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New thiourea and 1,3-thiazolidin-4-one derivatives effective on the HIV-1 virus

Running title: Evaluation of benzothiazol thiourea derivatives

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Abstract

Thiourea derivatives have been reported to possess many biological activities, among them antiviral and antitumoral properties. As part of our continuing effort to develop new active compounds, we report the synthesis and the evaluation of new fifteen thiourea derivatives with 1,3-benzothiazol-2-yl moiety, among them a group of biologically active (**1-7**) also underwent cyclisation to 1,3-thiazolidin-4-ones. Molecular structure of four compounds (**4**, **13**, **15** and **3a**) was determined by an X-ray crystallography. We here report the evaluation of their cytotoxicity against human leukaemia/lymphoma- and solid tumour-derived cell lines and of their antiviral activity against HIV-1 and representatives of ssRNA and dsDNA viruses. Derivative **5** showed an interesting activity against HIV-1 wild type and against variants carrying clinically relevant mutations. A colorimetric enzyme immunoassay clarified its mode of action as a non-nucleoside inhibitor of the reverse transcriptase.

Introduction

AIDS represents one of the major health problems worldwide, since that the WHO estimates for the 2015 approximately 36.7 million people living with Human Immunodeficiency virus (HIV-1) and 1.1 million people died from AIDS-related causes.

Antiretroviral therapy (HAART), actually based on combinations of drugs belonging to non-nucleoside reverse transcriptase (NNRTIs), nucleoside reverse transcriptase (NRTIs), protease (PR) and, more recently, integrase (IN) inhibitors, has improved the life quality and duration of AIDS patients. However, HIV-1 still remains an interesting biological target due to the side effects reported for clinically used drugs, to the rapid emergence of new drug-

resistant strains and to the difficulty to access the existing therapies for people in many countries [1,2].

Some thiourea compounds were reported to act as NNRTIs [3]. Their inhibitory activity is the result of binding into an allosteric hydrophobic pocket located near to the DNA polymerase active site, with the distortion of essential aspartyl residues [4]. Phenylethylthiourea compounds, such as the prototype thiazolylthiourea LY 73497, the its close derivative Trovirdine (TRV) and the new vaginal microbicide HI-443 are important representatives of this group (Figure 1) [5,6]. It was revealed that substitution of 2-pyridyl ring, as well as the presence of an ethyl spacer between phenyl and thiourea group are are important for the anti-HIV activity [7,8]. Another drug-candidate, an imidoylthiourea (ITU), in contrary to the voluminous and rigid structures of first-generation NNRTIs, has a flexible chemical arrangment based on an open chain. Thus it can adopt “horseshoe” or “U” conformation in the receptor binding site [9-11]. This torsional freedom enables to bind to a mutated NNRTI binding pocket (NNIBP), compensating the effects of these mutations. Since that the NNRTIs potency is considerably reduced by the development of viral strains with a mutated form of RT, the effective chemicals with high affinity for the mutated NNIBP are extensively designed. The thiourea derivatives are also recently discovered as HIV-1 capsid binders [12] or as inhibitors of the HIV-1 gp120-CD4 binding [13], offering potential additional targets to the combination chemotherapy.

Many other structural changes of 1,3-disubstituted (thio)ureas have been developed for novel potential antiviral agents. Bis(aryl)thioureas with amide functionality were considered as inhibitors of herpesviruses such as HSV, CMV and VZV [14]. A thiourea analogue with a pyridine-2-carboxamide moiety (ACH-806) has exhibited potent activity against the replication *in vivo* of genotype 1 HCV and in a proof-of-concept clinical trial [15]. The 2-methylbenzimidazol-1-ylthiourea derivative (MBZM-N-IBT) was found to inhibit the

Chikungunya virus (CHIKV) replication [16]. A benzimidazoleurea Frentizole (Figure 1) has been reported as immunosuppressive and antiviral agent [17]. Thiourea derivatives with substituted 1,2,4-triazole-3-thione group significantly inhibit Coxsackievirus B4 [18].

