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Original article

Synthesis, pharmacological and antiviral activity of 1,3-thiazepine derivatives

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ABSTRACT

The preparation of new fourteen thiourea and fourteen product of their condensation with 1,4-dibromobutane, viz. 1,3-thiazepine derivatives, of 10-isopropyl-8-methyl-4-aza-tricyclo[5.2.2.0^{2,6}]undec-8-ene-3,5-dione and 1-isopropyl-7-methyl-4-aza-tricyclo[5.2.2.0^{2,6}]undec-8-ene-3,5-dione is described. Elemental analysis, MS and ^{1}H NMR spectra confirmed the identity of the products. The molecular structure of linear disubstituted thiourea derivative and its cyclization product was determined by an X-ray crystal structure analysis. Two of new obtained compounds (**6b′** and **7a′**) were tested for their pharmacological activity on animal central nervous system (CNS) in behavioral animal tests. With relatively low acute toxicity (LD_{50} lower than 2000 mg kg^{-1} i.p.) they exhibited significant influence on spontaneous locomotor activity and body temperature. Additionally, compounds reduced number of the "head twitch" episodes after 5-hydroksytryptophan (5-HTP) administration.

New compounds were evaluated *in vitro* against representatives of different virus classes, such as a HIV-1 (Retrovirus), a HBV (Hepadnavirus) and the single-stranded RNA^{+} viruses Yellow fever virus (YFV) and Bovine viral diarrhea virus (BVDV), both belonging to Flaviridae. Three of new obtained compounds showed a modest activity against HIV-1 wt_{IIIB}, BVDV and YFV.

1. Introduction

Thiourea and urea derivatives show a broad spectrum of biological activities such as antibacterial, antiviral, anticancer, anticonvulsion, analgesic and HDL-elevating properties [1–12].

Numerous compounds containing thiourea group are selective ligands for 5-HT family receptors, including 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} [13–17].

The drug-elicited "head twitch" response (HTR) [18,19] is a selective behavioral model for 5-HT_2 agonist activity in the rodent, and several previous studies have established that direct and indirect 5-HT agonists induce this effect [20–27]. Additionally, 5-HT_2 receptor antagonists selectively block HTR [27–29], and their potency is highly correlated with the antagonist's affinity for 5-HT_2 receptors [20,30].

Furthermore, structural studies of active thiourea derivatives have shown that these compounds contain a central hydrophilic part and two hydrophobic moieties forming a butterfly-like conformation [31]. This conformation is a part of structure of NNRTIs (nucleoside reverse transcriptase inhibitors); anti-HIV agents (Scheme 1) [32]. The main target in contemporary drug discovery efforts against HIV-1 is RT (reverse transcriptase), a vital enzyme that is responsible for the reverse transcription of retroviral RNA to proviral DNA [33]. Non-nucleoside reverse transcriptase inhibitors (NNRTI's) inhibit HIV RT by altering either the conformation or mobility of RT by binding to a specific allosteric site near the polymerase site, thereby resulting in non-competitive inhibition of the enzyme [34,35].

Derivatives of 1,3-thiazepine (seven-membered cyclic thiourea derivatives) are important compounds, because of their biological activity as nitric oxide synthase inhibitors [36–39]. This is a rare system in heterocyclic chemistry.

1,3-Thiazepine ring is present in Omipatrilat which is currently in the phase IV of clinical trials. By inhibiting the activity of the angiotensin converting enzyme (ACE), which causes blood vessels to constrict, Omapatrilat lowers blood pressure. Another advantage of this drug is inhibition of enzyme known as neutral endopeptidase (NEP), which causes blood vessels to relax [40,41].

Here we report the synthesis of compounds which are composed of thiourea system and the products of their condensation, viz. 1,3-thiazepine system attached to polycyclic imide.

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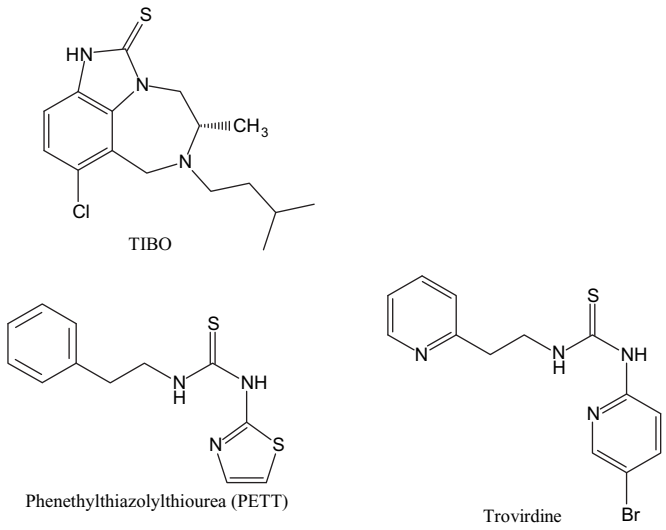


Scheme 1. Structures representative of NNRTIs.

Method of synthesis of 1,3-thiazepine ring connected to Diels–Alder product had been reported for authors [42].

In a previous study [43,44], we synthesized a series of urea and thiourea derivatives of tricyclic imide. Reported urea and thiourea derivatives exhibited activity on animal central nervous system (CNS) and characterized by low toxicity. This study is a continuation of our research.

2. Results and discussion

2.1. Chemistry

The preparation of new fourteen thiourea and fourteen product of their condensation with 1,4-dibromobutane, viz. 1,3-thiazepine derivatives, of 10-isopropyl-8-methyl-4-aza-tricyclo[5.2.2.$0^{2,6}$] undec-8-ene-3,5-dione and 1-isopropyl-7-methyl-4-aza-tricyclo[5.2.2.$0^{2,6}$]undec-8-ene-3,5-dione (Scheme 2) is described.

Anhydrides obtained in Diels–Alder reaction were used as starting materials. Anhydride (**a**) was obtained in the reaction of enantiomeric (*R*)-(−)-α-phellandrene with furan-2,5-dione [45]. The reaction of α-terpinene with furan-2,5-dione gave compound **b** [46].

Obtained tricyclic anhydrides were subjected to the reaction of hydrazine (80% aqueous solution), described previously [43,47].

In order to obtain corresponding thiourea derivatives of above compounds they were subjected to the reaction with appropriate isothiocyanates. Products were transformed into 1,3-thiazepine derivatives by condensation with 1,4-dibromobutane. The general synthesis pathway is given in Scheme 2.

Obtained compounds were purified by flash chromatography. Elemental analysis, MS, ^{1}H NMR and ^{13}C NMR spectra confirmed the identity of the products. The molecular structure of linear disubstituted thiourea derivative **7a** and its cyclization product **7a′** was determined by an X-ray crystal structure analysis (Figs. 1 and 2).

The crystal structure of **7a** is formed by of two crystallographically independent molecules, **A** and **B**, adopting different conformations of the thiourea fragment; the orientation of the N–H bonds is *trans* and *cis* in the molecules **A** and **B**, respectively (Fig. 1). These conformers show substantially different bond distances (Table 1). The disubstituted 1,3-thiazepine ring in **7a′** adopts slightly distorted chair conformation (Fig. 2).

2.2. Pharmacology

Two of the above compounds, **6b′** and **7a′**, were tested for their pharmacological activity.

They exhibited weak acute toxicity lower than 2000 mg kg^{-1} i.p. The progressive doses, calculated as a part of LD_{50} for compounds tested, were used in behavioral experiments.

The significant depressive action on spontaneous locomotor activity in mice was observed after administration of both investigated compounds at a dose of 0.1 LD_{50} ($p < 0.05$), but not at 0.05 LD_{50} (Fig. 3). Amphetamine hyperactivity was not changed by either of the compounds (Fig. 4).

These compounds did not produce any protection in clonic seizures and tonic convulsions evoked by pentetrazole and in abdominal constriction induced by i.p. administration of the acetic acid solution (Fig. 5).

The investigated substances did not affect motor coordination (rota rod and chimney tests) (Figs. 6 and 7).

Body temperature of normothermic mice was decreased significantly (within 30–90 min, **7a′**; 60–120 min, **6b′**) by both compounds (Fig. 8). 5-HT has been reported to play an important role in central regulation of body temperature [48]. The MAO (monoamine oxidase) type A inhibitors appear to be crucially involved in hypothermia [49]. As a result of MAO-inhibition, 5-HT levels in the body are increased and may precipitate a serotonin syndrome. Hypothermia in rodents has been reported for MAO type A enzyme inhibitors (antidepressant drugs), such as clorgyline [50] and harman (1-methyl-β-carboline) [51].

The "head twitch" responses after 5-hydroxtryptophan (5-HTP) administration were significantly decreased by **6b′** and markedly, but not significantly, by compound **7a′** (Fig. 9). The result seems to point out some connection with the 5-HT system. Since it appears that headshakes induced by 5-HTP are mediated by 5-HT_2 receptors, these data suggest that **6b′** and **7a′** may interact with 5-HT_2 receptors in the brain.

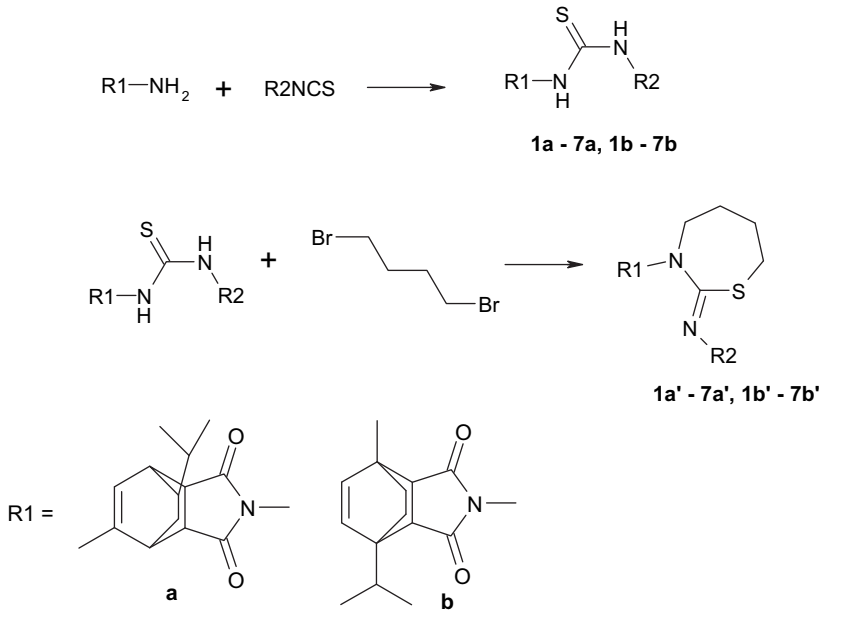
Based on our previous research on central nervous system (CNS) for urea [43] and thiourea [44] derivatives of tricyclic imide and described in this paper for cyclic derivatives of thiourea (1,3-thiazepine system), we could conclude that this group of compounds possesses significant influence on CNS of laboratory animals.

