Supporting Information

Discovery of Thiophene[3,2-d]pyrimidine Derivatives as Potent HIV-1 NNRTIs Targeting the Tolerant Region I of NNIBP

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Contents

1. Experimental section

All melting points were determined on a micro melting point apparatus (RY-1G, Tianjin TianGuang Optical Instruments) and are uncorrected. Proton nuclear magnetic resonance (^{1}H-NMR) and carbon nuclear magnetic resonance (^{13}C-NMR) spectra were recorded in DMSO-d_6 or $CDCl_3$ on a Bruker AV-400 spectrometer with tetramethylsilane (TMS) as the internal standard. Coupling constants are given in hertz, and chemical shifts are reported in δ values (ppm) from TMS; signals are abbreviated as s (singlet), d (doublet), t (triplet), q (quarter), and m (multiplet). A G1313A Standard LC Autosampler (Agilent) was used to collect samples for measurement of mass spectra. Analysis of sample purity was performed on a Shimadzu SPD-20A/20AV HPLC system using an Inertsil ODS-SP, 5 μm C18 column (150 mm × 4.6 mm). The temperature of the reaction mixture was monitored with a

mercury thermometer. All reactions were routinely monitored by thin layer chromatography (TLC) and spots were visualized with iodine vapor or by irradiation with UV light (λ = 254 nm). After completion of each reaction, the mixture was brought to ambient temperature *via* air-jet cooling. Flash column chromatography was performed on columns packed with Silica Gel (200-300 mesh), purchased from Qingdao Haiyang Chemical Company. Solvents were purified and dried with standard methods. Organic solutions were dried over anhydrous sodium sulfate and concentrated with a rotary evaporator under reduced pressure. Other reagents were obtained commercially and were used without further purification.

4-((2-chlorothieno[3,2-d]pyrimidin-4-yl)oxy)-3,5-dimethylbenzonitrile (5). A reaction mixture of 4-hydroxy-3,5-dimethylbenzonitrile (**4**, 1.5 g, 10 mmol) and potassium carbonate (1.7 g, 12 mmol) in 20 mL of DMF was stirred at room temperature for 15 min, and then 2,4-dichlorothiopheno[3,2-d]pyrimidine (0.21 g, 1 mmol) was added to it. Stirring was continued for an additional 1 h (monitored by TLC), then the mixture was poured into ice water and stirred for another 30 min. The formed precipitated white solid was collected by filtration, washed with cold water, and recrystallized in DMF-H_2O to provide the desired product **5** as a white solid in 92 % yield, mp: 258-260°C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.61 (d, J = 8.6 Hz, 1H, C_6-thienopyrimidine-H), 7.79 (s, 2H, C_3,C_5-Ph'-H), 7.70 (d, J = 7.7 Hz, 1H, C_7-thienopyrimidine-H), 2.14 (s, 6H). HRMS *m/z* $C_{15}H_{10}ClN_3OS$: calcd 315.0233, found 316.0326 $[M + H]^+$.

4-((2-((4-aminocyclohexyl)amino)thieno[3,2-d]pyrimidin-4-yl)oxy)-3,5-dimethylbenzonitrile (7). A solution of **5** (1.0 g, 3.17 mmol), *tert*-butyl (4-aminocyclohexyl)carbamate (0.82 g, 3.80 mmol), and anhydrous K_2CO_3 (0.87 g, 6.33 mmol) in 15 mL of DMF was heated at 120°C under magnetic stirring for 12 h (monitored by TLC). Then the solution was cooled to room temperature and 50 mL of ice water was added to it. Stirred for another15 minutes and the resulting precipitate was collected by filtration, and dried to give crude **6,** which was used directly in the next step without further purification. To a solution of **6** (1.26 g, 2.53 mmol) in DCM (5.0 mL) was added trifluoroacetic acid (TFA) (2.5 mL, 30 mmol) at ambient

