COOH], 100), 229.005 ([MH⁺-
H₃N(CH₂)₂COOH], 57.96), 188.054 ([M⁺(Cl³⁷)- C(S)NH(CH₂)₂COOH], 54.7), 187.046
15 ([MH⁺- C(S)NH(CH₂)₂COOH], 62.13).

HRMS: calcd. for C₁₄H₂₀N₂O₄SCl³⁵ (MH⁺, Cl⁺) 347.0832 found 347.0832.

Methyl 3-(amino-N-(4-chloro-2,5-dimethoxyphenyl)methanethioamino)propanoate (Compound 65): Compound **65** was obtained by reacting 1-chloro-2,5-dimethoxy-4-isothiocyantobenzene and methyl 3-aminopropanoate, was purified by
20 column chromatography (hexane/EtOAc 2:1 and was isolated as a white solid.

m.p. = 103-104 °C.

¹H NMR (300 MHz, acetone-*d*₆): δ = 8.47 (bs, 1H, *H*-N9), 7.94 (bs, 1H, *H*-C5), 7.6 (bs, 1H, *H*-N11), 7.07 (s, 1H, *H*-C2), 3.87 (q, 2H, *J* = 6.6 Hz, *H*-C12), 3.85 (s, 3H, *H*-C8), 3.83 (s, 3H, *H*-C7), 3.64 (s, 3H, *H*-C15), 2.71 (t, 2H, *J* = 6.6 Hz, *H*-C13).

¹³C NMR (200 MHz, acetone-*d*₆): δ = 181.85 (q, C10), 173.01 (q, C14), 149.42
25 (q, C4), 146.55 (q, C1), 127.79 (q, C6), 118.091 (q, C3), 114.21 (CH, C2), 110.57 (CH, C5), 56.90 (CH₃, C7+C8), 51.74 (CH₃, C15), 40.79 (CH₂, C12), 33.72 (CH₂, C13).

MS (CI⁺): *m/z* (%) = 335.063 (MH⁺(Cl³⁷), 14.55), 333.068 (MH⁺(Cl³⁵), 35.51), 332.062 (M, 10.43), 301.048 ([MH⁺-MeOH], 14.10), 231.997 ([M(Cl³⁷)-
30 HN(CH₂)₂COOCH₃], 36.11), 229.999 ([M- NH(CH₂)₂COOCH₃], 99.99), 228.993([M(Cl³⁵)-H₂N(CH₂)₂COOCH₃], 45.58).

HRMS: calcd. for $C_{13}H_{18}N_2O_4SCl^{35}$ (MH^+ , Cl^+) 333.0676 found 333.0684, calcd. for $C_{13}H_{18}N_2O_4SCl^{37}$ (MH^+ , Cl^+) 335.0646 found 335.0632, calcd. for $C_{13}H_{17}N_2O_4SCl^{35}$ (M , Cl^+) 332.0598 found 332.0623, calcd. for $C_9H_8NO_2SCl^{35}$ 228.9964 found 228.9925, calcd. for $C_9H_8NO_2SCl^{37}$ 230.9935 found 230.9931, calcd. for $C_9H_9NO_2SCl^{37}$ 232.0013 found 231.9974.

Ethyl 3-(amino-N-(4-chloro-2,5-dimethoxyphenyl)methanethioamino)propanoate (Compound 66): Compound 66 was obtained by reacting 1-chloro-2,5-dimethoxy-4-isothiocyanatobenzene and ethyl 3-aminopropanoate, was purified by column chromatography (hexane/EtOAc 2:1) and was isolated as a white solid.

m.p. = 84-85 °C.

1H NMR (300 MHz, acetone- d_6): δ = 8.48 (bs, 1H, $H-N9$), 7.96 (bs, 1H, $H-C5$), 7.62 (bt, 1H, $H-N11$), 7.09 (s, 1H, $H-C2$), 4.12 (q, 2H, J = 7.2 Hz, $H-C15$), 3.91-3.83 (q, 2H, J = 6.6 Hz, $H-C12$), 3.86 (s, 3H, $H-C8$), 3.84 (s, 3H, $H-C7$), 2.71 (t, 2H, J = 6.6 Hz, $H-C13$), 1.23 (t, 3H, J = 6.9 Hz, $H-C16$).

^{13}C NMR (200 MHz, acetone- d_6): δ = 181.84 (q, C10), 172.57 (q, C14), 149.42 (q, C4), 146.56 (q, C1), 127.81 (q, C6), 118.08 (q, C3), 114.22 (CH, C2), 110.59 (CH, C5), 60.86 (CH₂, C15), 56.92 (CH₃, C8+C7), 40.81 (CH₂, C12), 33.97 (CH₂, C13), 14.43 (CH₃, C16).

MS (Cl^+): m/z (%) = 349.08 ($MH^+(Cl^{37})$, 37.62), 347.083 ($MH^+(Cl^{35})$, 100), 346.077 (M^+ , 53.76), 315.005 ($[MH^+-MeOH]$, 72.43), 228.989 ($[MH^+-H_3N(CH_2)_2COOCH_2CH_3]$, 67.1), 187.019 ($[MH^+-C(S)NH(CH_2)_2COOCH_2CH_3]$, 66.67).

HRMS: calcd. for $C_{14}H_{20}N_2O_4SCl^{35}$ (MH^+ , Cl^+) 347.0832 found 347.0834, calcd. for $C_{14}H_{20}N_2O_4SCl^{37}$ (MH^+ , Cl^+) 349.0803 found 349.0804 calcd. for $C_{14}H_{19}N_2O_4SCl^{35}$ (M , Cl^+) 346.0754 found 346.077.

Propyl 4-(amino-N-(4-chloro-2,5-dimethoxyphenyl)methanethioamino)butanoate (Compound 67): Compound 67 was obtained by reacting 1-chloro-2,5-dimethoxy-4-isothiocyanatobenzene and propyl 3-aminopropanoate, was purified by column chromatography (hexane/EtOAc 3:1) and was isolated as a yellow oil in 49 % yield. 1H NMR (200 MHz, acetone- d_6): δ = 8.38 (bs, 1H, $H-N9$), 7.92 (bs, 1H, $H-C5$), 7.54 (bt, 1H, J = 4.6 Hz, $H-N11$), 7.05 (s, 1H, $H-C2$), 3.99 (t, 2H, J = 6.8 Hz, $H-C16$), 3.83 (s, 3H, $H-C8$), 3.83 (s, 3H, $H-C7$), 3.65 (q, 2H, J = 6.8 Hz, $H-C12$), 2.4 (t, 2H, J =

7.4 Hz, *H*-C14), 1.92 (quintet, 2H, *J* = 7.4 Hz, *H*-C13), 1.61 (sextet, 2H, *J* = 6.8 Hz, *H*-C17), 0.91 (t, 3H, *J* = 7.4 Hz, *H*-C18).

¹³C NMR (200 MHz, acetone-*d*₆): δ = 181.85 (q, C10), 173.46 (q, C15), 149.36 (q, C4), 146.67 (q, C1), 127.69 (q, C6), 118.15 (q, C3), 114.16 (CH, C2), 110.72 (CH, C5), 66.24 (CH₂, C16), 56.9 (CH₃, C7+C8), 44.43 (CH₂, C12), 31.93 (CH₂, C14), 25.09 (CH₂, C13), 22.62 (CH₂, C17), 10.62 (CH₃, C18).

MS (CI⁺): *m/z* (%) = 377.116 (MH⁺(Cl³⁷), 33.83), 375.119 (MH⁺, 87.86), 374.11 (M⁺, 21.21), 232.002 ([MH⁺(Cl³⁷)-H₂N(CH₂)₃COO(CH₂)₂CH₃], 29.84), 230.005 ([MH⁺-H₂N(CH₂)₃COO(CH₂)₂CH₃], 78.74), 228.997 ([MH⁺-H₃N(CH₂)₃COO(CH₂)₂CH₃], 49.73), 188.048 ([M⁺(Cl³⁷)-C(S)NH(CH₂)₃COO(CH₂)₂CH₃], 38.41), 187.049 ([MH⁺-C(S)NH(CH₂)₃COO(CH₂)₂CH₃], 33.01).

HRMS: calcd. for C₁₆H₂₄N₂O₄SCl³⁵ (MH⁺, Cl⁺) 375.1145 found 375.1187, C₁₆H₂₄N₂O₄SCl³⁷ (MH⁺, Cl⁺) 377.1116 found 377.1158, calcd. for C₉H₈NO₂SCl³⁵ 228.9964 found 228.997, calcd. for C₉H₉NO₂SCl³⁵ 230.0043 found 230.005, calcd. for C₉H₉NO₂SCl³⁷ 232.0013 found 232.0019.

Butyl 4-(amino-*N*-(4-chloro-2,5-dimethoxyphenyl)methanethioamino)butanoate (Compound 68): Compound 68 was obtained by reacting 1-chloro-2,5-dimethoxy-4-isothiocyanatobenzene and butyl 3-aminobutanoate, was purified by column chromatography (hexane/EtOAc 3:1) and was isolated as a colorless oil in 56 % yield. ¹H NMR (200 MHz, acetone-*d*₆): δ = 8.36 (bs, 1H, *H*-N9), 7.93 (bs, 1H, *H*-C5), 7.52 (bt, 1H, *J* = 4.8 Hz, *H*-N11), 7.05 (s, 1H, *H*-C2), 4.04 (t, 2H, *J* = 6.6 Hz, *H*-C16), 3.84 (s, 3H, *H*-C8), 3.83 (s, 3H, *H*-C7), 3.66 (q, 2H, *J* = 7.2 Hz, *H*-C12), 2.4 (t, 2H, *J* = 7.4 Hz, *H*-C14), 1.92 (quintet, 2H, *J* = 7.2 Hz, *H*-C13), 1.58 (quintet, 2H, *J* = 6.6 Hz, *H*-C17), 1.37 (sextet, 2H, *J* = 7 Hz, *H*-C18), 0.91 (t, 3H, *J* = 7.2 Hz, *H*-C19).

