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Original article

Synthesis of some novel thiourea derivatives obtained from 5-[(4-aminophenoxy)methyl]-4-alkyl/aryl-2,4-dihydro-3*H*-1,2,4-triazole-3-thiones and evaluation as antiviral/anti-HIV and anti-tuberculosis agents

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Abstract

As a continuation of our previous efforts on *N*-alkyl/aryl-*N*′-[4-(4-alkyl/aryl-2,4-dihydro-3*H*-1,2,4-triazole-3-thione-5-yl)phenyl]thioureas **1**–**19** and *N*-alkyl/aryl-*N*′-[4-(3-aralkylthio-4-alkyl/aryl-4*H*-1,2,4-triazole-5-yl)phenyl]thioureas **20**–**22**, a series of novel 5-[(4-aminophenoxy)-methyl]-4-alkyl/aryl-2,4-dihydro-3*H*-1,2,4-triazole-3-thiones **23**–**26** and several related thioureas, *N*-alkyl/aryl-*N*′-{4-[(4-alkyl/aryl-5-thioxo-4,5-dihydro-1*H*-1,2,4-triazol-3-yl)methoxy]phenyl}thioureas **27**–**42** were synthesized for evaluation of their antiviral potency. Structures of the synthesized compounds were confirmed by the use of ^{1}H NMR, ^{13}C NMR and HR-MS data. All compounds **1**–**42** were evaluated in vitro against HIV-1 (IIIB) and HIV-2 (ROD) strains in MT-4 cells, as well as other selected viruses such as HSV-1, HSV-2, Coxsackie virus B4, Sindbis virus and Varicella-zoster virus using HeLa, Vero, HEL and E6SM cell cultures, and anti-tuberculosis activity against *Mycobacterium tuberculosis* H37Rv. Compounds **4** and **5** showed weak activity against HSV-1, HSV-2 and TK^{-} HSV, whereas eight compounds showed marginal activity against Coxsackie virus B4. The most active derivative in this series was compound **38** which showed moderate protection against Coxsackie virus B4 with an MIC value of 16 μg/ml and a selectivity index of 5. This compound was also active against thymidine kinase positive Varicella-zoster virus (TK^{+} VZV, OKA strain) with an EC_{50} value of 9.9 μg/ml. Compound **38** was the most active compound with 79% inhibition against *M. tuberculosis* H37Rv.

Keywords: 1,2,4-Triazole; Thioureas; Antiviral activity; Anti-tuberculosis activity

1. Introduction

Although combination therapies have been proven to decrease HIV-related mortality, there is still a need for development of novel antiretroviral agents due to the emergence of multi-drug resistance, which is a major challenge to successful therapy for individuals infected with HIV. An essential step for the replication of the virus involves reverse transcription of retroviral RNA to proviral DNA by the enzyme reverse transcriptase (RT). In contrast to nucleoside analogues which inhibit HIV-1 RT competitively at the substrate binding site, non-nucleoside reverse transcriptase inhibitors (NNRTIs) exhibit their action by binding to a specific allosteric site, thereby resulting in non-competitive inhibition of this enzyme [1]. Examples of such non-nucleoside inhibitors of HIV-1 RT may include widely diverging chemical classes such as dipyridodiazepinones (nevirapine), bis-heteroarylpiperazines (delavirdine, U-90152), benzoxazinones (efavirenz; DMP-266), TIBO derivatives (9-Cl TIBO), 1-[(2-hydroxyethoxy)-methyl]-6-phenylthio)thymine (HEPT-S), α-anilinophenylacetamides (α-APAs) and phenylethylthiazolylthiourea (PETT) derivatives (LY73497 and trovirdine HCl) (Fig. 1) [2,3].

A literature survey revealed that molecular modeling studies for the non-nucleoside inhibitor (NNI)-binding pocket of

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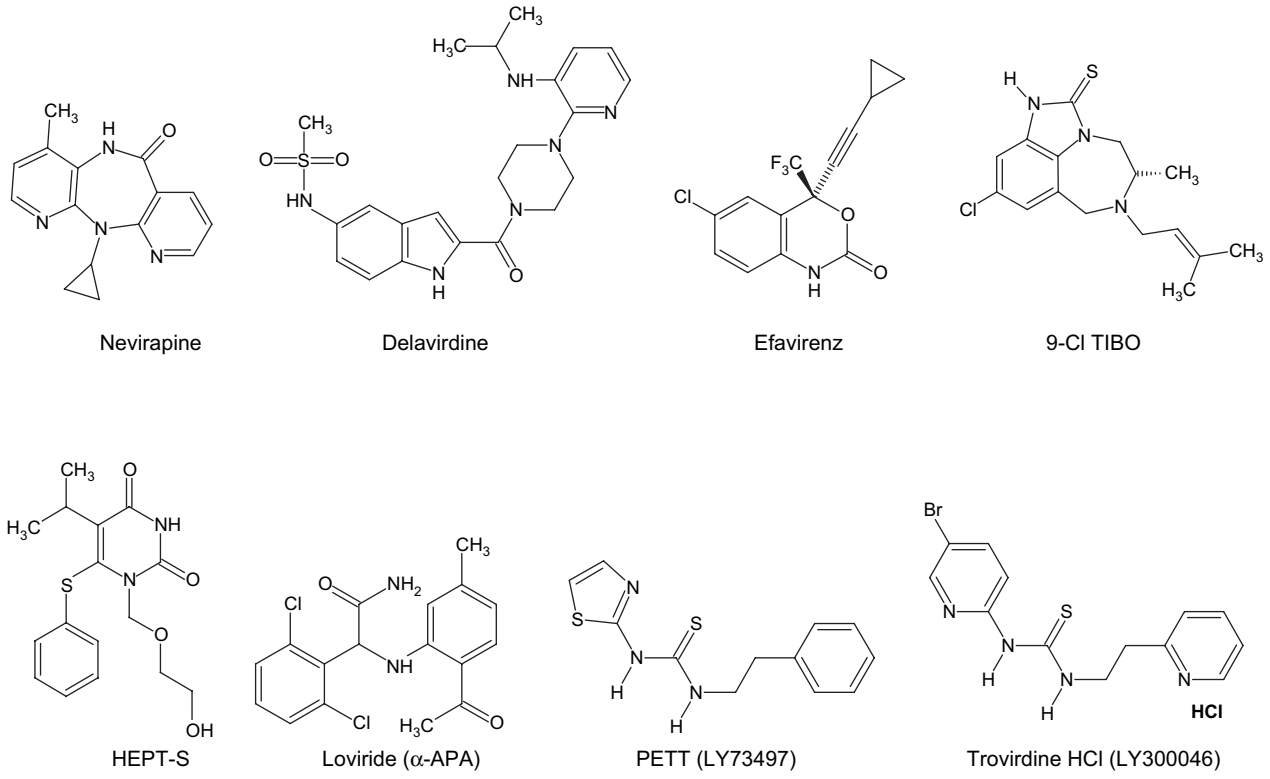


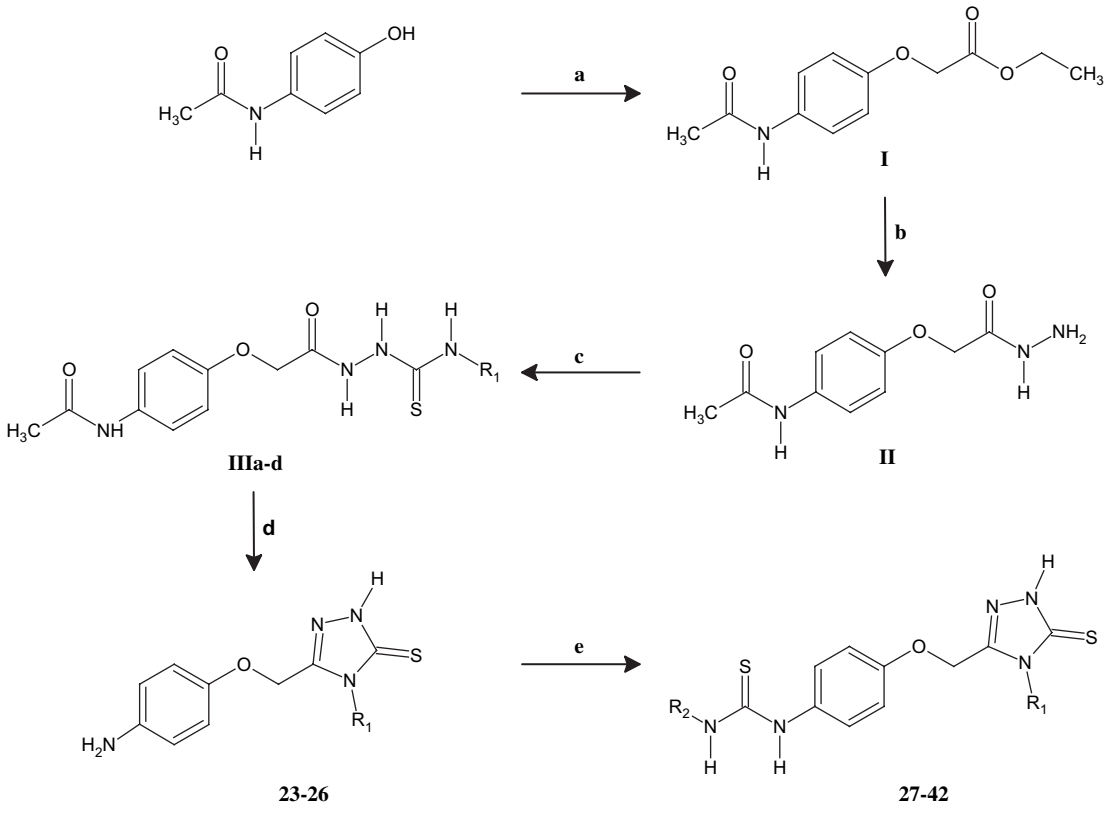
Fig. 1. Structures of the NNRTIs from different chemical classes.

