Synthesis and Biological Evaluation of New Conformationally Restricted *S*-DABO Hybrids as Non-nucleoside Inhibitors of HIV-1 Reverse Transcriptase

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Contents:

Chemical experiment

Melting points were measured on a SGWX-1 microscopic melting-point apparatus. ^{1}H-NMR and ^{13}C-NMR spectra on a Bruker AV 400MHz spectrometer were recorded in $CDCl_3$. Chemical shifts are reported in δ (ppm) units relative to the internal standard tetramethylsilane (TMS). Mass spectra were obtained on a Waters Quattro Micromass instrument using electrospray ionization (ESI) techniques. All chemicals and solvents used were of reagent grade and were purified and dried by standard methods before use. All air-sensitive reactions were carried out under a nitrogen atmosphere. All the reactions were monitored by thin layer chromatography (TLC) on pre-coated silica gel G plates at 254 nm under a UV lamp using ethyl acetate/hexane as eluent. Column chromatography separations were obtained on silica gel (300~400 mesh) using EtOAc and hexane as eluents.

General procedure for preparation of target compounds

To a solution of **7a~c** (10mmol) in anhydrous DMF (15 mL) were added appropriate aryl acetonitriles (12 mmol). After stirring at –10℃ for about 15 min, 60% NaH (0.48 g, 12 mmol) was added portion wise under a nitrogen atmosphere and maintained at this condition for 1h. Then, the resulting mixture was warmed slowly to room temperature and continued to react for 12~24 h. After that, additional NaH (0.48 g, 12 mmol) was added and the air was introduced, the mixture was stirred at room temperature for another 12~24 h to yield **8a~u**. Without purification, **8a~u** were hydrolyzed with 30% aqueous sodium hydroxide at room temperature for 4 h, and afterwards, the mixture was poured into 300 ml H_2O and neutralized with 3N HCl. The precipitate was collected and purified by column chromatography on silica gel (hexane : EtOAc = 8:1 to 1:1) to afforded the target compounds **1a~u**.

2-(2-(4-methoxybenzylthio)-5-methyl-6-oxo-1,6-dihydropyrimidin-4-yl)-2-(3-fluorophenyl)acetonitrile (**1a**) Yield 47%; white solid, mp 164.3-165.1 °C; ^{1}H NMR (CDC,l_3) δ (ppm): 2.07 (s, 3 H, CH_3), 3.78 (s, 3 H, OCH_3), 4.34 (d, 1 H, J = 14.0 Hz, CH_2), 4.44 (d, 1H, J = 13.6 Hz, CH_2), 5.22 (s, 1 H, CH), 6.78-7.33 (m, 8 H, Ph), 11.94 (s, 1 H, Py); ^{13}C NMR ($CDCl_3$) δ: 10.5, 34.5, 40.9, 55.3, 114.0, 116.2 (d, 2 C, $J_{C\text{-}C\text{-}F}$ = 21.8 Hz), 117.1, 117.7, 128.1, 128.9 (d, $J_{C4\text{-}F}$ = 3.3 Hz), 129.6 (d, 2 C, $J_{C3\text{-}F}$ = 8.3 Hz), 130.2, 155.5, 158.3, 159.1, 162.7 (d, $J_{C\text{-}F}$ = 247.2 Hz), 164.5; MS (ESI) *m/z* 396.15 $(M+H)^+$.

2-(2-(4-methoxybenzylthio)-5-methyl-6-oxo-1,6-dihydropyrimidin-4-yl)-2-(4-fluorophenyl)acetonitrile(**1b**) Yield 53%; pale yellow solid, mp 162.4-163.3 °C; ^{1}H NMR ($CDCl_3$) δ (ppm): 2.06 (s, 3 H, CH3), 3.78 (s, 3 H, OCH_3), 4.33 (d, 1 H, J = 13.6 Hz, CH_2), 4.45 (d, 1 H, J = 13.6 Hz, CH_2), 5.20 (s, 1 H, CH), 6.78-7.32 (m, 8 H, Ph), 12.60 (s, 1 H, Py);^{13}C NMR ($CDCl_3$) δ: 10.5, 34.4, 40.9, 55.2, 114.0, 116.2 (d, 2 C, $J_{C\text{-}C\text{-}F}$ = 21.9 Hz), 117.0, 117.7, 128.1, 128.9 (d, $J_{C4\text{-}F}$ = 3.2 Hz), 129.6 (d, 2 C, $J_{C3\text{-}F}$ = 8.3 Hz), 130.2, 155.6, 158.4, 159.1, 162.7 (d, $J_{C\text{-}F}$ = 247.2 Hz), 164.9; MS (ESI) *m/z* 396.16 $(M+H)^+$.