¹³C NMR (200 MHz, acetone-*d*₆): δ = 181.89 (q, C10), 173.44 (q, C15), 149.42 (q, C4), 146.67 (q, C1), 127.76 (q, C6), 118.21 (q, C3), 114.22 (CH, C2), 110.75 (CH, C5), 64.49 (CH₂, C16), 56.95 (CH₃, C7+C8), 44.47 (CH₂, C12), 31.99 (CH₂, C14), 31.40 (CH₂, C17), 25.10 (CH₂, C13), 19.72 (CH₂, C18), 13.94 (CH₃, C19).

MS (CI⁺): *m/z* (%) = 389.128 (MH⁺, 35.56), 388.12 (M⁺, 21.41), 357.083 ([MH⁺-MeOH], 54.21), 230.971 ([MH⁺-HN(CH₂)₃COO(CH₂)₃CH₃], 68.66), 189.023 ([MH⁺(Cl³⁷)-C(S)NH(CH₂)₃COO(CH₂)₃CH₃], 33.31), 187.019 ([MH⁺-C(S)NH(CH₂)₃COO(CH₂)₃CH₃], 100).

HRMS: calcd. for $C_{17}H_{25}N_2O_4SCl^{35}$ (M^+ , Cl^+) 388.1224 found 388.1198, calcd. for $C_{16}H_{26}N_2O_4SCl^{35}$ (MH^+ , Cl^+) 389.1302 found 389.1277, calcd. for $C_{17}H_{26}N_2O_4SCl^{37}$ (MH^+ , Cl^+) 391.1272 found 391.1272.

1-(3-(Propylcarbamoyl)propyl)-3-(4-chloro-2,5-dimethoxyphenyl)thiourea

5 **(Compound 69):** Compound **69** was obtained by reacting 1-chloro-2,5-dimethoxy-4-isothiocyanatobenzene and 4-amino-N-propylbutanamide, was purified by column chromatography (hexane/EtOAc 1:5) and was isolated as a colorless oil in 41 % yield.

1H NMR (300 MHz, acetone- d_6): δ = 8.44 (bs, 1H, $H-N9$), 8.00 (bs, 1H, $H-C5$), 7.86 (bt, 1H, J = 4.8 Hz, $H-N11$), 7.26 (bs, 1H, $H-C16$), 7.04 (s, 1H, $H-C2$), 3.83 (s, 3H, $H-C8$), 3.825 (s, 3H, $H-C7$), 3.63 (q, 2H, J = 6.9 Hz, $H-C12$), 3.12 (q, 2H, J = 6.9 Hz, $H-C17$), 2.28 (t, 2H, J = 7.2 Hz, $H-C14$), 1.91 (quintet, 2H, J = 6.9 Hz, $H-C13$), 1.47 (sextet, 2H, J = 6.9 Hz, $H-C18$), 0.86 (t, 3H, J = 7.5 Hz, $H-C19$).

^{13}C NMR (300 MHz, acetone- d_6): δ = 181.85 (q, C10), 173.17 (q, C15), 149.39 (q, C4), 146.5 (q, C1), 128.16 (q, C6), 117.86 (q, C3), 114.11 (CH, C2), 110.71 (CH, C5), 56.98 (CH_3 , C7+C8), 45.02 (CH_2 , C17), 41.67 (CH_2 , C12), 34.14 (CH_2 , C14), 25.79 (CH_2 , C13), 23.47 (CH_2 , C18), 11.71 (CH_3 , C19).

MS (Cl^+): m/z (%) = 373.127 (M^+ , 1.06), 232.002 ($[MH^+(Cl^{37})-H_2N(CH_2)_3CONH(CH_2)_2CH_3]$, 11.37), 230.997 ($[MH^+-HN(CH_2)_3CONH(CH_2)_2CH_3]$, 36.69), 228.994 ($[MH^+-H_3N(CH_2)_3CONH(CH_2)_2CH_3]$, 100).

20 HRMS: calcd. for $C_{16}H_{24}N_3O_3SCl^{35}$ (M^+ , Cl^+) 373.1227 found 373.1274, $C_9H_8NO_2SCl^{35}$ 228.9964 found 228.9937.

S-Propyl 4-(amino-N-(4-chloro-2,5-dimethoxyphenyl) methanethioamino) butanethioate (Compound 70): Compound **70** was obtained by reacting 1-chloro-2,5-dimethoxy-4-isothiocyanatobenzene and S-propyl 4-aminobutanethioate, was purified
25 by column chromatography (hexane/EtOAc 4:1) and was isolated as a colorless oil in 10 % yield.

1H NMR (300 MHz, acetone- d_6): δ = 8.36 (bs, 1H, $H-N9$), 7.93 (bs, 1H, $H-C5$), 7.53 (bt, 1H, $H-N11$), 7.06 (s, 1H, $H-C2$), 3.84 (s, 3H, $H-C8$), 3.835 (s, 3H, $H-C7$), 3.66 (q, 2H, J = 6.9 Hz, $H-C12$), 2.84 (t, 2H, J = 7.2 Hz, $H-C16$), 2.67 (t, 2H, J = 7.2 Hz, $H-C14$), 1.94 (quintet, 2H, J = 7.2 Hz, $H-C13$), 1.56 (sextet, 2H, J = 7.2 Hz, $H-C17$), 0.94 (t, 3H, J = 7.2 Hz, $H-C18$).

^{13}C NMR (300 MHz, acetone- d_6): δ = 198.9 (q, C15), 182.07 (q, C10), 149.5 (q, C4), 146.80 (q, C1), 127.86 (q, C6), 118.27 (q, C3), 114.3 (CH, C2), 110.87 (CH, C5), 56.99 (CH₃, C7+C8), 44.33 (CH₂, C12), 41.94 (CH₂, C14), 31.06 (CH₂, C16), 25.71 (CH₂, C13), 23.78 (CH₂, C17), 13.48 (CH₃, C18).

5 MS (CI⁺) m/z (%) = 391.09 (MH⁺, 3.85), 390.084 (M⁺, 6.61), 359.063 ([MH⁺-CH₃OH], 9.21), 316.045 ([MH⁺-S(CH₂)₂CH₃], 24.03), 314.047 ([M⁺-HS(CH₂)₂CH₃], 58.83), 285.027 ([M⁺-H⁺-COS(CH₂)₂CH₃], 33.83), 228.988 ([MH⁺-H₃N(CH₂)₃COS(CH₂)₂CH₃], 100).

10 HRMS: calcd. for C₁₆H₂₃N₂O₃S₂Cl³⁵ (M⁺, Cl⁺) 390.0839 found 390.0837, calcd. for C₁₆H₂₄N₂O₃S₂Cl³⁵ (MH⁺, Cl⁺) 391.0917 found 391.0897, calcd. for C₁₆H₂₃N₂O₃S₂Cl³⁵ (M⁺, Cl⁺) 392.0809 found 392.0819, calcd. for C₁₆H₂₄N₂O₃S₂Cl³⁷ (MH⁺, Cl⁺) 393.0887 found 393.0876.

Ethyl 4-(amino-N-(4-chloro-2,5-dimethoxyphenyl)methanethioamino)butanoate (Compound 71): Compound 71 was obtained by reacting 1-chloro-2,5-dimethoxy-4-isothiocyanatobenzene and ethyl 4-aminobutanoate, was purified by column chromatography (hexane/EtOAc 2:1) and was isolated as a yellowish oil in 59 % yield. ^1H NMR (300 MHz, acetone- d_6): δ = 8.39 (bs, 1H, H-N9), 7.88 (bs, 1H, H-C5), 7.53 (bt, 1H, J = 4.8 Hz, H-N11), 7.04 (s, 1H, H-C2), 4.07 (q, 2H, J = 7.2 Hz, H-C16), 3.83 (s, 3H, H-C8), 3.82 (s, 3H, H-C7), 3.65 (q, 2H, J = 6.6 Hz, H-C12), 2.38 (t, 2H, J = 7.2 Hz, H-C14), 1.89 (quintet, 2H, J = 7.2 Hz, H-C13), 1.2 (t, 3H, J = 7.2 Hz, H-C17).

^{13}C NMR (300 MHz, acetone- d_6): δ = 181.69 (q, C10), 173.37 (q, C15), 149.28 (q, C4), 146.65 (q, C1), 127.5 (q, C6), 118.21 (q, C3), 114.11 (CH, C2), 110.71 (CH, C5), 60.59 (CH₂, C16), 56.86 (CH₃, C7+C8), 44.37 (CH₂, C12), 31.89 (CH₂, C14), 24.99 (CH₂, C13), 14.47 (CH₃, C17).

25 MS (CI⁺): m/z (%) = 363.099 (MH⁺(Cl³⁷), 4.1), 361.099 (MH⁺, 19.15), 329.081 ([MH⁺-MeOH], 69.46), 228.985 ([MH⁺-H₃N(CH₂)₃COOCH₂CH₃], 100), 189.027 ([MH⁺(Cl³⁷)-C(S)NH(CH₂)₃COOCH₂CH₃], 17), 187.03 ([MH⁺-C(S)NH(CH₂)₃COOCH₂CH₃], 68.42).

30 HRMS: calcd. for C₁₅H₂₁N₂O₄SCl³⁵ (M⁺, Cl⁺) 360.0911 found 360.0920, calcd. for C₁₅H₂₂N₂O₄SCl³⁵ (MH⁺, Cl⁺) 361.0989 found 361.0995, calcd. for C₁₅H₂₂N₂O₄SCl³⁷ (MH⁺, Cl⁺) 363.0959 found 363.0988.

Ethyl 4-(3-(4-chloro-2,5-dimethoxyphenyl)ureido)butanoate (Compound 72):

Compound 72 was obtained by reacting 1-chloro-2,5-dimethoxy-4-cyanatobenzene and ethyl 4-aminobutanoate and was purified by chromatography (hexane/EtOAc 2:1), and was isolated as a white-yellow solid.

5 m.p. = 103 °C.

¹H NMR (300 MHz, acetone-*d*₆): δ = 8.23 (s, 1H, *H*-C5), 7.7 (bs, 1H, *H*-N9), 6.93 (s, 1H, *H*-C2), 6.48 (bt, 1H, *J* = 5.1 Hz, *H*-N11), 4.08 (q, 2H, *J* = 7.2 Hz, *H*-C16), 3.81 (s, 3H, *H*-C8), 3.8 (s, 3H, *H*-C7), 3.27 (q, 2H, *J* = 6.9 Hz, *H*-C12), 2.36 (t, 2H, *J* = 7.2 Hz, *H*-C14), 1.81 (quintet, 2H, *J* = 6.9 Hz, *H*-C13), 1.2 (t, 3H, *J* = 7.2 Hz, *H*-C17).

