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G. 知的所有権の出願・取得状況（予定を含む）

実績無し。

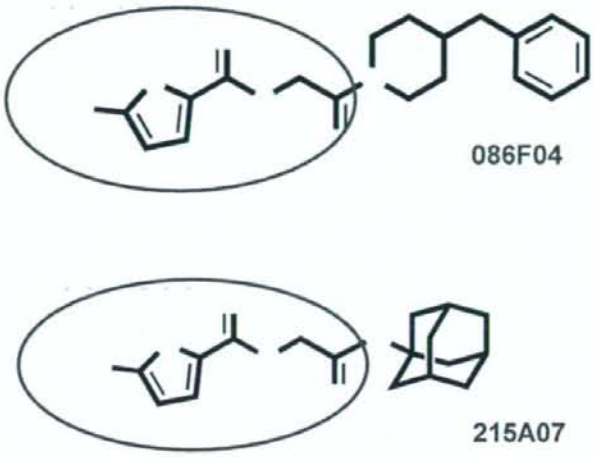


図 1. *都合により一部意図的に図を消去するなど修正を施しています。

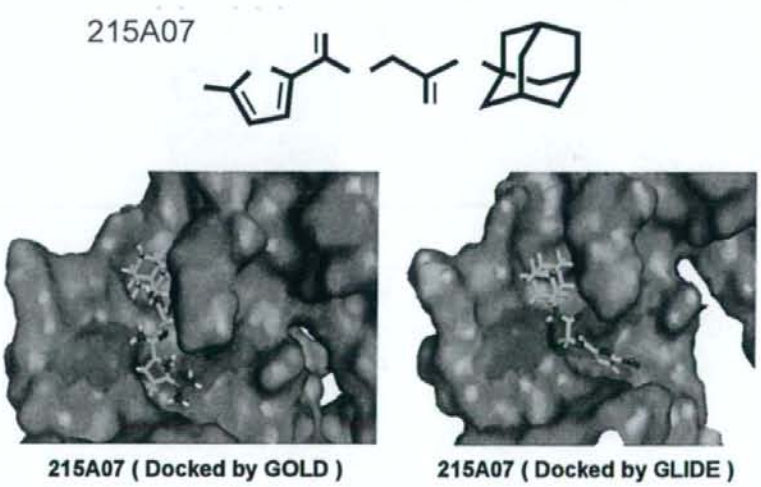


図 2. *都合により一部意図的に図を消去するなど修正を施しています。

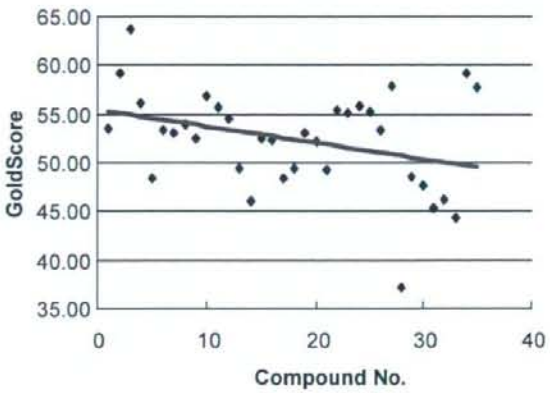

65.00
60.00
55.00
50.00
45.00
40.00
35.00
GoldScore
0
10
20
30
40
Compound No.

図3.

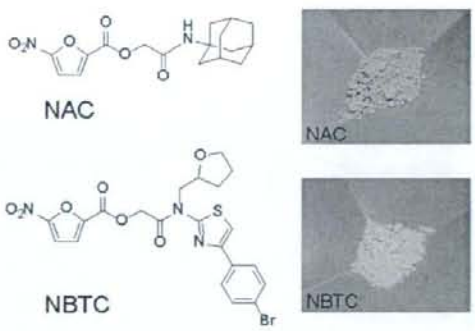

NAC
NBTC
Br
NAC
NBTC

図4.

