

Synthesis and Pharmacological Activity of Urea and Thiourea Derivatives of 4-Azatricyclo[5.2.2.0^{2,6}]undec-8-ene-3,5-dione

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A series of nineteen new thiourea and urea derivatives of 10-isopropyl-8-methyl-4-azatricyclo[5.2.2.0^{2,6}]undec-8-ene-3,5-dione, 1-isopropyl-7-methyl-4-azatricyclo[5.2.2.0^{2,6}]undec-8-ene-3,5-dione and 1,7,8,9,10-pentamethyl-4-azatricyclo[5.2.1.0^{2,6}]dec-8-ene-3,5-dione have been prepared and studied by ¹H-NMR. The compound **k1a** (1-(1,7,8,9,10-pentamethyl-3,5-dioxo-4-aza-tricyclo[5.2.1.0^{2,6}]dec-8-en-4-yl)-3-phenyl-urea) was tested for pharmacological activity on animal central nervous system (CNS). The activities of synthesized compounds were evaluated for their cytotoxicity and anti-HIV-1 activity in MT-4 cells. Antimicrobial activity of the newly obtained derivatives was tested against some Gram-positive and Gram-negative bacteria and fungi of the *Candida* species.

Key words urea derivative; thiourea derivative; pharmacological activity; cytotoxicity; antimicrobial activity

Thiourea and urea derivatives show a broad spectrum of biological activities as anti-HIV, antiviral, HDL-elevating, antibacterial, analgesic properties.^{1–4)}

Numerous compounds containing thiourea group are selective ligands for 5-HT family receptors, including 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C}.^{5–9)} The drug-elicited head twitch response (HTR)^{10,11)} is a selective behavioral model for 5-HT₂ agonist activity in rodents, and several previous studies have established that direct and indirect 5-HT agonists induce this effect.^{12–19)} Additionally, 5-HT₂ receptor antagonists selectively block HTR,^{19–21)} and their potency is highly correlated with the antagonist's affinity for 5-HT₂ receptors.^{12,22)}

Here we report the synthesis of compounds **h2**, **h2a**, **d2**, **d2a**, **k2**, **k2a**—**h4**, **h4a**, **d4**, **d4a**, **k4**, **k4a** which composed of thiourea or urea system attached to polycyclic imide.

Chemistry The preparation of new nineteen thiourea and urea derivatives of 10-isopropyl-8-methyl-4-azatricyclo[5.2.2.0^{2,6}]undec-8-ene-3,5-dione, 1-isopropyl-7-methyl-4-azatricyclo[5.2.2.0^{2,6}]undec-8-ene-3,5-dione and 1,7,8,9,10-pentamethyl-4-azatricyclo[5.2.1.0^{2,6}]dec-8-ene-3,5-dione (Chart 1) is described.

Imides obtained in Diels–Alder reaction were used as starting material. 10-Isopropyl-8-methyl-4-azatricyclo[5.2.2.0^{2,6}]undec-8-ene-3,5-dione was obtained in reaction of enantiomeric (*R*)-(–)- α -phellandrene with pyrrole-2,5-dione,²³⁾ 1-isopropyl-7-methyl-4-azatricyclo[5.2.2.0^{2,6}]undec-8-ene-3,5-dione in reaction of α -terpinene with pyrrole-2,5-dione²³⁾ and 1,7,8,9,10-pentamethyl-4-azatricyclo[5.2.1.0^{2,6}]dec-8-ene-3,5-dione in reaction 1,2,3,4,5-pentamethylcyclopentadiene with pyrrole-2,5-dione.²⁴⁾

The obtained tricyclic imides reacted with hydrazine (80% aqueous solution). Afterwards the compounds were subjected to the reaction with phenyl, 4-methoxyphenyl, cyclohexyl isocyanate or isothiocyanate in order to be transformed into the corresponding urea or thiourea derivatives.

All final compounds were characterized by ¹H-NMR spectra which corresponded with the proposed structures.

The general synthetic pathway is given in Chart 1.

Pharmacology Acute toxicity of tested compound was

lower than 2000 mg/kg i.p. and therefore LD₅₀=2000 mg/kg was accepted for the continuation of the studies.

Spontaneous motor activity, amphetamine-induced hyperactivity, motor coordination and body temperature, were not changed by **k1a**. This compound did not protect from clonic seizures and tonic convulsions evoked by pentetrazole and from abdominal constriction induced by i.p. administration of the acetic acid solution.

