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## DRUG SYNTHESIS

# SYNTHESIS AND BIOLOGICAL EVALUATION OF *N*-SUBSTITUTED POLYCYCLIC IMIDES DERIVATIVES

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**Abstract:** The preparation of 16 derivatives of 3,5,8-trioxo-4-azatricyclo- [5.2.2.0$^{2,6}$]undec-1-yl acetate and 8 derivatives of 1-isobutoxy-4-azatricyclo[5.2.2.0$^{2,6}$]undecane-3,5,8-trione was described. Substituents to the imide *N*-atom were alkyl-(aryl)piperazine fragments with an alkyl linker being propyl or butyl group. Selected newly obtained compounds were evaluated *in vitro* against anti-HIV-1 activity. A broad group of derivatives were tested for their antibacterial and antifungal activity. The pharmacological properties of butyl derivatives of imide **6** were evaluated in three behavioral tests in mice. The molecular structures of starting polycyclic 6-acetyl-imides, **1** and **5**, were determined by X-ray crystallography. Presented tests have not revealed any activity of the compounds, however, selected derivatives exerted no neurotoxicity in behavioral tests.

**Keywords**: polycyclic imide, antimicrobial activity, 4-azatricyclo[5.2.2.0$^{2,6}$]undecane-3,5,8-trione derivatives

Among numerous pharmacological properties of polycyclic imides, one of the leading is antimicrobial activity (1, 2). 4-Azatricyclo-8-ene-3,5-dione derivatives have been reported as antibacterial agents; it is supposed that their activity is connected mainly with the presence of complex phenyl moiety in their molecular structure (3-5). High-volume hydrophobic imide nucleus is also a part of antifungal (1) and anti-HIV agents (6-8). Furthermore, compounds of that group act as inhibitors of cell growth (9) and [$^3$H]-rimonabant binding agents (10). Introduction of electron-attracting groups, such as fluoride, increase antiviral (6, 11, 12) and antibacterial (13) properties of these compounds. Amphiphilic imide derivatives have ability to intercalate into phospholipid membranes and may consequently play a biological role, being potential pharmaceuticals (14). Literature survey revealed that modifications of imide structures lead to new anticancer agents (15, 16).

Alkylarylpiperazine imide derivatives are characterized as cytotoxic compounds (5, 17, 18). As reported, some of them act against viruses, such as Yellow Fever Virus (YFV), Bovine Viral Diarrhea Virus (BVDV) and Border Disease Virus (CVB-2) (5, 11, 17, 19). The presence of sulfur atom, either in a ring (11, 15, 20, 21) or in a chain (13, 20, 22) increases their biological activity. Moreover, polycyclic imides are widely known as anticonvulsant agents and ligands of the 5-HT receptors (23-25).

This work reports synthesis and a wide spectrum of antimicrobial activity screening of *N*-substituted derivatives purposely designed to combine a core of 4-azatricyclo-[5.2.2.0$^{2,6}$] undecane-3,5,8-trione with alkyl linker bearing arylpiperazine moiety, present in a structure of some antiretroviral agents (Fig. 1). Most of imide derivatives presented in this study were tested against HIV-1 virus, as well as bacterial strains and fungal species.

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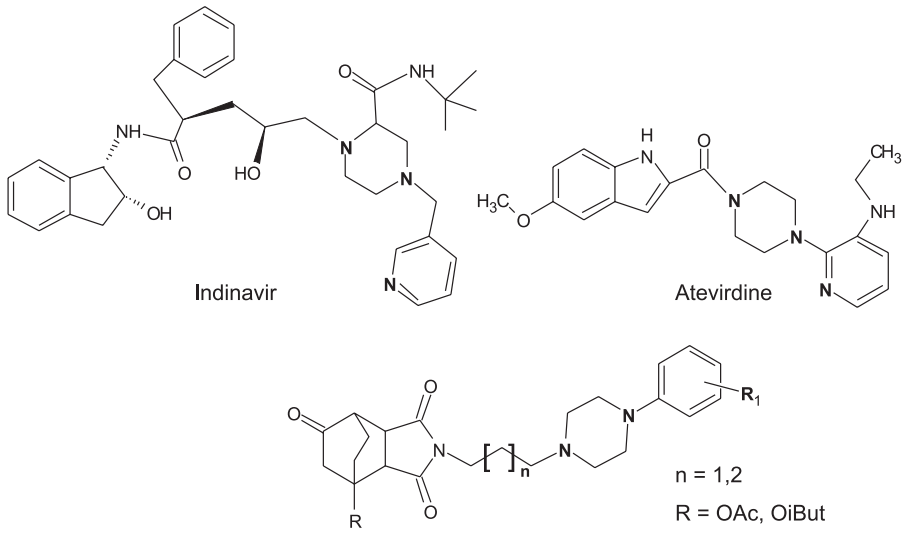



Figure 1. Chemical structures of indinavir, atevirdine and 4-azatricyclo-5.2.2.0$^{2,6}$]undecane-3,5,8-trione derivatives considered in this study

This paper is a continuation of our previous research in this field (5, 11, 17, 26). In addition, derivatives **7a**-**7g** (Scheme 1) were tested for their pharmacological properties in three behavioral tests in mice. The molecular structures of starting 6-acetyl-imides **1** and **5** were determined by X-ray crystal structure analysis.

## EXPERIMENTAL

### Chemistry

All chemicals and solvents were purchased from Aldrich (Vienna, Austria). Melting points were determined on Electrothermal Digital Melting Point Apparatus (Essex, UK) and are uncorrected. The $^1$H NMR spectra were recorded on a Bruker (Rheinstetten, Germany) spectrometer, operating at 400, 300 or 200 MHz. The chemical shift values are expressed in ppm relative to TMS as an internal standard. Elemental analyses were recorded on a CHN model 2400 Perkin-Elmer (Hitachi, Tokyo, Japan). Mass spectra were measured on a PE Biosystems Mariner spectrometer (Foster City, USA) with TOF detector. Methanol was used as a solvent. The spectra were performed in the positive ion mode with a declustering potential 140–300 V. TLC was carried out using silica gel 60 F254, layer thickness 0.25 mm (E. Merck, Darmstadt, Germany) and the results were visualized using UV lamp at 254 nm. Column chromatography was carried out using silica gel 60 (200–400 mesh, Merck).

**Preparation of 3,5-dioxo-4-azatricyclo[5.2.2.0$^{2,6}$]-undec-8-ene-1,8-diyl diacetate** (**1**)

In a round bottom flask maleimide (0.054 mole) was dissolved in warm isopropenyl acetate (15 cm$^3$), next, cyclohexane-1,3-dione (0.045 mole) was added. *p*-Toluenesulfonic acid was used as a catalyzing agent. A mixture was refluxed for 10 h. After that time, the liquid was evaporated, and the residue crystallized from hexane-ethyl acetate mixture (1:1 v/v).

Yield 43%, m.p. 151-153$^o$C. $^1$H NMR (400 MHz, DMSO, δ, ppm): 11.22 (s, 1H, NH), 5.77 (br.s, 1H, CH=), 3.77 (d, $J$ = 8.0 Hz, 1H, CH-C=O), 3.11 (dd, $J_1$ = 2.8 Hz, $J_2$ = 8.0 Hz, 1H, CH-C=O), 2.86 (m, 1H, CH-CH$_2$), 2.28 (m, 1H, CH), 2.12 (s, 3H, CH$_3$), 2.06 (s, 3H, CH$_3$), 1.73 (m, 2H, CH$_2$CH$_2$), 1.59 (m, 1H, CH). Analysis: for $C_{14}H_{15}NO_6$ (293.28): calcd. C 57.33, H 5.12, N 4.77%; found C 57.29, H 5.18, N 4.84%.

**Preparation of 3,5,8-trioxo-4-azatricyclo[5.2.2.0$^{2,6}$]undec-1-yl acetate** (**2**)

3,5-Dioxo-4-azatricyclo[5.2.2.0$^{2,6}$]undec-8-ene-1,8-diyl diacetate (0.02 mole) was hydrolyzed while heating in a mixture of anhydrous ethanol and 20% NH$_3$(aq) (5.3:1, v/v) for 1 h. After solvents evaporation, the solid residue was crystallized from anhydrous ethanol.

Yield 43%, m.p. 151-153$^o$C. $^1$H NMR (400 MHz, DMSO, δ, ppm): 11.44 (s, 1H, NH), 4.07 (dd, $J_1$ = 8.8 Hz, $J_2$ = 10.0 Hz, 1H, CH-C=O), 3.38 (dd, $J_1$ = 3.2 Hz,

$J_2$ = 10.0 Hz, 1H, CH-C=O), 2.69 (dd, $J_1$ = 18.0 Hz, $J_2$ = 19.2 Hz, 1H, CH-C=O), 2.56 (m, 2H, CH-$CH_2$, CH), 2.25 (dd, $J_1$ = 2.8 Hz, $J_2$ = 19.2 Hz, 1H, CH-C=O), 2.01 (s, 4H, $CH_3$, CH), 1.81 (m, 2H, $CH_2CH_2$). Analysis: for $C_{12}H_{13}NO_5$ (251.25): calcd. C 57.37, H 5.18, N 5.58%; found C 57.41, H 5.26, N 5.71%.

The synthesis of starting imides **5** and **6** was described previously (27). In this paper molecular structure of **5** is presented.

