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Synthesis, characterization and biological evaluation of 1,3-thiazolidine-4-ones derived from (2*S*)-2-benzoylamino-3-methylbutanohydrazide hydrazones

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ABSTRACT: Novel 2-aryl-5-non-substituted / methyl-1,3-thiazolidine-4-one derivatives **14-33** carrying L-valine core were synthesized by the reaction of acylhydrazones **4-13** with thioglycolic acid / thiolactic acid. Structures of all synthesized compounds **14-33** were confirmed by IR, ^{1}H-NMR and HR-MS analysis and ^{13}C-NMR were recorded for selected compounds **17, 21, 28** and **30**. None of the compounds **14-33** showed activity against HIV-1 (strain III_B) or HIV-2 (strain ROD) in an MT-4/MTT based assay. Compounds **14-33** were also screened against *Feline Corona Virus (FIPV), Feline Herpes Virus, HSV-1(KOS), HSV-1 (TK-KOS ACV^r), HSV-2 (G), Vaccinia virus, Vesicular stomatitis virus, Cytomegalovirus, Varicella-Zoster virus, Respiratory syncytial virus, Coxsackie B4 virus, Parainfluenza-3 virus, Reovirus-1, Sindbis virus and Punta Toro virus*, but none of them showed antiviral activity at subtoxic concentrations. Anti-HCV NS5B RdRp activity of some selected compounds from the series **14-33** were found to vary between 4.1-27 % at the concentration of 100 µM. *In vitro* antibacterial activity evaluation of selected compounds **16-23** and **25-32**, against *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Enterococcus faecalis* ATCC 29212, *Klebsiella pneumoniae* ATCC 4352, *Bacillus subtilis* ATCC 6633, *Staphylococcus epidermidis* ATCC 12228, MRSA and antifungal activity against *Candida albicans* ATCC 10231 resulted in the MIC values between 625->5000 µg/ml.

KEYWORDS: 4-Thiazolidinones; L-valine; antibacterial activity; anti-HIV activity; anti-HCV activity.

1. INTRODUCTION

Global summary of AIDS epidemic, including 38.0 millions of people living with HIV/AIDS and around 690,000 people died from AIDS-related ilnesses by the end of 2019, was declared by UNAIDS [1]. Antiretroviral treatment (ART), which was introduced as a sustainable and affordable treatment option for individuals infected with HIV in mid 1990s, has been found highly effective in suppressing HIV-1 RNA to undetectable levels [2, 3]. ART has strongly reduced the mortality from HIV infection; however, more than 10 million people affected by HIV have not started ART yet [4]. Standard ART comprises of two nucleoside reverse transcriptase inhibitors (NRTIs), or one NRTI and one nucleotide reverse transcriptase inhibitor (NtRTI), to which is then added one non-nucleoside reverse transcriptase inhibitor (NNRTI) or one protease inhibitor (PI) [5, 6]. Owing to the treatment-limiting side effects of older PIs; indinavir and nelfinavir, integrase strand transfer inhibitors (INSTIs) were added to the treatment regimens [7]. Despite this; difficulties with