10 ¹³C NMR (300 MHz, acetone-*d*₆): δ = 173.37 (q, C15), 155.94 (q, C10), 149.9 (q, C4), 142.46 (q, C1), 130.55 (q, C6), 113.08 (CH, C2), 113.04 (q, C3), 104.54 (CH, C5), 60.55 (CH₂, C16), 56.89 (CH₃, C8), 56.71 (CH₃, C7), 39.62 (CH₂, C12), 31.92 (CH₂, C14), 26.33 (CH₂, C13), 14.48 (CH₃, C17).

MS (CI⁺): *m/z* (%) = 344.118 (M⁺, 19.58), 299.107 ([MH⁺-EtOH], 3.82), 15 298.091 ([M⁺-EtOH], 8.7), 258.082 ([MH⁺-CH₂COOCH₂CH₃], 74.50), 213.025 ([M⁺-H⁺-HN(CH₂)₃COOCH₂CH₃], 88.92), 187.05 ([MH⁺-COHN(CH₂)₃COOCH₂CH₃], 44.48). HRMS: calcd. for C₂₀H₂₁N₂O₅Cl³⁵ 344.1139 found 344.1183.

Methyl 4-(amino-N-(4-chloro-2,5-dimethoxyphenyl) methanethioamino) butanoate (Compound 73): Compound 73 was obtained by reacting 1-chloro-2,5-
20 dimethoxy-4-isothiocyantobenzene and methyl 4-aminobutanoate, was purified by column chromatography (hexane/EtOAc 3:1) and was isolated as a yellowish solid in 20 % yield.

m.p. = 77 °C.

¹H NMR (300 MHz, acetone-*d*₆): δ = 8.38 (bs, 1H, *H*-N9), 7.91 (bs, 1H, *H*-C5),
25 7.54 (bt, 1H, *J* = 4.8 Hz, *H*-N11), 7.05 (s, 1H, *H*-C2), 3.83 (s, 3H, *H*-C8), 3.82 (s, 3H, *H*-C7), 3.64 (q, 2H, *J* = 6.6 Hz, *H*-C12), 3.61 (s, 3H, *H*-C16), 2.4 (t, 2H, *J* = 7.5 Hz, *H*-C14), 1.89 (quintet, 2H, *J* = 7.2 Hz, *H*-C13).

¹³C NMR (300 MHz, acetone-*d*₆): δ = 181.85 (q, C10), 173.83 (q, C15), 149.35 (q, C4), 146.7 (q, C1), 127.65 (q, C6), 118.2 (q, C3), 114.16 (CH, C2), 110.77 (CH, C5),
30 56.88 (CH₃, C7+C8), 51.59 (CH₃, C16), 44.36 (CH₂, C12), 31.61 (CH₂, C14), 25.00 (CH₂, C13).

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MS (CI⁺): m/z (%) = 349.077 (MH⁺(Cl³⁷), 10.74), 348.079 (M⁺(Cl³⁷), 14.61),
 347.084 (MH⁺, 32.55), 346.075 (M⁺, 25.20), 317.058 ([MH⁺(Cl³⁷)-MeOH], 30.86),
 315.063 ([MH⁺-MeOH], 85.08), 231.000 ([MH⁺(Cl³⁷)-H₃N(CH₂)₃COOCH₃], 37.03),
 230.009 ([MH⁺-H₂N(CH₂)₃COOCH₃], 40.03), 229.002 ([MH⁺-H₃N(CH₂)₃COOCH₃],
 5 100), 187.048 ([MH⁺-C(S)NH(CH₂)₃COOCH₃], 59.1).

HRMS: calcd. for C₁₄H₁₉N₂O₄SCl³⁵ (M⁺, Cl⁺) 346.0754 found 346.0750, calcd.
 for C₁₄H₂₀N₂O₄SCl³⁵ (MH⁺, Cl⁺) 347.0832 found 347.0845, calcd. for C₁₄H₂₀N₂O₄SCl³⁷
 (MH⁺, Cl⁺) 349.0803 found 349.0770.

Ethyl 4-(3-(4-chloro-2,5-dimethoxyphenyl)ureido)butanoate (Compound 74):

10 Compound **74** was obtained by reacting 1-chloro-2,5-dimethoxy-4-cyanatobenzene and
 methyl 4-aminobutanoate, was purified by chromatography (hexane/EtOAc 3:1), and
 was isolated as a white-yellow solid.

m.p. = 92 °C.

¹H NMR (300 MHz, acetone-*d*₆): δ = 8.24 (s, 1H, *H*-C5), 7.7 (bs, 1H, *H*-N9),
 15 6.93 (s, 1H, *H*-C2), 6.49 (bt, 1H, *J* = 4.8 Hz, *H*-N11), 3.81 (s, 3H, *H*-C8), 3.8 (s, 3H, *H*-
 C7), 3.61 (s, 3H, *H*-C16), 3.26 (q, 2H, *J* = 6.6 Hz, *H*-C12), 2.38 (t, 2H, *J* = 7.5 Hz, *H*-
 C14), 1.81 (quintet, 2H, *J* = 7.5 Hz, *H*-C13).

¹³C NMR (300 MHz, acetone-*d*₆): δ = 173.87 (q, C15), 155.98 (q, C10), 149.95
 (q, C4), 142.51 (q, C1), 130.59 (q, C6), 113.14 (q, CH, C2+C3), 104.61 (CH, C5), 56.93
 20 (CH₃, C8), 56.76 (CH₃, C7), 51.58 (CH₃, C16), 39.65 (CH₂, C12), 31.67 (CH₂, C14),
 26.34 (CH₂, C13).

MS (CI⁺): m/z (%) = 333.105 (MH⁺(Cl³⁷), 4.54), 332.098 (M⁺(Cl³⁷), 16.68),
 331.105 (MH⁺, 12.86), 330.1 (M⁺, 47.69), 299.08 ([MH⁺-MeOH], 13.05), 298.072 ([M⁺-
 MeOH], 23.79), 215.005 ([MH⁺-HN(CH₂)₃COOCH₃], 31.86), 212.997 ([MH⁺-
 25 H₃N(CH₂)₃COOCH₃], 100), 187.018 ([MH⁺-C(S)NH(CH₂)₃COOCH₃], 94.48).

HRMS: calcd. for C₁₄H₁₉N₂O₅Cl³⁵ (M⁺, Cl⁺) 330.0982 found 330.1, calcd. for
 C₁₄H₂₀N₂O₅Cl³⁵ (MH⁺, Cl⁺) 331.1061 found 331.1054, calcd. for C₁₄H₁₉N₂O₅Cl³⁷ (M⁺,
 Cl⁺) 332.0953 found 332.0982.

tert-Butyl 3-(3-(4-chloro-2,5-dimethoxyphenyl) thioureido)

30 ***propylpropylcarbamate (Compound 75):*** Compound **75** was obtained by reacting 1-
 chloro-2,5-dimethoxy-4-isothiocyanatobenzene and tert-butyl 3-

aminopropyl(propyl)carbamate and which was chromatographed (hexane/EtOAc 5:1). Pure white solid was obtained in the total yield of 2.2 % (2 steps).

m.p. = 86-87 °C.

¹H NMR (300 MHz, acetone-*d*₆) δ = 8.32 (bs, 1H, *H*-N9), 7.69 (bs, 1H, *H*-C5),
 5 7.61 (bs, 1H, *H*-N11), 7.06 (s, 1H, *H*-C2), 3.84 (s, 3H, *H*-C8), 3.82 (s, 3H, *H*-C7), 3.59
 (bq, 2H, *J* = 5.4 Hz, *H*-C12), 3.27 (t, 2H, *J* = 6.9 Hz, *H*-C14), 3.14 (t, 2H, *J* = 7.5 Hz,
H-C15), 1.8 (bs, 2H, *H*-C13), 1.54 (sextet, 2H, *J* = 7.5 Hz, *H*-C16), 1.41 (s, 9H, *H*-C20),
 0.85 (t, 3H, *J* = 7.5 Hz, *H*-C17).

¹³C NMR (300 MHz, acetone-*d*₆): δ = 181.82 (q, C10), 156.9 (q, C18), 149.57 (q,
 10 C4), 147.5 (q, C1), 127.69 (q, C6), 119.01 (q, C3), 114.44 (CH, C2), 111.57 (CH, C5),
 79.46 (q, C19), 56.97 (CH₃, C7+C8), 49.33 (CH₂, C14), 44.54 (CH₂, C15), 42.3 (CH₂,
 C12), 28.53 (CH₃, C20), 28.2 (CH₂, C13), 22.55 (CH₂, C16), 11.52 (CH₃, C17).

MS (CI⁺): *m/z* (%) = 447.18 (M⁺(Cl³⁷), 12.96), 446.184 (MH⁺, 16.59), 445.178
 (M⁺, 24.47), 414.16 ([MH⁺-MeOH], 40.89), 346 ([MH⁺-CH₂C(CH₃)₂-CO₂], 44.14), 348
 15 ([MH⁺(Cl³⁷)-CH₂C(CH₃)₂-CO₂], 17.84), 230.004 ([MH⁺-
 H₂N(CH₂)₃N(COOC(CH₃)₃)(CH₂)₂CH₃], 61.22), 228.996 ([MH⁺-
 H₃N(CH₂)₃N(COOC(CH₃)₃)(CH₂)₂CH₃], 100.02), 187.04 ([MH⁺-
 C(S)NH(CH₂)₃N(COOC(CH₃)₃)(CH₂)₂CH₃], 92.98).

HRMS: calcd. for C₂₀H₃₃N₃O₄SCl³⁵ (MH⁺, Cl⁺) 446.188 found 446.1843, calcd.
 20 for C₂₀H₃₂N₃O₄SCl³⁷ (M⁺, Cl⁺) 447.1773 found 447.1796, calcd. for C₂₀H₃₂N₃O₄SCl³⁵
 (M⁺, Cl⁺) 445.1802 found 445.1780.