HIV-1 RT might be an effective approach for new drug development. The need for a butterfly-like conformation in order for a compound to inhibit HIV-1 RT enzyme was first demonstrated using X-ray crystallographic data obtained from nevirapine [4]. As a result of such pharmacophoric modeling studies on the structures of existing NNRTIs, Bal et al. [5] recently reported a 3D pharmacophoric distance map which describes the structural requirements for a compound to show anti-HIV activity via inhibition of HIV-1 RT [6].

Ahgren et al. [7] reported a new class of NNRTIs, namely phenyl ethyl thiazolyl thiourea (PETT) analogues (Fig. 1). Discovery of these derivatives as potent inhibitors of HIV-1 was first reported by Bell et al. [8]. In this systematic search, pharmacophores essential for antiretroviral activity were identified. Disconnection of the bonds present in rigid tricyclic nucleus of the TIBO derivative led to the generation of simpler, open-chained structures, PETT (LY 73497) and trovirdine (LY300046). Many PETTs and analogous thiourea derivatives have been identified as NNRTIs [9–17]. There is an increasing concern on thioureas as some of them also have been reported as antiviral agents against several Herpes viruses such as HSV [18], VZV [19,20] and CMV [21–23].

In a previous study [24], we have synthesized a series of thioureas **1–22** (Fig. 2), in which a nitrogen-containing heterocycle is combined with thiourea moiety as in the case of non-nucleoside reverse transcriptase inhibitors (NNRTIs) phenylethylthiazolylthiourea (PETT) derivative LY 73497 and trovirdine (LY300046). The aim of the present work was to investigate antiviral properties of **1–22** and to modify their structure in the light of antiviral activity results of **1–22**. Based on the experience with this type of molecules, a certain degree of flexibility might be required for binding to HIV-1 RT. Following these observations, we have designed and synthesized compounds **23–42** in which more flexibility is ascertained by replacing the phenyl ring at the fifth position of 1,2,4-triazole ring with a phenoxymethyl moiety.

Tuberculosis is a chronic infection disease caused by several species of mycobacteria. The incidence of tuberculosis is increasing worldwide, partly due to poverty and inequity and partly to the HIV/AIDS pandemic, which greatly increases the risk of infectious diseases. During recent years, *Mycobacterium tuberculosis* has developed increased resistance against drugs. Some of these derivatives **1–3** and **21** have been reported to possess positive response against *M. tuberculosis* H37Rv [24]. Thiacetazone which possesses a thiosemicarbazone structure, has been reported as a tuberculostatic agent. Doub and coworkers [25] revealed anti-tuberculosis properties for a wide number of phenylthioureas. Isoxyl (ISO), a thiourea (thiocarlide; 4,4′-diisoamyloxythiocarbanilide), was reported to exhibit potent activity against *M. tuberculosis* H37Rv (MIC, 2.5 μg/ml), *Mycobacterium bovis* BCG (MIC, 0.5 μg/ml), *Mycobacterium avium* (MIC, 2.0 μg/ml), and *Mycobacterium aurum* A1 (MIC, 2.0 μg/ml), resulting in complete inhibition of mycobacteria [26]. It was also reported that combination therapy of INH (isonicotinic acid hydrazide) and ISO was more effective than monotherapy with either drug. In earlier studies, 3-thioxo/alkylthio-1,2,4-triazole derivatives [24] *N*-phenyl-*N*′-[4-(5-alkyl/arylamino-1,3,4-thiadiazole-2-yl)phenyl]thioureas [27] examined by the Tuberculosis Antimicrobial Acquisition

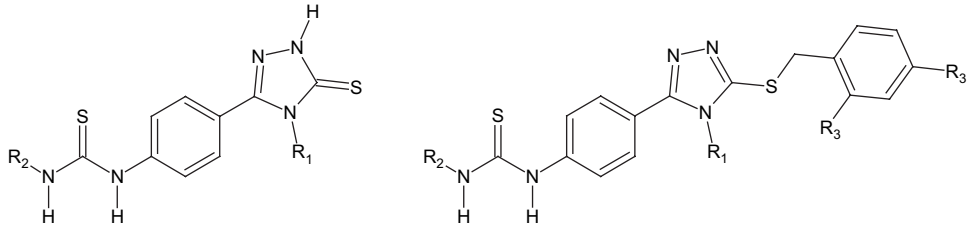


IIIa (R_1: CH_3);**IIIb** (R_1: C_2H_5); **IIIc** (R_1: $CH_2CH{=}CH_2$); **IIId** (R_1: C_6H_5);
23 (R_1: CH_3); **24** (R_1: C_2H_5); **25** (R_1: $CH_2CH{=}CH_2$); **26** (R_1: C_6H_5);
27 (R_1: CH_3; R_2: CH_3); **28** (R_1: CH_3; R_2: C_2H_5); **29** (R_1: CH_3; R_2: $CH_2CH{=}CH_2$); **30** (R_1: CH_3; R_2: C_6H_5);
31 (R_1: C_2H_5; R_2: CH_3); **32** (R_1: C_2H_5; R_2: C_2H_5); **33** (R_1: C_2H_5; R_2: $CH_2CH{=}CH_2$); **34** (R_1: C_2H_5; R_2: C_6H_5);
35 (R_1: $CH_2CH{=}CH_2$; R_2: CH_3); **36** (R_1: $CH_2CH{=}CH_2$; R_2: C_2H_5); **37** (R_1: $CH_2CH{=}CH_2$; R_2: $CH_2CH{=}CH_2$);
38 (R_1: $CH_2CH{=}CH_2$; R_2: C_6H_5); **39** (R_1: C_6H_5; R_2: CH_3); **40** (R_1: C_6H_5; R_2: C_2H_5); **41** (R_1: C_6H_5; R_2: $CH_2CH{=}CH_2$); **42** (R_1: C_6H_5; R_2: C_6H_5)

Scheme 1. Synthetic route to compounds **23–42**. *Reagents and conditions*: (**a**) Br–CH_2–COOEt/anhyd. K_2CO_3 – acetone, reflux; (**b**) $NH_2NH_2.H_2O$, reflux; (**c**) R_1–NCS/EtOH, reflux; (**d**) NaOH (2 N), reflux; (**e**) R_1–NCS/acetone, reflux.

and Coordinating Facility (TAACF), have been proved to possess considerable inhibition against *M. tuberculosis* H37Rv. These findings encouraged us to go further with our ongoing studies on thiourea derivatives and to evaluate anti-tuberculosis activity against *M. tuberculosis* H37Rv, besides antiviral activity.

This work describes synthesis and a wide spectrum antiviral activity screening of some novel 5-[(4-aminophenoxy)methyl]-4-alkyl/aryl-2,4-dihydro-3*H*-1,2,4-triazole-3-thiones **23–26** and several related thioureas, *N*-alkyl/aryl-*N*′-{4-[(4-alkyl/aryl-5-thioxo-4,5-dihydro-1*H*-1,2,4-triazol-3-yl)methoxy]phenyl}thioureas **27–42**, together with thiourea derivatives **1–22**



1 (R_1: CH_3; R_2: CH_3); **2** (R_1: CH_3; R_2: C_2H_5); **3** (R_1: CH_3; R_2: $CH_2CH{=}CH_2$); **4** (R_1: CH_3; R_2: C_6H_{11}); **5** R_1: (CH_3; R_2: C_6H_5);
6 (R_1: C_2H_5; R_2: $CH_2CH{=}CH_2$); **7** (R_1: C_2H_5; R_2: C_6H_{11}); **8** (R_1: $CH_2CH{=}CH_2$; R_2: CH_3); **9** (R_1: $CH_2CH{=}CH_2$; R_2: $CH_2CH{=}CH_2$); **10** (R_1: $CH_2CH{=}CH_2$; R_2: C_6H_5); **11** (R_1: C_6H_{11}; R_2: $CH_2CH{=}CH_2$); **12** (R_1: C_6H_{11}; R_2: C_6H_{11}); **13** (R_1: $CH_2CH_2C_6H_5$; R_2: $CH_2CH{=}CH_2$); **14** (R_1: C_6H_5; R_2: CH_3); **15** (R_1: C_6H_5; R_2: C_2H_5); **16** (R_1: C_6H_5; R_2: $CH_2CH{=}CH_2$); **17** (R_1: C_6H_5; R_2: C_6H_{11}); **18** (R_1: C_6H_5; R_2: C_6H_5); **19** (R_1: C_6H_5; R_2: C_6H_4Cl-*para*); **20** (R_1: CH_3; R_2: CH_3; R_3: H); **21** (R_1: CH_3; R_2: $CH_2CH{=}CH_2$; R_3: Cl); **22** (R_1: C_6H_5; R_2: $CH_2CH{=}CH_2$; R_3: Cl)

Fig. 2. General structure and substituents of compounds **1–22**, synthesized in a previous study [24].

synthesized previously [24]. Anti-tuberculosis activity of newly synthesized compounds **23**–**42** was also evaluated.