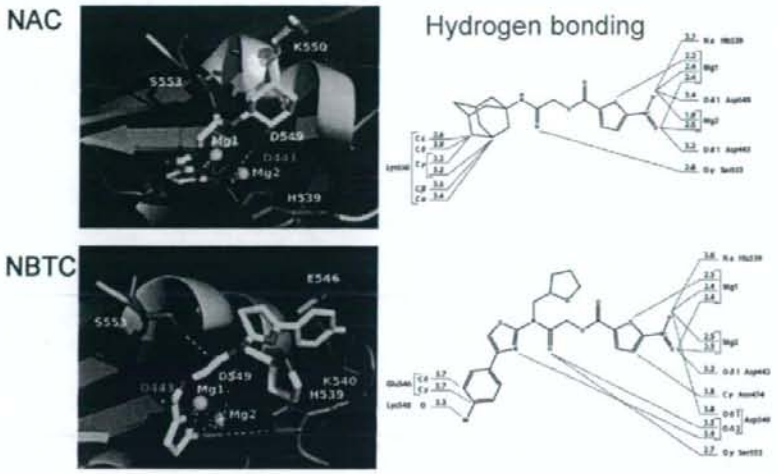

NAC
K550
S553
Mg1
D549
D443
Mg2
H539
Hydrogen bonding
NBTC
E546
S553
D549
D443
Mg1
K540
H539
Mg2

図5.

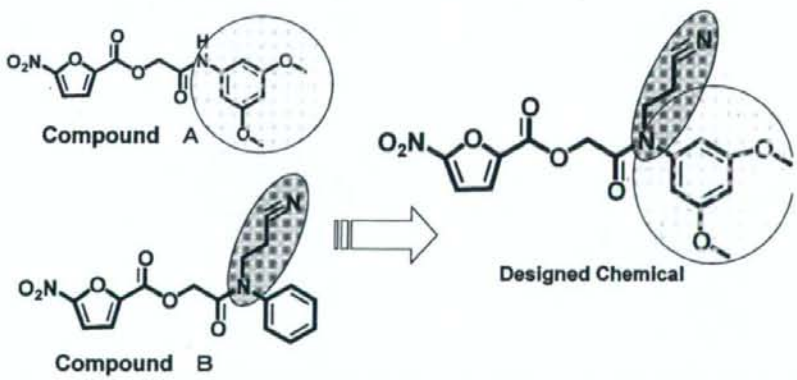

Compound A
Compound B
Designed Chemical

図6.

