SYNTHESIS OF AMINO DERIVATIVES OF 10-(DIPHENYLMETHYLENE)-4-AZATRICYCLO[5.2.1.0^{2,6}]DEC-8-ENE-3,5-DIONE AS POTENTIAL PSYCHOTROPIC AND/OR ANTI HIV AGENTS

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Abstract: A series of amino derivatives of 10-(diphenylmethylene)-4-azatricyclo[5.2.1.0^{2.6}]dec-8-ene-3,5dione, analogues of chlorpromazine and aminoperazine have been prepared. These compounds are expected to have antipsychotic and/or anti HIV activity. Molecular structure of III was confirmed by an X-ray structure analysis. The cytotoxicity and anti HIV activity of derivatives I–IV were determined.

Keywords: 10-(diphenylmethylene)-4-azatricyclo[5.2.1.0²⁶]dec-8-ene-3,5-dione; anti-HIV tests; X-ray structure analysis

Psychotropic (antipsychotic, neuroleptic) agents are primarily used in the therapy of schizophrenia, organic psychoses, the manic phase of manic-depressive illness and other acute or chronic idiopathic psychotic illnesses. Evidence supports the hypothesis that the etiology of psychotic disorders lies in neurochemical defects of dopaminergic and serotonergic pathways in the brain. The hypothesis is supported by the fact that the primary pharmacological action of antipsychotic agents is antagonism of dopamine and/or serotonin receptor in the central nervous system. It is also known that for typical antipsychotics (e.g. promazine, chlorpromazine, thioridazine, piperactazine, imipramine) a 2-carbon or 3-carbon alkyl connection between a heterocyclic ring and a terminal aminoalkyl or piperidine part is optimal for dopamine-receptor blockade and antipsychotic activity (1). What is more, a new phenothiazine derivative, aminoperazine, characterized by a cationic amphiphilic structure with a bulky hydrophobic core and a nitrogen-linked lateral positive chain, expresses anti HIV activity and might be used for boosting the immune response of vaccinated individuals and for restoring the immunity of immunocompromised patients (2).

Unfortunately, the therapeutic effects of classical neuroleptics frequently come with severe extrapyramidal side effects, therefore new drugs are still requested. Searching for compounds with pre-

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dictable neuroleptic activity, as a continuation of our previous investigations (3, 4), our attention was focused on amino derivatives of 10-(diphenylmeth-ylene)-4-azatricyclo [$5.2.1.0^{26}$] dec-8-ene-3,5-dione.

The test products were evaluated for their cytotoxicity and anti-HIV-1 activity in MT-4 cells using EFV as reference compound (Table 1). Investigations were performed in Dipartamento di Scienze e Tecnologie Biomediche, Universita di Cagliari, Monserrato, Italy.

The structure of all derivatives of compound **I** have been established on the basis of elemental analysis and 'H NMR. The general synthetic pathway is given in Scheme 1. Molecular structure of compound **III** was confirmed by an X-ray crystallography (Figure 1). Suitable crystal of dimensions $0.68 \times 0.26 \times 0.19$ mm was selected for the intensity measurement. Diffraction data were measured at 291 K on a KM4 diffractometer using variable scan speed in the ω -2 Θ scan mode and graphite monochromated CuK α radiation ($\lambda = 1.54178$ Å).

EXPERIMENTAL

Chemistry

Melting points were determined in a Kofler's apparatus and are uncorrected. The 'H NMR spectra were recorded on a Bruker AVANCE DMX400 spectrometer, at 400.13 MHz. The chemical shift



Scheme 1. Method of preparing the reported compounds.

values are expressed in ppm relative to TMS as an internal standard.

10-(diphenylmethylene)-4-azatricyclo[5.2.1.0^{2.6}]dec-8-ene-3,5-dione I was obtained according to the method described previously (5). General procedure for preparing of 4-(2-aminoethyl-10-(diphenylmethylene)-4-azatricyclo[5.2.1.0^{2.6}] dec-8-ene-3,5-diones II–VII.

A mixture of compound **I** (0.45 g, 0.0014 mol), the corresponding chloroethylamine or chloropropylamine (0.0028 mol), anhydrous K_2CO_3 (0.45 g) and catalytic amount of 98% 1,8-diazabicyclo [5.4.0] undec-7-ene were refluxed in acetone for 30–50 h. The reaction was monitored by TLC (silica gel, developing system: chloroform: methanol, 18: 1, v/v), and when completed, the mixture was filtered and the solvent was evaporated. The residue was crystallized from hexane.

