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SYNTHESIS AND PHARMACOLOGICAL ACTIVITY OF 1,8,11,11-TETRAMETHYL-4-AZATRICYCLO[5.2.2.0^{2,6}]UNDEC-8-ENE-3,5-DIONE DERIVATIVES

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Abstract: The synthesis and pharmacological activity of *N*-substituted derivatives of 1,8,11,11-tetramethyl-4-azatricyclo[5.2.2.0^{2,6}]undec-8-ene-3,5-dione (**1**) are described. The molecular structure of starting compound (**1**) was confirmed by elemental analysis, ^{13}C NMR and X-ray crystallography. The structures of derivatives were confirmed by ^{1}H NMR and mass spectra. The compounds were investigated for antibacterial activity, including Gram-positive cocci, Gram-negative rods, and antifungal activity. Studied compounds were evaluated also for their cytotoxicity and anti-HIV-1 activity in MT-4 cells.

Keywords: antimicrobial activity, 1,8,11,11-tetramethyl-4-azatricyclo[5.2.2.0^{2,6}]undec-8-ene-3,5-dione derivatives, cytotoxicity

Several imides and their derivatives have been found to possess a broad spectrum of biological activities. Many compounds of this class have antibacterial and antifungal (1-10), anticonvulsive (11), hypnotic, sedative (12, 13), antitumor (14-18), antiviral (19, 20) antioxidative (21) and anxiolytic (22) properties. Literature survey showed that cyclic imides and their derivatives have high affinity for α_1-adrenergic (23), dopaminergic (24) and serotoninergic receptors (25, 26).

Currently available drugs for the anti-HIV treatment are based on combination of two types of anti-HIV-1 agents: nucleoside reverse transcriptase inhibitors (RTIs) and protease inhibitors (27). The RTIs can be divided into nucleoside (NI) and non-nucleoside RT inhibitors (NNRTI). Several non-nucleoside inhibitors have been described, including nevirapine, thiobenzimidazolone (TIBO) derivatives, pyridinone derivatives and the bis(heteroaryl)piperazines (BHAPs), such as delavirdine and atevirdine (28). Another arylpiperazine, vicriviroc, is currently in phase III of clinical trials (29). The discovery of new BHAP analogs is actively proceeded (30, 31).

Cyclic imides, such as succinimides, maleimides, glutarimides, phthalimides, possess structural features, bearing potential biological activity and pharmaceutical use. Their molecules contain an imide fragment with the general structure -CO-N(R)-CO-, so that they are hydrophobic, neutral, and able to cross biological membranes *in vivo* (32). Imides with four methyl substituents at positions 1, 8 and 11 in their tricyclic compound are used as substrates for the synthesis of biologically active compounds (33, 34). Antimicrobial activity of derivatives of tricyclo[5.2.1.0^{2,6}]dec-8-ene-3,5-dione with methyl substituent was tested against selected Gram-positive and Gram-negative bacteria and fungi of the *Candida* species (3). Compounds showed significant activity in the above tests. It was expected that the imide **1** will have biological activity.

In the search for new compounds with antimicrobial properties, our attention was drawn to a group of imides with methyl substituents, alkylimides and *N*-aminoalkylimides. In this study, we have combined the structure of policyclic ring of 1,8,11,11-tetramethyl-4-azatricyclo[5.2.2.0^{2,6}]

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undec-8-ene-3,5-dione (**1**) with alkyl chains bearing different substituents. The molecular structure of **1** was determined by an X-ray crystal structure analysis. This work describes synthesis and a wide spectrum of antimicrobial, cytotoxicity and anti-HIV-1 activity of **1** and its *N*-substituted derivatives.

EXPERIMENTAL

All chemicals and solvents were purchased from Aldrich (Vienna, Austria). Flash chromatography was performed on Merck silica gel 60 (200–400 mesh) using the chloroform/methanol (9 : 1, v/v) mixture as eluent. Analytical TLC was carried out on silica gel F_{254} plates (0.25 mm thickness) (E. Merck, Darmstadt, Germany) and were visualized using an ultraviolet (UV) lamp at 254 nm.

Melting points were determined on Electrothermal digital melting point apparatus (Essex, UK) and are uncorrected. The NMR spectra were recorded on a Bruker AVANCE DMX 400 spectrometer (Rheinstetten, Germany), operating at 400 or 300 MHz (^{1}H NMR) and 300 MHz (^{13}C NMR). The chemical shift values are expressed in ppm relative to TMS as an internal standard. Elemental analyses were recorded with a Perkin-Elmer CHN model 2400 (Hitachi, Tokyo, Japan). Mass spectra ESI measurements were carried out on Waters ZQ Micromass instruments with quadrupole mass analyzer. The spectra were performed in the positive ion mode at a declustering potential of 40-60 V. The sample was previously separated on a UPLC column (C18) using UPLC ACQUITY™ system by Waters connected with DPA detector.

Diffraction data for X-ray structure analysis were collected for **1** at 293 K with a KM4 diffractometer, using graphite monochromated CuKα radiation (α = 1.54178 Å) and ω/2θ scan mode. Crystal structure was solved by the SHELXS-97 program and refined by full-matrix least squares on F^2 using the SHELXL-97 program [38]. All non-hydrogen atoms were refined with anisotropic displacement parameters. Position of amine H atom was found in the difference Fourier map. All remaining H atoms were positioned geometrically and allowed to ride on their parent atoms, with U_{iso}(H) = 1.2 or 1.5 U_{eq}(C). The experimental details and final atomic parameters for **1** have been deposited with the Cambridge Crystallographic Data Centre as supplementary material (CCDC ID: 846153). Copies of the data can be obtained free of charge on request *via* www.ccdc.cam.ac.uk/data_request/cif, or by e-mailing: data_request@ccdc. cam.ac.uk.

Synthesis of 1,8,11,11-tetramethyl-4-azatricyclo[5.2.2.0^{2,6}]undec-8-ene-3,5-dione (1)

A mixture of 1,3,5,5-tetramethylocyclohexa-1,3-diene (2.5 g, 0.018 mol) and maleimide (1.78 g, 0.018 mol) in benzene (10 mL) was refluxed for 1.5 h. Product **1** was filtered off and crystallized from benzene.

Yield 80%, m.p. 235–237°C; ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 9.13 (br. s, 1H, NH), 5.39 (s, 1H, CH=), 3.17 (dd, 1H, J_1 = 3.2 Hz, J_2 = 7.6 Hz, (HC)CH(C=O)), 2.39 (m, 2H, CH(C=O), $CHC(CH_3)_2$), 1.70 (s, 3H, CH_3), 1.28 (s, 3H, CH_3), 1.09 (d, 1H, J = 12.8 Hz, CH_2), 1.01 (s, 3H, CH_3), 0.96 (d, 1H, J = 12.8 Hz, CH_2), 0.81 (s, 3H, CH_3). ^{13}C NMR (300 MHz, DMSO, δ, ppm): 180.12 (C5), 178.84 (C3), 142.07 (C8), 126.97 (C9), 50.32 (C2), 49.72 (C10), 48.86 (C7), 44.09 (C6), 39.27 (C11), 34.44 (CH_3), 30.59 (CH_3), 29.31 (C1), 22.84 (CH_3). EMI MS: m/z = 232.3 $[M]^+$ (100%). Analysis: $C_{14}H_{19}NO_2$ (233.31): calcd. C 72.10, H 8.15, N 6.00%; found C 72.15, H 8.14, N 6.05 %.