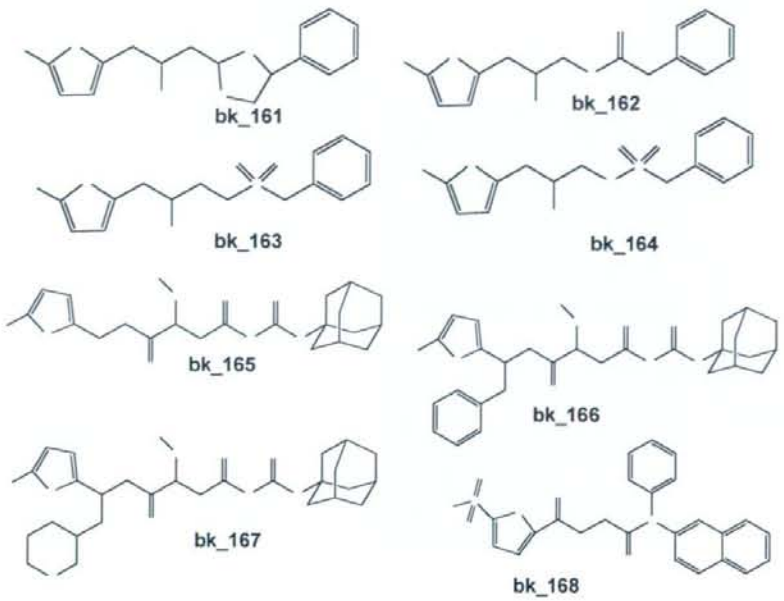


図７. *都合により一部意図的に図を消去するなど修正を施しています。

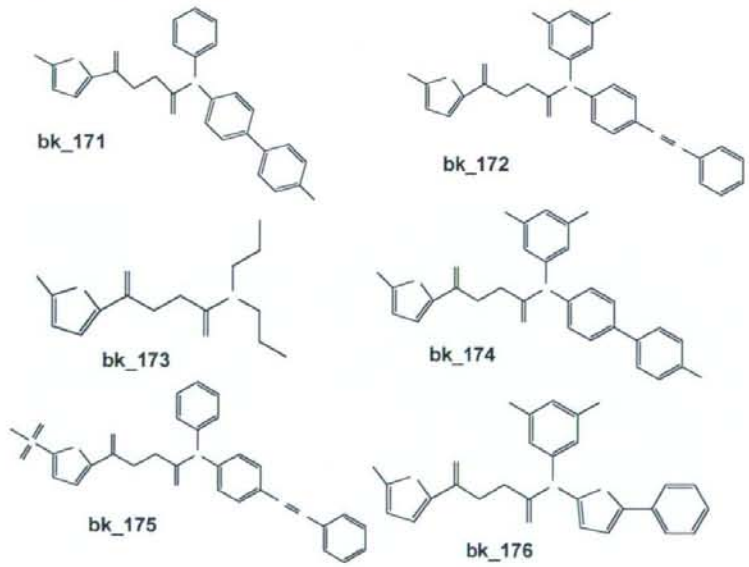


図８. *都合により一部意図的に図を消去するなど修正を施しています。

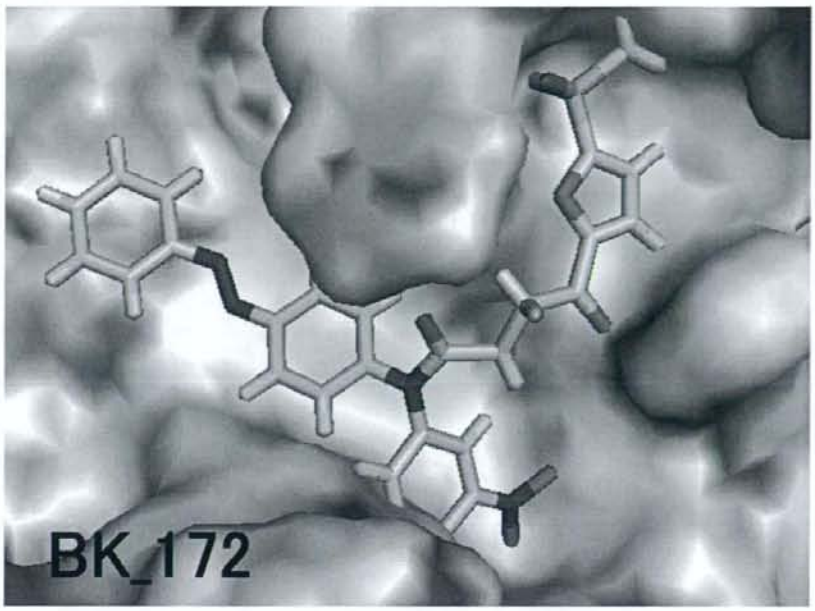
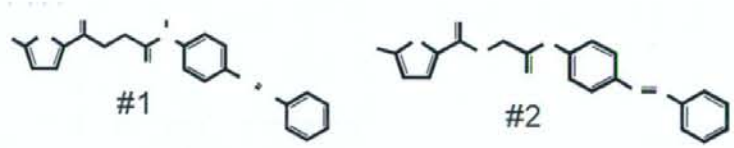


図９.



Procedure

① Build of chemical structure (ChemDraw) mol format saved
② Transform to pdb format (PRODRG Server)
③ Transform to mol2 format (nfutil)
④ Docking simulation (GOLD)
⑤ Select compound with high docking score
⑥ Evaluate the binding affinity (Original Orientation Software)