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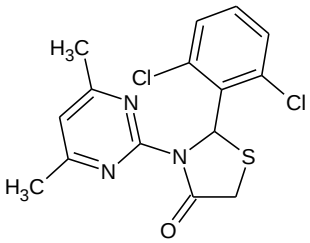
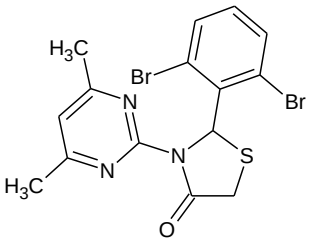
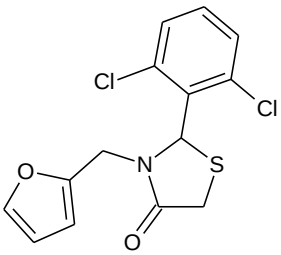
patient's adherence, adverse effects associated with long-term medication, interactions with other drugs, and emergence of virus drug resistance have not been overcome [8]. The efforts to increase ART's efficacy and overcome the problem of viral resistance has resulted in five FDA-approved NNRTIs for clinical use; nevirapine, delavirdine, efavirenz, etravirine and rilpivirine. NRTIs and NtRTIs interact with the catalytic site of the reverse transcriptase (RT) whereas NNRTIs, interact specifically with non-substrate binding site of the enzyme which is located at a close distance away from the substrate-binding site, and inhibit the replication of HIV-1, but not HIV-2 [5,6]. Bearing the chemical heterogeneity (dipyridodiazepinone derivative nevirapine, bis(hetero-aryl)piperazine derivative delavirdine, benzoxazinone derivative efavirenz, diarylpyrimidine derivatives etravirine and rilpivirine) but identical mechanism of action of NNRTIs in mind, new compounds with 1,3-thiazolidine-4-one core have been synthesized and evaluated for their anti-HIV activity and attracted much attention due to their significant activity [9-13]. Anti-HIV-1 activity of 1,3-thiazolidine-4-one compounds is associated with their "butterfly" like conformation. C-2 and N-3 substituted aryl groups, one of which is mostly substituted with halogens, are the pharmacophoric elements resembling the "wings" of the butterfly. The "body" of the butterfly has the hydrophilic features with the ability to form hydrogen bonds. This approach led to the discovery of 2,3-diaryl-4-thiazolidinones, a promising family of NNRTIs (Figure 1) [10, 14-20].



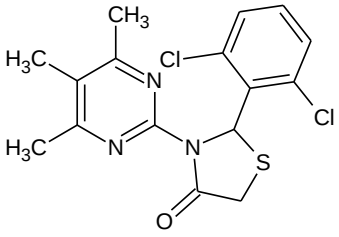
EC_{50}: 0.025 µM
[Ref. 10]



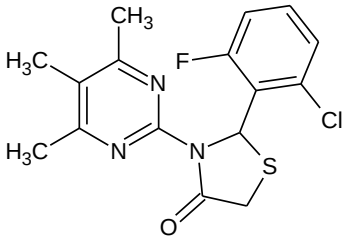
EC_{50}: 0.02 µM
[Ref. 18]



EC_{50}: 0.204 µM
[Ref. 14]



EC_{50}: 0.03 µM
[Ref. 16]



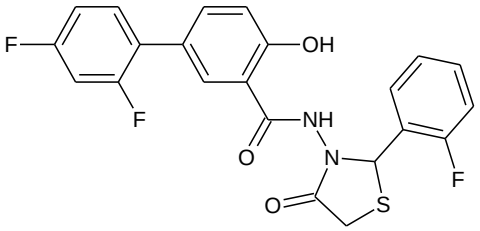
EC_{50}: 0.02 µM
[Ref. 16]

Figure 1. Chemical structures and anti-HIV activity of promising 2,3-diaryl-4-thiazolidinones.

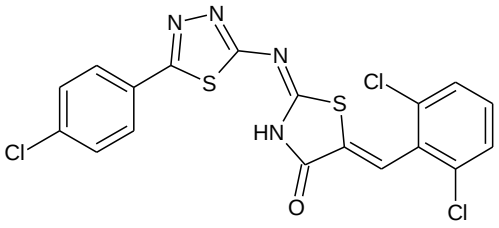
Co-infections of HIV and HCV are very common and constitute a burden. As HIV-1 increases viral load and persistence of HCV, the HCV's effect on HIV could be estimated due to enhanced predisposition of HCV-related liver diseases [21-23]. With the advent of HCV Direct-Acting Antivirals (DAAs) it was possible to succesfully treat both HCV monoinfections but also HIV and HCV coinfections. As prior studies demonstrated the potential of the thiazolidinone scaffold [24-30], especially by employing HCV NS5B (Figure 2) and considering their above-mentioned anti-HIV activity, it is conceivable that dual inhibitors could be developed.

