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ORIGINAL RESEARCH

Synthesis and biological evaluation of some new 1,3,4-thiadiazole and 1,2,4-triazole derivatives from *L*-methionine as antituberculosis and antiviral agents

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ABSTRACT

Some novel 1,3,4-thiadiazole [**5-8**] and 1,2,4-triazole [**9-12**] derivatives carrying amino acid moiety were synthesized starting from *L*-methionine. 1,3,4-Thiadiazole and 1,2,4-triazole scaffolds were prepared by cyclocondensation of the corresponding thiosemicarbazide and finally converted to their thiourea derivatives. Structures of the synthesized compounds [**4-12**] were confirmed by IR, ^{1}H-NMR and ^{13}C-NMR spectral data and elemental analysis. Synthesized compounds were evaluated for their antiviral and antibacterial activity. Of the screened compounds, *N*-{3-(methylsulfanyl)-1-[5-(phenylamino)-1,3,4-thiadiazole-2-yl]propyl}benzamide **[5]** was identified as the most potent inhibitor of *Influenza A H3N2 virus* with an EC_{50} value of 31.4 µM, which serves as a lead compound for prospective development. The antituberculosis activity screen of the synthesized compounds revealed 1-[4-(4-chloro-(3-trifluoromethyl)phenyl]-3-[3-(methylsulfanyl)-1-(4-phenyl-5-thioxo-4,5-dihydro-1H-1,2,4-triazole-3-yl)propyl]thiourea **[12]** as the most active compound against *M. tuberculosis H37Rv* strain (MIC : 30.88 µM) but the compound proved not selective.

Keywords: Thioureas; 1,3,4-thiadiazoles; 1,2,4-triazoles; antiviral activity; influenza; antituberculosis activity.

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1. INTRODUCTION

AIDS (Acquired Immune Deficiency Syndrome) is one of the most spread and most deadly diseases in the modern era. AIDS is the end-stage disease of HIV (human immunodeficiency virus) infection which was identified as a disease in 1981. HIV is a retrovirus which only replicates in certain human cells. *HIV-1 RT* (HIV-1 Reverse Transcriptase) plays a substantial role during HIV replication and it has been just over two decades since *HIV-1 RT* was recognised as one of the key macromolecules that have been targeted in the effort to design new antiretroviral drugs. Whereas nucleoside *RT* inhibitors such as zidovudine (AZT) act via the competitive inbition, non-nucleoside *RT* inhibitors (NNRTIs) interact with an allosteric site of *RT* and inhibit the enzyme non-competitively. NNRTIs have gained an increasingly

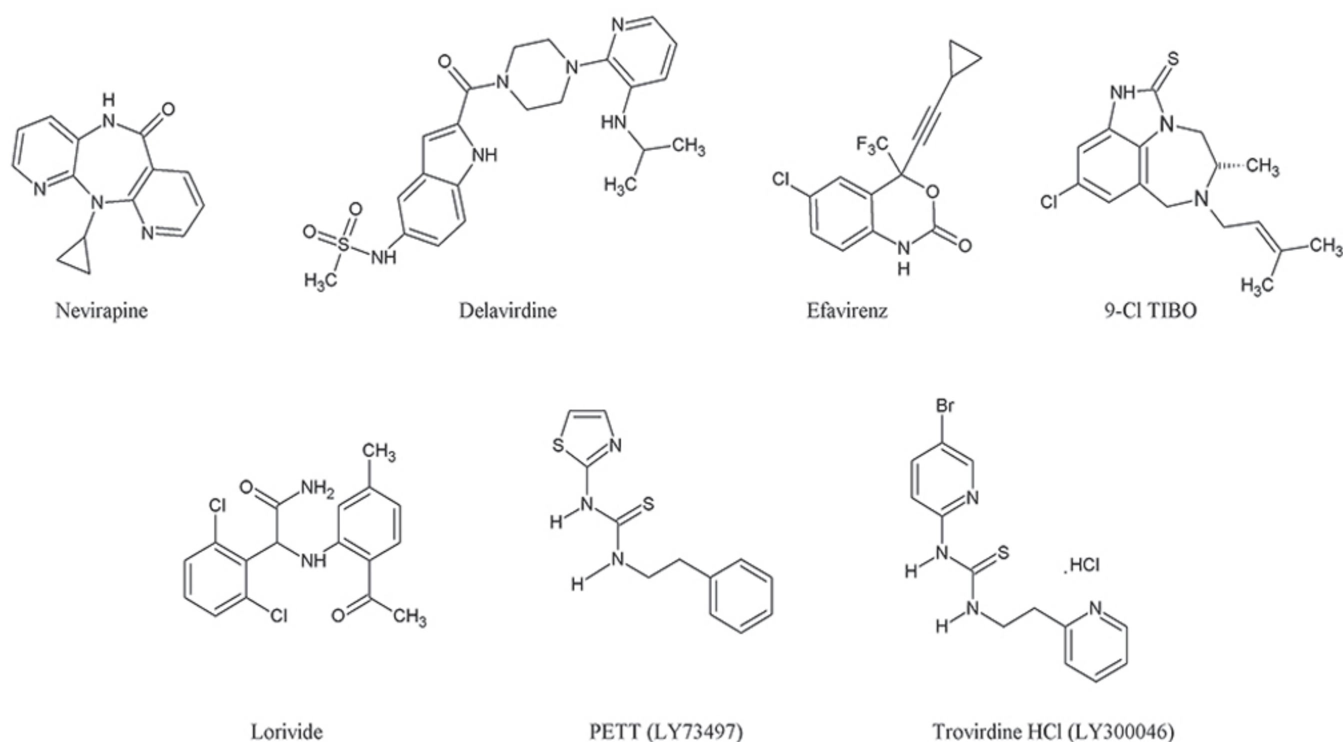


Figure 1: HIV-1 NNRTIs which belong to different chemical classes.

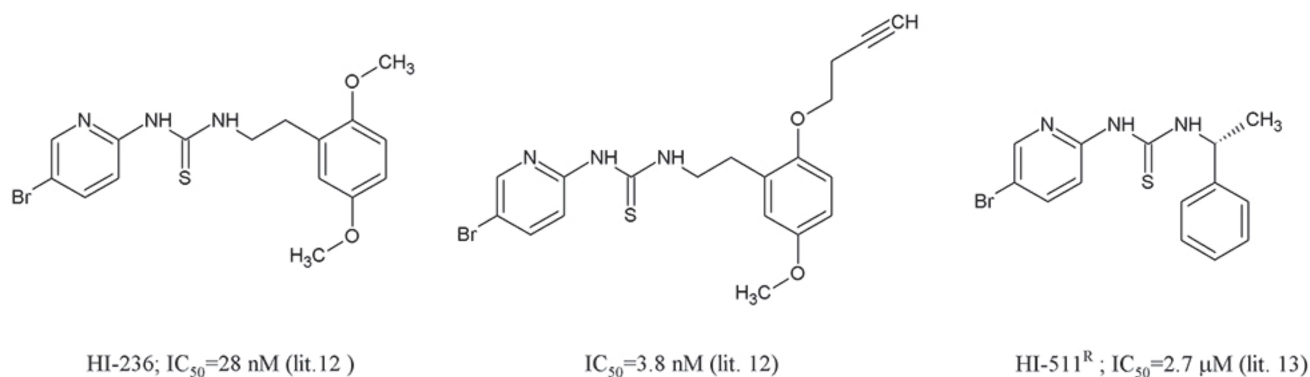


Figure 2: Some thiourea derivatives with promising *HIV-1 RT* inhibitory activity.

important role in antiretroviral therapy due to their high selectivity index and low cytotoxicity profile. Many compounds possess chemical diversity; dihydrodiazepinone derivatives (nevirapine), *bis*-heteroaryl piperazines (delavirdine), benzoxazinones (efavirenz), TIBO derivatives (tivirapine), α-APA derivatives (lorivide), PETT (phenylethylthiazolyl thiourea) derivatives (trovirdine) could be considered as NNRTIs (**Figure 1**) (1-4).

