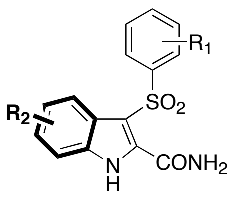
Supporting Information

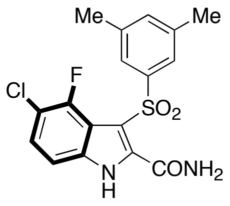
Indolyl Aryl Sulfones as HIV-1 Non-Nucleoside Reverse Transcriptase Inhibitors: Role of Two Halogen Atoms at the Indole Ring in Developing New Analogues with Improved Antiviral Activity

Giuseppe La Regina, Antonio Coluccia, Francesco Piscitelli, Alberto Bergamini, Anna Sinistro, Antonella Cavazza, Giovanni Maga, Alberta Samuele, Samantha Zanoli, Ettore Novellino, Marino Artico, and Romano Silvestri

Table of Contents Graphic



R_1 = H, 3,5-Me_2; R_2 = 5,6-Cl_2, 5,7-Cl_2, 4,5-F_2, 5,6-F_2, 5,7-F_2, 4-Cl,5-F, 6-Cl,5-F, 4-F,5-Cl, 6-F,5-Cl



16
WT(IIIB) ED_{50} = 0.5 nM;
Y181C ED_{50} = 4 nM;
K103N RT IC_{50} = 6 nM.

Contents

- Preparative, physicochemical and spectral data of derivatives **7-17**, **19-22**, **25-31**, **33-43** and **45-55**.
- Cell-based antiviral assay procedures.
- Enzymatic assay procedures.
- References in Supporting Information.
- Elemental analysis of compounds **6-17**.

Chemistry

5,6-Dichloro-3-[(3,5-dimethylphenyl)sulfonyl]-1*H*-indole-2-carboxamide (7). Was prepared as **6** starting from **45**. Yield 43%, mp 280-282 °C (from ethanol). ^{1}H NMR (DMSO-d_6): δ 2.31 (s, 6H), 7.28 (s, 1H), 7.64 (s, 2H), 7.74 (s, 1H), 8.15 (s,1H), 8.29 (broad s, 1H, disappeared on treatment with D_2O), 8.47 (broad s, 1H, disappeared on treatment with D_2O, 1H), 13.2 ppm (broad s, 1H, disappeared on treatment with D_2O). IR (nujol): ν 1270, 1660, 3160 cm^{-1}. Anal. ($C_{17}H_{14}Cl_2N_2O_3S$ (397.27)) C, H, N, Cl, S.

5,7-Dichloro-3-[(3,5-dimethylphenyl)sulfonyl]-1*H*-indole-2-carboxamide (8). Was prepared as **6** starting from **46**. Yield 74%, mp >300 °C (from aqueous DMF). ^{1}H NMR (DMSO-d_6): δ 2.31 (s, 6H), 7.25 (s, 1H), 7.53 (d, J = 1.3 Hz, 1H), 7.69 (s, 2H), 7.85 (d, J = 1.9 Hz, 1H), 8.14 (broad s, 1H, disappeared on treatment with D_2O), 8.38 (broad s, 1H, disappeared on treatment with D_2O), 13.30 ppm (broad s, 1H, disappeared on treatment with D_2O). IR (nujol): ν 1140, 1280, 1660, 3120, 3230, 3250 cm^{-1}. Anal. ($C_{17}H_{14}Cl_2N_2O_3S$ (397.27)) C, H, N, Cl, S.

4,5-Difluoro-3-(phenylsulfonyl)-1*H*-indole-2-carboxamide (9). Was prepared as **6** starting from **47**. Yield 60%, mp 295-295 °C (from ethanol). ^{1}H NMR (DMSO-d_6): δ 7.26 (m, 2H), 7.58 (m, 3H), 8.02 (m, 2H), 8.11 (broad s, 1H, disappeared on treatment with D_2O), 8.46 (broad s, 1H, disappeared on treatment with D_2O), 13.09 ppm (broad s, 1H, disappeared on treatment with D_2O). IR (nujol): ν 1145, 1327, 1674, 3249, 3366 cm^{-1}. Anal. ($C_{15}H_{10}F_2N_2O_3S$ (336.31)) C, H, N, F, S.

4,5-Difluoro-3-[(3,5-dimethylphenyl)sulfonyl]-1*H*-indole-2-carboxamide (10). Was prepared as **6** starting from **48**. Yield 43%, mp 298-300 °C (from aqueous DMF). ^{1}H NMR (DMSO-d_6): δ 2.31 (s, 6H), 7.29 (m, 3H), 7.67 (m, 2H), 8.05 (broad s, 1H, disappeared with D_2O), 8.41 (broad s, 1H, disappeared on treatment with D_2O), 12.90 ppm (broad s, 1H, disappeared on treatment with D_2O). IR (nujol): ν 1070, 1680, 3400 cm^{-1}. Anal. ($C_{17}H_{14}F_2N_2O_3S$ (364.37)) C, H, N, F, S.

5,6-Difluoro-3-(phenylsulfonyl)-1*H*-indole-2-carboxamide (11). Was prepared as **6** starting from **49**. Yield 52%, mp >300 °C (from aqueous DMF). ^{1}H NMR (DMSO-d_6): δ 7.45-7.70 (m, 4H), 7.91 (m, 1H), 8.02-8.08 (m, 2H), 8.23 (broad s, 1H, disappeared with D_2O), 8.45 (broad s, 1H, disappeared on treatment with D_2O), 13.00 ppm (broad s, 1H, disappeared on treatment with D_2O). IR (nujol): ν 903, 1137, 1598, 1670, 3242, 3465 cm^{-1}. Anal. ($C_{15}H_{10}F_2N_2O_3S$ (336.31)) C, H, N, F, S.