2-(2-(4-methoxybenzylthio)-5-methyl-6-oxo-1,6-dihydropyrimidin-4-yl)-2-(2-chlorophenyl)acetonitrile(**1c**) Yield 52%; white solid, mp 166.8-167.9 °C; ^{1}H NMR ($CDCl_3$) δ (ppm): 2.08 (s, 3 H, CH_3), 3.78 (s, 3 H, OCH_3), 4.23 (d, 1 H, J = 13.6 Hz, CH_2), 4.32 (d, 1 H, J = 14.0 Hz, CH_2), 5.63 (s, 1 H, CH), 6.77-7.40 (m, 8 H, Ph), 12.77 (s, 1 H, Py); ^{13}C NMR ($CDCl_3$) δ: 10.5, 34.3, 38.5, 55.2, 114.0, 117.2, 117.9, 127.5, 128.2, 129.7, 130.1, 130.2, 154.5, 158.1, 159.0, 165.0; MS (ESI) *m/z* 412.09 $(^{35}M+H)^+$, 414.04 $(^{37}M+H)^+$.

2-(2-(4-methoxybenzylthio)-5-methyl-6-oxo-1,6-dihydropyrimidin-4-yl)-2-(3-chlorophenyl)acetonitrile(**1d**) Yield 58%; white solid, mp 151.5-152.5 °C; ^{1}H NMR ($CDCl_3$) δ (ppm): 2.08 (s, 3 H, CH_3), 3.78 (s, 3 H, OCH_3), 4.33 (d, 1 H, J = 14.0 Hz, CH_2), 4.44 (d, 1 H, J = 14.0 Hz, CH_2), 5.20 (s, 1 H, CH), 6.78-7.37 (m, 8 H, Ph), 12.40 (s, 1 H, Py);^{13}C NMR ($CDCl_3$) δ: 10.6, 34.5, 41.2, 55.2, 114.0, 117.3, 126.0, 128.0, 130.2, 134.8, 135.1, 155.1, 158.5, 159.1, 164.7; MS (ESI) *m/z* 412.09 $(^{35}M+H)^+$, 414.10 $(^{37}M+H)^+$.

2-(2-(4-methoxybenzylthio)-5-methyl-6-oxo-1,6-dihydropyrimidin-4-yl)-2-(4-chlorophenyl)acetonitrile(**1e**) Yield 65%; white solid, mp 175.1-176.0 °C;^{1}H NMR ($CDCl_3$) δ (ppm): 2.07 (s, 3 H, CH_3), 3.79 (s, 3 H, OCH_3), 4.30 (d, 1 H, J = 14.0 Hz, CH_2), 4.45 (d, 1 H, J = 13.6 Hz, CH_2), 5.20 (s, 1 H, CH), 6.78-7.33 (m, 8 H, Ph), 12.63 (s, 1 H, Py); ^{13}C NMR ($CDCl_3$) δ: 10.5, 34.4, 41.0, 55.2, 114.0, 117.0, 117.5, 128.1, 129.1, 129.4, 130.2, 131.5, 134.8, 155.4, 158.5, 159.0, 164.9; MS (ESI) *m/z* 412.14 (^{35}M+H)$^+$, 414.03 (^{37}M+H)$^+$.

2-(2-(4-methoxybenzylthio)-5-methyl-6-oxo-1,6-dihydropyrimidin-4-yl)-2-(2-bromophenyl)acetonitrile(**1f**) Yield 48%; white solid, mp 174.1-175.0 °C; ^{1}H NMR ($CDCl_3$) δ (ppm): 2.09 (s, 3 H, CH_3), 3.78 (s, 3 H, OCH_3), 4.21 (d, 1 H, J = 14.0 Hz, CH_2), 4.31 (d, 1 H, J = 13.6 Hz, CH_2), 5.61 (s, 1 H, CH), 6.77-7.59 (m, 8 H, Ph), 12.31 (s, 1 H, Py); ^{13}C NMR ($CDCl_3$) δ: 10.6, 34.4, 41.2, 55.3, 114.0, 117.1, 118.2, 123.4, 128.1, 128.2, 130.2, 133.1, 154.5, 158.0, 159.1, 164.6; MS (ESI) *m/z* 456.00 (^{79}M+H)$^+$, 457.95 (^{81}M+H)$^+$.