PETT derivatives were introduced as a new class of NNRTIs in 1995 by Ahgren *et al*. (5) but the first scientific effort that led to the discovery of PETT derivatives was from Bell *et al*. (6). Within the context of Bell's work; 9-Cl TIBO, a classical NNRTI compound, was selected as the starting compound and by dismantling 9-Cl TIBO's tricyclic ring system new basic PETT derivatives were obtained. In contrast to 9-Cl TIBO, PETT derivatives have a flexible chemical structure based on an open chain. Since the first representative analogues of PETT derivatives; [PETT (LY 73749) and Trovirdine (LY 300046) were shown to exhibit high antiviral activity], the amount of the work invested in targeting the design and the discovey of new PETT analogues has been accelerated (7-11).

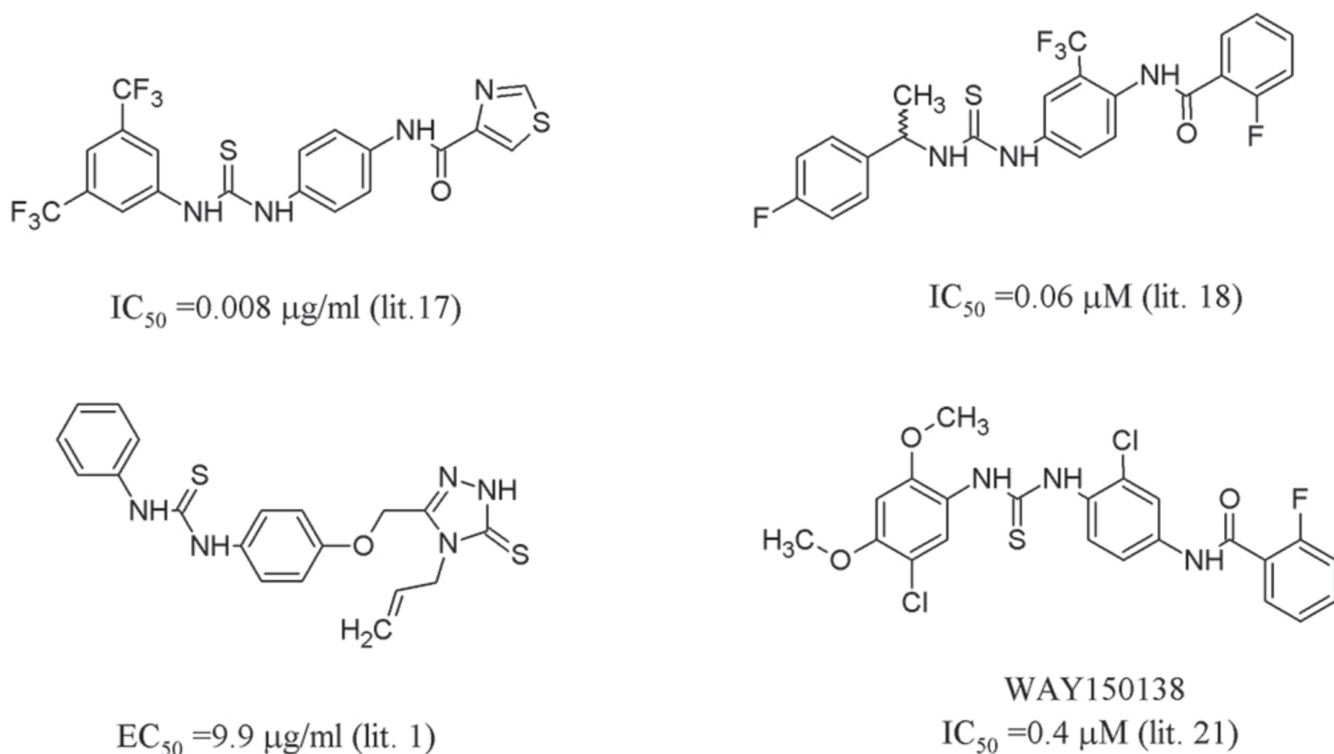


Figure 3: Several thiourea derivatives with antiviral activity.

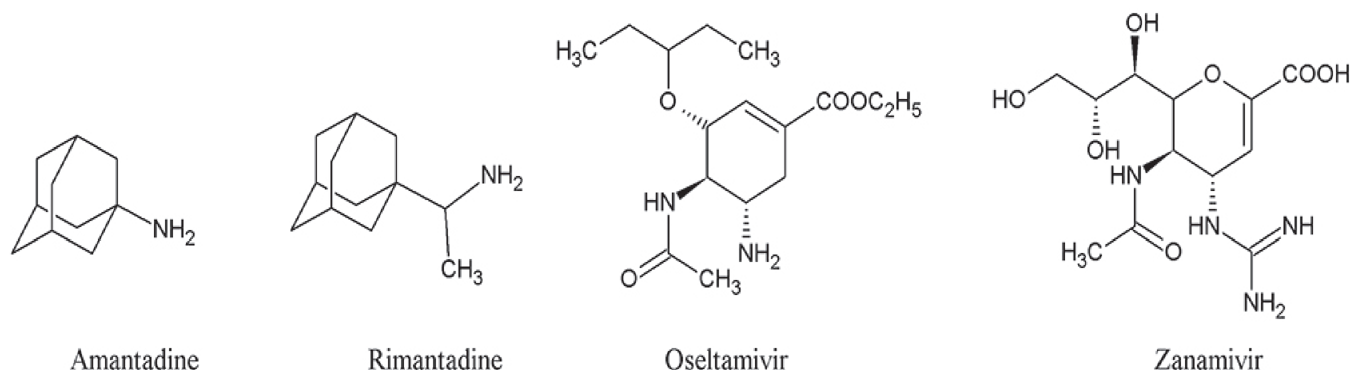


Figure 4: Current anti-Influenza virus drugs.

The compounds carrying –NH–CS–NH– pharmacophore have been demonstrated to exhibit antiviral activity. Further structural modification of the thiourea compounds led to the discovery of even more HIV-1 Reverse Transcriptase (HIV-1 RT) inhibitors. Hunter *et al*. (12) synthesized thiourea derivatives involving HI-236, a NNRTI with PETT moiety, and by this approach they further improved the activity of HI-236 (IC_{50}=28nM) further. Venkatachalam *et al*. (13) reported the inhibitory profile of HI-511^R that inhibits the HIV-strain A17 double mutant containing RT mutations K103N and Y181C with an IC_{50} value of 2.7 μM, whereas the IC_{50} values of nevirapine, delavirdine, and trovirdine against this NNI-resistant HIV-1 strain were reported as >100 μM. The literature focussed on the *HIV-1 RT* inhibitory activity of thiourea derivatives (**Figure 2**) formed the basis of our present work. Our aim was to synthesize thiourea analogues bearing a flexible amino acid core and evaluate them for their anti-HIV activity. The target compounds were synthesized through the reaction of 5-[1-amino-3-(methylsulfanyl)propyl]-*N*-phenyl-1,3,4-thiadiazole-2-amine or 5-[1-amino-3-(methylsulfanyl) propyl]-4-phenyl-2,4-dihydro-3*H*-1,2,4-triazole-3-thione