1-(4-Chloro-2,5-dimethoxyphenyl)-3-(3-(propylamino)propyl)thiourea

(Compound 76): Compound 76 was obtained by dissolving Compound 75 in Et₂O and
 adding 1 ml of concentrated HCl 37 %. The solution was stirred for 15 minutes and
 25 additional concentrated HCl was added. The aqueous phase was separated and
 concentrated to afford Compound 76 as a white solid in 92 % yield.

m.p. = 116-117 °C.

¹H NMR (300 MHz, MeOD): δ = 7.58 (bs, 1H, *H*-C5), 7.09 (s, 1H, *H*-C2), 3.83
 (s, 3H, *H*-C8), 3.82 (s, 3H, *H*-C7), 3.74 (t, 2H, *J* = 6.6 Hz, *H*-C12), 3.07 (t, 2H, *J* = 6.9
 30 Hz, *H*-C16), 2.99 (t, 2H, *J* = 7.8 Hz, *H*-C14), 1.99 (quintet, 2H, *J* = 6.6 Hz, *H*-C13),
 1.75 (sextet, 2H, *J* = 7.8 Hz, *H*-C17), 1.03 (t, 3H, *J* = 7.5 Hz, *H*-C18).

^{13}C NMR (200 MHz, MeOD): δ = 182.53 (q, C10), 150.24 (q, C4), 148.39 (q, C1), 127.16 (q, C6), 120.78 (q, C3), 115.03 (CH, C2), 112.37 (CH, C5), 57.3 (CH₃, C8), 57.12 (CH₃, C7), 50.76 (CH₂, C16), 45.96 (CH₂, C14), 41.84 (CH₂, C12), 27.55 (CH₃, C13), 20.76 (CH₂, C17), 11.29 (CH₃, C18).

5 MS (CI⁺): m/z (%) = 348.138 (MH⁺(Cl³⁷), 1.29), 346.134 (MH⁺, 6.75), 314.115 ([MH⁺-MeOH], 24.53), 229.998 ([MH⁺-H₂N(CH₂)₃NH(CH₂)₂CH₃], 85.89), 228.991 ([MH⁺-H₃N(CH₂)₃NH(CH₂)₂CH₃], 100), 188.057 ([M⁺(Cl³⁷)-C(S)NH(CH₂)₃NH(CH₂)₂CH₃], 25.24), 187.057 ([MH⁺-C(S)NH(CH₂)₃NH(CH₂)₂CH₃], 78.74).

10 HRMS: calcd. for C₁₅H₂₅N₃O₂SCl³⁵ (MH⁺, Cl⁺) 346.1356 found 346.1341.

EXAMPLE 5

Mechanistic Insights

Without being bound to any particular theory, from a mechanistic point of view,
15 it is suggested that the compounds described herein bind *bona fide* the NNRTI-binding hydrophobic pocket of HIV-1 RT, which is not found in other DNA polymerases such as the control KF DNA polymerase, used in the selectivity assays conducted. Such compounds should possess some hydrophobic properties to enable binding to this pocket and this feature may also favor their ability to penetrate cells.

20 To pinpoint the molecular interactions of Compounds **3** and **20** with HIV-1 RT, the docking conformation of these molecules onto the crystal structure of one of the two HIV-1 RT used in these studies (1dtq) was visually analyzed. It has been shown that both compounds fit into the NNRTI hydrophobic pocket interacting with several RT residues (see, FIGs. 6A-B). Specifically, Compound **20** formed two hydrogen bonds
25 with HIV-1 RT, via the hydrogen atoms attached to the two thiourea nitrogens of the compound, which interact with carbonyl oxygen of two different RT residues: One hydrogen bond was formed with Glu138 side chain, located in the p51 subunit of HIV-1 RT, while the other hydrogen bond was formed with the carbonyl backbone of Lys 101, which is located in the p66 subunit (See, FIG. 6B). Notably, the interaction with
30 Lys101 of HIV-1 RT was experimentally-observed for several NNRTIs and further supports the accuracy of the suggested interaction model presented herein. Compound

3 formed also two hydrogen bonds: one with Glu138 of the p51 RT subunit and another one with the hydroxyl group of Tyr318 in the larger (p66) subunit (see, FIG. 6A).

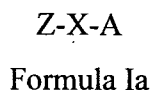
Tyr318 is highly conserved in HIV-1 RT and interacts within a 4 Å contact distance with most NNRTIs that have been shown to bind the hydrophobic pocket. The importance of a Tyr at position 318 was evident from a mutagenesis analysis, which showed that only when replacing Tyr318 with either Trp or Phe (i.e. Y318F or Y318W RT mutants), the activity of the enzyme substantially retained. While the Y318F mutant still retained a substantial sensitivity to the majority of the NNRTIs tested, the Y318W mutant showed varying degrees of resistance to different NNRTIs. The mutation E138K in HIV-1 RT has also been reported and led to resistance to derivatives of [2',5'-bis-O-(tert-butyldimethylsilyl)-3'-spiro-5''-(4''-amino-1'',2''-oxathiole-2'',2''-dioxide)]-beta-D-pentofuranosyl, (TSAO), while retaining sensitivity to other NNRTIs, such as nevirapine.

Although the invention has been described in conjunction with specific embodiments thereof, it is evident that many alternatives, modifications and variations will be apparent to those skilled in the art. Accordingly, it is intended to embrace all such alternatives, modifications and variations that fall within the spirit and broad scope of the appended claims.

All publications, patents and patent applications mentioned in this specification are herein incorporated in their entirety by reference into the specification, to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated herein by reference. In addition, citation or identification of any reference in this application shall not be construed as an admission that such reference is available as prior art to the present invention. To the extent that section headings are used, they should not be construed as necessarily limiting.

WHAT IS CLAIMED IS:

1. A compound having the general Formula Ia:



or a pharmaceutically acceptable salt thereof;

wherein:

Z is selected from the group consisting of a substituted or non-substituted aryl and heteroaryl, wherein when substituted, the substituent is selected from the group consisting of halide, alkoxy, hydroxy, thioalkoxy, thioalkyl, alkyl, haloalkyl, amine, sulfonamide, sulfinyl, sulfonyl, carboxylate, cyano, nitro, amide, and OCO_2R^4 ;

X is selected from the group consisting of $\text{NR}^5\text{-C(=W)-NR}^6$ and $\text{N=CR}^7\text{-NR}^6$, whereas W is selected from the group consisting of N, O, S, =NCN , =CHNO_2 and NR^{11} ;

A is $\text{R}^8\text{-Y-R}^9$,

wherein Y is selected from the group consisting of -O- , -N- , -S- , $\text{-CR}^{10}\text{R}^{11}$, -NR^{10} , -SC(=O)- , -C(=O)S- , -OC(=S)- , -C(=S)O- , -SC(=S)- , -C(=S)S- , -S(=O)- , S(=O)_2 , $\text{-NR}^{10}\text{S(=O)-}$, -S(=O)NR^{10} , $\text{-S(=O)}_2\text{NR}^{10}$, $\text{-NR}^{10}\text{S(=O)}_2$, -C(=O)- , -C(=O)O- , -OC(=O)- , -C(=O)NR^{11} , $\text{-R}^{11}\text{NC(=O)-}$, -OC(=O)O- , aryl and heterocyclic;

R^7 is selected from the group consisting of -SC(=O)R^{12} , -C(=O)SR^{12} , -SR^{12} , -S(=O)R^{12} , -OC(=O)R^{12} , -C(=O)OR^{12} , $\text{-NR}^{13}\text{C(=O)R}^{12}$, $\text{-C(=O)NR}^{12}\text{R}^{13}$, -OR^{12} and $\text{-NR}^{12}\text{R}^{13}$;

R^8 and R^9 are each independently a linear or branched, saturated or unsaturated substituted or non-substituted hydrocarbon chain having 1-10 carbon atoms in its backbone, which when substituted, the substituent is selected from the group consisting of alkyl and cycloalkyl; and

R^4 , R^5 , R^6 , R^{10} , R^{11} , R^{12} and R^{13} are each independently hydrogen or alkyl;

with the proviso that:

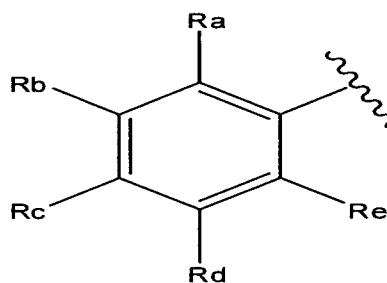
when X is $\text{NR}^5\text{-C(=W)-NR}^6$ and W is sulfur, A is other than $(\text{CH}_2)_3\text{O}(\text{CH}_2)_2\text{CH}_3$;

when A is $(\text{CH}_2)_3\text{O}(\text{CH}_2)_2\text{CH}_3$ and X is $\text{NR}^5\text{-C(=W)-NR}^6$, W is other than sulfur;

and

when X is $\text{NR}^5\text{-C(=W)-NR}^6$ and W is sulfur and A is $(\text{CH}_2)_3\text{O}(\text{CH}_2)_2\text{CH}_3$, Z is selected from the group consisting of a substituted or non-substituted heteroaryl and a substituted aryl, wherein at least one substituent on said aryl is other than alkoxy, methyl, and halide, the compound being capable of interacting with a hydrophobic binding site within at least one of the catalytic domains of said reverse transcriptase.

2. The compound of claim 1, wherein Z is a substituted or non-substituted aryl having formula II:



Formula II

or a pharmaceutically acceptable salt thereof;

wherein:

Ra-Re are each independently selected from the group consisting of halide, alkoxy, hydroxy, thioalkoxy, thioalkyl, alkyl, haloalkyl, amine, sulfonamide, sulfinyl, sulfonyl, carboxylate, cyano, nitro, amide, and OCO_2R^4 ,

wherein at least one of said Ra-Rd is selected from the group consisting of halide, alkoxy, alkyl, carboxylate, cyano, nitro, haloalkyl and amine.

3. The compound of claim 2, wherein Ra is selected from the group consisting of alkoxy, alkyl and halide.

4. The compound of any of claims 2 and 3, wherein Rc is selected from the group consisting of hydrogen, halide and nitro.