**General procedure for preparation of alkyl derivatives 3, 4 and 7**

An appropriate imide (0.01 mole) was dissolved in 2-butanone (30 $cm^3$), next, anhydrous $K_2CO_3$ (0.01 mole) and 1-bromo-3-chloropropane (0.02 mole) or 1,4-dibromo-butane (0.02 mole) were added, respectively. The mixture was refluxed for 24-38 h. When the reaction was completed, the mixture was filtered off and the solvent was evaporated. The residue was purified by column chromatography (chloroform : methanol, 9 : 0.5, v/v).

**4-(3-Chloropropyl)-3,5,8-trioxo-4-azatricyclo[5.2.2.0$^{2,6}$]undec-1-yl acetate** (**3**)

Yield 85%, m.p. 128-129°C. $^1$H NMR (400 MHz, $CDCl_3$, δ, (ppm): 4.26 (dd, $J_1$ = 1.6 Hz, $J_2$ = 10.0 Hz, 1H, CH-C=O), 3.61 (m, 2H, $NCH_2$), 3.47 (t, 1H, $J$ = 6.4 Hz, CH-Cl), 3.31 (t, 1H, $J$ = 6.4 Hz, CH-Cl), 3.20 (dd, $J_1$ = 3.2 Hz, $J_2$ = 9.6 Hz, 1H, CH-C=O), 2.90 (d, $J$ = 2.8 Hz, 1H, CH-C=O), 2.75 (m, 1H, CH-$CH_2$), 2.69 (dd, $J_1$ = 1.6 Hz, $J_2$ = 19.6 Hz, 1H, CH-C=O), 2.33 (dd, $J_1$ = 3.2 Hz, $J_2$ = 19.6 Hz, 1H, CH), 2.13 (s, 3H, $CH_3$), 2.07 (m, 1H, CH), 1.95 (m, 4H, $CH_2CH_2$, $CH_2CH_2CH_2$). Analysis: for $C_{15}H_{18}ClNO_5 \times {}^1/_2H_2O$ (336.78): calcd. C 53.49, H 5.69, N 4.16%; found C 53.43, H 5.57, N 4.23%.

**4-(4-Bromobutyl)-3,5,8-trioxo-4-azatricyclo[5.2.2.0$^{2,6}$]undec-1-yl acetate** (**4**)

Yield 85%, m.p. 111-112°C. $^1$H NMR (400 MHz, $CDCl_3$, δ, ppm): 4.25 (dd, $J_1$ = 2.0 Hz, $J_2$ = 10.0 Hz, 1H, CH-C=O), 3.50 (m, 2H, $NCH_2$), 3.40 (t, 2H, $J$ = 6.4 Hz, $CH_2Br$), 3.21 (dd, $J_1$ = 3.6 Hz, $J_2$ = 10.0 Hz, 1H, CH-C=O), 2.91 (d, $J$ = 2.8 Hz, 1H, $CH_2$-C=O), 2.77 (m, 1H, CH-$CH_2$), 2.69 (dd, $J_1$ = 2.0 Hz, $J_2$ = 19.6 Hz, 1H, CH-C=O), 2.34 (dd, $J_1$ = 3.2 Hz, $J_2$ = 19.6 Hz, 1H, CH), 2.14 (s, 3H, $CH_3$), 1.97 (m, 3H, $CH_2CH_2$, CH), 1.73 (m, 4H, $CH_2CH_2CH_2CH_2$). Analysis: for $C_{16}H_{20}BrNO_5$ (386.25): calcd. C 49.75, H 5.22, N 3.63%; found C 49.80, H 5.27, N 3.62.

**4-(4-Bromobutyl)-1-isobutoxy-4-azatricyclo[5.2.2.0$^{2,6}$]-undecane-3,5,8-trione** (**7**)

Yield 70%, m.p. 78.5-80°C. $^1$H NMR (200 MHz, $CDCl_3$, δ, ppm: 3.45 (m, 5H, $NCH_2$, OCH, $CH_2Br$), 3.28 (dd, $J_1$ = 2.2 Hz, $J_2$ = 9.4 Hz, 1H, CH-C=O), 3.16 (m, 2H, OCH, CH-C=O), 2.85 (m, 1H, CH-$CH_2$), 2.52 (dd, $J_1$ = 2.2 Hz, $J_2$ = 19.4 Hz, 1H, CH-C=O), 2.4 (dd, $J_1$ = 3.0 Hz, $J_2$ = 19.2 Hz, 1H, CH-C=O), 1.89 (m, 9H, $CH_2CH_2$, $CH_2CH_2$, $CH_2CH_2CH_2CH_2$, $CH(CH_3)_2$), 0.97 (d, $J$ = 1.6 Hz, 3H, CH-$CH_3$), 0.94 (d, $J$ = 1.8 Hz, 3H, CH-$CH_3$). Analysis: for $C_{18}H_{26}BrNO_4$ (400.32): calcd. C 54.00, H 6.55, N 3.50%; found C 53.90, H 6.38, N 3.32%.

**General procedure for preparation of 4-arylpiperazinyl derivatives of N-substituted imides 3a-3g, 4a-4g** and **7a-7g**

To a mixture of *N*-halogenoalkyl imide derivative (0.01 mole), a powdered anhydrous $K_2CO_3$ (0.01 mole), and a catalytic amount of KI in 2-butanone (30 $cm^3$), an appropriate amine was added. The reaction mixture was refluxed for 24-36 h. Then, an inorganic residue was filtered off and the solvent was evaporated. The obtained mixture was purified by column chromatography, eluent: chloroform : methanol 9 : 0.5, v/v.

Obtained compounds were converted into their hydrochlorides. The solid product was dissolved in methanol saturated with gaseous HCl. The hydrochloride was precipitated by addition of diethyl ether. The crude product was crystallized from methanol/ethyl ether. The elemental analyses and $^1$H NMR spectra, as well as melting points are given for hydrochlorides (except for compounds **3b, 3d, 3e, 4a, 4b, 4g, 7b, 7d-f**). MS data and yields are presented for crude products.

**4-{3-[4-(2-Metoxyphenyl)piperazin-1-yl]propyl}-3,5,8-trioxo-4-azatricyclo[5.2.2.0$^{2,6}$]undec-1-yl acetate** (**3a**)

Yield 65%, m.p. 56-57°C. $^1$H NMR (400 MHz, $CDCl_3$, δ, ppm): 7.05 (m, 4H, $H_{arom}$), 4.23 (d, $J$ = 10.0 Hz, 1H, CH-C=O), 3.85 (s, 3H, $OCH_3$), 3.57 (t, $J$ = 6.8 Hz, 2H, $NCH_2$), 3.21 (m, 4H, $N(CH_2)_{2\ piperazine}$-Ph), 2.90 (m, 1H, CH-C=O), 2.74 (m, 6H, $CH_2$-$N(CH_2)_{2\ piperazine}$, CH-C=O, CH-$CH_2$), 2.68 (m, 1H, CH-C=O), 2.51 (m, 2H, $CH_2N$), 2.36 (dd, $J_1$ = 2.8 Hz, $J_2$ = 19.2 Hz, 1H, CH), 2.13 (s, 3H, $CH_3$), 1.95 (m, 5H, $CH_2CH_2$, $CH_2CH_2$, CH). Analysis: for: $C_{26}H_{33}N_3O_6 \times HCl$ (520.02): calcd. C 60.05, H 6.59, N 8.08%; found C 60.14, H 6.58, N 8.23%.

**3,5,8-Trioxo-4-[3-(4-pyrimidin-2-ylpiperazin-1-yl)propyl]-4-azatricyclo[5.2.2.0$^{2,6}$]undec-1-yl acetate** (**3b**)

Yield 65%, m.p. 88-90°C. $^1$H NMR (400 MHz, $CDCl_3$, δ, ppm: 8.28 (d, $J$ = 4.8 Hz, 2H, 2×$H_{\alpha\ arom}$),

6.47 (t, $J$ = 4.4 Hz, 1H, $H_{\beta\ arom}$), 4.21 (dd, $J_1$ = 2.0 Hz, $J_2$ = 10.0 Hz, 1H, CH-C=O), 3.84 (m, 4H, $N(CH_2)_{2\ piperazine}$-Ph), 3.55 (t, $J$ = 6,8 Hz, 2H, $NCH_2$), 3.19 (dd, $J_1$ = 2.0 Hz, $J_2$ = 10.0 Hz, 1H, CH-C=O), 2.88 (d, $J$ = 2.8 Hz, 1H, CH-C=O), 2.73 (m, 1H, CH-$CH_2$), 2.36 (dd, $J_1$ = 2.0 Hz, $J_2$ = 19.6 Hz, 1H, CH-C=O), 2.51 (m, 4H, $CH_2$-$N(CH_2)_{2\ piperazine}$), 2.36 (m, 3H, $CH_2N$, CH), 2.11 (s, 3H, $CH_3$), 1.91 (m, 3H, $CH_2CH_2$, CH), 1.76 (m, 2H, $CH_2CH_2CH_2$). Analysis: for $C_{23}H_{29}N_5O_5 \times H_2O$ (473.53): calcd. C 58.34, H 6.60, N 14.79%; found C 58.18, H 6.23, N 14.44%.