5,6-Difluoro-3-[(3,5-dimethylphenyl)sulfonyl]-1*H*-indole-2-carboxamide (12). Was prepared as **6** starting from ethyl **50**. Yield 62%, mp 266-269 °C (from aqueous DMF). ^{1}H NMR (DMSO-*d*$_6$): δ 2.31 (s, 6H), 7.27 (m, 1H), 7.50 (dd, *J* = 7.0 and 10.5 Hz, 1H), 7.65 (m, 2H), 7.90 (dd, *J* = 7.9 and 11.0 Hz, 1H), 8.20 (broad s, 1H, disappeared with D_2O), 8.43 (broad s, 1H, disappeared on treatment with D_2O), 13.00 ppm (broad s, 1H, disappeared on treatment with D_2O). IR (nujol): ν 1150, 1600, 1660, 3120, 3440 cm^{-1}. Anal. ($C_{17}H_{14}F_2N_2O_3S$ (364.37)) C, H, N, F, S.

5,7-Difluoro-3-[(3,5-dimethylphenyl)sulfonyl]-1*H*-indole-2-carboxamide (13). Was prepared as **6** starting from **51**. Yield 75%, mp >300 °C (from aqueous DMF). ^{1}H NMR (DMSO-*d*$_6$): δ 2.32 (s, 6H), 7.25 (m, 2H), 7.53 (dd, *J* = 1.9 and 9.1 Hz, 1H), 7.69 (m, 2H), 8.16 (broad s, 1H, disappeared on treatment with D_2O), 8.34 (broad s, 1H, disappeared on treatment with D_2O), 13.41 ppm (broad s, 1H, disappeared on treatment with D_2O). IR (nujol): ν 850, 1110, 1280, 1300, 1660, 3340 cm^{-1}. Anal. ($C_{17}H_{14}F_2N_2O_3S$ (364.37)) C, H, N, F, S.

4-Chloro-5-fluoro-3-[(3,5-dimethylphenyl)sulfonyl]-1*H*-indole-2-carboxamide (14). Was prepared as **6** starting from **52**. Yield 46%, mp 279-280 °C (from ethanol). ^{1}H NMR (DMSO-*d*$_6$): δ 2.37 (s, 6H), 7.23 (s, 1H), 7.30 (t, *J* = 9.5 Hz, 1H), 7.46 (dd, *J* = 4.3 and 8.8 Hz, 1H), 7.69 (s, 2H), 8.04 (broad s, 1H, disappeared on treatment with D_2O), 8.42 (broad s, 1H, disappeared on treatment with D_2O), 12.55 ppm (broad s, 1H, disappeared on treatment with D_2O). IR (nujol): ν 800, 1140, 1280, 1640, 3280, 3320, 3400 cm^{-1}. Anal. ($C_{17}H_{14}ClFN_2O_3S$ (380.82)) C, H, N, Cl, F, S.

6-Chloro-5-fluoro-3-[(3,5-dimethylphenyl)sulfonyl]-1*H*-indole-2-carboxamide (15). Was prepared as **6** starting from **53**. Yield 50%, mp 262-264 °C (from ethanol). ^{1}H NMR (DMSO-*d*$_6$): δ 2.25 (s, 6H), 7.20 (s, 1H), 7.59 (s, 2H), 7.60 (d, *J* = 9.8 Hz, 1H), 7.84 (d, *J* = 10.0 Hz, 1H), 8.16 (broad s, 1H, disappeared on treatment with D_2O), 8.38 (broad s, 1H, disappeared on treatment with D_2O), 12.50 ppm (broad s, 1H, disappeared on treatment with D_2O). IR (nujol): ν 850, 1130, 1300, 1670, 3120, 3310 cm^{-1}. Anal. ($C_{17}H_{14}ClFN_2O_3S$ (380.82)) C, H, N, Cl, F, S.

5-Chloro-4-fluoro-3-[(3,5-dimethylphenyl)sulfonyl]-1*H*-indole-2-carboxamide (16). Was prepared as **6** starting from **54**. Yield 95%, mp >300 °C (from ethanol). ^{1}H NMR (DMSO-*d*$_6$): δ 2.32 (s, 6H), 7.32 (m, 3H), 7.67 (s, 2H), 8.13

(broad s, 1H, disappeared on treatment with D_2O), 8.50 (broad s, 1H, disappeared on treatment with D_2O), 12.90 ppm (broad s, 1H, disappeared on treatment with D_2O). IR (nujol): ν 680, 780, 1140, 1290, 1310, 1670, 3200, 3310 cm^{-1}. Anal. ($C_{17}H_{14}ClFN_2O_3S$ (380.82)) C, H, N, Cl, F, S.

5-Chloro-6-fluoro-3-[(3,5-dimethylphenyl)sulfonyl]-1*H*-indole-2-carboxamide (17). Was prepared as **6** starting from **55**. Yield 65%, mp 270-274 °C (from ethanol). ^{1}H NMR (DMSO-*d*$_6$): δ 2.32 (s, 6H), 7.28 (s, 1H), 7.50 (d, *J* = 9.4 Hz, 1H), 7.64 (s, 2H), 8.09 (d, *J* = 7.20 Hz, 1H), 8.23 (broad s, 1H, disappeared on treatment with D_2O), 8.43 (broad s, 1H, disappeared on treatment with D_2O), 12.45 ppm (broad s, 1H, disappeared on treatment with D_2O). IR (nujol): ν 880, 1120, 1300, 1670, 3200, 3320 cm^{-1}. Anal. ($C_{17}H_{14}ClFN_2O_3S$ (380.82)) C, H, N, Cl, F, S.