Comparison of urea and thiourea derivatives of tricyclic imide shows that urea derivatives have influence on "head twitch response" only. However thiourea and 1,3-thiazepine derivatives were active in "head twitch" test similarly to urea derivatives and have influence on spontaneous motor activity and decrease body temperature of normothermic mice.

Based on performed research we could conclude that urea derivatives of polycyclic imides were less active than thiourea and cyclic thiourea (1,3-thiazepine system). Comparison of the activity on CNS of thiourea and 1,3-thiazepine derivatives could lead to the conclusion that there are no differences between free thiourea system and the system inbuilt in 1,3-thiazepine ring.

2.3. Cytotoxicity and antiviral activity

Title compounds were evaluated in cell-based assays for cytotoxicity and antiviral activity against viruses representative of two of the three genera of the Flaviviridae family, i.e. Flaviviruses (Yellow Fever Virus, YFV) and Pestiviruses (Bovine Viral Diarrhoea Virus, BVDV), as Hepaciviruses can hardly be used in routine cell-based assays. Title compounds were also tested against representatives of other virus families. Among ssRNA^{+} were a retrovirus (Human Immunodeficiency Virus Type 1, HIV-1) and two Picornaviruses (Coxsackie Virus Type B2, CVB-2 and Poliovirus Type-1, Sabin strain, Sb-1); among ssRNA^{-} were a Paramixoviridae



Scheme 2. Synthesis of studied compounds.

(Respiratory Syncytial Virus, RSV) and a Rhabdoviridae (Vesicular Stomatitis Virus, VSV) representative. Among double-stranded RNA (dsRNA) viruses was a Reoviridae representative (Reo-1). Two representatives of DNA virus families were also included: Herpes Symplex Type-1, HSV-1 (Herpesviridae) and Vaccinia Virus, VV (Poxviridae).

AZT (3′-azido-thymidine), NM 108 (2′-β-methyl-guanosine), NM 176 (2′-ethynyl-D-citidine), NM 299 (6-azauridine), M 5255 (Mycophenolic Acid) and ACG (acycloGuanosine) were used as reference inhibitors of $ssRNA^+$, $ssRNA^-$ and DNA viruses, respectively.

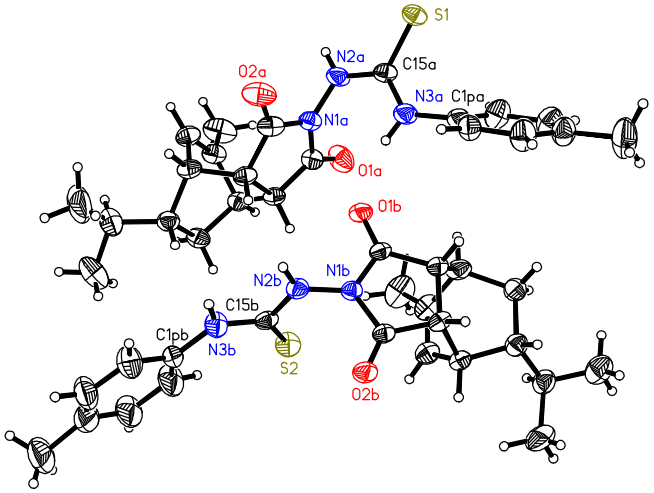


Fig. 1. Perspective view of two symmetry independent molecules **7a**.

Unfortunately, none of the compounds showed any activity against any viruses tested; with the exception of compounds **2a**, **1b** and **2b** (Tables 2–4), that showed a modest activity against HIV-1 wt_{IIIB}, BVDV and YFV ($EC_{50} = 30$, 52 and 41 μM, respectively).

3. Experimental protocol

3.1. Chemistry

Melting points were determined in a Kofler's apparatus and are uncorrected. The NMR spectra were recorded on a Bruker AVANCE DMX400 spectrometer, operating at 300 MHz (^{1}H NMR) and 75 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 CHN model 2400 Perkin–Elmer. Mass spectral ESI

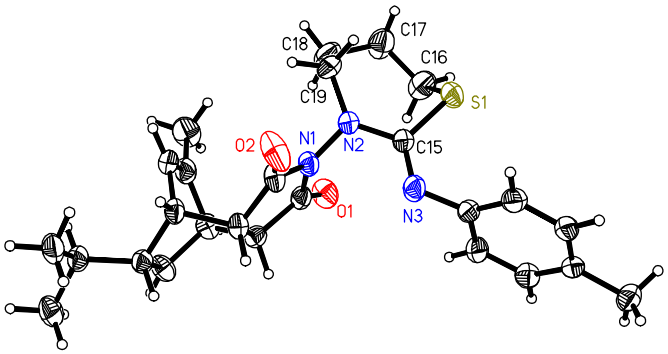


Fig. 2. Perspective view of molecule **7a**′. Bond distances of 1,3-thiazepine ring are: S1–C15 1.768(4), S1–C16 1.828(7), C16–C17 1.503(9), C17–C18 1.528(9), C18–C19 1.509(9), C19–N2 1.457(6), N2–C15 1.388(6) Å.

Table 1
Bond lengths (Å) within the thiourea fragment of two conformers of **7a**.

	S=C15	C15–N3	C15–N2	N2–N1	N3–C1 p
mol. **A** – *trans*	1.689(5)	1.321(7)	1.346(7)	1.395(6)	1.423(7)
mol. **B** – *cis*	1.631(6)	1.362(7)	1.373(6)	1.364(6)	1.422(7)

measurements were carried out on Waters ZQ Micro-mass instruments with quadrupol 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.

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 F_{254} (Merck) plates (0.25 mm thickness).

X-ray crystallography: intensity measurements were carried out at 295 K with a KM4 diffractometer, using graphite monochromated CuKα radiation ($\lambda = 1.54178$ Å) and $\omega/2\theta$ scan mode. Structures were solved by the SHELXS-97 program and refined by full-matrix least squares on F^2 using the SHELXL-97 program [52]. Non-hydrogen atoms were refined anisotropically. Hydrogen atoms were positioned geometrically and C–H bond 'riding' model was used in the refinement. The experimental details and final atomic parameters for **7a** and **7a′** have been deposited with the Cambridge Crystallographic Data Centre as supplementary material; No CCDC 721055 and CCDC 721056, respectively. Copies of the data can be obtained free of charge on request to The Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (www: http://www.ccdc.cam.ac.uk).

3.2. Thiourea derivatives of 10-isopropyl-8-methyl-4-azatricyclo[5.2.2.$0^{2,6}$]undec-8-ene-3,5-dione (**1a**–**7a**) and 1-isopropyl-7-methyl-4-azatricyclo[5.2.2.$0^{2,6}$] undec-8-ene-3,5-dione (**1b**–**7b**)

General procedure: A solution of 4-amino-10-isopropyl-8-methyl-4-azatricyclo[5.2.2.$0^{2,6}$]undec-8-ene-3,5-dione or 4-amino-1-isopropyl-7-methyl-4-azatricyclo[5.2.2.$0^{2,6}$]undec-8-ene-3,5-dione (0.0025 mol, 0.62 g) in acetonitrile (10 mL) was treated with appropriate isothiocyanate (0.003 mol) and the mixture was refluxed for 6 h. Then solvent was removed on rotary evaporator. The residue was purified by column chromatography (chloroform:methanol; 9.5:0.5 vol.). The compound was crystallized from ethanol.

3.2.1. 1-Allyl-3-(10-isopropyl-8-methyl-3,5-dioxo-4-azatricyclo[5.2.2.$0^{2,6}$] undec-8-en-4-yl)-thiourea (**1a**)

Yield 67%. Mp. 165 °C. ^{1}H NMR ($CDCl_3$) δ (ppm): 0.73 (d, 3H, CH_3, $J = 8.8$ Hz); 0.90 (d, 3H, CH_3, $J = 8.8$ Hz); 1.04–1.15 (m, 2H, CH_2); 1.34–1.422 (m, 1H, CH); 1.75 (d, 3H, CH_3, $J = 2.4$ Hz); 1.8–1.88 (m, 1H, CH); 2.91–3.01 (m, 3H, CH, CH–C=O); 3.18–3.21 (n, 1H, CH–C=O); 4.1–4.16 (m, 1H, CH_2); 4.26–4.31 (m, 1H, CH_2); 5.17–5.26 (m, 2H, CH_2=); 5.69 (d, 1H, CH, $J = 8$ Hz); 5.78–5.91 (m, 1H, CH=); 6.33 (s, 1H, NH); 8.07 (s, 1H, NH). ^{13}C NMR ($CDCl_3$) δ: 20.61, 21.16, 21.24, 30.76, 33.43, 35.51, 37.98, 42.53, 44.56, 45.54, 49.09, 117.40, 132.78, 134.25, 135.64, 175.43, 175.80, 181.63. Anal. Calcd for $C_{18}H_{25}N_3O_2S$: C 62.22, H 7.25, N 12.09. Found: C 62.00, H 7.26, N 12.08. ESI MS: $m/z = 370.2$ $[M + Na]^+$ (100%).

3.2.2. 1-Benzyl-3-(10-isopropyl-8-methyl-3,5-dioxo-4-azatricyclo[5.2.2.$0^{2,6}$] undec-8-en-4-yl)-thiourea (**2a**)

Yield 67%. Mp. 165 °C. ^{1}H NMR ($CDCl_3$) δ (ppm): 0.78 (d, 3H, CH_3, $J = 8.8$ Hz); 0.86 (d, 3H, CH_3, $J = 8.8$ Hz); 0.96–1.04 (m, 2H, CH_2); 1.28–1.36 (m, 1H, CH); 1.46 (d, 3H, CH_3, $J = 1.2$ Hz); 1.69–1.82 (m, 1H, CH); 2.84–2.94 (m, 3H, CH, CH–C=O); 3.09–3.12 (m, 1H, CH–C=O); 4.61–4.89 (m, 1H, CH_2); 5.29 (d, 1H, CH, $J = 7.6$ Hz); 6.37 (s, 1H, NH); 7.3–7.37 (m, 5H, $CH_{arom,}$); 7.96 (s, 1H, NH). ^{13}C NMR ($CDCl_3$) δ: 20.62, 21.16, 21.28, 30.78, 33.42, 35.52, 37.96, 42, 53, 40.56, 45.50, 49.83, 128.42 (2C), 128.68 (2C), 129.12 (2C), 136.63, 137.37, 173.15, 173.72, 181.70. Anal. Calcd for $C_{18}H_{25}N_3O_2S$: C 62.22, H 7.25, N 12.09. Found: C 62.00, H 7.26, N 12.08. ESI MS: $m/z = 370.2$ $[M + Na]^+$ (100%).