**4-{3-[4-(2-Hydroxyphenyl)piperazin-1-yl]propyl}-3,5,8-trioxo-4-azatricyclo[5.2.2.0$^{2,6}$]-undec-1-yl acetate** (**3c**)

Yield 60%, m.p. 160-162°C. $^1$H NMR (300 MHz, $CDCl_3$, δ, ppm): 7.00 (m, 4H, $H_{arom}$), 4.24 (dd, $J_1$ = 2.1 Hz, $J_2$ = 9.9 Hz, 1H, CH-C=O), 3.56 (t, $J$ = 7.2 Hz, 2H, $NCH_2$), 3.20 (dd, $J_1$ = 3.3 Hz, $J_2$ = 9.9 Hz, 1H, CH-C=O), 2.89 (m, 6H, $N(CH_2)_{2\ piperazine}$-Ph, CH-C=O, CH-$CH_2$), 2.75 (m, 1H, CH-C=O), 2.65 (m, 2H, $CH_2$-$N(CH_2)_{piperazine}$), 2.38 (m, 3H, N-$CH_{2\ piperazine}$, CH), 2.13 (s, 3H, $CH_3$), 1.97 (m, 4H, $CH_2N$, $CH_2CH_2$), 1.73 (m, 2H, $CH_2CH_2CH_2$), 1.25 (m, 1H, CH). Analysis: $C_{25}H_{31}N_3O_6 \times HCl \times H_2O$ (524.03): calcd. C 57.30, H 6.54, N 8.02%; found C 57.31, H 6.82, N 7.80%.

**3,5,8-Trioxo-4-[3-(4-pyridin-2-ylopiperazin-1-yl)propyl]-4-azatricyclo[5.2.2.0$^{2,6}$]undec-1-yl acetate** (**3d**)

Yield 70%, m.p. 113-115°C. $^1$H NMR (400 MHz, $CDCl_3$, δ, ppm): 8.17 (m, 1H, $H_{\alpha\ arom}$), 7.54 (t, $J$ = 14.0 Hz, 1H, $H_{\gamma\ arom}$), 6.70 (m, 2H, 2×$H_{\beta\ arom}$), 4.25 (dd, $J_1$ = 1.6 Hz, $J_2$ = 10.0 Hz, 1H, CH-C=O), 3.69 (m, 5H, $N(CH_2)_{2\ piperazine}$-Ph, CH-C=O), 3.30 (dd, $J_1$ = 2.8 Hz, $J_2$ = 9.6 Hz, 1H, CH-C=O), 3.05 (m, 3H, $NCH_2$, CH-$CH_2$), 2.88 (d, $J$ = 2.4 Hz, 1H, CH-C=O), 2.74 (m, 4H, $CH_2$-$N(CH_2)_{2\ piperazine}$), 2.37 (dd, $J_1$ = 2.8 Hz, $J_2$ = 19.6 Hz, 1H, CH), 2.14 (s, 5H, $CH_2N$, $CH_3$), 2.01 (m, 5H, $CH_2CH_2$, $CH_2CH_2$, CH). Analysis: for $C_{24}H_{30}N_4O_5$ (454.52): calcd. C 63.42, H 6.65, N 12.33%; found C 63.01, H 6.68, N 11.96%.

**3,5,8-Trioxo-4-[3-(4-phenylpiperazin-1-yl)propyl]-4-azatricyclo-[5.2.2.0$^{2,6}$]undec-1-yl acetate** (**3e**)

Yield 65%, m.p. 121-123°C. $^1$H NMR (300 MHz, $CDCl_3$, δ, ppm): 7.29 (m, 3H, $H_{\gamma\ arom}$, 2×$H_{\alpha\ arom}$), 6.94 (m, 2H, 2×$H_{\beta\ arom}$), 4.24 (dd, $J_1$ = 2.1 Hz, $J_2$ = 9.9 Hz, 1H, CH-C=O), 3.60 (m, 2H, $NCH_2$), 3.42 (m, 4H, $N(CH_2)_{2\ piperazine}$-Ph), 3.26 (dd, $J_1$ = 3.0 Hz, $J_2$ = 9.6 Hz, 1H, CH-C=O), 2.91 (d, $J$ = 3.0 Hz, 3H, CH-C=O, $NCH_2$), 2.76 (m, 5H, $N(CH_2)_{2\ piperazine}$, CH-$CH_2$), 2.38 (dd, $J_1$ = 3.3 Hz, $J_2$ = 19.5 Hz, 1H, CH-C=O), 2.14 (s, 3H, $CH_3$), 1.98 (m, 6H, $CH_2CH_2$, $CH_2CH_2$, $CH_2CH_2CH_2$). Analysis: for $C_{25}H_{31}N_3O_5$ (453.53): calcd. C 66.21, H 6.89, N 9.27%; found C 65.98, H 6.89, N 9.25%.

**4-{3-[4-(4-Fluorophenyl)piperazin-1-yl]propyl}-3,5,8-trioxo-4-azatricyclo[5.2.2.0$^{2,6}$]undec-1-yl acetate** (**3f**)

Yield 68%, m.p. 151-153°C. $^1$H NMR (300 MHz, $CDCl_3$, δ, ppm): 6.91 (m, 4H, $H_{arom}$), 4.23 (dd, $J_1$ = 2.4 Hz, $J_2$ = 9.9 Hz, 1H, CH-C=O), 3.57 (t, $J$ = 7.2 Hz, 2H, $NCH_2$), 3.20 (m, 5H, $N(CH_2)_{2\ piperazine}$-Ph, CH-C=O), 2.90 (m, 1H, CH-C=O), 2.74 (m, 6H, $(CH_2)_{2\ piperazine}$, CH-C=O, CH-$CH_2$), 2.46 (t, $J$ = 7.2 Hz, 2H, $CH_2N$), 2.36 (dd, $J_1$ = 3.3 Hz, $J_2$ = 19.5 Hz, 1H, CH), 2.13 (s, 3H, $CH_3$), 1.99 (m, 3H, $CH_2CH_2$, CH), 1.83 (m, 2H, $CH_2CH_2CH_2$). Analysis: for $C_{25}H_{30}FN_3O_5 \times HCl$ (507.98): calcd. C 59.11, H 6.15, N 8.27%; found C 58.86, H 6.30, N 8.19%.

**4-[3-(4-Benzylpiperazin-1-yl)propyl]-3,5,8-trioxo-4-azatricyclo-[5.2.2.0$^{2,6}$]undec-1-yl acetate** (**3g**)

Yield 60%, m.p. 147-149°C. $^1$H NMR (300 MHz, $CDCl_3$, δ, ppm): 7.32 (m, 5H, $H_{arom}$), 4.20 (dd, $J_1$ = 2.1 Hz, $J_2$ = 9.9 Hz, 1H, CH-C=O), 3.53 (m, 4H, $CH_2$-Ph, $NCH_2$), 3.16 (dd, $J_1$ = 3.6 Hz, $J_2$ = 9.9 Hz, 1H, CH-C=O), 2.89 (m, 1H, CH-C=O), 2.76 (m, 2H, $N(CH_2)_{piperazine}$-Ph), 2.68 (dd, $J_1$ = 2.4 Hz, $J_2$ = 19.2 Hz, 1H, CH-C=O), 2.55 (m, 7H, $N(CH_2)_{piperazine}$ -Ph, $CH_2$-$N(CH_2)_{2\ piperazine}$, CH-$CH_2$), 2.36 (m, 3H, $CH_2N$, CH), 2.13 (s, 3H, $CH_3$), 1.96 (m, 3H, $CH_2CH_2$, CH), 1.73 (m, 2H, $CH_2CH_2CH_2$). Analysis: $C_{26}H_{33}N_3O_5 \times HCl \times {}^1/_2H_2O$ (513.03): calcd. C 60.87, H 6.88, N 8.20%; found C 60.95, H 7.02, N 8.00%.

**4-{4-[4-(2-Metoxyphenyl)piperazin-1-yl]butyl}-3,5,8-trioxo-4-azatricyclo[5.2.2.0$^{2,6}$]undec-1-yl acetate** (**4a**)

Yield 70%, m.p. 123-124°C. $^1$H NMR (400 MHz, DMSO, δ, ppm): 6.96 (m, 4H, $H_{arom}$), 4.15 (d, $J$ = 9.6 Hz, 1H, CH-C=O), 3.78 (s, 3H, $OCH_3$), 3.46 (m, 6H, $N(CH_2)_{2\ piperazine}$-Ph, $NCH_2$), 3.12 (m, 4H, CH-$CH_2$, CH-C=O, $CH_2$-C=O), 2.86 (m, 6H, $CH_2$-$N(CH_2)_{2\ piperazine}$, $CH_2N$), 2.64 (m, 1H, CH), 2.19 (m, 1H, CH), 2.04 (s, 3H, $CH_3$), 1.86 (m, 2H, $CH_2CH_2$), 1.45 (m, 4H, $CH_2CH_2CH_2CH_2$). Analysis: for $C_{27}H_{35}N_3O_6$ (497.58): ESI MS m/e (%): 498.23 $[M + H]^+$ (100).