Only the head-twitch responses to 5-HTP were significantly decreased by 77.88% (*p*<0.05), from 14.13±4.24 to 3.125±1.007, by **k1a** (Fig. 1). The result seems to point out some connection with 5-HT the system. Since it appears that head shakes induced by 5-HTP are mediated by 5-HT₂ recep-

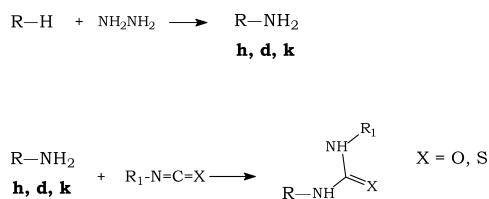
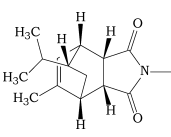
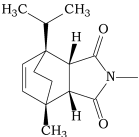
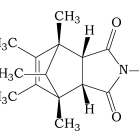


Chart 1. Synthesis of the Obtained Compounds **h2**, **h2a**, **d2**, **d2a**, **k2**, **k2a**—**h4**, **h4a**, **d4**, **d4a**, **k4**, **k4a**

Table 1. Structure of the Investigated Compounds **h2**, **h2a**, **d2**, **d2a**, **k2**, **k2a**—**h4**, **h4a**, **d4**, **d4a**, **k4**, **k4a**

	R		
R ₁			
	h1 , ^{a)} h1a ^{b)}	d1 , h1a	k1 , k1a
	h2 , h2a	d2 , d2a	k2 , k2a
	h3 , h3a	d3 , d3a	k3 , k3a

a) X=S; b) a for X=O.

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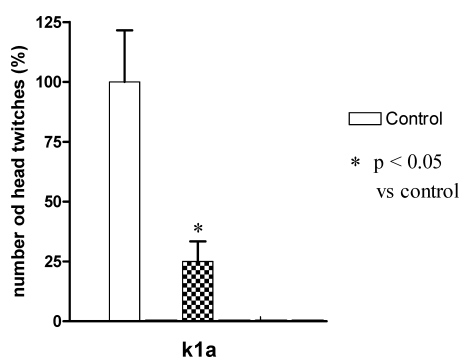


Fig. 1. The Influence of **k1a** on the Head Twitch Responses Evoked by 5-Hydroxytryptophan (5-HTP) (180 mg/kg i.p.)

The results are expressed as mean $\pm$ S.E.M. ($n=8$).

Table 2. Cytotoxicity and Anti-HIV Activity of Compounds (**h**, **h2**, **h2a**, **d**, **d2**, **d2a**, **k**, **k2**, **k2a**—**h4**, **h4a**, **d4**, **d4a**, **k4**, **k4a**)

Compound	CC ₅₀ ^{a)}	EC ₅₀ ^{b)}
	MT-4	HIV-1
h	>100	>100
h1a	>100	62
h1	48	>48
h2a	46	>46
h2	52	>52
h3a	63	>63
h3	47	>47
d	>100	>100
d1a	>100	>100
d1	60	>60
d2a	57	>57
d2	76	>76
d3a	72	>72
d3	53	>53
k	>100	>100
k1a	>42	>42
k2	55	>55
k3a	12	>12
k3	48	>48
EFV	35	0.003

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 cytopathogenicity, as determined by the MTT method.

tors, these data suggest that **k1a** significantly interact with 5-HT₂ receptors in the brain.

Antiviral Activities The synthesized compounds were evaluated for their cytotoxicity and anti-HIV-1 activity in MT-4 cells. None of the synthesized compounds showed activity against HIV-1.

Microbiology All newly synthesized compounds were tested for their antibacterial and antifungal activities. Gram-negative and Gram-positive bacterial strains and *Candida albicans* used in this study have common application in the antimicrobial activity tests for many substances like antibiotics, disinfectants and antiseptic drugs or in research on new antimicrobial agents.^{25–27} All synthesized compounds were completely inactive against tested microorganisms.

Experimental

Chemistry Melting points were determined in a capillary on Kofler's apparatus and are uncorrected. The ¹H-NMR spectra were recorded with

Bruker DMX400 spectrometer, operating at 400.13 MHz for ¹H or with a Varian UNITYplus-200 spectrometer, operating at 199.97 MHz for ¹H. The chemical shift values, expressed in ppm, were referenced downfield to TMS at ambient temperature. Microanalysis was performed at the Microanalysis Laboratory of Warsaw Technical University and all values were within $\pm 0.4\%$ of the calculated compositions. Flash chromatography was performed on Merck silica gel 60 (200–400 mesh) using chloroform/methanol (19:1 vol.) mixture as eluent. Analytical TLC was carried out on silica gel F₂₅₄ (Merck) plates (0.25 mm thickness).