The prevelance of bacterial co-infections with HIV have been increasing [31]. Tuberculosis is one of the leading causes of death in HIV-infected individuals [32]. Candidiasis, cryptococcosis, histoplasmosis, *Pneumocystis jirovicii* pneumonia, syphilis, pneumopathies, bacterial infections including *Pseudomonas species* and *Escherichia coli* sepsis are the other opportunistic infections that remain challenges to HIV(+) patients survival [33].

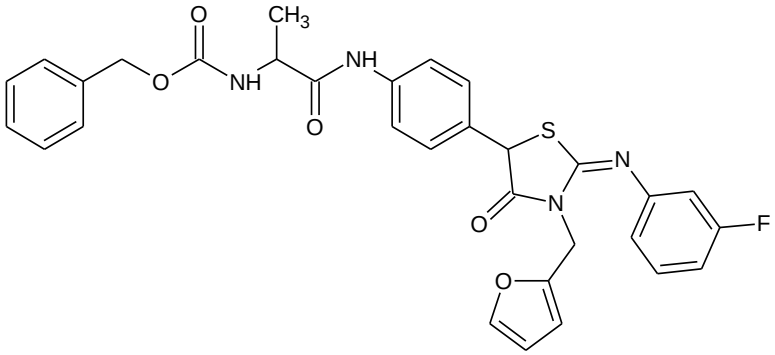
Considering the critically important phenomenon that reveals the urgent need for dual acting inhibitors to treat HIV and HIV-aligned co-infections, we synthesized twenty new 1,3-thiazolidine-4-one derivatives and evaluated them for their antiviral and antibacterial activity potential.



IC_{50} (HCV NS5B RdRp inhibitor) = 48 µM
[Ref. 27]



IC_{50} (HCV NS5B RdRp inhibitor) = 5.6 µM
[Ref. 28]



BMS-858
EC50 (HCV NS5A) = 0.57-1 µM
[Ref. 30]

Figure 2. Chemical structures of 4-thiazolidinones with anti-HCV activity.

2. RESULTS AND DISCUSSION

2.1. Chemistry

Compound **1** was prepared by benzoylation of *L*-valine. Compound **2** was obtained by esterification of compound **1**. Compound **3** was obtained by heating compound **2** with hydrazine hydrate [34]. (2*S*)-2-(benzoylamino)-3-methyl-*N*'-(arylmethylene)butanohydrazides (**4-13**) were obtained by refluxing compound **3** and appropriate aldehydes in ethanolic medium [35]. Compounds **14-23** and **24-33** were synthesized by refluxing compounds **4-13** in dry benzene using a Dean–Stark water separator with thioglycolic acid and thiolactic acid, respectively. The stepwise reaction procedure was outlined in Figure 3.

Purity of compounds was assessed through HPLC data and compounds **14-33** were characterized based on their IR, ^{1}H-NMR, and HR-FAB Mass Spectral data. ^{13}C-NMR spectra were recorded for some representative compounds of our series. Physical and chromatographic data for compounds **14-33** are given in Table 1.

More than one peak were evaluated in the HPLC chromatograms of our pure compounds **14-18**, **23**, **25**, **27**, **28**, and **31-33** of which identical UV spectra were determined. For example; two peaks were evaluated in the HPLC chromatogram of compound **17** (Figure 4a) and the UV spectra (Figure 4b) obtained through these two peaks were found identical. All these data could be considered by means of diastereoisomerism due to chiral carbon atom in the thiazolidinone ring [11, 36]. Since our starting material was *L*-valine (*S*-valine) for compounds **14-23** and as a new chiral center was formed at the C2 atom of thiazolidinone ring, two diastereomers *S,R* and *S,S* arose. In case of compounds **24-33**, two new chiral centers at C2 and C5 atoms of thiazolidinone ring were formed and diastereoisomeric forms (*S,R,R*), (*S,R,S*), (*S,S,R*), and (*S,S,S*) might arise, which were analyzed by HPLC.