**3,5,8-Trioxo-4-[4-(4-pyrimidin-2-ylpiperazin-1-yl)-butyl]-4-azatricyclo[5.2.2.0$^{2,6}$]undec-1-yl acetate** (**4b**)

Yield 75%, m.p. 129-131°C. $^1$H NMR (400 MHz, $CDCl_3$, δ, ppm): 8.35 (m, 2H, $H_{\alpha\ arom}$), 6.49 (t,

*J* = 4.4 Hz, 1H, $H_{\beta\ arom}$), 4.24 (dd, $J_1$ = 1.6 Hz, $J_2$ = 10,0 Hz, 1H, CH-C=O), 3.89 (m, 3H, $NCH_2$, CH-C=O), 3.55 (m, 4H, $N(CH_2)_{2\ piperazine}$-Ph), 3.23 (dd, $J_1$ = 3.2 Hz, $J_2$ = 9.6 Hz, 1H, CH-C=O), 2.90 (d, *J* = 2.8 Hz, 1H, CH-C=O), 2.61 (m, 6H, $CH_2$-N($\underline{CH_2}$)$_{2\ piperazine}$, $CH_2N$), 2.36 (m, 1H, $CH_2\underline{CH}$), 2.13 (s, 3H, $CH_3$), 1.93 (m, 4H, $CH_2\underline{CH_2}$, $\underline{CH_2}CH_2$), 1.56 (m, 4H, $CH_2\underline{CH_2}\underline{CH_2}CH_2$). Analysis: for $C_{24}H_{31}N_5O_5$ (469.53): ESI MS m/e (%): 470.2 $[M + H]^+$ (100).

**4-{4-[4-(2-Hydroxyphenyl)piperazin-1-yl]butyl}-3,5,8-trioxo-4-azatricyclo[5.2.2.0$^{2,6}$]-undec-1-yl acetate** (**4c**)

Yield 65%, m.p. 72-73°C. $^1$H NMR (400 MHz, DMSO, δ, ppm): 10.98 (br s, 1H, OH), 6.87 (m, 3H, $H_{\beta\ arom}$, 2×$H_{\alpha\ arom}$), 6.76 (m, 1H, $H_{\gamma\ arom}$), 4.16 (d, *J* = 9.6 Hz, 1H, CH-C=O), 3.44 (m, 7H, $NCH_2$, $N(CH_2)_{2\ piperazine}$-Ph, CH-C=O), 3.09 (m, 7H, N($\underline{CH_2}$)$_{2\ piperazine}$, $CH_2N$, CH-C=O), 2.75 (m, 1H, $CH_2\underline{CH}$), 2.22 (m, 1H, CH-C=O), 2.04 (s, 3H, $CH_3$), 1.87 (m, 2H, $CH_2\underline{CH_2}$), 1.64 (m, 2H, $\underline{CH_2}CH_2$), 1.48 (m, 2H, $CH_2\underline{CH_2}CH_2CH_2$), 1.09 (t, *J* = 6.8 Hz, 2H, $CH_2CH_2\underline{CH_2}CH_2$). Analysis: for $C_{26}H_{33}N_3O_6 \times HCl \times H_2O$ (538.04): calcd. C 58.04, H 6.74, N 7.81%; found C 58.39, H 6.93, N 7.45%.

**3,5,8-Trioxo-4-[4-(4-pyridin-2-ylpiperazin-1-yl)butyl]-4-azatricyclo[5.2.2.0$^{2,6}$]undec-1-yl acetate** (**4d**)

Yield 65%, m.p. 86-87°C. $^1$H NMR (400 MHz, DMSO, δ, ppm): 8.15 (m, 1H, $H_{\alpha\ arom}$), 7.58 (t, *J* = 7.6 Hz, 1H, $H_{\gamma\ arom}$), 6.91 (m, 1H, $H_{\beta\ arom}$), 6.71 (m, 1H, $H_{\beta\ arom}$), 4.15 (d, *J* = 9.6 Hz, 1H, CH-C=O), 3.48 (m, 2H, $NCH_2$), 3.56 (m, 7H, $N(CH_2)_{2\ piperazine}$-Ph, $CH_2N$, CH-C=O), 2.89 (m, 4H, $CH_2$-N($\underline{CH_2}$)$_{2\ piperazine}$), 2.77 (m, 1H, $CH_2$-C=O), 2.64 (m, 1H, $CH_2\underline{CH}$), 2.60 (m, 1H, CH-C=O), 2.19 (d, *J* = 19.2 Hz, 1H, CH), 2.03 (s, 4H, $CH_3$, CH), 1.86 (m, 2H, $\underline{CH_2}CH_2$), 1.49 (m, 4H, $CH_2\underline{CH_2}\underline{CH_2}CH_2$). Analysis: for $C_{25}H_{32}N_4O_5 \times HCl \times 1^1/_2H_2O$ (532.02): calcd. C 56.44, H 6.82, N 10.53%; found C 56.66, H 6.84, N 10.42%.

**3,5,8-Trioxo-4-[4-(4-phenylpiperazin-1-yl)butyl]-4-azatricyclo-[5.2.2.0$^{2,6}$]undec-1-yl acetate** (**4e**)

Yield 60%, m.p. 146-148°C. $^1$H NMR (400 MHz, $CDCl_3$, δ, ppm): 7.27 (m, 2H, 2×$H_{\alpha\ arom}$), 6.90 (m, 3H, 2×$H_{\beta\ arom,}$ $H_{\gamma\ arom}$), 4.24 (dd, $J_1$ = 1.6 Hz, $J_2$ = 10.0 Hz, 1H, CH-C=O), 3.51 (m, 2H, $NCH_2$), 3.33 (m, 4H, $N(CH_2)_{2\ piperazine}$-Ph), 3.23 (dd, $J_1$ = 2.8 Hz, $J_2$ = 9.6 Hz, 1H, CH-C=O), 2.90 (d, *J* = 2.8 Hz, 1H, CH-C=O), 2.75 (m, 5H, $CH_2$-N($\underline{CH_2}$)$_{2\ piperazine}$, $CH_2\underline{CH}$), 2.68 (m, 1H, CH-C=O), 2.57 (m, 2H, $CH_2N$), 2.34 (dd, $J_1$ = 2.8 Hz, $J_2$ = 19.2 Hz, 1H, CH), 2.13 (s, 3H, $CH_3$), 1.97 (m, 3H, $CH_2\underline{CH_2}$, CH), 1.49 (m, 4H, $CH_2\underline{CH_2}\underline{CH_2}CH_2$). Analysis: for $C_{26}H_{33}N_3O_5 \times HCl$, (504.04): calcd. C 61.96, H 6.80, N 8.34%; found C 62.21, H 6.98, N 8.32%.

**4-{4-[4-(4-Fluorophenyl)piperazin-1-yl]butyl}-3,5,8-trioxo-4-azatricyclo[5.2.2.0$^{2,6}$]undec-1-yl acetate** (**4f**)

Yield 70%, m.p. 195-196°C. $^1$H NMR (400 MHz, DMSO, δ, ppm): 7.06 (m, 4H, $H_{arom}$), 4.15 (d, *J* = 9.2 Hz, 1H, CH-C=O), 3.69 (m, 2H, $NCH_2$), 3.47 (m, 3H, 2×CH-C=O, $CH_2\underline{CH}$), 3.35 (m, 4H, $N(CH_2)_{2\ piperazine}$-Ph), 3.10 (m, 5H, N($\underline{CH_2}$)$_{2\ piperazine}$, CH-C=O), 2.75 (d, *J* = 18.4 Hz, 1H, CH), 2.62 (m, 2H, $CH_2N$), 3.23 (dd, $J_1$ = 2.0 Hz, $J_2$ = 19.2 Hz, 1H, CH), 2.04 (s, 4H, $CH_3$, CH), 1.87 (m, 1H, CH), 1.63 (m, 2H, $CH_2\underline{CH_2}CH_2CH_2$), 1.49 (m, 2H, $CH_2CH_2\underline{CH_2}CH_2$). Analysis: for $C_{26}H_{32}FN_3O_5 \times HCl$ (522.01): calcd. C 59.82, H 6.37, N 8.05%; found C 59.96, H 6.38, N 8.06%.

**4-[4-(4-Benzylpiperazin-1-yl)butyl]-3,5,8-trioxo-4-azatricyclo-[5.2.2.0$^{2,6}$]-undec-1-yl acetate** (**4g**)

Yield 58%, m.p. 69-70°C. $^1$H NMR (400 MHz, $CDCl_3$, δ, ppm): 7.30 (m, 5H, $H_{arom}$), 4.23 (dd, $J_1$ = 1.6 Hz, $J_2$ = 9.6 Hz, 1H, CH-C=O), 3.61 (m, 2H, $CH_2$-Ph), 3.49 (m, 2H, $NCH_2$), 3.24 (dd, $J_1$ = 6.8 Hz, $J_2$ = 10.0 Hz, 1H, CH-C=O), 2.89 (m, 2H, CH-C=O, $CH_2\underline{CH}$), 2.73 (m, 8H, $N(CH_2)_{4\ piperazine}$), 2.61 (m, 2H, $CH_2N$), 2.32 (dd, $J_1$ = 2.8 Hz, $J_2$ = 19.2 Hz, 1H, CH-C=O), 2.13 (s, 3H, $CH_3$), 1.96 (m, 4H, $CH_2\underline{CH_2}$, $\underline{CH_2}CH_2$), 1.57 (m, 4H, $CH_2\underline{CH_2}\underline{CH_2}CH_2$). Analysis: for $C_{27}H_{35}N_3O_5 \times 3^1/_2H_2O$ (544.64): calcd. C 59.54, H 6.77, N 7.72%; found C 59.40, H 6.48, N 7.50%.