It was proved that the presence of (hetero)aromatic rings such as pyrazole [19], chloroquinoline [20], thiazole [21], acridine [22] or podophyllotoxin [23] at different positions of the thiourea branch, enhances cytotoxicity and anticancer properties of a compound. Recently, the combination of a halogeno-substituted benzothiazole with the thiourea moiety, as well as its oxidative cyclisation to N-bis-benzothiazoles, have delivered new potent antitumor agents, effective against human cancer cell lines [24].

1,3-Thiazolidinones, the products of the thiourea branch cyclisation, are a promising group of bioactive compounds, that exhibit diverse applications in medicinal chemistry, acting as anti-HIV [25], antimicrobial [26,27], antimalarial [28], antinflammatory [29] and anticancer agents [30,31]. The mechanism of anti-HIV action of studied 2,3-diaryl-1,3-thiazolidin-4-one derivatives is attributed to the inhibition of HIV-1 RT, with a simultaneous minimal toxicity to MT-4 cells [32,33]. Substituted 2-imino-4-thiazolidinones, derivatives of benzothiazole, are known for antiproliferative activity through their ability to inhibit heat shock protein 70 (Hsp70) [30]. Thiophene ring-containing thiazolidinones show anticancer properties *via* induction of apoptosis or contribution in cell cycle arrest in melanoma cells [31].

These findings encouraged us to go further with our ongoing studies on the thiourea derivatives, since that many biological properties have been already discovered by our team among this group of compounds [34-39]. In the present study we have synthesized new thiourea and thiazolidinone derivatives with 1,3-benzothiazol-2-yl scaffold. All synthesized compounds were tested for their anti-HIV activity, some of them also for other antiviral activities. Antiproliferative potential of compounds was also examined against human leukaemia/lymphoma- and solid tumour-derived cell lines.

Methods and Materials

Chemistry

The starting amine was commercially available (Koch-Light Laboratories Ltd). Isothiocyanates were supplied from Alfa Aesar or Sigma Aldrich. Organic solvents (acetonitrile, chloroform and methanol) were supplied from POCh (Polskie Odczynniki Chemiczne). Prior usage, dried acetonitrile was kept in crown cap bottles over anhydrous phosphorus pentoxide (Carl Roth). The NMR spectra were recorded on Varian VNMRS 300 Oxford NMR spectrometer, operating at 300 MHz (1H NMR) and 75.4 MHz (13C NMR). Chemical shifts (δ) were expressed in parts per million relative to tetramethylsilane used as the internal reference. Mass spectral ESI measurements were carried out on Waters ZQ Micro-mass instruments with quadruple mass analyzer. The spectra were performed in the negative or positive ion mode at a declustering potential of 40–60 V. The sample was previously separated on a UPLC column (C18) using UPLC ACQUITYTM system by Waters connected with DPA detector. Flash chromatography was performed on Merck silica gel 60 (230–400 mesh) using chloroform eluent.

Analytical TLC was carried out on silica gel F254 (Merck) plates (0.25 mm thickness).

The diffraction data for 4, 13 and 15 were collected at 120(2) K and at room temperature for 3a on a SuperNova diffractometer (Oxford Diffraction) equipped with the microfucus X-ray source (Cu Kα, λ = 1.54184 Å) and CCD detector. The CRYSALIS program system was used for data collection, cell refinement and data reduction. The data were corrected for Lorentz and polarization effects. A multi-scan absorption correction was applied. The structure was solved using direct methods implemented in the SHELXS-97, and refined by the full-matrix least-squares on F^2 with the SHELXL-97 program [40]. All non-H atoms were refined with anisotropic displacement parameters. The H-atoms attached to carbon were positioned geometrically and refined using the riding model with $U_{iso}(H) = a \cdot U_{eq}(C)$, where

a = 1.2, except for $-CH_3$ groups in 13 (*a* = 1.3), and in 4 and 3a (*a* = 1.5). The nitrogen bonded H-atoms were found in the difference-Fourier map and refined with isotropic displacement parameters. The final structural parameters can be retrieved from the Cambridge Structural Database (CSD) (deposition numbers: CCDC).