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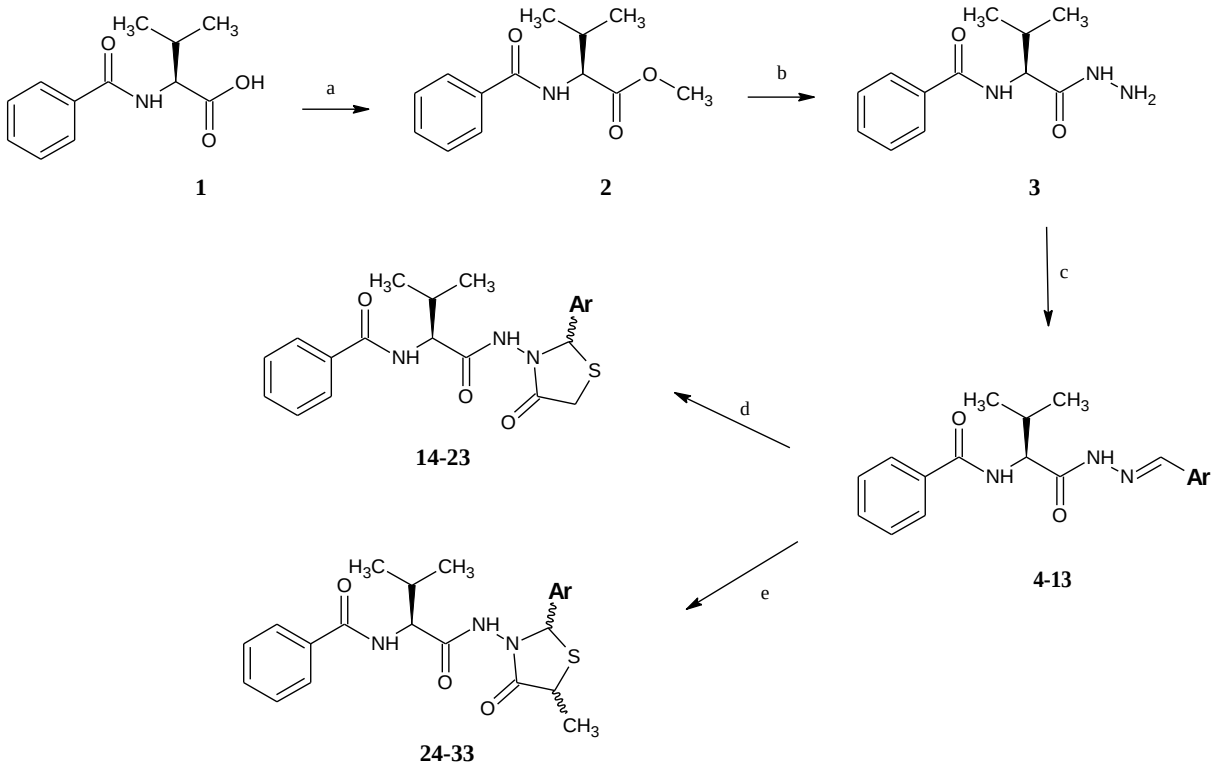

Compound ID	Ar	Compound ID	Ar
4, **14**, **24**	4-chlorophenyl	**9**, **19**, **29**	2,6-difluorophenyl
5, **15**, **25**	2,6-dichlorophenyl	**10**, **20**, **30**	2-fluoro-6-chlorophenyl
6, **16**, **26**	2-fluorophenyl	**11**, **21**, **31**	4-trifluoromethylphenyl
7, **17**, **27**	3-fluorophenyl	**12**, **22**, **32**	4-methoxyphenyl
8, **18**, **28**	4-fluorophenyl	**13**, **23**, **33**	2,4,6-trimethylphenyl

Figure 3. Synthetic route to compounds **1-33**. Key to reagents: (a) MeOH/H_2SO_4, reflux; (b) $NH_2NH_2.H_2O$, reflux; (c) R-CHO/EtOH, reflux; (d) $HSCH_2COOH$/dry benzene, reflux; (e) $HSCH(CH_3)COOH$/dry benzene, reflux.