5. The compound of any of claims 2-4, wherein Rd is selected from the group consisting of hydrogen, alkyl, halide, alkoxy and haloalkyl.

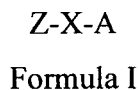
6. The compound of any of claims 1-5, wherein X is said $\text{NR}^5\text{-C(=W)-NR}^6$.
7. The compound of claim 6, wherein W is S or O.
8. The compound of any of claims 1-7, wherein Y is selected from the group consisting of -O-, -N-, -S-, $\text{-CR}^{10}\text{R}^{11}$ -, -NR^{10} -, -SC(=O)- , -C(=O)S- , -OC(=S)- , -C(=S)O- , -SC(=S)- , -C(=S)S- , -S(=O)- , S(=O)_2 -, $\text{-NR}^{10}\text{S(=O)-}$, -S(=O)NR^{10} -, $\text{-S(=O)}_2\text{NR}^{10}$ -, $\text{-NR}^{10}\text{S(=O)}_2$ -, -C(=O)- , -C(=O)O- , -OC(=O)- , -C(=O)NR^{11} -, $\text{-R}^{11}\text{NC(=O)-}$ and -OC(=O)O- .
9. The compound of any of claims 1-8, wherein R^8 and R^9 are each independently a linear, saturated, non-substituted hydrocarbon chain having 1-10 carbon atoms in its backbone.
10. The compound of claim 9, wherein said hydrocarbon chain has 2-4 carbon atoms.
11. The compound of any of claims 2-10, wherein Z has said general formula II, wherein Ra is methoxy, Rb is hydrogen, Rc is chloro, Rd is methoxy and Re is hydrogen.
12. The compound of claim 11, wherein A is said $\text{NR}^5\text{-C(=W)-NR}^6$, W is O or S and each of R^5 and R^6 is hydrogen.
13. A pharmaceutical composition comprising the compound of any of claims 1-12 and a pharmaceutically acceptable carrier.
14. The pharmaceutical composition of claim 13, being packaged in a packaging material and identified in print, in or on said packaging material, for use in the treatment of a viral infection caused by a retrovirus.

15. A process of synthesizing the compound of any of claims 1-12, the process comprising:

reacting a compound having the formula $Z-R^1$ with a compound having the formula $A-R^2$, wherein R^2 is amine and R^1 is selected such that upon reacting with said amine, said X is formed.

16. A method of inhibiting an activity of a reverse transcriptase, the method comprising contacting said reverse transcriptase with a compound selected from the group consisting of:

(i) a compound having the general Formula I:



or a pharmaceutically acceptable salt thereof;

wherein:

Z is selected from the group consisting of a substituted or non-substituted aryl and heteroaryl, wherein when substituted, the substituent is selected from the group consisting of halide, alkoxy, hydroxy, thioalkoxy, thioalkyl, alkyl, haloalkyl, amine, sulfonamide, sulfinyl, sulfonyl, carboxylate, cyano, nitro, amide, and OCO_2R^4 ;

X is selected from the group consisting of $NR^5-C(=W)-NR^6$ and $N=CR^7-NR^6$, whereas W is selected from the group consisting of N, O, S, $=NCN$, $=CHNO_2$ and NR^{11} ;

A is R^8-Y-R^9 ,

wherein Y is selected from the group consisting of $-O-$, $-N-$, $-S-$, $-CR^{10}R^{11}-$, $-NR^{10}-$, $-SC(=O)-$, $-C(=O)S-$, $-OC(=S)-$, $-C(=S)O-$, $-SC(=S)-$, $-C(=S)S-$, $-S(=O)-$, $S(=O)_2$, $-NR^{10}S(=O)-$, $-S(=O)NR^{10}-$, $-S(=O)_2NR^{10}-$, $-NR^{10}.S(=O)_2-$, $-C(=O)-$, $-C(=O)O-$, $-OC(=O)-$, $-C(=O)NR^{11}-$, $-R^{11}NC(=O)-$, $-OC(=O)O-$, aryl and heterocyclic;;

R^7 is selected from the group consisting of $SC(=O)R^{12}$, $C(=O)SR^{12}$, SR^{12} , $S(=O)R^{12}$, $OC(=O)R^{12}$, $C(=O)OR^{12}$, $NR^{13}C(=O)R^{12}$, $C(=O)NR^{12}R^{13}$, OR^{12} and $NR^{12}R^{13}$;

R^8 and R^9 are each independently a linear or branched, saturated or unsaturated substituted or non-substituted hydrocarbon chain having 1-10 carbon atoms in its

backbone, which when substituted, the substituent is selected from the group consisting of alkyl and cycloalkyl; and

$R^4, R^5, R^6, R^{10}, R^{11}, R^{12}$ and R^{13} are each independently hydrogen or alkyl;

(ii) a compound having the general Formula III:



Formula III

or a pharmaceutically acceptable salt thereof;

wherein

B^1 and B^2 are each independently selected from the group consisting of a substituted or unsubstituted heteroaryl and heteroalicyclic, which, when substituted, the substituent is selected from the group consisting of halide, alkoxy, hydroxy thioalkoxy, thioalkyl, alkyl, haloalkyl, amine, sulfonamide, sulfinyl, sulfonyl, carboxylate, cyano, nitro, amide, and OCO_2R^{15} ,

D is selected from a group consisting of O-, -N-, -S-, $-CR^{16}R^{17}$ -, $-NR^{16}$ -, $-SC(=O)$ -, $-C(=O)S$ -, $-OC(=S)$ -, $-C(=S)O$ -, $-SC(=S)$ -, $-C(=S)S$ -, $-S(=O)$ -, $S(=O)_2$ -, $-NR^{16}S(=O)$ -, $-S(=O)NR^{16}$ -, $-S(=O)_2NR^{16}$ -, $-NR^{16}S(=O)_2$ -, $-C(=O)$ -, $-C(=O)O$ -, $-OC(=O)$ -, $-C(=O)NR^{17}$ -, $-R^{17}NC(=O)$ - and $-OC(=O)O$ -, aryl and heterocyclic; and

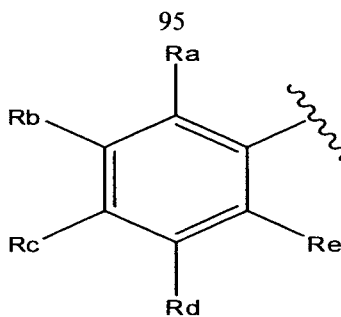
R^{15}, R^{16} and R^{17} are each independently hydrogen or alkyl; and

(iii) a compound selected from the group consisting of Compounds **1**, **4**, **5**, **6**, **8** and **13**,

the compound being capable of interacting with a hydrophobic binding site within at least one of the catalytic domains of said reverse transcriptase.

17. The method of claim 16, wherein said compound has said general Formula I.

18. The method of claim 17, wherein Z is a substituted or non-substituted aryl having formula II:



Formula II

or a pharmaceutically acceptable salt thereof;

wherein:

Ra-Re are each independently selected from the group consisting of halide, alkoxy, hydroxy, thioalkoxy, thioalkyl, alkyl, haloalkyl, amine, sulfonamide, sulfinyl, sulfonyl, carboxylate, cyano, nitro, amide, and OCO_2R^4 ,

provided that at least one of said Ra-Rd is selected from the group consisting of halide, alkoxy, alkyl, carboxylate, cyano, nitro, haloalkyl and amine.

19. The method of claim 18, wherein Ra is selected from the group consisting of alkoxy, alkyl and halide.

20. The method of any of claims 18 and 19, wherein Rc is selected from the group consisting of hydrogen, halide and nitro.

21. The method of any of claims 18-20, wherein Rd is selected from the group consisting of hydrogen, alkyl, halide, alkoxy and haloalkyl.

22. The method of any of claims 17-21, wherein X is said $\text{NR}^5\text{-C(=W)-NR}^6$.

23. The method of claim 22, wherein W is S or O.

24. The method of any of claims 17-23, wherein Y is selected from the group consisting of $-\text{O}-$, $-\text{N}-$, $-\text{S}-$, $-\text{CR}^{10}\text{R}^{11}-$, $-\text{NR}^{10}-$, $-\text{SC(=O)}-$, $-\text{C(=O)S}-$, $-\text{OC(=S)}-$, $-\text{C(=S)O}-$, $-\text{SC(=S)}-$, $-\text{C(=S)S}-$, $-\text{S(=O)}-$, S(=O)_2 , $-\text{NR}^{10}\text{S(=O)}-$, $-\text{S(=O)NR}^{10}-$, $-\text{S(=O)}_2\text{NR}^{10}-$, -

$\text{NR}^{10}\text{-S(=O)}_2\text{-}$, -C(=O)- , -C(=O)O- , -OC(=O)- , $\text{-C(=O)NR}^{11}\text{-}$, $\text{-R}^{11}\text{NC(=O)-}$, -OC(=O)O- , aryl and heterocyclic.

25. The method of any of claims 17-24, wherein R^8 and R^9 are each independently a linear, saturated, non-substituted hydrocarbon chain having 1-10 carbon atoms in its backbone.

26. The method of claim 25, wherein said hydrocarbon chain has 2-4 carbon atoms.

27. The method of any of claims 18-26, wherein Z has said general formula II, wherein Ra is methoxy, Rb is hydrogen, Rc is chloro, Rd is methoxy and Re is hydrogen.

28. The method of claim 27, wherein A is said $\text{NR}^5\text{-C(=W)-NR}^6$, W is O or S and each of R^5 and R^6 is hydrogen.

29. The method of claim 16, wherein said compound has said general Formula III.

30. The method of claim 29, wherein said reverse transcriptase is wild type HIV-1 reverse transcriptase or a mutant thereof and at least one heteroatom in said B^1 and B^2 is capable of interacting with Glu138 of the p51 subunit in said HIV-1 reverse transcriptase and/or Tyr318 in the p66 subunit in said HIV-1 reverse transcriptase.

31. The method of claim 30, wherein said compound is Compound 3.

32. The method of any of claims 16-31, wherein said reverse transcriptase is selected from the group consisting of a wild type reverse transcriptase and a mutant thereof.