**1-Isobutoxy-4-{4-[4-(2-metoxyphenyl)piperazin-1-yl]butyl}-4-azatricyclo[5.2.2.0$^{2,6}$]undecane-3,5,8-trione** (**7a**)

Yield 50%, m.p. 168-170°C. $^1$H NMR (200 MHz, $CDCl_3$, δ, ppm): 6.94 (m, 4H, $H_{arom}$), 3.86 (s, 3H, $OCH_3$), 3.50 (m, 3H, OCH, $NCH_2$), 3.27 (m, dd, $J_1$ = 2.2 Hz, $J_2$ = 9.8 Hz, 1H, CH-C=O), 3.16 (m, 6H, OCH, $\underline{CH}$-$CH_2$, $N(CH_2)_{2\ piperazine}$-Ph), 2.85 (m, 1H, CH-C=O), 2.63 (m, 4H, $CH_2$-N($\underline{CH_2}$)$_{2\ piperazine}$), 2.50 (dd, $J_1$ = 2.0 Hz, $J_2$ = 19.6 Hz, 1H, CH-C=O), 2.39 (t, *J* = 7.1 Hz, 2H, $CH_2N$), 2.24 (dd, $J_1$ = 2.4 Hz, $J_2$ = 19.2 Hz, 1H, CH-C=O), 2.09 (m, 5H, $\underline{CH_2}CH_2$, $CH_2\underline{CH_2}$, $\underline{CH}(CH_3)_2$), 1.51 (m, 4H, $CH_2\underline{CH_2}\underline{CH_2}CH_2$), 0.97 (d, *J* = 1.8 Hz, 3H, CH-$\underline{CH_3}$), 0.94 (d, *J* = 1.6 Hz, 3H, CH-$\underline{CH_3}$). Analysis: for $C_{29}H_{41}N_3O_5 \times 2\ HCl \times {}^1/_2H_2O$ (593.60): calcd. C 58.70, H 7.47, N 7.08%; found C 58.47, H 7.50, N 6.69%.

**1-Isobutoxy-4-[4-(4-pyrimidin-2-ylpiperazin-1-yl)butyl]-4-azatricyclo-[5.2.2.0$^{2,6}$]-undecane-3,5,8-trione** (**7b**)

Yield 30%, m.p. 79-80°C. $^1$H NMR (200 MHz, $CDCl_3$, δ, ppm): 8.30 (d, $J$ = 46 Hz, 2H, $H_{\alpha\ arom}$), 6.48 (dd, $J_1$ = 2.0 Hz, $J_2$ = 19.6 Hz, 1H, $H_{\beta\ arom}$), 3.84 (m, 4H, $N(CH_2)_{2\ piperazine}$-Ph), 3.49 (m, 3H, OCH, $NCH_2$), 328 (dd, $J_1$ = 2.2 Hz, $J_2$ = 9.6 Hz, 1H, CH-C=O), 3.18 (m, 2H, OCH, CH-C=O), 2.86 (m, 1H, CH-$CH_2$), 2.49 (m, 7H, $CH_2$-$N(\underline{CH_2})_{2\ piperazine}$, CH-C=O, $CH_2N$), 2.24 (dd, $J_1$ = 2.6 Hz, $J_2$ = 19.4 Hz, 1H, CH-C=O), 1.83 (m, 9H, $\underline{CH_2}CH_2$, $CH_2\underline{CH_2}$, $CH_2\underline{CH_2}\underline{CH_2}CH_2$, $CH(CH_3)_2$), 0.97 (d, $J$ = 1.6 Hz, 3H, CH-$\underline{CH_3}$), 0.94 (d, $J$ = 1.6 Hz, 3H, CH-$\underline{CH_3}$). Analysis: for $C_{26}H_{37}N_4O_5 \times 1^1/_2H_2O$ (510.65): calcd. C 61.15, H 7.90, N 13.72%; found C 61.33, H 7.61, N 13.72%.

**4-{4-[4-(2-Hydroxyphenyl)piperazin-1-yl]butyl}-1-isobutoxy-4-azatricyclo-[5.2.2.0$^{2,6}$]-undecane-3,5,8-trione** (**7c**)

Yield 40%, m.p. 220-222°C. $^1$H NMR (400 MHz, DMSO, δ, ppm): 6.85 (m, 4H, $H_{arom}$), 5.90 (br. s, 1H, OH), 3.41 (m, 6H, $N(CH_2)_{2\ piperazine}$-Ph, OCH, CH-C=O), 3.33 (m, 3H, CH-C=O, $NCH_2$), 3.15 (m, 7H, OCH, $CH_2N$, $N(\underline{CH_2})_{2\ piperazine}$), 2.55 (m, 1H, $\underline{CH}$-$CH_2$), 2.04 (m, 4H, $CH_2$-C=O, $\underline{CH_2}CH_2$), 1.78 (m, 2H, $CH_2\underline{CH_2}$), 1.61 (m, 3H, $CH_2\underline{CH_2}CH_2CH_2$, $\underline{CH}(CH_3)_2$), 1.45 (m, 2H, $CH_2CH_2\underline{CH_2}CH_2$), 0.89 (dd, $J_1$ = 4.4 Hz, $J_2$ = 6.4 Hz, 6H, CH-$(\underline{CH_3})_2$). Analysis: for $C_{28}H_{39}N_3O_5 \times 2$ HCl × $H_2O$ (588.59): calcd. C 57.24, H 7.38, N 7.15%; found C 57.33, H 7.06, N 7.14%.

**1-Isobutoxy-4-[4-(4-pyridin-2-ylpiperazin-1-yl)butyl]-4-azatricyclo-[5.2.2.0$^{2,6}$]undcane-3,5,8-trione** (**7d**)

Yield 45%, m.p. 77.5-79°C. $^1$H NMR (200 MHz, $CDCl_3$, δ, ppm): 8.19 (dd, $J_1$ = 1.6 Hz, $J_2$ = 5.2 Hz, 1H, $H_{\alpha\ arom}$), 7.49 (m, 1H, $H_{\gamma\ arom}$), 6.65 (m, 2H, $H_{\beta\ arom}$), 3.57 (m, 7H, $N(CH_2)_{2\ piperazine}$-Ph, OCH, $NCH_2$), 3.29 (m, 1H, CH-C=O), 3.18 (m, 3H, OCH, 2×CH-C=O), 2.85 (m, 2H, CH-C=O, $\underline{CH}$-$CH_2$), 2.55 (m, 6H, $CH_2$-$N(\underline{CH_2})_{2\ piperazine}$, $CH_2N$), 1.74 (m, 9H, $\underline{CH_2}CH_2$, $CH_2CH_2$, $CH_2\underline{CH_2}\underline{CH_2}CH_2$, $\underline{CH}(CH_3)_2$), 0.96 (dd, $J_1$ = 2.0 Hz, $J_2$ = 6.8 Hz, 6H, CH-$(\underline{CH_3})_2$). Analysis: for $C_{27}H_{38}N_4O_4 \times H_2O$ (500.63): calcd. C 64.78, H 8.05, N 11.19%; found C 65.34, H 8.24, N 11.04%.

**1-Isobutoxy-4-[4-(4-phenylpiperazin-1-yl)butyl]-4-azatricyclo[5.2.2.0$^{2,6}$]-undecane-3,5,8-trione** (**7e**)

Yield 40%, m.p. 127-128°C. $^1$H NMR (200 MHz, $CDCl_3$, δ, ppm): 7.25 (m, 2H, 2×$H_{\alpha\ arom}$), 6.88 (m, 3H, 2×$H_{\beta\ arom}$, $H_{\gamma\ arom}$), 3.50 (m, 3H, OCH, $NCH_2$), 3.27 (dd, $J_1$ = 2.0 Hz, $J_2$ = 9.8 Hz, 1H, CH-C=O), 3.16 (m, 6H, $N(CH_2)_{2\ piperazine}$-Ph, OCH, CH-C=O), 2.85 (m, 1H, CH-$CH_2$), 2.58 (m, 4H, $CH_2$-$N(\underline{CH_2})_{2\ piperazine}$), 2.41 (m, 3H, CH-C=O, $CH_2N$), 2.24 (dd, $J_1$ = 2.4 Hz, $J_2$ = 19.6 Hz, 1H, CH-C=O), 1.76 (m, 9H, $\underline{CH_2}CH_2$, $CH_2\underline{CH_2}$, $CH_2\underline{CH_2}\underline{CH_2}CH_2$, $\underline{CH}(CH_3)_2$), 0.97 (d, $J$ = 1.8 Hz, 3H, CH-$\underline{CH_3}$), 0.94 (d, $J$ = 1.8 Hz, 3H, CH-$\underline{CH_3}$). Analysis: for $C_{28}H_{39}N_3O_4$ (481.65): calcd. C 69.82, H 8.16, N 8.73%; found C 70.21, H 7.98, N 8.69%.