Crystal Data

Crystal system monoclinic, space group $P21/c$, unit cell dimensions a = 13.578(3), b = 7.644(2), c = 12.432(2) Å, β = 93.73(3)°, V = 1287.6(5) Å^3; Z = 4, d_c = 1.204 g/cm^3, μ = 0.637 mm^{-1}, F(000) = 504. A crystal of dimensions 0.39 × 0.30 × 0.25 mm was used for intensity measurements. Within the θ range 3.26–80.24° [0 = h = 17, -9 = k = 0, -15 = l = 15] 2877 reflections were collected. The 2781 unique reflections [R(int) = 0.0124] were used for the refinement of 158 parameters, including extinction coefficient [x = 0.0057(5)]. Final R indices on F^2 for 2184 observed reflections [$I > 2\sigma(I)$] were: $R1$ = 0.0394, $wR2$ = 0.1126, goodness-of-fit 1.049, and largest difference peak/hole 0.21/-0.17 e Å^{-3}.

Synthesis of 4-(4-bromobutyl)-1,8,11,11-tetramethyl-4-azatricyclo[5.2.2.0^{2,6}]undec-8-ene-3,5-dione (2)

A mixture of imide **1** (1 g, 0.0043 mol), dibromobutane (2.7 g, 0.012 mol) and anhydrous K_2CO_3 (1 g, 0.0072 mol) in acetonitrile (100 mL) was refluxed for 8 h. The inorganic precipitate was filtered off, the solvent was evaporated.

Yield 82%, oil; ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 5.37 (s, 1H, CH=), 3.39 (m, 4H, CH_2Br, NCH_2), 3.14 (dd, 1H, J_1 = 3.3 Hz, J_2 = 7.8 Hz, (HC)CH(C=O)), 2.43(dd, 1H, J_1 = 1.2 Hz, J_2 = 3.3 Hz, $CHC(CH_3)$), 2.39 (d, 1H, J = 7.8 Hz, CH(C=O)), 2.01 (m, 2H, CH_2CH_2Br), 1.72 (s, 3H, CH_3), 1.63 (d, 2H, NCH_2CH_2), 1.34 (s, 3H, CH_3), 1.21 (d, 1H, J = 12.8 Hz, CH_2), 1.06 (s, 3H, CH_3), 1.00 (d, 1H, J =

12.8 Hz, CH_2), 0.85 (s, 3H, CH_3). EMI MS: m/z = 368.1 $[M]^+$ (100%).

Synthesis of 4-(3-chloropropyl)-1,8,11,11-tetramethyl-4-azatricyclo[5.2.2.$0^{2,6}$]undec-8-en-3,5-dione (3)

A mixture of imide 1 (0.6 g, 0.0026 mol), 1-bromo-3-chloropropane (1.28 g, 0.0081 mol) and anhydrous K_2CO_3 (0.6 g, 0.0043 mol) in acetonitrile (60 mL) was refluxed for 25 h. The inorganic precipitate was filtered off, the solvent was evaporated.

Yield 74%, oil; ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 5.37 (s, 1H, CH=), 3,53 (t, 2H, *J* = 6.8 Hz, NCH_2), 3.42 (t, 2H, *J* = 8.8 Hz, CH_2Cl), 3.17 (dd, 1H, J_1 = 3.2 Hz, J_2 = 8 Hz, (HC)CH=O), 2.48 (m, 1H, CHC=O), 2.42 (br. d, 1H, $CHC(CH_3)_2$, *J* = 8.0 Hz), 1.94 (t, 2H, *J* = 6.8 Hz, $CH_2CH_2CH_2$), 1.74 (s, 3H, CH_3), 1.37 (s, 3H, CH_3), 1.17 (d, 1H, *J* = 12.8 Hz, CH_2), 1.08 (s, 3H, CH_3), 1.04 (d, 1H, *J* = 12.8 Hz, CH_2), 0.87 (s, 3H, CH_3). ESI MS: m/z = 309.8 $[M]^+$ (100%).

General procedures for the preparation of arylpiperazine derivatives 2a–j and 3a-j

A mixture of compound **2** (0.3 g; 0.0008 mol) or **3** (0.5 g; 0.0016 mol), an appropriate amine (0.0015 or 0.0047 mol), anhydrous K_2CO_3 (0.3 g, 0.0022 mol or 0.5 g; 0.0036 mol) and catalytic amount of KI (0.2 g; 0,0012 mol) was dissolved in acetonitrile (50 mL) and refluxed for 12–74 h. The solvent was evaporated, then the residue was purified by column chromatography (dichloromethane/methanol, 9.5 : 0.5, v/v) to give compounds **2a** – **2j** and **3a** – **3j**, respectively.

4-{4-[4-(2-Methoxyphenyl)piperazin-1-yl]butyl}-1,8,11,11-tetramethyl-4-azatricyclo[5.2.2.$0^{2,6}$]undec-8-ene-3,5-dione (2a)

Yield 60%, m.p. 149 – 151°C; ^{1}H NMR (400 MHz, DMSO, δ, ppm): 10.46 (br. s, 1H, HCl), 6.95 (m, 4H, $CH_{arom.}$), 5.40 (s, 1H, CH=), 3.79 (s, 3H, OCH_3), 3.48 (d, 4H, *J* = 10.8 Hz, $N(CH_2CH_2)_2N$), 3.30 (t, 2H, *J* = 6.9 Hz, NCH_2), 3.23 (dd, 1H, J_1 = 2.9 Hz, J_2 = 7.6 Hz, (HC)CH(C=O)), 3.11 (m, 6H, $N(CH_2CH_2)_2N$, CH_2N), 2.55 (d, 1H, CH(C=O)), 2.50 (s, 2H, CH_2N), 2.32 (s, 1H, $CHC(CH_3)_2$), 1.66 (s, 3H, CH_3), 1.63 (m, 2H, CH_2 CH_2N), 1.42 (m, 2H, NCH_2 CH_2), 1.28 (s, 3H, CH_3), 1.18 (d, 1H, *J* = 12.0 Hz, CH), 1.05 (s, 3H, CH_3), 0.91 (d, 1H, *J* = 12.7 Hz, CH), 0.81 (s, 3H, CH_3), 0.86 (s, 3H, CH_3). EMI MS: m/z = 480.3 $[M + H]^+$ (100%).

1,8,11,11-Tetramethyl-4-[4-(4-pyrimidin-2-ylpiperazin-1-yl)butyl]-4-azatricyclo[5.2.2.$0^{2,6}$]undec-8-ene-3,5-dione (2b)

Yield 68%, m.p. 160 – 162°C; ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 12.86 (br. s, 1H, HCl), 8.68 (m, 2H, $CH_{arom.}$), 7.09 (m, 1H, CH_{arom}), 5.41 (s, 1H, CH=), 5.15 (br. d, 2H, *J* = 13.8 Hz, $N(CH_2CH_2)_2N$), 4.20 (br. t, 2H, *J* = 11.7 Hz, NCH_2,), 3.73 (br. d, 2H, *J* = 10.2 Hz, $N(CH_2CH_2)_2N$,), 3.40 (t, 2H, *J* = 6.6 Hz, CH_2N, 3.29 (m, 4H, $N(CH_2CH_2)_2N$), 3.22 (dd, 1H, J_1 = 3.3 Hz, J_2 = 7.8 Hz, (HC)CHC=O)), 2.45 (m, 2H, CHC=O, $CHC(CH_3)_2$), 1.85 (m, 2H, CH_2CH_2N), 1.72 (s, 3H, CH_3), 1.58 (m, 2H, NCH_2CH_2), 1.35 (s, 3H, CH_3), 1.17 (d, 1H, *J* = 6.9 Hz, CH_2), 1.08 (s, 3H, CH_3), 1.03 (d, 1H, *J* = 12.9 Hz, CH_2). EMI MS: m/z = 452.58 $[M + H]^+$ (100%).