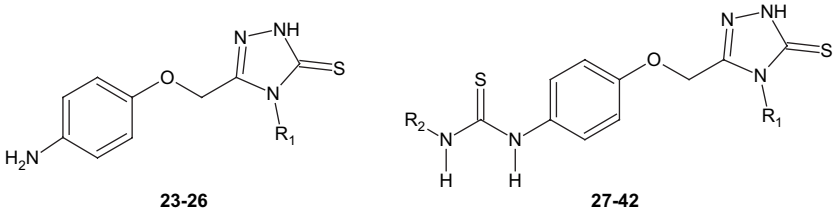
2. Chemistry

Synthesis of **23**–**46** required stepwise reactions starting with etherification of *N*-(4-hydroxyphenyl)acetamide with ethyl α-bromoacetate in the presence of anhydrous potassium carbonate using dry acetone as reaction medium to give compound **I**. The choice of *N*-(4-hydroxyphenyl)acetamide as the starting compound provided a protected amino functionality during the following two steps (Scheme 1) and allowed to introduce different groups at R_1 and R_2 positions. Hydrazinolysis of **I** with three-fold equimolar amount of hydrazine hydrate gave 2-[4-(acetylamino)phenoxy]acetohydrazide **II**, which was then reacted with alkyl or aryl isothiocyanates to yield 1-[2-(4-acetamidophenoxy)acetyl]-4-alkyl/aryl thiosemicarbazides **IIIa**–**d** as described. Cyclocondensation of compounds **IIIa**–**d** to give 5-[(4-aminophenoxy)methyl]-4-methyl-2,4-dihydro-3*H*-1,2,4-triazole-3-thione derivatives **23**–**26** was performed by refluxing **IIIa**–**d** in aqueous alkaline medium. During the ring closure, acetylamino group was hydrolyzed to give a primary amino function which provided a nucleophilic centre for the synthesis of disubstituted thiourea derivatives. The final step comprised the reaction of the amines **23**–**26** with various alkyl or aryl isothiocyanates in dry acetone yielding the corresponding title compounds 1-alkyl/aryl-3-{4-[(4-alkyl/aryl-5-thioxo-4,5-dihydro-1*H*-1,2,4-triazol-3-yl)-methoxy]-phenyl}thiourea derivatives **27**–**42** in yields between 41 and 88%. Synthetic route for compounds **23**–**46** is depicted in Scheme 1.

This type of reactions was reported to be performed in certain dry solvents or mixtures [28–30]. In the present study, dry acetone [31] was tried and found to be useful [32,33]. However, the yields of thioureas **27**–**42** seemed to be affected by the possible formation of several by-products such as urethans [25]. Another yield-limiting factor might be the side reaction of isothiocyanates with heterocyclic nitrogen at the second position of 1,2,4-triazoline ring present in the **23**–**26** series [30]. Nevertheless, spectral findings such as the ^{1}H NMR resonances at 13–14 ppm due to heterocyclic NH–CS function and the lack of resonances attributable to NH_2 function at around 4.68–4.80 ppm, supported the regioselectivity of this reaction.

All the new compounds **23**–**42** were characterized by their melting points, and spectral data (^{1}H NMR, ^{13}C NMR and HR-MS). These physical and spectral data of the synthesized compounds are presented in Tables 1 and 2. The ^{1}H NMR spectra showed $-O-CH_2-$ chemical shifts at 4.75–5.24 ppm for all compounds. Resonances due to the $-NH_2$ function present in **23**–**26** were observed at 4.68–4.80 ppm. These signals disappeared in compounds **27**–**42** as evidence for the formation of thiourea group and instead, resonances for thiourea NH groups

Table 1
Physical constants and HR-MS data for the synthesized compounds **23**–**42**



Compound	R_1	R_2	Yield (%)	M.p.(°C)	Formula	log *P*[a]	HR–MS (m/z)	
							calculated	found
23	CH_3	–	76	143	$C_{10}H_{12}N_4OS$	1.58	236.0732 (M^+)	236.0740 (EI)
24	C_2H_5	–	52	160–161	$C_{11}H_{14}N_4OS$	2.01	250.0888 (M^+)	250.0902 (EI)
25	$CH_2CH{=}CH_2$	–	88	117	$C_{12}H_{14}N_4OS$	2.14	262.0888 (M^+)	262.0892 (EI)
26	C_6H_5	–	72	162	$C_{15}H_{14}N_4OS$	2.80	298.0888 (M^+)	298.0891 (EI)
27	CH_3	CH_3	46	200–202	$C_{12}H_{15}N_5OS_2$	1.98	309.0718 (M^+)	309.0685 (EI)
28	CH_3	C_2H_5	41	140–141	$C_{13}H_{17}N_5OS_2$	2.39	323.0874 (M^+)	323.0865 (EI)
29	CH_3	$CH_2CH{=}CH_2$	64	164–166	$C_{14}H_{17}N_5OS_2$	2.51	335.0874 (M^+)	335.0864 (EI)
30	CH_3	C_6H_5	71	183–185	$C_{17}H_{17}N_5OS_2$	3.39	372.0947 (MH^+)	372.0952 (FAB)
31	C_2H_5	CH_3	39	188–190	$C_{13}H_{17}N_5OS_2$	2.37	323.0874 (M^+)	323.0873 (EI)
32	C_2H_5	C_2H_5	39	153–155	$C_{14}H_{19}N_5OS_2$	2.76	337.1031 (M^+)	337.1030 (EI)
33	C_2H_5	$CH_2CH{=}CH_2$	72	218–220	$C_{15}H_{19}N_5OS_2$	2.88	349.1031 (M^+)	349.1001 (EI)
34	C_2H_5	C_6H_5	46	180–182	$C_{18}H_{19}N_5OS_2$	3.72	385.1104 (MH^+)	386.1094 (FAB)
35	$CH_2CH{=}CH_2$	CH_3	63	142	$C_{14}H_{17}N_5OS_2$	2.51	335.0874 (M^+)	335.0884 (EI)
36	$CH_2CH{=}CH_2$	C_2H_5	57	110–113	$C_{15}H_{19}N_5OS_2$	2.89	349.1031 (M^+)	349.1039 (EI)
37	$CH_2CH{=}CH_2$	$CH_2CH{=}CH_2$	74	135	$C_{16}H_{19}N_5OS_2$	2.98	361.1031 (M^+)	361.1036 (EI)
38	$CH_2CH{=}CH_2$	C_6H_5	51	137–140	$C_{19}H_{19}N_5OS_2$	3.84	398.1104 (MH^+)	398.1111 (FAB)
39	C_6H_5	CH_3	41	219–221	$C_{17}H_{17}N_5OS_2$	3.12	372.0953 (MH^+)	372.0913 (FAB)
40	C_6H_5	C_2H_5	47	217–220	$C_{18}H_{19}N_5OS_2$	3.49	386.1110 (MH^+)	386.1071 (FAB)
41	C_6H_5	$CH_2CH{=}CH_2$	82	160	$C_{19}H_{19}N_5OS_2$	3.60	397.1031 (M^+)	397.1013 (EI)
42	C_6H_5	C_6H_5	77	120–122	$C_{22}H_{20}N_5OS_2$	4.43	434.1110 (M^+)	434.1124 (EI)

[a] Calculation of log *P* values were performed using ALOGPS 2.102 log *P*/log *S* calculation software http://www.vcclab.org.