temperature, and the solution was stirred for 3 h (monitored by TLC). Then, the solution was alkalized to pH 9 with saturated sodium bicarbonate solution and washed with saturated salt water (20 mL). The aqueous phase was extracted with DCM (3 × 5 mL). The combined organic phase was dried over anhydrous Na_2SO_4, filtered and purified by silica gel flash column chromatography to afford **7** as a white solid. Yield: 56%, mp: 185-190℃. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.72 (d, J = 5.4 Hz, 1H, C_6-thienopyrimidine-H), 7.36 (s, 2H, C_3,C_5-Ph'-H), 7.13 (d, J = 5.4 Hz, 1H, C_7-thienopyrimidine-H), 4.78 (s, 1H, NH), 3.06 (q, J = 7.3 Hz, 1H), 2.80-2.96 (m, 3H), 2.09 (s, 6H), 1.95-1.97 (m, 4H), 1.07-1.35 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 164.2, 161.7, 158.9, 152.4, 132.0, 131.3, 122.2, 117.6, 116.8, 113.9, 108.2, 59.3, 48.9, 44.7, 35.5, 29.4, 28.1, 15.3. HRMS *m/z* $C_{21}H_{23}N_5OS$: calcd 393.1623, found 394.1740 $[M + H]^+$.

General procedure for the preparation of final compounds 8a-e and 9a-e.

Compound **7** was dissolved in anhydrous DCM (10 mL) in the presence of TEA (1.2 eq), followed by addition of the appropriate substituted acyl chloride and sulfonyl chloride (1.2 eq). The reaction mixture was stirred at ambient temperature for 3-5 h (monitored by TLC), and then the reaction was added 10 mL DCM. The organic phase was washed with saturated sodium chloride (3 × 5 mL), then dried over anhydrous Na_2SO_4, filtered, and purified by flash column chromatography. The product was recrystallized from ethyl acetate/petroleum ether to afford the target compounds **8a-e** and **9a-e**.

***N*-(4-((4-(4-cyano-2,6-dimethylphenoxy)thieno[3,2-d]pyrimidin-2-yl)amino) cyclohexyl)benzamide (8a).** Recrystallized from EA/PE as a white solid, 67% yield, mp: 201-203℃. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.80 (d, J = 5.4 Hz, 1H, C_6-thienopyrimidine-H), 7.75-7.78 (m, 2H), 7.48-7.50 (m, 1H), 7.38-7.46 (m, 4H), 7.22 (d, J = 5.4 Hz, 1H, C_7-thienopyrimidine-H), 5.99 (s, 1H), 3.93 (s, 1H), 3.38-3.55 (m, 4H), 2.18 (s, 6H), 2.02-2.10 (m, 4H), 1.24-1.30 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 166.9, 165.3, 162.7, 160.1, 153.3, 134.7, 133.1, 132.2, 131.4, 128.5, 126.8, 123.2, 118.8, 109.4, 60.4, 49.8, 48.2, 31.7, 31.5, 16.4. HRMS *m/z* $C_{28}H_{27}N_5O_2S$: calcd 497.1885, found 498.1963 $[M + H]^+$, 520.1775 $[M + Na]^+$. HPLC purity: 98.97%.

***N*-(4-((4-(4-cyano-2,6-dimethylphenoxy)thieno[3,2-d]pyrimidin-2-yl)amino) cyclohexyl)-4-fluorobenzamide (8b).** Recrystallized from EA/PE as a white solid, 70% yield, mp: 220-223°C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.21 (d, J = 5.4 Hz, 1H, C_6-thienopyrimidine-H), 7.85-7.96 (m, 2H), 7.73 (s, 2H, C_3,C_5-Ph''-H), 7.27-7.31 (m, 3H), 5.98 (s, 1H), 3.71 (s, 1H), 3.23-3.30 (m, 1H), 2.14 (s, 6H), 1.83-2.00 (m, 4H), 1.32-1.47 (m, 5H). ^{13}C NMR (100 MHz, DMSO): δ 165.4, 164.9, 162.9, 162.4, 160.5, 153.5, 133.2, 131.7 (d, J = 2.8 Hz), 130.3 (d, J = 8.9 Hz), 123.7, 119.0, 115.6 (d, J = 21.6 Hz), 109.1, 60.2, 49.4, 48.5, 31.3, 16.2. HRMS *m/z* $C_{28}H_{26}FN_5O_2S$: calcd 515.1791, found 516.1867 $[M + H]^+$. HPLC purity: 97.93%.

