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Synthesis of thiourea derivatives of 1H-isoindole-1,3 (2H)-dione as potential antiviral agents

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1. INTRODUCTION

The first antiretroviral agents approved for the treatment of AIDS were nucleosidic inhibitors of reverse transcriptase such as Zidovudine. Later, the human immunodeficiency virus (HIV) protease inhibitors (*e.g.* saquinavir) were introduced as therapeutics. Recently, a third class of antiretroviral agents, the non-nucleosidic reverse trancriptase inhibitors (NNRTIs) (*i.e.* nevirapine, delavirdine, efavirenz) have been marketed (1).

From all the active compounds, some common characteristics have emerged. First, NNRTIs interact with a non-substrate binding site that is located in the close vicinity of the substrate binding site of HIV-1. As a consequence, all these compounds are deprived of activity against HIV-2.

Furthermore, structural studies have shown that these derivatives contain a central hydrophilic parts and two hydrophobic moieties, generally an aromatic cycle forming a butterfly-like conformation (2). Finally, most of these compounds contain urea or thiourea function in their structure (3).

Searching for new compounds with predictable antiviral properties, our attention was drawn to a group of thiourea derivatives of 1H-isoindole-1,3 (2H)-dione. Imides obtained in Diels-Alder reaction were used as starting materials. These were reacted with hydrazine (80% aqueous solution). The compounds were subjected to the reaction with benzyl, allyl, benzoyl isothiocyanate to be transformed into the corresponding thiourea derivatives.

All of the final compounds were characterized by ^{1}H NMR spectra which were in accordance with the proposed structures.

The general synthetic pathway is given in Scheme 1.

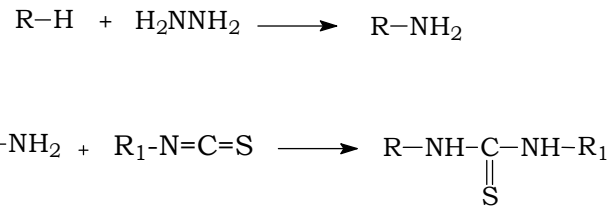
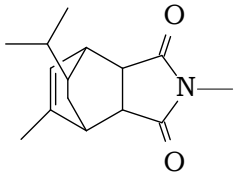
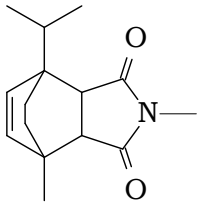
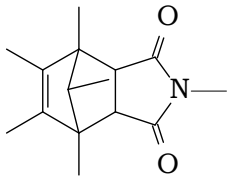
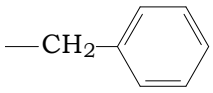
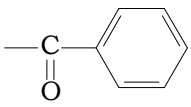


Table 1. Structure of the reported compounds.

R / R_1			
-NH_2	**h1**	**d1**	**k1**
—CH_2—CH=CH_2	**h2**	**d2**	**k2**
—CH_2— 	**h3**	**d3**	**k3**
—C(=O)— 	**h4**	**d4**	**k4**

2. EXPERIMENTAL

Chemistry. Melting points were determined in a capillary on Kofler's apparatus and are uncorrected. The ^{1}H NMR spectra were recorded in Warsaw Medical University, Pharmacy Department on a Bruker AVANCE DMX400 spectrometer, operating at 400.13 MHz for ^{1}H or in the Department of Chemistry, Warsaw University on a Varian UNITYplus-200 spectrometer,

operating at 199.97 MHz for ^{1}H. 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 ± 0.4 % of the calculated compositions.

2-Amino derivatives

General Procedure: A mixture of appropriate imide (4, 5) (0.1 mole) and hydrazine (80% water solution) (50 ml) was refluxed for 5 h. The solvents were evaporated. The residue was purified by a column chromatography (silica gel) to give compounds (**h1, d1, k1**).