**4-{4-[4-(4-Fluorophenyl)piperazin-1-yl]butyl}-1-isobutoxy-4-azatricyclo-[5.2.2.0$^{2,6}$]-undecane-3,5,8-trione** (**7f**)

Yield 45%, m.p. 86.5-88°C. $^1$H NMR (200 MHz, $CDCl_3$, δ, ppm): 6.92 (m, 4H, $H_{arom}$), 3.45 (m, 3H, OCH, $NCH_2$), 3.27 (dd, $J_1$ = 2.4 Hz, $J_2$ = 9.4 Hz, 1H, CH-C=O), 3.14 (m, 6H, $N(CH_2)_{2\ piperazine}$-Ph, OCH, CH-C=O), 2.85 (m, 1H, $\underline{CH}$-$CH_2$), 2.58 (m, 4H, $\underline{CH_2}$-$N(CH_2)_{2\ piperazine}$), 2.42 (m, 3H, CH-C=O, $CH_2N$), 2.24 (dd, $J_1$ = 2.6 Hz, $J_2$ = 19.4 Hz, 1H, CH-C=O), 1.75 (m, 9H, $\underline{CH_2}CH_2$, $CH_2\underline{CH_2}$, $CH_2\underline{CH_2}\underline{CH_2}CH_2$, $\underline{CH}(CH_3)_2$), 0.97 (d, $J$ = 1.8 Hz, 3H, CH-$\underline{CH_3}$), 0.94 (d, $J$ = 1.6 Hz, 3H, CH-$\underline{CH_3}$). Analysis: for $C_{28}H_{38}FN_3O_4$ (499.64): calcd. C 67.31, H 7.67, N 8.41%; found C 67.48, H 7.67, N 8.37&.

**4-[4-(4-Benzylpiperazin-1-yl)butyl]-1-isobutoxy-4-azatricyclo[5.2.2.0$^{2,6}$]undecane-3,5,8-trione** (**7g**)

Yield 38%, m.p. 259-260°C. $^1$H NMR (200 MHz, $CDCl_3$, δ, ppm): 7.23 (m, 5H, $H_{arom}$), 3.48 (m, 5H, OCH, $NCH_2$, $CH_2$-Ph), 3.15 (m, 3H, OCH, CH-C=O, $\underline{CH}$-$CH_2$), 2.84 (m, 1H, CH-C=O), 2.41 (m, 11H, $N(CH_2)_{4\ piperazine}$, $CH_2N$, CH-C=O), 1.90 (m, 6H, $\underline{CH_2}CH_2$, $CH_2\underline{CH_2}$, CH-C=O, $\underline{CH}(CH_3)_2$), 1.47 (m, 4H, $CH_2\underline{CH_2}\underline{CH_2}CH_2$), 0.97 (d, $J$ = 1.6 Hz, 3H, CH-$\underline{CH_3}$), 0.94 (d, $J$ = 1.6 Hz, 3H, CH-$\underline{CH_3}$). Analysis: for $C_{29}H_{41}N_3O_4 \times 2$ HCl × $^1/_2H_2O$ (577.59): calcd. C 60.30, H 7.68, N 7.28%; found C 60.24, H 7.53, N 7.08%. ESI MS m/z (%): 496.2 $(M + H)^+$ (100); 497.3 $(M + 2H)^+$ (37); 518.3 $(M + Na)^+$ (8).

**X-ray crystallography**

Crystal data for **1** and **5** are listed in Table 1. Intensity measurements were carried out at 295 K with a KM4 diffractometer (Oxford Diffraction) using graphite monochromated $CuK_\alpha$ radiation (λ = 1.54178 Å) and ω/2θ scan mode. Structure was solved by the SHELXS-97 program and refined by full-matrix least-squares on $F^2$ using the SHELXL-97 program (28). The non-H atoms were refined anisotropically. The H-atoms were positioned geometrically and the 'riding' model for the C–H bonds was used in the refinement. The methyl H-atoms in **1** are disordered over two sites rotated by 60 degrees one from another (SOF's 0.5). The N-bonded H-atoms were located in the difference

Fourier maps. The experimental details and final atomic parameters for **1** and **5** have been deposited with the Cambridge Crystallographic Data Centre as supplementary material.

**Cell-based assays**

Compounds for cytotoxicity and antiviral activity assays were dissolved in DMSO at concentration of 100 mM and then diluted in culture medium. Cell line and viruses were purchased from American Type Culture Collection (ATCC). The absence of mycoplasma contamination was checked periodically by the Hoechst staining method. Cell line supporting the multiplication of Human Immunodeficiency Virus type-1 (HIV-1) was the CD4+ human T cells containing an integrated HTLV-1 genome (MT-4).

**Cytotoxicity assays**

Cytotoxicity assays were run in parallel with antiviral assays. Exponentially growing MT-4 cells were seeded at an initial density of $1 \times 10^5$ cells/mL in 96-well plates in RPMI-1640 medium, supplemented with 10% fetal bovine serum (FBS), 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-diphenyl-tetrazolium bromide (MTT) method (29).

**Antiviral assay**

The activity of compounds against HIV-1 was based on inhibition of virus-induced cytopathogenicity in MT-4 cell acutely infected with a multiplicity of infection (m.o.i.) of 0.01. Briefly, 50 mL of RPMI containing $1 \times 10^4$ MT-4 cells were added to each well of flat-bottom microtitre trays, containing 50 mL of RPMI without or with serial dilutions of test compounds. Then, 20 µL of a HIV-1 suspension containing 100 $CCID_{50}$ were added. After a 4-day incubation at 37°C, cell viability was determined by the MTT method (29).

**Linear regression analysis**

The extent of cell growth/viability and viral multiplication, at each drug concentration tested, were expressed as percentage of untreated controls. Concentrations resulting in 50% inhibition ($CC_{50}$ or $EC_{50}$) were determined by linear regression analysis.

**Antibacterial and antifungal activity**

The antibacterial activity of compounds was tested against collection strains representative of Gram-positive bacteria (*S. aureus* DSM 2569) and Gram-negative bacteria (*P. aeruginosa* DSM 1117). Antifungal activity was tested against collection strains representative of yeasts (*C. albicans* DSM 1386) and moulds (*A. niger* DSM 1988).

**Antibacterial and antifungal assays**

The antibacterial and antifungal activities were evaluated by determining the minimum inhibitory concentration (MIC) by the broth microdilution procedure.

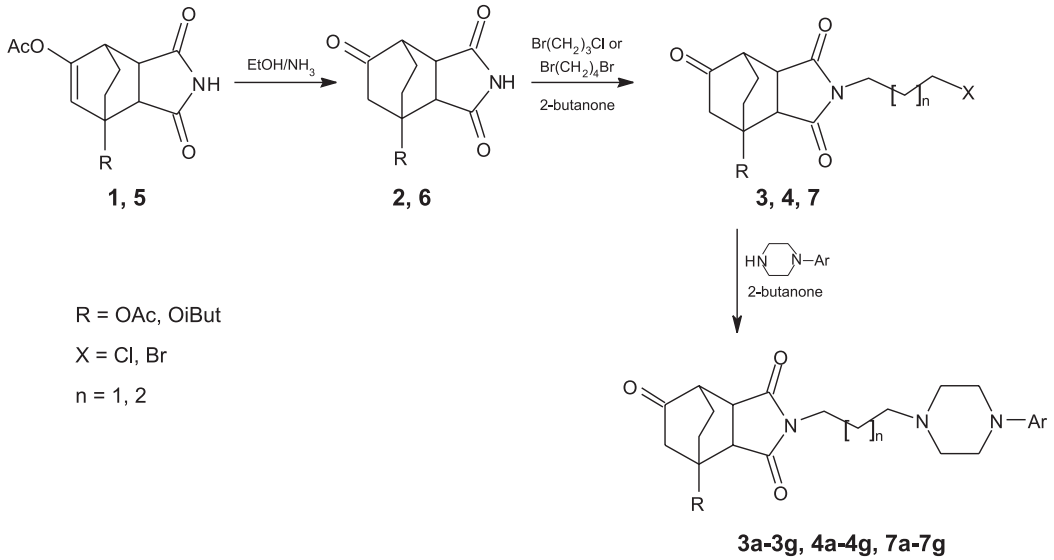
Bacterial strains were grown on Tryptic soy agar at 37°C for 1 day. Cell suspensions of these recent cultures were prepared in sterile 0.85% saline solution by 4-5 colonies. The turbidity of the suspensions was adjusted to the McFarland 0.5 standard. Suspensions were diluted in cation-supplemented Mueller-Hinton broth. For each microorganism, 100 µL of the fivefold serial dilutions of the compounds in cation-supplemented Mueller-Hinton broth and 100 µL of inoculum were added to each well of a microdilution plate (final titre $5 \times 10^5$ CFU/mL). The inoculated plates were incubated at 37°C in non-$CO_2$ incubator and humid atmosphere. The MICs were determined after 16-20 h (30).

Fungal strains were grown on Sabouraud's dextrose agar at 35°C for 1-5 days. Suspensions of these recent cultures were prepared in sterile saline solution (NaCl 0.85%). Suspensions were then diluted in Sabouraud's dextrose broth. Hundred µL of the fivefold serial dilutions of the compounds in Sabouraud's dextrose broth and 100 µL of inoculum were added to each well of a microdilution plate (*C. albicans* $1 \times 10^4$ cell/mL; *A. niger* OD600 0.05). The inoculated plates were incubated at 35°C in non-$CO_2$ incubator and humid atmosphere. The MICs were determined after 24 and 48 h.

The concentration of each inoculum was confirmed by viable counts on agar plates by plating the appropriate dilution of the growth control well, immediately after inoculation, and incubating until visible growth. MIC corresponded to the lowest concentration of an antimicrobial compound that showed complete growth inhibition.