4-bromo-*N*-(4-((4-(4-cyano-2,6-dimethylphenoxy)thieno[3,2-d]pyrimidin-2-yl) amino)cyclohexyl)benzamide (8c). Recrystallized from EA/PE as a white solid, 61% yield, mp: 219-221°C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.81 (d, J = 5.4 Hz, 1H, C_6-thienopyrimidine-H), 7.61 (d, J = 8.1 Hz, 2H, C_2,C_6-Ph'-H), 7.53 (d, J = 8.0 Hz, 2H, C_3,C_5-Ph'-H), 7.43 (s, 2H, C_3,C_5-Ph''-H), 7.21 (d, J = 5.4 Hz, 1H, C_7-thienopyrimidine-H), 5.97 (d, J = 8.0 Hz, 1H), 4.81 (s, 1H), 3.91 (s, 1H), 2.18 (s, 6H), 1.93-2.08 (m, 5H), 1.22-1.31 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 165.9, 165.3, 162.7, 160.1, 153.3, 135.1, 133.5, 133.1, 132.2, 131.7, 128.5, 126.0, 123.2, 118.8, 109.4, 49.7, 48.4, 31.4, 16.4. HRMS *m/z* $C_{28}H_{26}BrN_5O_2S$: calcd 575.0991, found 578.1056 $[M + 3]^+$. HPLC purity: 96.12%.

4-cyano-*N*-(4-((4-(4-cyano-2,6-dimethylphenoxy)thieno[3,2-d]pyrimidin-2-yl) amino)cyclohexyl)benzamide (8d). Recrystallized from EA/PE as a white solid, 66% yield, mp: 141-146°C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.81 (d, J = 5.3 Hz, 1H, C_6-thienopyrimidine-H), 7.61 (d, J = 8.0 Hz, 2H, C_2,C_6-Ph'-H), 7.54-7.58 (m, 2H), 7.42 (s, 2H, C_3,C_5-Ph''-H), 7.20 (d, J = 5.4 Hz, 1H, C_7-thienopyrimidine-H), 5.96 (s, 1H), 4.82 (s, 1H), 3.91-3.92 (m, 1H), 2.18 (s, 6H), 1.90-2.01 (m, 4H), 1.20-1.37 (m, 5H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 165.7, 165.0, 162.7, 160.4, 154.2, 135.7, 133.5, 133.4, 132.2, 131.6, 128.5, 126.7, 123.2, 118.9, 109.4, 49.2, 48.4, 31.4, 16.2. HRMS *m/z* $C_{29}H_{26}N_6O_2S$: calcd 522.1838, found 523.1916 $[M + H]^+$. HPLC purity: 98.69%.

***N*-(4-((4-(4-cyano-2,6-dimethylphenoxy)thieno[3,2-d]pyrimidin-2-yl)amino) cyclohexyl)-3-(trifluoromethyl)benzamide (8e).** Recrystallized from EA/PE as a

white solid, 58% yield, mp: 113-116°C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.81 (d, J = 5.3 Hz, 1H, C_6-thienopyrimidine-H), 7.61-7.63 (m, 1H), 7.50-7.57 (m, 3H), 7.42 (s, 2H, C_3,C_5-Ph''-H), 7.21 (d, J = 5.3 Hz, 1H, C_7-thienopyrimidine-H), 5.96 (s, 1H), 4.80 (s, 1H), 3.88-3.89 (m, 1H), 2.18 (s, 6H), 1.94-2.03 (m, 4H), 1.75-1.83 (m, 2H), 1.22-1.30 (m, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 165.7, 162.6, 160.4, 154.4, 135.3, 134.5, 133.7, 133.0, 132.1, 131.7, 128.5, 126.3, 123.2, 119.2, 109.4, 49.3, 48.4, 31.4, 16.3. HRMS *m/z* $C_{29}H_{26}F_3N_5O_2S$: calcd 565.1759, found 566.1836 $[M + H]^+$. HPLC purity: 97.14%.