Preparation of 1-(1,3-benzothiazol-2-yl)-3-thiourea derivatives (1-15).

A solution of 1,3-benzothiazol-2-amine (0.0027 mol, 0.40 g) in dry acetonitrile (20 mL) was treated with appropriate isothiocyanate (mol. 1:1). The mixture was heated under reflux condenser for 30 h. After solvent evaporating, the residue was either crystallized from acetonitrile or purified by column chromatography (chloroform). The details of the synthesis of each compound are provided in the "*Supporting information"*.

Preparation of 2-(1,3-benzothiazol-2-ylimino)-1,3-thiazolidin-4-ones (1a–7a).

A solution of an appropriate thiourea derivative **1-7** (0.001 mol) was heated with chloroacetamide (0.003 mol) in dried acetonitrile (20 mL) for 36h. After the solvent has been evaporated, the solid residue was purified by column chromatography (chloroform). The details of the synthesis of each compound are provided in the "*Supporting information"*.

Cells and viruses

Cell lines were purchased from American Type Culture Collection (ATCC). Cell lines supporting the multiplication of RNA and DNA viruses were the following: CD4+ human T-cells containing an integrated HTLV-1 genome (MT-4); Baby Hamster Kidney (BHK-21) [ATCC CCL 10 (C-13) Mesocricetus auratus] and Monkey kidney (Vero 76) [ATCC CRL 1587 Cercopithecus Aethiops]. Human Immunodeficiency Virus type-1 (HIV-1) IIIB laboratory strain was obtained from the supernatant of the persistently infected H9/IIIB cells

(NIH 1983). Viruses representative of ssRNA+ were: i) Flaviviridae: yellow fever virus (YFV) [strain 17-D vaccine (Stamaril Pasteur J07B01)], ii) Picornaviridae: enterovirus B [coxsackie type B5 (CV-B5), strain Ohio-1 (ATCC VR-29)], and enterovirus C [poliovirus type-1 (Sb-1), Sabin strain Chat (ATCC VR-1562)]. Viruses representative of ssRNA- were: iii) Paramyxoviridae: human respiratory syncytial virus (RSV) [strain A2 (ATCC VR-1540)]; iv) Rhabdoviridae: vesicular stomatitis virus (VSV) [lab strain Indiana (ATCC VR 1540)]. DNA virus representatives were: v) Poxviridae: vaccinia virus (VV) [vaccine strain Elstree-Lister (ATCC VR-1549)]; vi) Herpesviridae: human herpes 1 (HSV-1) [strain KOS (ATCC VR-1493)].

Mutants carrying NNRTI mutations used: Y181C mutant (NIH N119) of HIV-1 derives from an AZT-sensitive clinical isolate passaged initially in CEM and then in MT-4 cells, in the presence of Nevirapine (up to 10 µM); K103N + Y181C mutant (NIH A17) derives from an IIIB strain passaged in H9 cells in the presence of BI-RG 587 (up to 1 µM); K103R + V179D + P225H mutant (EFVR) derives from an IIIB strain passaged in MT-4 cells in the presence of Efavirenz (up to 2 µM). Mutants carrying NRTI mutations used: AZTR strain (67N, 70R, 215F, 219Q); MDR strain (74V, 41L, 106A, 215Y).