Ethyl pyruvate 3,4-difluorophenylhydrazone (19). Was prepared following the above procedure as we previously reported.[1S]

Ethyl pyruvate 2,4-difluorophenylhydrazone (20). Was prepared as previously reported.[2S]

Ethyl pyruvate 3-chloro-4-fluorophenylhydrazone (21). Was prepared as **18** starting from 3-chloro-4-fluoroaniline. Yield 17%, mp 89-91 °C (from aqueous ethanol). ^{1}H NMR (DMSO-*d*$_6$): δ 1.36 (t, *J* = 7.1 Hz, 3H), 2.06 (s, 3H), 4.22 (q, *J* = 7.1 Hz, 2H), 7.17-7.39 (m, 3H), 9.96 ppm (broad s, 1H, disappeared on treatment with D_2O). IR (nujol): ν 780, 1220, 1260, 1700, 3100 cm^{-1}.

Ethyl pyruvate 3-fluoro-4-chlorophenylhydrazone (22). Was prepared following the above procedure as we previously reported.[3S]

Ethyl 5,6-dichloro-1*H*-indole-2-carboxylate (23). Was prepared according to Lit.[4S]

Ethyl 4,5-difluoro-1*H*-indole-2-carboxylate (25) and ethyl 5,6-difluoro-1*H*-indole-2-carboxylate (26). Were prepared as **24** from ethyl pyruvate 3,4-difluorophenylhydrazone (**19**), as we previously reported.[1S]

Ethyl 5,7-difluoro-1*H*-indole-2-carboxylate (27). Was prepared as **24** using **20**. Yield 15%, mp 175-177 °C (from ethanol). ^{1}H NMR (DMSO-*d*$_6$): δ 1.30 (t, *J* = 7.1 Hz, 3H), 4.36 (q, *J* = 7.1 Hz, 2H), 7.16 (m, 3H), 12.54 ppm (broad s, disappeared on treatment with D_2O, 1H). IR (nujol): ν 830, 1080, 1240, 1680, 3260 cm^{-1}.

Ethyl 4-chloro-5-fluoro-1*H*-indole-2-carboxylate (28) and ethyl 6-chloro-5-fluoro-1*H*-indole-2-carboxylate (29). Were prepared as **24** using **21**. The crude product was purified by silica gel column chromatography (*n*-hexane-ethyl acetate 2:1 as eluent). The first eluates furnisehd **29**, yield 61%, mp 198-200 °C (from ethanol). ^{1}H NMR (DMSO-d_6): δ 1.35 (t, J = 7.1 Hz, 3H), 4.35 (q, J = 7.1 Hz, 2H), 7.15 (d, J = 0.8 Hz, 1H), 7.57 (dd, J = 0.8 and 6.4 Hz, 1H), 7.68 (d, J = 10.0 Hz, 1H), 12.05 ppm (broad s, 1H, disappeared on treatment with D_2O). IR (nujol): ν 770, 820, 1240, 1700, 3310 cm^{-1}. Further elution with the same solvent afforded **28**, yield 18%, mp 183-186 °C (from ethanol). ^{1}H NMR (DMSO-d_6): δ 1.36 (t, J = 7.1 Hz, 3H), 4.36 (q, J = 7.1 Hz, 2H), 7.13 (d, J = 0.8 Hz, 1H), 7.31 (t, J = 9.0 Hz, 1H), 7.45 (ddd, J = 0.8, 4.1 and 9.0 Hz, 1H), 12.12 (broad s, 1H, disappeared on treatment with D_2O). IR (nujol): ν 780, 1220, 1260, 1700, 3100 cm^{-1}.

Ethyl 5-chloro-4-fluoro-1*H*-indole-2-carboxylate (30) and ethyl 5-chloro-6-fluoro-1*H*-indole-2-carboxylate (31). Were prepared as **24** using **22**, as we previously reported.[3S]

Ethyl 5,6-dichloro-3-[(3,5-dimethylphenyl)thio]-1*H*-indole-2-carboxylate (33). Was prepared as **32** using *N*-[(3,5-dimethylphenyl)thio]succinimide.[14] Yield 90%, mp 198-200 °C (from ethanol). ^{1}H NMR (DMSO-d_6): δ 1.26 (t, J = 7.1 Hz, 3H), 2.14 (s, 6H), 4.35 (q, J = 7.1 Hz, 2H), 6.74 (s, 2H), 6.78 (s, 1H), 6.99 (s,1H), 7.73 (s, 1H). IR (nujol): ν 1240, 1650, 3240 cm^{-1}.

Ethyl 5,7-dichloro-3-[(3,5-dimethylphenyl)thio]-1*H*-indole-2-carboxylate (34). Was prepared as **32** using **24** and *N*-[(3,5-dimethylphenyl)thio]succinimide. Yield 80% as crude product. Due to difficulty encountered in the purification of this compound, it was used as crude product.

Ethyl 4,5-difluoro-3-(phenylthio)-1*H*-indole-2-carboxylate (35). Was prepared as **32** using **25**. Yield 77%, mp 171-174 °C (from ethanol). ^{1}H NMR (DMSO-d_6): δ 1.24 (t, J = 7.1 Hz, 3H), 4.31 (q, J = 7.1 Hz, 2H), 7.02-7.42 (m, 7H), 12.7 ppm (broad s, 1H, disappeared on treatment with D_2O). IR (nujol): ν 799, 1258, 1671, 3282 cm^{-1}.

Ethyl 4,5-difluoro-3-[(3,5-dimethylphenyl)thio]-1*H*-indole-2-carboxylate (36). Was prepared as **32** using **25** and *N*-[(3,5-dimethylphenyl)thio]succinimide. Yield 82%, mp 148-150 °C (from ethanol). ^{1}H NMR (DMSO-d_6): δ 1.28 (t, J = 7.1 Hz, 3H), 2.14 (s, 6H) 4.34 (q, J = 7.1 Hz, 2H), 6.70 (m, 3H), 7.36 (m, 2H), 12.4 ppm (broad s, 1H, disappeared on treatment with D_2O). IR (nujol): ν 1270, 1680, 3300 cm^{-1}.