3.2.3. 1-(4-Fluorophenyl)-3-(10-isopropyl-8-methyl-3,5-dioxo-4-aza-tricyclo [5.2.2.$0^{2,6}$]undec-8-en-4-yl)-thiourea (**3a**)

Yield 82%. Mp. 166.7 °C. ^{1}H NMR ($CDCl_3$) δ (ppm): 0.82 (d, 3H, CH_3, $J = 6.8$ Hz); 0.91 (d, 3H, CH_3, $J = 6.4$ Hz); 1.06–1.13 (m, 2H, CH_2); 1.34–1.37 (m, 1H, CH); 1,69 (s, 3H, CH_3); 1.71–1.85 (m, 1H, CH); 2.92–3.0 (m, 3H, CH, CH–C=O); 3.2 (d, 1H, CH–C=O, $J = 6$ Hz); 5.7 (d, 1H, CH=, $J = 5.6$ Hz); 7.0–7.08 (m, 2H, $CH_{arom,}$); 7.35–7.38 (m, 2H, $CH_{arom,}$); 8.15 (s, 1H, NH). ^{13}C NMR ($CDCl_3$) δ: 20.62, 21.16, 21.28, 30.78, 33.42, 35.52, 37.96, 40.56, 42.53, 45.50, 116.29, 116.59, 127.31 (2C), 132.93, 136.24, 137.02, 163.02, 174.06, 174.56, 181.89. Anal. Calcd for $C_{21}H_{24}FN_3O_2S$: C 62.82, H 6.03, N 10.47. Found: C 62.92, H 6.09, N 10.49. ESI MS: $m/z = 418.1$ $[M + H]^+$ (100%).

3.2.4. 1-(4-Chlorophenyl)-3-(10-isopropyl-8-methyl-3,5-dioxo-4-aza-tricyclo [5.2.2.$0^{2,6}$]undec-8-en-4-yl)-thiourea (**4a**)

Yield 82%. Mp. 179.5 °C. ^{1}H NMR ($CDCl_3$) δ (ppm): 0.82 (d, 3H, CH_3, $J = 6.4$ Hz); 0.91 (d, 3H, CH_3, $J = 6.4$ Hz); 1.06–1.13 (m, 2H, CH_2); 1.35–1.36 (m, 1H, CH); 1,69 (s, 3H, CH_3); 1.75–1.84 (m, 2H, CH, NH); 2.92–2.98 (m, 3H, CH, CH–C=O); 3.2 (d, 1H, CH–C=O, $J = 5.6$ Hz); 5.69 (d, 1H, CH=, $J = 5.2$ Hz); 7.29–7.36 (m, 4H, $CH_{arom,}$); 8.38 (s, 1H, NH). ^{13}C NMR ($CDCl_3$) δ: 20.61, 21.17, 21.22, 30.73, 33.42,

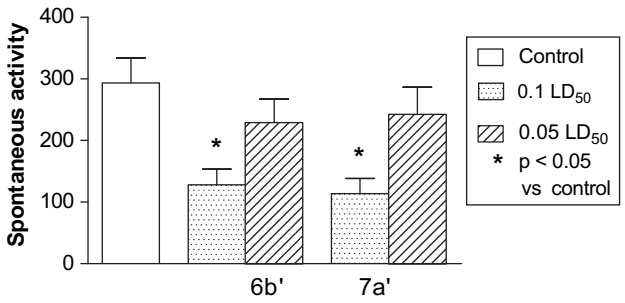


Fig. 3. The influence of **6b′**and **7a′** on the spontaneous motor activity.

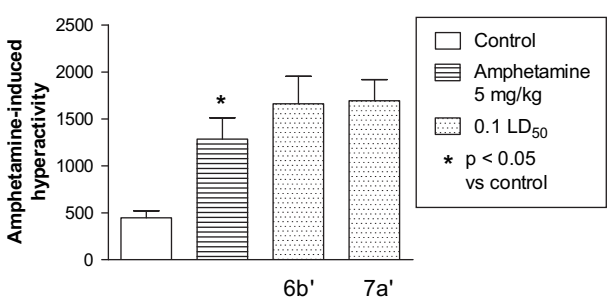


Fig. 4. The influence **6b′** and **7a′** on amphetamine-induced hyperactivity.

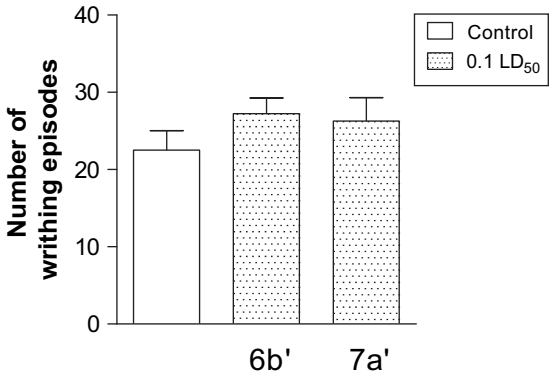


Fig. 5. The influence of **6b**′ and **7a**′ on nociceptive reactions studied in the acetic acid (0.6%) induced writhing test.

35.51, 37.98, 42.55, 44.55, 45.54, 122.50 (2C), 126.53 (2C), 129.66, 132.79, 135.64, 142.59, 175.45, 175.80, 181.62. Anal. Calcd for $C_{21}H_{24}ClN_3O_2S$: C 60.35, H 5.79, N 10.05. Found: C 60.32, H 5.92, N 10.09. ESI MS: $m/z = 424.1$ $[M + Na]^+$ (100%).

*3.2.5. 1-(4-Iodophenyl)-3-(10-isopropyl-8-methyl-3,5-dioxo-4-aza-tricyclo [5.2.2.0^{2,6}]undec-8-en-4-yl)-thiourea (**5a**)*

Yield 82%. Mp. 181 °C. ^{1}H NMR ($CDCl_3$) δ (ppm): 0.82 (d, 3H, CH_3, $J = 6.4$ Hz); 0.91 (d, 3H, CH_3, $J = 6.4$ Hz); 1.06–1.14 (m, 2H, CH_2); 1.35–1.36 (m, 1H, CH); 1,69 (s, 3H, CH_3); 1.72–1.85 (m, 2H, CH, NH); 2.92–2.98 (m, 3H, CH, CH–C═O); 3.2 (d, 1H, CH–C═O, $J = 5.6$ Hz); 5.69 (d, 1H, CH═, $J = 5.2$ Hz); 7.1 (d, 2H, CH_{arom}, $J = 8.4$ Hz); 7.6 (d, 2H, CH_{arom}, $J = 8.4$ Hz) 8.4 (s, 1H, NH). ^{13}C NMR ($CDCl_3$) δ: 20.64, 21.17, 21.18, 30.76, 33.38, 35.53, 37.93, 42.51, 44.53, 45.54, 91.08, 126.23 (2C), 135.76, 136.49, 137.22, 138.38 (2C), 174.14, 174.68, 181.22. Anal. Calcd for $C_{21}H_{24}IN_3O_2S$: C 49.51, H 4.75, N 8.25. Found: C 49.58, H 4.77, N 8.29. ESI MS: $m/z = 532.1$ $[M + Na]^+$ (100%).

*3.2.6. 1-(3,4-Dichlorophenyl)-3-(10-isopropyl-8-methyl-3,5-dioxo-4-aza-tricyclo [5.2.2.0^{2,6}]undec-8-en-4-yl)-thiourea (**6a**)*

Yield 82%. Mp. 154 °C. ^{1}H NMR ($CDCl_3$) δ (ppm): 0.83 (d, 3H, CH_3, $J = 6.4$ Hz); 0.91 (d, 3H, CH_3, $J = 6.4$ Hz); 1.07–1.14 (m, 2H, CH_2); 1.35–1.37 (m, 1H, CH); 1,7 (s, 3H, CH_3); 1.74–1.85 (m, 2H, CH, NH); 2.93–3.01 (m, 3H, CH, CH–C═O); 3.2 (d, 1H, CH–C═O, $J = 5.6$ Hz); 5.69 (d, 1H, CH═, $J = 5.6$ Hz); 7.24–7.36 (m, 3H, $CH_{arom,}$); 7.6 (s, 1H, $CH_{arom,}$) 8.56 (s, 1H, NH). ^{13}C NMR ($CDCl_3$) δ: 20.64, 21.17, 21.18, 30.76, 33.38, 35.53, 37.93, 42.51, 44.53, 45.54, 123.52, 125.88, 130.19, 130.69, 132.93, 136.28, 136.96, 137.04, 174.46, 175.00, 181.18. Anal. Calcd for $C_{21}H_{23}Cl_2N_3O_2S$: C 55.75, H 5.12, N 9.29. Found: C 55.80, H 5.21, N 9.31. ESI MS: $m/z = 473.7$ $[M + Na]^+$ (100%).

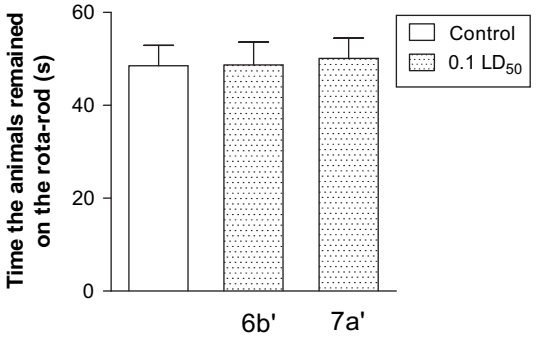


Fig. 6. The influence of **6b**′ and **7a**′ on motor coordination evaluated in rota-rod test.

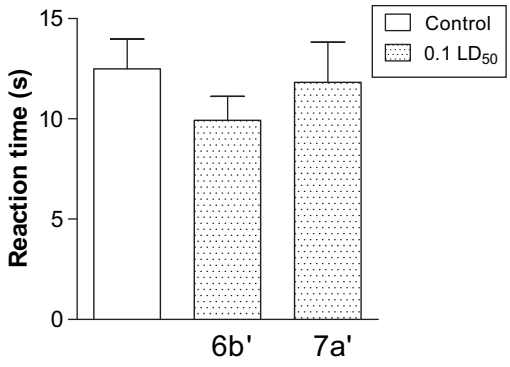


Fig. 7. The influence of **6b**′ and **7a**′ on motor coordination evaluated in chimney test.