1,8,11,11-Tetramethyl-4-[4-(4-phenylpiperazin-1-yl)butyl]-4-azatricyclo[5.2.2.$0^{2,6}$]undec-8-ene-3,5-dione (2c)

Yield 72%, oil; ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 7.25 (m, 2H, $CH_{arom.}$), 6.93 (m, 1H, $CH_{arom.}$), 6.90 (m, 1H, $H_{arom.}$), 6.85 (m, 1H, $CH_{arom.}$), 5.38 (br. s, 1H, CH=), 3.41 (br. t, 2H, *J* = 6.6 Hz, NCH_2), 3.21 (m, 4H, $N(CH_2CH_2)_2N$), 3.16 (dd, 1H, J_1 = 3.30 Hz, J_2 = 7.80 Hz, (CH)CH=O), 2.62 (m, 4H, $N(CH_2CH_2)_2N$), 2.47 (dd, 1H, J_1 = 1.50 Hz, J_2 = 3.30 Hz, CHC=O), 2.40 (m, 3H, CH_2N, $CHC(CH_3)_2$), 1.73 (d, 3H, CH_3), 1.49 (m, 4H, CH_2CH_2N, NCH_2CH_2), 1.37 (s, 3H, CH_3), 1.17 (br. d, 1H, *J* = 12.9 Hz, CH_2,), 1.08 (s, 3H, CH_3), 1.03 (br. d, 1H, *J* = 12.9 Hz, CH_2,), 0.87 (s, 3H, CH_3). EMI MS: m/z = 450.3 $[M + H]^+$ (100%).

1,8,11,11-Tetramethyl-4-[4-(4-pyridin-2-ylpiperazin-1-yl)butyl]-4-azatricyclo[5.2.2.$0^{2,6}$]undec-8-ene-3,5-dione (2d)

Yield 67%, m.p. 159 – 161°C; ^{1}H NMR (300 MHz, MeOD, δ, ppm): 8.17 (m, 1H, $CH_{arom.}$), 8.01 (m, 1H, $CH_{arom.}$), 7.51 (m, 1H, $CH_{arom.}$), 7.16 (m, 1H, $CH_{arom.}$), 5.43 (br. s, 1H, CH=), 4.41 (m. 2H, NCH_2), 3.76 (m, 1H, (HC)CHC=O), 3.44 (t, 2H, *J* = 6.9 Hz, CH_2N), 3.31 (m, 8H, $H_{piper.}$), 3.58 (d, 1H, *J* = 7.50 Hz, CHC=O), 2.42 (dd, 1H, J_1 = 1.50 Hz, J_2 = 2.40 Hz, $CHC(CH_3)_2$), 1.78 (m, 2H, CH_2CH_2N), 1.74 (s, 3H, CH_3), 1.60 (m, 2H, NCH_2CH_2), 1.35 (s, 3H, CH_3), 1.25 (br. d, 1H, *J* = 12.9 Hz, CH_2), 1.12 (s, 3H, CH_3), 1.02 (d, 1H, *J* = 12.9 Hz, CH_2), 0.86 (s, 3H, CH_3). EMI MS: m/e = 451.3 $[M + H]^+$ (100%).

4-{4-[4-(4-Fluorophenyl)piperazin-1-yl]butyl}-1,8,11,11-tetramethyl-4-azatricyclo[5.2.2.$0^{2,6}$]undec-8-ene-3,5-dione (2e)

Yield 64%, m.p. 182 – 184°C; ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 13.41 (br. s, 1H, HCl), 7.94 (m, 2H, $CH_{arom.}$), 7.21 (m, 2H, $CH_{arom.}$), 5.42 (s, 1H, CH=), 4.78 (t, 2H, *J* = 11.7 Hz, NCH_2), 4.24 (m, 2H,

$N(CH_2CH_2)_2N$), 3.66 (br. t, 4H, J = 9.9 Hz, $N(CH_2CH_2)_2N$), 3.42 (m, 2H, $N(CH_2CH_2)_2N$), 3.23 (m, 3H, CHC=O, (HC)CHC=O, $CHC(CH_3)_2$), 2.44 (m, 2H, CH_2N), 1.84 (m, 2H, CH_2CH_2N), 1.71 (s, 3H, CH_3), 1.63 (m, 2H, NCH_2CH_2), 1.33 (s, 3H, CH_3), 1.16 (d, 1H, J = 12.9 Hz, CH_2), 1.07 (s, 3H, CH_3), 0.99 (d, 1H, J = 12.9 Hz, CH_2), 0.85 (s, 3H, CH_3). EMI MS: m/e = 468.3 $[M + H]^+$ (100%).

4-[4-(4-Benzylpiperazin-1-yl)butyl]-1,8,11,11-tetramethyl-4-azatricyclo[5.2.2.0^{2,6}]undec-8-ene-3,5-dione (2f)

Yield 58%, m.p. 179 – 181°C; ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 7.70 (m, 2H, $CH_{arom.}$), 7.51 (m, 3H, $CH_{arom.}$), 5.45 (br. s, 1H, CH=), 4.27 (m, 2H, NCH_2), 4.18 (m, 2H, CH_2), 3.51 (m, 8H, $H_{piper.}$), 3.27 (dd, 1H, J_1 = 3.3 Hz, J_2 = 7.8 Hz, (HC)CH=O), 3.21 (m, 3H, CH=O, CH_2N), 2.48 (m, 1H, $CHC(CH_3)_2$), 1.85 (m, 2H, CH_2CH_2N), 1.74 (s, 3H, CH_3), 1.63 (m, 2H, NCH_2CH_2), 1.37 (s, 3H, CH_3), 1.20 (br. d, 1H, J = 12.9 Hz, CH_2), 1.10 (s, 3H, CH_3), 1.05 (br. d, 1H, J = 12.9 Hz, CH_2), 0.88 (s, 3H, CH_3). EMI MS: m/z = 464.3 $[M + H]^+$ (100%).

4-{4-[4-(4-Chlorophenyl)piperazin-1-yl]butyl}-1,8,11,11-tetramethyl-4-azatricyclo[5.2.2.0^{2,6}] undec-8-ene-3,5-dione (2g)

Yield 73%, m.p. 194 – 196°C; ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 13.42 (br. s, 1H, HCl), 7.72 (m, 2H, $CH_{arom.}$), 7.42 (m, 2H, $CH_{arom.}$), 5.38 (s, 1H, CH=), 4.62 (br. s, 2H, NCH_2), 4.06 (br. s, 2H, $N(CH_2CH_2)N$), 3.58 (m, 4H, $N(CH_2CH_2)_2N$), 3.38 (br. s, 2H, $N(CH_2CH_2)N$), 3.17 (br. s, 3H, (HC)CH(C=O), CHC=O, $CHC(CH_3)_2$), 2.40 (m, 2H, CH_2N), 1.80 (m, 2H, CH_2CH_2N), 1.67 (s, 3H, CH_3), 1.58 (m, 2H, NCH_2CH_2), 1.29 (m, 3H, CH_3), 1.12 (d, 1H, J = 12.9 Hz, CH_2), 1.02 (s, 3H, CH_3), 0.97 (d, 1H, J = 12.8 Hz, CH_2), 0.80 (s, 3H, CH_3). EMI MS: m/z = 484.34 $[M]^+$ (100%).

4-{4-[4-(3-Methoxyphenyl)piperazin-1-yl]butyl}-1,8,11,11-tetramethyl-4-azatricyclo[5.2.2.0^{2,6}] undec-8-ene-3,5-dione (2h)

Yield 60%, oil; ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 7.14 (t, 1H, J = 8.1 Hz, $CH_{arom.}$), 6.51 (m, 1H, $CH_{arom.}$), 6.42 (m, 2H, $CH_{arom.}$), 5.36 (s, 1H, CH=), 3.76 (s, 3H, OCH_3), 3.39 (br. t, 2H, J = 6.3 Hz, NCH_2), 3.24 (m, 4H, $N(CH_2CH_2)_2N$), 3.15 (dd, 1H, J_1 = 3.3 Hz, J_2 = 7.8 Hz, (HC)CHC=O), 2.65 (m, 4H, $N(CH_2CH_2)_2N$), 2.45 (dd, 1H, J_1 = 1.5 Hz, J_2 = 3.3 Hz, CHC=O), 2.40 (m, 3H, $CHC(CH_3)_2$, CH_2N), 1.71 (s, 3H, CH_3), 1.49 (m, 4H, CH_2CH_2N, NCH_2CH_2), 1.35 (s, 3H, CH_3), 1.15 (d, 1H, J = 12.9 Hz, CH_2), 1.06 (s, 3H, CH_3), 1.01 (d, 1H, J = 12.9 Hz, CH_2), 0.85 (s, 3H, CH_3). EMI MS: m/z = 480.3 $[M + H]^+$ (100%).