(1*R*,2*S*,6*R*,7*R*,10*R*) 4-Amino-10-isopropyl-8-methyl-4-aza-tricyclo[5.2.2.0^{2,6}]undec-8-ene-3,5-dione (h**)** This compound has been synthesized as described previously.²⁸⁾

(1*S*,2*R*,6*S*,7*R*) 4-Amino-1,7,8,9,10-pentamethyl-4-azatricyclo[5.2.1.0^{2,6}]dec-8-ene-3,5-dione (k**) and (1*S*,2*R*,6*S*,7*S*) 4-Amino-1-isopropyl-7-methyl-4-azatricyclo[5.2.2.0^{2,6}]undec-8-ene-3,5-dione (**d**). General Procedure** A mixture of imide (0.01 mol) and hydrazine (80% water solution) (50 ml) was refluxed for 5 h. After solvents were evaporated, the residue was purified by a column chromatography (silica gel) to give compound (**d** or **k**). The product was recrystallized from heptane.

(1*S*,2*R*,6*S*,7*S*) 4-Amino-1-isopropyl-7-methyl-4-azatricyclo[5.2.2.0^{2,6}]undec-8-ene-3,5-dione (d**)** Yield 2.18 g (88%). mp 147 °C. ¹H-NMR (200 MHz, CDCl₃) δ : 0.98 (d, $J=7.2$ Hz, 3H, CH₃), 1.11 (d, $J=6.8$ Hz, 3H, CH₃), 1.23–1.36 (m, 2H, CH₂), 1.4–1.46 (m, 2H, CH₂), 1.48 (s, 3H, CH₃), 2.53–2.62 (m, 2H, CH), 2.57 (d, $J=8$ Hz, 1H, CH), 4.24 (s, 2H, NH₂), 5.88 (d, $J=8.4$ Hz, 1H, CH=), 5.96 (d, $J=8.4$ Hz, 1H, CH=). C₁₄H₂₀O₂N₂ (248.32): Calcd C 67.71, H 8.12, N 11.28; Found C 67.64, H 8.08, N 11.36.

(1*S*,2*R*,6*S*,7*R*) 4-Amino-1,7,8,9,10-pentamethyl-4-azatricyclo[5.2.1.0^{2,6}]dec-8-ene-3,5-dione (k**)** Yield 2.23 g (90%). mp 171–172 °C. ¹H-NMR (200 MHz, CDCl₃) δ : 0.61 (d, $J=6.4$ Hz, 3H, CH₃); 1.34 (s, 6H, CH₃); 1.48 (s, 6H, CH₃); 1.54 (q, 1H, CH); 2.87 (s, 2H, CH); 3.49 (s, 1H, NH₂). C₁₄H₂₀O₂N₂ (248.32): Calcd C 67.71, H 8.12, N 11.18; Found: C 67.58, H 8.03, N 11.31.

Urea or Thiourea Derivatives of (1*R*,2*S*,6*R*,7*R*,10*R*) 10-Isopropyl-8-methyl-4-azatricyclo[5.2.2.0^{2,6}]undec-8-ene-3,5-dione (h1**, **h1a**—**h3**, **h3a**), (1*S*,2*R*,6*S*,7*S*) 1-Isopropyl-7-methyl-4-azatricyclo[5.2.2.0^{2,6}]undec-8-ene-3,5-dione (**d1**, **d1a**—**d3**, **d3a**) and (1*S*,2*R*,6*S*,7*R*) 1,7,8,9,10-Pentamethyl-4-azatricyclo[5.2.1.0^{2,6}]dec-8-ene-3,5-dione (**k1**, **k1a**—**k3**, **k3a**). General Procedure** A solution of 4-aminoimide (**h**) or (**d**) or (**k**) (0.01 mol) in acetonitrile (6 cm³) was treated with phenyl, 4-methoxyphenyl, cyclohexylisocyanate or isothiocyanate (0.011 mol) and the mixture was refluxed for 6 h. The precipitate was filtered and then washed with ether to give compounds (**h1**, **h1a**—**h3**, **h3a**), (**d1**, **d1a**—**d3**, **d3a**), (**k1**, **k1a**—**k3**, **k3a**). The product was recrystallized from ethanol.

(1*R*,2*S*,6*R*,7*R*,10*R*) 1-(10-Isopropyl-8-methyl-3,5-dioxo-4-aza-tricyclo[5.2.2.0^{2,6}]undec-8-en-4-yl)-3-phenyl-thio-urea (h1**)** Yield 2.93 g (67%). mp 188 °C. ¹H-NMR (400 MHz, CDCl₃) δ : 0.803 (d, $J=6.2$ Hz, 3H, CH₃), 0.9 (d, $J=6.4$ Hz, 3H, CH₃), 0.94–1.45 (m, 2H, CH), 1.34–1.41 (m, 1H, CH), 1.66 (s, 3H, CH₃), 1.78–1.88 (m, 1H, CH–CH₃), 2.75 (m, 1H, CH), 2.91–2.98 (m, 3H, CH₂, CH), 5.61 (d, $J=4.4$ Hz, 1H, CH), 7.14–7.36 (m, 5H, C_{arom}), 9.88 (s, 1H, NH), 10.22 (s, 1H, NH). C₂₁H₂₅O₃N₃S (387.53): Calcd C 65.08, H 7.54, N 10.84; Found C 65.0, H 7.65, N 10.76.