Table 1. Physical and chromatographic data for compounds **14-33.**

Comp	Retention time (min) Method S_1	Retention time (min) Method S_2	Comp	Retention time (min) Method S_1	Yield %	M.p. (°C) Recryst. solvent	Comp	Retention time (min) Method S_2	Yield %	M.p. (°C) Recrys. solvent
3	4.108	1.88								
4	4.19/7.90		**14**	13.52 / 16.95	45	188-191 (EtOH:DMF, 99:1)	**24**	11.17	43	143 / 169-171 (Et_2O)
5	8.13	11.12	**15**	7.93 / 20.07	10	226 (EtOH)	**25**	12.36 / 13.62	89	112 (Petr. ether)
6	6.86	5.46	**16**	10.65 / 17.27	54	146 (EtOH:DMF, 99:1)	**26**	7.61	23	86 (EtOH)
7	9.11	5.54	**17**	13.51 / 14.22	38	186-188 (EtOH:DMF, 99:1)	**27**	7.93 / 8.71	23	137 (EtOH)
8	9.83	5.13	**18**	13.75 / 15.98	49	207-210 (EtOH)	**28**	7.46 / 8.41	8	179-182 (Petr. ether)
9	10.39	6.11	**19**	18.78	43	237 (MeOH)	**29**	7.80	44	185 (EtOH)
10	9.19	8.33	**20**	21.15	42	195 (MeOH)	**30**	10.27	42	214-219 (EtOH)
11	9.36	11.87	**21**	12.98	15	172-176 (MeOH:DMF, 99:1)	**31**	15.93 / 18.86	44	95 & 115 (Petr. ether)
12	11.82	4.53	**22**	16.00	38	235-238 (EtOH)	**32**	6.33 / 7.23	88	227-229 (Petr. ether)
13	6.65	20.15	**23**	7.22 / 11.03	15	172-176 (MeOH:DMF, 99:1)	**33**	23.33 / 25.07	59	168-172 (Petr. ether)

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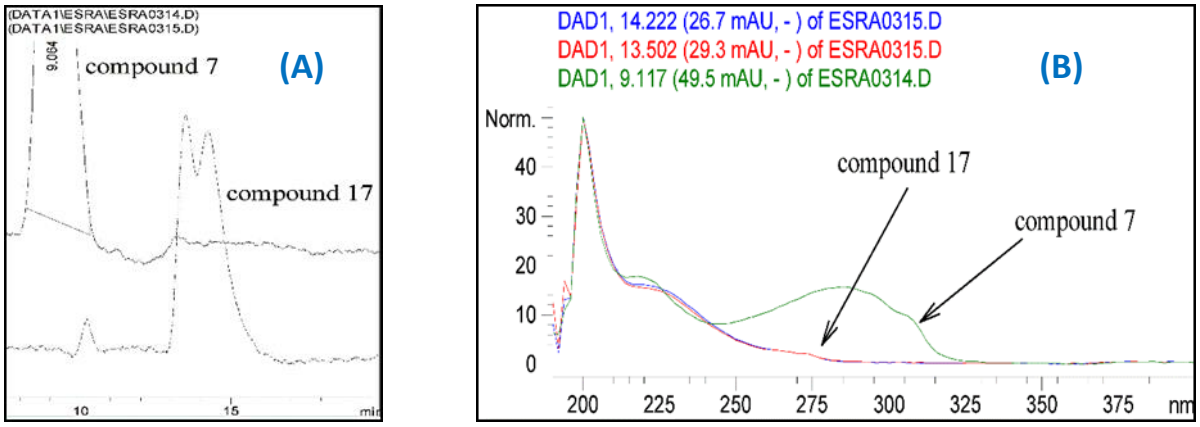

Figure 4. (a) Overlaid HPLC chromatograms of compounds **7** and **17.** (b) Overlaid UV spectra of compounds **7** and **17.**