Table 2
^{1}H NMR spectral data of compounds **23–42**

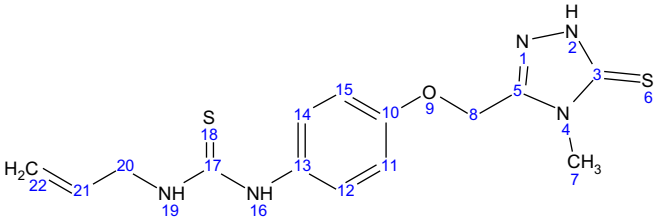
Compound	^{1}H NMR (δ)
23	3.43 (s, 3H, >N-CH_3); 4.68 (b, 2H, Ar-NH_2); 5.00 (s, 2H, $-OCH_2-$); 6.48 (d, 2H, $J = 8.78$ Hz, Ar-H); 6.72 (d, 2H, $J = 8.78$ Hz, Ar-H).
24	1.25 (t, 3H, >N$-CH_2-CH_3$), 4.00 (q, 2H, >N$-CH_2-CH_3$); 4.72 (b, 2H, Ar-NH_2); 5.00 (s, 2H, $-OCH_2-$); 6.45 (d, 2H, $J = 8.78$ Hz, Ar-H); 6.72, (d, 2H, $J = 8.78$ Hz, Ar-H).
25	4.62 (s, 2H, NH$-CH_2-$CH=CH_2); 4.70–4.80 (b, 2H, Ar-NH_2); 4.95 (s, 2H, $-OCH_2-$); 5.0 (d, 1H, $-CH_2-$CH=CH_2, $J = 17.0$ Hz, *trans*); 5.20 (d, 1H, $-CH_2-$CH=CH_2, $J = 10.5$ Hz, *cis*); 5.78–5.95 (m, 1H, NH$-CH_2-$CH=CH_2); 6.45 (d, 2H, $J = 9.00$ Hz, Ar-H); 6.72 (d, 2H, $J = 8.70$ Hz, Ar-H).
26	4.68 (b, 2H, Ar-NH_2); 4.75 (s, 2H, $-OCH_2-$); 6.35 (d, 2H, $J = 8.78$ Hz, Ar-H); 6.45 (d, 2H, $J = 8.78$ Hz, Ar-H); 7.40–7.50 (m, 5H, Ar-H).
27	2.89 (d, 3H, NH$-CH_3$); 3.51 (s, 3H, >N$-CH_3$) 5.22 (s, 2H, $-OCH_2-$); 7.05 (d, 2H, $J = 8.78$ Hz, *o*-OCH_2); 7.25 (d, 2H, $J = 8.78$ Hz, *m*-OCH_2); 7.50 (s, 1H, thiourea NH) 9.40 (s, 1H, thiourea N′H); 13.90 (s, 1H, triazole NH).
28	1.10 (t, 3H, NH$-CH_2-CH_3$); 3.51 (m, 2H, Ar-NH–CS–NH$-CH_2-CH_3$); 3.57 (s, 3H, >N$-CH_3$); 5.23 (s, 2H, $-OCH_2-$); 7.05 (d, 2H, $J = 8.78$ Hz, *o*-OCH_2); 7.27 (d, 2H, $J = 8.78$ Hz, *m*-OCH_2); 7.70 (s, 1H, thiourea NH); 9.30 (s, 1H, thiourea N′H); 13.98 (s, 1H, triazole NH).
29	3.46 (s, 3H, >N$-CH_3$); 4.07 (s, 2H, $-CH_2-$CH=CH_2); 5.03–5.09 (m, 2H, $-CH_2-$CH=CH_2); 5.18 (s, 2H, $-OCH_2-$); 5.82–6.00 (m, 1H, $-CH_2-CH$=CH_2); 7.02 (d, 2H, $J = 8.78$ Hz, *o*-OCH_2); 7.25 (d, 2H, $J = 8.78$ Hz, *m*-OCH_2), 7.70 (s, 1H, thiourea NH); 9.36 (s, 1H, thiourea N′H); 13.80 (s, 1H, triazole NH).
30	3.52 (s, 2H, >N$-CH_3$); 5.24 (s, 2H, $-OCH_2$); 7.05–7.47 (m, 9H, Ar-H); 9.67 (s, 1H, thiourea N′H); 9.72 (s, 1H, thiourea NH); 13.85 (s, 1H, triazole NH)
31	1.29 (t, 3H, >N$-CH_2CH_3$); 2.91 (d, 3H, NH$-CH_3$); 4.03 (q, 2H, >N$-CH_2CH_3$); 5.17 (s, 2H, $-OCH_2$); 7.05 (d, 2H, $J = 8.78$ Hz, *o*-OCH_2); 7.25 (d, 2H, $J = 8.78$ Hz, *m*-OCH_2); 7.51 (b, 1H, thiourea NH); 9.56 (b, 1H, thiourea N′H); 13.92 (s, 1H, triazole NH)
32	1.12 (t, 3H, NH$-CH_2-CH_3$); 1.27 (t, 3H, >N$-CH_2-CH_3$); 3.47 (m, 2H, NH$-CH_2-$CH$_3$); 4.03 (q, 2H, >N$-CH_2CH_3$); 5.22 (s, 2H, $-OCH_2$); 7.06 (d, 2H, $J = 8.78$ Hz, *o*-OCH_2); 7.26 (d, 2H, $J = 8.78$ Hz, *m*-OCH_2); 7.51 (s, 1H, thiourea NH); 9.30 (s, 1H, thiourea N′H); 13.98 (s, 1H, triazole NH).
33	1.29 (t, 3H, >N$-CH_2-CH_3$); 4.03 (q, 2H, >N$-CH_2-$CH$_3$); 4.13 (s, 2H, $-CH_2-$CH=CH_2) 5.10 (d, 1H, $-CH_2-$CH=CH_2, $J = 17.0$ Hz, *trans*); 5.24 (m, 3H, $-OCH_2-$ and $-CH_2-$CH=CH_2, *cis*); 5.80–6.00 (m, 1H, >N$-CH_2-CH$=CH_2); 7.06 (d, 2H, $J = 8.78$ Hz, *o*-OCH_2); 7.30 (d, 2H, $J = 8.78$ Hz, *m*-OCH_2); 7.67 (s, 1H, thiourea NH); 9.42 (s, 1H, thiourea N′H); 13.97 (s, 1H, triazole NH).
34	1.29 (t, 3H, >N$-CH_2-CH_3$); 4.08 (q, 2H, >N$-CH_2CH_3$); 5.24 (s, 2H, $-OCH_2$); 7.04–7.47 (m, 9H, Ar-H); 9.66 and 9.71 (s, 2H, *NH*–CS–*NH*); 13.98 (s, 1H, triazole NH)
35	2.84 (d, 3H, NH$-CH_3$); 4.65 (s, 2H, $-CH_2-$CH=CH_2); 5.04 (d, 1H, $-CH_2-$CH=CH_2, $J = 17.0$ Hz, *trans*); 5.12 (s, 2H, $-OCH_2-$); 5.17 (d, 1H, $J = 11.1$ Hz, $-CH_2-$CH=CH_2, *cis*); 5.80–6.00 (m, 1H, $-CH_2-CH$=CH_2); 6.97 (d, 2H, $J = 8.78$ Hz, *o*-OCH_2); 7.20 (d, 2H, $J = 8.78$ Hz, *m*-OCH_2), 7.45 (s, 1H, thiourea NH); 9.30 (s, 1H, thiourea N′H); 13.85 (s, 1H, triazole NH)
36	1.05 (m, 3H, NH$-CH_2-CH_3$); 3,42 (m, 2H, NH$-CH_2-$CH$_3$); 4.64 (s, 2H, $-CH_2-$CH=CH_2); 5.05 (d, 1H, $-CH_2-$CH=CH_2, $J = 17.57$ Hz, *trans*); 5.12 (s, 2H, $-OCH_2-$); 5.17 (d, 1H, $J = 9.95$ Hz, $-CH_2-$CH=CH_2, *cis*); 5.80–6.00 (m, 1H, $-CH_2-CH$=CH_2); 6.96 (d, 2H, $J = 8.78$ Hz, *o*-OCH_2); 7.21 (d, 2H, $J = 8.78$ Hz, *m*-OCH_2); 7.45 (s, 1H, thiourea NH); 9.30 (s, 1H, thiourea N′H); 13.85 (s, 1H, triazole NH)
37	4.19 (s, 2H, NH$-CH_2-$CH=CH_2); 4.71 (d, 2H, >N$-CH_2-$CH=CH_2); 5,01–5.07 (d, 2H, >N$-CH_2-$CH=CH_2 and NH$-CH_2-$CH=CH_2 $J = 17.0$ Hz, *trans*); 5.12 (s, 2H, $-OCH_2-$); 5.15–5.19 (d, 2H, >N$-CH_2-$CH=CH_2 and NH$-CH_2-$CH=CH_2, $J = 11.1$ Hz, *cis*); 5.80–6.00 (m, 2H, >N$-CH_2-CH$=CH_2 and NH$-CH_2-CH$=CH_2); 7.01 (d, 2H, $J = 8.78$ Hz, *o*-OCH_2); 7.26 (d, 2H, $J = 8.78$ Hz, *m*-OCH_2); 7.70 (s, 1H, thiourea NH); 9.40 (s, 1H, thiourea N′H); 13.96 (s, 1H, triazole NH)
38	4.71 (d, 2H, $-CH_2-$CH=CH_2); 5.05 (d, 1H, $-CH_2-$CH=CH_2, $J = 17.0$ Hz, *trans*); 5.18 (s, 2H, $-OCH_2-$); 5.21 (d, 1H, $J = 11.1$ Hz, >N$-CH_2-$CH=CH_2, *cis*); 5.80–6.00 (m, 1H, >N$-CH_2-CH$=CH_2); 7.02–7.47 (m, 9H, Ar-H); 9.66 and 9.71 (s, 2H, *NH*-CS-*NH*); 13.99 (s, 1H, triazole NH)
39	2.91 (d, 3H, NH$-CH_3$); 4.96 (s, 2H, $-OCH_2-$); 6.80 (d, 2H, $J = 8.78$, *o*-OCH_2); 7.18 (d, 2H, $J = 8.78$, *m*-OCH_2); 7.46–7.57 (m, 6H, Ar-H and thiourea NH); 9.35 (b, 1H, thiourea N′H)
40	1.09 (t, 3H, $-CH_2-CH_3$); 3.44 (q, 2H, $-CH_2-$CH$_3$); 4.95 (s, 2H, $-OCH_2-$); 6.80 (d, 2H, $J = 8.78$ Hz, *o*-OCH_2); 7.17 (d, 2H, $J = 8.78$ Hz, *m*-OCH_2); 7.46–7.57 (m, 5H; Ar-H); 7.57 (s, 1H, thiourea NH); 9.25 (s, 1H, thiourea N′H); 13.97 (s, 1H, triazole NH)
41	4.97 (d, 2H, $-CH_2-$CH=CH_2); 5.10 (d, 1H, $-CH_2-$CH=CH_2, $J = 17.0$ Hz, *trans*); 5.16 (d, 1H, $J = 11.1$ Hz, $-CH_2-$CH=CH_2, *cis*); 5.82–5.95 (m, 1H, >N$-CH_2-CH$=CH_2); 6.83 (d, 2H, $J = 8.78$ Hz, *o*-OCH_2); 7.22 (d, 2H, $J = 8.78$ Hz, *m*-OCH_2); 7.29–7.59 (m, 5H, Ar-H); 7.69 (s, 1H, thiourea NH); 9.38 (s, 1H, Ar-thiourea N′H)
42	4.91 (s, 2H, $-OCH_2-$); 6.76 (d, 2H, $J = 8.78$ Hz, *o*-OCH_2); 7.23 (d, 2H, $J = 8.78$ Hz, *m*-OCH_2); 7.04–7.51 (m, 10H, $-C_6H_5$); 9.56 (s, 1H, thiourea N′H); 9.63 (s, 1H, thiourea NH); 13.98 (s, 1H, triazole NH)

were observed at 7.50–9.72 ppm (R_2-*NH*CSNH–$C_6H_4OCH_2-$) and 9.30–9.71 ppm (R_2-NHCS*NH*–$C_6H_4OCH_2-$). Triazole NH resonances between 13.80 and 13.98 ppm, were observed to be shifted to downfield because of strong intermolecular hydrogen bonding as previously reported [34] The N–H protons of compounds **23–26**, **39** and **41** exchanged with deuterium in the solvent used for obtaining the ^{1}H NMR spectra. Remaining chemical shifts were also recorded at expected values.