(1*R*,2*S*,6*R*,7*R*,10*R*) 1-(10-Isopropyl-8-methyl-3,5-dioxo-4-aza-tricyclo[5.2.2.0^{2,6}]undec-8-en-4-yl)-3-phenyl-urea (h1a**)** Yield 2.89 g (78%). mp 143 °C. ¹H-NMR (400 MHz, CDCl₃) δ : 0.81 (d, $J=6.4$ Hz, 3H, CH₃), 0.89 (d, $J=6.2$ Hz, 3H, CH₃), 1.1–1.12 (m, 2H, CH), 1.31–1.33 (m, 1H, CH), 1.71 (s, 3H, CH₃), 1.76–1.82 (m, 1H, CH), 2.76–2.84 (m, 2H, CH₂), 2.92 (s, 1H, CH), 3.17 (s, 1H, CH), 4.92 (s, 1H, NH), 5.63 (d, $J=5.4$ Hz, 1H, CH), 7.14–7.37 (m, 5H, C_{arom}), 8.66 (s, 1H, NH). C₂₁H₂₅O₃N₃ (367.44): Calcd C 68.64, H 6.86, N 11.44; Found C 68.68, H 7.65, N 11.53.

(1*R*,2*S*,6*R*,7*R*,10*R*) 1-(10-Isopropyl-8-methyl-3,5-dioxo-4-aza-tricyclo[5.2.2.0^{2,6}]undec-8-en-4-yl)-3-(4-methoxy-phenyl)-thiourea (h2**)** Yield 3.34 g (81%). mp 186 °C. ¹H-NMR (400 MHz, CDCl₃) δ : 0.81 (d, $J=4$ Hz, 3H, CH₃), 0.90 (d, $J=4$ Hz, 3H, CH₃), 1.07–1.08 (m, 2H, CH), 1.34–1.45 (m, 1H, CH), 1.71 (s, 3H, CH₃), 1.78–1.83 (m, 1H, CH), 2.85–2.95 (m, 3H, CH, CH₂), 3.19 (s, 1H, CH), 3.78 (s, 3H, O–CH₃), 5.67 (s, 1H, CH), 6.87–6.89 (m, 2H, C_{arom}), 7.28–7.3 (m, 2H, C_{arom}), 7.94 (s, 1H, NH), 8.32 (s, 1H, NH). C₂₂H₂₇O₃N₃S (413.53): Calcd C 63.90, H 6.58, N 10.16; Found C 63.82, H 6.30, N 10.23.

(1*R*,2*S*,6*R*,7*R*,10*R*) 1-(10-Isopropyl-8-methyl-3,5-dioxo-4-aza-tricyclo[5.2.2.0^{2,6}]undec-8-en-4-yl)-3-(4-methoxy-phenyl)-urea (h2a**)** Yield 3.21 g (81%). mp 108 °C. ¹H-NMR (400 MHz, CDCl₃) δ : 0.8 (d, $J=4.4$ Hz, 3H, CH₃), 0.91 (d, $J=8$ Hz, 3H, CH₃), 1.04–1.05 (m, 2H, CH), 1.34–1.45 (m, 1H, CH), 1.72 (s, 3H, CH₃), 1.79–1.84 (m, 1H, CH), 2.77–2.95 (m,

3H, CH, CH₂), 3.2 (s, 1H, CH), 3.79 (s, 3H, O-CH₃), 5.7 (d, *J*=5.2 Hz, 1H, CH), 6.81–6.88 (m, 3H, NH, C_{arom}), 7.22–7.24 (m, 2H, C_{arom}). C₂₂H₂₇O₄N₃ (397.46): Calcd C 66.48, H 6.85, N 10.57; Found C 66.40, H 6.86, N 10.44.

(1R,2S,6R,7R,10R) 1-Cyclohexyl-3-(10-isopropyl-8-methyl-3,5-dioxo-4-aza-tricyclo[5.2.2.0^{2,6}]undec-8-en-4-yl)-thiourea (h3) Yield 2.8 g (72%). mp 201 °C. ¹H-NMR (400 MHz, CDCl₃) δ: 0.81 (d, *J*=6 Hz, 3H, CH₃), 0.92 (d, *J*=7.2 Hz, 3H, CH₃), 1.07–1.15 (m, 6H, CH, CH_{cyclohexyl}), 1.35–1.38 (m, 1H, CH), 1.62–1.86 (m, 4H, C_{cyclohexyl}), 1.78 (s, 3H, CH₃), 1.98–12.05 (m, 1H, CH), 2.9–3.01 (m, 3H, CH, CH₂), 3.2 (s, 1H, CH), 4.12 (s, 1H, NH), 5.72 (d, *J*=4 Hz, 1H, CH), 6.22 (s, 1H, NH). C₂₁H₃₁O₂N₃S (389.55): Calcd C 64.75, H 7.02, N 10.79; Found C 65.02, H 7.07, N 10.95.