1,8,11,11-Tetramethyl-4-{4-[4-(2-methylphenyl) piperazin-1-yl]butyl}-4-azatricyclo[5.2.2.0^{2,6}] undec-8-ene-3,5-dione (2i)

Yield 67%, m.p. 174 – 176°C; ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 11.58 (br. s, 1H, HCl), 7.16 (m, 2H, CH_{arom}), 7.06 (m, 2H, CH_{arom}), 5.40 (s, 1H, CH=), 3.61 (m, 4H, $N(CH_2CH_2)_2N$), 3.41 (t, 2H, J = 6.6 Hz, NCH_2), 3.23 (dd, 1H, J_1 = 3.3 Hz, J_2 = 7.8 Hz, (HC)CHC=O,), 3.07 (m, 6H, $CH_2N(CH_2CH_2)_2N$), 2.45 (m, 2H, CHC=O, $CHC(CH_3)_2$), 2.26 (s, 3H, CH_3), 1.92 (m, 2H, CH_2CH_2N), 1.71 (s, 3H, CH_3), 1.59 (m, 2H, $CH_2CH_2CH_2N$), 1.34 (s, 3H, CH_3), 1.17 (d, 1H, CH_2, J = 12.9 Hz), 1.07 (s, 3H, CH_3), 1.01 (d, 1H, J = 12.9 Hz, CH_2), 0.85 (s, 3H, CH_3). EMI MS: m/z = 465.57 $[M + 2H]^+$ (100%).

4-{4-[4-(2-Fluorophenyl)piperazin-1-yl]butyl}-1,8,11,11-tetramethyl-4-azatricyclo[5.2.2.0^{2,6}] undec-8-ene-3,5-dione (2j)

Yield 69%, m.p. 152 – 154°C; ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 13.20 (br. s, 1H, HCl); 7.58 (t, 1H, J = 7.8 Hz, $CH_{arom.}$), 7.19 (m, 3H, $CH_{arom.}$), 5.40 (s, 1H, CH=), 4.22 (t, 2H, J = 11.4 Hz, NCH_2), 3.54 (m, 4H, $N(CH_2CH_2)_2N$), 3.41 (t, 1H, J = 6.6 Hz, (HC)CHC=O), 3.19 (m, 6H, $N(CH_2CH_2)_2N$, CH_2N), 2.45 (m, 2H, CHC=O, $CHC(CH_3)_2$), 1.87 (m, 2H, CH_2CH_2N), 1.71 (s, 3H, CH_3), 1.59 (m, 2H, NCH_2CH_2), 1.34 (s, 3H, CH_3), 1.16 (d, 1H, J = 12.9 Hz, CH_2), 1.07 (s, 3H, CH_3), 1.00 (d, 1H, J = 12.9 Hz, CH_2), 0.85 (s, 3H, CH_3). EMI MS: m/z = 468.3 $[M + H]^+$ (100%).

4-{3-[4-(2-Methoxyphenyl)piperazin-1-yl]propyl} -1,8,11,11-tetramethyl-4-azatricyclo[5.2.2.0^{2,6}] undec-8-ene-3,5-dione (3a)

Yield 67%, m.p. 232 – 234°C; ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 5.37 (s, 1H, CH=), 3.53 (t, 2H, J = 6.8 Hz, NCH_2), 3.42 (t, 2H, J = 8.8 Hz, CH_2Cl), 3.17 (dd, 1H, J_1 = 3.2 Hz, J_2 = 8 Hz, (HC)CH=O), 2.48 (m, 1H, CHC=O), 2.42 (br. d, 1H, J = 8.0 Hz, $CHC(CH_3)_2$), 1.94 (t, 2H, J = 6.8 Hz, $CH_2CH_2CH_2$), 1.74 (s, 3H, CH_3),1.37 (s, 3H, CH_3), 1.17 (d, 1H, J = 12.8 Hz, CH_2), 1.08 (s, 3H, CH_3), 1.04 (d, 1H, J = 12.8 Hz, CH_2), 0.87 (s, 3H, CH_3). EMI MS: m/z = 309.8 $[M]^+$ (100%).

1,8,11,11-Tetramethyl-4-[3-(4-pyrimidin-2-ylpiperazin-1-yl)propyl]-4-azatricyclo[5.2.2.0^{2,6}] undec-8-ene-3,5-dione (3b)

Yield 65%, oil; ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 8.74 (br. s, 1H, HCl), 8.30 (m, 2H, $CH_{arom.}$),

6.48 (m, 1H, $CH_{arom.}$), 5.38 (s, 1H, CH=), 4.50 (t, 3H, J = 5.1 Hz, NCH_2, (HC)CH(C=O), 3.47 (t, 2H, J = 6.7 Hz, CH_2N), 3.18 (dd, 1H, J_1 = 3.3 Hz, J_2 = 7.8 Hz, CHC=O), 2.47 (m, 9H, $H_{piper.}$, $CHC(CH_3)_2$), 1.77 (m, 2H, $CH_2CH_2CH_2$), 1.73 (d, J = 1.5 Hz, 3H, CH_3), 1.37 (s, 3H, CH_3), 1.17 (d, 1H, J = 12.8 Hz, CH_2), 1.08 (s, 3H, CH_3), 1.03 (d, 1H, J = 12.5 Hz, CH_2), 0.87 (s, 3H, CH_3). EMI MS: m/z = 438.3 $[M + H]^+$ (100%).

1,8,11,11-Tetramethyl-4-[3-(4-phenylpiperazin-1-yl)propyl]-4-azatricyclo[5.2.2.0^{2,6}]undec-8-ene-3,5-dione (3c)

Yield 70%, m.p. 234 – 236°C; ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 13.03 (br. s, 1H, HCl), 7.30 (m, 2H, $CH_{arom.}$), 6.96 (m, 3H, $CH_{arom.}$), 5.42 (s, 1H, CH=), 3.59 (m, 10H, $H_{piper.}$, NCH_2), 3.23 (dd, 1H, J_1 = 3.3 Hz, J_2 = 7.8 Hz, (HC)CH=O), 2.94 (m, 1H, CHC=O), 2.48 (m, 2H, CH_2N), 2.18 (m, 1H, $CHC(CH_3)_2$), 1.76 (s, 3H, CH_3), 1.65 (m, 2H, $CH_2CH_2CH_2$), 1.37 (s, 3H, CH_3), 1.21 (d, 1H, J = 12.9 Hz, CH_2), 1.09 (s, 3H, CH_3), 1.05 (d, 1H, J = 13.2 Hz, CH_2), 0.88 (s, 3H, CH_3). EMI MS: m/z = 436.3 $[M + H]^+$ (100%).