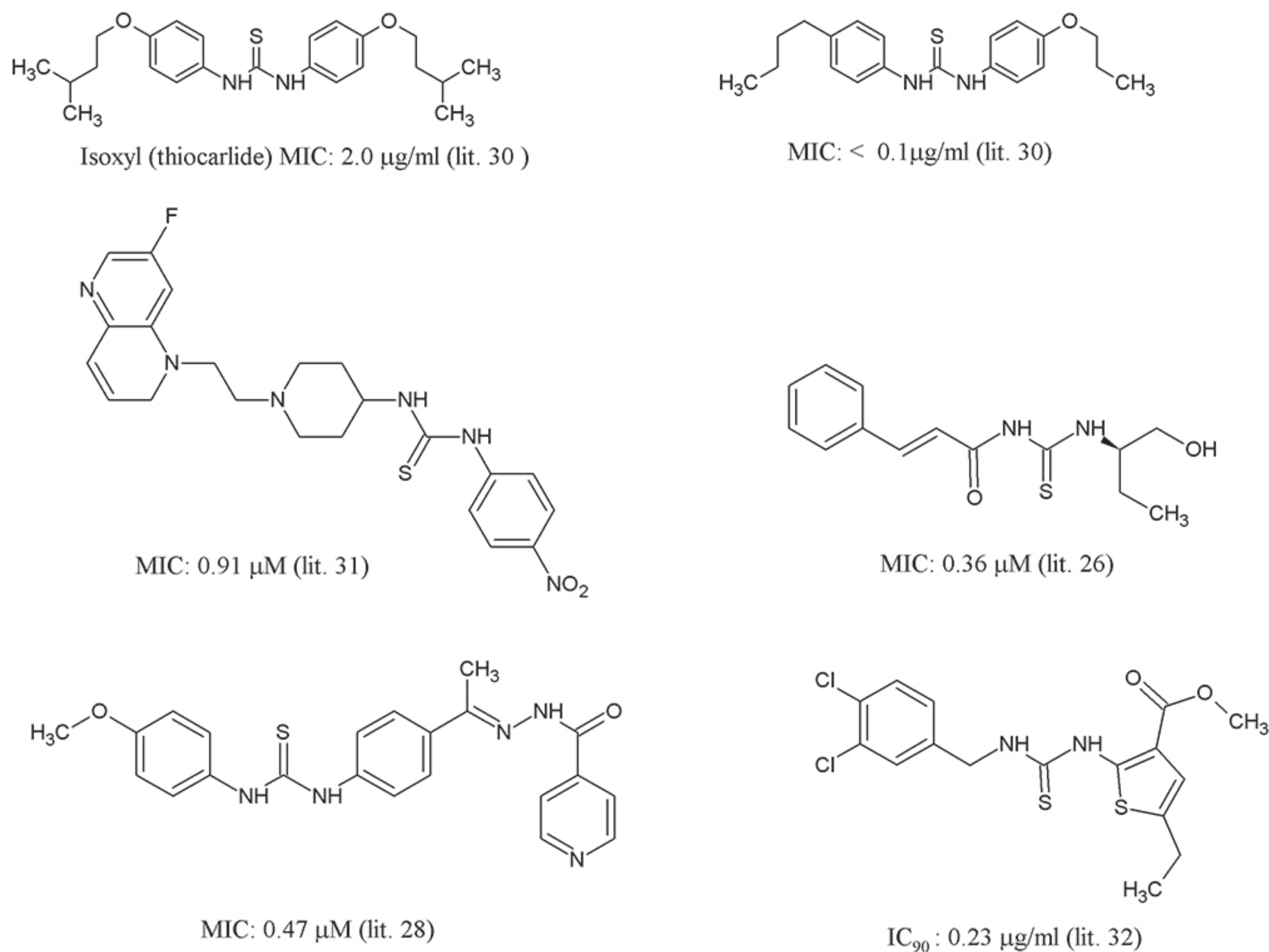
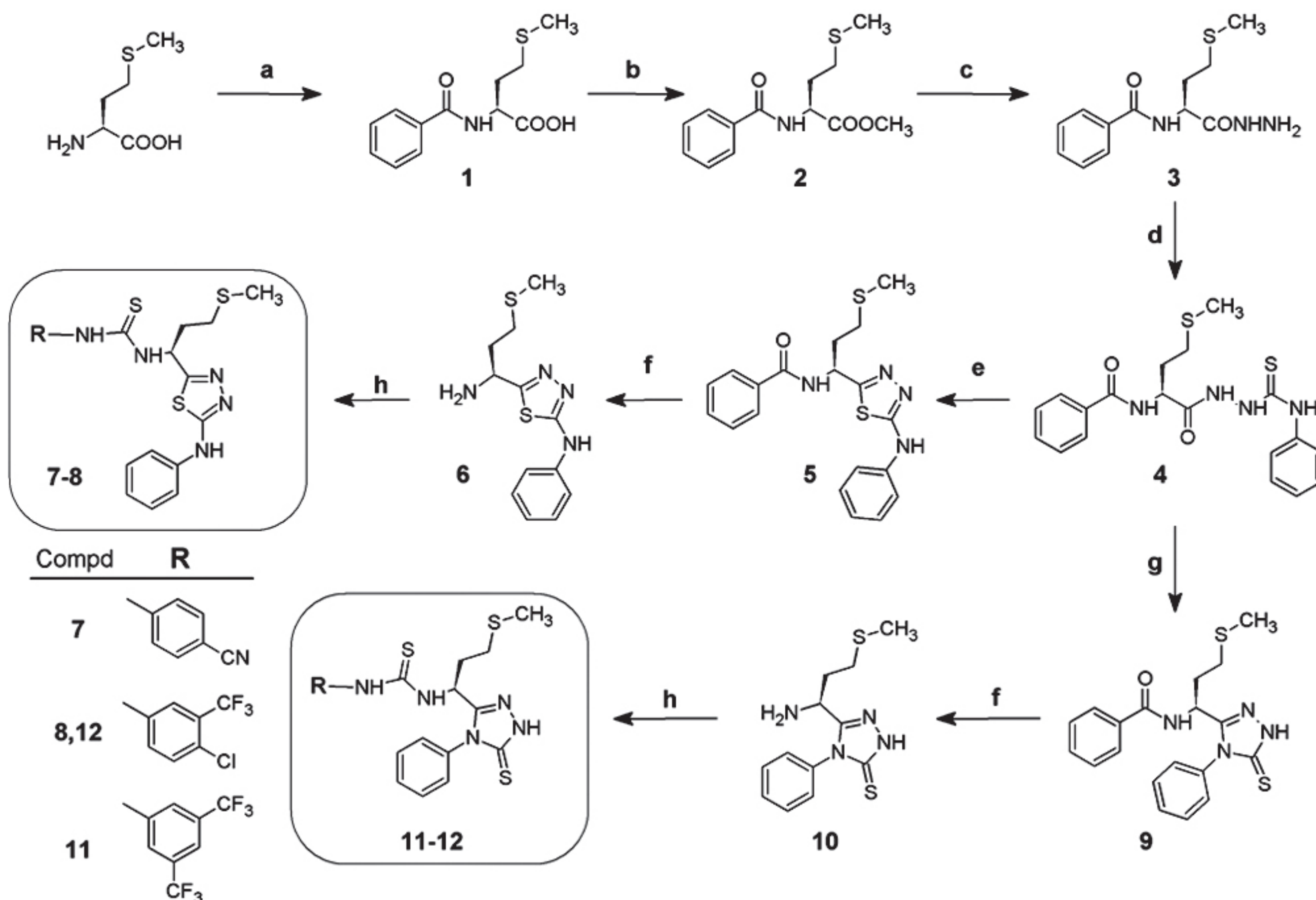


Figure 5: Antituberculosis activity of some thiourea derivatives against *M. tuberculosis* H37Rv strain.

with substituted phenyl isothiocyanates and evaluated for their anti-HIV activity using the strains HIV-1 (III_B) and HIV-2 (ROD).

Due to the fact of recurrent or persistent coinfections with the GB virus C (formerly known as hepatitis G virus), *hepatitis B virus* (HBV), *hepatitis C virus* (HCV), *herpes simplex virus type 2* (HSV-2) that increase morbidity and mortality among *HIV*-infected individuals by increasing the HIV viral load (14-16) antiviral effects of thioureas against various types of viruses have also been investigated. In this regard, potent and selective inhibitors of *cytomegalovirus* (CMV) (17), *Varicella-zoster virus* (VZV) (18), and *herpes simplex virus type-1* (HSV-1) (19-21) were developed (**Figure 3**). In our previous work on thiourea derivatives (1), we reported the synthesis and antiviral evaluation of *N*-phenyl-*N′*-{4-[(4-allyl-5-thioxo-4,5-dihydro-1*H*-1,2,4-triazol-3-yl)methoxy]phenyl}thiourea which showed moderate protection against *Coxsackie virus B4* with an MIC value of 16 μg/ml (40.25 μM, SI=5) and thymidine kinase wild-type *varicella-zoster virus* (TK^+ VZV, OKA strain) with an EC_{50} value of 9.9 μg/ml (**Figure 3**). Antiviral activity of *N*-cyclohexyl-*N′*-[4-(4-methyl-2,4-dihydro-*3H*-1,2,4-triazole-3-thione-5-yl)phenyl]thiourea (1) against herpes simplex virus type-1 (HSV-1), HSV-2 and HSV-1 TK^- KOS with a minimum inhibitory concentration (MIC) value of 48 μg/ml was also demonstrated. Antiviral activity of our synthesized compounds **1-12** were screened against various types of viruses.