2-(2-(4-methoxybenzylthio)-5-methyl-6-oxo-1,6-dihydropyrimidin-4-yl)-2-(3-bromophenyl)acetonitrile(**1g**) Yield 52%; yellow solid, mp 169.0-170.3 °C; ^{1}H NMR ($CDCl_3$) δ (ppm): 2.07 (s, 3 H, CH_3), 3.78 (s, 3 H, OCH_3), 4.33 (d, 1 H, J = 13.6 Hz, CH_2), 4.44 (d, 1 H, J = 13.6 Hz, CH_2), 5.20 (s, 1 H, CH), 6.78-7.53 (m, 8 H, Ph), 12.62 (s, 1 H, Py); ^{13}C NMR ($CDCl_3$) δ: 10.6, 34.5, 41.1, 55.2, 114.0, 117.3, 123.1, 126.5, 128.0, 130.2, 130.7, 130.8, 155.1, 158.6, 159.1, 164.8; MS (ESI) *m/z* 456.00 (^{79}M+H)$^+$, 458.08 (^{81}M+H)$^+$.

2-(2-(4-methoxybenzylthio)-5-ethyl-6-oxo-1,6-dihydropyrimidin-4-yl)-2-(2-fluorophenyl)acetonitrile(**1h**) Yield 47%; white solid, mp 175.5-177.4 °C; ^{1}H NMR ($CDCl_3$) δ (ppm): 1.06 (t, 3 H, J = 7.4 Hz, CH_3), 2.49-2.59(m, 2 H, CH_2), 3.79 (s, 3 H, OCH_3), 4.31 (d, 1 H, J = 13.8 Hz CH_2), 4.38 (d, 1 H, J = 13.7 Hz CH_2), 5.54 (s, 1 H, CH), 6.79-7.54 (m, 8 H, Ph), 12.28 (s, 1 H, Py); ^{13}C NMR ($CDCl_3$) δ: 12.7, 18.7, 34.6 (d, $J_{C3\text{-}F}$ = 4.5 Hz), 34.4, 55.3, 114.0, 115.7 (d, 2 C, $J_{C\text{-}C\text{-}F}$ = 21.8 Hz), 117.5, 120.8, 120.9, 123.4, 124.8 (d, $J_{C4\text{-}F}$ = 3.5 Hz), 128.2, 130.23 130.3 (d, $J_{C4\text{-}F}$ = 2.4 Hz), 130.7 (d, 2 C, $J_{C3\text{-}F}$ = 8.2 Hz,), 154.0, 158.3, 159.1, 159.4 (d, $J_{C\text{-}F}$ = 246.8 Hz); MS (ESI) *m/z* 410.14 (M+H)$^+$.

2-(2-(4-methoxybenzylthio)-5-ethyl-6-oxo-1,6-dihydropyrimidin-4-yl)-2-(3-fluorophenyl)acetonitrile(**1i**) Yield 60%; white solid, mp 128.6-130.5 °C; ^{1}H NMR ($CDCl_3$) δ (ppm): 1.07 (t, 3 H, J = 7.4 Hz CH_3), 2.44-2.58(m, 2 H, CH_2), 3.78 (s, 3 H, OCH_3), 4.31 (d, 1 H, J = 13.6 Hz, CH_2), 4.42 (d, 1 H, J = 13.6 Hz, CH_2), 5.23 (s, 1 H, CH), 6.77-7.36 (m, 8 H, Ph), 12.42 (s, 1 H, Py); ^{13}C NMR ($CDCl_3$) δ: 12.9, 18.9, 34.4, 40.8, 55.2, 114.0, 115.0, 115.2, 115.8 (d, 2C, $J_{C\text{-}C\text{-}F}$ = 20.9 Hz), 117.6, 123.2, 123.6 (d, 1C, $J_{C4\text{-}F}$ = 3.1 Hz), 128.2, 130.2, 130.7 (d, 1C, $J_{C3\text{-}F}$ =8.2 Hz), 135.6 (d, $J_{C3\text{-}F}$ = 7.5 Hz), 154.6, 158.6, 159.1, 162.9 (d, 1C, $J_{C\text{-}F}$ = 246.8 Hz), 164.4; MS (ESI) *m/z* 410.23(M+H)$^+$.