Cytotoxicity assays

Exponentially growing MT-4 cells were seeded at an initial density of $4x10^5$ cells/ml in 96-well plates in RPMI-1640 medium, supplemented with 10% fetal bovine serum (FBS), 100 units/mL penicillin G and 100 µg/mL streptomycin. BHK cells were seeded in 24-well plates at an initial density of $6x10^5$ and $1x10^6$ cells/mL, respectively, in Minimum Essential Medium with Earle's salts (MEM-E), L-glutamine, 1mM sodium pyruvate and 25mg/L kanamycin, supplemented with 10% fetal bovine serum (FBS). Vero-76 cells were seeded in 24-well plates at an initial density of $4x10^5$ cells/mL, in Dulbecco's Modified Eagle Medium

(D-MEM) with L-glutamine and 25mg/L kanamycin, supplemented with 10% FBS. 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 48-96 hrs at 37 °C by MTT method for MT-4 and BHK [41]. Cell viability was determined after 48-96 hrs at 37 °C by the crystal violet staining method for Vero-76.

Antiviral assays

Compound's activity against HIV-1 was based on inhibition of virus-induced cytopathogenicity in exponentially growing MT-4 cell acutely infected with a multiplicity of infection (m.o.i.) of 0.01. Briefly, 50 µL of RPMI containing $1x10^4$ MT-4 cells were added to each well of flat-bottom microtitre trays, containing 50 µL of RPMI without or with serial dilutions of test samples. Then, 20 µL of a HIV-1 suspension containing 100 CCID50 were added. After a 4-day incubation at 37 °C, cell viability was determined by the MTT method. Compound's activity against YFV was based on inhibition of virus-induced cytopathogenicity in BHK-21 cells acutely infected with a m.o.i. of 0.01. Briefly, BHK cells were seeded in 96-well plates at a density of $5x10^4$ cells/well 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 maintenance medium [MEM-Earl with L-glutamine, 1mM sodium pyruvate and 0.025g/L kanamycin, supplemented with 0.5% inactivated FBS]. At the same time, 50 µL of maintenance medium, without or with serial dilutions of test compounds, were added. After a 3 day incubation at 37°C, cell viability was determined by the MTT method. Compound's activity against CV-B5, Sb-1, VV, VSV, HSV-1 and RSV was determined by plaque reduction assays in infected cell monolayers. Briefly, Vero-76 monolayers were infected for 2 hours with 250 µL of proper virus dilutions; following removal of unadsorbed virus, 500 µL

of maintenance medium containing 0.75% methyl-cellulose, without or with serial dilutions of test compounds, were added. Cultures were incubated at 37°C for 2 (Sb-1 and VSV), 3 (CV-B5, 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. The extent of cell growth/viability and viral multiplication, at each sample concentration tested, were expressed as percentage of untreated controls. Concentrations resulting in 50% inhibition (CC_{50} or EC_{50}) were determined by linear regression analysis.

Reverse transcriptase Assay

The colorimetric Reverse Transcriptase Assay (Roche, Cat. 11468120910) was used for the quantitative determination of retroviral reverse transcriptase activity, using efavirenz as reference compound.

Antiproliferative assays

Cell lines derived from human haematological tumours were: CD4+ human T-cells containing an integrated HTLV-1 genome (MT-4); CD4+ human acute T-lymphoblastic leukaemia (CCRF-CEM); human splenic B-lymphoblastoid cells (WIL-2NS); human acute B-lymphoblastic leukaemia (CCRF-SB). Cell lines derived from human solid tumours were: human lung carcinoma (SK-MES-1); prostate carcinoma (DU-145); human cervix adenocarcinoma (Hela). Normal tissues foreskin fibroblasts (CRL-7065) were also used.

Cells were seeded at an initial density of 1×10^5 cells/ml in 96 well plates in RPMI-1640 medium supplemented with 10% fetal calf serum (FCS), 100 units/ml penicillin G and 100 μg/ml streptomycin. Cell cultures were then incubated at 37 °C in a humidified, 5% CO_2 atmosphere in the absence or presence of serial dilutions of test compounds. Cell viability was determined after 96 hrs at 37 °C by the MTT method.