Seasonal H3N2 and H1N1 (except for 1918 H1N1 and 2009 H1N1) are low-virulence viruses that cause primarily inflammation, congestion and epithelial necrosis of trachea, bronchi and bronchioles (22). Antiviral chemotherapy (**Figure 4**) against influenza (23) comprises M2 proton channel inhibitors (amantadine and rimantadine) and



Scheme 1: Synthetic route to compounds 1-12.

Reagents and conditions: **(a)** NaOH / H_2O, benzoyl chloride; **(b)** Methanol, H_2SO_4 (conc.), reflux; **(c)** Methanol, $N_2H_4.H_2O$, reflux; **(d)** Ethanol / phenyl isothiocyanate, reflux; **(e)** H_2SO_4 (conc.), rt; **(f)** 50% H_2SO_4, reflux; **(g)** 2N NaOH, reflux; **(h)** Acetonitrile / R-NCS, reflux.

neuraminidase inhibitors (zanamivir and oseltamivir). These compounds are not structurally related to the 1,3,4-thiadiazole-based compound described here.

A recent report on HIV-tuberculosis co-infection has proposed that HIV-1 facilitates *Mycobacterium tuberculosis* to grow in co-infected individuals while *M. tuberculosis* facilitates HIV-1 replication via increasing expression of co-receptors on CD4 T-cells (24). The preferred clinical recommendation for HIV-tuberculosis (TB) co-infected patients includes efavirenz-based antiretroviral therapy together with standard antituberculosis drugs (25). Standard antituberculosis drug regimen comprises of first line drugs isoniazid, rifampicin, ethambutol, pyrazinamide and second line drugs ethionamide, prothionamide, thiacetazone, isoxyl (thiocarlide), amikacin, kanamycin, capreomycin, and some fluoroquinolone derivatives (ofloxacin, levofloxacin, moxifloxacin and gatifloxacin). Many compounds with thiourea moiety (1, 26-30) have been synthesized drawing their inspiration from the second line antituberculosis pro-drug isoxyl (thiocarlide). Besides thiourea derivatives (26, 31-35), various compounds bearing heterocycles, 1,3,4-thiadiazole (36-38) and 1,2,4-triazole (39,40) have been reported to possess antituberculosis and antibacterial properties. We decided to evaluate our thiourea-thiadiazole hybrid or thiourea-triazole hybrid compounds for their antibacterial and antiviral activity.

2. RESULT AND DISCUSSION

2.1. Chemistry

N-Benzoyl methionine **(1),** 2-(benzoylamino)-4-(methylthio)butyric acid methyl ester **(2)** and 2-(benzoylamino)-4-(methylthio)butyric acid hydrazide **(3)** were prepared through the procedure reported in the literature (41). The synthetic route for compounds **1-12** is presented in Scheme 1. Purities of compounds **4-12** were

Table 1. Anti-HIV evaluation of selected compounds against HIV-1(III_B) and HIV-2 (ROD) in MT4 cells.

Compound	CC_{50}[a] (μM)	EC_{50}[b] (μM)	
		HIV-1(III_B)	HIV-2(ROD)
4	>300	>300	>300
5	>300	>300	>300
6	241	>241	>241
7	201	>201	>201
8	20.4	>20.4	>20.4
9	>325	>325	>325
10	>445	>445	>445
11	3.3	>3.3	>3.3
12	22.2	>22.2	>22.2
nevirapine	>15.0	0.18	>15.0
zidovudine	>95	0.008	0.003
dideoxycytidine	>95	0.76	0.90
didanosine	>210	8.8	16.0

[a] 50% Cytotoxic concentration, as determined by measuring the cell viability with the colorimetric formazan-based MTS assay.
[b] 50% Effective concentration, or concentration producing 50% inhibition of virus-induced cytopathic effect, as determined by measuring the cell viability with the colorimetric formazan-based MTS assay

Table 2. Antiviral evaluation and in vitro cytotoxicity in HEL (Human Embryonic Lung) cell culture.

Compound	Minimum cytotoxic concentration (μM)	EC_{50} (μM)				
		Herpes simplex virus-1 (KOS)	Herpes simplex virus-2 (G)	Vaccinia virus	Vesicular stomatitis virus	Herpes simplex virus-1 (TK- KOS ACVr)
4	>100	>100	>100	>100	>100	>100
5	≥20	>20	>20	>20	>20	>20
6	>100	>100	>100	>100	>100	>100
7	>100	>100	>100	>100	>100	>100
8	100	>20	>20	>20	>20	>20
9	>100	>100	>100	>100	>100	>100
10	>100	>100	>100	>100	>100	>100
11	4	>0.8	>0.8	>0.8	>0.8	>0.8
12	100	>20	>20	>20	>20	>20
Brivudin	>250	0.08	250	29	>250	>250
Cidofovir	>250	4	1	10	>250	6
Acyclovir	>250	0.9	0.4	>250	>250	>250
Ganciclovir	>100	0.08	0.03	>100	>100	20

assessed through HPLC analysis. The synthesized compounds were characterized by their IR and ^{1}H-NMR spectroscopy data. ^{13}C-NMR and elemental analysis data are provided for some selected compounds.

Table 3. Antiviral evaluation and in vitro cytotoxicity in CRFK (Crandell Rees Feline Kidney) cell culture.

Compound	CC_{50} (μM)	EC_{50} (μM)	
		Feline Corona Virus (**FIPV**)	Feline Herpes Virus
4	>100	>100	>100
5	>100	>100	>100
6	>100	>100	>100
7	>100	>100	>100
8	11	>4	>4
9	>100	>100	>100
10	>100	>100	>100
11	0.6	>0.16	>0.16
12	10	>4	>4
HHA (μg/ml)	>100	13.3	6.5
UDA (μg/ml)	>100	7.8	2.4
Ganciclovir	>100	>100	4.7

By condensing the hydrazide **3** with phenyl isothiocyanate in ethanol, 1-[2-(benzoylamino)-4-(methylthio)butyryl]-4-phenyl thiosemicarbazide **4** was synthesized. Absorption band at 1172 cm^{-1}, which was attributed to the C=S streching vibrations, was observed in the IR spectra of compound **4**. Cyclization of compound **4,** using concentrated H_2SO_4 provided *N*-{3-(methylsulfanyl)-1-[5-(phenylamino)-1,3,4-thiadiazole-2-yl]propyl}benzamide 5. The signal belonging to thiadiazole -NH- at 10.28 ppm proved the formation of 1,3,4-thiadiazole ring. Compound **6** was synthesized via hydrolysis of compound **5** in acidic medium. The diagnostic broad singlet signal for primary aliphatic amine was observed at 5.42-5.47 ppm. 5-[1-Amino-3-(methylsulfanyl)propyl]-*N*-phenyl-1,3,4-thiadiazole-2-amine **6** was reacted with 4-cyanophenyl isothiocyate and 4-chloro-3-(trifluoromethyl)phenyl isothiocyanate to afford the corresponding thiourea derivatives **7** and **8**. Following ^{1}H-NMR analysis for compound **7**, the doublet signal at 9.09 ppm was assigned to NH proton attached to the asymmetric carbon atom of *L*-methionine moiety (42), whereas the same proton belonging to compound **8** was observed as a singlet at 8.09 ppm.

We succeeded in the synthesis of *N*-[3-(methyllsulfanyl)-1-(4-phenyl-5-thioxo-4,5-dihydro-1*H*-1,2,4-triazole-3-yl) propyl]benzamide **9** and its derivative **10** attempted by subsequent removal of the protecting amide group. The triazole ring closure for compounds **9** and **10** were established on the basis of ^{1}H-NMR data, corresponding the signal at 13.80 ppm due to triazole NH. The diagnostic

Table 4. Antiviral evaluation and in vitro cytotoxicity in HeLa cell culture.

Compound	CC_{50} (µM)	Minimum cytotoxic concentration	EC_{50} (µM)					
			Vesicular stomatitis virus		**Coxsackie virus B4**		**Respiratory syncytial virus**	
			Visual CPE score	MTS	Visual CPE score	MTS	Visual CPE score	MTS
4	>100	>100	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
5	>100	>100	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
6	>100	>100	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
7	48.8	100	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
8	2.2	4	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
9	>100	>100	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
10	>100	>100	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
11	0.3	0.8	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
12	0.8	0.8	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
DS-5000 (µg/ml)	>100	>100	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
(S)-DHPA	>250	>250	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
Ribavirin	>250	>250	50	12.1	50	28.5	10	4.6

[*] N.A. : Not active at highest concentration tested or at subtoxic concentration.