2-(2-(4-methoxybenzylthio)-5-ethyl-6-oxo-1,6-dihydropyrimidin-4-yl)-2-(2-chlorophenyl)acetonitrile(**1j**) Yield 54%; pale yellow solid, mp 178.0-179.3 °C; ^{1}H NMR ($CDCl_3$) δ (ppm): 1.04 (t, 3 H, J = 7.4 Hz, CH_3), 2.44-2.61(m, 2 H, CH_2), 3.78 (s, 3 H, OCH_3), 4.23 (d, 1 H, J =13.8 Hz, CH_2), 4.34 (d, 1 H, J = 13.8 Hz, CH_2), 5.66 (s, 1 H, CH), 6.78-7.40 (m, 8 H, Ph), 12.32 (s, 1 H, Py); ^{13}C NMR ($CDCl_3$) δ: 12.55, 18.87, 34.33, 38.11, 55.26, 113.99, 117.46, 123.75, 127.46, 128.28, 129.75, 130.12, 130.21, 130.68,154.01, 158.18, 159.04, 164.40; MS (ESI) *m/z* 426.15 (^{35}M+H)$^+$, 428.14 (^{37}M+H)$^+$.

2-(2-(4-methoxybenzylthio)-5-ethyl-6-oxo-1,6-dihydropyrimidin-4-yl)-2-(4-bromophenyl)acetonitrile(**1k**) Yield 53%; white solid, mp 173.0-174.0 °C; ^{1}H NMR ($CDCl_3$) δ (ppm): 1.08 (t, 3 H, J = 7.4 Hz, CH_3), 2.44-2.64(m, 2 H, CH_2), 3.79 (s, 3 H, OCH_3), 4.26 (d, 1 H, J = 13.8 Hz, CH_2), 4.44 (d, 1 H, J = 13.9 Hz, CH_2), 5.20 (s, 1 H, CH), 6.77-7.49 (m, 8 H, Ph), 12.35 (s, 1 H, Py); ^{13}C NMR ($CDCl_3$) δ: 13.0, 18.9, 34.3, 40.5, 55.3, 114.0, 117.6, 122.8, 123.1, 128.2, 129.5, 130.2, 132.3, 132.4, 154.7, 158.6, 159.1, 164.3; MS (ESI) *m/z* 470.19 (^{79}M+H)$^+$, 472.16 (^{81}M+H)$^+$.

2-(2-(4-methoxybenzylthio)-5-isopropyl-6-oxo-1,6-dihydropyrimidin-4-yl)-2-(4-fluorophenyl)acetonitrile(**1l**) Yield 40%; white solid, mp 149.9-151.2 °C; ^{1}H NMR ($CDCl_3$) δ (ppm): 1.19 (d, 3 H, J = 6.8 Hz, CH_3), 1.19 (d, 3 H, J = 6.8 Hz, CH_3), 2.87-2.93(m, 1 H, CH), 3.78 (s, 3H, OCH_3), 4.30 (d, 1 H, J = 13.9 Hz, CH_2), 4.45 (d, 1 H, J = 14.0 Hz, CH_2), 5.33 (s, 1 H, CH), 6.78-7.30 (m, 8 H, Ph), 12.52 (s, 1 H, Py); ^{13}C NMR ($CDCl_3$) δ: 19.2, 19.6, 28.3, 34.3, 40.9, 55.2, 114.0, 116.1 (d, 2C, $J_{C\text{-}C\text{-}F}$ = 21.8 Hz), 118.1, 125.5, 128.4, 129.5 (d, 2 C, $J_{C3\text{-}F}$ = 21.8 Hz), 129.6, 130.2, 154.6, 158.5, 159.0, 162.6 (d, 1C, $J_{C\text{-}F}$ = 247.1 Hz), 163.9; MS (ESI) *m/z* 424.22 (M+H)$^+$.