Ethyl 5,6-difluoro-3-(phenylthio)-1*H*-indole-2-carboxylate (37). Was prepared as **32** using **26**. Yield 78%, mp 153-155 °C (from ethanol). ^{1}H NMR (DMSO-d_6): δ 1.24 (t, *J* = 7.0 Hz, 3H), 4.30 (q, *J* = 7.0 Hz, 2H), 7.08-7.16 (m, 3H), 7.23 (m, 2H), 7.30 (m, 1H), 7.47 (m, 1H), 12.57 ppm (broad s, 1H, disappeared on treatment with D_2O). IR (nujol): ν 1238, 1682, 3275 cm^{-1}.

Ethyl 4,5-difluoro-3-[(3,5-dimethylphenyl)thio]-1*H*-indole-2-carboxylate (38). Was prepared as **32** using **25** and *N*-[(3,5-dimethylphenyl)thio]succinimide. Yield 80%, mp 171-174 °C (from ethanol). ^{1}H NMR (DMSO-d_6): δ 1.26 (t, *J* = 7.1 Hz, 3H), 2.15 (s, 6H), 4.32 (q, *J* = 7.1 Hz, 2H), 6.74 (m, 2H), 6.77 (m, 1H), 7.30 (dd, *J* = 8.0 and 10.7 Hz, 1H), 7.48 (dd, *J* = 6.9 and 10.7 Hz, 1H), 12.30 ppm (broad s, 1H, disappeared on treatment with D_2O). IR (nujol): ν 1300, 1440, 1680, 3280 cm^{-1}.

Ethyl 5,7-difluoro-3-[(3,5-dimethylphenyl)thio]-1*H*-indole-2-carboxylate (39). Was prepared as **32** using **27** and *N*-[(3,5-dimethylphenyl)thio]succinimide. Yield 77% as crude product. Due to difficulty encountered in the purification of this compound, it was used as crude product.

Ethyl 4-chloro-5-fluoro-3-[(3,5-dimethylphenyl)thio]-1*H*-indole-2-carboxylate (40). Was prepared as **32** using **28** and *N*-[(3,5-dimethylphenyl)thio]succinimide. Yield 77%, mp 182-184 °C (from ethanol). ^{1}H NMR (DMSO-d_6): δ 1.24 (t, *J* = 7.1 Hz, 3H), 2.12 (s, 6H), 4.34 (q, *J* = 7.1 Hz, 2H), 6.61 (s, 2H), 6.69 (s, 1H), 7.32 (t, *J* = 9.5 Hz, 1H), 7.54 (dd, *J* = 4.4 and 9.0 Hz, 1H), 12.45 (broad s, 1H, disappeared on treatment with D_2O). IR (nujol): ν 710, 1240, 1660, 3280 cm^{-1}.

Ethyl 6-chloro-5-fluoro-3-[(3,5-dimethylphenyl)thio]-1*H*-indole-2-carboxylate (41). Was prepared as **32** using **29** and *N*-[(3,5-dimethylphenyl)thio]succinimide. Yield 61%, mp 186-190 °C (from ethanol). ^{1}H NMR ($CDCl_3$): δ 1.34 (t, *J* = 7.1 Hz, 3H), 2.19 (s, 6H), 4.42 (q, *J* = 7.1 Hz, 2H), 6.76 (s, 1H), 6.78 (s, 2H), 7.30 (d, *J* = 9.2 Hz, 1H), 7.48 (d, *J* = 5.9 Hz, 1H), 9.34 (broad s, 1H, disappeared on treatment with D_2O, 1H). IR (nujol): ν 850, 1630, 1670, 3260 cm^{-1}.

Ethyl 5-chloro-4-fluoro-3-[(3,5-dimethylphenyl)thio]-1*H*-indole-2-carboxylate (42). Was prepared as **32** using **30** and *N*-[(3,5-dimethylphenyl)thio]succinimide. Yield 51%, mp 149-151 °C (from ethanol). ^{1}H

NMR (DMSO-d_6): δ 1.19 (t, *J* = 7.1 Hz, 3H), 2.07 (s, 6H), 4.26 (q, *J* = 7.1 Hz, 2H), 6.40-7.35 ppm (m, 5H). IR (nujol): ν 780, 1130, 1700, 3400 cm^{-1}.

Ethyl 5-chloro-6-fluoro-3-[(3,5-dimethylphenyl)thio]-1*H*-indole-2-carboxylate (43). Was prepared as **32** using **31** and *N*-[(3,5-dimethylphenyl)thio]succinimide. Yield 77%, mp 170-173 °C (from ethanol). ^{1}H NMR (DMSO-d_6): δ 1.19 (t, *J* = 7.1 Hz, 3H), 2.07 (s, 6H), 4.26 (q, *J* = 7.1 Hz, 2H), 6.67 (s, 2H), 6.70 (s, 1H) 7.38 (d, *J* = 9.5 Hz, 1H), 7.42 (d, *J* = 7.2 Hz, 1H), 12.25 ppm (broad s, 1H, disappeared on treatment with D_2O). IR (nujol): ν 1270, 1660, 3280 cm^{-1}.

Ethyl 5,6-dichloro-3-[(3,5-dimethylphenyl)sulfonyl]-1*H*-indole-2-carboxylate (45). Was prepared as **44** using **33**. Yield 72%, mp 161-163 °C (from ethanol). ^{1}H NMR (DMSO-d_6): δ 1.25 (t, *J* = 6.9 Hz, 3H), 2.30 (s, 3H), 4.34 (q, *J* = 6.9 Hz, 2H), 7.26 (s, 1H), 7.58 (s, 2H), 7.78 (s, 1H), 8.39 (s, 1H) 12.50 ppm (broad s, 1H, disappeared on treatment with D_2O). IR (nujol): ν 1734, 3215. cm^{-1}.