Tiourea derivatives

General Procedure: A solution of 2-amino derivatives of of 8-isopropyl-5-methyl-4,7-ethano-3a,4,7,7a-tetrahydro-1H-isoindole-1,3(2H)-dione (**h1**) or 7-methylo-4-isopropylo-4,7-ethano-3a,4,7,7a-tetrahydro-1H-isoindole-1,3(2H)-dione (**d1**) or 4,5,6,7,8-pentamethyl-4,7-methano-3a,4,7,7a-tetrahydro-1H-isoindole-1,3(2H)-dione (**k1**) (0.01 mole) in acetonitrile (6 cm^3) was treated with allyl, benzyl, benzoylisothiocyanate (0.011mole) and the mixture was stirred for 6h. The precipitate was filtered off and washed with ether to give compounds (**h2-h4**), (**d2-d4**), (**k2-k4**).

h1 m.p. 92^{o}C, 70%; ^{1}H NMR ($CDCl_3$) δ(ppm): 0.80 (d, 3H, CH_3 J = 6.4Hz); 0.89 (d, 3H, CH_3 J = 8Hz); 1.1-1.12 (m, 2H, CH); 1.31-1.33 (m, 1H, CH); 1.71 (s, 3H, CH_3); 1.76-1.82 (m, 1H, CH-CH_3), 2.76-2.84 (m, 2H, CH_2); 2.92 (s, 1H, CH); 3.17 (s, 1H, CH); 3.47 (s, 2H, NH_2); 5.63 (d, 1H, C_5 J = 4Hz). *Anal.* Calcd for $C_{14}H_{20}O_2N_2$ (248.32g): C 67.71, H 8.12, N 11.28. Found: C 67.83, H 8.24, N 11.27.

h2 m.p. 145-146 ^{0}C, 67%; ^{1}H NMR ($CDCl_3$) δ(ppm): 0.82 (d, 3H, CH_3 J = 6Hz); 0.88 (d, 3H, CH_3 J = 6Hz); 0.98-1.11 (m, 2H, CH); 1.2-1.35 (m, 1H, CH); 1.74 (s, 3H, CH_3); 1.85-2.0 (m, 2H, CH), 2.76-2.90 (m, 2H, CH); 3.1-3.15 (m, 1H, CH); 4.18(m, 2H, CH_2); 5.22 (t, 2H,=CH J = 14,8Hz); 5.68 (d, 1H, CH J = 4Hz); 5.82-5.9 (d, 1H, HC= J =6.4Hz); 6.37 (s, 1H, NH); 7.78 (s, 1H, NH). *Anal.* Calcd for $C_{18}H_{25}O_2N_3S$ (247.47g): C 62.22, H 7.25, N 12.09. Found: C 62.0, H 7.26, N 12.08.

h3 m.p. 147-148 ^{0}C, 80%; ^{1}H NMR ($CDCl_3$) δ(ppm): 0.82 (m, 3H, CH_3); 0.89 (m, 3H, CH_3); 1.01-1.15 (m, 2H, CH); 1.31-1.33 (m, 1H, CH); 1.58 (s, 3H, CH_3); 1.55-1.81 (m, 1H, CH), 2.61-2.64(2H, m, CH_2), 2.76-2.87 (m, 2H, CH); 2.92 (s, 1H, CH); 3.17 (s, 1H, CH); 4.77-4.89 (m, 1H, CH), 6.24 (1H, s, NH), 7.31-7.37 (m, 5H, $CH_{arom.}$), 7.83 (s, 1H, NH). *Anal.* Calcd for $C_{22}H_{27}O_2N_3S$ (397.53g): C 66.47, H 6.85, N 10.53. Found: C 66.56, H 6.65, N 10.41.