***N*-(4-(*N*-(4-((4-(4-cyano-2,6-dimethylphenoxy)thieno[3,2-d]pyrimidin-2-yl) amino)cyclohexyl)sulfamoyl)phenyl)acetamide (9a).** Recrystallized from EA/PE as a white solid, 69% yield, mp: 252-254°C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.15 (d, J = 8.6 Hz, 2H, C_3,C_5-Ph'-H), 7.79 (d, J = 5.4 Hz, 1H, C_6-thienopyrimidine-H), 7.76 (d, J = 8.6 Hz, 2H, C_2,C_6-Ph'-H), 7.43 (s, 2H, C_3,C_5-Ph''-H), 7.16 (d, J = 5.4 Hz, 1H, C_7-thienopyrimidine-H), 4.78 (d, J = 7.6 Hz, 1H), 3.10-3.13 (m, 1H), 2.15 (s, 6H), 2.06 (s, 3H), 1.86-1.99 (m, 5H), 1.10-1.18 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 168.7, 165.3, 162.8, 160.4, 153.1, 140.7, 135.2, 133.4, 132.4, 132.0, 128.4, 127.6, 123.2, 118.9, 109.5, 106.2, 60.4, 58.6, 52.3, 49.2, 32.4, 31.2, 25.6, 16.2. HRMS *m/z* $C_{29}H_{30}N_6O_4S_2$: calcd 590.1770, found 591.1837 $[M + H]^+$. HPLC purity: 99.08%.

***N*-(4-((4-(4-cyano-2,6-dimethylphenoxy)thieno[3,2-d]pyrimidin-2-yl)amino) cyclohexyl)-4-fluorobenzenesulfonamide (9b).** Recrystallized from EA/PE as a white solid, 76% yield, mp: 187-190°C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.86-7.95 (m, 2H), 7.79 (d, J = 5.4 Hz, 1H, C_6-thienopyrimidine-H), 7.41 (s, 2H, C_3,C_5-Ph''-H), 7.14-7.24 (m, 3H), 4.72 (s, 1H), 3.07-3.09 (m, 1H), 2.15 (s, 6H), 1.81-1.95 (m, 5H), 1.08-1.14 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 166.2, 165.3, 163.7, 162.7, 160.0, 153.2, 137.4, 135.1, 133.0, 132.2, 129.5 (d, J = 9.2 Hz), 123.2, 118.7, 116.2 (d, J = 22.6 Hz), 109.5, 60.4, 52.2, 49.2, 32.4, 31.3, 16.4. HRMS *m/z* $C_{27}H_{26}FN_5O_3S_2$: calcd 551.1461, found 552.2164 $[M + H]^+$. HPLC purity: 98.49%.

4-bromo-*N*-(4-((4-(4-cyano-2,6-dimethylphenoxy)thieno[3,2-d]pyrimidin-2yl) amino)cyclohexyl)benzenesulfonamide (9c). Recrystallized from EA/PE as a white solid, 73% yield, mp: 235-237°C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.79 (d, J = 5.4 Hz,

1H, C_6-thienopyrimidine-H), 7.76 (d, *J* = 8.6 Hz, 2H, C_2,C_6-Ph'-H), 7.66 (d, *J* = 8.6 Hz, 2H, C_3,C_5-Ph'-H), 7.42 (s, 2H, C_3,C_5-Ph''-H), 7.18 (d, *J* = 5.4 Hz, 1H, C_7-thienopyrimidine-H), 4.76 (d, *J* = 7.5 Hz, 1H), 3.07-3.09 (m, 1H), 2.15 (s, 6H), 1.81-1.95 (m, 5H), 1.08-1.23 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$): *δ* 165.2, 162.6, 160.0, 153.2, 140.4, 135.1, 133.0, 132.4, 132.2, 128.4, 127.4, 123.2, 118.7, 109.5, 106.5, 60.4, 58.4, 52.3, 49.2, 32.4, 31.2, 16.4. HRMS *m/z* $C_{27}H_{26}BrN_5O_3S_2$: calcd 611.0660, found 614.0731 $[M + 3]^+$. HPLC purity: 96.77%.