Ethyl 5,7-dichloro-3-[(3,5-dimethylphenyl)sulfonyl]-1*H*-indole-2-carboxylate (46). Was prepared as **44** using **34**. Yield 50%. ^{1}H NMR ($CDCl_3$): δ 1.41 (t, *J* = 7.1 Hz, 3H), 2.25 (s, 6H), 4.45 (q, *J* = 7.1 Hz, 2H), 7.17 (s, 1H), 7.45 (d, *J* = 1.4 Hz, 1H), 7.62 (s, 1H), 8.50 (d, *J* = 1.0 Hz, 1H), 9.57 (broad s, 1H, disappeared on treatment with D_2O). IR (nujol): ν 1140, 1220, 1720, 3280 cm^{-1}.

Ethyl 4,5-difluoro-3-(phenyl)sulfonyl-1*H*-indole-2-carboxylate (47). Was prepared as **44** using **35**. Yield 65%, mp 205-207 °C (from ethanol). ^{1}H NMR (DMSO-d_6): δ 1.36 (t, *J* = 7.1 Hz, 3H), 4.44 (q, *J* = 7.1 Hz, 2H), 7.35 (m, 2H), 7.62 (m, 3H), 8.03 ppm (m, 2H).

Ethyl 4,5-difluoro-3-[(3,5-dimethylphenyl)sulfonyl]-1*H*-indole-2-carboxylate (48). Was prepared as **44** using **36**. Yield 37%, mp 176-178 °C (from ethanol). ^{1}H NMR (DMSO-d_6): δ 1.39 (t, *J* = 7.1 Hz, 3H), 2.34 (s, 6H), 4.45 (q, *J* = 7.1 Hz, 2H), 7.31 (m, 3H), 7.68 ppm (m, 2H). IR (nujol): ν 1260, 1750, 3320 cm^{-1}.

Ethyl 5,6-difluoro-3-(phenyl)sulfonyl-1*H*-indole-2-carboxylate (49). Was prepared as **44** using **37**. Yield 52%, mp 184-187 °C (from ethanol). ^{1}H NMR (DMSO-d_6): δ 1.31 (t, *J* = 7.0 Hz, 3H), 4.46 (q, *J* = 7.0 Hz, 2H), 7.52-7.62 (m, 3H), 7.65 (m, 1H), 8.02 (m, 2H), 8,14 (m, 1H), 13.30 ppm (broad s, 1H, disapeared on treatment with D_2O. IR (nujol): ν 869, 1312, 1709, 3281 cm^{-1}.

Ethyl 5,6-difluoro-3-[(3,5-dimethylphenyl)sulfonyl]-1*H*-indole-2-carboxylate (50). Was prepared as **44** using **38**. Yield 68%, mp 232-235 °C (from ethanol). ^{1}H NMR (DMSO-d_6): δ 1.29 (t, *J* = 7.1 Hz, 3H), 2.32 (s, 6H), 4.37 (q, *J* = 7.1 Hz, 2H), 7.28 (m, 1H), 7.55 (dd, *J* = 7.1 and 10.5 Hz, 1H), 7.62 (m, 2H), 8.13 (dd, *J* = 8.0 and 10.5 Hz, 1H), 13.04 ppm (broad s, 1H, disappeared on treatment with D_2O). IR (nujol): ν 850, 1200, 1740, 3300 cm^{-1}.

Ethyl 5,7-difluoro-3-[(3,5-dimethylphenyl)sulfonyl]-1*H*-indole-2-carboxylate (51). Was prepared as **44** using **39**. Yield 58%, mp 208-210 °C (from ethanol). ^{1}H NMR (DMSO-d_6): δ 1.32 (t, *J* = 7.1 Hz, 3H), 2.33 (s, 6H), 4.38 (q, *J* = 7.1 Hz, 2H), 7.29 (s, 1H), 7.40 (d, *J* = 10.1 Hz, 1H), 7.65 (s, 2H), 7.73 (d, *J* = 9.6 Hz, 1H), 13.80 ppm (broad s, 1H, disappeared on treatment with D_2O). IR (nujol): ν 900, 1150, 1220, 1760, 3300 cm^{-1}.

Ethyl 4-chloro-5-fluoro-3-[(3,5-dimethylphenyl)sulfonyl]-1*H*-indole-2-carboxylate (52). Was prepared as **44** using **40**. Yield 54%, mp 232-234 °C (from ethanol). ^{1}H NMR (DMSO-d_6): δ 1.37 (t, *J* = 7.1 Hz, 3H), 2.32 (s, 6H), 4.46 (q, *J* = 7.1 Hz, 2H), 7.27 (s, 1H), 7.40 (d, *J* = 9.2 Hz, 1H), 7.55 (dd, *J* = 4.3 and 9.0 Hz, 1H), 7.68 ppm (s, 2H). IR (nujol): ν 780, 850, 1050, 1100, 1280, 1740, 3200 cm^{-1}.

Ethyl 6-chloro-5-fluoro-3-[(3,5-dimethylphenyl)sulfonyl]-1*H*-indole-2-carboxylate (53). Was prepared as **44** using **41**. Yield 60%, mp 233-235 °C (from ethanol). ^{1}H NMR (DMSO-d_6): δ 1.27 (t, *J* = 7.1 Hz, 3H), 2.30 (s, 6H), 4.34 (q, *J* = 7.1 Hz, 2H), 7.26 (s, 1H), 7.60 (s, 2H), 7.70 (d, *J* = 6.5 Hz, 1H), 8.08 (d, *J* = 10.3 Hz, 1H), 12.50 (broad s, 1H, disappeared on treatment with D_2O). IR (nujol): ν 1130, 1710, 3200 cm^{-1}.

