Electronic Supplementary Material (ESI) for Medicinal Chemistry Communications.

Novel Anti-HIV-1 NNRTIs Based on a Pyrazolo[4,3-d]isoxazole Backbone Scaffold: Design, Synthesis and Insights into the Molecular Basis of Action

Sobhi M. Gomha[a], Mohamed G. Badrey[b], Mohamed M. Abdalla[c], Reem K. Arafa*[d]

[a] Dr. Sobhi M. Gomha, Department of Chemistry, Faculty of Science, Cairo University, Giza, Egypt

[b] Dr. Mohamed G. Badrey, Chemistry Department; Faculty of Science; Fayoum University, El-Fayoum, Egypt

[c] Dr. Mohamed M. Abdalla, Research Unit, Saco Pharm. Co., 6th October City, Egypt

[d] Dr. Reem K. Arafa, Pharmaceutical Chemistry Department, Faculty of Pharmacy, Cairo University, Cairo, Egypt,P.O. Box 11562, Cairo, Egypt

** **Corresponding author:** Reem. K. Arafa, E-mail: rkarafa@cu.edu.eg. Tel: +2-01002074028.*

Experimental Procedures

Chemistry

Melting points were measured on Electrothermal IA 9000 series digital melting point apparatus. The IR spectra were recorded in potassium bromide discs on a Pye Unicam SP 3300 and Shimadzu FT IR 8101 PC infrared spectrophotometer. ^{1}H NMR and / ^{13}C NMR spectra were recorded in deuterated dimethyl sulfoxide (DMSO-d_6) using a Varian Gemini 300 NMR spectrometer (300 MHz for ^{1}HNMR). Mass spectra were recorded on a Shimadzu GCMS-QP1000 EX mass spectrometer at 70 eV. Elemental analysis was carried out at the Microanalytical Centre of Cairo University, Giza, Egypt. All reactions were followed by TLC (Silica gel, Merck). 4-(4-Chlorobenzylidene)-3-phenylisoxazol-5(4*H*)-one (**1**),[1] *N*-phenyl 2-oxopropane-hydrazonyl chloride (**5**),[2] 2-hydrazinyl-4-methyl-5-(phenyldiazenyl)thiazole (**10**)[3] were prepared as reported in the literature.

4.1.1. Synthesis of 4-(4-chlorophenyl)-3-phenyl-4*H*-pyrazolo[4,3-d]isoxazole-5(6*H*)-carbothioamide (3).

To a mixture of 4-(4-chlorobenzylidene)-3-phenylisoxazol-5(4*H*)-one **1** (2.83 g, 10 mmol) and thiosemicarbazide (0.92 g, 10 mmol) in ethanol (20 mL), a catalytic amount of triethylamine was added, then heated under reflux for 6 h. The resulting solid was collected, washed with ethanol and recrystallized from acetic acid to give pure product of compound **3**. White solid, 70% yield; mp = 175-177 °C; IR (KBr): $\tilde{\nu}$ 3435, 3280, 3162 (NH_2 and NH), 1597 (C=N, C=C stretch., NH bend.) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 4.72 (s, 1H, pyrazole-H), 7.09-7.82 (m, 9H, Ar-H), 8.13 (s, br, 2H, NH_2), 11.40 (s, 1H, NH); MS *m/z* (%): 358 (M^++2, 10), 356 (M^+, 14), 301 (22), 213 (46), 138 (50), 117 (29), 60 (100). Anal. calcd. for $C_{17}H_{13}ClN_4OS$ (356.83): C, 57.22; H, 3.67; N, 15.70. Found C, 57.16; H, 3.63; N, 15.51%.

4.1.2. General method for the synthesis of 4-(4-chlorophenyl)-5-(4-methyl-5-(phenyldiazenyl)thiazol -2-yl)-3-phenyl-5,6-dihydro-4*H*-pyrazolo[4,3-d]isoxazole (7a-f).