2-(2-(4-methoxybenzylthio)-5-isopropyl-6-oxo-1,6-dihydropyrimidin-4-yl)-2-(2-chlorophenyl)acetonitrile(**1m**) Yield 37%; white solid, mp 174.0-175.4 °C; ^{1}H NMR ($CDCl_3$) δ (ppm): 1.11 (d, 3 H, J = 6.8 Hz, CH_3), 1.36 (d, 3 H, J = 6.8 Hz, CH_3), 2.82-2.89(m, 1 H, CH), 3.78 (s, 3 H, OCH_3), 4.20 (d, 1 H, J = 13.6 Hz, CH_2), 4.35 (d, 1 H, J = 13.6 Hz, CH_2), 5.76 (s, 1 H, CH), 6.77-7.42 (m, 8 H, Ph), 12.76 (s, 1 H, Py); ^{13}C NMR ($CDCl_3$) δ: 19.0, 19.5, 28.6, 34.2, 38.5, 55.3, 114.0, 117.4, 126.1, 127.4, 128.4, 129.8, 130.1, 130.2, 130.6, 131.8, 132.9, 153.6, 158.2, 159.0; MS (ESI) *m/z* 440.15(^{35}M+H)$^+$, 442.16(^{37}M+H)$^+$.

2-(2-(4-methoxybenzylthio)-5-isopropyl-6-oxo-1,6-dihydropyrimidin-4-yl)-2-(4-chlorophenyl)acetonitrile(**1n**) Yield 44%; pale yellow solid, mp 217.5-218.5 °C; ^{1}H NMR ($CDCl_3$) δ (ppm): 1.21 (d, 3 H, J = 6.8 Hz, CH_3), 1.30 (d, 3 H, J = 6.8 Hz, CH_3), 2.89-2.96(m, 1 H, CH), 3.79 (s, 3 H, OCH_3), 4.28 (d, 1 H, J = 13.6 Hz, CH_2), 4.45 (d, 1 H, J = 13.6 Hz, CH_2), 5.32 (s, 1 H, CH), 6.77-7.33 (m, 8 H, Ph), 12.08 (s, 1 H, Py); ^{13}C NMR ($CDCl_3$) δ: 19.3, 19.7, 28.4, 34.3, 40.9, 55.3, 114.0, 117.8, 125.7, 128.3, 129.0, 129.3, 130.2, 132.2, 134.6, 154.3, 158.5, 159.0, 163.6; MS (ESI) *m/z* 440.21 (^{35}M+H)$^+$, 442.10(^{37}M+H)$^+$.

2-(2-(4-methoxybenzylthio)-5-isopropyl-6-oxo-1,6-dihydropyrimidin-4-yl)-2-(2-bromophenyl)acetonitrile(**1o**) Yield 38%; white solid, mp 169.9-170.8 °C; ^{1}H NMR ($CDCl_3$) δ (ppm): 1.11 (d, 3 H, J = 6.8 Hz, CH_3), 1.37 (d, 3 H, J = 6.8 Hz, CH_3), 2.82-2.89 (m, 1 H, CH), 3.78 (s, 3 H, OCH_3), 4.18 (d, 1 H, J = 13.8 Hz, CH_2), 4.34 (d, 1 H, J = 13.9 Hz, CH_2), 5.75 (s, 1 H, CH), 6.78-7.59 (m, 8 H, Ph), 12.73 (s, 1 H, Py); ^{13}C NMR ($CDCl_3$) δ: 19.0, 19.4, 28.6, 34.1, 41.2, 55.2, 113.9, 117.4, 123.4, 126.0, 128.0, 128.5, 130.2, 130.3, 130.8, 133.1, 133.5, 153.8, 158.3, 159.0, 164.1; MS (ESI) *m/z* 484.03 (^{79}M+H)$^+$, 485.97 (^{81}M+H)$^+$.