Ethyl 5-chloro-4-fluoro-3-[(3,5-dimethylphenyl)sulfonyl]-1*H*-indole-2-carboxylate (54). Was prepared as **44** using **42**. Yield 59%, mp 224-226 °C (from ethanol). ^{1}H NMR (DMSO-d_6): δ 1.34 (t, *J* = 7.1 Hz, 3H), 2.28 (s, 6H), 4.40 (q, *J* = 7.1 Hz, 2H), 7.24 (s, 1H), 7.32 (d, *J* = 8.8 Hz, 1H), 7.37 (dd, *J* = 6.0 and 8.8 Hz, 1H), 7.62 ppm (s, 2H). IR (nujol): ν 780, 1130, 1700, 3400 cm^{-1}.

Ethyl 5-chloro-6-fluoro-3-[(3,5-dimethylphenyl)sulfonyl]-1*H*-indole-2-carboxylate (55). Was prepared as **44** using **43**. Yield 83%, mp 236-240 °C (from ethanol). ^{1}H NMR (DMSO-d_6): δ 1.21 (t, *J* = 7.1 Hz, 3H), 2.28 (s, 6H), 4.40 (q, *J* = 7.1 Hz, 2H), 7.22 (s, 1H), 7.48 (d, *J* = 9.27 Hz, 1H), 7.55 (s, 2H), 8.28 (d, *J* = 7.4 Hz, 1H), IR (nujol): ν 680, 850, 1050, 1130, 1710, 3200 cm^{-1}.

Cell Based Antiviral Assay Procedures

Cells. C8166 and MT4 are CD4+ T-cell lines containing Human T cell Lymphotropic Virus-I (HTLV-I) genome of which only the tat gene is expressed. Both cell lines are highly sensitive to HIV-1. After HIV-1 infection cell clusters characteristic of intact C8166 and MT4 rapidly disintegrate, syncytia appear and the cells die. Cell cultures were checked periodically for the absence of mycoplasma contamination with a MycoTect Kit (Gibco). Normal human lymphocytes and macrophages were obtained as follows. Peripheral blood obtained from healthy HIV-negative donors was enriched for peripheral blood mononuclear cells (PBMC) by centrifugation over Ficoll Hypaque gradient. PBMC were then further enriched for lymphocytes or monocytes by elutriation.[5S] Cells obtained by this method are >90% pure as determined by cytofluorimetric assay (FACS). Cells were grown at 37 °C in 5% CO_2 atmosphere in RPMI 1640 medium and supplemented with 10% fetal calf serum (FCS), 100 IU/mL penicillin G, and 100 μg/mL streptomycin.

Viruses. A laboratory lymphocyte-tropic strain of HIV (HTLV-IIIB also called HIV-IIIB) and two primary isolates (HIV-112 and HIV-AB1) were used to infect MT-4, C8166 and lymphocytes. A laboratory monocyte-tropic strain of HIV-1 (HTLV-III-Ba-L, also called HIV-$1_{\text{Ba-L}}$) was used to infect macrophages. Both the laboratory strains are available through the AIDS Research and Reference Reagent Program (NIH, Bethesda, MD). The primary isolates of HIV-1 were obtained from two HIV-1-Ab seropositive individuals who had experienced a virologic failure (plasma HIV RNA levels >500 copies/mL on at least two consecutive measurements) during HAART including zidovudine, lamivudine, efavirenz, tenofovir plus, at least, one protease inhibitor. Isolation of these strains from the plasma of infected individuals was performed in PBMC cultures; the supernatants of these coltures were used as a source of virus.

The Y181C and the K103N-Y181C mutant strains were obtained from AIDS Research and Reference Reagent Program (NIH, Bethesda, MD). The Y81C mutant (HIV-1 119) was obtained from CEM cells infected with AZT-sensitive HIV-1 patient isolate A018. Resistance was selected for in vitro by culturing the AZT-sensitive A018 isolate in the presence of nevirapine. The K103N-Y181C mutant (HIV-1 IIIB

A17) highly resistant to inhibition by RT non-nucleoside inhibitors, including pyridinone derivatives, BI-RG-587, and TIBO, was obtained by reapeted passages in H9 cells.

HIV Titration. Titration to determine the infectivity of laboratory viral strains and the primary isolates was performed in C8166 cells (HIV-IIIB), lymphocytes (HIV-112 and HIV-AB1) and macrophages (HIV-$1_{\text{Ba-L}}$).[6S,7S] The titer of virus stocks, expressed as 50% tissue culture infectious dose ($TCID_{50}$), was determined as previously described.[7S]