A mixture of carbothioamide **3** (0.356 g, 1 mmol) and the appropriate hydrazonyl halides **5a-f** (1 mmol) in dioxane (20 mL) containing TEA (0.5 mL) was refluxed for 4h (monitored by TLC),

allowed to cool and the solid formed was filtered off, washed with EtOH, dried and recrystallized from DMF to give **7a-f.**

4.1.2.1. 4-(4-Chlorophenyl)-5-(4-methyl-5-(phenyldiazenyl)thiazol-2-yl)-3-phenyl-5,6-dihydro-4*H*-pyrazolo[4,3-d]isoxazole (7a).

Red solid, 75% yield; mp 147-149 °C; IR (KBr) $\tilde{\nu}$ 3424 (NH), 1600 (C=N, C=C stretch., NH bend.) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 2.60 (s, 3H, CH_3), 4.75 (s, 1H, pyrazole-H), 7.04-7.89 (m, 14H, Ar-H),10.52 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 12.11 (CH_3), 66.59 (CH), 104.52, 111.92, 118.39, 121.32, 123.43, 125.42, 127.62, 129.39, 130.62, 132.39, 135.32, 137.26, 138.42, 143.21, 151.34, 154.85, 160.36, 168.71 (Ar-C); MS, *m/z* (%) 500 (M^++2, 2), 498 (M^+, 7), 433 (42), 322 (43), 211 (18), 111 (28), 77 (100). Anal. calcd. for $C_{26}H_{19}ClN_6OS$ (498.99): C, 62.58; H, 3.84; N, 16.84; found: C, 62.50; H, 3.71; N, 16.66%.

4.1.2.2. 4-(4-Chlorophenyl)-5-(4-methyl-5-(p-tolyldiazenyl)thiazol-2-yl)-3-phenyl-5,6-dihydro-4*H*-pyrazolo[4,3-d]isoxazole (7b).

Red solid, 78% yield; mp 167-169 °C; IR (KBr) $\tilde{\nu}$ 3423 (NH), 1605 (C=N, C=C stretch., NH bend.) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 2.38 (s, 3H, CH_3), 2.57 (s, 3H, CH_3), 4.72 (s, 1H, pyrazole-H), 7.17-7.86 (m, 13H, Ar-H), 10.63 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 12.14, 23.18 (2 CH_3), 63.32 (CH), 106.26, 113.50, 117.16, 120.23, 120.83, 126.73, 127.10, 129.18, 131.76, 133.04, 137.32, 137.70, 138.32, 140.46, 144.39, 150.49, 157.34, 167.85 (Ar-C); MS, *m/z* (%) 513 (M^+, 10), 426 (39), 336 (42), 200 (27), 111 (38), 91 (100). Anal. calcd. for $C_{27}H_{21}ClN_6OS$ (513.01): C, 63.21; H, 4.13; N, 16.38; found: C, 63.29; H, 4.10; N, 16.23%.

4.1.2.3. 4-(4-Chlorophenyl)-5-(5-((4-chlorophenyl)diazenyl)-4-methylthiazol-2-yl)-3-phenyl-5,6-dihydro-4*H*-pyrazolo[4,3-d]isoxazole (7c).

Red solid, 76% yield; mp 194-196 °C; IR (KBr) $\tilde{\nu}$ 3414 (NH), 1605 (C=N, C=C stretch., NH bend.) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 2.60 (s, 3H, CH_3), 4.73 (s, 1H, pyrazole-H), 7.10-7.89 (m, 13H, Ar-H), 10.79 (s, 1H, NH); MS, *m/z* (%) 533 (M^+, 8), 471 (39), 336 (32), 251 (38), 111 (100), 91 (80).

Anal. calcd. for $C_{26}H_{18}Cl_2N_6OS$ (533.43): C, 58.54; H, 3.40; N, 15.75; found: C, 58.42; H, 3.25; N, 15.54%.

4.1.2.4. 5-(5-((4-Bromophenyl)diazenyl)-4-methylthiazol-2-yl)-4-(4-chlorophenyl)-3-phenyl-5,6-dihydro-4H-pyrazolo[4,3-d]isoxazole (7d).