2-(2-(4-methoxybenzylthio)-5-isopropyl-6-oxo-1,6-dihydropyrimidin-4-yl)-2-(3-bromophenyl)acetonitrile(**1p**) Yield 37%; white solid, mp 176.5-177.9 °C; ^{1}H NMR ($CDCl_3$) δ (ppm): 1.23 (d, 3 H, J = 6.8 Hz, CH_3), 1.30 (d, 3 H, J = 6.8 Hz, CH_3), 2.88-2.95 (m, 1 H, CH), 3.78 (s, 3 H, OCH_3), 4.31 (d, 1 H, J = 14 Hz, CH_2), 4.43 (d, 1 H, J = 14 Hz, CH_2), 5.33 (s, 1 H, CH), 6.79-7.51 (m, 8 H, Ph), 12.66 (s, 1 H, Py); ^{13}C NMR ($CDCl_3$) δ: 19.2, 19.6, 28.4, 34.3, 41.1, 55.2, 114.0, 117.6, 123.1, 125.7, 126.4, 128.3, 130.2, 130.6, 130.7, 131.8, 135.7, 154.1, 158.7, 159.0, 164.0; MS (ESI) *m/z* 484.04 (^{79}M+H)$^+$, 486.02 (^{81}M+H)$^+$.

2-(2-(4-methoxybenzylthio)-5-methyl-6-oxo-1,6-dihydropyrimidin-4-yl)-2-(2,6-dichlorophenyl)acetonitrile(**1q**) Yield 54%; white solid, mp 178.1-178.9 °C; ^{1}H NMR ($CDCl_3$) δ (ppm): 1.89 (s, 3 H, CH_3), 3.78 (s, 3 H, OCH_3), 4.23 (d, 1 H, J = 13.8 Hz, CH_2), 4.35 (d, 1 H, J = 13.6 Hz, CH_2), 6.07 (s, 1 H, CH), 6.77-7.38 (m, 7 H, Ph),12.79 (s, 1 H, Py); ^{13}C NMR ($CDCl_3$) δ: 10.0, 34.1, 39.2, 55.2, 113.9, 115.3, 117.5, 128.2, 129.1, 129.4, 130.2, 130.6, 136.1, 153.5, 157.4, 159.0, 165.1; MS (ESI) *m/z* 446.17 (^{35}M+H)$^+$, 448.15($^{35+37}$M+H)$^+$, 450.19(^{37}M+H)$^+$.

2-(2-(4-methoxybenzylthio)-5-methyl-6-oxo-1,6-dihydropyrimidin-4-yl)-2-(4-bromo-2-fluorophenyl)acetonitrile(**1r**) Yield 52%; yellow solid, mp 149.7-151.0 °C; ^{1}H NMR ($CDCl_3$) δ (ppm): 2.09 (s, 3 H, CH_3), 3.80 (s, 3 H, OCH_3), 4.28 (d, 1 H, J = 13.8 Hz, CH_2), 4.40 (d, 1 H, J = 13.8 Hz, CH_2), 5.44 (s, 1 H, CH), 6.80-7.40 (m, 7 H, Ph), 12.17 (s, 1 H, J = 5.2 Hz, Py); ^{13}C NMR ($CDCl_3$) δ: 10.2, 34.4, 34.4, 55.3, 114.1, 116.7, 117.7, 119.3, 119.5, 119.7, 119.8, 123.4, 123.4, 128.0, 128.3, 128.3, 130.1, 131.2, 131.3, 153.8, 157.9, 158.4, 159.1, 164.5; MS (ESI) *m/z* 474.12 (^{79}M+H)$^+$, 476.07 (^{81}M+H)$^+$.

2-(2-(4-methoxybenzylthio)-5-methyl-6-oxo-1,6-dihydropyrimidin-4-yl)-2-(2-chloro-6-fluorophenyl)acetonitrile(**1s**) Yield 40%; white solid, mp 127.5-128.8 °C; ^{1}H NMR ($CDCl_3$) δ (ppm): 2.04 (s, 3 H, CH_3), 3.78 (s, 3 H, OCH_3), 4.27 (s, 3 H, CH_2), 5.72 (s, 1 H, CH_2), 6.77-7.33 (m, 7 H, Ph), 12.61 (s, 1 H, Py); ^{13}C NMR ($CDCl_3$) δ: 10.2, 34.2, 35.0, 55.3, 114.0, 115.1 (d, 1C, $J_{C\text{-}C\text{-}F}$ = 22.2 Hz), 115.6, 117.8, 119.8 (d, 1C, $J_{C\text{-}C\text{-}F}$ = 15.4 Hz), 125.7 (d, 1C, $J_{C4\text{-}F}$ = 3.3 Hz), 128.1, 130.2, 130.9 (d, 1C, $J_{C3\text{-}F}$ = 9.7 Hz), 134.7 (d, 1C, $J_{C3\text{-}F}$ = 4.6 Hz), 153.4, 157.9, 159.1, 161.7 (d, 1C, $J_{C\text{-}F}$=252.6 Hz), 164.9; MS (ESI) *m/z* 430.02 (^{35}M+H)$^+$, 431.97 (^{37}M+H)$^+$.