*3.2.7. 1-(4-Methylphenyl)-3-(10-isopropyl-8-methyl-3,5-dioxo-4-azatricyclo [5.2.2.0^{2,6}]undec-8-en-4-yl)-thiourea (**7a**)*

Yield 82%. Mp. 195 °C. ^{1}H NMR 300 MHz ($CDCl_3$) δ (ppm): 0.76 (d, 3H, CH_3, $J = 6.3$ Hz); 0.84 (d, 3H, CH_3, $J = 6.3$ Hz); 0.98–1.07 (m, 2H, CH_2); 1.25–1.29 (m, 1H, CH); 1,58 (s, 3H, CH_3); 1.62–1.79 (m, 2H, CH, NH); 2.28 (s, 3H, CH_3); 2.81–2.92 (m, 3H, CH, CH–C═O); 3.15 (d, 1H, CH–C═O, $J = 6.3$ Hz); 5.63 (d, 1H, CH═, $J = 6.3$ Hz); 7.12–7.22 (m, 4H, $CH_{arom,}$); 8.05 (s, 1H, NH). ^{13}C NMR ($CDCl_3$) δ: 20.61, 21.17, 21.22, 22.73, 30.73, 33.42, 35.51, 37.98, 42.55, 44.55, 45.54, 125.41 (2C), 130.55 (2C), 133.76, 136.23, 137.04, 137.82, 173.66, 174.10, 181.75. Anal. Calcd for $C_{22}H_{27}N_3O_2S$: C 66.47, H 6.85, N 10.57. Found: C 66.56, H 6.91, N 10.67. ESI MS: $m/z = 398.28$ $[M + H]^+$ (100%).

Crystal data: crystal system monoclinic, space group $P2_1$, unit cell dimensions $a = 14.056(3)$, $b = 10.933(2)$, $c = 15.955(3)$ Å, $\beta = 111.06(3)°$, $V = 2288.1(8)$ Å^3; $Z = 4$, $d_c = 1.154$ g cm^{-3}, $\mu = 1.415$ mm^{-1}, $F(000) = 848$. A crystal of dimensions $0.5 \times 0.32 \times 0.21$ mm was used for intensity measurements. Within the θ range 2.97–75.14° [$-16 \le h \le 16$, $-13 \le k \le 13$, $0 \le l \le 19$] 9034 reflections were collected. The 8898 unique reflections [R(int) = 0.0476] were used for the refinement of 513 parameters, including extinction coefficient [$x = 0.0022(3)$]. Final R indices on F^2 for 3456 observed reflections [$I > 2\sigma(I)$] were: $R1 = 0.0544$, $wR2 = 0.1454$, goodness-of-fit 0.964, and largest difference peak/hole 0.29/−0.23 e Å^{-3}.

*3.2.8. 1-(Allyl)-3-(1-isopropyl-7-methyl-3,5-dioxo-4-azatricyclo[5.2.2.0^{2,6}] undec-8-en-4-yl)-thiourea (**1b**)*

Yield 84%. Mp. 135 °C. ^{1}H NMR ($CDCl_3$) δ (ppm): 0.99 (d, 3H, CH_3, $J = 6.9$ Hz); 1.04 (d, 3H, CH_3, $J = 6.9$ Hz); 1.25–1.37 (m, 2H, CH_2); 1.48 (s, 3H, CH_3); 1.52–1.58 (m, 2H, CH_2); 2.48–2.57 (m, 1H, CH); 2.65 (d, 1H, CH–C═O, $J = 8.1$ Hz); 3.08 (d, 1H, CH–C═O, $J = 8.1$ Hz); 4.19 (s, 2H, CH_2); 5.79–5.86 (m, 1H, CH═); 6.03 (dd, 2H, CH═, $J = 11.3$ Hz); 6.29 (s, 1H, NH); 7.7 (s, 1H, NH). ^{13}C NMR ($CDCl_3$) δ: 17.01, 18.57,

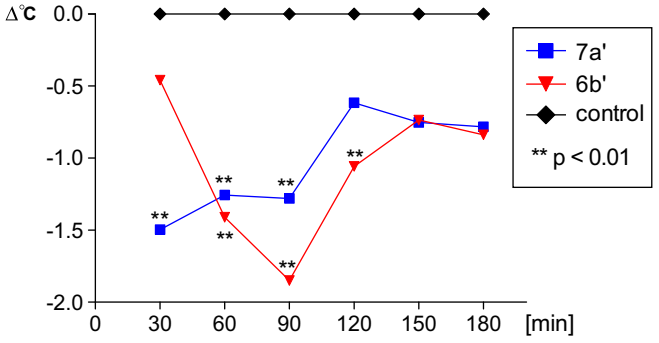


Fig. 8. The influence of **6b**′ and **7a**′ on the body temperature of mice. *Note*: each point represents the mean for a group of 10 mice.

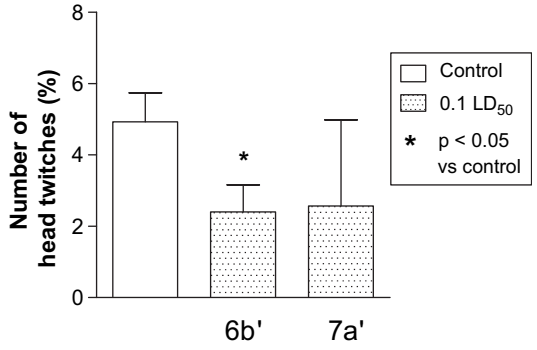


Fig. 9. The influence of **6b′** and **7a′** on the head twitch response evoked by 5–hydroxytryptophan (5-HTP). The results are expressed as mean ±S.E.M. ($n = 10$).

22.69, 23.15, 29.81, 34.31, 37.29, 40.61, 44.12, 45.42, 49.09, 117.4, 134.25, 136.54, 137.27, 174.02, 174.55, 182.23. Anal. Calcd for $C_{18}H_{25}N_3O_2S$: C 62.22, H 7.25, N 10.09. Found: C 62.0, H 7.26, N 12.08. ESI MS: $m/z = 358.2$ $[M + Na]^+$ (100%).

3.2.9. 1-(4-Benzylphenyl)-3-(1-isopropyl-7-methyl-3,5-dioxo-4-aza-tricyclo [5.2.2.0^{2,6}]undec-8-en-4-yl)-thiourea (**2b**)

Yield 84%. Mp. 208 °C. ^{1}H NMR ($CDCl_3$) 300 MHz δ (ppm): 0.74 (d, 3H, CH_3, $J = 5.1$ Hz); 1.1 (d, 3H, CH_3, $J = 5.1$ Hz); 1.12–1.26 (m, 2H, CH_2); 1.28 (s, 3H, CH_3); 1.38–1.45 (m, 2H, CH_2); 2.26–2.33 (m, 1H, CH_2); 2.51 (d, 1H, CH–C═O, $J = 5.7$ Hz); 2.58 (d, 1H, CH–C═O, $J = 5.7$ Hz); 3.14 (s, 1H, NH); 4.5–4.69 (m, 2H, CH_2); 5.89 (dd, 2H, CH═, $J = 13.1$ Hz); 7.19–7.29 (m, 4H, $CH_{arom,}$); 7.68 (s, 1H, NH). ^{13}C NMR ($CDCl_3$) δ: 16.99, 18.49, 22.55, 23.01, 29.66, 34.08, 37.27, 44.11, 45.24, 49.00, 49.83, 128.42 (2C), 128.68 (2C), 129.12 (2C), 136.63, 137.37, 173.15, 173.72, 181.70. Anal. Calcd for $C_{22}H_{27}N_3O_2S$: C 66.47, H 6.85, N 10.57. Found: C 66.52, H 6.89, N 10.67. ESI MS: $m/z = 420.2$ $[M + Na]^+$ (100%).

3.2.10. 1-(4-Fluorophenyl)-3-(1-isopropyl-7-methyl-3,5-dioxo-4-aza-tricyclo [5.2.2.0^{2,6}]undec-8-en-4-yl)-thiourea (**3b**)

Yield 84%. Mp. 142 °C. ^{1}H NMR ($CDCl_3$) δ (ppm): 0.98 (d, 3H, CH_3, $J = 6.8$ Hz); 1.1 (d, 3H, CH_3, $J = 6.8$ Hz); 1.27–1.41 (m, 2H, CH_2); 1.47 (s, 3H, CH_3); 1.49–1.56 (m, 2H, CH_2); 1.89 (s, 1H, NH); 2.48–2.55 (m, 1H, CH_2); 2.76 (d, 1H, CH–C═O, $J = 8$ Hz); 3.12 (d, 1H, CH–C═O, $J = 8$ Hz); 5.99 (dd, 2H, CH═, $J = 12.8$ Hz); 7.0–7.09 (m, 2H, $CH_{arom,}$); 7.36–7.39 (m, 2H, $CH_{arom,}$); 8.04 (s, 1H, NH). ^{13}C NMR ($CDCl_3$) δ: 17.02, 18.57, 22.79, 23.18, 29.88, 34.33, 37.16, 44.02, 45.52, 49.23, 116.29, 116.59, 127.31 (2C), 132.93, 136.24, 137.02, 163.02, 174.06, 174.56, 181.89. Anal. Calcd for $C_{21}H_{24}FN_3O_2S$: C 62.82, H 6.03, N 10.47. Found: C 62.98, H 6.11, N 10.52. ESI MS: $m/z = 424.1$ $[M + Na]^+$ (100%).

Table 2
Cytotoxicity and anti-HIV-1 activity of compounds (**1a**–**7a**, **1a′**–**7a′**).

Cmpds	MT-4 CC_{50}[a]	HIV-1 EC_{50}[b]	Cmpds	MT-4 CC_{50}[a]	HIV-1 EC_{50}[b]
1a	>100	>100	**1a′**	59	>59
2a	54	**30**	**2a′**	47	>47
3a	69	>69	**3a′**	49	>49
4a	45	>45	**4a′**	30	>30
5a	45	>45	**5a′**	22	>22
6a	41	>41	**6a′**	100	>100
7a	Nt	nt	**7a′**	nt	nt
EFV	35	0.003	**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.

Table 3
Cytotoxicity and anti-HIV-1 activity of compounds (**1b**–**7b**, **1b′**–**7b′**).