**Pharmacology**

The experiments were carried out on male Albino Swiss mice (20–24 g) kept at a room temperature of 18–20°C on a natural day-night cycle, with free access to food and water. Permission to carry out animal tests and experiments was issued by the Ethical Board at the Medical University of Lublin. The investigated compounds were administered intraperitoneally (*i.p.*) as suspensions in 1% Tween 80 at a constant volume of 0.1 mL/10 g body




Scheme 1. Synthesis of *N*-substituted derivatives of 3,5,8-trioxo-4-azatricyclo-[5.2.2.0$^{2,6}$]undec-1-yl acetate (**2**) and 1-isobutoxy-4-azatricyclo[5.2.2.0$^{2,6}$]undecane-3,5,8-trione (**6**)

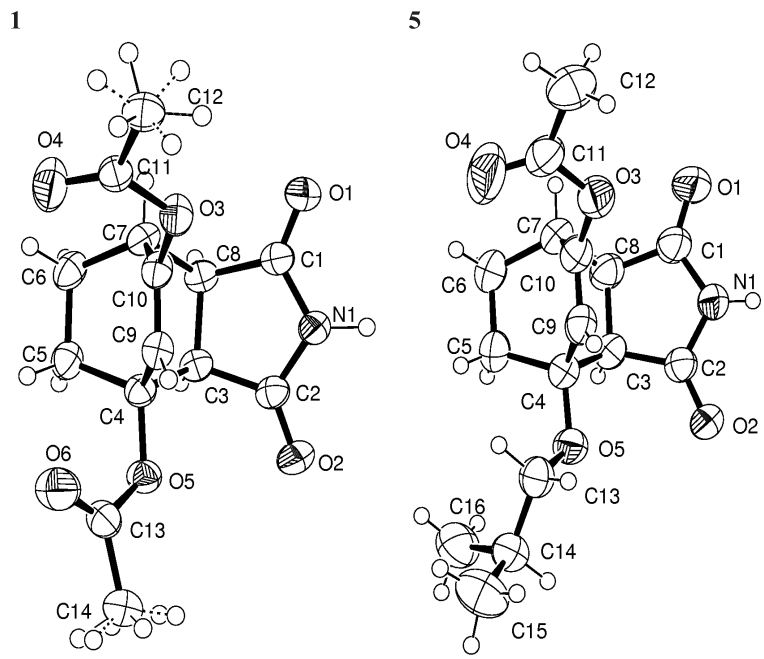



Figure 2. The ortep view of molecules of starting imides **1** and **5**. The C–H bonds in one of the disordered positions of methyl groups in **1** were marked as dashed lines.

weight of mice. Control animals received the same volume of a solvent. The compounds were administered in doses equivalent to 0.1 of their $LD_{50}$. Each experimental group consisted of eight animals.

Motor coordination in a "chimney test" was measured according to the method of Boissier et al. (31), at 30 min after administration of the investigated compounds in a dose equivalent to 0.1 of their $LD_{50}$. The mice had to climb up backwards in a plastic tube (inner diameter: 3 cm, length: 25 cm). The mice that were unable to perform the three tasks within 60 s were considered to display motor

Table 1. Crystal data and structure refinement parameters for crystals of **1** and **5**.

| Compound | **1** | **5** |
|---|---|---|
| CCDC No.* | 902021 | 902022 |
| Empirical formula | $C_{14}$ $H_{15}$ N $O_6$ | $C_{16}$ $H_{21}$ N $O_5$ |
| Formula weight | 293.27 | 307.34 |
| Crystal system, space group | Monoclinic, $P2_1/n$ | Orthorhombic, $P2_12_12_1$ |
| Unit cell dimensions a (Å) | 8.547(2) | 7.569(2) |
| b (Å) | 17.250(3) | 12.618(3) |
| c (Å) | 9.202(2) | 16.578(3) |
| β (°) | 103.26(3) | 90 |
| Volume (Å$^3$) | 1320.5(5) | 1583.3(6) |
| Z, Calculated density (g/cm$^3$) | 4, 1.475 | 4, 1.289 |
| Absorption coefficient (mm$^{-1}$) | 0.989 | 0.795 |
| F(000) | 616 | 656 |
| Crystal size (mm) | 0.30 × 0.30 × 0.20 | 0.30 × 0.25 × 0.20 |
| θ range for data collection (°) | 5.13 – 80.20 | 4.40 – 79.83 |
| Limiting indices | $-10 \le h \le 10$, $0 \le k \le 22$, $0 \le l \le 11$ | $-9 \le h \le 9$, $0 \le k \le 16$, $0 \le l \le 21$ |
| Refl. collected / unique | 3051 / 2878 [$R_{int}$ = 0.0211] | 3663 / 3420 [$R_{int}$ = 0.0175] |
| Data / parameters | 2878 / 191 | 3420 / 203 |
| Goodness-of-fit on $F^2$ | 1.055 | 1.032 |
| Final $R$ indices [$I > 2\sigma(I)$] | $R_1$ = 0.0376, $wR_2$ = 0.1091 | $R_1$ = 0.0402, $wR_2$ = 0.1128 |
| Extinction coeff. | — | 0.015(1) |
| Max and min Δρ (e Å$^{-3}$) | 0.29 and -0.23 | 0.34 and -0.15 |

* Data deposited with the Cambridge Crystallographic Data Centre as supplementary material. Copies of the data can be obtained free of charge on request *via* www.ccdc.cam.ac.uk/data_request/cif, or by emailing data_request@ccdc.cam.ac.uk.

impairment. The motor impairment was quantified as percentage of animals which failed to complete the test.

Spontaneous locomotor activity. Locomotor activity of mice was measured by automatic photoresistor actometers (DIGISCAN Optical Animal Activity Monitoring System, Omnitech Electronics, Inc., Columbus). Thirty minutes after the administration of the investigated compounds in a dose equivalent to 0.1 of their $LD_{50}$, the animals were placed in the actometers for 60 min and the total distance, horizontal activity and vertical activity were recorded automatically.

Anxiolytic activity was assessed by a "four-plate" test in mice according to Aron et al. (32), at 30 min after injection of the investigated compounds in a dose of 0.1 of their $LD_{50}$. The number of punished crossings was counted for 1 min.

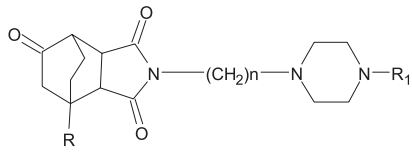
Antidepressant properties were assessed by a "forced swimming" test according to Porsolt et al. (33), at 30 min after administration of investigated compounds in doses of 0.1 of their $LD_{50}$. The mice were individually placed and forced to swim in a glass cylinder (27 × 16 cm) containing 15 cm of water (25°C). The mice were left in the cylinder for 6 min. After the first 2 min, the total duration of immobility was measured during a 4-min test. A mouse was judged to be immobile when it remained floating passively, making slow movements to keep its head above the water.

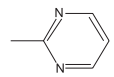
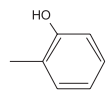
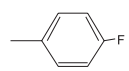
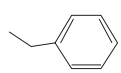
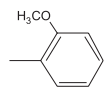
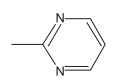
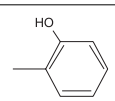
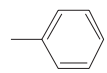
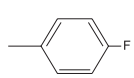
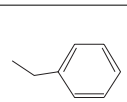
## RESULTS AND DISCUSSION

### Chemistry

In order to check the impact of different substituents at N-4 atom on antimicrobial activity, series of alkyl derivatives of 3,5,8-trioxo-4-azatricyclo[5.2.2.0$^{2,6}$]undec-1-yl acetate and 1-isobutoxy-4-azatricyclo[5.2.2.0$^{2,6}$]undecane-3,5,8-trione was designed and synthesized. Two polycyclic imides **2**

Table 2. Substituents $R_1$ in the molecules of (4-aryl-piperazin-1-ylalkyl) derivatives of 3,5,8-trioxo-4-azatricyclo-[5.2.2.0$^{2,6}$]undec-1-yl acetate (**3**, **4**) and 1-isobutoxy-4-azatricyclo- [5.2.2.0$^{2,6}$]undecane-3,5,8-trione (**7**).




| Compound | n | R | $R_1$ |
|---|---|---|---|
| **3a** | 3 | OAc |  |
| **3b** | 3 | OAc |  |
| **3c** | 3 | OAc |  |
| **3d** | 3 | OAc |  |
| **3e** | 3 | OAc |  |
| **3f** | 3 | OAc |  |
| **3g** | 3 | OAc |  |
| **4a, 7a** | 4 | OAc, OiBut |  |
| **4b, 7b** | 4 | OAc, OiBut |  |
| **4c, 7c** | 4 | OAc, OiBut |  |
| **4d, 7d** | 4 | OAc, OiBut |  |
| **4e, 7e** | 4 | OAc, OiBut |  |
| **4f, 7f** | 4 | OAc, OiBut |  |
| **4g, 7g** | 4 | OAc, OiBut |  |

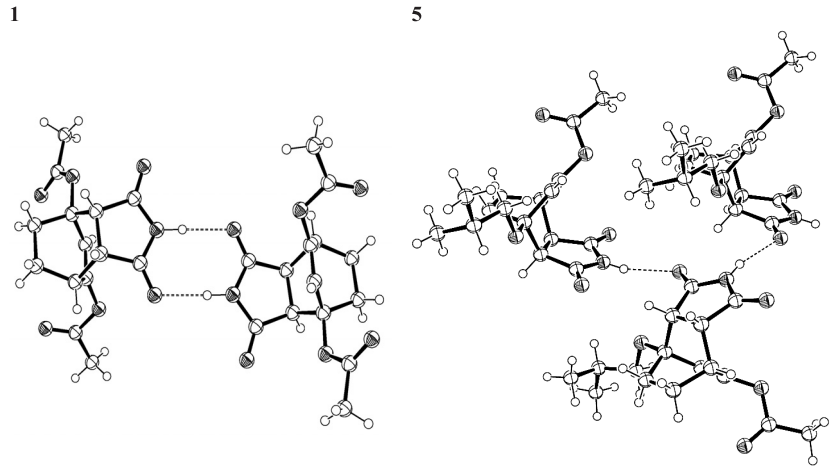



Figure 3. Hydrogen bonded dimer and chain in **1** and **5**, respectively.