Table 5. Antiviral evaluation and in vitro cytotoxicity in Vero cell culture.

Compound	Minimum cytotoxic concentration (µM)	EC_{50} (µM)				
		Para influenza-3 virus	Reovirus-1	Sindbis virus	Coxsackie virus B4	Punta Toro virus
4	>100	>100	>100	>100	>100	>100
5	≥20	>20	>20	>20	>20	>20
6	>100	>100	>100	>100	>100	>100
7	100	>20	>20	>20	>20	>20
8	20	>4	>4	>4	>4	>4
9	>100	>100	>100	>100	>100	>100
10	>100	>100	>100	>100	>100	>100
11	0.8	>0.16	>0.16	>0.16	>0.16	>0.16
12	20	>4	>4	>4	>4	>4
DS-5000 (µg/ml)	>100	>100	>100	20	100	100
(S)-DHPA	>250	250	250	>250	>250	>250
Ribavirin	>250	50	>250	>250	>250	112

signal for the primary aliphatic amine moiety of compound **10** was observed at 3.35 ppm. 5-[1-Amino-3-(methylsulfanyl)propyl]-4-phenyl-2,4-dihydro-3*H*-1,2,4-triazole-3-thione **10** was reacted with 3,5-bis-(trifluoromethyl)phenyl isothiocyate and 4-chloro-3-(trifluoromethyl)phenyl isothiocyate to synthesize the corresponding thiourea derivatives **11** and **12**. Structures of novel thioureas (compounds **7**, **8**, **11** and **12)** carrying amino acid scaffolds were confirmed by IR and NMR spectroscopy analysis that were consistent with the literature data (42).

2.2. Antiviral, Antibacterial and Antituberculosis Activity

Anti-HIV activity and cytotoxicity data were obtained with the synthesized compounds **1-12** using the strains HIV-1 (III_B) and HIV-2 (ROD) in an MT-4/MTT based assay. None of the tested compounds showed antiviral activity at subtoxic concentrations (**Table 1**).

Antiviral activity of our synthesized compounds **1-12** were also screened against *Feline Corona virus (FIPV), Feline Herpes virus, Influenza A H1N1, Influenza A H3N2, Influenza B, Herpes simplex (HSV-1 and HSV-2), Herpes simplex virus-1 TK$^-$ KOS ACV^r, Vaccinia virus,*and *Vesicular stomatitis virus, Coxsackie virus B4, respiratory syncytial virus (RSV), Parainfluenza-3, Reovirus-1, Sindbis virus, Punta Toro virus* in CRFK, HEL, HeLa and Vero cell cultures. Antiviral activity data against the viruses mentioned above are tabulated in **Table 2-5**. Among all tested compounds, *N*-{3-(methylsulfanyl)-1-[5-(phenylamino)-1,3,4-thiadiazole-2-yl]propyl}benzamide **(5)** was found active with an EC_{50} value of 20-40 µM against *Influenza A H3N2 virus* (**Table 6-7**).Table 7 summarizes the confirmative anti-*Influenza A H3N2 virus* activity data appertaining to compound **5**

The antituberculosis activity of compounds **5-9**, **12** were tested against *M. tuberculosis H37Rv* strain. Compound **12** was determined as the most active compound with an MIC value of 30.88 µM (**Table 8**). Compounds **5** and **8** were shown to inhibit *M. tuberculosis H37Rv* strain with the MIC values of 79.50 and 61.77 µM. The MIC for **6**, **7** and **9** were 228.23, 145.24 and 166.44 µM. When the compounds were evaluated for cytotoxicity in Vero cells, they proved non selective as MCC (minimum cytotoxic concentration) values were lower than MIC values (**Table 8**). Compounds **5, 7, 9** were also evaluated for their antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus epidermidis* and *Candida albicans*. None of them showed antibacterial activity against these microorganisms.

3. CONCLUSION

A thiourea derivative, compound **12**, was identified as the most active one against *M. Tuberculosis* H37Rv with an

Table 6. Antiviral evaluation and in vitro cytotoxicity in MDCK (Madin-Darby Canine Kidney) cell cultures.

Compound	CC_{50} (µM)	Minimum cytotoxic concentration	EC_{50} (µM)					
			Influenza A H1N1 subtype		Influenza A H3N2 subtype		Influenza B	
			Visual CPE score	MTS	Visual CPE score	MTS	Visual CPE score	MTS
4	>100	>100	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
5	**>100**	**100**	N.A.	N.A.	**20**	**17.3**	N.A.	N.A.
6	23	20	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
7	79	100	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
8	2.7	≥0.8	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
9	>100	>100	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
10	37.5	100	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
11	0.3	0.8	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
12	0.8	0.8	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
Oseltamivir carboxylate	>100	>100	20	28.6	20	26.1	12	6.8
Ribavirine	>100	>100	9	10.1	9	5.2	9	6.3
Amantadine	>500	>500	12	14.7	20	15.6	>500	>500
Rimantadine	246	500	3	2.8	20	11.4	>100	>100

* N.A. : Not active at highest concentration tested or at subtoxic concentration.

Table 7. Anti-Influenza virus activity and cytotoxicity of compound **5** in MDCK cell cultures.

Compound	CC_{50} (µM)	Minimum cytotoxic concentration	EC_{50} (µM)					
			Influenza A H1N1 subtype		Influenza A H3N2 subtype		Influenza B	
			Visual CPE score	MTS	Visual CPE score	MTS	Visual CPE score	MTS
5	>100	>100	**50**	**42**	**45**	**31.4**	N.A.	N.A.
Oseltamivir carboxylate	>100	>100	3	4.7	9	9	100	66.1
Ribavirine	>100	≥100	9	8	9	8.1	20	9.8
Amantadine	>500	>500	34	127	2	1.7	N.A.	N.A.
Rimantadine	305	500	10	15.4	0.09	0.08	N.A.	N.A.

MIC value of 30.88 µM. Nevertheless, further studies on compound **12** was not intended due to its low selectivity index. The preliminary determination of a broad spectrum antiviral profile of compounds **4-12** revealed compound **5**, a thiadiazole-based compound, with an EC_{50} value of 31.4 µM activity against *Influenza A H3N2 virus*. Development of this scaffold, to achieve a highly selective and active lead, will be the subject of our future work.

Table 8. The antituberculosis activity of compounds **5-9, 12**.

Compound	**MIC**[a] vs. *M. tuberculosis H37Rv* (µM)	**MCC**[b] (µM)
5	79.50	≥20
6	228.23	>100
7	145.25	100
8	61.77	20
9	166.44	>100
12	**30.88**	20
Isoniazid	0.073-1.45	-
Rifampin	0.061-0.61	-
Ethambutol	6.12-24.47	-
Thiacetazone	0.42	-

[a] Minimum Inhibitor Concentration.
[b] Minimum Cytotoxic Concentration was determined on Vero cells.

4. MATERIALS AND METHOD

4.1. Synthetic Chemistry

All solvents and reagents were obtained from commercial sources and used without purification. All melting points (ºC, uncorrected) were determined using Kleinfeld SMP-II basic model melting point apparatus. Elemental analyses were obtained using Elementar Analysensysteme GmbH vario MICRO CHNS and are consistent with the assigned structures. Infrared spectra were recorded on a Shimadzu FTIR 8400S and data are expressed in wavenumbers ν (cm^{-1}). NMR spectra were recorded on Brüker AVANCE-DPX 400 at 400 MHz for ^{1}H-NMR and 100 MHz for ^{13}C-NMR (Decoupled), the chemical shifts were expressed in δ (ppm) downfield from tetramethylsilane (TMS) using DMSO-d_6 as solvent. The liquid chromatographic system consists of an Agilent technologies 1100 series instrument equipped with a quaternary solvent delivery system and a model Agilent series G1315 A photodiode array detector. A Rheodyne syringe loading sample injector with a 50 µl sample loop was used for the injection of the analytes. Chromatographic data were collected and processed using Agilent Chemstation Plus software. The separation was performed at ambient temperature by using a reversed phase Kromasil 5C-18 (4.6x250 mm, 5 µm particle size) column. All experiments were performed in gradient mode. The mobile phase was prepared by mixing acetonitrile and bidistilled water (50 : 50 v/v during 0-3 min, 75 : 25 v/v during 3-5 min, 100 : 0 v/v during 5-7 min, 100 : 0 v/v during 7-12 min, 75 : 25 v/v during 12-15 min, 50 : 50 v/v during 15-18 min) and filtered through a 0.45 µm pore filter

and subsequently degassed by ultrasonication, prior to use. Solvent delivery was employed at a flow rate of 1 ml.min^{-1}. Detection of the analytes was carried out at 254 and 280 nm.