Cmpds	MT-4 CC_{50}[a]	HIV-1 EC_{50}[b]	Cmpds	MT-4 CC_{50}[a]	HIV-1 EC_{50}[b]
1b	>100	>100	**1b′**	58	>58
2b	58	>58	**2b′**	>100	>100
3b	nt	nt	**3b′**	86	>86
4b	nt	nt	**4b′**	32	>32
5b	>100	>100	**5b′**	59	>59
6b	59	>59	**6b′**	>100	>100
7b	nt	nt	**7b′**	nt	nt
EFV	35	0.003	**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.

3.2.11. 1-(4-Chlorophenyl)-3-(1-isopropyl-7-methyl-3,5-dioxo-4-aza-tricyclo [5.2.2.0^{2,6}]undec-8-en-4-yl)-thiourea (**4b**)

Yield 84%. Mp. 142 °C. ^{1}H NMR ($CDCl_3$) δ (ppm): 0.92 (d, 3H, CH_3, $J = 6.8$ Hz); 1.1 (d, 3H, CH_3, $J = 6.8$ Hz); 1.26–1.41 (m, 2H, CH_2); 1.47 (s, 3H, CH_3); 1.49–1.63 (m, 2H, CH_2); 2.49–2.54 (m, 1H, CH_2); 2.76 (d, 1H, CH–C═O, $J = 8$ Hz); 3.12 (d, 1H, CH–C═O, $J = 8$ Hz); 6.01 (dd, 2H, CH═, $J = 12.8$ Hz); 7.27–7.28 (m, 2H, $CH_{arom,}$); 7.36–7.42 (m, 2H, $CH_{arom,}$); 8.66 (s, 1H, NH). ^{13}C NMR ($CDCl_3$) δ: 17.04, 18.59, 22.73, 23.20, 29.90, 34.35, 37.21, 44.06, 45.54, 49.25, 126.07 (2C), 130.03 (2C), 132.60, 135.61, 136.29, 137.05, 174.03, 174.35, 181.50. Anal. Calcd for $C_{21}H_{24}ClN_3O_2S$: C 60.35, H 5.79, N 10.05. Found: C 60.47, H 5.82, N 10.08. ESI MS: $m/z = 418.1$ $[M + H]^+$ (100%).

3.2.12. 1-(4-Iodophenyl)-3-(1-isopropyl-7-methyl-3,5-dioxo-4-aza-tricyclo [5.2.2.0^{2,6}]undec-8-en-4-yl)-thiourea (**5b**)

Yield 84%. Mp. 140.8 °C. ^{1}H NMR ($CDCl_3$) δ (ppm): 0.89 (d, 3H, CH_3, $J = 6.8$ Hz); 1.1 (d, 3H, CH_3, $J = 6.8$ Hz); 1.25–1.36 (m, 2H, CH_2); 1.47 (s, 3H, CH_3); 1.4–1.53 (m, 2H, CH_2); 2.5–2.59 (m, 3H, CH_2, CH–C═O); 2.95 (d, 1H, CH–C═O, $J = 7.6$ Hz); 5.93 (dd, 2H, CH═, $J = 13.4$ Hz); 7.34–7.36 (m, 2H, $CH_{arom,}$); 7.59–7.61 (m, 2H, $CH_{arom,}$); 9.11 (s, 1H, NH). ^{13}C NMR ($CDCl_3$) δ: 17.04, 18.59, 22.74, 23.06, 29.87, 34.35, 37.18, 44.03, 45.53, 49.23, 91.08, 126.23 (2C), 135.76, 136.49, 137.22, 138.38 (2C), 174.14, 174.68, 181.22. Anal. Calcd for $C_{21}H_{24}IN_3O_2S$: C 49.51, H 4.75, N 8.25. Found: C 49.56, H 4.76, N 8.25. ESI MS: $m/z = 508.2$ $[M - H]^-$ (100%).

3.2.13. 1-(3,4-Dichlorophenyl)-3-(1-isopropyl-7-methyl-3,5-dioxo-4-aza-tricyclo [5.2.2.0^{2,6}]undec-8-en-4-yl)-thiourea (**6b**)

Yield 84%. Mp. 143 °C. ^{1}H NMR ($CDCl_3$) δ (ppm): 1.0 (d, 3H, CH_3, $J = 7.2$ Hz); 1.13 (d, 3H, CH_3, $J = 6.8$ Hz); 1.27–1.42 (m, 2H,

Table 4
Cytotoxicity, anti-HIV-1, anti-BVDV and anti-YFV activity of compounds (**1b** and **2b**).

Compds	MT-4 CC_{50}[a]	HIV-1 EC_{50}[b]	BVDV EC_{50}[c]	YFV EC_{50}[d]
1b	>100	>100	**52**	>100
2b	58	>58	>100	**41**
EFV	35	0.003		
NM–108			1.8	**2.5**

[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.
[c] Compound concentration (μM) required to achieve 50% protection of MDBK cells from the BVDV (Bovine Viral Diarrhea Virus) – induced cytopathogenicity, as determined by the MTT method.
[d] Compound concentrarion (μM) required to achieve 50% protection of BHK (Kidney fibroblast) cells from the YFV (Yellow Fever Virus) – induced cytopathogenicity, as determined by the MTT method.

CH_2); 1.48 (s, 3H, CH_3); 1.49–1.57 (m, 2H, CH_2); 1.96 (s, 1H, NH); 2.47–2.59 (m, 1H, CH_2); 2.78 (d, 1H, CH–C═O, $J = 8.4$ Hz); 3.14 (d, 1H, CH–C═O, $J = 8$ Hz); 6.02 (dd, 2H, CH═, $J = 13$ Hz); 7.28–7.44 (m, 4H, $CH_{arom,}$); 8.22 (s, 1H, NH). ^{13}C NMR ($CDCl_3$) δ: 17.05, 18.59, 22.74, 23.31, 29.93, 34.33, 37.24, 44.12, 45.6, 49.31, 123.52, 125.88, 130.19, 130.69, 132.93, 136.28, 136.96, 137.04, 174.46, 175.00, 181.18. Anal. Calcd for $C_{21}H_{23}$ $Cl_2N_3O_2S$: C 55.75, H 5.12, N 9.29. Found: C 55.78, H 5.18, N 9.31. ESI MS: $m/z = 474.2$ $[M + Na]^+$ (100%).

3.2.14. 1-(4-Methylphenyl)-3-(1-isopropyl-7-methyl-3,5-dioxo-4-aza-tricyclo [5.2.2.0^{2,6}]undec-8-en-4-yl)-thiourea (**7b**)

Yield 84%. Mp. 142 °C. ^{1}H NMR ($CDCl_3$) δ (ppm): 0.96 (d, 3H, CH_3, $J = 7.2$ Hz); 1.08 (d, 3H, CH_3, $J = 7.2$ Hz); 1.2–1.37 (m, 2H, CH_2); 1.46 (s, 3H, CH_3); 1.399–1.54 (m, 2H, CH_2); 2.32 (s, 3H, CH_3); 2.47–2.57 (m, 1H, CH); 2.72 (d, 1H, CH–C═O, $J = 8.1$ Hz); 3.08 (d, 1H, CH–C═O, $J = 8.4$ Hz); 5.94 (dd, 2H, CH═, $J = 10.5$ Hz); 7.17 (d, 2H, $CH_{arom,}$ $J = 8.1$ Hz); 7.25 (d, 2H, $CH_{arom,}$ $J = 8.7$ Hz); 7.59 (s, 1H, NH); 8.26 (s, 1H, NH). ^{13}C NMR ($CDCl_3$) δ: 17.03, 18.59, 21.27, 22.73, 23.19, 29.85, 34.38, 37.29, 43.97, 45.49, 49.20, 125.41 (2C), 130.55 (2C), 133.76, 136.23, 137.04, 137.82, 173.66, 174.10, 181.75. Anal. Calcd for $C_{23}H_{28}$ N_3O_2S: C 66.47, H 6.85, N 10.57. Found: C 66.67, H 6.91, N 10.62. ESI MS: $m/z = 398.16$ $[M + Na]^+$ (100%).

3.3. 1,3-Thiazepine derivatives (**1a′**–**7a′**, **1b′**–**7b′**)

General procedure: Sodium hydride dispersion (60%) in mineral oil (0.44 g, ~10 mmol) was added in a single portion to a stirred solution of thiourea derivative (10 mmol) in anhydrous *N*,*N*-dimethylformamide at room temperature. After hydrogen evolution ceased, 1,4-dibromobutane (15 mmol) was added to the reaction mixture, after 5 and 15 min., respectively. The mixture was stirred for 6 h. Evaporation in vacuum gave a residue which was then purified by column chromatography (chloroform was used as eluent). The compound was crystallized from methanol.

3.3.1. 10-Isopropyl-8-methyl-4-{2-[(Z)-allylimino]-1,3-thiazepan-3-yl}-4-aza-tricyclo [5.2.2.0^{2,6}]undec-8-ene-3,5-dione (**1a′**)

Yield 66%. Mp. 112 °C. 0.82 (d, 3H, CH_3, $J = 4.4$ Hz); 0.84 (d, 3H, CH3, $J = 4.4$ Hz); 1.27–1.33 (m, 2H, CH_2); 1.44–1.49 (m, 3H, CH_2, CH); 1.68 (s, 3H, CH_3); 1.68–1.7 (m, 2H, CH_2); 2.3–2.16 (m, 2H, CH_2); 2.53–2.59 (m, 2H, CH_2); 2.8–2.99 (m, 2H, CH–C═O); 3.569–3.87 (m, 2H, CH_2); 3.92–4.05 (m, 2H, CH_2); 4.94–5.03 (m, 1H, CH_2═); 5.16–5.25 (m, 1H, CH_2═); 5.75–5.82 (m, 1H, CH═); 5.95 (dd, 2H, CH═, $J_1 = J_2 = 8.4$ Hz). ^{13}C NMR ($CDCl_3$) δ: 20.61, 21.16, 21.24, 27.54, 28.13, 30.76, 33.43, 34.54, 35.51, 37.98, 42.53, 44.56, 45.54, 49.09, 54.22, 117.4, 134.25, 136.54, 137.81, 163.01, 174.02, 174.45. Anal. Calcd for $C_{22}H_{31}N_3O_2S$: C 65.8, H 7.78, N 10.46. Found: C 65.6, H 7.8, N 10.32. ESI MS: $m/z = 402.2$ $[M + H]^+$ (100%).