Red solid, 73% yield; mp 172-174 °C; IR (KBr) $\tilde{\nu}$ 3429 (NH), 1603 (C=N, C=C stretch., NH bend.) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 2.62 (s, 3H, CH_3), 4.74 (s, 1H, pyrazole-H), 7.13-7.91 (m, 13H, Ar-H), 10.74 (s, 1H, NH); MS, *m/z* (%) 577 (M^+, 17), 466 (76), 390 (62), 258 (68), 111 (84), 89 (100), 75 (94). Anal. calcd. for $C_{26}H_{18}BrClN_6OS$ (577.88): C, 54.04; H, 3.14; N, 14.54; found: C, 54.01; H, 3.06; N, 14.35%.

4.1.2.5. 4-(4-Chlorophenyl)-5-(5-((4-methoxyphenyl)diazenyl)-4-methylthiazol-2-yl)-3-phenyl-5,6-dihydro-4H-pyrazolo[4,3-d]isoxazole (7e).

Red solid, 72% yield; mp 167-169 °C; IR (KBr) $\tilde{\nu}$ 3432 (NH), 1606 (C=N, C=C stretch., NH bend.) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 2.61 (s, 3H, CH_3), 3.58 (s, 3H, OCH_3), 4.72 (s, 1H, pyrazole-H), 7.12-7.84 (m, 13H, Ar-H), 10.76 (s, 1H, NH); MS, *m/z* (%) 529 (M^+, 61), 447 (49), 362 (88), 294 (73), 158 (69), 77 (100). Anal. calcd. for $C_{27}H_{21}ClN_6O_2S$ (529.01): C, 61.30; H, 4.00; N, 15.89; found: C, 61.13; H, 4.18; N, 15.64%.

4.1.2.6. 4-(4-Chlorophenyl)-5-(4-methyl-5-((4-nitrophenyl)diazenyl)thiazol-2-yl)-3-phenyl-5,6-dihydro-4H-pyrazolo[4,3-d]isoxazole (7f).

Brown solid, 78% yield; mp 181-183 °C; IR (KBr) $\tilde{\nu}$ 3435 (NH), 1601 (C=N, C=C stretch., NH bend.) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 2.61 (s, 3H, CH_3), 4.76 (s, 1H, pyrazole-H), 7.16-7.86 (m, 13H, Ar-H), 10.78 (s, 1H, NH); MS, *m/z* (%) 543 (M^+, 48), 472 (69), 333 (75), 278 (62), 111 (60), 77 (100). Anal. calcd. for $C_{26}H_{18}ClN_7O_3S$ (543.98): C, 57.41; H, 3.34; N, 18.02; found: C, 57.32; H, 3.30; N, 17.88%.

4.1.3. Synthesis of 4-(4-chlorophenyl)-5-(4-methylthiazol-2-yl)-3-phenyl-5,6-dihydro-4*H*-pyrazolo [4,3-d]isoxazole (9).

A mixture of **3** (1.78 g, 5 mmol) and chloroacetone **8** (2.30 g, 5 mmol) in absolute EtOH (30 mL) was refluxed for 4h. The product started to separate out during the course of reaction. The crystalline solid was filtered, washed with water, dried and recrystallized from EtOH to give the target thiazole **9** in a pure form**.** Yellow crystals, 74% yield; mp 187-189 °C; IR (KBr) $\tilde{\nu}$ 3434 (NH), 1597 (C=N, C=C stretch., NH bend.) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 2.57 (s, 3H, CH_3), 4.73 (s, 1H, pyrazole-H), 6.82 (s, 1H, thiazole-H5), 7.13-7.86 (m, 9H, Ar-H), 10.55 (s, 1H, NH); MS, *m/z* (%) 396 (M^++2, 5), 394 (M^+, 17), 231 (64), 162 (59), 105 (74), 77 (100). Anal. calcd. for $C_{20}H_{15}ClN_4OS$ (394.88): C, 60.83; H, 3.83; N, 14.19; found: C, 60.80; H, 3.78; N, 14.03%.