(1R,2S,6R,7R,10R) 1-Cyclohexyl-3-(10-isopropyl-8-methyl-3,5-dioxo-4-aza-tricyclo[5.2.2.0^{2,6}]undec-8-en-4-yl)-urea (h3a) Yield 2.46 g (66%). mp 107 °C. ¹H-NMR (400 MHz, CDCl₃) δ: 0.83 (d, *J*=6.8 Hz, 3H, CH₃), 0.93 (d, *J*=8 Hz, 3H, CH₃), 1.08–1.14 (m, 6H, CH, CH_{cyclohexyl}), 1.26–1.34 (m, 5H, CH, CH_{cyclohexyl}), 1.84 (s, 3H, CH₃), 1.86–1.92 (m, 1H, CH), 2.95 (m, 1H, CH), 3.02 (s, 2H, CH₂), 3.23 (s, 1H, CH), 4.93 (s, 1H, NH), 5.77 (d, *J*=4 Hz, 1H, CH), 8.66 (s, 1H, NH). C₂₁H₃₁O₃N₃ (373.48): Calcd C 67.53, H 8.37, N 11.25; Found C 67.94, H 8.39, N 10.88.

(1S,2R,6S,7S) 1-(1-Isopropyl-7-methyl-3,5-dioxo-4-aza-tricyclo[5.2.2.0^{2,6}]undec-8-en-4-yl)-3-phenyl-thiourea (d1) Yield 2.87 g (75%). mp 108 °C. ¹H-NMR (400 MHz, CDCl₃) δ: 0.98 (d, *J*=6.8 Hz, 3H, CH₃), 1.12 (d, *J*=6.4 Hz, 3H, CH₃), 1.26–1.37 (m, 2H, CH₂), 1.49 (s, 3H, CH₃), 1.51–1.57 (m, 2H, CH₂), 2.53–2.58 (m, 1H, CH), 2.76 (d, *J*=8 Hz, 1H, CH), 3.12 (d, *J*=8.4 Hz, 1H, CH), 5.95 (d, *J*=8.4 Hz, 1H, CH=), 6.03 (d, *J*=8.4 Hz, 1H, CH=), 7.28–7.43 (m, 5H, CH_{arom}), 7.53 (s, 1H, NH), 8.17 (s, 1H, NH). C₂₁H₂₅O₃N₃S (383.50): Calcd C 65.77, H 6.57, N 10.96; Found C 65.93, H 6.55, N 10.97.

(1S,2R,6S,7S) 1-(1-Isopropyl-7-methyl-3,5-dioxo-4-aza-tricyclo[5.2.2.0^{2,6}]undec-8-en-4-yl)-3-phenyl-urea (d1a) Yield 2.71 g (74%). mp 171 °C. ¹H-NMR (400 MHz, CDCl₃) δ: 1.03 (d, *J*=6.8 Hz, 3H, CH₃), 1.14 (d, *J*=6.8 Hz, 3H, CH₃), 1.36–1.46 (m, 2H, CH₂), 1.54 (s, 3H, CH₃), 1.56–1.69 (m, 2H, CH₂), 2.52–2.59 (m, 1H, CH), 2.89 (d, *J*=8 Hz, 1H, CH), 3.28 (d, *J*=8.4 Hz, 1H, CH), 6.21 (d, *J*=8.4 Hz, 1H, CH=), 6.29 (d, *J*=8.4 Hz, 1H, CH=), 7.05–7.47 (m, 7H, CH_{arom}, NH), C₂₁H₂₅O₃N₃ (367.44): Calcd C 68.64, H 6.86, N 11.44; Found C 68.50, H 6.71, N 11.37.

(1S,2R,6S,7S) 1-(1-Isopropyl-7-methyl-3,5-dioxo-4-aza-tricyclo[5.2.2.0^{2,6}]undec-8-en-4-yl)-3-(4-methoxy-phenyl)-thiourea (d2) Yield 2.56 g (62%). mp 126 °C. ¹H-NMR (400 MHz, CDCl₃) δ: 0.98 (d, *J*=3.6 Hz, 3H, CH₃), 1.11 (d, *J*=3.6 Hz, 3H, CH₃), 1.27–1.40 (m, 2H, CH₂), 1.49 (s, 3H, CH₃), 1.58–1.53 (m, 2H, CH₂), 2.55–2.57 (m, 1H, CH), 2.75 (d, *J*=6 Hz, 1H, CH), 3.11 (d, *J*=6 Hz, 1H, CH), 3.81 (s, 3H, O-CH₃), 5.93 (d, 1H, CH=), 5.99 (d, 1H, CH=), 6.91–6.93 (m, 2H, CH_{arom}), 7.30–7.36 (m, 3H, CH_{arom}, NH), 8.0 (s, 1H, NH). C₂₂H₂₇O₃N₃S (413.53): Calcd C 63.9, H 6.58, N 10.16; Found C 64.26, H 6.64, N 10.14.