^{13}C NMR data of the representative thiourea derivative **29** also supported the carbon framework of triazole ring and thiourea moieties by displaying high accuracy between experimental and calculated ^{13}C chemical shifts (Table 3). Calculation of the ^{13}C NMR chemical shifts were performed using ACD/CNMR Predictor software available online at ACD/I-Lab Interactive Laboratory website (http://www.acdlabs.com/ilab). As shown in Table 3, thiocarbonyl (C=S) carbon present in triazoline ring and thiourea function resonated at 168.39 ppm and 180.52 ppm, whereas these values were predicted as 168.40 ppm and 180.80 ppm, respectively. Remaining resonances were also observed to be consistent with calculated values.

High resolution mass spectra (HR-MS) confirmed the molecular weights and empirical formula of compounds **23–42**, with less than 8 mmu bias between calculated and experimental *m*/*z* values of either molecular or fragment ions (Table 1). Ionization

Table 3
Assignment of ^{13}C NMR spectrum of compound **29**



Carbon no.	Chemical shift (ppm)	
	Calculated[a]	Observed
3	168.40	168.39
5	148.55	149.07
7	37.67	30.89
8	60.82	61.05
10	155.56	155.12
11, 15	115.51	115.61
12, 14	122.20	126.59
13	132.84	134.05
17	180.80	180.52
20	47.39	41.01
21	132.11	129.13
22	121.89	124.39

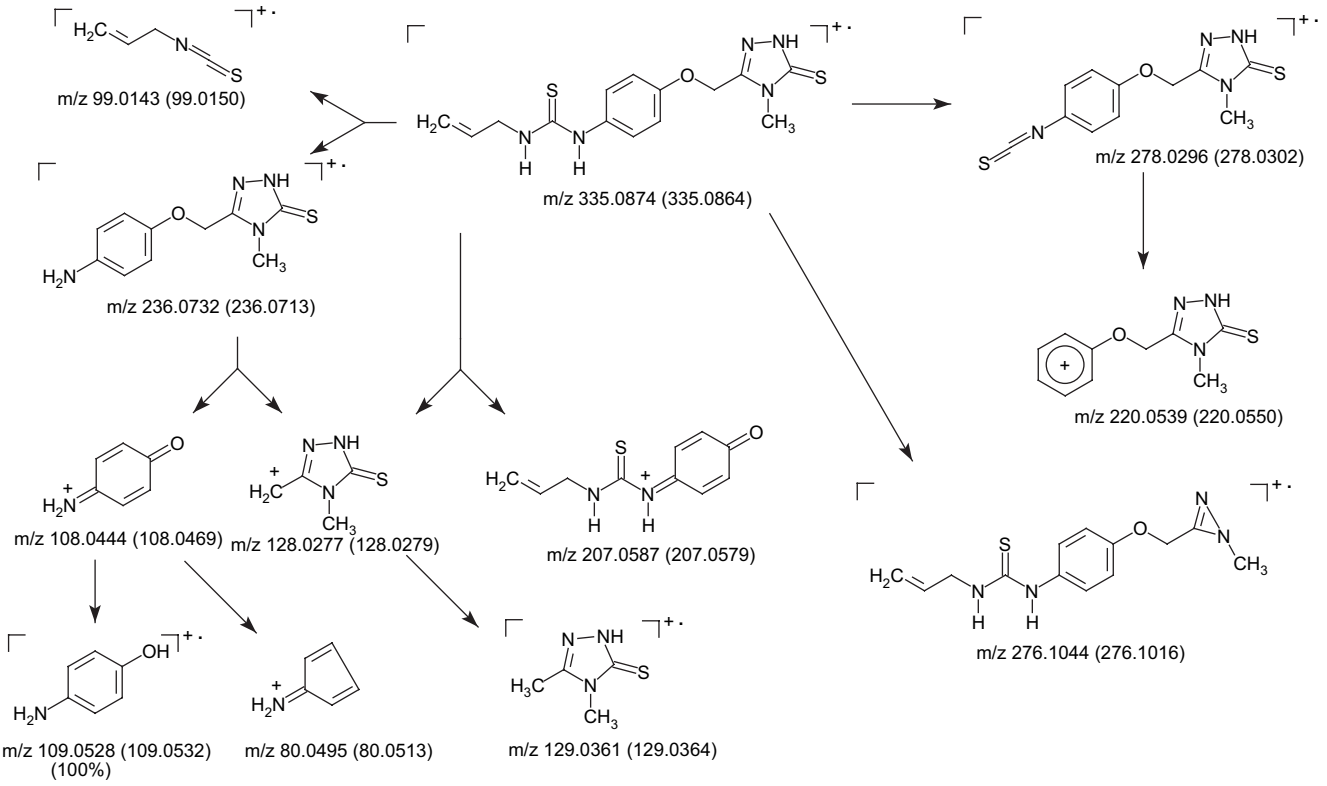
[a] Calculation of the ^{13}C NMR chemical shifts were performed using ACD/CNMR Predictor software available online at ACD/I-Lab Interactive Laboratory website http://www.acdlabs.com/ilab.

mode was electron impact (EI) in most cases, whereas some compounds **30**, **34**, **38**–**42**, especially the ones with an aryl moiety on terminal thiourea nitrogen, did not give molecular ion peaks using this technique. These compounds were analyzed using fast atomic bombardment (FAB) procedure giving exact MH^+ peaks instead of M^+ in 3-nitrobenzyl alcohol matrix. Fragmentation pattern for a representative compound **29** which is given in Scheme 2, also supported the expected structures. First fragmentation was cleavage of thiourea moiety yielding isothiocyanate fragment at *m/z* 278.0302 via allylamine loss, or, formation of amine fragment at *m/z* 236.0713 by the expulsion of allyl isothiocyanate which was detected at *m/z* 99.0150. Characteristic fragmentations for 1,2,4-triazoline ring were also observed. Main fragmentation product was observed as 4-aminophenol radical cation, giving the base peak at *m/z* 109.0532. Other fragmentations were observed to be consistent with the expected structures for compounds **23**–**42**.

3. Biological activity

3.1. Antiviral activity

For each compound, the minimum inhibitory concentration (MIC) and the minimal cytotoxic concentration (MCC) or the 50% cytotoxic concentration (CC_{50}) were obtained. Thiourea derivatives **1**–**22** were evaluated for their anti-HIV activity. None of the synthesized compounds showed any specific



Scheme 2. HR-EI mass spectral fragmentation of **29** (experimental values in brackets).

Table 4
Cytotoxicity and anti-HIV activity of compounds **1**–**42** in MT-4 cells

Compound	Anti-HIV activity				Cytotoxicity CC_{50} (μg/ml)[b]
	IC_{50}[a] (μg/ml)		Max. protection (%)		
	HIV-1 (III_B)	HIV-2 (ROD)	HIV-1 (III_B)	HIV-2 (ROD)	
1	>80.30	>80.30	1	0	80.30 ± 7.50
2	>82.37	>82.37	3	0	82.37 ± 5.10
3	>78.20	>78.20	9.5	1	78.20 ± 14.73
4	>8.82	>8.82	8	0	8.82 ± 2.34
5	>42.80	>42.80	35	10	42.80 ± 21.31
6	>56.97	>56.97	8.5	0	56.97 ± 20.30
7	>2.58	>2.58	21	3	2.58 ± 0.31
8	>80.90	>80.90	9	0	80.90 ± 14.71
9	>65.43	>65.43	6.5	0	65.43 ± 5.43
10	>19.40	>19.40	30.5	0	19.40 ± 8.34
11	>10.06	>10.06	4	0	10.06 ± 1.78
12	>1.71	>1.71	3	1	1.71 ± 1.06
13	>13.03	>13.03	9	0	13.03 ± 0.49
14	>94.40	>94.40	23.5	0	94.40 ± 18.99
15	>91.27	>91.27	5.5	3	91.27 ± 13.13
16	>55.13	>55.13	13.5	0	55.13 ± 29.95
17	>3.99	>3.99	16	3	3.99 ± 0.85
18	>28.57	>28.57	25	0	28.57 ± 6.77
19	>10.29	>10.29	4.5	31	10.29 ± 2.02
20	>75.40	>75.40	2.5	6	75.40 ± 16.83
21	>116.00	>116.00	0.5	5	116.00
22	>65.30	>65.30	1.5	0	65.30 ± 50.9
23	>125	>125	2.5	4	≥119
24	>125	>125	6	8	>125
25	>125	>125	3.5	9	>125
26	>125	>125	2	2.5	>125
27	>112	>125	1	1	≥112
28	>107	>125	1.5	2	≥107
29	>107	>114	1.5	3	≥107
30	>98.90	>87.80	3.5	6.5	≥87.80
31	>68.10	>77.90	1.5	2	76.78 ± 8.60
32	>88.30	>91.10	9.5	8	94.18 ± 5.63
33	>72.30	>71.40	3	2	75.88 ± 4.71
34	>27.20	>31.40	26.5	25.5	31.93 ± 3.86
35	>101	>110	1	3.5	108.25 ± 5.44
36	>76.50	>86.20	4	2.5	90.03 ± 10.82
37	>66.50	>69.70	3	1	71.50 ± 4.18
38	>20.20	>20.30	19	22.5	23.20 ± 4.04
39	>83.50	>113	0.5	2	≥83.50
40	>15.00	>19.40	1	11	22.03 ± 6.27
41	>92.30	>103	1.5	0.5	107.08 ± 12.87
42	>30.60	>41.50	5.5	4.5	44.15 ± 10.36