Anti-HIV Assays. The activity of test compounds against multiplication of HIV-1 in MT-4 and C8166 cells was based on inhibition of virus-induced cytopathogenicity. Briefly, 150 μL of culture medium containing 2×10^4 cells were added to each well flat-bottom microtiter trays containing 50 μL of culture medium with or without various concentrations of test compounds. Then 50 μL of HIV suspension (100 $TCID_{50}$) were added. After 5 days incubation at 37 °C, the viral activity of compounds was evaluated by quantifying the HIV induced cytopathogenicity by means of the 3-(4,5-dimethylthiazol-1-yl)-2,5-diphenyltetrazolium bromide (MTT) method.[8S] Protection (%) from the viral induced cytopathogenicity in HIV infected cells as mesured by the MTT assay was calculated as follows: $(OD_T)_{HIV} - (OD_C)_{HIV} : (OD_C)_{mock} - (OD_C)_{HIV}$, whereby $(OD_T)_{HIV}$ is the optical density measured with a given concentration of cysteamine in HIV infected cells, $(OD_C)_{HIV}$ is the optical density measured for the control untreated HIV infected cells, $(OD_C)_{mock}$ is the optical density measured for the control untreated mock infected cells. The optical density at 540/690 nm was measured using a plate reader. For evaluation of antiviral activity in acutely infected lymphocytes, PHA stimulated lymphocytes were washed twice with PBS, counted, distributed into 15 mL polyethylene tubes at a concentration of 6×10^6 cells/mL and exposed to 100 $TCID_{50}$ of HIV in the presence or the absence of antiviral compounds. After 2 h incubation in 5% CO_2 at 37 °C, the cells were washed twice in PBS and inoculated in each well of the 48-well plates (Costar, Cambridge, MA) at a concentration of 10^6 cells/mL of complete medium supplemend with 10 units/mL of recombinant Interleukin-2 (Collaborative Research Incorporated, Bedford, MA). The cells were subsequently cultured in the presence of the same concentrations of drugs as before. Half the volume of supernatant in each well was replaced every 3-4 days. The antiviral activity of the compounds was assessed by measuring HIV-p24 antigen production in the supernatants of infected cultures as previously described[9S] by using a commercially available HIV-antigen kit (Abbott, Pomezia, Italy). The assay to evaluate anti-HIV drug efficacy in acutely infected mature macrophages has been previously described.[9S] Briefly, 10^5 macrophages were suspended in complete medium. The cells were exposed to 100 $TCID_{50}$ of HIV, in the presence or in the absence of the compounds and incubated at 37 °C in a CO_2 incubator. Two hours after the viral exposure, the macrophages were extensively washed to remove excess virus and then cultured under the same conditions and drug concentrations before. Cell were washed and fed every seven days. The antiviral activity of the compounds was assessed by measuring HIV-p24 antigen production in the supernatants of infected curltures.

Cytotoxicity. The cytotoxicity of test compounds was evaluated in parallel with their antiviral activity and was based on the viability of mock-infected cells, as monitored by the MTT method. The 50% effective dose (ED_{50}) and 50% cytotoxic dose (TD_{50}) values were calculated from pooled values in the effective dynamic range of the antiviral activity and cytotoxicity assays (5-95%) using the median effect equation as previously described.[10S] The selectivity index (SI) was calculated ad TD_{50}:ED_{50} ratio.

Enzymatic Assay Procedures

Chemicals. [^{3}H]dTTP (40 Ci/mmol) was from Amersham and unlabelled dNTP's from Boehringer. Whatman was the supplier of the GF/C filters. All other reagents were of analytical grade and purchased from Merck or Fluka.

Nucleic acid substrates. The homopolymer poly(rA) (Pharmacia) was mixed at weight ratios in nucleotides of 10:1, to the oligomer oligo$(dT)_{12-18}$ (Pharmacia) in 20 mM Tris-HCl (pH 8.0), containing 20 mM KCl and 1 mM EDTA, heated at 65 °C for 5 min and then slowly cooled at room temperature.

Expression and purification of recombinant HIV-1 RT forms. The coexpression vectors pUC12N/p66(His)/p51with the wild-type or the mutant forms of HIV-1 RT p66 were kindly provided by Dr. S. H. Hughes (NCI-Frederick Cancer Research and Development Center). Proteins were expressed in *E. coli* and purified as described.[11S]

HIV-1 RT RNA-dependent DNA polymerase activity assay. RNA-dependent DNA polymerase activity was assayed as follows: a final volume of 25 μl contained reaction buffer (50 mM Tris-HCl pH 7.5, 1 mM DTT, 0.2 mg/ml BSA, 4% glycerol), 10 mM $MgCl_2$, 0.5 μg of poly(rA)/oligo$(dT)_{10:1}$ (0.3 μM 3'-OH ends), 10 μM [3H]-dTTP (1Ci/mmol) and 2-4 nM RT. Reactions were incubated at 37°C for the indicated time. 20 μl-Aliquots were then spotted on glass fiber filters GF/C which were immediately immersed in 5% ice-cold TCA. Filters were washed twice in 5% ice-cold TCA and once in ethanol for 5 min, dried and acid-precipitable radioactivity was quantitated by scintillation counting.

Inhibition assays. Reactions were performed under the conditions described for the HIV-1 RT RNA-dependent DNA polymerase activity assay. Incorporation of radioactive dTTP into poly(rA)/oligo(dT) at different substrate (nucleic acid or dTTP) concentrations was monitored in the presence of increasing fixed amounts of inhibitor. Data were then plotted according to Lineweaver-Burke and Dixon. For K_i determination, an interval of inhibitor concentrations between 0.2 K_i and 5 K_i was used.

References

(1S) Silvestri, R.; Artico, M.; La Regina, G.; De Martino, G. Synthetic approaches to difluoroindolecarboxylic acid ethyl esters. *Arkivoc* **2004**, *V*, 26-31.

(2S) (a) Silvestri, R.; De Martino, G.; La Regina, G.; Artico, M.; Massa, S.; Vargiu, L.; Mura, M.; Loi, A. G.; Marceddu, T.; La Colla, P. Novel indolyl aryl sulfones active against HIV-1 carrying NNRTI resistance mutations: Synthesis and SAR studies. *J. Med. Chem.* **2003**, *46*, 2482-93. (b) La Colla, P.; Artico, M.; Sommadossi, J. P. Use of phenylindoles for the treatment of HIV. PCT Int. Appl. **2002**, WO 2002083126.

(3S) Silvestri, R.; De Martino, G.; Sbardella, G. A simplified synthesis of ethyl 5-chloro-6-fluoro-1*H*-indole-2-carboxylate and ethyl 5-chloro-4-fluoro-1*H*-indole-2-carboxylate. *Org. Prep. Proc. Int. (OPPI)* **2002**, *34*, 507-510.