h4 m.p. 188-189 ^{0}C, 85%; ^{1}H NMR ($CDCl_3$) δ(ppm): 0.82 (d, 3H, CH_3, 6.4Hz); 0.91 (d, 3H, CH_3 J = 8Hz); 1.08-1.11 (m, 2H, CH); 1.25-1.35 (m, 1H, CH); 1.78 (s, 3H, CH_3); 1.78-1.84 (m, 1H, CH), 2.98 (m, 3H, CH); 3.23 (s, 1H, CH); 5.37 (s, 1H, CH), 7.53-7.85 (m, 5H, $CH_{arom.}$), 9.26 (s, 1H, NH), 11.92 (s, 1H, NH). *Anal.* Calcd for $C_{22}H_{25}O_3N_3S$ (411.51g): C 64.59, H 6.12, N 10.21. Found: C 63.96, H 5.84, N 9.98.

d1 m.p. 147^{0}C, 72%; ^{1}H NMR ($CDCl_3$) δ(ppm): 0.98 (d, 3H, CH_3 J = 7.2Hz); 1.11 (d, 3H, CH_3 J = 6.8Hz); 1.23-1.36 (m, 2H, CH_2); 1.4-1.46 (m, 2H, CH_2); 1.48 (s, 3H, CH_3); 2.53-2.62 (m, 2H, CH); 2.57 (d, 1H, CH J = 8Hz); 4.24 (s, 2H, NH_2); 5.88 (d, 1H, CH J = 8.4Hz); 5.96 (d, 1H, CH J = 8.4Hz). *Anal.* Calcd for $C_{14}H_{20}O_2N_2$ (248.32g): C 67.71, H 8.12, N 11.28. Found: C 67.64, H 8.08, N 11,36.

d2 m.p. 132-133 ^{0}C, 72%; ^{1}H NMR ($CDCl_3$) δ(ppm): 0.97 (d, 3H, CH_3 J = 6.8Hz); 1.01 (d, 3H, CH_3 J = 6.4Hz); 1.26-1.43 (m, 2H, CH_2); 1.43-1.57 (m, 2H, CH_2); 1.48 (s, 3H, CH_3); 2.49-2.57 (m, 1H, CH); 2.73 (d, 1H, CH J = 7.2Hz); 3.10 (d, 1H, CH J = 6.4Hz); 4.18 (m, 2H, CH_2); 5.22 (t, 2H, =CH_2 J = 14.8Hz); 5.82-5.90 (m, 1H, CH=); 5.97 (d, 1H, CH J = 6.4Hz); 6.05 (d, 1H, CH J = 6.4Hz); 6.34 (s, 1H, NH); 7.85 (s, 1H, NH). *Anal.* Calcd for $C_{18}H_{25}O_3N_3S$ (347.47g): C 62.22, H 7.25, N 12.09. Found: C 62.59, H 7.24, N 12.25.

d3 m.p. 198^{0}C, 75%; ^{1}H NMR ($CDCl_3$) δ(ppm): 0.87 (d, 3H, CH_3 J = 6.8Hz); 1.05 (d, 3H, CH_3 J = 5.2Hz); 1.18-1.27 (m, 2H, CH_2); 1.38 (s, 3H, CH_3); 1.43-1.50 (m, 2H, CH_2); 2.36-2.38 (m, 1H, CH-CH_3); 2.65 (d, 1H, C_{3a} J = 8Hz); 3.01 (d, 1H, C_{7a} J = 8Hz); 4.63-4.76 (m, 2H, CH_2); 5.45 (d, 2H, C_5, C_6 J = 13.2Hz); 6.2 (s, 1H, NH); 7.26-7.34 (m, 5H, $C_{arom.}$); 7.77 (s, 1H, NH). *Anal.* Calcd for $C_{20}H_{25}O_2N_3S$ (397.53g): C 66.47, H 6.85, N 10.57. Found: C 66.47, H 7.13, N 10.66.