4-cyano-*N*-(4-((4-(4-cyano-2,6-dimethylphenoxy)thieno[3,2-d]pyrimidin-2-yl)amino)cyclohexyl)benzenesulfonamide (9d). Recrystallized from EA/PE as a white solid, 70% yield, mp: 258-260°C. ^{1}H NMR (400 MHz, $CDCl_3$): *δ* 7.79 (d, *J* = 5.3 Hz, 1H, C_6-thienopyrimidine-H), 7.76 (d, *J* = 8.6 Hz, 2H, C_2,C_6-Ph'-H), 7.62-7.65 (m, 2H), 7.42 (s, 2H, C_3,C_5-Ph''-H), 7.18 (d, *J* = 5.1 Hz, 1H, C_7-thienopyrimidine-H), 4.71 (d, *J* = 7.3 Hz, 1H), 3.07-3.09 (m, 1H), 2.17 (s, 6H), 1.92-2.04 (m, 5H), 1.12-1.24 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$): *δ* 165.2, 162.2, 154.1, 140.4, 135.8, 133.4, 132.4, 132.0, 128.4, 127.6, 123.2, 119.1, 118.7, 109.5, 106.1, 60.4, 58.7, 52.3, 49.6, 32.4, 31.8, 16.2. HRMS *m/z* $C_{28}H_{26}N_6O_3S_2$: calcd 558.1508, found 559.2297 $[M + H]^+$. HPLC purity: 97.20%.

***N*-(4-((4-(4-cyano-2,6-dimethylphenoxy)thieno[3,2-d]pyrimidin-2yl)amino)cyclohexyl)-3-(trifluoromethyl)benzenesulfonamide (9e).** Recrystallized from EA/PE as a white solid, 65% yield, mp: 128-130°C. ^{1}H NMR (400 MHz, $CDCl_3$): *δ* 8.16 (s, 1H, C_2-Ph'-H), 8.08 (d, *J* = 8.1 Hz, 1H, C_4-Ph'-H), 7.83 (d, *J* = 8.0 Hz, 1H, C_6-Ph'-H), 7.79 (d, *J* = 5.4 Hz, 1H, C_6-thienopyrimidine-H), 7.69 (t, *J* = 7.9 Hz, 1H, C_5-Ph'-H), 7.42 (s, 2H, C_3,C_5-Ph''-H), 7.19 (d, *J* = 5.4 Hz, 1H, C_7-thienopyrimidine-H), 4.76 (d, *J* = 7.8 Hz, 1H), 3.15-3.20 (m, 1H), 2.15 (s, 6H), 1.81-1.95 (m, 5H), 1.08-1.14 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$): *δ* 165.1, 164.2, 162.7, 159.9, 153.2, 142.7, 133.0, 132.2, 131.6, 130.0 (d, *J* = 7.3 Hz), 129.2 (d, *J* = 3.7 Hz), 124.5, 123.9, 123.2, 121.8, 118.7, 109.5, 106.5, 52.5, 49.1, 32.4, 31.2, 16.4. HRMS *m/z* $C_{28}H_{26}F_3N_5O_3S_2$: calcd 601.1429, found 602.2193 $[M + H]^+$. HPLC purity: 96.16%.

2. Antiviral activity assays and cytotoxicity assays.

***In vitro* anti-HIV activities assays**

The anti-HIV activity and cytotoxicity of the newly synthesized compounds were evaluated with WT HIV-1 (strain HIV-IIIB), five single RT mutant strains of HIV-1 IIIB (L100I, K103N, E138K, Y181C, Y188L), two double RT mutant strains of HIV-1 IIIB (F227L/V106A and RES056), and HIV-2 (strain ROD) in MT-4 cell cultures using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method as reported previously[1]. At the starting of the experiment, stock solutions (10×final concentration) of test compounds were added in 25 μL volumes to two series of triplicate wells in order to allow simultaneous evaluation of their effects on mock- and HIV-infected cells. Serial five-fold dilutions of the test compounds (final 200 μL volume per well) were made directly in flat-bottomed 96-well microtiter trays, including untreated control HIV-1 and mock-infected cell samples for each sample, with a Biomek 3000 robot (Beckman Instruments, Fullerton, CA). HIV-1 (IIIB) and mutant HIV-1 strains (L100I, K103N, E138K, Y181C, Y188L, F227L/V106A, and RES056) or HIV-2 (ROD) stock (50 μL at 100-300 $CCID_{50}$) (50% cell culture infectious dose) or culture medium was added to either the infected or mock-infected wells of the microtiter tray. Mock-infected cells were used to evaluate the effect of test the newly synthesized compounds on uninfected cells in order to evaluate their cytotoxicity. Exponentially growing MT-4 cells were centrifuged for 5 min at 1000 rpm and the supernatant was discarded. The MT-4 cells were resuspended at 6×10^5 cells/mL, and 50 μL aliquots were transferred to the microtiter tray wells. At five days after infection, the viability of mock- and HIV-infected cells was determined spectrophotometrically with the MTT assay.