(1S,2R,6S,7S) 1-(1-Isopropyl-7-methyl-3,5-dioxo-4-aza-tricyclo[5.2.2.0^{2,6}]undec-8-en-4-yl)-3-(4-methoxy-phenyl)-urea (d2a) Yield 3.13 g. mp 145 °C. ¹H-NMR (400 MHz, CDCl₃) δ: 1.02 (d, *J*=6.8 Hz, 3H, CH₃), 1.14 (d, *J*=6.8 Hz, 3H, CH₃), 1.34–1.45 (m, 2H, CH₂), 1.53 (s, 3H, CH₃), 1.59–1.65 (m, 2H, CH₂), 2.52–2.57 (m, 1H, CH), 2.87 (d, *J*=8.4 Hz, 1H, CH), 3.27 (d, *J*=8.8 Hz, 1H, CH), 3.81 (s, 3H, O-CH₃), 5.95 (d, *J*=8.4 Hz, 1H, CH=), 6.25 (d, *J*=8.4 Hz, 1H, CH=), 6.81–6.92 (m, 3H, CH_{arom}, NH), 7.20–7.37 (m, 2H, CH_{arom}), 10.65 (s, 1H, NH). C₂₂H₂₇O₄N₃ (397.46): Calcd C 66.48, H 6.85, N 10.57; Found C 66.76, H 6.58, N 10.47.

(1S,2R,6S,7S) 1-Cyclohexyl-3-(1-isopropyl-7-methyl-3,5-dioxo-4-aza-tricyclo[5.2.2.0^{2,6}]undec-8-en-4-yl)-thiourea (d3) Yield 2.56 g (66%). mp 112 °C. ¹H-NMR (200 MHz, DMSO) δ: 1.01 (d, *J*=6.8 Hz, 3H, CH₃), 1.11 (d, *J*=6.4 Hz, 3H, CH₃), 1.15–1.43 (m, 10H, CH₂cyclohexyl), 1.49 (s, 3H, CH₃), 1.52–1.57 (m, 2H, CH₂), 1.7–1.74 (m, 2H, CH_{cyclohexyl}), 2.50–2.55 (m, 1H, CH), 2.71 (d, *J*=8.4 Hz, 1H, CH), 3.08 (d, *J*=4 Hz, 1H, CH), 4.0 (s, 1H, NH), 5.98 (d, *J*=8 Hz, 1H, CH=), 6.08 (d, *J*=8 Hz, 1H, CH=), 7.32 (s, 1H, NH). C₂₁H₃₁O₂N₃S (389.55): Calcd C 67.75, H 8.02, N 10.79; Found C 67.69, H 7.80, N 11.24.

(1S,2R,6S,7S) 1-Cyclohexyl-3-(1-isopropyl-7-methyl-3,5-dioxo-4-aza-tricyclo[5.2.2.0^{2,6}]undec-8-en-4-yl)-urea (d3a) Yield 2.31 g (62%). mp 203 °C. ¹H-NMR (400 MHz, CDCl₃) δ: 0.98 (d, *J*=6.8 Hz, 3H, CH₃), 1.10 (d, *J*=6.4 Hz, 3H, CH₃), 1.06–1.34 (m, 4H, CH₂ CH_{cyclohexyl}), 1.28–1.38 (m, 6H, CH₂ CH_{cyclohexyl}), 1.35 (s, 3H, CH₃), 1.59–1.7 (m, 2H, CH₂), 1.91–1.94 (m, 2H, C_{cyclohexyl}), 2.52–2.59 (m, 1H, CH), 2.67 (d, *J*=8 Hz, 1H, CH), 3.05 (d, *J*=8 Hz, 1H, CH), 4.80 (s, 1H, NH), 5.97 (d, *J*=8.4 Hz, 1H, CH=), 6.05 (d, *J*=8.4 Hz, 1H, CH=), 6.35 (s, 1H, NH). C₂₁H₃₁O₃N₃ (373.48): Calcd C 67.53, H 8.37, N 11.25; Found C 67.67, H 8.32, N 11.22.

(1S,2R,6S,7R) 1-(1,7,8,9,10-Pentamethyl-3,5-dioxo-4-aza-tricyclo-

[5.2.1.0^{2,6}]dec-8-en-4-yl)-3-phenyl-thiourea (k1) Yield 3.18 g (83%). mp 191 °C. ¹H-NMR (400 MHz, CDCl₃) δ: 0.62 (d, *J*=8 Hz, 3H, CH₃), 1.35 (s, 6H, CH₃), 1.48 (s, 6H, CH₃), 1.61 (q, 1H, CH), 3.0 (s, 2H, CH), 7.27–7.38 (m, 5H, CH_{arom}), 7.71 (s, 1H, NH), 8.10 (s, 1H, NH). C₂₁H₂₅O₂N₃S (383.50): Calcd C 65.77, H 6.57, N 10.96; Found C 65.74, H 6.5, N 10.84.