Results

The synthetic route for the preparation of title derivatives of 1,3-benzothiazol-2-amine is shown in the Figure 2. First, the starting compound was heated with appropriate isothiocyanates in acetonitrile medium to give 1-(1,3-benzothiazol-2-yl)thiourea derivatives **1-15**. To provide structural diversity, isothiocyanates with aryl (**1-4**, **6**, **7**, **9-12, 15**), alicyclic (**8**), alkyl (**13**) or alkylaryl (**5**, **14**) substituents were introduced. In the second step, previously obtained biologically active (cytotoxic) compounds **1-7** was directly cyclized to the corresponding iminothiazolidinones **1a-7a** by treating these intermediates with chloroacetamide in acetonitrile. According to literature data [42-45], this synthetic route usually gives a mixture of two isomeric forms, that differ in substitutions at thiazolidinone N2 and N3 nitrogen atoms (Figure 3). Under reaction conditions proposed in this paper, the cyclization led to the formation of one product only, the 2-iminothiazolidin-4-one derivative. This regioselectivity could be explained by the use of the polar aprotic solvent (acetonitrile) and by the reactive halo acetic acid derivative as a substrate, as well as high temperature of the reaction. Finally, the nature of both terminal functionalities at the nitrogen atoms determined the type of the product - bulky polar benzothiazole ring prefers substitution at N2 of the 2-imino-4-thiazolidinone [43].

The structures of newly synthesized compounds were verified by spectral analyses, and molecular structure of the molecules **4**, **13** and **15**, and of the 2-imino-4-thiazolidinone derivative **3a** was confirmed by an X-ray crystallography (Figures 4 and 5). The conformation adopted by the molecules of **4**, **13** and **15** is stabilized by the intramolecular N-H...N hydrogen bond between the Z oriented benzothiazole and thiourea fragments (see Table 1S of *Supporting information*). This hydrogen bonding causes coplanarity of thiourea and thiazole fragments, and exposes the N1-H, S1 and S2 atoms for intermolecular contacts, e.g. hydrogen bonds with receptors.

The antiviral activity of newly synthesized thiourea and thiazolidinone derivatives was evaluated against HIV-1 in cell based assay. In parallel the cytotoxicity was investigated against the cell line supporting the multiplication of HIV-1, the CD4+ human T-cells derived from human haematological tumours, containing an integrated HTLV-1 genome (MT-4). Some derivatives were also tested their antiviral potency against representative of several RNA and DNA virus families. Results are reported in the Table 1.

The 3-(1-phenylethyl)thiourea (**5**) showed anti-HIV-1 activity (with EC_{50} value of 1.0 μM), with a moderate cytotoxicity against the MT-4 cells. Since a critical issue in clinical management of HIV-1 disease is the development of drug resistant strains, compound **5** was further tested against a set of viruses possessing mutations that confer resistance to NRTI and NNRTI inhibitors, and that often appear during HAART therapy, reducing its effectiveness [46]. Interestingly, the activity against AZT^R and MDR strains is comparable with those of HIV-1 wild type, while it resulted less or not active against NNRTI-resistant mutants (Table 2). Results suggest its mode of action as an inhibitor of the reverse transcriptase.

The derivative **5** was tested in a colorimetric enzyme immunoassay to evaluate its ability to inhibit the reverse transcriptase of HIV-1. Results confirmed it as a good inhibitor of the reverse transcriptase, with a 60% of inhibition at 10μM and a 85% at 50μM (Figure 5). Regarding the other tested viruses, no relevant activities were found.

With the exception of **5**, none of the other tested compounds showed selective anti-HIV-1 activity; however compounds **1**, **2**, **3**, **4**, **6**, **7** turned out cytotoxic for exponentially growing MT4 cells in the low micromolar range ($CC_{50} \leq 10$ μM). The antiproliferative activity against CD4+ human T cell line derived from a haematological human tumor, prompted us to evaluate the antiproliferative activity of them also for a panel of other human haematological and solid tumours and for cell lines derived from normal human tissues (Table 1).