d4 m.p. 144-145^{0}C, 62%; ^{1}H NMR ($CDCl_3$) δ(ppm): 1.01 (d, 3H, CH_3 J = 6.8Hz); 1.12 (d, 3H, CH_3 J = 6.8Hz); 1.26-1.34 (m, 2H, CH_2); 1.51 (s, 3H, CH_3); 1.51-1.6 (m, 2H, CH_2); 2.53-2.58 (m, 1H, CH-CH_3); 2.78 (d, 1H, C_{3a} J = 7.2Hz); 3.15 (d, 1H, C_{7a} J = 6.4Hz); 6.07 (m, 2H, C_5, C_6); 7.21-7.485 (m, 5H, $C_{arom.}$); 9.22 (s, 1H, NH); 11.87 (s, 1H, NH). *Anal.* Calcd for $C_{20}H_{23}O_3N_3S$ (411.51g): C 64.21, H 6.12, N 10.21. Found: C 63.90, H 6.21, N 9.95.

k1 m.p. 171^{0}C, 66%; ^{1}H NMR ($CDCl_3$) δ(ppm): 0.61 (d, 3H, CH_3 J = 6.4Hz); 1.34 (s, 6H, CH_3); 1.48 (s, 6H, CH_3); 1.54 (q, 1H, CH); 2.87 (s, 2H, CH); 3.49 (s, 1H, NH_2). *Anal.* Calcd for $C_{14}H_{20}O_2N_2$ (248.32g): C 67.71, H 8.12, N 11.18. Found: C 67.58, H 8.03, N 11.31.

k2 m.p. 136-137 ^{0}C, 63%; ^{1}H NMR ($CDCl_3$) δ(ppm): 0.61 (d, 3H, CH_3 J = 8Hz); 1.34 (s, 6H, CH_3); 1.41 (s, 6H, CH_3); 1.53 (m, 1H, CH); 2.87 (m, 2H, CH); 4.24 (s, 2H, CH_2); 5.20 (t, 2H, =CH J = 14Hz); 5.87-5.82 (m, 1H, CH=); 6.12 (s, 1H,

NH); 7.68 (s, 1H, NH). *Anal.* Calcd for $C_{18}H_{25}O_2N_3S$ (447.47g): C 62.22, H 7.25, N 12.09. Found: C 62.21, H 7.21, N 12.0.

k3 m.p. 104^{0}C, 83%; ^{1}H NMR ($CDCl_3$) δ(ppm): 0.57 (d, 3H, CH_3 J = 8Hz); 1.32 (s, 6H, CH_3); 1.38 (s, 6H, CH_3); 1.41-1.48 (m, 1H, CH); 2.78 (s, 2H, CH); 4.82 (d, 2H, CH_2 J = 4Hz); 6.10 (s, 1H, NH); 7.29-7.35 (m, 5H, $C_{arom.}$); 7.48 (s, 1H, NH). *Anal.* Calcd for $C_{23}H_{33}O_2N_3S$ (415.59g): C 66.47, H 8.0, N 10.11. Found: C 66.07, H 7.89, N 10.07.

k4 m.p. 110^{0}C, 64%; ^{1}H NMR ($CDCl_3$) δ(ppm): 0.62 (d, 3H, CH_3 J = 6.4Hz); 1.35 (s, 6H, CH_3); 1.55 (s, 6H, CH_3); 1.57-1.65 (m, 1H, CH); 3.07 (s, 2H, CH); 7.52-7.84 (m, 5H, $C_{arom.}$); 9.27 (s, 1H, NH); 11.70 (s, 1H, NH). *Anal.* Calcd for $C_{23}H_{31}O_3N_3$ (429.57g): C 64.31, H 7.27, N 9.78. Found: C 64.21, H 7.21, N 9.70.

The antiviral investigations for the compounds were performed in Dipartamento di Scienze e Tecnologie Biomediche, Universita di Cagliari, Monserato, Italy.