II. Yield 65%, m.p. 175–159°C, ¹H NMR (400.13 MHz, CDCl₃) δ (ppm): 7.32–7.27 (m, 6H, H_{arom}), 7.07–7.05 (m, 4H, H_{arom}), 6.31 (s, 2H, CH=), 3.90 (m, 2H, =CH–*CH*), 3.48 (dd, $J_1 = J_2 = 6.8$ Hz, 2H, CH–C=O), 3.42 (m, 2H, N–CH₂), 2.34 (t, J = 6 Hz, 2H, CH₂–N), 2.21 (s, 6H, N(CH₃)₂); C₂₆H₂₆N₂O₂ × 5 1/2 H₂O (497.6): calcd: C 62.76, H 7.49, N 5.63; found: C 62.69, H 7.11, N 5.27.

III. Yield 68%, m.p. 94–96°C, hexane; ¹H NMR (400.13 MHz, CDCl₃) δ (ppm): 7.32–7.27 (m, 6H, H_{arom}), 7.07–7.05 (m, 4H, H_{arom}), 6.31 (s, 2H, CH=), 3.89 (m, 2H, =CH–*CH*), 3.46 (dd, $J_1 = J_2 = 7$ Hz, 2H, CH–C=O), 3.41 (m, 2H, N–CH₂), 2.54–2.46 (m, 6H, N(CH₂)₂, CH₂–N), 0.99 (t, J = 6 Hz, 6H, N(CH₃)₂), C₂₈H₃₀N₂O₂ (426.57): calcd.: C



Figure 1. Perspective drawing of the molecule of III.

78.84, H 7.09, N 6.57; found: C 78.88, H 7.10, N 6.57.

Crystal data for **III**: $C_{28}H_{30}N_2O_2$, $M_w = 426.54$, crystal system orthorhombic, space group $P2_12_12_1$, unit cell dimensions: a = 6.0732(7) A, b = 16.883(3)A, c = 22.952(3) A, $V = 2353.4(6) \text{ A}^3$, Z = 4, F(000) = 912, $D_{calc} = 1.204$ g cm⁻³, $\mu = 0.593$ mm⁻¹. In the Θ range 4.66–80.30°, 5245 reflections were collected of which 4981 were independent [R9int) = 0.0264] and 2915 observed $[I > 2\sigma(I)]$. The structure was solved by direct methods using SHELXS-93 program (6). Refinement was performed by the fullmatrix least-squares method on F² using SHELXL-97 program (7). The non-hydrogen atoms were refined with anisotropic displacement parameters. The H-atom positions were calculated from the geometry and were given isotropic factors of 1.2 Uea of the bonded C-atoms; for the C-H bonds 'riding model was used in the refinement. For 285 parameters refined, final discrepancy factors are R1 = 0.0377, wR2 = 0.1023 for observed reflections, and R1 = 0.1036, wR2 = 0.1214 for all data, S = 1.056. Residual peaks on final difference map were -0.195 to 0.177 e Å³.

Crystallographic data have been deposited with the Cambridge Crystallographic Data Centre as CCDC No. 606658. Copies of the data can be obtained on application to The Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (Fax: +44-1223-336-033; e-mail: deposit@ccdc.cam.ac.uk or www: http://www.ccdc.cam.ac.uk).

IV. Yield 70%, m.p. 155.5–157.5°C, ¹H NMR (400.13 MHz, CDCl₃) δ (ppm): 7.31–7.27 (m, 6H, H_{arom}), 7.07–7.05 (m, 4H, H_{arom}), 6.31 (s, 2H, CH=), 3.89 (m, 2H, =CH–*CH*), 3.50 (dd, $J_1 = J_2 = 6.6$ Hz, 2H, CH–C=O), 3.40 (m, 2H, N–CH₂), 2.37–2.34 (m, 6H, CH₂N_{piper}, H_{α piper}), 1.50 (m, 4H, H_{β piper}), 1.38 (m, 2H, H_{γ piper}), C₂₉H₃₁ClN₂O₂ × 2/3H₂O (487.05): calcd.: C 71.52, H 6.69, N 5.75; found: C 71.17, H 6.27, N 5.79.

V. Yield 50%, m.p. 142–144°C, ¹H NMR (400.13 MHz, CDCl₃) δ (ppm): 7.32–7.28 (m, 6H, H_{arom}), 7.07–7.06 (m, 4H, H_{arom}), 6.32 (s, 2H, CH=), 3.91 (m, 2H, =CH–*CH*), 3.64–3.63 (m, 4H, H_{β} m_{orph}), 3.50 (dd, $J_1 = J_2 = 8$ Hz, 2H, CH–C=O), 3.42

Table 1. Cytotoxicity and anti-HIV activity of compounds I-IV.