図１０.*都合により一部意図的に図を消去するなど修正を施しています。

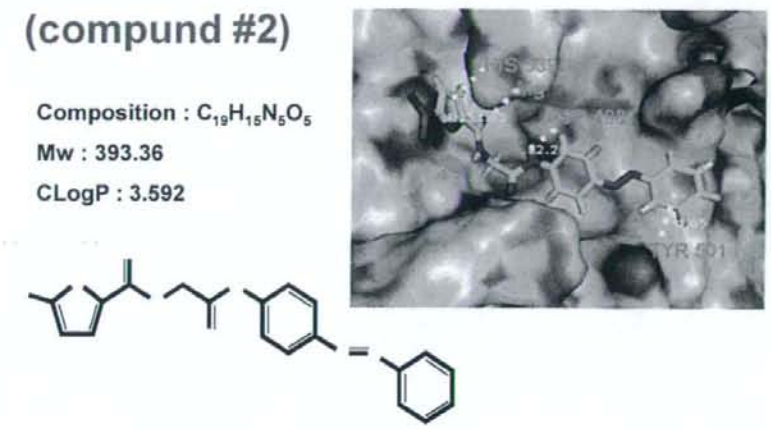


図１１.*都合により一部意図的に図を消去するなど修正を施しています。

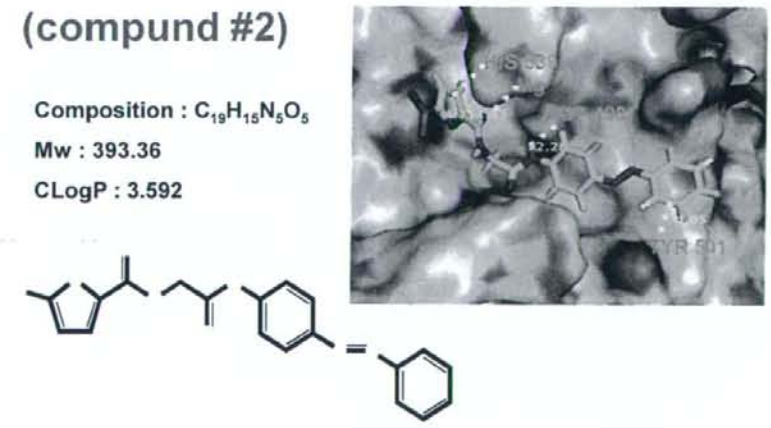


図１２.*都合により一部意図的に図を消去するなど修正を施しています。

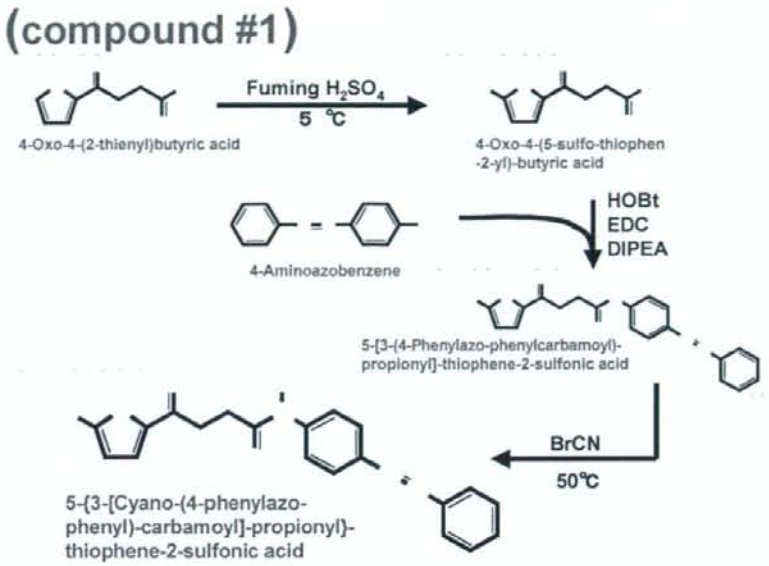


図１３.*都合により一部意図的に図を消去するなど修正を施しています。

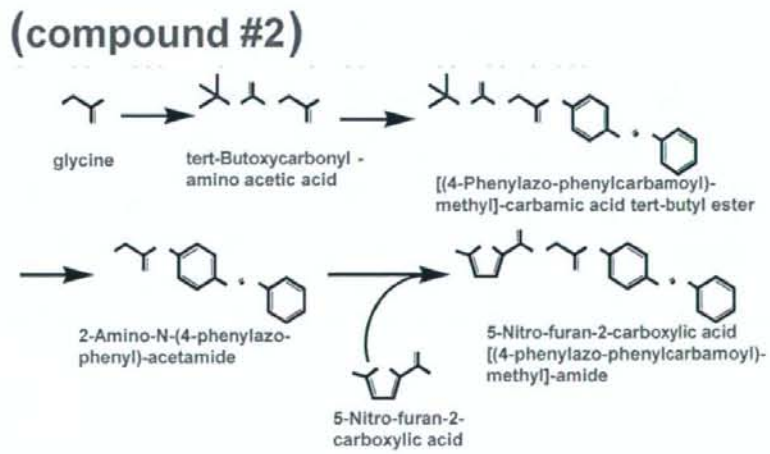


図１４.*都合により一部意図的に図を消去するなど修正を施しています。

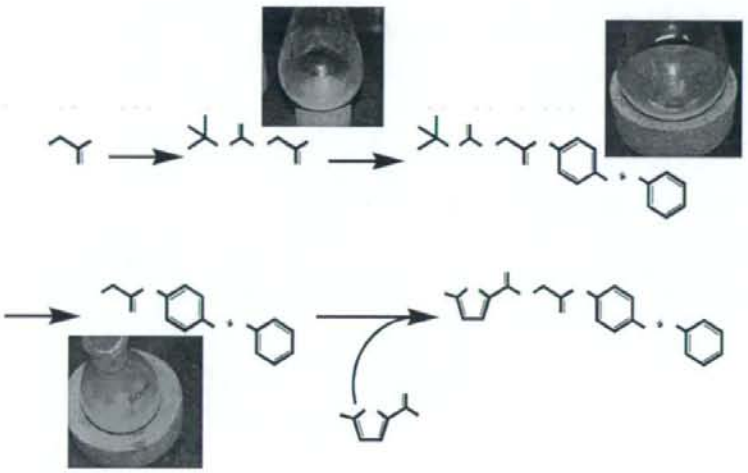