1,8,11,11-Tetramethyl-4-[3-(4-pyridin-2-ylpiperazin-1-yl)propyl]-4-azatricyclo[5.2.2.0^{2,6}]undec-8-ene-3,5-dione (3d)

Yield 67%, m.p. 229 – 231°C; ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 12.16 (br. s, 1H, HCl), 8.0 (m, 2H, $CH_{arom.}$), 7.01 (m, 2H, $CH_{arom.}$), 5.36 (s, 1H, CH=), 4.50 (m, 2H, NCH_2), 4.02 (m, 4H, $N(CH_2CH_2)_2N$), 3.69 (m, 4H, $N(CH_2CH_2)_2N$), 3.46 (m, 1H, (HC)CH(C=O), 3.23 (m, 1H, CHC=O), 3.09 (m, 2H, CH_2N), 2.37 (s, 1H, $CHC(CH_3)_2$), 2.05 (m, 2H, $CH_2CH_2CH_2$), 1.67 (s, 3H, CH_3), 1.26 (s, 3H, CH_3), 1.12 (d, 1H, J = 10.2 Hz, CH_2), 1.02 (s, 3H, CH_3), 0.93 (d, 1H, J = 12.9 Hz, CH_2), 0.79 (s, 3H, CH_3). EMI MS: m/z = 438.57 $[M + 2H]^+$ (100%).

4-{3-[4-(4-Fluorophenyl)piperazin-1-yl]propyl}-1,8,11,11-tetramethyl-4-azatricyclo[5.2.2.0^{2,6}]undec-8-ene-3,5-dione (3e)

Yield 63%, m.p. 264 – 266°C; ^{1}H NMR (300 MHz, DMSO, δ, ppm): 11.01 (br. s, 1H, HCl), 7.06 (m, 4H, $CH_{arom.}$), 5.46 (s, 1H, CH=), 3.87 (s, 9H, $H_{piper.}$, (HC)CH=O), 3.74 (m, 2H, NCH_2), 3.47 (m, 1H, CHC=O), 3.36 (br. t, 2H, J = 6.6 Hz, CH_2N), 2.33 (m, 1H, $CHC(CH_3)_2$), 1.87 (m, 2H, $CH_2CH_2CH_2$), 1.69 (s, 3H, CH_3), 1.29 (s, 3H, CH_3), 1.21 (d, 1H, J = 12.6 Hz, CH_2), 1.06 (s, 3H, CH_3), 0.93 (d, 1H, J = 12.6 Hz, CH_2), 0.83 (s, 3H, CH_3). EMI MS: m/z = 454.1 $[M + H]^+$ (100%).

4-[3-(4-Benzylpiperazin-1-yl)propyl]-1,8,11,11-tetramethyl-4-azatricyclo[5.2.2.0^{2,6}]undec-8-ene-3,5-dione (3f)

Yield 58%, m.p. 244 – 246°C; ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 13.55 (br. s, 1H, HCl), 7.59 (m, 1H, $CH_{arom.}$), 7.41 (m, 3H, $CH_{arom.}$), 7.19 (m, 1H, $CH_{arom.}$), 5.37 (s, 1H, CH=), 4.20 (m, 2H, NCH_2), 3.93 (m, 3H, CH_2, (HC)CH=O), 3.48 (m, 8H, $H_{piper.}$), 3.15 (dd, 1H, J_1 = 3.0 Hz, J_2 = 7.8 Hz, CHC=O), 2.97 (m, 2H, CH_2N), 2.40 (m, 1H, $CHC(CH_3)_2$), 1.74 (m, 2H, $CH_2CH_2CH_2$), 1.69 (s, 3H, CH_3), 1.27 (s, 3H, CH_3), 1.11 (d, 1H, J = 14.3 Hz, CH_2), 1.01 (s, 3H, CH_3), 1.97 (d, 1H, J = 13.1 Hz, CH_2), 0.80 (s, 3H, CH_3). EMI MS: m/z = 450.3 $[M + H]^+$ (100%).

4-{3-[4-(4-Chlorophenyl)piperazin-1-yl]propyl}-1,8,11,11-tetramethyl-4-azatricyclo[5.2.2.0^{2,6}]undec-8-ene-3,5-dione (3g)

Yield 70%, m.p. 194 – 196°C; ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 7.90 (m, 1H, $CH_{arom.}$), 7.51 (m, 1H, $CH_{arom.}$), 7.38 (m, 1H, $CH_{arom.}$), 7.07 (m, 1H, $CH_{arom.}$), 5.44 (d, 1H, J = 8.4 Hz, CH=), 4.26 (m, 2H, NCH_2), 3.64 (m, 8H, $H_{piper.}$), 3.35 (m, 1H, (HC)CHC=O), 3.24 (m, 1H, CHC=O), 2.96 (m, 2H, CH_2N), 2.47 (s, 1H, $CHC(CH_3)_2$), 2.07 (m, 2H, $CH_2CH_2CH_2$), 1.77 (s, 3H, CH_3), 1.36 (s, 3H, CH_3), 1.19 (d, 1H, J = 2.4 Hz, CH_2), 1.08 (s, 3H, CH_3), 1.02 (d, 1H, J = 12.9 Hz, CH_2), 0.87 (s, 3H, CH_3). EMI MS: m/z = 470.3 $[M]^+$ (100%).

4-{3-[4-(3-Methoxyphenyl)piperazin-1-yl]propyl}-1,8,11,11-tetramethyl-4-azatricyclo[5.2.2.0^{2,6}]undec-8-ene-3,5-dione (3h)

Yield 58%, m.p. 139 – 141°C; ^{1}H NMR (400 MHz, DMSO, δ, ppm): 10.87 (br. s, 1H, HCl), 7.15 (t, 1H, J = 8.1 Hz, $CH_{arom.}$), 6.51 (m, 3H, $CH_{arom.}$), 5.45 (s, 1H, CH=), 3.80 (br. d, 2H, J = 10.3 Hz, NCH_2), 3.72 (s, 3H, OCH_3), 3.45 (m, 2H, $N(CH_2CH_2)N$), 3.35 (t, 2H, J = 6.4 Hz, $N(CH_2CH_2)N$), 3.24 (dd, 1H, J_1 = 2.6 Hz, J_2 = 7.4 Hz, (HC)CHC=O), 3.10 (m, 6H, $N(CH_2CH_2)_2N$, CH_2N), 2.56 (d, 1H, J = 7.7 Hz, CHC=O), 2.32 (m, 1H, $CHC(CH_3)_2$), 1.86 (t, 2H, J = 7.0 Hz, $CH_2CH_2CH_2$), 1.68 (s, 3H, CH_3), 1.28 (s, 3H, CH_3), 1.20 (d, 1H, J = 12.7 Hz, CH_2), 1.08 (s, 3H, CH_3), 0.91 (d, 1H, J = 12.7 Hz, CH_2), 0.82 (s, 3H, CH_3). EMI MS: m/z = 466.44 $[M + H]^+$ (100%).

1,8,11,11-Tetramethyl-4-{3-[4-(2-methylphenyl)piperazin-1-yl]propyl}-4-azatricyclo[5.2.2.0^{2,6}]undec-8-ene-3,5-dione (3i)

Yield 68%, m.p. 227 – 229°C; ^{1}H NMR (400 MHz, DMSO, δ, ppm): 10.80 (br. s, 1H, HCl), 7.17 (m, 2H, $CH_{arom.}$), 7.01 (m, 2H, $CH_{arom.}$), 5.45 (s, 1H,

Table 1. Antibacterial and antifungal activites of 1,8,11,11-tetramethyl-4-azatricyclo[5.2.2.$0^{2,6}$]undec-8-en-3,5-dione (**1**) and some of its derivatives. Diameter of the growth inhibition zone [mm] and Minimal Inhibitory Concentration (MIC in parentheses) [μg/mL] are given.