Synthesis of 2-(benzoylamino)-4-(methylthio)butyric acid [1], 2-(benzoylamino)-4-(methylthio)butyric acid methyl ester [2], 2-(benzoylamino)-4-(methylthio) butyric acid hydrazide [3]

Synthesis of compounds **1**, **2** and **3** was performed according to the literature method (41). Compound **1,** M.p. 88-90ºC; (lit.41: 88-90ºC), compound **2,** M.p. 86-88ºC (lit. 41: 86-88 ºC), compound **3,** M.p. 180ºC (lit. 41: 180ºC).

1-[2-(Benzoylamino)-4-(methylthio)butyryl]-4-phenylthiosemicarbazide [4]

Compound **3** was heated with phenyl isothiocyanate under reflux for 2h in ethanol. The crude product was filtered and crystallized from ethanol. Yield 80%. **M.p.** 154-157ºC. **HPLC**, t_R (min): 6.78. **IR**, ν (cm^{-1}): 3205 and 3171 (NH str), 1695 (C=O str), 1633 (amide C=O str), 1172 (C=S str). **^{1}H NMR**: **δ** (ppm) 2.04-2.08 (m, *5*H, **H_3C**-S-CH_2-**CH_2**-CH-), 2.52-2.59 (q, 2H, H_3C-S-**CH_2**-CH_2-CH-), 4.37 (s, 1H, H_3C-S-CH_2-CH_2-**CH-**), 7.14 (m, 1H, Ar-H), 7.32 (t, *J=10 Hz*, *J=10 Hz*, 2H, Ar-H), 7.45-7.58 (m, 5H, Ar-H), 7.83 (d, *J=9.2 Hz*, 2H, Ar-H), 8.92 (s, 1H, N^4-H), 9.30 (s, 1H, Ar-CONH-), 9.66 (s, 1H, N^2-H), 10.49 (s, 1H, N^1-H). **^{13}C NMR**, **δ** (ppm): 15.21(**H_3C**-S-CH_2-CH_2-CH-), 30.24 (H_3C-S-**CH_2**-CH_2-CH-), 30.97 (H_3C-S-CH_2-**CH_2**-CH-), 53.28 (H_3C-S-CH_2-CH_2-**CH-**), 124.88 (CSNH-ArC), 125.74 (CSNH-ArC), 128.27 (ArC), 128.91 (CSNH-ArC), 129.03 (ArC), 132.57 (ArC), 133.80 (ArC), 139.50 (CSNH-ArC), 168.69 (-CONHNH-), 171.90 (ArCONH-), 180.79 (C=S). Anal. calc. for $C_{19}H_{22}N_4O_2S_2$ (402.53): C, 56.69; H, 5.51; N, 13.92; S, 15.93%. Found C, 56.29; H, 5.47; N, 13.79; S, 15.73%.

***N*-{3-(Methylsulfanyl)-1-[5-(phenylamino)-1,3,4-thiadiazole-2-yl]propyl}benzamide [5]**

Cyclization of compound **4** by using concentrated H_2SO_4 and neutralization with cold 2N NaOH gave compound **5**. The crude product was filtered and crystallized from methanol. Yield 14%. **M.p.** 207ºC. **HPLC**, t_R (min): 7.83. **IR**, ν (cm^{-1}): 3225 (NH str), 3030 (=C-H str), 1637 (amide C=O str). **^{1}H NMR**, **δ** (ppm): 2.1 (s, 3H, **H_3C**-S-CH_2-CH_2-CH-), 2.3 (m, *2*H, H_3C-S-CH_2-**CH_2**-CH-), 2.6 (m, 2H, H_3C-S-**CH_2**-CH_2-CH-), 5.45 (q, 1H, H_3C-S-CH_2-CH_2-**CH-**), 6.95 (t, *J=10 Hz*, *J=10 Hz*, 1H, Ar-H), 7.29 (t, *J=10.8 Hz*, *J=10.8 Hz*, 2H, Ar-H), 7.46-7.58 (m, 5H, Ar-H), 7.87 (d, *J=9.2 Hz*, 2H, Ar-H), 9.10 (d, *J=11.2 Hz*, 1H, Ar-CONH-), 10.28 (s, 1H, thiadiazole -NH-). **^{13}C NMR**, **δ** (ppm): 15.37 (**H_3C**-S-CH_2-CH_2-CH-), 30.58 (H_3C-S-CH_2-**CH_2**-CH-), 33.27 (H_3C-S-**CH_2**-CH_2-CH-), 49.02 (H_3C-S-CH_2-CH_2-**CH-**), 118.02, 122.49, 128.15. 129.06, 129.76, 132.33, 134.42, 141.33, 162.37, 165.31 (ArC), 167.10 (Ar-CONH-). Anal. calc. for $C_{19}H_{20}N_4OS_2$. H_2O (402.53): C, 56.69; H, 5.51; N, 13.92; S, 15.93%. Found C, 57.70; H, 4.87; N, 14.40; S, 16.71%.

5-[1-Amino-3-(methylsulfanyl)propyl]-*N*-phenyl-1,3,4-thiadiazole-2-amine [6]

Compound **5** was refluxed with 50 % H_2SO_4 at 110-130ºC for 30 hours and neutralized using 2N NaOH. The crude product was washed with distilled water and crystallized from methanol. Yield 5%. **M.p.** 136-140ºC. **HPLC**, t_R (min): 3.22. **IR,** ν (cm^{-1}): 3230, 3186 and 3124 (N-H str), 3030 (=C-H str), 1608 (C=N str). **^{1}H NMR**, **δ** (ppm): 1.85-1.92 (m, 3H, **H_3C**-S-CH_2-CH_2-CH-), 2.03-2.09 (m, 3H, H_3**C**-S-CH_2-**CH_2**-CH-), 2.26-2.38 (m, 2H, H_3C-S-**CH_2**-CH_2-CH-), 3.5 (s, 1H, H_3C-S-CH_2-CH_2-**CH-**), 5.42-5.47 (brs, -NH_2), 7.43-7.48 (m, 2H, Ar-H), 7.58-7.61 (m, 3H, Ar-H), 10.28 (s, 1H, thiadiazole-NH-). Anal. calc. for $C_{12}H_{16}N_4S_2$ (280.41): C, 51.40; H, 5.75; N, 19.98; S, 22.87%. Found C, 51.83; H, 4.81; N, 18.96; S, 23.48%.

Synthesis of 1-{3-(methylsulfanyl)-1-[5-(phenylamino)-1,3,4-thiadiazole-2-yl]propyl}-3-(substituted phenyl) thioureas [7-8]

To the stirred solution of compound **6** in acetonitrile was added an equimolar amount of 4-cyanophenylisothiocyate or 4-chloro-3-(trifluoromethyl)phenylisothiocyate in acetonitrile. The reaction mixture was heated at 140 ºC under stirring. The progress of reaction was monitored by TLC. Acetonitrile was evaporated under vacuum and the solid precipitated was recrystallized from appropriate solvents.