[a] Concentration required to protect MT-4 cells against the cytopathogenicity of HIV by 50%.
[b] Concentration required to reduce MT-4 cell viability by 50%.

activity against HIV-1 (III_B) or HIV-2 (strain ROD) in MT-4 cells. Based on the experience with this type of molecules, it was considered that a certain degree of flexibility might be required for binding to HIV-1 RT. Following these observations, we have designed and synthesized compounds **27**–**42**, in which more flexibility is ascertained by replacing the phenyl ring on the fifth position of 1,2,4-triazole ring with a phenoxymethyl moiety. Anti-HIV screening results of compounds **1**–**42** are given in Table 4. However, they were also found inactive, probably due to their inability to exist in butterfly-like conformation as explained in a similar case by Chaouni-Benabdallah et.al. [35].

The compounds were also evaluated for in vitro antiviral activity against Herpes simplex virus [HSV-1 (strain KOS), thymidine kinase deficient (TK^-) strain of HSV-1 resistant to acyclovir (ACV^R), HSV-2 (G)], Varicella-zoster virus (VZV) [OKA strain and a thymidine kinase deficient (TK^-) VZV (07/1 strain)], Cytomegalovirus (CMV) [AD-169 and Davis strains], Vaccinia virus (VV), Vesicular stomatitis virus (VSV) in HEL and Embryonic skin muscle (E6SM) cell cultures; VSV, Coxackie virus B4, Respiratory syncytial virus (RSV) in HeLa cell cultures and Parainfluenza-3 virus, Sindbis virus, Coxackie virus B4 and Punta Toro virus in Vero cell cultures. Results are presented in Tables 5–9.

As a result of antiviral screening of **1**–**42**, none of the evaluated compounds showed specific antiviral effects (i.e., minimal antivirally effective concentration less than one-fifth of minimal cytotoxic concentration) against any of the viruses examined. However, some of the compounds showed moderate activity against certain viruses. Among the derivatives screened, compound **4** which had a MCC value of 400 μg/ml, showed weak activity against the herpes simplex family [HSV-1 (KOS), HSV-2 (G) and HSV-1 TK^- (ACV^R)] with the MIC value of 48 μg/ml. Compound **5** also had a weak activity against HSV-1 TK^- (ACV^R) with the MIC value of 48 μg/ml (Table 6). Compounds **33** and **42** possessed an MIC value of 48 μg/ml against Sindbis virus whereas MCC for these compounds in HEL cell cultures were 400 and 80 μg/ml, respectively (Table 7). Many thiourea derivatives from **27**–**42** series were observed to have weak activity against Coxackie virus B4, but at a subtoxic concentration (Table 7). Compounds **26**, **29**, **32**, **33**, **34**, **37**, **41** and **42** had the same MIC value of 48 μg/ml, whereas their MCC were 400 μg/ml (selectivity index = 8.33) except for compounds **34** and **42** whose MCC values were 80 μg/ml (selectivity index = 5). Compound **38**, bearing an allyl group at N-4 of 1,2,4-triazole ring and a phenyl moiety at the terminal nitrogen of the thiourea, was identified as the most active derivative and the MIC value of this compound was 16 μg/ml against Coxackie virus B4, whereas its MCC was 80 μg/ml (selectivity index = 5). This compound was also active against thymidine kinase positive Varicella-zoster virus (TK^+ VZV, OKA strain) with an EC_{50} value of 9.9 μg/ml; whereas it showed only a weak activity against the thymidine kinase deficient (TK^-) VZV (07/1 strain) with an EC_{50} of 26.8 μg/ml (Table 9). On the other hand, compound **38** possessed low toxicity on human embryonic lung (HEL) cells (MCC of >100 μg/ml).

3.2. Anti-tuberculosis activity

Compounds **II**, **IIIa**–**d** and **23**–**42** were also tested for in vitro anti-tuberculosis activity against *M. tuberculosis* H37Rv using the BACTEC 12B medium in a broth microdilution assay, the Microplate Alamar Blue Assay (MABA) [36,37]. Rifampicin was used as the standard in the antimycobacterial assays. Triazoles **24**–**26** and thiourea derivatives **27**, **28**, **31**, **32** and **36** were completely inactive against

Table 5
Cytotoxicity and antiviral activity of compounds **1**–**42** in HeLa cell cultures

Compound	Minimum cytotoxic concentration[a] (μg/ml)	Minimum inhibitory concentration[b] (μg/ml)		
		Vesicular stomatitis virus	Coxsackie virus B4	Respiratory syncytial virus
1	400	>80	>80	>80
2	400	>80	>80	>80
3	400	>80	>80	>80
4	80	>16	>16	>16
5	80	>16	>16	>16
6	400	>80	>80	>80
7	≥400	>400	>400	>400
8	400	>80	>80	>80
9	400	>80	>80	>80
10	400	>80	>80	>80
11	80	>16	>16	>16
12	16	>3.2	>3.2	>3.2
13	400	>80	>80	>80
14	≥400	>400	>400	>400
15	≥80	>80	>80	>80
16	≥400	>400	>400	>400
17	400	>80	>80	>80
18	80	>80	>80	>80
19	80	>16	>16	>16
20	80	>16	>16	>16
21	400	>80	>80	>80
22	80	>16	>16	>16
23	>400	>400	>400	>400
24	≥400	>400	>400	>400
25	>400	>400	>400	>400
26	>400	>400	>400	>400
27	>400	>400	>400	>400
28	400	>80	>80	>80
29	400	>80	>80	>80
30	>400	>400	>400	>400
31	400	>80	>80	>80
32	400	>80	>80	>80
33	400	>80	>80	>80
34	400	80	>80	>80
35	400	>80	>80	>80
36	400	>80	>80	>80
37	400	>80	>80	>80
38	80	>16	>16 (80)	>16
39	400	>80	>80	>80
40	400	>80	>80	>80
41	400	>80	>80	>80
42	80	>16	>16 (80)	>16
Brivudin	≥400	>400	>400	>400
(S)-DHPA	>400	>400	>400	>400
Ribavirin	>400	48	240	9.6

[a] Required to cause a microscopically detectable alteration of normal cell morphology.
[b] Required to reduce virus-induced cytopathogenicity by 50%.

M. tuberculosis H37Rv at 6.25 μg/mL, whereas the remaining compounds exhibited varying degrees of inhibition in the primary screen. None of the tested compounds were considered for further evaluation as they exhibited less than 90% inhibition in the primary screen (MIC > 6.25 mg/mL). Compound **38** was the most active compound with 79% inhibition against *M. tuberculosis* H37Rv. Compounds **30**, **33**, **34** and **37** also had positive responses exhibiting inhibition percentages of 70, 75, 76 and 59, respectively.

Table 6
Cytotoxicity and antiviral activity of compounds **1**–**42** in HEL and E6SM cell cultures

Compound	Minimum cytotoxic concentration[a] (μg/ml)	Minimum inhibitory concentration[b] (μg/ml)				
		Herpes simplex virus-1 (KOS)	Herpes simplex virus-2 (G)	Vaccinia virus	Vesicular stomatitis virus	Herpes simplex virus-1 TK^- KOS ACV^r
1	400	240	240	240	>80	>80
2	≥400	240	240	240	>400	240
3	400	240	240	240	>80	240
4	400	48	48	80	>80	48
5	400	>80	>80	>80	>80	48
6	400	240	240	240	>80	240
7	400	>80	>80	>80	>80	>80
8	400	240	240	240	>80	240
9	≥80	>80	>80	>80	>80	240
10	80	>16	>16	>16	>16	>16
11	80	>16	>16	>16	>16	>16
12	16	>3.2	>3.2	>3.2	>3.2	>3.2
13	400	>80	>80	>80	>80	>80
14	400	240	>80	>80	>80	240
15	≥3.2	>3.2	>3.2	>3.2	>3.2	>3.2
16	≥80	>80	>80	>80	>80	>80
17	≥16	>16	>16	>16	>16	>16
18	≥80	>80	>80	>80	>80	>80
19	≥16	>16	>16	>16	>16	>16
20	≥16	>16	>16	>16	>16	>16
21	80	>16	>16	>16	>16	>16
22	≥16	>16	>16	>16	>16	>16
23	>400	400	80	240	>400	80
24	>400	>400	240	240	>400	240
25	>400	>400	240	>400	>400	>400
26	>400	>400	240	>400	>400	>400
27	>400	>400	240	>400	>400	>400
28	>400	>400	240	>400	>400	240
29	>400	>400	240	>400	>400	240
30	400	>80	>80	>80	>80	>80
31	>400	>400	>400	>400	>400	>400
32	>400	>400	240	240	>400	240
33	>400	>400	240	240	>400	240
34	400	>80	>80	>80	>80	>80
35	>400	>400	>400	>400	>400	>400
36	>400	>400	>400	>400	>400	>400
37	>400	240	240	240	>400	240
38	400	>80	>80	>80	80	>80
39	>400	>400	>400	>400	>400	>400
40	>400	>400	>400	>400	>400	>400
41	>400	240	240	240	240	240
42	400	>80	>80	>80	>80	>80
Brivudin	>400	0.0768	80	3.2	>400	>400
Ribavirin	>400	9.6	48	48	240	48
Acyclovir	>400	0.384	0.384	>400	>400	48
Gancyclovir	>100	0.032	0.032	>100	>100	2.4

[a] Required to cause a microscopically detectable alteration of normal cell morphology.
[b] Required to reduce virus-induced cytopathogenicity by 50%.