(4S) Ethyl 5,6-dichloroindole-2-carboxylate was prepared according to: Gagliardi, S.; Nadler, G.; Consolandi, E.; Parini, C.; Morvan, M.; Legave, M. N.; Belfiore, P.; Zocchetti, A.; Clarke, G. D.; James, I.; Nambi, P.; Gowen, M.; Farina, C. 5-(5,6-Dichloro-2-indolyl)-2-methoxy-2,4-pentadienamides: novel and selective inhibitors of the vacuolar H^+-ATPase of osteoclasts with bone antiresorptive activity. *J. Med. Chem.* **1998**, *41*, 1568-1573.

(5S) Placido, R.; Mancino, G.; Amendola, A.; Mariani, F.; Venditti, S.; Piacentini, M.; Sanduzzi. A.; Bocchino, M. L.; Zembala, M.; Colizzi, V. Apoptosis of human monocytes/macrophages in Mycobacterium tubercolosis infection. *J. Pathol.* **1997**, *181*, 31-38.

(6S) Andreoni, M.; Sarmati, L.; Parisi, S.G.; Ercoli, L.; Rocchi G. Efficient and reproducible new semimicromethod for the detection and tritation of HIV in human plasma. *J. Med. Virol.* **1992,** *38*, 207-213.

(7S) Bergamini, A.; Capozzi, M.; Ghibelli, L.; Dini, L.; Salanitro, A.; Milanese, G.; Wagner, T.; Beninati, S.; Pesce, C. D.; Amici, C.; Rocchi G. Cystamine potently suppresses in vitro HIV replication in acutely and chronically infected human cells. *J. Clin. Invest.* **1994**, *93*, 2251-2257.

(8S) Pauwels, R.; Balzarini, J.; Baba, M.; Snoeck, R.; Schols, D.; Hederwijn, P.; Desmyster, J.; De Clercq, E. A rapid and automated tetrazolium-based colorimetric assay for the detection of anti HIV compounds. *J. Virol. Methods*, **1988**, *20*, 309-321.

(9S) Perno, C. F.; Yarchoan, R.; Cooney, D. A.; Hartman, N. R.; Gartner, S.; Popovic, M.; Hao, Z.; Gerrard, T. L.; Wilson, Y. A.; Johns, D. G. Inhibition of human immunodeficiency virus (HIV-17, HTLV-III-Ba-L) replication in fresh and cultured human peripheral blood monocytes/macrophages by azidothymidine and related 2’,3’-didesoxynucleosides. *J. Exp. Med.* **1988**, *168*, 1111-1125.

(10S) Chou, T. C. Derivation and properties of Micaelis-Menten type and Hill type equation for reference ligands. *J. Theor. Biol.* **1976**, *59*, 253-276.

(11S) Maga, G.; Amacker, M.; Ruel, N.; Hubsher, U.; Spadari, S. Resistance to nevirapine of HIV-1 reverse transcriptase mutants: loss of stabilizing interactions and thermodynamic or steric barriers are induced by different single amino acid substitutions. *J. Mol. Biol.* **1997**, *274*, 738-747.

Appendix. Elemental analyses of derivatives **6-17**.

6. Calcd: C, 48.79; H, 2.73; Cl, 19.20; N, 7.59; S, 8,68.
Found: C, 48.54; H, 2.65; Cl, 19.06; N, 7.44; S, 8.50.

7. Calcd: C, 51.40; H, 3.55; Cl, 17.85; N, 7.05; S, 8,07.
Found: C, 51.18; H, 3.44; Cl, 17.64; N, 6.88; S, 7.92.

8. Calcd: C, 51.40; H, 3.55; Cl, 17.85; N, 7.05; S, 8.07.
Found: C, 51.32; H, 3.51; Cl, 17.77; N, 6.94; S, 7.91.

9. Calcd: C, 53.57; H, 3.00; F, 11.30; N, 8.33; S, 9.53.
Found: C, 53.42; H, 2.92; F, 11.25; N, 8.18; S, 9.41.

10. Calcd: C, 56.04; H, 3.87; F, 10.43; N, 7.69; S, 8.80.
Found: C, 55.88; H, 3.82; F, 10.28; N, 7.44; S, 8.67.

11. Calcd: C, 53.57; H, 3.00; F, 11.30; N, 8.33; S, 9.53.
Found: C, 53.45; H, 2.90; F, 11.12; N, 8.06; S, 9.27.

12. Calcd: C, 56.04; H, 3.87; F, 10.43; N, 7.69; S, 8.80.
Found: C, 55.85; H, 3.75; F, 10.12; N, 7.44; S, 8.52.

13. Calcd: C, 56.04; H, 3.87; F, 10.43; N, 7.69; S, 8.80.
Found: C, 55.88; H, 3.81; F, 10.24; N, 7.38; S, 8.62.

14. Calcd: C, 53.62; H, 3.71; Cl, 9.31; F, 4.99; N, 7.36; S, 8,42.
Found: C, 53.45; H, 3.66; Cl, 9.20; F, 4.75; N, 7.25; S, 8,27.

15. Calcd: C, 53.62; H, 3.71; Cl, 9.31; F, 4.99; N, 7.36; S, 8.42.
Found: C, 53.38; H, 3.55; Cl, 9.06; F, 4.74; N, 7.08; S, 8.18.

16. Calcd: C, 53.62; H, 3.71; Cl, 9.31; F, 4.99; N, 7.36; S, 8.42.
Found: C, 53.44; H, 3.67; Cl, 9.11; F, 4.70; N, 7.18; S, 8.25.

17. Calcd: C, 53.62; H, 3.71; Cl, 9.31; F, 4.99; N, 7.36; S, 8.42.
Found: C, 53.50; H, 3.69; Cl, 9.21; F, 4.85; N, 7.18; S, 8.22.