Interestingly, all tested compounds showed antiproliferative activity with CC_{50} values comparable to that obtained with MT-4 cells. Particularly derivatives **1**, **2**, **6** and **7** showed more interesting values, but they did not result highly selective, showing cytotoxic also against the "normal" CRL7065 cell lines, even if with values slightly lower.

Discussion

Novel 1,3-benzothiazol-2-yl derivatives of thiourea and their 2-iminothiazolidin-4-one analogs have been designed and synthesized. The 3-(1-phenylethyl)thiourea (**5**) showed a potent anti-HIV-1 activity, targeting the reverse transcriptase of HIV-1, while other derivatives (**1**, **2**, **3**, **4**, **6**, **7**) turned out cytotoxic for the exponentially growing MT4 cells. They also showed antiproliferative properties against cells derived from other human haematological and solid tumours. This could be a good starting point for the development of novel and more active compounds for the treatment of AIDS, for which there is a continued need.

A close overview of the structures of presented 2-aminobenzothiazoles (Table 1, Figure 2) revealed that their biological potency is strongly bound to the nature of the substituent of the thiourea. The anti-HIV activity of the compound **5** is directly influenced by its flexibility due to the presence of an alkylphenyl spacer, attached to the heteroaryl core. From this point of view, the compound is an analog of other known NNRTIs such as (hetero)aryletylthioureas LY 73497, Trovirdine , HI-443, as well as the (phenyl)thiazole connections in the mentioned LY 73497 or Frentizole (Figure 1). On the contrary, the higher rigidity of the other synthesized benzothiazoles resulted in their inactivity in antiviral tests.

A remarkable cytotoxicity was observed within the group of substituted phenylthioureas bearing electron-withdrawing (**1**, **2**, **2a**, **6**, **7**) or weakly electron-donating (**3**, **4**) functionalities on the benzene ring. On the contrary, the introduction of electronegative atoms

such as chlorine or fluorine (compounds **9**, **10**, **15**) or the adding of strongly electron-donating groups to a phenyl ring (**11**) led to the reduction of cytotoxicity of 2-aminobenzothiazole derivatives. The same effect was achieved by connection of the thiourea branch with an alkyl (**13**, **14**) or alicyclic (**8**) group.

The cyclization of the compounds **1-7** resulted in an overall decrease of the biological activities. Indeed, anti-HIV activity of **5** is totally lost in the derivative **5a** and the compounds **1a-7a** have lost their cytotoxicity on the MT4 cells. Only **2a** remains cytotoxic even if with a lower value (CC_{50} from 1.6 to 9 μM). Compound **2a** seems to be less cytotoxic also in the other antiproliferative assays, when compared with others and with its linear analog **2**; interestingly it showed no cytotoxicity against the normal cells CRL7065.

Obtained results are in contrary to our previous reports on this class of compounds [31, 34], in which the highest activity was observed for mono- and dihalogenothioureas.

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Supporting information

A supporting information file is available and contains: the synthetic details of the preparation of 1-(1,3-benzothiazol-2-yl)-3-thiourea derivatives (**1-15**) and of 2-(1,3-benzothiazol-2-ylimino)-1,3-thiazolidin-4-ones (**1a–7a**); the geometry of the intramolecular N-H...N hydrogen bonds in molecules **4**, **13** and **15** (Table 1S); the 1H NMR and 13C NMR spectra of all newly synthesized derivatives.

Figure legends

Figure 1. Chemical structures of (thio)urea derivatives with antiviral activity.

Figure 2. Scheme of the synthesis of thioureas **1-15** and 1,3-thiazolidin-4-one derivatives **1a-7a**.

Figure 3. Regioselectivity of the 2-iminothiazolidin-4-one synthesis.

Figure 4. Perspective view of molecules **4** (two molecules in the asymmetric unit, A) and **13** (B).

Figure 5. Perspective view of molecules **15 (**A**)** and **3a (**B**).**

Figure 6. % of Reverse Transcriptase inhibition of derivative **5** and efavirenz (as reference compound), used at different concentrations in a colorimetric enzyme immunoassay. Results represent the mean ± S.D. of three independent determinations.