Strain	Compound Diameter of growth inhibitory area (mm) and MIC (μg/mL)													
	1		**2d**		**2f**		**3f**		**3h**		**3j**		Ciprofloxacin		Fluconazole	
Staphylococcus aureus NCTC 4163	21	(> 400)	11	(> 400)	16	(200)	11	(400)	15	(> 400)	12	(> 400)	26	(0.5)	nt	
Staphylococcus aureus ATCC 25923	30	(> 400)	11	(> 400)	13	(400)	11	(400)	12	(> 400)	13	(> 400)	26	(0.5)	nt	
Staphylococcus aureus ATCC 6538	28	(> 400)	12	(> 400)	15	(400)	13	(400)	15	(> 400)	13	(> 400)	28	(0.5)	nt	
Staphylococcus aureus ATCC 29213	25	(> 400)	13	(> 400)	16	(400)	15	(400)	16	(> 400)	15	(> 400)	22	(0.5)	nt	
*Staphylococcus epidermidis*ATCC 12228	22	(> 400)	13	(> 400)	-	(400)	-	(>400)	14	(400)	-	(> 400)	30	(0.5)	nt	
Bacillus subtilis ATCC 6633	20	(> 400)	15	(400)	18	(400)	14	(400)	18	(400)	17	(> 400)	40	(< 0.125)	nt	
Bacillus cereus ATCC 11778	18	(> 400)	12	(> 400)	16	(400)	13	(400)	14	(> 400)	11	(> 400)	20	(1)	nt	
Enterococcus hirae ATCC 10541	20	(> 400)	-	(> 400)	12	(400)	-	(>400)	-	(> 400)	-	(> 400)	-	(4)	nt	
Micrococcus luteus ATCC 9341	20	(> 400)	14	(> 400)	14	(400)	13	(400)	13	(400)	18	(> 400)	22	(4)	nt	
Micrococcus luteus ATCC 10240	15	(> 400)	20	(400)	20	(200)	17	(400)	19	(400)	15	(400)	24	(2)	nt	
Escherichia coli ATCC 10538	-				-		-		11	(> 400)	-		34	(<0.125)	nt	
Escherichia coli ATCC 25922	-				-		-		11	(> 400)	12	(> 400)	35	(<0.125)	nt	
Escherichia coli NCTC 8196	-				-		-		11	(> 400)	11	> 400)	35	(<0.125)	nt	
Proteus vulgaris NCTC 4635	16	(> 400)			na		na		na		na		36	(<0.125)	nt	
Pseudomonas aeruginosa ATCC 15442	16	(> 400)			na		na		na		na		25	(0.5)	nt	
Pseudomonas aeruginosa NCTC 6749	17	(> 400)			na		na		na		na		26	(0.5)	nt	
Pseudomonas aeruginosa ATCC 27853	15	(> 400)			na		na		na		na		23	(1)	nt	
Bordetella bronchiseptica ATCC 4617	18	(> 400)			na		na		na		na		31	(1)	nt	
Candida albicans ATCC 10231	-				na		na		na		na		nt		22	1
Candida albicans ATCC 90028	-				na		na		na		na		nt		32	1
Candida parapsilosis ATCC 22019	-				na		na		na		na		nt		22	2

na = not active, nt = not tested

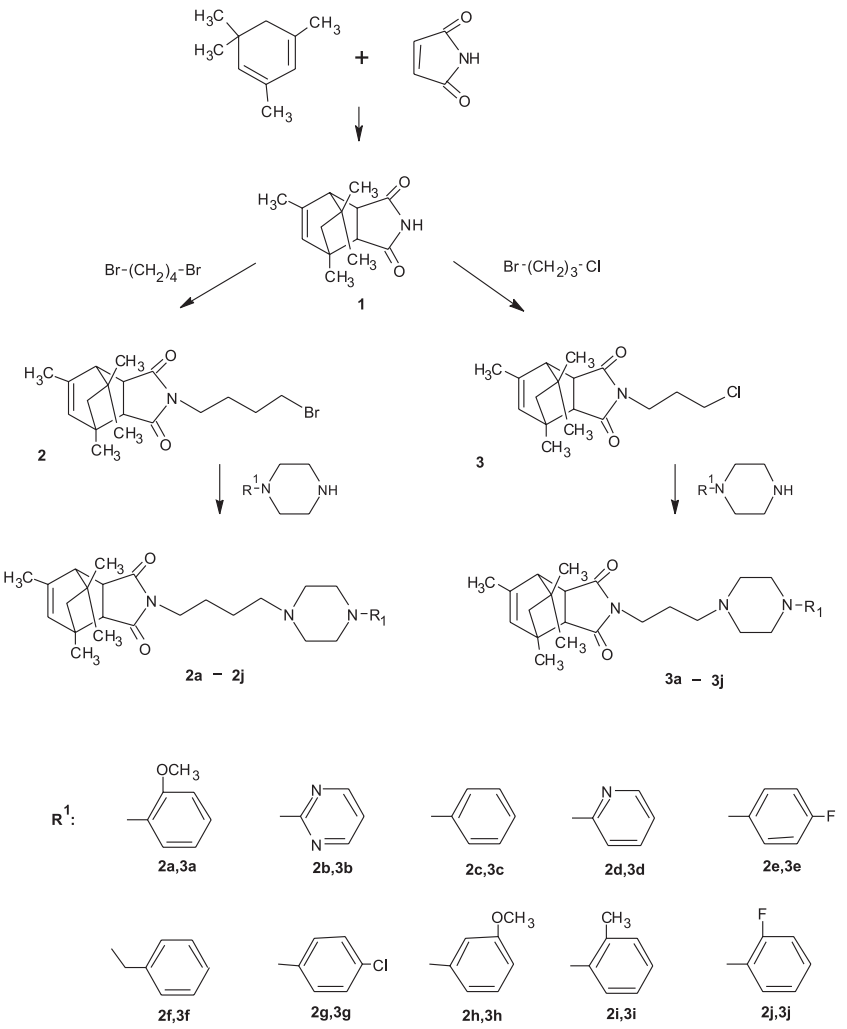
CH=), 3.50 (m, 8H, $H_{piper.}$), 3.36 (t, 2H, J = 6.5 Hz, NCH_2), 3.25 (dd, 1H, J_1 = 2.8 Hz, J_2 = 7.5 Hz, (HC)CHC=O), 3.04 (m, 2H, CH_2N), 2.57 (d, 1H, J = 7.7 Hz, CHC=O), 2.33 (br. s, 1H, $CHC(CH_3)_2$), 2.25 (s, 3H, CH_3), 1.87 (m, 2H, $CH_2CH_2CH_2$), 1.69 (s, 3H, CH_3), 1.29 (s, 3H, CH_3), 1.20 (br. d, 1H, J = 12.7 Hz, CH_2), 1.06 (s, 3H, CH_3), 0.92 (br. d, 1H, J = 12.7 Hz, CH_2), 0.82 (s, 3H, CH_3). EMI MS: m/z = 451.40 $[M + 2H]^+$ (100%).

4-{3-[4-(2-Fluorophenyl)piperazin-1-yl]propyl}-1,8,11,11-tetramethyl-4-azatricyclo[5.2.2.0^{2,6}]undec-8-ene-3,5-dione (3j)

Yield 67%, m.p. 264 – 266°C; ^{1}H NMR (400 MHz, DMSO, δ ppm): 10.27 (br. s, 1H, HCl), 7.13 (m, 4H, $CH_{arom.}$), 5.44 (s, 1H, CH=), 3.49 (m, 2H, NCH_2), 3.35 (m, 8H, $H_{piper.}$), 3.24 (m, 1H, (HC)CHC=O), 3.14 (m, 2H, CN_2N), 2.49 (br. s, 1H, CHC=O), 2.33 (br. s, 1H, $CHC(CH_3)_2$), 1.84 (m, 2H, $CH_2CH_2CH_2$), 1.69 (s, 3H, CH_3), 1.29 (s, 3H, CH_3), 1.21 (br. d, 1H, J = 12.7 Hz, CH_2), 1.08 (s, 3H, CH_3), 1.93 (br. d, 1H, J = 12.7 Hz, CH_2), 0.82 (s, 3H, CH_3). EMI MS: m/z = 455.37 $[M + 2H]^+$ (100%).