4. Experimental protocols

4-(Acetylamino)phenol (Paracetamol) was a gift from Drogsan Pharmaceuticals. Ethyl bromoacetate, hydrazine hydrate, methyl-, ethyl-, allyl- and phenyl-isothiocyanate were

Table 7
Cytotoxicity and antiviral activity of compounds **1–42** in Vero cell cultures

Compound	Minimum cytotoxic concentration[a] (μg/ml)	Minimum inhibitory concentration[b] (μg/ml)				
		Parainfluenza-3 virus	Reo virus-1	Sindbis virus	Coxsackie virus B4	Punta Toro virus
1	400	>80	>80	>80	>80	>80
2	400	>80	>80	>80	>80	>80
3	400	>80	>80	>80	>80	>80
4	80	>16	>16	>16	>16	>16
5	400	>80	>80	>80	>80	>80
6	≥80	>80	>80	>80	>80	80
7	400	>80	>80	>80	>80	>80
8	≥80	>80	>80	>80	>80	>80
9	≥80	>80	>80	>80	>80	>80
10	80	>16	>16	>16	>16	>16
11	≥16	>16	>16	>16	>16	>16
12	16	>3.2	>3.2	>3.2	>3.2	>3.2
13	≥80	>80	>80	>80	>80	>80
14	≥80	>80	>80	>80	>80	>80
15	≥80	>80	>80	>80	>80	>80
16	≥400	>400	>400	>400	>400	>400
17	400	>80	>80	>80	>80	>80
18	80	>16	>16	>16	>16	>16
19	≥16	>16	>16	>16	>16	>16
20	80	>16	>16	>16	>16	>16
21	80	>16	>16	>16	>16	>16
22	≥16	>16	>16	>16	>16	>16
23	16	>3.2	>3.2	>3.2	>3.2	>3.2
24	80	>16	>16	>16	>16	>16
25	400	>80	>80	>80	>80	>80
26	400	>80	>80	>80	48	>80
27	400	>80	>80	>80	>80	>80
28	400	>80	>80	>80	>80	>80
29	400	>80	>80	>80	48	>80
30	400	>80	>80	>80	>80	>80
31	400	>80	>80	>80	>80	>80
32	400	>80	>80	>80	48	>80
33	400	>80	>80	48	48	>80
34	≥80	>80	>80	>80	48	>80
35	400	>80	>80	>80	>80	>80
36	400	>80	>80	>80	80	>80
37	400	>80	>80	>80	48	>80
38	80	>16	>16	>16	16	>16
39	400	>80	>80	>80	>80	>80
40	400	>80	>80	>80	80	>80
41	400	>80	>80	>80	48	>80
42	80	>16	>16	>16 (48)	>16 (48)	>16
Brivudin	>400	>400	>400	>400	>400	>400
(S)-DHPA	>400	>400	>400	>400	>400	>400
Ribavirin	>400	48	48	240	>400	48

[a] Required to cause a microscopically detectable alteration of normal cell morphology.
[b] Required to reduce virus-induced cytopathogenicity by 50%.

purchased from Fluka. Sodium hydroxide, anhydrous potassium carbonate and all solvents were supplied from Merck.

^{1}H and ^{13}C NMR spectra were obtained using a Bruker AVANC-DPX 400 instrument. HR-EI-mass spectra were performed using a Jeol JMS-700 instrument.

SMILES were generated from the structures using the ACD/ChemSketch version 8.0 molecular editor (http://www.acdlabs.com) and then log *P* values were calculated using ALOGPS 2.102 log *P*/log *S* calculation software [38, 39]. The calculated log *P* values for all the compounds are given in Table 1.

4.1. Ethyl(4-acetamidophenoxy)acetate (*I*)

4-(Acetylamino)phenol (0.04 mol) was refluxed in acetone containing anhydrous K_2CO_3 (0.06 mol) for 4 h; then ethyl bromoacetate (0.042 mol) was added dropwise for 1 h and after that the reaction mixture was heated for 8 h. At the end of this duration the flask content was filtered and evaporated to get the crude product. The crude product was recrystallized from ethanol. M.p. 100–103 °C (lit. 103 °C) [40], yield 63%.

Table 8
Cytotoxicity and antiviral activity of compounds **23**–**42** against Cytomegalovirus (CMV) in human embryonic lung (HEL) cells

Compound	Antiviral activity EC_{50}[a] (μg/ml)		Cytotoxicity (μg/ml)	
	AD-169 strain	Davis strain	Cell morphology (MCC)[b]	Cell growth (CC50)[c]
23	>100	>100	>100	>50
24	>100	>100	>100	>50
25	>100	>100	>100	>50
26	>100	>100	>100	>50
27	>100	>100	>100	>50
28	>100	>100	>100	>50
29	>100	>100	>100	>50
30	63	49	>100	>50
31	63	>100	>100	>50
32	49	55	>100	>50
33	45	45	>100	>50
34	41	37	>100	>50
35	>100	>100	>100	>50
36	>100	>100	>100	>50
37	100	77	>100	>50
38	45	45	>100	42
39	>100	>100	>100	>50
40	>100	>100	>100	>50
41	>100	>100	>100	>50
42	>20	37	≥100	>50
Ganciclovir	0.64	1.9	>400	87
Cidofovir	0.075	0.64	>400	≥18

[a] Effective concentration required to reduce virus plaque formation by 50%. Virus input was 20 plaque forming units (PFU).
[b] Minimum cytotoxic concentration that causes a microscopically detectable alteration of cell morphology.
[c] Cytotoxic concentration required to reduce cell growth by 50%.

4.2. 2-(4-Acetamidophenoxy)acetohydrazide (*II*)

Compound **II** was prepared by refluxing ethyl[4-(acetylamino)phenoxy]acetate (**I**) (0.015 mol) with hydrazine hydrate (80%) (0.045 mol) in ethanol for 3 h. The reaction mixture was allowed to cool and the precipitate washed with ice-cold water and recrystallized from ethanol. M.p. 194–196 °C (lit. 194–195 °C) [40], yield 80%.

4.3. General procedure for the synthesis of 1-[2-(4-acetamidophenoxy)acetyl]-4-alkyl/aryl thiosemicarbazides (*IIIa–d*)

2-(4-Acetamidophenoxy)acetohydrazide (**II**) (0.018 mol) was dissolved in boiling ethanol and equimolar amounts of methyl-isothiocyanate (**IIIa**), ethyl-isothiocyanate (**IIIb**), allyl-isothiocyanate (**IIIc**) and phenyl-isothiocyanate (**IIId**) were added and refluxed for 4 h. The flask content was allowed to cool and the filtered precipitates were washed with boiling ethanol.

4.3.1. 1-[2-(4-Acetamidophenoxy)acetyl]-4-methyl thiosemicarbazide *IIIa*

$C_{12}H_{16}N_4O_3S$; yield 87%. M.p. 222–226 °C. ^{1}H NMR (DMSO-d_6) δ: 1.97 (s, 3H, $-CO\mathit{CH}_3$); 2.46 (m, DMSO); 2.84 (d, 3H, $-NH\mathit{CH}_3$); 3.32 (s, DMSO–H_2O); 4.48 (s, 2H, $-O\mathit{CH}_2-$); 6.87 (d, 2H, $J = 9.37$ Hz, *m*-NHAc); 7.45 (d, 2H, $J = 8{,}78$ Hz, *o*-NHAc); 7.94 (s, 1H, $-CS\mathit{NH}-CH_3$); 9.24 (s, 1H, $-CONH\mathit{NH}-$), 9.78 (s, 1H, $-CO\mathit{NH}-CH_3$); 10.00 (s, 1H, $-CO\mathit{NH}NH-$). HR-MS (EI^+), *m*/*z* (calculated/found): 296.0943/296.0953.