Compound	^a CC ₅₀	^b EC ₅₀
	MT-4	HIV-1
Ι	51	> 51
II	26	> 26
III	27	> 27
IV	38	> 38
EFV	35	0.004

^a Compound concentration (μ M) required to reduce the viability of mock-infected MT-4 cells by 50%, as determined by the MTT method. ^b Compound concentration (μ M) required to achieve 50% protection of MT-4 cells from the HIV-1 induced cytopathogenecity, as determined by the MTT method.

(m, 2H, N–CH₂), 2.44–2.39 (m, 6H, CH₂–N_{morph}, $H_{\alpha \text{ morph.}}$), $C_{28}H_{28}N_2O_2$ (440.55): calcd.: C 76.34, H 6.41, N 6.36; found: C 76.58, H 6.11, N 6.05.

VI. Yield 63%, m.p. 145–147.5°C, 'H NMR (400.13 MHz, CDCl₃) δ (ppm): 7.32–7.27 (m, 5H, H_{arom}), 7.07–7.05 (m, 5H, H_{arom}), 6.30 (s, 2H, CH=), 3.90 (m, 2H, =CH–*CH*), 3.51–3.39 (m, 3H, CH–C=O, N–CH₂), 3.19–3.14 (m, 1H, N–CH₂), 2.90–2.84 (m, 1H, CH₂–N), 2.18 (s, 6H, N(CH₃)₂); 0.87–0.85 (m, 3H, –CH₂–, N–CH₂), C₂₇H₂₈N₂O₂ × 1/2 H₂O (421.55): calcd.: C 76.93, H 6.93, N 6.64; found: C 77.16, H 6.90, N 6.71.

VII. Yield 45%, oil; ¹H NMR (400.13 MHz, CDCl₃) δ (ppm): 7.33–7.27 (m, 6H, H_{arom}), 7.07–7.05 (m, 4H, H_{arom}), 6.33 (dd, $J_1 = J_2 = 1.8$ Hz, 2H, CH=), 3.90 (m, 2H, =CH–*CH*), 3.41–3.38 (m, 4H, NCH₂, CH–C=O), 2.32–2.23 (m, 6H, CH₂–N_{piper}, H_{α piper}), 1.69–1.61 (m, 2H, –CH2–), 1.58–1.53 (m, 4H, H_β _{piper}), 1.42–1.41 (m, 2H, H_{γ piper}), C₃₀H₃₄Cl₂N₂O₂ × 9H₂O (687.67): calcd.: C 52.40, H 7.62, N 6.07; found: C 52.21, H 7.30, N 6.28.

Antiviral assay procedures

COMPOUNDS. The compounds were dissolved in DMSO at 200 mM and then diluted into a culture medium.

CELLS AND VIRUSES. MT-4 cells were grown at 37°C in a 5% CO₂ atmosphere in RPMI 1640 medium, supplemented with 10% fetal calf serum (FCS), 100 IU/mL penicillin G, and 100 μ g/mL streptomycin. Cell cultures were checked periodically for the absence of mycoplasma contamination with a Myco Test Kit (Gibco). Human immunodeficiency viruses HIV-1, III_Bstrain were obtained from supernatants of persistently infected H9/III_B cells. HIV-1 stock solutions had titers of 4.5 × 10⁶ and 1.4 × 10⁵ 50% cell culture infectious dose (CCID₅₀)/mL, respectively.

ANTI-HIV ASSAYS. Activity of the compound against HIV-1 multiplication in acutely infected cells was based on the inhibition of virusinduced cytopathicity in MT-4 cells. Briefly, 50 µL of culture medium containing 1×10^4 cells was added to each well of flat-bottom microtiter trays containing 50 µL of culture medium with or without various concentrations of the test compounds. Then 20 µL of an HIV suspension containing 100 (HIV-1) was added. After 4-day incubation at 37°C, the number of viable cells was determined by the 3-(4,5dimethylthiazol-1-yl)-2,5-diphenyltetrazolium bromide (MTT) method (8). Cytotoxicity of the compounds was evaluated in parallel with their antiviral activity. It was based on the viability of mock-infected cells, as monitored by MTT method.

CONCLUSIONS

Six new compounds were obtained. The activities of the synthesized compounds were evaluated for their cytotoxicity and anti-HIV-1 activity in MT-4 cells. None of the tested compounds showed any activity against HIV-1.

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