33. The method of claim 32, wherein said reverse transcriptase is an HIV-1 reverse transcriptase or a mutant thereof.

34. The method of claim 32, wherein said reverse transcriptase is an HIV-2 reverse transcriptase or a mutant thereof.

35. The method of any of claims 16-34, wherein said compound is characterized by a specific inhibition of said reverse transcriptase, as determined by the percent of residual activity of a cellular DNA polymerase in the presence of said compound divided by the percent of residual activity of said reverse transcriptase in the presence of said compound.

36. A pharmaceutical composition comprising, as an active ingredient, the compound as described in claims 16-31 and a pharmaceutically acceptable carrier, the composition being packaged in a packaging material and identified in print, in or on said packaging material, for use in the treatment of a viral infection caused by a retrovirus.

37. Use of a compound as described in any of claims 16-31 in the preparation of a medicament for treating a viral infection caused by a retrovirus.

38. A compound as described in any of claims 16-31, being identified for use in the treatment of a viral infection caused by a retrovirus.

39. A method of treating a viral infection caused by a retrovirus, the method comprising administering to a subject in need thereof a therapeutically effective amount of the compound as described in any of claims 16-31.

40. The method, use, compound or composition of any of claims 36-39, wherein said retrovirus is HIV-1.

41. The method, use, compound or composition of any of claims 36- 39, wherein said retrovirus is selected from the group consisting of HIV-1 and HIV-2.

42. The pharmaceutical composition of any of claims 13, 14 and 36, further comprising at least one agent capable of treating a viral infection caused by a retrovirus.

43. The use or compound of claims 37 and 38, wherein said compound is utilized in combination with at least one agent capable of treating a viral infection caused by a retrovirus.

44. The method of claim 39, further comprising administering to the subject a therapeutically effective amount of at least one agent capable of treating a viral infection caused by a retrovirus.

45. The method, use, compound or composition of any of claims 42-44, wherein said agent is selected from the group consisting of a protease inhibitor, a reverse transcriptase inhibitor, an integrase inhibitor and any combination thereof.

46. A method of identifying a candidate compound for inhibiting an activity of a reverse transcriptase, the method comprising computationally identifying a compound which is capable of specifically binding to a three-dimensional structure of an active site cavity of at least two crystalline forms of a reverse transcriptase, thereby identifying the candidate compound for inhibiting an activity of the type reverse transcriptase.

47. The method of claim 46, wherein said reverse transcriptase is selected from the group consisting of a HIV-1 reverse transcriptase and a HIV-2 reverse transcriptase.

48. The method of claim 46, wherein said reverse transcriptase is a wild type HIV-1 reverse transcriptase.

49. The method of any of claims 46 and 48, wherein said reverse transcriptase is a wild type HIV-1 reverse transcriptase and said crystalline forms of said HIV-1 reverse transcriptase have the pdb entry codes 1fk9 and 1dtq.

50. The method of any of claims 46-49, wherein said computationally identifying comprises:

- (a) obtaining at least two crystalline forms of said reverse transcriptase;
- (b) detecting a location of an active site in each of said crystalline forms of said reverse transcriptase;
- (c) generating a protomol for said active site in each said crystalline forms of said reverse transcriptase;
- (d) performing a docking process of compounds from a chemical library onto said active sites using said protomol; and
- (e) identifying a compound that both spatially and chemically fits to said three-dimensional structure of said active site cavities of each crystalline form of said reverse transcriptase.

51. The method of any of claims 46-50, further comprising:
biologically assaying said candidate compound for its activity in inhibiting a catalytic activity of said reverse transcriptase.

52. The method of claim 51, further comprising biologically assaying said candidate compound for its activity in treating a viral infection caused by a retrovirus.

53. The method of any of claims 51 and 52, being for identifying candidate compounds for treating a viral infection caused by a retrovirus.

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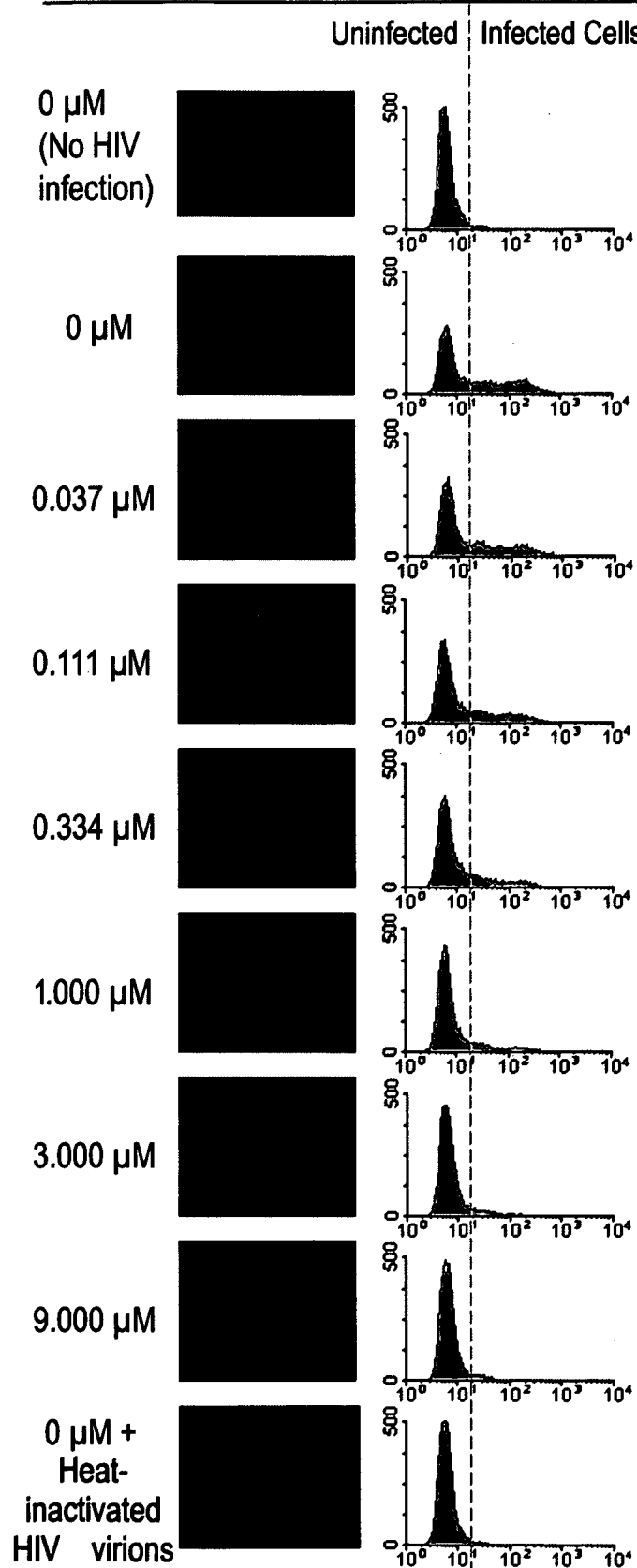
Concentration Microscopy Flow cytometry