4.3.2. 1-[2-(4-Acetamidophenoxy)acetyl]-4-ethyl thiosemicarbazide *IIIb*

$C_{13}H_{18}N_4O_3S$;. yield %87. M.p. 182–184 °C. ^{1}H NMR (DMSO-d_6) δ: 1.97 (s, 3H, $-CO\mathit{CH}_3$); 2.46 (m, DMSO); 3.59 (d, 3H, $-NH\mathit{CH}_2-$); 3.32 (s, DMSO–H_2O); 4.54 (s, 2H, $-O\mathit{CH}_2-$); 6.95 (d, 2H, $J = 9.37$ Hz, *m*-NHAc); 7.47 (d, 2H, $J = 8{,}78$ Hz, *o*-NHAc); 7.95 (s, 1H, $-CS\mathit{NH}-CH_3$); 9.18 (s, 1H, $-CONH\mathit{NH}-$), 9.82 (s, 1H, $-CO\mathit{NH}-CH_3$); 10.05 (s, 1H, $-CO\mathit{NH}NH-$).

4.3.3. 1-[2-(4-Acetamidophenoxy)acetyl]-4-allyl thiosemicarbazide *IIIc*

$C_{14}H_{18}N_4O_3S$; yield 87%. M.p. 196–200 °C. ^{1}H NMR (DMSO-d_6) δ: 1.96 (s, 3H, $-CO\mathit{CH}_3$); 2.46 (m, DMSO); 4.06 (b, 2H, $-\mathit{CH}_2-CH{=}CH_2$); 3.32 (s, DMSO–H_2O); 4.49 (s, 2H, $-O\mathit{CH}_2-$); 5.01 (d, 1H, $J = 10.54$ Hz, $-CH_2-CH{=}\mathit{CH}_2$ *cis*); 5.09 (d, 1H, $J = 17.57$ Hz, $-CH_2-CH{=}\mathit{CH}_2$ *trans*); 5.71–5.83 (m, 1H, $-CH_2-\mathit{CH}{=}CH_2$); 6.86 (d, 2H, $J = 9.37$ Hz, *m*-NHAc); 7.43 (d, 2H, $J = 8{,}78$ Hz, *o*-NHAc); 8.15 (s, 1H,$-CS\mathit{NH}-CH_3$); 9.30 (s, 1H, $-CONH\mathit{NH}-$); 9.78 (s, 1H, $-CO\mathit{NH}-CH_3$); 10.03 (s, 1H, $-CO\mathit{NH}NH-$).

4.3.4. 1-[2-(4-Acetamidophenoxy)acetyl]-4-phenyl thiosemicarbazide *IIId*

$C_{17}H_{18}N_4O_3S$; yield 85%. M.p. 190–192 °C (lit.190–191 °C) [40]. ^{1}H NMR (DMSO-d_6) δ: 1.97 (s, 3H, $-CO\mathit{CH}_3$); 2.47 (m, DMSO); 3.31 (s, DMSO–H_2O); 4.54 (s, 2H, $-O\mathit{CH}_2-$); 6.90 (d, 2H, $J = 8.78$ Hz, *m*-NHAc); 7.11–7.16, 7.28–7.39 (m, 5H, Ar-H) 7.45 (d, 2H, $J = 9.37$ Hz, *o*-NHAc); 9.63 (s, 2H, $-\mathit{NH}CS\mathit{NH}$-Ar); 9.78 (s, 1H, $-CO\mathit{NH}-CH_3$); 10.22 (s, 1H, $-CO\mathit{NH}NH-$).

4.4. General procedure for the synthesis of 5-[(4-aminophenoxy)methyl]-4-methyl/ethyl/allyl/phenyl-2,4-dihydro-3H-1,2,4-triazole-3-thiones (*23–26*)

Compounds **23**–**26** were prepared by refluxing **IIIa**–**d** (0.01 mol) in sodium hydroxide solution (2 N) for 4 h and followed by treatment of sodium salts with HCl to give desired compounds. Compounds **23**–**26** were recrystallized from ethanol.

4.5. General procedure for the synthesis of N-alkyl/aryl-N′-{4-[(4-alkyl/aryl-5-thioxo-4,5-dihydro-1H-1,2,4-triazol-3-yl)methoxy]phenyl}thioureas (*27–42*)

Compounds **27**–**42** (0.0015 mol) were prepared by refluxing **23**–**26** in dry acetone for 6 h with equimolar amounts of appropriate isothiocyanates. The reaction mixtures were then evaporated and the crude products of **26**–**29**, **31**, **32**, **35**–**38**,

Table 9
Cytotoxicity and antiviral activity of compounds **23**–**42** against varicella-zoster virus (VZV) in human embryonic lung (HEL) cells

Compound	Antiviral activity EC_{50}[a] (μg/ml)		Cytotoxicity (μg/ml)	
	TK^+ VZV OKA strain	TK^- VZV 07/1 strain	Cell morphology (MCC)[b]	Cell growth (CC_{50})[c]
23	>100	>100	>100	>50
24	>100	>100	>100	>50
25	>100	>100	>100	>50
26	>100	>100	>100	>50
27	>100	>100	>100	>50
28	>100	>100	>100	>50
29	>100	>100	>100	>50
30	33	38	≥100	>50
31	>100	88	>100	>50
32	64	58	>100	>50
33	41	42	>100	>50
34	29	34	≥100	>50
35	>100	>100	>100	>50
36	64	64	>100	>50
37	41	46	>100	>50
38	9.9	26.8	≥100	42
39	>100	>100	>100	>50
40	>100	>100	>100	>50
41	>100	>100	>100	>50
42	26	39	>100	>50
Acyclovir	0.17	16	400	200
Brivudin	0.02	25	400	128

[a] Effective concentration required to reduce virus plaque formation by 50%. Virus input was 20 plaque forming units (PFU).
[b] Minimum cytotoxic concentration that causes a microscopically detectable alteration of cell morphology.
[c] Cytotoxic concentration required to reduce cell growth by 50%.

42 were recrystallized from ethanol, **30**, **33**, **34**, **39** were washed with boiling ethanol and **40**, **41** were recrystallized from methanol.

4.6. In vitro antiviral assays

4.6.1. Inhibition of HIV-induced cytopathicity in MT-4 cells

Evaluation of the antiviral activity of the compounds against HIV-1 strain III_B and HIV-2 strain (ROD) in MT-4 cells was performed using the MTT assay as previously described [41]. Stock solutions (10 × final concentration) of test compounds were added in 25 μl volumes to two series of triplicate wells so as to allow simultaneous evaluation of their effects on mock- and HIV-infected cells at the beginning of each experiment. Serial five-fold dilutions of test compounds were made directly in flat-bottomed 96-well microtiter trays using a Biomek 2000 robot (Beckman instruments, Fullerton, CA). Untreated control HIV- and mock-infected cell samples were included for each sample.

HIV-1(IIIB) [42] or HIV-2 (ROD) [43] stock (50 μl) at 100–300 $CCID_{50}$ (cell culture infectious dose) or culture medium was added to either the infected or mock-infected wells of the microtiter tray. Mock-infected cells were used to evaluate the effect of test compound on uninfected cells in order to assess the cytotoxicity of the test compound. Exponentially growing MT-4 cells [44] were centrifuged for 5 minutes at 1000 rpm and the supernatant was discarded. The MT-4 cells were resuspended at 6×10^5 cells/ml, and 50 μl volumes were transferred to the microtiter tray wells. Five days after infection, the viability of mock- and HIV-infected cells was examined spectrophotometrically by the MTT assay.

The MTT assay is based on the reduction of yellow coloured 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Acros Organics, Geel, Belgium) by mitochondrial dehydrogenase of metabolically active cells to a blue-purple formazan that can be measured spectrophotometrically. The absorbances were read in an eight-channel computer-controlled photometer (Multiscan Ascent Reader, Labsystems, Helsinki, Finland), at two wavelengths (540 and 690 nm). All data were calculated using the median OD (optical density) value of tree wells. The 50% cytotoxic concentration (CC_{50}) was defined as the concentration of the test compound that reduced the absorbance (OD540) of the mock-infected control sample by 50%. The concentration achieving 50% protection from the cytopathic effect of the virus in infected cells was defined as the 50% effective concentration (EC_{50}).

4.6.2. Antiviral assays

The antiviral assays, other than HIV-1, were based on inhibition of virus-induced cytopathicity in either E6SM cells (HSV-1, HSV-2, VV, VSV), human embryonic lung (HEL) cells [Varicella-zoster virus VZV), HCMV], HeLa cells (Respiratory syncytial virus) or Vero cells (Coxsackie B4 virus, Parainfluenza-3 virus, Sindbis virus, Punta Toro virus, Reovirus-1), following previously established procedures [45–47]. Briefly, confluent cell cultures in microtiter 96-well plates were inoculated with 100 $CCID_{50}$ of virus, one $CCID_{50}$ being the virus dose required to infect 50% of the cell cultures. After a 1 h virus adsorption period, residual virus was removed, and the cell cultures were incubated in the presence of varying concentrations (400, 200, 100, … μg/ml) of the test compounds. Viral cytopathicity was recorded as soon as it reached completion in the control virus-infected cell cultures that had not been treated with the test compounds.

4.7. In vitro evaluation of antimycobacterial activity against M. tuberculosis H37Rv

Primary screening was conducted at 6.25 μg/ml against *M. tuberculosis* H37Rv in BACTEC 12B medium using the BACTEC 460 radiometric system [36]. Compounds effecting <90% inhibition in the primary screening (MIC > 6.25 μg/ml) were not evaluated further. Compounds demonstrating at least 90% inhibition in the primary screening were re-tested at lower concentration (MIC) in a broth microdilution assay Alamar Blue. The MIC was defined as the lowest concentration inhibiting 99% of the inoculum.

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