Acknowledgements

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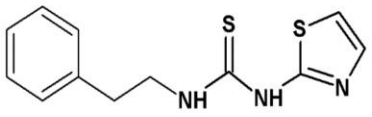
Table 1. Antiviral activity of derivatives against RNA and DNA viruses and cytotoxicity against: the cell lines used in the assays; human leukaemia/lymphoma-derived cell lines; solid tumour-derived cell lines.

	HIV-1	MT-4 cells	YFV	BHK cells	CV-B5, Sb-1, RSV, VSV, VV, HSV-1	Vero-76 cells	CCRF-CEM	WIL-2NS	CCRF-SB	SK-MES1	DU145	Hela	CRL7065
	EC_{50}[a]	CC_{50}[b]	EC_{50}[c]	CC_{50}[d]	EC_{50}[e]	CC_{50}[f]	CC_{50}[g]						
1	>8.4	8.4 ± 0.9	>100	>100	>76	76	8.9 ± 0.7	9.0 ± 0.8	6.6 ± 0.6	7.2 ± 0.6	8.0 ± 0.9	6.8 ± 0.9	14
2	>1.6	1.6 ± 0.1	>15	15	>12	12	2.8 ± 0.3	2.3 ± 0.3	1.7 ± 0.2	8.0 ± 0.7	2.8 ± 0.2	9.9 ± 1.1	11
3	>8.6	8.6 ± 0.5	-	-	-	-	9.6 ± 0.9	10	8.9 ± 1.0	11	11	26	11
4	>9.7	9.7 ± 1.0	>9	9 ± 0.8	>12	12	11	13	8.7 ± 0.8	18	13	22	25
5	**1.0 ± 0.1**	38	>100	>100	>100	>100							
6	>9.5	9.5 ± 0.9	>100	>100	>100	>100	4.0 ± 0.6	9.0 ± 1.0	4.8 ± 0.5	15	6.6 ± 0.9	14	28
7	>5.8	5.8 ± 0.6	-	-	-	-	9.0 ± 0.8	9.0 ± 1.4	7.4 ± 0.6	8.5 ± 0.8	6.8 ± 0.7	8.6 ± 0.5	20
8	>46	46	-	-	-	-							
9	>14	14	-	-	-	-							
10	>15	15	>100	>100	>100	>100							
11	>68	68	>100	>100	>100	>100							
12	>100	>100	>100	>100	>2	2.0 ± 0.1							
13	>100	100	>100	>100	>80	80							
14	>67	67	-	-	-	-							
15	>27	27	-	-	-	-							
1a	>100	100	-	-	-	-							
2a	>9.0	9.0 ± 0.9	-	-	-	-	25 ± 3.0	17 ± 2.5	9.5 ± 1.5	53	50	53	>100
3a	>86	86	-	-	-	-							
4a	>100	>100	-	-	-	-							
5a	>84	84	-	-	-	-							
6a	>29	29	-	-	-	-							
7a	>67	67	-	-	-	-							
References													
Efavirenz	0.002 ± 0.0002	40											
2'-C-methyl-guanosine			1.9 ± 0.1	>10									
Vincristine							0.003 ± 0.005	0.003 ± 0.005	0.002 ± 0.003	0.01 ± 0.002	0.01 ± 0.002	0.07 ± 0.005	>20

Data represent mean values for three independent determinations. Standard deviations are reported for the more active compounds. Also for the others values the variation was less than 15%.