4.1.4. Alternate synthesis of 7a (Method A)

To a solution of **9** (0.349 g, 1 mmol) in EtOH (20 mL) was added sodium acetate trihydrate (0.138 g, 1 mmol), and the reaction mixture was cooled to 0-5 ^{o}C in an ice bath. To the resulting cold solution was added portionwise a cold solution of benzenediazonium chloride [prepared by diazotizing aniline] (1 mmol) dissolved in hydrochloric acid (6 M, 1 mL) with a solution of sodium nitrite (0.07 g, 1 mmol) in water (2 mL)]. After complete addition of the diazonium salt, the reaction mixture was stirred for a further 30 min in an ice bath. The solid that separated was filtered off, washed with water and finally recrystallized from DMF to give product proved to be identical in all respects (mp, mixed mp and IR spectra) with compound **7a** which was obtained from reaction of compound **3** and **5a**.

4.1.5. Alternate synthesis of 7a (Method B)

Equimolar amounts of **1** (0.283 g, 1 mmol) and 2-hydrazinyl-4-methyl-5-(phenyldiazenyl)thiazole (**10**) (0.233 g, 1 mmol) in 2-propanol (10 mL), was refluxed for 2 hr then cooled to room temperature. The solid precipitated was filtered off, washed with water, dried and recrystallized from DMF to give the corresponding product, **7a** which were identical in all respects (m.p., mixed m.p. and IR spectra) with those obtained from reaction of **3** with **5a** but in 78% yield**.**

4.1.6. Synthesis of 4-(4-chlorophenyl)-3-phenyl-4*H*-pyrazolo[4,3-d]isoxazole-5(6*H*)-carbohydrazonamide (12).

To carbothioamide **3** (3.56 g, 10 mmol) in dry EtOH (10 mL) was added hydrazine hydrate (80%, 5 mL). The reaction mixture was kept under reflux for 20 h, and then cooled. The precipitated solid was filtered off and crystallized from DMF to give **12**. Pale yellow solid, 68% yield as; mp 316-318 °C. IR (KBr): $\tilde{\nu}$ 3429, 3322, 3178 (NH_2 and NH), 1602 (C=N, C=C stretch., NH bend.) cm^{-1}; ^1H NMR (DMSO-d_6): δ 4.86 (s, 1H, pyrazole-H), 5.64 (s, 2H, NH_2), 7.13-7.79 (m, 11H, ArH and NH_2), 10.16 (s, br, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 61.26 (CH), 104.66, 120.54, 120.89, 125.38, 128.98, 130.16, 134.23, 136.65, 137.36, 138.14, 149.48, 155.37 (Ar-C); MS *m/z* (%): 356 (M^++2, 22), 354 (M^+, 60), 286 (34), 165 (78), 111 (62), 89 (100), 75 (98). Anal. Calcd. for $C_{17}H_{15}ClN_6O$ (354.79): C, 57.55; H, 4.26; N, 23.69. Found C, 57.33; H, 4.04; N, 23.51%.

4.1.7. General method for the synthesis of 4-(4-chlorophenyl)-5-(6-methyl-5-(phenyldiazenyl)-1,2-dihydro-1,2,4-triazin-3-yl)-3-phenyl-5,6-dihydro-4*H*-pyrazolo[4,3-d]isoxazole (14a-c).

A mixture of carbohydrazonamide **3** (0.354 g, 1 mmol) and the appropriate hydrazonoyl halides **5a-c** (1 mmol) in dioxane (20 mL) containing TEA (0.5 mL) was refluxed for 4-6h (monitored by TLC), allowed to cool and the solid formed was filtered off, washed with EtOH, dried and recrystallized from DMF to give **14a-c.**

4.1.7.1. 4-(4-Chlorophenyl)-5-(6-methyl-5-(phenyldiazenyl)-1,2-dihydro-1,2,4-triazin-3-yl)-3-phenyl -5,6-dihydro-4*H*-pyrazolo[4,3-d]isoxazole (14a).