Table 3. Selected bond lengths and dihedral angles in the molecules of **1** and **5** (Å, °).

| Bond [Å] | **1** | **5** | Torsion [°] | **1** | **5** |
|---|---|---|---|---|---|
| C1–O1 | 1.212(2) | 1.204(3) | O4–C11–O3–C10 | -2.1(2) | 6.3(4) |
| C2–O2 | 1.206(2) | 1.217(2) | C11–O3–C10–C9 | -103.4(2) | -107.7(2) |
| N1–C1 | 1.373(2) | 1.378(3) | C9–C4–O5–C13 | 53.8(2) | 51.2(2) |
| N1–C2 | 1.381(2) | 1.368(3) | C10–C9–C4–O5 | 177.4(1) | 178.0(2) |
| C9–C10 | 1.316(2) | 1.317(3) | C4–O5–C13–O6[1]/C14[5] | 5.9(2) | 144.7(2) |
| C11–O4 | 1.187(2) | 1.177(3) | | | |
| C13–O6 | 1.200(2) | — | | | |

and **6** were prepared by hydrolysis of compounds **1** and **5**, respectively (Scheme 1). The next step of the synthesis was their alkylation with 1-bromo-3-chloropropane or 1,4-dibromobutane, in which the respective halogenoalkyl derivatives were obtained. They were condensed with appropriate aryl-piperazines to give derivatives **3a**-**3g**, **4a**–**4g** and **7a**-**7g** (Table 2). The structure of all newly synthesized compounds have been established on the basis of $^1$H-NMR spectra, elemental analysis and/or MS. For biochemical studies free bases were converted into their hydrochlorides.

**Molecular and crystal structure**

The molecular and crystal structures of the starting compounds **1** and **5** have been determined by an X-ray structural analysis (Fig. 2, Table 1). Compound **1** crystallizes in the noncentrosymmetric space group $P2_12_12_1$, while **5** in the centrosymmetric $P2_1/n$. The bond lengths and angles are within normal ranges (Table 3). The hydrocarbon skeleton is rigid and both molecules have very similar conformation of the common molecular part including the acetoxy substituent at the C10 atom. However, the presence of voluminous isobutyl substituent in **5** causes more hydrophobic character of the molecule and relatively lower density is observed for **5** in comparison to **1**, despite of higher molecular mass (1.289 *vs.* 1.475 g/cm$^3$ and 307.34 *vs.* 293.27 g/mol for **5** and **1**, respectively; Table 1). The introduction of different substituent at the C4 atom influences also the molecular association. The molecules interact through the intermolecular N–H…O hydrogen bonds forming dimers and chains in the crystals of **1** and **5**, respectively (Fig. 3, Table 4). The acetoxy C=O bonds in **1** are both oriented in opposite direction to the N–H vector. One of them forms two weak C–H...O hydrogen bonds. The other is involved in orthogonal dipole...dipole interaction with imide carbonyl group (34) (Table 4). In crystal of **5**, the

Table 4. Geometric parameters of hydrogen bonds and carbonyl...carbonyl interactions in the crystals of **1** and **5** (Å, °).

| Crystal | D–H….A | D–H [Å] | H….A [Å] | D….A [Å] | < DHA [°] |
|---|---|---|---|---|---|
| **1** | N1–H….O1$^{i}$ | 0.97 | 1.94 | 2.905 (2) | 174 |
| **1** | C12–H…O4$^{ii}$ | 0.96 | 2.56 | 3.369 (2) | 142 |
| **1** | C8–H…O4$^{iii}$ | 0.98 | 2.56 | 3.519 (2) | 166 |
| **5** | N1–H….O2$^{iv}$ | 0.82 | 2.09 | 2.893 (2) | 165 |
| **5** | C3-H3….O2$^{v}$ | 0.98 | 2.60 | 3.444 (3) | 144 |
| **5** | C14–H…O4$^{vi}$ | 0.98 | 2.59 | 3.232 (3) | 123 |
| **5** | C8–H…O4$^{vii}$ | 0.98 | 2.71 | 3.453 (4) | 133 |
| Crystal | C=O….C* =O* | dist. O…..C* [Å] | < COC* [°] | < OC*O* [°] | |
| **1** | C13 = O6…C1$^{viii}$ = O1$^{viii}$ | 3.026(2) | 140.1(1) | 80.9(1) | |
| | | < C*O*C [°] | < O*CO [°] | < COC*O* [°] | |
| | | 74.7(1) | 20.9(1) | 132.3(2) | |

Symmetry codes: $^{i}$ $-x+1, -y+1, -z+1$; $^{ii}$ $x+1/2, -y+3/2, z+1/2$; $^{iii}$ $x+1/2, -y+3/2, z-1/2$; $^{iv}$ $x-1/2, -y+3/2, -z+1$; $^{v}$ $x+1/2, -y+3/2, -z+1$; $^{vi}$ $-x+5/2, -y+2, z-1/2$; $^{vii}$ $-x+2, y-1/2, -z+3/2$; $^{viii}$ $x-1, y, z$.

Table 5. Cytotoxicity and anti-HIV-1 activity of compound **7e**.

| Compound | **MT-4**[a] | **HIV-1**[b] |
|---|---|---|
| | $CC_{50}$ [μM] | $EC_{50}$ [μM] |
| **7e** | 84 | > 84 |
| **EFV** | 37 | 0.002 ± 0.001 |

[a] Compounds concentration (μM) required to reduce the viability of mock-infected MT-4 cells by 50%, as determined by the MTT method. [b] Compounds concentration (μM) required to achieve 50% protection of MT-4 cells from the HIV-1 induced cytopathogenicity, as determined by the MTT method.

acetoxy C=O bond plays very similar role as in **1** and it acts also as a double C–H...O hydrogen bond acceptor (Table 4). The ether substituent is not involved in strong intermolecular interactions.

**Antimicrobial activity**

Nineteen compounds, derivatives of imides **2** and **6** (**2, 3a, 3b, 3d, 3e, 4a, 4b, 4c, 4d, 4e, 4f, 4g, 6, 7, 7a, 7b, 7d, 7e, 7f**), were evaluated *in vitro* for their cytotoxicity and anti-HIV-1 (human immunodeficiency virus type 1) activity (Table 5). However, none of them showed selective antiviral activity.

In addition to the antiviral activity, selected eleven compounds (**2, 3a, 3b, 3d, 3e, 4a, 4b, 4c, 4d, 4f, 4g**) were tested *in vitro* against representative strains of Gram-positive and Gram-negative bacteria (*Staphylococcus aureus, Pseudomonas aeruginosa*), yeasts and moulds (*Candida albicans* and *Aspergillus niger*). Gram-negative rods as well as Gram-positive strains and fungal organisms were resistant to all tested agents. Their minimal inhibitory concentration (MIC) values for all compounds were above 100 μM. Moreover, none of title compounds turned out to be active against HIV-1 virus.

**Pharmacological activity**

As a part of present investigation, the therapeutic potential of derivatives **7a-7g** was screened in a dose equivalent to 0.1 of $LD_{50}$ in a functional test of neurotoxicity and in two models of anxiety and depression in mice (data for $LD_{50}$ are not presented in this paper). No neurotoxic effects were detected in the "chimney test" for any of the 7 investigated compounds, nor did any of them (administrated in the screening dose) show anxiolytic properties in the "four-plate" test and antidepressant-like action in Porsolt's test. All tested compounds have not presented neurotoxicity.

## CONCLUSIONS

Twenty six new compounds were obtained; among them 19 were tested for cytotoxicity and

anti-HIV-1 activity, and 11 for antibacterial and antifungal properties. The activity of 7 derivatives on CNS was screened. Molecular structures of two starting polycyclic imides were presented. N-substituted derivatives expressed no activity in given pharmacological tests, however, as buspiron analogues they could be tested for their 5-$HT_{1A}$ and 5-$HT_{2A}$ receptor activity. Previously synthesized polycyclic imide derivatives, containing the same arylpiperazinyl fragments as in the structures of compounds presented in this work, express high affinity to 5-$HT_{1A}$ receptor (35). On the other hand, it was proved that high hydrophobic imide volume resulted in the receptor's affinity decreasing (36). As a result it could be expected that derivatives with *o*-methoxy substituents (e.g. **3a**, **4a**, **7a**) express higher affinity to 5-HT receptors in comparison with other synthesized compounds. *In vivo* tests could also revealed if more hydrophobic isobutoxy imide derivatives have higher affinity than acetoxy ones.

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