2-(2-(4-methoxybenzylthio)-5-methyl-6-oxo-1,6-dihydropyrimidin-4-yl)-2-(2,4-difluorophenyl)acetonitrile(**1t**) Yield 51%; yellow solid, mp 168.7-169.5 °C; ^{1}H NMR ($CDCl_3$) δ (ppm): 2.08 (s, 3 H, CH_3), 3.79 (s, 3 H, OCH_3), 4.31 (d, 1 H, J = 13.9 Hz, CH_2), 4.40 (d, 1 H, J = 13.8 Hz, CH_2), 5.46 (s, 1 H, CH), 6.80-7.50 (m, 7 H, Ph), 12.63 (s, 1 H, Py); ^{13}C NMR ($CDCl_3$) δ: 10.1, 10.2, 34.2, 34.2, 34.4, 55.3, 103.9, 104.2, 104.4, 112.1,112.1, 112.3, 112.3, 114.0, 116.4, 116.5, 116.6, 116.6, 117.0, 117.4, 130.1, 131.0, 131.1, 131.1, 131.2, 154.3, 158.5, 159.1, 160.7, 160.8, 161.8, 161.9, 164.8; MS (ESI) *m/z* 414.18 (M+H)$^+$.

2-(2-(4-methoxybenzylthio)-5-methyl-6-oxo-1,6-dihydropyrimidin-4-yl)-2-(3,5-difluorophenyl)acetonitrile(**1u**) Yield 60%; white solid, mp 146.3-147.3 °C; ^{1}H NMR ($CDCl_3$) δ (ppm): 2.07 (s, 3 H, CH_3), 3.78 (s, 3 H, OCH_3), 4.32 (d, 1 H, J = 13.8 Hz, CH_2), 4.43 (d, 1 H, J = 13.8 Hz, CH_2), 5.20 (s, 1 H, CH), 6.79-7.29 (m, 7 H, Ph), 12.70 (s, 1 H, Py); ^{13}C NMR ($CDCl_3$) δ: 10.6, 34.5, 41.2, 55.2, 104.20, 104.4, 104.7, 111.0, 111.03, 111.1, 111.2, 114.0, 116.8, 117.5, 127.9, 130.2, 136.3, 136.4, 136.5, 154.6, 158.8, 159.2, 161.9, 162.0, 164.4, 164.5, 164.7; MS (ESI) *m/z* 414.18 (M+H)$^+$.

Biological methods

Anti-HIV activity assay

The anti-HIV activity and cytotoxicity of the compounds were evaluated against wild-type HIV-1 strain III_B in MT-4 cell cultures using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method[1]. Stock solutions (10 × final concentration) of test compounds were added in 25 µL volumes to two series of triplicate wells to allow simultaneous evaluation of their effects on mock- and HIV-infected cells at the beginning of each experiment. Serial five-fold dilutions of test compounds were made directly in flat-bottomed 96-well microtiter trays by adding 100 µL medium to the 25 µL stock solution and transferring 25 µL of this solution to another well that contained 100 µL medium using a Biomek 3000 robot (Beckman instruments, Fullerton, CA). Untreated control HIV-1 and mock-infected cell samples were included for each sample.