図１５.*都合により一部意図的に図を消去するなど修正を施しています。

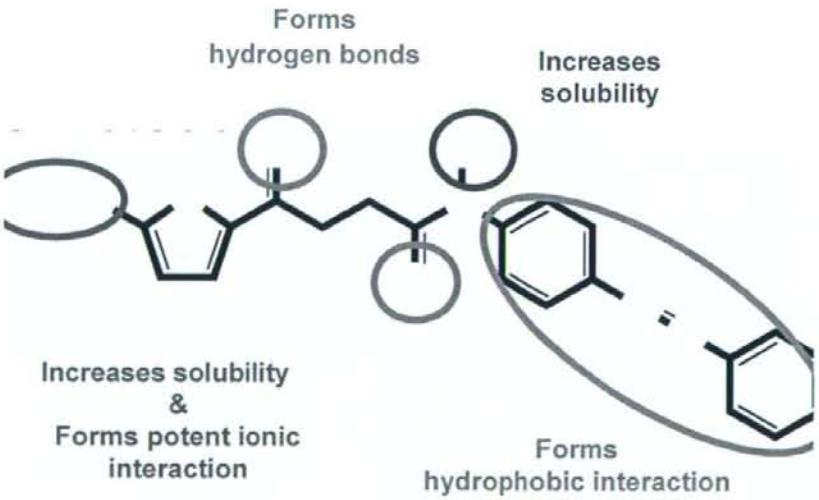


図１６.*都合により一部意図的に図を消去するなど修正を施しています。

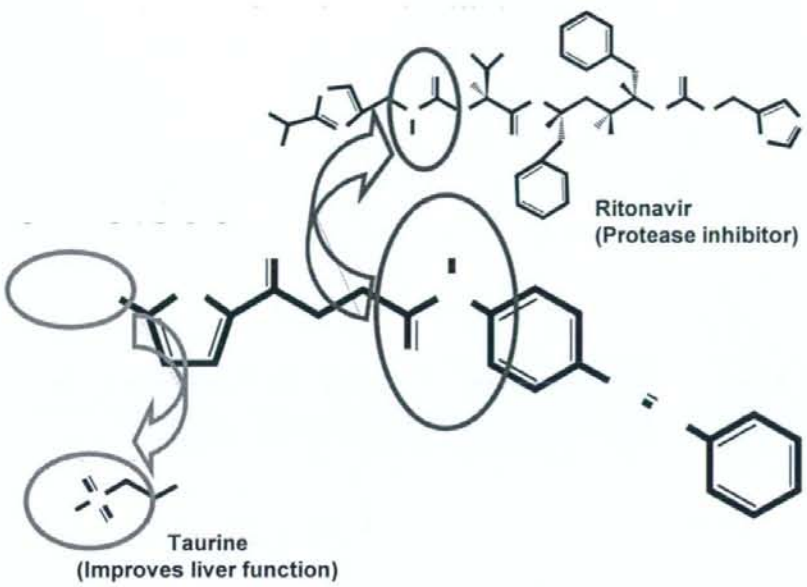


図１７.*都合により一部意図的に図を消去するなど修正を施しています。

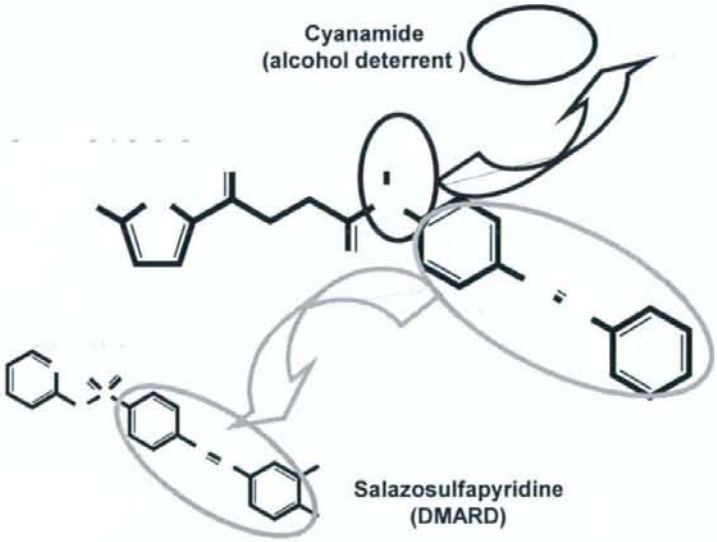


図１８.*都合により一部意図的に図を消去するなど修正を施しています。

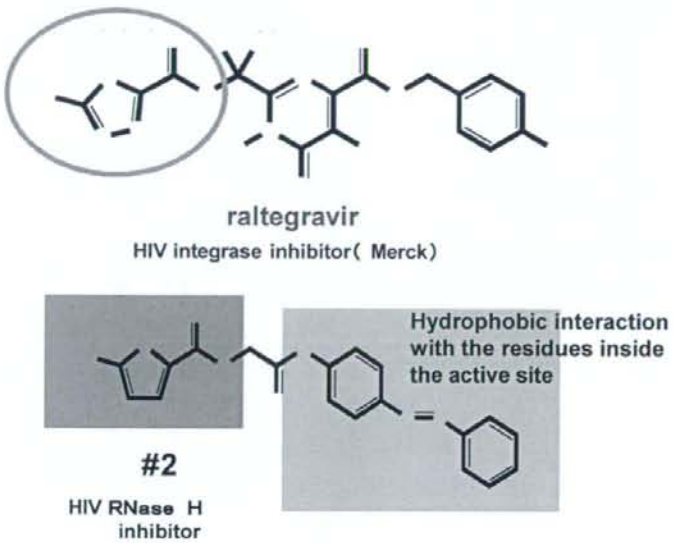


図１９.*都合により一部意図的に図を消去するなど修正を施しています。

III. 平成１８～２０年度
業績一覧

研究成果の刊行に関する一覧表

主任研究者　駒野　淳

書籍:なし

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IV. 平成１８～２０年度
刊行物別刷（抜粋）

Derivatives of 5-Nitro-furan-2-carboxylic Acid Carbamoylmethyl Ester Inhibit RNase H Activity Associated with HIV-1 Reverse Transcriptase

Hideyoshi Fuji,[†,‡] Emiko Urano,[†,§,⊥] Yuko Futahashi,[∥] Makiko Hamatake,[∥] Junko Tatsumi,[‡] Tyuji Hoshino,[‡] Yuko Morikawa,[§] Naoki Yamamoto,[∥] and Jun Komano*[,⊥]