3.3.2. 10-Isopropyl-8-methyl-4-{2-[(Z)-benzylimino]-1,3-thiazepan-3-yl}-4-aza-tricyclo[5.2.2.0^{2,6}]undec-8-ene-3,5-dione (**2a′**)

Yield 38%. Mp. 107.1 °C. ^{1}H NMR ($CDCl_3$) δ (ppm): 0.82 (d, 3H, CH_3, $J = 6.6$ Hz); 0.91 (d, 3H, CH_3, $J = 6.6$ Hz); 1.03–1.17 (m, 2H, CH_2); 1.25–1.28 (m, 1H, CH); 1.32–1.44 (m, 1H, CH); 1.72–1.87 (m, 2H, CH_2); 1,75 (s, 3H, CH_3); 2.04–2.12 (m, 2H, CH_2); 2.74 (d, 1H, CH–C═O, $J = 3.6$ Hz); 2.74 (d, 1H, CH–C═O, $J = 3.6$ Hz); 2.82–2.89 (m, 2H, CH); 3.14–3.22 (m, 2H, CH_2); 3.49–3.82 (m, 2H, CH_2); 4.78–4.92 (m, 2H, CH_2); 5.70 (d, 1H, CH═, $J = 6$ Hz); 7.27–7.42 (m, 5H, $CH_{arom,}$). ^{13}C NMR ($CDCl_3$) δ: 20.62, 21.16, 21.28, 27.56, 28.13, 30.78, 33.42, 34.54, 35.52, 37.96, 42.53, 40.56, 45.50, 49.83, 54.23, 123.11, 128.42, 128.67 (2C), 129.12 (2C), 136.63, 137.37, 162.8, 173.15, 173.71. Anal. Calcd for $C_{26}H_{33}N_3O_2S$: C 69.15, H 7.36, N 9.3. Found: C 69.18, H 7.43, N 9.31. ESI MS: $m/z = 452.3$ $[M + H]^+$ (100%).

3.3.3. 10-Isopropyl-8-methyl-4-{2-[(Z)-4-fluorophenylimino]-1,3-thiazepan-3-yl}-4-azatricyclo[5.2.2.0^{2,6}]undec-8-ene-3,5-dione (**3a′**)

Yield 45%. Mp. 119.6 °C. ^{1}H NMR ($CDCl_3$) δ (ppm): 0.80 (d, 3H, CH_3, $J = 6.8$ Hz); 0.9 (d, 3H, CH_3, $J = 6.8$ Hz); 1.01–1.14 (m, 2H, CH_2); 1.25–1.33 (m, 1H, CH); 1.36–1.38 (m, 1H, CH); 1.62–1.86 (m, 2H, CH_2); 1,77 (s, 3H, CH_3); 1.9–2.0 (m, 2H, CH_2); 2.72 (d, 1H, CH–C═O, $J = 2.4$ Hz); 2.74 (d, 1H, CH–C═O, $J = 2.8$ Hz); 2.81–2.6 (m, 2H, CH); 3.08–3.19 (m, 2H, CH_2); 3.83–3.92 (m, 2H, CH_2); 5.73 (d, 1H, CH═, $J = 5.6$ Hz); 6.57–6.64 (m, 2H, $CH_{arom,}$); 6.88–7.0 (m, 2H, $CH_{arom,}$). ^{13}C NMR ($CDCl_3$) δ: 20.62, 21.16, 21.28, 27.53, 28.14, 30.79, 33.42, 34.54, 35.52, 37.96, 40.56, 42.56, 44.53, 45.50, 54.45, 115.21 (2C), 122, 84 (2C), 135.92, 136.75, 161.80, 162.82, 174.05, 174.39. Anal. Calcd for $C_{25}H_{30}FN_3O_2S$: C 65.91, H 6.64, N 9.22. Found: C 66.02, H 6.68, N 9.22. ESI MS: $m/z = 456.3$ $[M + H]^+$ (100%).

3.3.4. 10-Isopropyl-8-methyl-4-{2-[(Z)-4-chlorophenylimino]-1,3-thiazepan-3-yl}-4-azatricyclo[5.2.2.0^{2,6}]undec-8-ene-3,5-dione (**4a′**)

Yield 45%. Mp. 164 °C. ^{1}H NMR ($CDCl_3$) δ (ppm): 0.82 (d, 3H, CH_3, $J = 6.4$ Hz); 0.91 (d, 3H, CH_3, $J = 6.4$ Hz); 1.05–1.15 (m, 2H, CH_2); 1.25–1.28 (m, 1H, CH); 1.36–1.38 (m, 1H, CH); 1.73–1.86 (m, 2H, CH_2); 1,77 (s, 3H, CH_3); 2.03–2.04 (m, 2H, CH_2); 2.73 (d, 1H, CH–C═O, $J = 2.4$ Hz); 2.76 (d, 1H, CH–C═O, $J = 2.8$ Hz); 2.81–2.86 (m, 2H, CH); 3.09–3.19 (m, 2H, CH_2); 3.83–3.92 (m, 2H, CH_2); 5.73 (d, 1H, CH═, $J = 5.6$ Hz); 6.57–6.81 (m, 2H, $CH_{arom,}$); 7.23–7.32 (m, 2H, $CH_{arom,}$). ^{13}C NMR ($CDCl_3$) δ: 20.61, 21.17, 21.22, 27.45, 28.17, 30.73, 33.42, 34.56, 35.51, 37.98, 42.55, 44.55, 45.54, 49.05, 54.12, 124.19 (2C), 128.64, 135.51, 135.95, 136.75, 148.35, 153.68, 173.54, 174.32. Anal. Calcd for $C_{25}H_{30}ClN_3O_2S$: C 63.61, H 6.41, N 8.9. Found: C 63.72, H 6.45, N 8.93. ESI MS: $m/z = 471.8$ $[M + H]^+$ (100%).

3.3.5. 10-Isopropyl-8-methyl-4-{2-[(Z)-4-iodophenylimino]-1,3-thiazepan-3-yl}-4-azatricyclo[5.2.2.0^{2,6}]undec-8-ene-3,5-dione (**5a′**)

Yield 43%. Mp. 163.1 °C. ^{1}H NMR ($CDCl_3$) δ (ppm): 0.82 (d, 3H, CH_3, $J = 6.4$ Hz); 0.91 (d, 3H, CH_3, $J = 6.9$ Hz); 1.05–1.15 (m, 2H, CH_2); 1.3–1.42 (m, 1H, CH); 1.62–1.65 (m, 1H, CH); 1.73–1.85 (m, 2H, CH_2); 1,79 (s, 3H, CH_3); 2.0–2.02 (m, 2H, CH_2); 2.81 (d, 1H, CH–C═O, $J = 2.4$ Hz); 2.84 (d, 1H, CH–C═O, $J = 2.8$ Hz); 2.92–2.98 (m, 2H, CH); 3.04–3.2 (m, 2H, CH_2); 3.7–3.78 (m, 2H, CH_2); 5.73 (d, 1H, CH═, J═6 Hz); 6.49–6.55 (m, 2H, $CH_{arom,}$); 7.5–7.62 (m, 2H, $CH_{arom,}$). ^{13}C NMR ($CDCl_3$) δ: 20.64, 21.17, 21.18, 27.56, 28.17, 30.76, 33.38, 34.54, 35.53, 37.93, 42.51, 44.53, 45.54, 49.29, 54.23, 86.56, 124.70 (2C), 135.52, 135.89, 137.58 (2C), 153.61, 173.69, 174.11. Anal. Calcd for $C_{25}H_{30}IN_3O_2S$: C 53.29, H 5.37, N 7.46. Found: C 53.35, H 5.43, N 7.46. ESI MS: $m/z = 564.2$ $[M + H]^+$ (100%).

3.3.6. 10-Isopropyl-8-methyl-4-{2-[(Z)-3,4-dichlorophenylimino]-1,3-thiazepan-3-yl}-4-azatricyclo[5.2.2.0^{2,6}]undec-8-ene-3,5-dione (**6a′**)

Yield 45%. Mp. 95.3 °C. ^{1}H NMR ($CDCl_3$) δ (ppm): 0.81 (d, 3H, CH_3, $J = 6.4$ Hz); 0.9 (d, 3H, CH_3, $J = 6.4$ Hz); 1.05–1.11 (m, 2H, CH_2); 1.25–1.28 (m, 1H, CH); 1.47–1.49 (m, 1H, CH); 1.73–1.85 (m, 2H, CH_2); 1,77 (s, 3H, CH_3); 2.04–2.08 (m, 2H, CH_2); 2.76 (d, 1H, CH–C═O, $J = 2.4$ Hz); 2.79 (d, 1H, CH–C═O, $J = 2.8$ Hz); 2.85–2.89 (m, 2H, CH); 3.08–3.12 (m, 2H, CH_2); 3.84–3.86 (m, 2H, CH_2); 5.74 (d, 1H, CH═, $J = 5.6$ Hz); 6.76–6.78 (m, 1H, $CH_{arom,}$); 7.01–7.02 (m, 2H, $CH_{arom,}$); 7.27–7.29 (m, 2H, $CH_{arom,}$). ^{13}C NMR ($CDCl_3$) δ: 20.64, 21.17, 21.18, 27.48, 28.12, 30.76, 33.38, 34.52, 35.53, 37.93, 42.51, 44.53, 45.54, 49.02, 54.24, 122.59, 124.76, 130.25, 132.29, 135.94, 136.74, 149.41, 154.68, 173.48, 173.98. Anal. Calcd for $C_{25}H_{29}Cl_2N_3O_2S$: C 59.28, H 5.77, N 8.3. Found: C 59.29, H 5.77, N 8.35. ESI MS: $m/z = 506.03$ $[M]^+$ (100%).