(1S,2R,6S,7R) 1-(1,7,8,9,10-Pentamethyl-3,5-dioxo-4-aza-tricyclo[5.2.1.0^{2,6}]dec-8-en-4-yl)-3-phenyl-urea (k1a) Yield 3.9 g (82%). mp 240 °C. ¹H-NMR (400 MHz, CDCl₃) δ: 0.62 (d, *J*=6.4 Hz, 3H, CH₃), 1.35 (s, 6H, CH₃), 1.55 (s, 6H, CH₃), 1.57–1.65 (m, 1H, CH), 3.07 (s, 2H, CH), 7.51–7.84 (m, 5H, CH_{arom}), 9.25 (s, 1H, NH), 11.82 (s, 1H, NH). C₂₁H₂₅O₃N₃ (367.44): Calcd C 68.64, H 6.86, N 11.44; Found C 68.9, H 6.75, N 11.46.

(1S,2R,6S,7R) 1-(4-Methoxy-phenyl)-3-(1,7,8,9,10-pentamethyl-3,5-dioxo-4-aza-tricyclo[5.2.1.0^{2,6}]dec-8-en-4-yl)-thiourea (k2) Yield 2.84 g (69%). mp 127 °C. ¹H-NMR (400 MHz, CDCl₃) δ: 0.61 (d, *J*=12 Hz, 3H, CH₃), 1.34 (s, 6H, CH₃), 1.53 (s, 6H, CH₃), 1.6 (q, 1H, CH), 3.03 (s, 2H, CH), 3.79 (s, 3H, O-CH₃), 6.89 (d, *J*=12 Hz, 2H, CH_{arom}), 7.27 (d, *J*=12 Hz, 2H, CH_{arom}), 7.9 (s, 1H, NH), 8.12 (s, 1H, NH). C₂₂H₂₇O₃N₃S (413.53): Calcd C 62.9, H 6.58, N 10.16; Found C 62.78, H 6.29, N 10.07.

(1S,2R,6S,7R) 1-(4-Methoxy-phenyl)-3-(1,7,8,9,10-pentamethyl-3,5-dioxo-4-aza-tricyclo[5.2.1.0^{2,6}]dec-8-en-4-yl)-urea (k2a) Yield 3.13 g (79%). mp 205 °C. ¹H-NMR (400 MHz, CDCl₃) δ: 0.62 (d, *J*=8 Hz, 3H, CH₃), 1.33 (s, 6H, CH₃), 1.54 (s, 6H, CH₃), 1.65 (s, 1H, CH), 2.95 (s, 2H, CH), 3.74 (s, 3H, O-CH₃), 4.81 (s, 1H, NH), 6.75 (d, 2H, CH_{arom}), 7.17 (s, 1H, NH), 7.17–7.23 (m, 2H, CH_{arom}). C₂₂H₂₇O₄N₃ (397.46): Calcd C 66.48, H 6.85, N 10.57; Found C 66.52, H 6.47, N 10.47.

(1S,2R,6S,7R) 1-Cyclohexyl-3-(1,7,8,9,10-pentamethyl-3,5-dioxo-4-aza-tricyclo[5.2.1.0^{2,6}]dec-8-en-4-yl)-thiourea (k3) Yield 3.07 g (72%). mp 127 °C. ¹H-NMR (400 MHz, CDCl₃) δ: 0.61 (d, *J*=8 Hz, 3H, CH₃), 1.15–1.27 (m, 6H, CH_{cyclohexyl}), 1.37 (s, 6H, CH₃), 1.53 (s, 6H, CH₃), 1.58 (q, 1H, CH), 1.70–1.73 (m, 2H, CH_{cyclohexyl}), 2.03–12.06 (m, 2H, CH_{cyclohexyl}), 3.02 (s, 2H, CH), 4.13 (s, 1H, NH), 6.13 (s, 1H, NH). C₂₁H₃₁O₂N₃S (389.55): Calcd C 64.75, H 8.02, N 10.79; Found C 64.96; H 8.02, N 10.72.