[a]Compound concentration (μM) required to achieve 50% protection of MT-4 cells from HIV-1 induced cytopathogenicity, determined by the MTT method. [b]Compound concentration (μM) required to reduce the proliferation of mock-infected MT-4 cells by 50%, determined by the MTT method. [c]Compound concentration (μM) required to achieve 50% protection of BHK cells from YFV-induced cytopathogenicity, determined by the MTT method. [d,f]Compound concentration (μM) required to reduce the viability of mock-infected BHK[(d)] and Vero-76[(f)] cells by 50%, determined by the MTT method. [e]Compound concentration (μM) required to reduce the plaque number of CV-B5, Sb-1, RSV, VSV, VV, HSV-1 by 50% in VERO-76 monolayers. [g]Compound concentration (μM) required to reduce cell proliferation by 50%, as determined by the MTT method, under conditions allowing untreated controls to undergo at least three consecutive rounds of multiplications.



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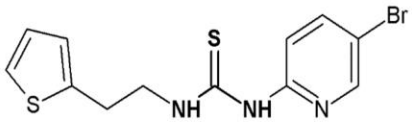

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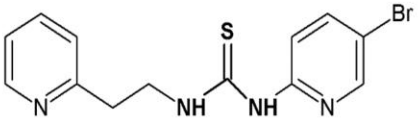

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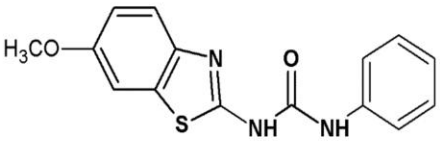

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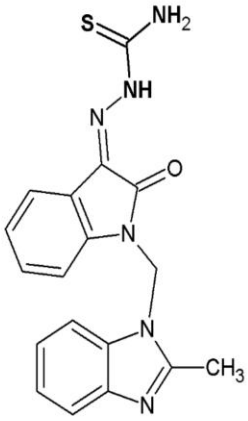

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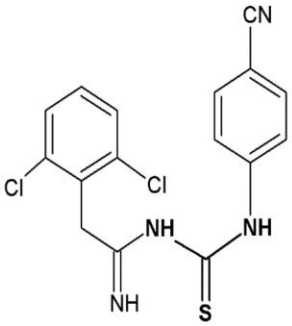

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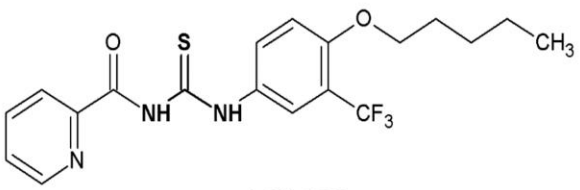

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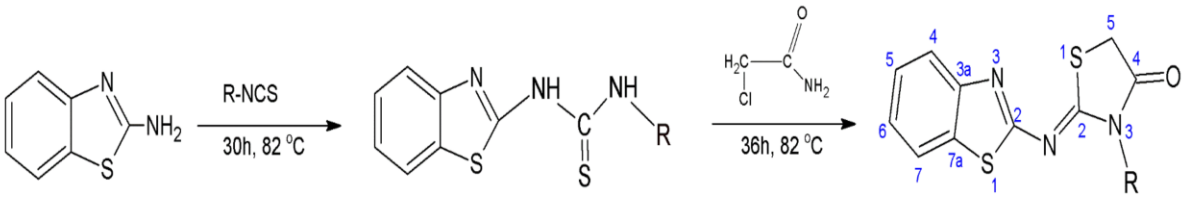

1-15 **1a-7a**

R:	4-CN-Ph (**1**,**1a**)	norbornyl (**8**)
	2-Br-Ph (**2**,**2a**)	3-Cl-4-F-Ph (**9**)
	4-C_4H_9-2-CH_3-Ph (**3**,**3a**)	3-Cl-4-Cl-Ph (**10**)
	4-CH_3-Ph (**4**,**4a**)	4-OCH_3-Ph (**11**)
	-$CH(CH_3)$-Ph (**5**,**5a**)	4-NO_2-Ph (**12**)
	4-I-Ph (**6**,**6a**)	metallil (**13**)
	3-CF_3-Ph (**7**,**7a**)	methylfuryl (**14**)
		2-F-Ph (**15**)