*3.3.7. 10-Isopropyl-8-methyl-4-{2-[(Z)-4-methylphenylimino]-1,3-thiazepan-3-yl}-4-azatricyclo[5.2.2.0^{2,6}]undec-8-ene-3,5-dione (**7a'**)*

Yield 45%. Mp. 148 °C. ^{1}H NMR ($CDCl_3$) δ (ppm): 0.82 (d, 3H, CH_3, $J = 6.4$ Hz); 0.84 (d, 3H, CH_3, $J = 6.4$ Hz); 0.95–1.08 (m, 2H, CH_2); 1.15–1.28 (m, 1H, CH); 1.34–1.5 (m, 1H, CH); 1.64–1.67 (m, 2H, CH_2); 1,7 (s, 3H, CH_3); 2.22 (s, 3H, CH_3); 2.73 (d, 1H, CH–C═O, $J = 2.4$ Hz); 2.76 (d, 1H, CH–C═O, $J = 2.8$ Hz); 2.77–2.83 (m, 2H CH_2); 2.89–3.03 (m, 2H, CH); 3.06–3.12 (m, 2H, CH_2); 3.58–3.72 (m, 2H, CH_2); 5.66 (d, 1H, CH═, $J = 5.6$ Hz); 6.47–6.55 (m, 2H, $CH_{arom,}$); 6.95–6.97 (m, 2H, $CH_{arom,}$). ^{13}C NMR ($CDCl_3$) δ: 20.61, 21.17, 21.22, 22.73, 27.54, 28.17, 30.73, 33.42, 34.54, 35.51, 37.98, 42.55, 44.55, 45.54, 49.28, 54.15, 122.05, 139.25 (2C), 132.42, 135.91, 136.70, 147.26, 152.74, 173.66, 174.10. Anal. Calcd for $C_{26}H_{33}N_3O_2S$: C 69.15, H 7.36, N 9.3. Found: C 69.23, H 7.42, N 8.99. ESI MS: $m/z = 452.18$ $[M + H]^+$ (100%).

Crystal data: crystal system orthorhombic, space group $P2_12_12_1$, unit cell dimensions $a = 8.203(2)$, $b = 12.925(3)$, $c = 23.349(5)$ Å, $V = 2475.5(10)$ Å^3; $Z = 4$, $d_c = 1.198$ g cm^{-3}, $\mu = 1.366$ mm^{-1}, $F(000) = 948$. A crystal of dimensions $0.4 \times 0.38 \times 0.1$ mm was used for intensity measurements. Within the θ range 3.91–80.27° [$0 \le h \le 10$, $0 \le k \le 16$, $0 \le l \le 29$] 3033 reflections were collected. The 3022 unique reflections [R(int) = 0.10] were used for the refinement of 290 parameters, including extinction coefficient [$x = 0.0053(7)$]. Final R indices on F^2 for 1609 observed reflections [$I > 2\sigma(I)$] were: $R1 = 0.0432$, $wR2 = 0.1254$, goodness-of-fit 1.038, and largest difference peak/hole 0.27/−0.25 e Å^{-3}.

*3.3.8. 1-Isopropyl-7-methyl-4-{2-[(Z)-4-allylimino]-1,3-thiazepan-3-yl}-4-aza-tricyclo [5.2.2.0^{2,6}]undec-8-ene-3,5-dione (**1b'**)*

Yield 62%. Mp. 123 °C. ^{1}H NMR ($CDCl_3$) δ (ppm): 0.98 (d, 3H, CH_3, $J = 6.8$ Hz); 1.09 (d, 3H, CH_3, $J = 6.8$ Hz); 1.27–1.33 (m, 2H, CH_2); 1.44–1.49 (m, 3H, CH_2, CH); 1.48 (s, 3H, CH_3); 1.68–1.7 (m, 2H, CH_2); 2.3–2.16 (m, 2H, CH_2); 2.53–2.59 (m, 2H, CH_2); 2.8–2.99 (m, 2H, CH–C═O); 3.569–3.87 (m, 2H, CH_2); 3.92–4.05 (m, 2H, CH_2); 4.94–5.03 (m, 1H, CH_2═); 5.16–5.25 (m, 1H, CH_2═); 5.75–5.82 (m, 1H, CH═); 5.95 (dd, 2H, CH═, $J_1 = J_2 = 8.4$ Hz). ^{13}C NMR ($CDCl_3$) δ: 18.27, 19.91, 22.69, 23.15, 23.53, 24.50, 29.52, 29.81, 34.31, 37.29, 40.61, 44.12, 45.42, 49.09, 117.4, 134.25, 136.54, 137.81, 163.01, 174.02, 174.45. Anal. Calcd for $C_{22}H_{31}N_3O_2S$: C 65.8, H 7.78, N 10.46. Found: C 65.88, H 7.82, N 10.52. ESI MS: $m/z = 402.22$ $[M + H]^+$ (100%).

*3.3.9. 1-Isopropyl-7-methyl-4-{2-[(Z)-4-benzylimino]-1,3-thiazepan-3-yl}-4-aza-tricyclo [5.2.2.0^{2,6}]undec-8-ene-3,5-dione (**2b'**)*

Yield 62%. Mp. 179 °C. ^{1}H NMR ($CDCl_3$) δ (ppm): 0.98 (d, 3H, CH_3, $J = 6.4$ Hz); 1.06 (d, 3H, CH_3, $J = 6.8$ Hz); 1.23–1.33 (m, 2H, CH_2); 1.44–1.49 (m, 3H, CH_2, CH); 1.5 (s, 3H, CH_3); 1.73–1.76 (m, 2H, CH_2); 1.86–1.89 (m, 2H, CH_2); 2.57–2.62 (m, 2H, CH_2); 2.77 (d, 1H, CH–C═O, $J = 6.4$ Hz); 2.98 (d, 1H, CH–C═O, $J = 6.4$ Hz); 3.51–3.54 (m, 2H, CH_2); 4.74–4.96 (m, 2H, CH_2); 6.0 (dd, 2H, CH═, $J_1 = J_2 = 7.2$ Hz); 7.32–7.38 (m, 5H, $CH_{arom,}$). ^{13}C NMR ($CDCl_3$) δ: 16.99, 18.45, 22.54, 23.01, 27.12, 28.13, 29.65, 29.97, 34.08, 34.54, 37.20, 44.11, 45.23, 49.00, 49.85, 123.11, 128.42, 128.67 (2C), 129.12 (2C), 136.63, 137.37, 162.8, 173.15, 173.71. Anal. Calcd for $C_{26}H_{33}N_3O_2S$: C 69.15, H 7.36, N 9.3. Found: C 69.18, H 7.42, N 9.36. ESI MS: $m/z = 474.3$ $[M + Na]^+$ (100%).

*3.3.10. 1-Isopropyl-7-methyl-4-{2-[(Z)-4-fluorophenylimino]-1,3-thiazepan-3-yl}-4-azatricyclo[5.2.2.0^{2,6}]undec-8-ene-3,5-dione (**3b'**)*

Yield 62%. Mp. 138 °C. ^{1}H NMR ($CDCl_3$) δ (ppm): 0.93 (d, 3H, CH_3, $J = 6.8$ Hz); 1.06 (d, 3H, CH_3, $J = 6.8$ Hz); 1.18–1.3 (m, 4H, CH_2); 1.36–1.43 (m, 3H, CH_2, CH); 1.44 (s, 3H, CH_3); 1.64–1.69 (m, 2H, CH_2); 1.89–1.96 (m, 2H, CH_2); 2.46–2.56 (m, 2H, CH_2); 2.93 (d, 1H, CH–C═O, $J = 8.4$ Hz); 2.96 (d, 1H, CH–C═O, $J = 8.4$ Hz); 3.71–3.76 (m, 2H, CH_2); 5.96 (dd, 2H, CH═, $J_1 = J_2 = 8.8$ Hz); 6.52–6.61 (m, 2H, $CH_{arom,}$), 6.84–6.88 (m, 2H, $CH_{arom,}$). ^{13}C NMR ($CDCl_3$) δ: 17.03, 18.61, 22.89, 23.26, 27.28, 28.28, 29.55, 29.97, 33.43, 34.56, 37.18, 44.04, 45.55, 49.27, 54.45, 115.21 (2C), 122, 84 (2C), 135.92, 136.75, 161.80, 162.82, 174.05, 174.39. Anal. Calcd for $C_{25}H_{30}$ FN_3O_2S: C 65.91, H 6.64, N 9.22. Found: C 66.03, H 6.72, N 9.33. ESI MS: $m/z = 456.62$ $[M + H]^+$ (100%).

*3.3.11. 1-Isopropyl-7-methyl-4-{2-[(Z)-4-chlorophenylimino]-1,3-thiazepan-3-yl}-4-azatricyclo[5.2.2.0^{2,6}]undec-8-ene-3,5-dione (**4b'**)*

Yield 82%. Mp. 138 °C. ^{1}H NMR ($CDCl_3$) δ (ppm): 1.0 (d, 3H, CH_3, $J = 6.8$ Hz); 1.1 (d, 3H, CH_3, $J = 6.8$ Hz); 1.25–1.41 (m, 2H, CH_2); 1.47–1.55 (m, 3H, CH_2, CH); 1.47 (s, 3H, CH_3); 1.7–1.77 (m, 2H, CH_2); 1.99–2.02 (m, 2H, CH_2); 2.55–2.63 (m, 2H, CH_2); 3.02 (d, 1H, CH–C═O, $J = 14.4$ Hz); 3.06 (d, 1H, CH–C═O, $J = 14.4$ Hz); 3.78–3.82 (m, 2H, CH_2); 5.95 (dd, 2H, CH═, $J_1 = 8.4$ Hz, $J_2 = 8.8$ Hz); 6.59–6.81 (d, 2H, $CH_{arom,}$), 7.19–7.23 (m, 2H, $CH_{arom,}$). ^{13}C NMR ($CDCl_3$) δ: 17.05, 18.60, 22.77, 23.25, 27.12, 28.13, 29.42, 29.97, 33.47, 34.54, 37.18, 44.04, 45.55, 49.05, 124.19 (2C), 128.64 (2C), 135.51, 135.95, 136.75, 148.35, 153.68, 173.54, 174.32. Anal. Calcd for $C_{25}H_{30}$ ClN_3O_2S: C 63.61, H 6.41, N 8.9. Found: C 63.72, H 6.44, N 8.92. ESI MS: $m/z = 472.18$ [M] (100%).

*3.3.12. 1-Isopropyl-7-methyl-4-{2-[(Z)-4-iodophenylimino]-1,3-thiazepan-3-yl}-4-azatricyclo[5.2.2.0^{2,6}]undec-8-ene-3,5-dione (**5b'**)*

Yield 78%. Mp. 188 °C. ^{1}H NMR ($CDCl_3$) δ (ppm): 0.93 (d, 3H, CH_3, $J = 6.8$ Hz); 1.04 (d, 3H, CH_3, $J = 6.8$ Hz); 1.16–1.28 (m, 2H, CH_2); 1.36–1.48 (m, 3H, CH_2, CH); 1.43 (s, 3H, CH_3); 1.59–1.7 (m, 2H, CH_2); 1.9–1.95 (m, 2H, CH_2); 2.46–2.56 (m, 2H, CH_2); 2.93 (d, 1H, CH–C═O, J = 7.6 Hz); 2.95 (d, 1H, CH–C═O, $J = 7.6$ Hz); 3.71–3.76 (m, 2H, CH_2); 5.96 (dd, 2H, CH═, $J_1 = 8.8$ Hz, $J_2 = 8.8$ Hz); 6.35–6.45 (d, 2H, $CH_{arom,}$), 7.45–7.47 (m, 2H, $CH_{arom,}$). ^{13}C NMR ($CDCl_3$) δ: 17.04, 18.62, 22.79, 23.15, 23.27, 27.54, 28.13, 29.65, 29.95, 33.23, 34.54, 37.18, 44.03, 45.56, 49.29, 86.56, 124.70 (2C), 135.52, 135.89, 137.58 (2C), 153.61, 173.69, 174.11. Anal. Calcd for $C_{25}H_{30}IN_3O_2S$: C 53.29, H 5.37, N 7.46. Found: C 53.33, H 5.42, N 7.52. ESI MS: $m/z = 563.85$ [M] (100%).