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

Cells and Viruses. MT-4 cells were grown at 37^{o}C in a 5% CO_2 atmosphere in RPMI 1640 medium, supplemented with 10% fetal calf serum (FCS), 100IU/mL penicillin G, and 100μg/mLstreptomycin. Cell cultures were checked periodically for the absence of mycoplasma contamination wuth a Myco Tect 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 $4.5x10^6$ and $1.4x10^5$ 50% cell culture infectious dose ($CCID_{50}$)/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 cytopathicity in MT-4 cells. Briefly, 50μL of culture medium containing 1x 10^4 cells was added to each well of flat-bottom microtiter trays containing 50μL of culture medium with or without various concentrations of the test compounds. Then 20μL of an HIV suspention containing 100 (HIV-1) was added. After 4-day incubation at 37^{o}C, the number of viable cells was determined by the 3-(4,5-dimethylthiazol-1-yl)-2,5-diphenyltetrazolium bromide (MTT) method (6). 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.

Table 2. Cytotoxicity and anti-HIV activity of h1-h4a, d1-d6a, k1-k6a compounds.

Compound	aCC50	bEC50
	MT-4	HIV-1
h1	>100	>100
d1	>100	>100
d2	50	50
d4	>100	>100
k1	>100	>100
k3	20	>20
k4	>100	>100
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 cytopathogeneticy, as determined by the MTT method

3. RESULTS AND DISCUSSION

12 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 synthesized compounds showed any activity against HIV-1. Compound **k4** has very low cytotoxicity.

4. REFERENCES

[1] A. Gładysz, *Współczesne leczenie zakażenia HIV i AIDS*. Volumed, Wrocław, 2000
[2] Ding J., Das K., Moereels H., Kaymans L., Andries K., Janssen P.A.J., Huges S.H., Arnold E., *Nat. Struct. Biol*., 2, 407 (1995)
[3] De Clercq E., *Clin. Microbiol. Rev*., 10, 674, (1997)
[4] Kossakowski J., Kuśmierczyk J., *Pharmazie*, 55, 1, (2000)
[5] Zawadowski T., Kossakowski J., *Polish pat.* 168611, (1994)
[6] Pauwels P., Balzarini J., Baba M., Snoeck R., Schols D., Herdewijn P., Desmyster J., De Clercq E., *J. Virol. Methods* 20, 309, (1988)

CURRICULA VITAE

Professor Jerzy Kossakowski was born in 1943. He studied at Warsaw University. In 1967 he obtained M.Sc. title, and started to work as scientific assistant in the Chair and Department of General Chemistry, the Medical University in Warsaw. In 1975 he presented the thesis "Synthesis of new derivatives of isovisnagine and khellin with expected pharmacological activity" and obtained the Ph.D. in pharmacy. Synthesis in the field of new derivatives of coumarins, benzofurans and benzopirans resulted in many papers and habilitation "Searching for new compounds affecting the circulation system – in the group of derivatives of furobenzopiranone, benzofuran and benzopiranone" presented in 1989. In April 1993 was appointed to an Assistant Professor post at the Ist Faculty of Medicine, the Medical University of Warsaw.
Scientific activity of Professor comprises investigation of relationship between pharmacological activity and chemical structure of anxiolytic, antidepressants and β-blockers. Professor's scientific output consists of 70 papers, 7 patents and 100 communications. Professor Kossakowski is a member of the Polish Pharmaceutical Society.

Marta Struga was born in Dwikozy in 1971. She studied Chemistry (1990–1995) at Maria Curie-Skłodowska University in Lublin and was graduated in 1995 receiving M.Sc. Then she started to work as scientific assistant in Chair and Department of Organic Chemistry, Faculty of Pharmacy of Medical University in Lublin. In 2001 she presented the thesis "Synthesis of 1,2,4-triazole derivatives in the nucleophilic substitution reactions" and obtained the Ph.D. in pharmacy. In 2001 she started to work as a lecturer in Chair and Department of Medical Chemistry, the Medical University in Warsaw. Fields of interest: organic synthesis, synthesis of anxiolytic, antidepressive and β-adrenolytic compounds. During the time she was a co-author of 5 publications and 20 posters.