HIV-1(III_B)[2] at 100-300 $CCID_{50}$ (50% cell culture infectious dose-50%) or culture medium was added to either the infected or mock-infected wells of the microtiter plate. Mock-infected cells were used to evaluate the effect of test compound on uninfected cells in order to assess the cytotoxicity of the test compound. Exponentially growing MT-4 cells were centrifuged for 5 min at 220 g and the supernatant was discarded. The MT-4 cells were resuspended at 6 × 10^5 cells/mL, and 50 µL volumes were transferred to the microtiter tray wells. Five days after infection, the viability of mock- and HIV-infected cells was examined spectrophotometrically by the MTT assay. The 50% cytotoxic concentration (CC_{50}) was defined as the concentration of the test compound that reduced the viability of the mock-infected MT-4 cells by 50%. The concentration achieving 50% protection from the cytopathic effect of the virus in infected cells was defined as the 50% effective concentration (EC_{50}).

Reverse transcriptase assay

Recombinant wild type p66/p51 HIV-1 RT was expressed and purified as described by Auwerx et al[3]. The RT assay is performed with the EnzCheck Reverse Transcriptase Assay kit (Molecular Probes, Invitrogen), as described by the manufacturer. The assay is based on

the dsDNA quantitation reagent PicoGreen. This reagent shows a pronounced increase in fluorescence signal upon binding to dsDNA or RNA-DNA heteroduplexes. Single-stranded nucleic acids generate only minor fluorescence signal enhancement when a sufficiently high dye:base pair ratio is applied[4]. This condition is met in the assay.

A poly(rA) template of approximately 350 bases long, and an oligo(dT)16 primer, are annealed in a molar ratio of 1:1.2 (60 min. at room temperature). Fifty-two mg of the RNA/DNA is brought into each well of a 96-well plate in a volume of 20 µl polymerization buffer (60 mM Tris-HCl, 60 mM KCl, 8 mM $MgCl_2$, 13 mM DTT, 100 µM dTTP, pH 8.1). Five µl of RT enzyme solution, diluted to a suitable concentration in enzyme dilution buffer (50 mM Tris-HCl, 20% glycerol, 2 mM DTT, pH 7.6), is added. The reactions are incubated at 25°C for 40 minutes and then stopped by the addition of EDTA (15 mM fc). Heteroduplexes are then detected by addition of PicoGreen. Signals are read using an excitation wavelength of 490 nm and emission detection at 523 nm using a spectrofluorometer (Safire2, Tecan). To test the activity of compounds against RT, 1 µl of compound in DMSO is added to each well before the addition of RT enzyme solution. Control wells without compound contain the same amount of DMSO. Results are expressed as relative fluorescence i.e. the fluorescence signal of the reaction mix with compound divided by the signal of the same reaction mix without compounds.

Docking study

Molecular modeling was carried out with the Tripos molecular modeling package Sybyl-1.2. The new *S*-DABO derivative **1s** for docking was built with standard bond lengths and angles using Sybyl-1.2/base Builder and fully optimized with the tripos force field for 1000 generations, with an energy gradient of 0.001 kcal/(molÅ) and Gasteiger–Hückel charge. The geometry of the non-nucleoside inhibitor binding site (NNIBS) of RT was defined by using the TNK-651: RT (wt) complex filed in the Brookhaven Protein Data Bank [complex] (PDB entry code: 1RT2) by selecting all the residues within of the co-crystallized inhibitor. Prior to docking, the protein was prepared by removing water molecules, the ligand (TNK-651), and other unnecessary small molecules from the crystal structure of the complex. Hydrogens were added to the unfilled valences of HEPT and of the amino acids according to standard geometries of the Tripos force field. The atomic charges were recalculated using the Kollman all-atom approach for the protein and the Gasteiger–Hückel approach for the ligand. Simultaneously, the dock protocol was generated according to the ligand with residue setting in 0.5 and boad setting in 0. Surflex-Dock default settings were used for other parameters, such as the number of starting conformations per molecule (set to 0), the size to expand search grid (set to 6), 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 on all single bonds and move flexibly within the tentative binding pocket. The binding interaction energy was calculated to include van der Waals, electrostatic, and torsional energy terms defined in the Tripos force field. The structure optimization was performed for 20,000 generations using a genetic algorithm, and the 20-best-scoring ligand–protein complexes were kept for further analyses. The $\log(Kd)^2$ values of the 20-best-scoring complexes, which represented the binding affinities of ligand with RT, ranged a wide scope of functional classes. Therefore, only the highest-scoring 3D structural model of the ligand-bound RT was chosen to define the binding interaction.[5]

References

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