*3.3.13. 1-Isopropyl-7-methyl-4-{2-[(Z)-3,4-dichlorophenylimino]-1,3-thiazepan-3-yl}-4-azatricyclo[5.2.2.0^{2,6}]undec-8-ene-3,5-dione (**6b'**)*

Yield 80%. Mp. 144 °C. ^{1}H NMR ($CDCl_3$) δ (ppm): 0.93 (d, 3H, CH_3, $J = 5.1$ Hz); 1.03 (d, 3H, CH_3, $J = 5.1$ Hz); 1.19–1.3 (m, 2H, CH_2); 1.36–1.49 (m, 3H, CH_2, CH); 1.44 (s, 3H, CH_3); 1.63–1.7 (m, 2H, CH_2); 1.92–1.96 (m, 2H, CH_2); 2.45–2.56 (m, 2H, CH_2); 2.94 (d, 1H, CH–C═O, $J = 6.3$ Hz); 2.97 (d, 1H, CH–C═O, J = 6.3 Hz); 3.67–3.71 (m, 2H, CH_2); 2.95 (dd, 2H, CH═, $J_1 = J_2 = 6.3$ Hz); 6.54 (d, 1H, $CH_{arom,}$ $J = 1.5$ Hz), 6.8 (s, 1H, $CH_{arom,}$); 7.1–7.23 (m, 2H, $CH_{arom.}$). ^{13}C NMR ($CDCl_3$) δ: 17.01, 18.62, 22.81, 23.17, 27.15, 28.00, 29.42, 29.99, 33.48, 34.54, 37.21, 44.07, 45.26, 49.02, 122.59, 124.76, 130.21, 130.25, 132.29, 135.94, 136.74, 149.41, 154.68, 173.48, 173.98. Anal. Calcd for $C_{25}H_{29}$ $Cl_2N_3O_2S$: C 59.28, H 5.77, N 8.3. Found: C 59.29, H 5.82, N 8.31. ESI MS: $m/z = 507.25$ $[M + H]^+$

*3.3.14. 1-Isopropyl-7-methyl-4-{2-[(Z)-4-methylphenylimino]-1,3-thiazepan-3-yl}-4-azatricyclo[5.2.2.0^{2,6}]undec-8-ene-3,5-dione (**7b'**)*

Yield 85%. Mp. 142.9 °C. ^{1}H NMR ($CDCl_3$) δ (ppm): 0.91 (d, 3H, CH_3, $J = 6.8$ Hz); 1.04 (d, 3H, CH_3, $J = 6.8$ Hz); 1.18–1.33 (m, 2H, CH_2); 1.4–1.49 (m, 3H, CH_2, CH); 1.44 (s, 3H, CH_3); 1.64–1.69 (m, 2H, CH_2); 1.9–1.95 (m, 2H, CH_2); 2.22 (s, 3H, CH_3), 2.48–2.57 (m, 2H, CH_2); 2.92 (d, 1H, CH–C═O, $J = 6$ Hz); 2.94 (d, 1H, CH–C═O, $J = 6$ Hz); 3.71–3.76 (m, 2H, CH_2); 5.91 (dd, 2H, CH═, $J_1 = 6.3$ Hz, $J_2 = 6.6$ Hz); 6.49–6.55 (d, 2H, $CH_{arom.}$), 6.98–7.05 (m, 2H, $CH_{arom.}$). ^{13}C NMR ($CDCl_3$) δ: 17.05, 18.62, 21.10, 22.88, 23.27, 27.79, 28.33, 29.82, 29.95, 33.17, 34.57, 37.15, 43.99, 45.56, 49.28, 54.15, 122.05, 139.25 (2C), 132.42, 135.91, 136.70, 147.26, 152.74, 173.66, 174.10. Anal. Calcd for $C_{26}H_{33}N_3O_2S$: C 69.15, H 7.36, N 9.3. Found: C 69.23, H 7.39, N 9.32. ESI MS: $m/z = 552.18$ $[M + H]^+$ (100%).

3.4. Pharmacology

The experiments were performed on male Albino Swiss mice (18–30 g). The animals were kept 8–10 to a cage, at room temperature of 20 ± 1 °C, on a 12:12 h dark–light cycle. Standard food (LSM, Motycz, Poland) and water were available *ad libitum*. The investigated compounds **6b**′ and **7a**′, were administered intraperitoneally (ip.) at a 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. The controls received the equivalent volume of the solvent.

All tests performed, suggested by Vogel and Vogel [53] are generally accepted as basic in investigation of the central activity by behavioral methods.

The acute toxicity of the compound was assessed in mice acc. to Litchfield and Wilcoxon method [54], as the LD_{50} calculated on mortality within 48 h. The compound was injected in doses equivalent to 0.1 LD_{50} (200 mg kg^{-1}). 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*
  b) *amphetamine-induced hyperactivity:* mice received subcutaneously (s.c.) 5 mg kg^{-1} of amphetamine 30 min before the test;
- *nociceptive reactions* were studied in *the acetic acid (0.6%) induced writhing test* [55]. 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 test [56] and chimney test [57];
- *body temperature* in normothermic mice was measured in the rectum by thermistor thermometer;
- *pentylenetetrazole (110 mg kg^{-1}, 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), acc. to Corne et al. [18]. Mice received 5-HTP (180 mg kg^{-1}, 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).

Statistics. Obtained data were calculated by χ^2 test with Yates correction (pentylenetetrazole-induced seizures), Dunnett's test, followed by one-way ANOVA (body temperature) and Students *t*-test (other tests).

3.5. Cytotoxicity and antiviral activity assays

3.5.1. Compounds

Compounds were dissolved in DMSO at 100 mM and then diluted in culture medium.

3.5.2. Cells and viruses

Cell lines were purchased from American Type Culture Collection (ATCC). The absence of mycoplasma contamination was checked periodically by the Hoechst staining method. Cell lines supporting the multiplication of RNA viruses were the following: $CD4^+$ human T-cells containing an integrated HTLV-1 genome (MT-4), Madin Darby Bovine Kidney (MDBK), Baby Hamster Kidney (BHK-21) and Monkey kidney (Vero 76) cells.

3.5.3. Cytotoxicity assays

For cytotoxicity tests, run in parallel with antiviral assays, MDBK, BHK and Vero 76 cells were resuspended in 96 multiwell plates at an initial density of 6×10^5, 1×10^6 and 5×10^5 cells mL^{-1}, respectively, in maintenance medium, with or without serial dilutions of test compounds. Cell viability was determined after 48–120 h at 37 °C in a humidified CO_2 (5%) atmosphere by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) method [58]. The cell number of Vero 76 monolayers was determined by staining with the crystal violet dye.

For cytotoxicity evaluations, exponentially growing cells derived from human haematological 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^{-1} in 96 well plates in RPMI-1640 medium, supplemented with 10% fetal calf serum (FCS), 100 units mL^{-1} penicillin G and 100 μg mL^{-1} 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 MTT method.

3.5.4. 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 1×10^4 MT-4 were added to each well of flat-bottom microtitre trays containing 50 μL of RPMI, with or without 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.

Activity of compounds against Yellow Fever Virus (YFV) and Reo Virus type-1 (Reo-1) was based on inhibition of virus-induced cytopathogenicity in acutely infected BHK-21 cells. Activities against Bovine Viral Diarrhoea Virus (BVDV), in infected MDBK cells, were also based on inhibition of virus-induced cytopathogenicity.

BHK and MDBK cells were seeded in 96-well plates at a density of 5×10^4 and 3×10^4 cells per well, respectively, and were allowed to form confluent monolayers by incubating overnight in growth medium at 37 °C in a humidified CO_2 (5%) atmosphere. Cell monolayers were then infected with 50 μL of a proper virus dilution (in serum-free medium) to give an m.o.i. = 0.01. One hr later, 50 μL of MEM Earle's medium, supplemented with inactivated foetal calf serum (FCS), 1% final concentration, without or with serial dilutions of test compounds, were added. After 3–4 days incubation at 37 °C, cell viability was determined by the MTT method.

Activity of compounds against Coxsackie virus, B-2 strain (CVB-2), Polio Virus type-1 (Polio-1), Sabin strain, Vesicular Stomatitis Virus (VSV), Vaccinia Virus (VV), Herpes Virus 1 (HSV-1) and against Respiratory Syncytial Virus (RSV), A-2 strain, in infected Vero 76 cells, was determined by plaque reduction assays in Vero 76 cell monolayers. To this end, Vero 76 cells were seeded in 24-well plates at a density of 2×10^5 cells per well and were allowed to form confluent monolayers by incubating overnight in growth medium at 37 °C in a humidified CO_2 (5%) atmosphere. Then, monolayers were infected with 250 μL of proper virus dilutions to give 50–100 PFU/well. Following removal of unadsorbed virus, 500 μL of Dulbecco's modified Eagle's medium, supplemented with 1% inactivated FCS and 0.75% methyl cellulose, with or without serial dilutions of test compounds, were added. Cultures were incubated at 37 °C for 2 (Sb-1 and VSV), 3 (CVB-2, VV and HSV-1) or 5 days (RSV) and then fixed with PBS containing 50% ethanol and 0.8% crystal violet, washed and air-dried. Plaques were then counted. 50% effective concentrations (EC_{50}) were calculated by linear regression technique.

AZT (3′-azido-thymidine), NM 108 (2′-β-methyl-guanosine), NM 176 (2′-ethynyl-D-citidine), NM 299 (6-azauridine), M 5255 (mycophenolic acid) and ACG (acycloGuanosine) were used as reference inhibitors of $ssRNA^+$, $ssRNA^-$ and DNA viruses, respectively.

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