Microbiology

Microorganisms used in this study included: Gram-positive bacteria: *Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus* ATCC 29213, *Staphylococcus aureus* ATCC 6538P, *Staphylococcus aureus* NCTC 4163, *Bacillus subtilis* ATCC 6633, *Enterococcus hirae* ATCC 10541; Gram-negative bacteria: *Escherichia coli* ATCC 25922,



Scheme 1. Synthesis of the arylpiperazine derivatives **2** and **3**

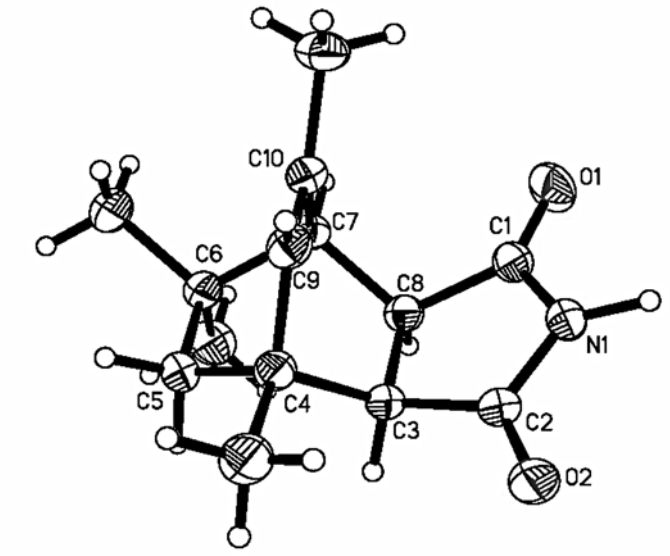


Figure 1. Molecular structure and atom numbering scheme of **1** with displacement ellipsoids drawn at 50% probability level

Table 2. Cytotoxicity and anti-HIV-1 activity of compounds (**1** and **2a**-**2j**, **3a**-**3j**.

Compd.	MT-4	HIV-1	Compd.	MT-4	HIV-1
	CC_{50} [a]	EC_{50} [b]		CC_{50} [a]	EC_{50} [b]
1 >	100 >	100	**3a**	51	> 51
2a	nt	nt	**3b**	nt	nt
2b	63	> 63	**3c**	24	> 24
2c	nt	nt	**3d**	67	> 67
2d	nt	nt	**3e**	30	> 30
2e	22	> 22	**3f**	90	> 90
2f	49	> 49	**3g**	22	> 22
2g	25	> 25	**3h**	32	> 32
2h	nt	nt	**3i**	21	> 21
2i	20	> 20	**3j**	43	> 43
2j	35	> 35	**EFV**	35	0.003

[a] Compound concentration (μM) required to reduce the viability of mock-infected MT-4 cells by 50%, as determined by the MTT method. [b]Compound concentration (μM) required to achieve 50% protection of MT-4 cells from the HIV-1 induced cytopathogenicity, as determined by the MTT method. nt = not tested

Escherichia coli ATCC 10538, *Escherichia coli* NCTC 8196, *Provulgaris* NCTC 4635, *Pseudomonas aeruginosa* ATCC 15442, *Pseudomonas aeruginosa* NCTC 6749, *Bordetella bronchiseptica* ATCC 4617; fungi: *Candida albicans* ATCC 10231, *Candida albicans* ATCC 90028, and *Candida parapsilosis* ATCC 22019. Other microorganisms used were obtained from the collection of the Department of Pharmaceutical Microbiology, Medical University of Warsaw, Poland.

Media, growth conditions, and antimicrobial activity assays

Antimicrobial activity was examined by the disc diffusion method and the minimal inhibitory concentration (MIC) method under standard condi-

tions using Mueller-Hinton II agar medium (Becton Dickinson) according to the guidelines established by the Clinical and Laboratory Standarts Institute (CLSI, 2006). Atifungal activites were assessed using Roswell Park Memorial Institute (RPMI) medium (Sigma).

For the disc diffusion assay, the solution of tested agents was prepared in water (imide **1**), ethanol or dimethyl sulfoxide (DMSO). Sterile filter paper disc (9 mm diameter, Whatman No. 3 chromatography paper) was dripped with test compound solution to load 400 µg of a given compound per disc. The results were read following 24–48 h incubation at 30°C for fungi and 18 h incubation at 35°C for bacteria strains.

For MIC determinations, concentrations of tested compounds in solid medium ranged from 6.25 to 400 µg/mL. Agar plates (with MH II medium for bacteria and RPMI for fungi) were inoculated using 2 µL aliquots. The final inoculum of studied organism were 10^4 CFU/mL (colony forming units per mL), except final inoculum of *E. Hirae* ATCC 10541 was 10^5 CFU/mL. Results of antimicrobial activity were read after 18 h incubation at 35°C.

Antiviral assay procedures

Antiviral investigations of compounds were performed in the Dipartamento di Scienze e Tecnologie Biomediche, Universita di Cagliari, Monserato, Italy.

Compounds

Compounds were solubilized in DMSO at 200 mM and then diluted into a culture medium.

Cell and viruses

Cell lines were purchased from American Type Culture Collection (ATCC). Absence of mycoplasma contamination was checked periodically by Hoechst staining method. Cell lines supporting the multiplication of RNA viruses were $CD4^+$ human T-cells containing an integrated HTLV-1 genome (MT-4).

Cytotoxicity assays

For cytotoxicity evaluations, exponentially growing cells derived from human hematological tumors [$CD4^+$ human T-cells containing an integrated HTLV-1 genome (MT-4)] were seeded at an initial density of 1×10^5 cells/mL in 96 well plates in RPMI-1640 medium, supplemented with 10% fetal calf serum (FCS), 100 units/mL penicillin G and 100 µg/mL streptomycin. Cell cultures were then incubated at 37°C in a humidified 5% CO_2 atmosphere in the absence or presence of serial dilutions of test compounds. Cell viability was determined after 96 h at 37°C by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method (39).

Antiviral assay

Activity of compounds against human immunodeficiency virus type-1 (HIV-1) was based on inhibition of virus-induced cytopathogenicity in MT-4 cells acutely infected with a multiplicity of infection (m.o.i.) of 0.01. Briefly, 50 µL of RPMI containing 2×10^4 MT-4 were added to each well of flat-bottom microtitre trays containing 50 µL of RPMI, without or with serial dilutions of test compounds. Then, 20 µL of an HIV-1 suspension containing 100 $CCID_{50}$ were added. After a 4-day incubation, cell viability was determined by the MTT method (39).

RESULTS AND DISCUSSION

Chemistry

The starting compound, 1,8,11,11-tetramethyl-4-azatricyclo[5.2.2.$0^{2,6}$]undec-8-ene-3,5-dione (**1**), was obtained in Diels-Alder reaction of 1,3,5,5-tetramethylocyclohexa-1,3-diene with maleimide. The molecular structure of **1** was established by X-ray crystallography (Fig. 1) and ^{13}C NMR. Imide **1** was subjected to the reaction with 1,4-dibromobutane or 1-bromo-3-chloropropane in anhydrous medium, to obtain compounds **2** and **3**, respectively. Next, **2** and **3** were condensed with selected arylpiperazines to form series of derivatives **2a-2j** and **3a-3j** (Scheme 1). Obtained *N*-substituted derivatives were purified by column chromatography. The MS and ^1H NMR spectra confirmed the identity of the products.

Microbiology

In the present study, derivatives of **1** were tested *in vitro* against bacteria, yeasts, and HIV-1 virus. Their cytotoxicity was also examinated. All obtained compounds were tested against bacteria, including Gram-positive cocci, Gram-negative rods and *Candida albicans*. Microorganisms used in this study have common aplication in the antimicrobial tests for many substances like antibiotics, disinfectants and antiseptic drugs or in research on new antimicrobial agents (3, 5, 37). All compounds were screened for their antimicrobial activity by the disc diffusion method (35). Compounds showing significant activity were next examined for their minimal inhibitory concentration (MIC) (36). Prelimary antimicrobial test by disc diffusion method showed

activity of compounds **1, 2d, 2f, 3f, 3h** and **3j** against Gram-positive bacteria. Gram-negative rods and *Candida albicans* were resistant to all tested compounds. Test results of activity are summarized in Table 1.