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Received August 28, 2008

The RNase H activity associated with human immunodeficiency virus type 1 (HIV-1) is an attractive target for an antiretroviral drug development. We screened 20000 small-molecular-weight compounds for RNase H inhibitors and identified a novel RNase H-inhibiting structure characterized by a 5-nitro-furan-2-carboxylic acid carbamoylmethyl ester (NACME) moiety. Two NACME derivatives, 5-nitro-furan-2-carboxylic acid adamantan-1-carbamoylmethyl ester (compound **1**) and 5-nitro-furan-2-carboxylic acid [[4-(4-bromo-phenyl)-thiazol-2-yl]-(tetrahydro-furan-2-ylmethyl)-carbamoyl]-methyl ester (compound **2**), effectively blocked HIV-1 and MLV RT-associated RNase H activities with IC_{50}s of 3−30 μM but had little effect on bacterial RNase H activity in vitro. Additionally, 20−25 μM compound **2** effectively inhibited HIV-1 replication. An in silico docking simulation indicated that the conserved His539 residue, and two metal ions in the RNase H catalytic center are involved in RNase H inhibition by NACME derivatives. Taken together, these data suggest that NACME derivatives may be potent lead compounds for development of a novel class of antiretroviral drugs.

Introduction

Highly Active Anti-Retroviral Therapy, a combination of antiretroviral drugs, has become the standard treatment for HIV-infected individuals. However, the emergence of drug-resistant viruses is problematic because there are few alternative treatment regimens. HIV-1 has three known enzymes, protease, reverse transcriptase (RT), and integrase (IN). The RT, a heterodimer of p51 and p66 subunits, has three enzymatic functions: RNA- and DNA-dependent DNA polymerase and RNase H activity. Inhibitors of the protease, RT-associated polymerase, and IN activities are now in clinical use; however, a suitable inhibitor of the RT-associated RNase H activity has not been found. Thus, developing a highly specific inhibitor against HIV-1 RT-associated RNase H activity could provide another option for treatment of HIV-1-infected individuals.

RNase H activity is a ribonuclease activity that recognizes RNA hybridized to DNA. The RT-associated RNase H activity resides in the carboxy-terminal region of p66 and is essential for synthesis of double-stranded DNA from the HIV-1 single-stranded RNA genome. The RNase H activity is also involved in HIV-1 drug resistance to RT inhibitors as well as generating a diversity of viruses in vivo by homologous recombination.[1−7] Thus, RT-associated RNase H activity is another attractive target for development of a novel class of antiretroviral drugs.[8] Dual inhibitors that target RNase H and IN or RT-associated polymerase activities have been reported because these enzymes possess structural similarities.[9−11] Such inhibitors should be more effective than inhibitors that merely target RNase H activity.

The difficulty in developing an RNase H inhibitor for the treatment of HIV-1 infection lies in the specificity and toxicity of the drug. Although several RNase H inhibitors with different structures have been reported,[9,12−20] the 50% inhibitory concentration (IC_{50}) of many previous derivatives are on the order of micromolar concentration and they often lack sufficient specificity for the HIV-1 RT-associated RNase H activity. Most problematically, they often display cytotoxicity to mammalian cells. Many RNase H inhibitors are assumed to bind to the catalytic center and interact with Mg^{2+} ions. The toxicity of such inhibitors may be due to inhibition of other cellular proteins that require divalent metal cations to function.

More chemical compounds need to be tested in order to find a novel RNase H inhibitor with a chemical structure that can be used to design a potent and specific HIV-1 specific RNase H inhibitor. For a structure−function based approach to drug design, the chemical−enzyme interactions are examined by in silico docking simulations and the models critically assessed by comparing them to data obtained from experiments. This approach is possible because the crystallized protein structures of the HIV-1 RT and RNase H proteins from diverse organisms have been solved. Indeed, structure-based drug design has been successful in identifying several of the drugs currently available for HIV-1 treatment including protease inhibitors. Molecular docking simulations can reveal the molecular details of the interaction between HIV-1 RT and RNase H inhibitors.

In this study, we screened 20000 small molecular weight compounds to find chemicals that suppress the HIV-1 RT-associated RNase H activity. We found that derivatives of 5-nitro-furan-2-carboxylic acid carbamoylmethyl ester inhibit retroviral RNase H activity in vitro. One of the derivatives was

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[#] Abbreviations: HIV-1, human immunodeficiency virus type 1; RT, reverse transcriptase; NACME, 5-nitro-furan-2-carboxylic acid carbamoylmethyl ester; compound 1, 5-nitro-furan-2-carboxylic acid adamantan-1-carbamoylmethyl ester; compound 2, 5-nitro-furan-2-carboxylic acid [[4-(4-bromo-phenyl)-thiazol-2-yl]-(tetrahydro-furan-2-ylmethyl)-carbamoyl]-methyl ester.

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