1-{3-(Methylsulfanyl)-1-[5-(phenylamino)-1,3,4-thiadiazole-2-yl]propyl}-3-(4-cyanophenyl)thiourea [7]

The crude product was crystallized from methanol. Yield 62%. **M.p.** 171ºC. **HPLC,** t_R (min): 7.54. **IR**, ν (cm^{-1}): 3207 and 3165 (N-H str), 3030 1606 (C=N str), 1176 (C=S str). **^{1}H NMR**, **δ** (ppm): 2.06-2.39 (m, *5*H, **H_3C**-S-CH_2-**CH_2**-CH-), 2.56-2.63 (m, 2H, H_3C-S-**CH_2**-CH_2-CH-), 4,01 (s, 1H, H_3C-S-CH_2-CH_2-**CH**-), 6.95-7.30 (m, 2H, Ar-H), 7.44-7.60 (m, 3H, Ar-H), 7.77-7.88 (m, 4H, Ar-H), 9.09 (d, *J: 7.8 Hz,* 1H, -NH-) 10.16 (s, 1H, thiadiazole -NH-), 11.52 (s, 1H, -NH-). **^{13}C NMR**, **δ** (ppm): 15.34 (**H_3C**-S-CH_2-CH_2-CH-), 30.57 (H_3C-S-CH_2-**CH_2**-CH-), 33.24 (H_3C-S-**CH_2**-CH_2-CH-), 49.01 (H_3C-S-CH_2-CH_2-**CH-**), 106.93 (CN), 118.01 and 119.53, 122.10 and 122.47, 128.16, 129.06 and 129.76, 132.33, 133.80 and 134.42, 141.33,

162.35, 165.28 (ArC), 167.08 (Ar-CONH-), 189.13 (thiourea C=S).

1-{3-(Methylsulfanyl)-1-[5-(phenylamino)-1,3,4-thiadiazole-2-yl]propyl}-3-(4-chloro-3-(trifluoromethyl) phenyl)thiourea [8]

The crude product was crystallized from ethanol. Yield 52%. **M.p.** 192ºC. **HPLC, t_R** (min): 8.15. **IR,** ν (cm^{-1}): 3225 and 3122 (N-H str), 1606 (C=N str), 1111 (C=S str). **^{1}H NMR**, **δ** (ppm): 1.32-1.98 (m, 5H, **H_3C**-S-CH_2-**CH_2**-CH-), 2.72 and 2.88 (s and s, 2H, H_3C-S-**CH_2**-CH_2-CH-), 3.98 (m, 2H, CH_3-**CH_2**-OH), 4.50 (m, 1H, H_3C-S-CH_2-CH_2-**CH-**), 6.92-7.37 (m, 5H, Ar-H), 7.52-7.64 (m, 3H, Ar-H), 8.03 (s, 1H, -NH-) 9.86 (s, 1H, -NH-), 10.37 (s, 1H, thiadiazole -NH-).

***N*-[3-(Methyllsulfanyl)-1-(4-phenyl-5-thioxo-4,5-dihydro-1*H*-1,2,4-triazole-3-yl)propyl]-benzamide [9]**

Compound **4** was refluxed with 2N NaOH at 90-110ºC for 10 hours and neutralized using 2N HCl. The crude product was washed with distilled water and crystallized from methanol. Yield 72%. **M.p.** 63ºC. **HPLC**, **t_R** (min): 6.15. **IR,** ν (cm^{-1}): 3232 and 3188 (N-H str), 1606 (C=N str), 1170 and 1128 (C=S str). **^{1}H NMR**, **δ** (ppm): 2.08 (q, 5H, **H_3C**-S-CH_2-**CH_2**-CH-), 2.40-2.60 (q, 2H, H_3C-S-**CH_2**-CH_2-CH-) 5.01 (q, 1H, H_3C-S-CH_2-CH_2-**CH-**), 7.41-7.68 (m, 10H, Ar-H), 8.75 (s, 1H, Ar-CONH-), 13.8 (s, 1H, triazole -NH-). **^{13}C NMR**, **δ** (ppm): 14.91 (**H_3C**-S-CH_2-CH_2-CH-), 30.19 (H_3C-S-CH_2-**CH_2**-CH-), 31.66 (H_3C-S-**CH_2**-CH_2-CH-), 45.11 (H_3C-S-CH_2-CH_2-**CH-**), 128.09, 128.83, 130.00 and 130.08, 132.12, 134.09, 134.15, 152.98 (ArC), 166.81 (ArCONH-), 168.85 (triazol C=S). Anal. calc. for $C_{19}H_{20}N_4OS_2$ (384.52): C, 59.35; H, 5.24; N, 14.57; S, 16.68%. Found C, 59.06; H, 5.24; N, 14.77; S, 17.81%.

5-[1-Amino-3-(methylsulfanyl)propyl]-4-phenyl-2,4-dihydro-3*H*-1,2,4-triazole-3-thione [10]

Compound **9** was refluxed with 50 % H_2SO_4 at 90-100ºC for 20 hours and neutralized using 2N NaOH. The oily crude product was triturated with ice-cold petroleum ether several times following the trituration process including ice-cold ethanol 50% and then compound **10** was obtained as an oil through extraction using chloroform. Yield 20%. **HPLC**, **t_R** (min): 7.12. **^{1}H NMR,** **δ** (ppm): 1.76-1.95 (m, 5H, **H_3C**-S-CH_2-**CH_2**-CH-), 2.44 (t, *J: 7.1 Hz, J:7.1 Hz,* 2H, H_3C-S-**CH_2**-CH_2-CH-), 3.35 (s, -NH_2-, 2H), 3.73-3.76 (m, 1H, H_3C-S-CH_2-CH_2-**CH-**), 7.43-7.48 (m, 2H, Ar-H), 7.58-7.61 (m, 3H, Ar-H), 13.80 (s, 1H, triazole -NH-). **^{13}C NMR**, **δ** (ppm): 14.84 and 15.38 (**H_3C**-S-CH_2-CH_2-CH-), 27.10 (H_3C-S-CH_2-**CH_2**-CH-), 30.40 (H_3C-S-**CH_2**-CH_2-CH-), 46.28 (H_3C-S-CH_2-CH_2-**CH-**), 128.78 and 128.87, 131.04, 131.11, 131.15, 134.46, 151.29 (ArC), 160.51 (triazole C=S).

1-[4-(Substituted phenyl)]-3-[3-(methylsulfanyl)-1-(4-phenyl-5-thioxo-4,5-dihydro-1*H*-1,2,4-triazole-3-yl) propyl]thioureas [11-12]

Into the stirred solution of compound **10** in acetonitrile was added an equimolar amount of 3,5-bis-(trifluoromethyl) phenylisothiocyate or 4-chloro-3-(trifluoromethyl)phenyl isothiocyate in acetonitrile. The reaction mixture was heated at 140ºC under stirring. The reaction progress was monitored by TLC. Acetonitrile was evaporated under vacuum and the oily crude product was extracted with chloroform.

1-[4-((3,5-Bis(trifluoromethyl)phenyl)]-3-[3-(methylsulfanyl)-1-(4-phenyl-5-thioxo-4,5-dihydro-1*H*-1,2,4-triazole-3-yl)propyl]thiourea [11]

Yield 26%. **HPLC**, **t_R** (min): 10.29. **^{1}H NMR**, **δ** (ppm): 1.76-1.95 (m, 5H, **H_3C**-S-CH_2-**CH_2**-CH-), 2.44 (t, *J: 7.1 Hz, J:7.1 Hz,* 2H, H_3C-S-**CH_2**-CH_2-CH-), 3.20 (s, 1H, H_3C-S-CH_2-CH_2-**CH-**), 7.80 (s, 3H, Ar-H), 8.20 (s, 5H, Ar-H), 10,70 (brs, 2H, -NH-), 13.80 (s, 1H, triazole -NH-). **^{13}C NMR**, **δ** (ppm): 14.84 and 15.38 (**H_3C**-S-CH_2-CH_2-CH-), 27.10 (H_3C-S-CH_2-**CH_2**-CH-), 30.40 (H_3C-S-**CH_2**-CH_2-CH-), 50.00 (H_3C-S-CH_2-CH_2-**CH-**), 118.85, 118.89 and 118.93, 120.23, 122.90, 125.71, 128.42, 131.10, 131.43 and 131.76, 132.10, 142.44 (ArC), 180.00 (C=S).