Red solid, 72% yield; mp 188-190 °C; IR (KBr) $\tilde{\nu}$ 3426 (NH), 1590 (C=N, C=C stretch., NH bend.) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 2.53 (s, 3H, CH_3), 4.69 (s, 1H, pyrazole-H), 7.21-7.83 (m, 15H, Ar-H and NH), 8.69 (s, 1H, NH), 12.15 (s, 1H, NH); MS, *m/z* (%) 498 (M^++2, 6), 496 (M^+, 15), 451 (12), 277 (17), 165 (69), 111 (68), 75 (100). Anal. calcd. for $C_{26}H_{21}ClN_8O$ (496.95): C, 62.84; H, 4.26; N, 22.55; found: C, 62.81; H, 4.16; N, 22.28%.

4.1.7.2. 4-(4-Chlorophenyl)-5-(6-methyl-5-(p-tolyldiazenyl)-1,2-dihydro-1,2,4-triazin-3-yl)-3-phenyl -5,6-dihydro-4*H*-pyrazolo[4,3-d]isoxazole (14b).

Red solid, 70% yield; mp 171-173 °C; IR (KBr) $\tilde{\nu}$ 3432 (NH), 1589 (C=N, C=C stretch., NH bend.) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 2.55 (s, 3H, CH_3), 2.45 (s, 3H, CH_3), 4.72 (s, 1H, pyrazole-H), 7.13-7.89 (m, 14H, Ar-H and NH), 8.71 (s, 1H, NH), 12.11 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 11.48, 22.41 (2 CH_3), 60.39 (CH), 102.38, 107.63, 118.53, 119.46, 120.52, 121.24, 123.36, 126.18, 128.06, 128.26, 130.76, 133.58, 137.14, 137.56, 139.32, 146.37, 153.44, 164.68 (Ar-C); MS, *m/z* (%) 512 (M^++2, 13), 510 (M^+, 40), 423 (15), 248 (18), 165 (60), 111 (78), 75 (100). Anal. calcd. for $C_{27}H_{23}ClN_8O$ (510.98): C, 63.46; H, 4.54; N, 21.93; found: C, 63.31; H, 4.48; N, 21.67%.

4.1.7.3. 4-(4-Chlorophenyl)-5-(5-((4-chlorophenyl)diazenyl)-6-methyl-1,2-dihydro-1,2,4-triazin-3-yl)-3-phenyl-5,6-dihydro-4*H*-pyrazolo[4,3-d]isoxazole (14c).

Red solid, 75% yield; mp 206-207 °C; IR (KBr) $\tilde{\nu}$ 3432 (NH), 1590 (C=N, C=C stretch., NH bend.) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 2.55 (s, 3H, CH_3), 4.76 (s, 1H, pyrazole-H), 7.18-7.94 (m, 14H, Ar-H and NH), 8.74 (s, 1H, NH), 12.23 (s, 1H, NH); MS, *m/z* (%) 533 (M^++2, 6), 531 (M^+, 11), 314 (15), 216 (50), 165 (43), 111 (56), 69 (100). Anal. calcd. for $C_{26}H_{20}Cl_2N_8O$ (531.40): C, 58.77; H, 3.79; N, 21.09; found: C, 58.54; H, 3.69; N, 21.01%.

1. Ablajan, K. and Xiamuxi, H. The convenient synthesis of 4-arylmethylidene-4,5-dihydro-3-phenylisoxazol-5-ones. *Chinese Chem. Lett.* **2011**, *22*, 151-154.

2. Eweiss, N. F. and Osman, A. Synthesis of heterocycles. Part II: New routes to acetylthiadiazolines and alkylazothiazoles. *J. Heterocycl. Chem.* **1980**, *17*, 1713-1717.

3. Emam, H. A.; Zohdi, Hs. F.; Abdelhamid, A. O. Reactions with hydrazonyl halides Part 15: A synthetic approach to 2,3-dihydrothiazoles. *J. Chem. Res.*, **1998**, *1*, 169-179.