The MTT assay is based on the reduction of yellow-colored MTT (Acros Organics, Geel, Belgium) by mitochondrial dehydrogenase of metabolically active cells to form a blue-purple formazan that can be measured spectrophotometrically. The absorbances were read in an eight-channel computer-controlled photometer (Multiscan Ascent Reader, Labsystems, Helsinki, Finland), at the wavelengths of 540 and 690 nm. All data were calculated using the median optical density (OD) value of

two or three wells. The 50% effective antiviral concentration (EC_{50}) was defined as the concentration of the test compound affording 50% protection from viral cytopathogenicity and the 50% cytotoxic concentration (CC_{50}) was defined as the compound concentration that reduced the absorbance (OD_{540}) of mock-infected cells by 50%.

Recombinant HIV-1 reverse transcriptase (RT) inhibitory assays

The HIV-1 RT inhibition assay was evaluated with an RT assay kit (produced by Roche). All the reagents for performing the RT reaction are contained in the kit and the procedure for assaying HIV-1 RT inhibition was carried out as described in the kit protocol[2].

Briefly, the reaction mixture containing HIV-1 RT enzyme, reconstituted template and viral nucleotides [digoxigenin (DIG)-dUTP, biotin-dUTP and dTTP] in the incubation buffer with or without inhibitors was incubated for 1 h at 37ºC. Then, the reaction mixture was transferred to a streptavidin-coated microtitre plate (MTP) and incubated for another 1 h at 37ºC. The biotin-labeled dNTPs that were incorporated into the cDNA chain in the presence of RT were bound to streptavidin. The unbound dNTPs were washed with washing buffer, and anti-DIG-POD was added to the MTPs.

After incubation for 1 h at 37ºC, the DIG-labeled dNTPs incorporated in cDNA were bound to the anti-DIG-POD antibody. The unbound anti-DIG-PODs were washed out and the peroxide substrate (ABST) solution was added to the MTPs. The absorbance of the sample was determined at OD_{405} nm using a microtiter plate ELISA reader. The IC_{50} values correspond to the concentrations of the inhibitors required to inhibit biotin-dUTP incorporation by 50%.

3. Molecular simulations

Molecular simulations was performed with the Tripos molecular modeling package Sybyl-X 2.0. All the molecules for docking were built using standard bond lengths and angles from Sybyl-X 2.0/Base Builder and were optimized using the Tripos force field for 1000 generations two times or more, until the minimized

conformers of the ligand were the same. The flexible docking method (Surflex-Dock) docks the ligand automatically into the ligand-binding site of the receptor by using a protocol-based approach and an empirically derived scoring function. The protocol is a computational representation of a putative ligand that binds to the intended binding site and is a unique and essential element of the docking algorithm. The scoring function in Surflex-Dock, containing hydrophobic, polar, repulsive, entropic, and solvation terms, was trained to estimate the dissociation constant (Kd) expressed as -log $(Kd)^2$. The protein was prepared by removing the ligand, water molecules and other unnecessary small molecules from the crystal structure of the ligand HIV-1 RT complex (PDB code: 3M8Q) before docking; polar hydrogen atoms and charges were added to the protein. Surflex-Dock default settings were used for other parameters, such as the maximum number of rotatable bonds per molecule (set to 100), and the maximum number of poses per ligand (set to 20). During the docking procedure, all of the single bonds in residue side-chains inside the defined RT binding pocket were regarded as rotatable or flexible, and the ligand was allowed to rotate at all single bonds and to move flexibly within the tentative binding pocket. The atomic charges were recalculated using the Kollman all-atom approach for the protein and the Gasteiger-Hückel approach for the ligand. The binding interaction energy was calculated, including van der Waals, electrostatic, and torsional energy terms defined in the Tripos force field. The structure optimization was performed for 10,000 generations using a genetic algorithm, and the 20-best-scoring ligand-protein complexes were kept for further analysis. The -log $(Kd)^2$ values of the 20-best-scoring complexes, representing the binding affinities of ligand with RT, encompassed a wide range of functional classes (10^{-2}-10^{-9}). Therefore, only the highest-scoring 3D structural model of ligand-bound RT was chosen to define the binding interaction.

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