1-[4-(4-Chloro-(3-trifluoromethyl)phenyl]-3-[3-(methylsulfanyl)-1-(4-phenyl-5-thioxo-4,5-dihydro-1*H*-1,2,4-triazole-3-yl)propyl]thiourea [12]

Yield 80%. **HPLC**, **t_R** (min): 10.07. **^{1}H NMR**, **δ** (ppm): 2.31-2.71 (s, 8H, , H_3C-S-CH_2-CH_2-CH-), 6.66-6.97 (m, 4H, Ar-H), 7.23-7.90 (m, 4H, Ar-H), 8.28 (s, 1H, -NH-) 9.23 (s, 1H, -NH-), 11.09 (s, 1H, triazole -NH-). **^{13}C NMR**, **δ** (ppm): 14.84 and 15.38 (**H_3C**-S-CH_2-CH_2-CH-), 27.10 (H_3C-S-CH_2-**CH_2**-CH-), 30.40 (H_3C-S-**CH_2**-CH_2-CH-), 46.28 (H_3C-S-CH_2-CH_2-**CH-**), 113.74 and 113.79, 117.67, 120.01, 122.80, 123.87, 125.45, 128.05 and 128.37, 133.24, 148.54 (ArC), 180.02 (C=S).

4.2. In Vitro Antiviral Assays

4.2.1. 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 (43, 44). Stock solutions (10 x 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 5-fold dilutions of test compounds were made directly in flat-bottomed 96-well microtiter trays using a Biomek 3000 robot (Beckman instruments, Fullerton, CA). Untreated control HIV-and mock-infected cell samples were included for each sample. HIV-1(III_B) (45) or HIV-2 (ROD) (46) 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 (47) were centrifuged for 5 minutes at 220 g and the supernatant was discarded. The MT-4 cells were resuspended at 6 x 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 (Infinite M1000, Tecan, Mechelen, Belgium), at two wavelenghths (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 (OD_{540}) 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}).

4.2.2. Antiviral assays other than HIV

The antiviral assays, other than HIV-1, were based on inhibition of virus-induced cytopathicity in HEL cells (*HSV-1(KOS), HSV-1(TK⁻KOS ACV^r), HSV-2(G), Vaccinia virus, Vesicular stomatitis virus*), Hela cells (*Vesicular stomatitis virus, Respiratory syncytial virus, Coxsackie B4 virus*) and Vero cells (*Parainfluenza-3 virus, Reovirus-1, Sindbis virus, Coxsackie B4 virus, Punta Toro virus*), following previously established procedures (48-50).

Briefly, confluent cell cultures in microtiter 96-well plates were inoculated with 100 $CCID_{50}$ of virus, 1 $CCID_{50}$ 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.

4.2.3. Anti- Influenza assays

The test compounds were evaluated for anti-influenza virus activity by a cytopathic effect (CPE) reduction assay (51, 52). MDCK cells were infected with one of three influenza virus strains: A/H1N1, A/H3N2 and B. After 72 h incubation at 35ºC, microscopy followed by the MTS cell viability test were performed, to determine the following parameters for antiviral and cytotoxic activity: EC_{50}, MCC, and CC_{50}. MDCK cells, seeded into 96-well plates, were exposed to serial dilutions of the compounds, together with the influenza virus (multiplicity of infection: 0.0004 PFU per cell). After three days incubation at 35ºC, microscopy was performed for visual scoring of the CPE and cytotoxic effects. The data were confirmed by the spectrophotometric MTS[3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxy-phenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium] cell viability assay.

4.3. Antimicrobial Activity

The antimicrobial activities of all compounds were evaluated in the Department of Genetics and Bioengineering, Faculty of Engineering and Architecture, Yeditepe University. The antibacterial and antifungal activities of the compounds were evaluated against eight microbial cultures isolates of six bacteria and one yeast species by micro-well dilution assay as described below (53). Microorganisms were provided by the Department of Genetics and Bioengineering, Faculty of Engineering and Architecture, Yeditepe University, Istanbul, Turkey. The microorganisms used were *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus epidermidis* and *Candida albicans*. Ampicillin, Cefepime and Amphotericin B were used as the positive reference compounds for bacteria and yeast.

4.3.1. Micro-well dilution assay

The sensitivity of the bacterial strains towards the compounds was quantitatively evaluated from the minimal inhibitory concentration (MIC) values obtained by the

micro-well dilution method. The inocula of the bacterial strains were prepared from 12-h broth cultures and suspensions were adjusted to 0.5 McFarland standard turbidity. Compounds dissolved in DMSO were first prepared at the highest concentration to be tested (200 µg/ml), and then serial two-fold dilutions were made in order to obtain a concentration range from 6.25 to 200 µg/ml, in 15-ml sterile test tubes containing nutrient broth. The 96-well plates were prepared by dispensing into each well 95 µl of nutrient broth and 5 µl of the inoculum. 200 µl of nutrient broth without inoculum was transferred into the first wells as positive control. Aliquots, (100 µl) taken from the 200-µg/ml stock solution, were added to the second well.

100 µl from the respective serial dilutions was transferred into 5 consecutive wells. The last well containing 195 µl of nutrient broth without compound and 5 µl of the inoculum on each strip was used as negative control. Contents of each well were mixed on a plate shaker at 300 rpm for 20 s and then incubated at appropriate temperatures for 24 h. Microbial growth in each medium was determined by reading the absorbance (Abs) at 630 nm using the ELx 800 universal microplate reader (Biotek Instrument inc, Highland Park, Vermont, USA) and confirmed by plating 5-µl samples from clear wells on nutrient agar medium. The MIC was defined as the lowest concentration of the compounds to inhibit the growth of microorganisms. Cefepime (Maxipime®, Bristol-Myers Squibb, Istanbul, Turkey) at the concentration range of 500–7.8 µg/ml was prepared in nutrient broth and used as standard drug for positive control.

4.4. Anti-tuberculosis Activity

The antituberculosis activity of compounds was tested against *M. tuberculosis H37Rv* strain. For the minimal inhibitory concentration (MIC) determination, the compounds were dissolved in dimethyl sulfoxide (DMSO) and serial two fold dilutions were done in Middlebrook 7H9 Broth containing glycerol. Microorganisms were suspended in Middlebrook 7H9 broth to match the turbidity of 0.5 McFarland (1.5 x10^8 *cfu/ml)* and 1/10 dilution was prepared from this suspension and used as inoculum. The tested final concentrations ranged between 512 to 0.5 µg/ml. To make sure that DMSO did not show any inhibitory activity, controls prepared with serial dilutions of DMSO were also tested. Tubes were incubated at 37°C for 24 hours and then examined for turbidity. MIC was determined if turbidity was observed in the positive control tube containing no compound and no turbidity in the negative control tube containing no microorganism (54-56).

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L-Metiyoninden hareketle elde edilen bazı yeni 1,3,4-tiyadiazol ve 1,2,4-triazol türevlerinin sentezi, antitüberküler ve antiviral etkilerinin değerlendirilmesi

ÖZET

Bu çalışma kapsamında, *L*-metiyonin'den hareketle bazı yeni 1,3,4-tiyadiazol **[5-8]** ve 1,2,4-triazol **[9-12]** türevi bileşikler sentezlenmiştir. İlgili tiyosemikarbazit'in siklokondensasyonu ile elde edilen 1,3,4-tiyadiazol ve 1,2,4-triazol bileşikleri, yeni tiyoüre türevlerinin sentez başlangıç maddelerini de oluşturmaktadır. Sentezlenen bileşiklerin **[4-12]** yapıları IR, ^{1}H-NMR ve ^{13}C-NMR spektroskopisi yöntemleriyle aydınlatılmıştır. Elde edilen bileşikler antiviral ve antibakteriyel etkinlikleri açısından değerlendirilmişlerdir. *N*-{3-(Metilsülfanil)-1-[5-(fenilamino)-1,3,4-tiyadiazol-2-il] propil}benzamit'in **[5]**, *Influenza A H3N2* virüsüne karşı EC_{50}: 31.4 µM derişimde etkinlik gösterdiği saptanmış ve devam eden çalışmalarda geliştirilmek üzere önder bileşik olarak tanımlanmıştır. Antitüberküloz etki çalışmaları sonucunda 1-[4-(4-kloro-(3-trifluorometil)fenil]-3-[3-(metilsülfanil)-1-(4-fenil-5-tiyokso-4,5-dihidro-1*H*-1,2,4-triazol-3-il)propil] tiyoüre'nin **[12]** *M. tuberculosis H37Rv* suşuna karşı MİK : 30.88 µM derişimde etkinlik gösterdiği belirlenmiştir.

Anahtar Kelimeler: Tiyoüre; 1,3,4-tiyadiazol; 1,2,4-triazol; antiviral etki; influenza; antitüberküler etki.

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