The next step was evaluation of compound's MIC values for standard strains. The most active was compound **1**. This simple imide is soluble in water which simplifies the research. In general, compounds which have amphiphilic properties like compound **1** are active. In the molecule of imide **1** hydrophobic part consists of methyl groups connected to a cyclic imide. Carbonyl groups and *N* atom of the cyclic imide are the hydrophilic part of the generally hydrophobic imide. The activity of the titled imide confirms a propriety of research on methyl derivatives of N-substituted tricyclo[5.2.1.$0^{2,6}$]dec-8-ene-3,5-dione (37).

The microbiological activity is definitely lower for alkilaryl/heteroaryl-piperazine *N*-substituted imides (Table 1).

Antiviral activities

The cytotoxicity and anti HIV-1 activity of derivatives **1, 2, 3, 2a-2j, 3a-3j** were determined. However, none of the studied compounds showed any activity against HIV-1. The results are summarized in Table 2. The highest cytotoxicity > 100 μM has imide **1** and the lowest 20 μM was for compound **2i**.

The observed values for compounds **2e, 2g, 2i, 3c, 3e, 3g, 3h, 3i** were lower than for the standard efavirenz (EFV). The lowest cytotoxicity was observed for the compounds having butyl or propyl linker connected to phenylpiperazine, 4-fluorophenylpiperazine, 4-chlorophenylpiperazine, 3-methoxyphenylpiperazine or 2-methylphenylpiperazine fragments.

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REFERENCES

1. Zentz F., Valla A., Le Guillou R., Labia R., Mathot A.-G., Sitrot D.: Farmaco 57, 421 (2002).
2. Albuquerque J.F.C., Rocha Filho J.A., Brandao S.S.F., Lima M.C.A., Ximenes E.A., Galdino S.L., Pitta I.R. et al.: Farmaco 54, 77 (1999).
3. Struga M., Kossakowski J., Stefańska J., Zimniak A., Koziol A.E.: Eur. J. Med. Chem. 43, 1309 (2008).
4. Khalil A., Berghot M., Gouda M.: Chem. Pap. – Chem. Zvesti 64, 637 (2010).
5. Stefanska J., Bielenica A., Struga M., Tyski S., Kossakowski J., Loddo R., Ibba C., Collu D., Marongiu E., La Colla P.: Cent. Eur. J. Biol. 4, 362 (2009).
6. Foroumadi A., Emami S., Hassanzadeh A., Rajaee M., Sokhanvar K., Moshafi MH., Shafiee A.: Bioorg. Med. Chem. Lett. 15, 4488 (2005).
7. Pinna G.A., Loriga G., Murineddu G., Grella G., Mura M., Vargiu L., Murgioni Ch., La Colla P.: Chem. Pharm. Bull. 49, 1406 (2001).
8. Stratford E.S., Curley Jr. R.W.: J. Med. Chem. 26, 1463 (1983).
9. Duarte F.S., Andrade E.S., Vieira R.A., Uieara M., Nunes R.J., De Lima T.C.M.: Bioorg. Med. Chem. 14, 5397 (2006).
10. Koziol A.E., Chilmonczyk Z., Cybulski J.: Acta Cryst. E62, 05616 (2006).
11. Kumar S., Rajkumar S.V.: Eur. J. Cancer 42, 1612 (2006).
12. Nakamura T., Noguchi T., Kobyashi H., Miyachi H., Hashimoto Y.: Chem. Pharm. Bull. 54, 1709 (2006).
13. Corral L.G., Kaplan G.: Ann. Rheum. Dis. 58 (Suppl I) 107 (1999).
14. DiPardo R.M., Patane M.A., Newton R.C., Price R., Broten T.P., Chang R.S.L., Ransom R.W. et al.: Bioorg. Med. Chem. Lett. 11, 1959 (2001).
15. Brana M.F., Dominguez D., Saez B., Romerdahl C., Robinson S., Barlozzari T.: Bioorg. Med. Chem. Lett. 11, 3027 (2001).
16. Richter S., Parolin C., Palumbo M., Palu G.: Curr. Drug. Targets Infect. Disord. 4, 111 (2004).
17. Chan T.M., Cox K., Feng W., Miller M.W., Westton D., McCombie S.W.: U.S. Pat. 2006223821 (2006).
18. Kimura M., Masuda T., Yamada K., Kawakatsu N., Kubota N., Mitani M., Kishii K. et al.: Bioorg. Med. Chem. Lett. 14, 4287 (2004).
19. Chilmonczyk Z., Les A., Wozniakowska A., Cybulski J., Koziol A.E., Gdaniec M.: J. Med. Chem. 38, 1701 (1995).
20. Cho K.-Y., Sung S.-J., Lee S., Chang H.-S., Park J.-K.: Mater. Sci. Eng. C 24, 195 (2004).
21. Hoogboom J., Rasing T., Rowa, A.E., Nolte R.J.M.: J. Mater. Chem. 16, 1305 (2006).
22. Mehdipour-Ataei S., Hatami M.: Eur. Polymer J. 41, 2010 (2005).

23. Cecchetti V., Schiaffella F., Tabarrini O.; Fravolini A.: Bioorg. Med. Chem. Lett. 10, 465 (2000).
24. Dutta A.K., Venkataraman S.K., Fei X.S., Kolhatkar R., Zhang S., Reith M.E.A.: Bioorg, Med. Chem. 12, 4361 (2004).
25. Betti L., Zanelli M., Giannaccini G., Manetti F., Schenone S., Strappaghetti G.: Bioorg. Med. Chem. 14, 2828 (2006).
26. Obniska J., Kolaczkowski M., Bojarski A.J., Duszynska B.: Eur. J. Med. Chem. 41, 874 (2006).
27. Carpenter C.C., Fischl M.A., Hammer S.M., Hirsch M.S., Jacobsen D.M., Katzenstein D.A., Montaner J.S. et al.: J. Am. Med. Assoc. 280, 78 (1998).
28. Romero D.L., Morge R.A., Genin M.J., Biles C., Busso M., Resnick L., Althaus I.W. et al.: J. Med. Chem. 36, 1505 (1993).
29. Psomas K.C., Corbeau P., Reynes J.: Antibiotiques 12, 27 (2010).
30. Pinna G.A., Loriga G., Murineddu G., Grella G., Mura M., Vargiu L., Murgioni C., La Colla P.: Chem. Pharm. Bull. 49, 1406 (2001).
31. Tagat J.R., McCombie S.W., Nazareno D., Labroli M.A., Xiao Y., Steensma R.W., Strizki J.M. et al.: J. Med. Chem. 47, 2405 (2004).
32. Haergreaves M.K., Pritchard J.G., Dave H.R.: Chem. Res. 70, 439 (1970).
33. Struga M., Kossakowski J., Kedzierska E., Fidecka S., Stefanska J.: Chem. Pharm. Bull. 55, 796 (2007).
34. Kossakowski J., Kusmierczyk J.: Pharmazie 55, 5 (2000).
35. Clinical and Laboratory Standards Institute. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard M7-A7. Clinical and Laboratory Standards Institute, Wayne, PA, USA, (2006).
36. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Disc Susceptibility Tests; Approved Standard M2-A9. Clinical and Laboratory Standards Institute, Wayne, PA, USA, (2006).
37. Struga M., Krawiecka M., Kossakowski J., Stefanska J., Mirosław B., Koziol A.E.: J. Chin. Chem. Soc. 55, 1258 (2008).
38. G. M. Sheldrick: Acta Cryst. A 64, 112 (2008).
39. Pauwels R., Balzarini J., Baba M., Snoeck R., Schols D., Hardewijn P., Desmyter J., De Clercq E.: J. Virol. Methods 20, 309 (1988).

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