(1S,2R,6S,7R) 1-Cyclohexyl-3-(1,7,8,9,10-pentamethyl-3,5-dioxo-4-aza-tricyclo[5.2.1.0^{2,6}]dec-8-en-4-yl)-urea (k3a) Yield 2.72 g (73%). mp 256 °C. ¹H-NMR (400 MHz, CDCl₃) δ: 0.62 (d, *J*=8 Hz, 3H, CH₃), 1.09–1.14 (m, 6H, CH_{cyclohexyl}), 1.33 (s, 6H, CH₃), 1.51 (s, 6H, CH₃), 1.60 (q, 1H, CH), 1.67–1.7 (m, 2H, CH_{cyclohexyl}), 1.90–1.93 (m, 2H, CH_{cyclohexyl}), 2.96 (s, 2H, CH), 4.77 (s, 1H, NH), 6.38 (s, 1H, NH). C₂₁H₃₁O₃N₃ (373.48): Calcd C 67.53, H 8.37, N 11.25; Found C 67.62, H 8.17, N 11.23.

Pharmacology The experiments were performed on male Albino Swiss mice (18–30 g). 8–10 animals were kept in a cage, at room temp. of 20±1 °C, on a 12:12 h dark–light cycle. Standard food (Bacutil, Motycz) and water were available *ad libitum*. The investigated substance, marked as **k1a**, was administered i.p. in the volume of 10 ml/kg⁻¹ as suspensions in aqueous solution of 0.5% methylcellulose (tylose). The compounds were injected 60 min before the test. Controls received the equivalent volume of the solvent.

All performed tests, suggested by Vogel and Vogel²⁹) are generally accepted as basic in investigation of the central activity by behavioral methods.

The acute toxicity of the compound was assessed in mice according to Litchfield and Wilcoxon method,³⁰) as the LD₅₀ calculated from mortality within 48 h. In addition, the activity of compounds was assessed in the following test:

- Locomotor activity was measured in photoresistor actometers for single mice for 30 min as a) spontaneous activity and b) amphetamine-induced hyperactivity: mice received subcutaneously (s.c.) 5 mg/kg of amphetamine 30 min before the test.

- Nociceptive reactions were studied in 0.6% acetic acid-induced writhing test.³¹) The number of writhing episodes was measured for 10 min starting 5 min after i.p. administration of acid solution.

- Motor coordination was evaluated in rota rod test³²) and chimney test.³³)

- Body temperature in normothermic mice was measured in the rectum by thermistor thermometer.

- Pentylene-tetrazole (110 mg/kg, s.c.)-induced convulsions were evaluated as the number of mice with clonic seizures, tonic convulsions and dead animals.

- “Head twitch” responses after 5-hydroxytryptophan (5-HTP), according to Corne *et al.*³⁴) Mice received 5-HTP (180 mg/kg, i.p.) and the number of head twitches was recorded in 6 two-minute intervals (4–6, 14–16, 24–26, 34–36, 44–46, 54–56 min).

The compound was injected in doses equivalent to 0.1 LD₅₀ (200 mg/kg).

Statistics Obtained data were calculated by Student's *t*-test and χ^2 test

with Yates correction (pentylentetrazole-induced seizures).

Antiviral Assay Procedures The antiviral investigations of the compounds were performed in Dipartimento di Scienze e Tecnologie Biomediche, Università di Cagliari, Monserrato, Italy.

Compounds Compounds were solubilized 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 µg/ml streptomycin. Cell cultures were checked periodically for the absence of mycoplasma contamination with a Myco Tect Kit (Gibco). Human immunodeficiency viruses HIV-1, III_B strain were obtained from supernatants of persistently infected H9/III_B cells. HIV-1 stock solutions had titers of 4.5×10^6 and 1.4×10^5 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 virus-induced cytopathogenicity in MT-4 cells. Briefly, 50 µl of culture medium containing 1×10^4 cells was added to each well of flat-bottom microtiter plates containing 50 µl of culture medium with or without various concentrations of compounds. Then 20 µl of an HIV suspensions (containing the appropriate amount of CCID₅₀ to cause complete cytopathogenicity at day 4) was added. After incubation at 37 °C, the number of viable cells was determined by the 3-(4,5-dimethylthiazol-1-yl)-2,5-diphenyltetrazolium bromide (MTT) method.³⁵ 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.

Microbiology Microorganisms used in this study were as follows: *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus* ATCC 29213, *Staphylococcus aureus* ATCC 6538P, *Staphylococcus aureus* NCTC 4163, *Escherichia coli* ATCC 25922, *Escherichia coli* ATCC 10538, *Enterococcus hirae* ATCC 10541, *Pseudomonas aeruginosa* ATCC 15442, *Pseudomonas aeruginosa* NCTC 6749, *Bordetella bronchiseptica* ATCC 4617, *Candida albicans* ATCC 10231. 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 Antibacterial activity was examined by the disc-diffusion method under standard conditions using Mueller-Hinton II agar medium (Becton Dickinson) according to the guidelines established by the NCCLS (CLSI).³⁶ Antifungal activities were assessed using YNB-agar medium.

For the disc-diffusion assay, the solution of tested agents was prepared in ethanol or dimethylsulfoxide. Sterile filter paper discs (9 mm diameter, Whatman No 3 chromatography paper) were 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 bacterial.

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