FIG. 1A

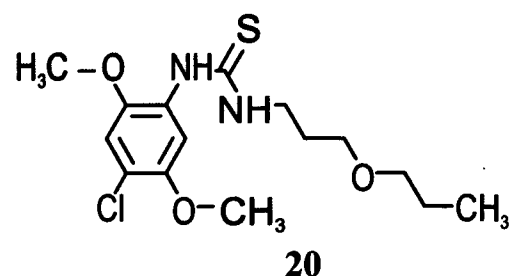


FIG. 1B

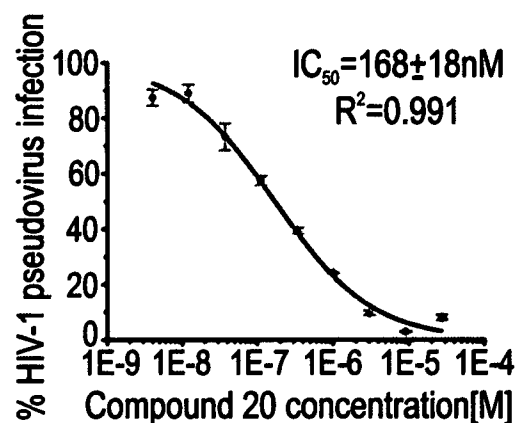


FIG. 1C

CompoundId	3	20
IC ₅₀ [nM]	374	168
CC ₅₀ [nM]	>138000	58000
TI	> 446	345

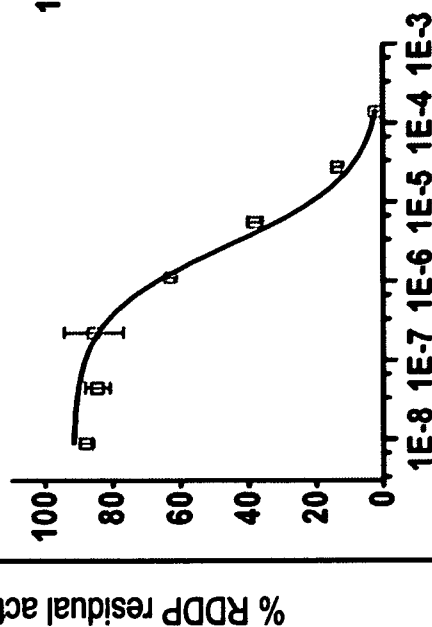
FIG. 1D

HIV-1 RT wild type

Compound 3

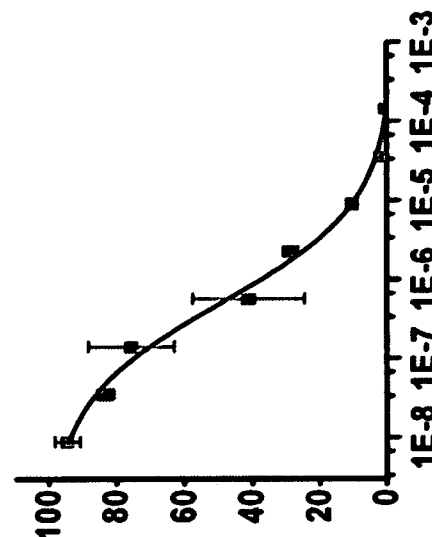
$$IC_{50} = 2.8 \pm 0.3 \mu M$$

$$s = 0.9 \pm 0.1, R^2 = 0.995$$

Compound 20

$$IC_{50} = 0.51 \pm 0.17 \mu M$$

$$s = 0.7 \pm 0.2, R^2 = 0.991$$

Nevirapine

$$IC_{50} = 1.7 \pm 0.2 \mu M$$

$$s = 1.1 \pm 0.1, R^2 = 0.991$$

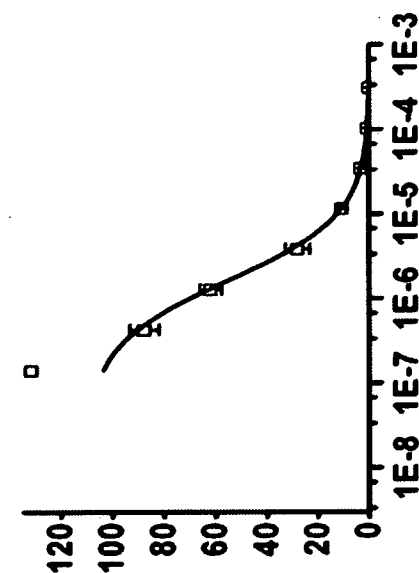


FIG. 2

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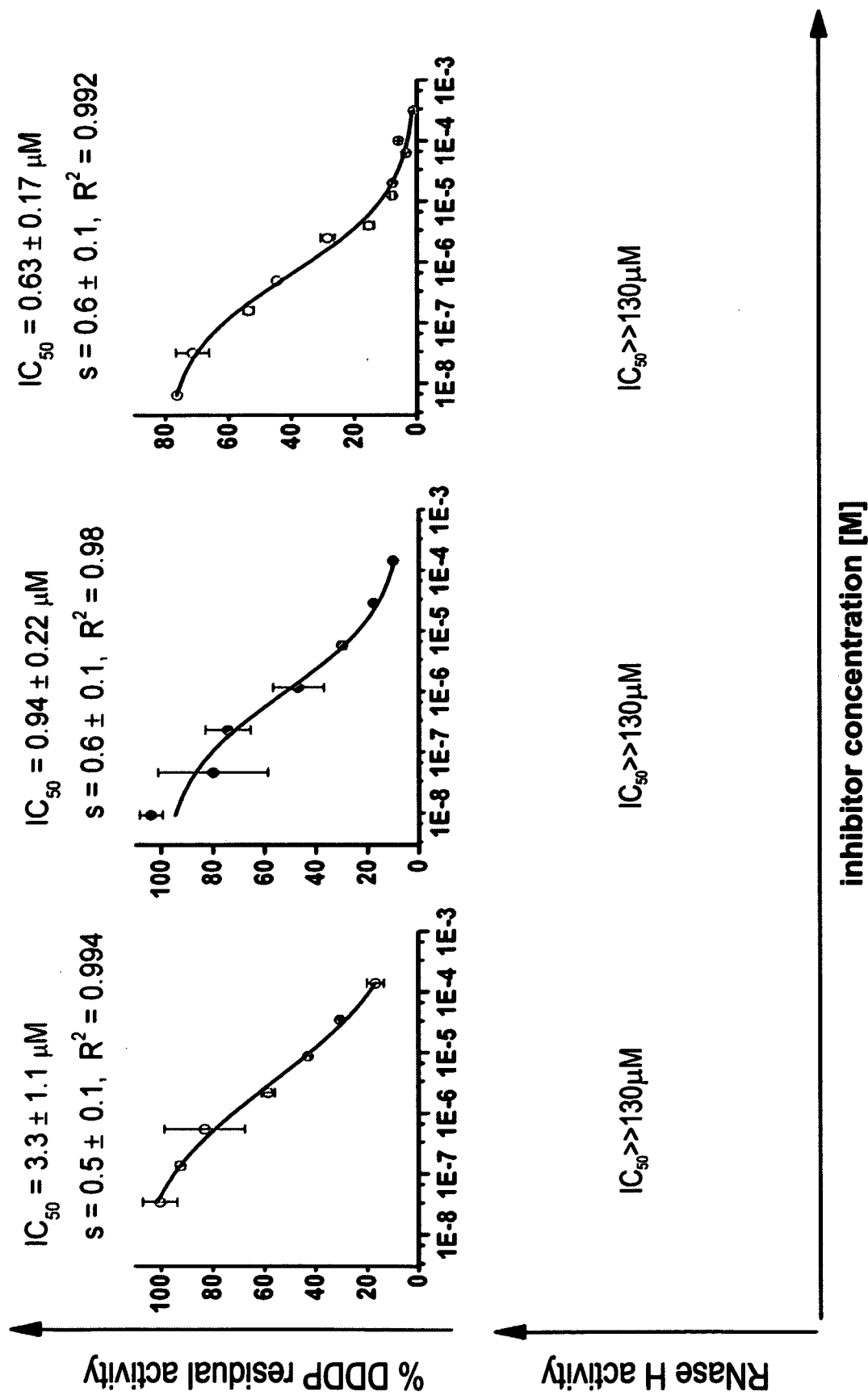


FIG. 2 cont.

HIV-1 Y181C RT

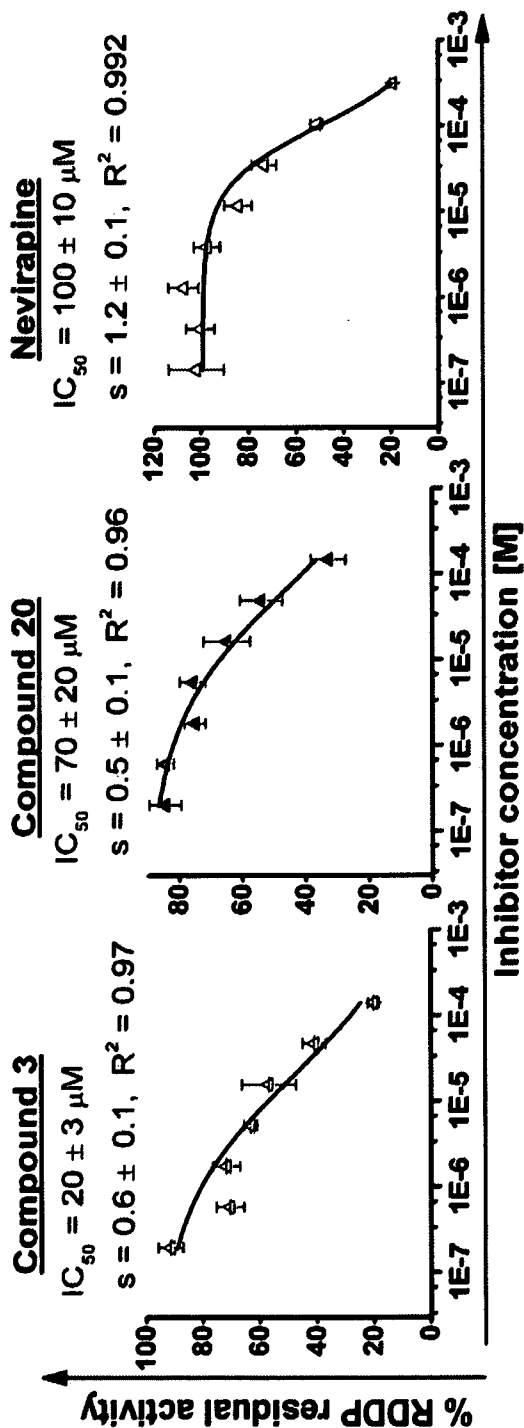


FIG. 3A

HIV-2 RT

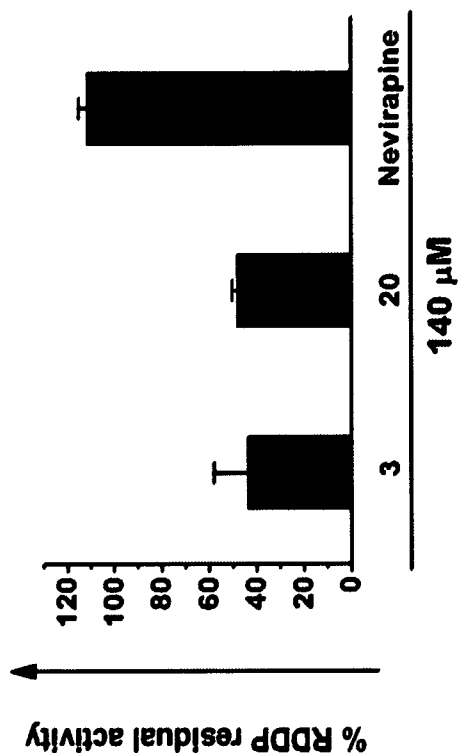


FIG. 3B

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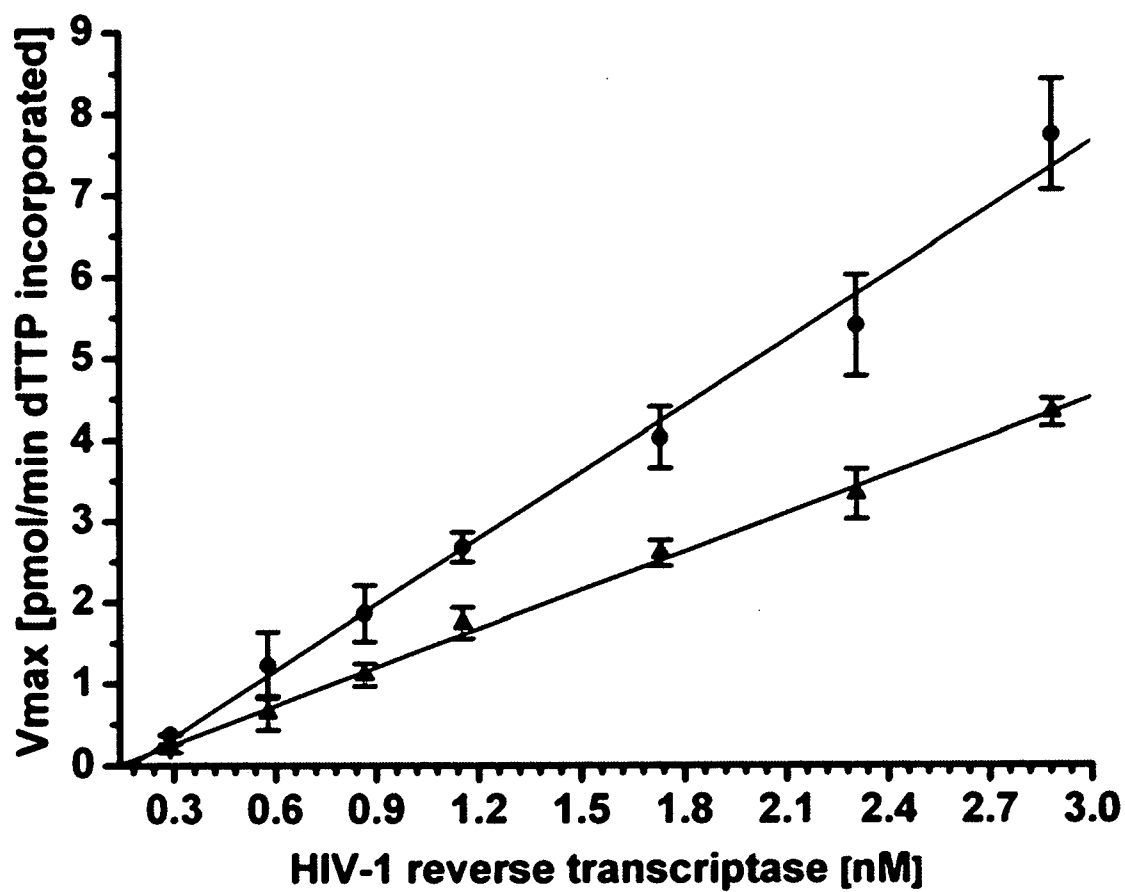
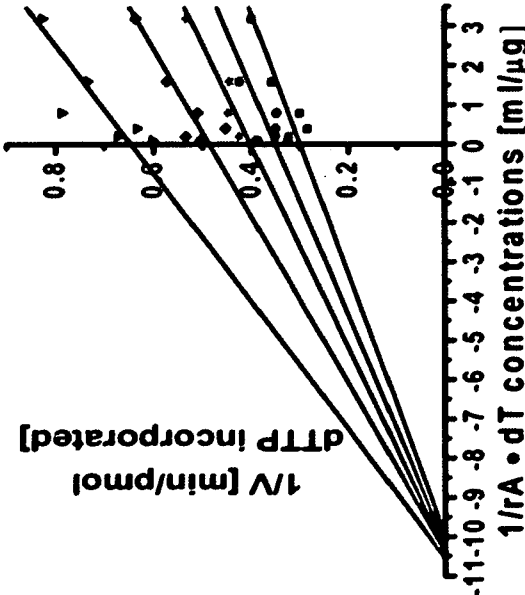
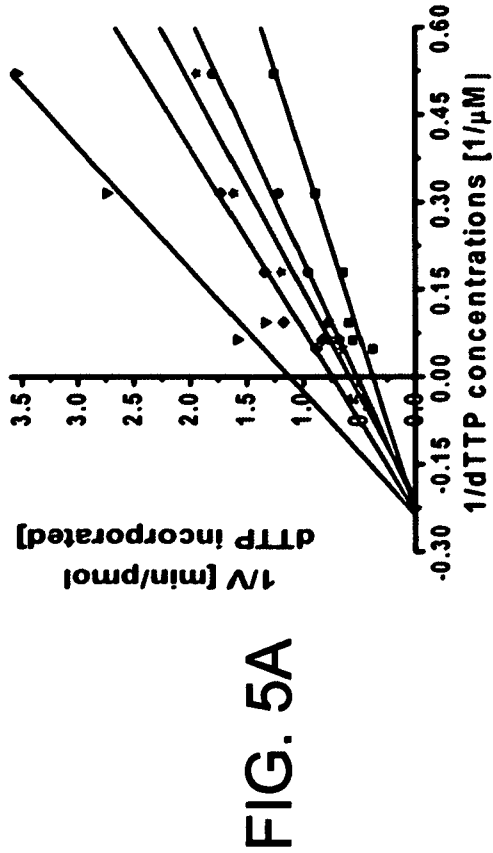
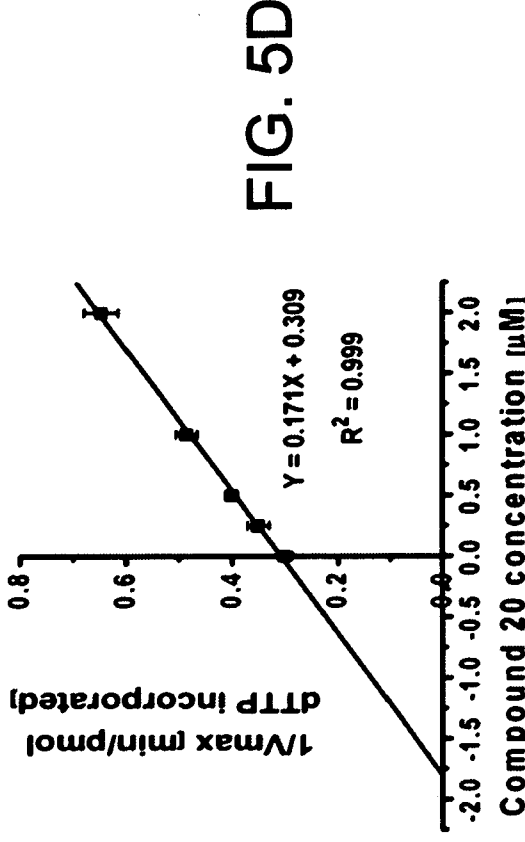
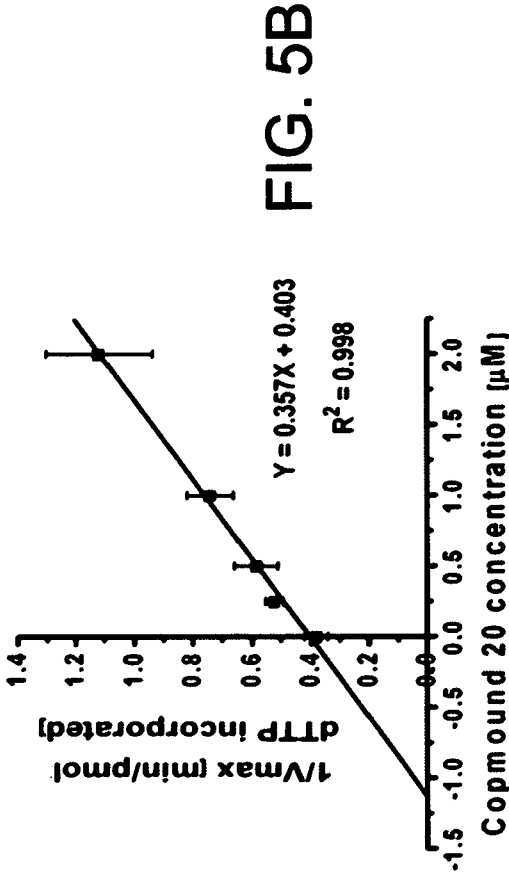


FIG. 4



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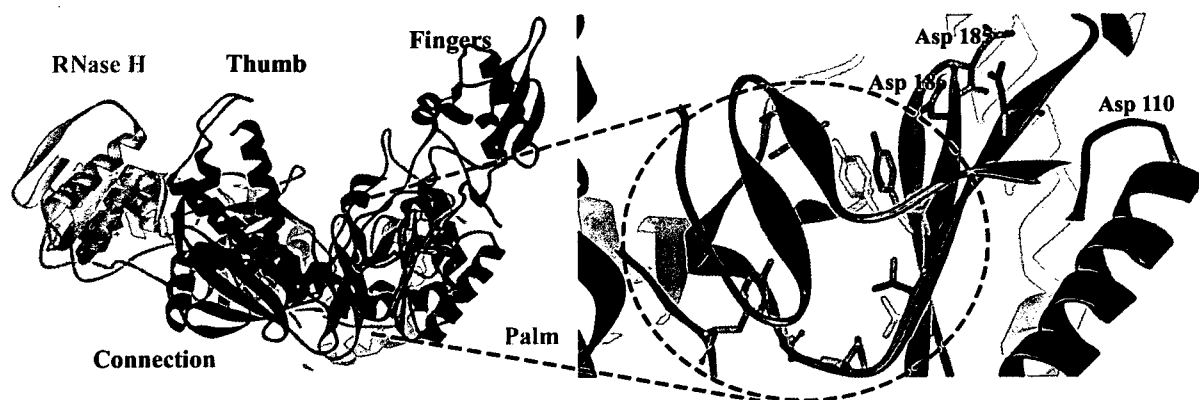
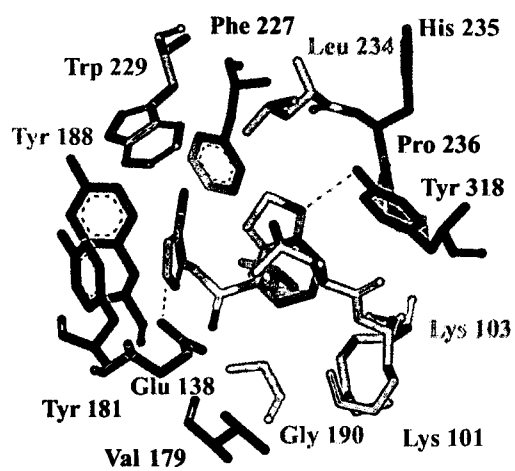
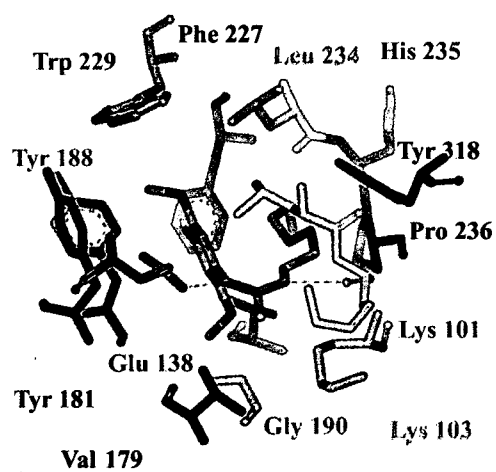


FIG.6A



Compound 3



Compound 20

FIG. 6B