Interestingly, for compounds **24** and **31**, two melting points were determined for each and we assumed that the diastereomers, which were crystallized from same solution, formed two separate crystal lattices. In the IR spectra of compounds **14-33**, the bands between 1676-1666 cm^{-1} which were attributed to C=O stretching bands of acylhydrazones **4-13**, were not detected [35]. New C=O bands observed between ranges 1705-1728 cm^{-1} in the IR spectra of 1,3-thiazolidine-4-ones **14-33** respectively, provided confirmatory evidence for thiazolidine-4-one ring closure [37]. Compound **17** has two bands at 1728 cm^{-1} and 1716 cm^{-1} that were assigned to C=O bands of the mentioned ring. Compound **26** also showed a similar IR spectroscopy profile as compound **17** based on the two C=O bands.

Signals in ^{1}H-NMR spectra at about 3.52-3.95 ppm that were attributed to the CH_2 protons at the position 5 of the 1,3-thiazolidine-4-one ring, supported the exact structures of compounds **14-23.** The two singlet signals between ranges 5.69-6.65 ppm and 5.82-6.76 ppm were associated with the CH proton at position 2 of the 1,3-thiazolidine-4-one ring of compounds **14-22** with the exception of compound **23** demonstrating six singlet signals between 5.73-6.90 ppm [26, 38]. In the ^{1}H-NMR spectra of most of the compounds belonging to the series 5-methyl-1,3-thiazolidinones (compounds **24-33)** –SCH**-** protons were determined together with the chiral proton of valine moiety between 4.02-4.37 ppm. The methyl protons, attached to the –SCH**-** adduct of 5-methyl-1,3-thiazolidine-4-one ring, resonated between 1.09-1.61 ppm in accordance with literature [39]. Moreover in the ^{1}H-NMR spectra of all thiazolidinones (**14-33**) two sets of singlet, doublet or distorted multiplet signals were observed for most of the protons, and this aspect was presumably due to magnetically inequivalent protons on chiral center, that occured following nucleophilic addition of thioglycolic acid to -N=CH- adduct.

For the reprensentative compounds **17, 21, 28** and **30**; ^{13}C-NMR spectra were recorded. The methylene, methyn and carbonyl carbons of thiazolidine-4-one ring were observed in accordance with the literature; for compounds **17** and **21** experimentally detected resonances assigned to 4-thiazolidinone C5 δ 29.57– 29.61 ppm, 4-thiazolidinone C2 δ 61.15-61.14 ppm, and endocyclic C=O δ 170.14 and 170.61–170.13 ppm [40]. For compounds **28** and **30** experimentally detected resonances assigned to methyl carbon on 4-thiazolidinone C5 δ 20.43 -18.87 and 18.95 ppm, 4-thiazolidinone C5 δ 38.52- 38.79 and 38.96 ppm, 4-thiazolidinone C2 δ 60.14 and 60.45 - 53.87, 54.17 and 54.43 ppm, and endocyclic C=O δ 171.89 and 172.02- 171.07 ppm. The ^{13}C-NMR spectral data also supported the existence of diastereomers by showing duplicate signals for some of the carbons of the framework.

The compounds **14-33** were subjected to HR-MS analysis by using FAB method to confirm their molecular weights; observed $(M+Na)^+$ and $(M+H)^+$ ions for compounds **14-33** confirmed their calculated molecular weights.

2.2. Biological evaluation

(2*S*)-3-[2-(Benzoylamino)-3-methylbutanamido]-2-aryl-5-non substituted / methyl-1,3-thiazolidinones **14-33** were synthesized via (2*S*)-2-(benzoylamino)-3-methyl-*N*'-(arylmethylene)butanohydrazides **4-13** three of which (**6**, **9** and **10**) were shown to possess anti-HIV-1(III_B)activity with an IC_{50}=123.8 µM and a SI>3, IC_{50}= 12.1 µM and SI>29, IC_{50}= 17.4 µM) and SI>19, respectively [35]. The idea of synthesizing the series of 1,3-

thiazolidine-4-one compounds by using compounds **4-13** as starting materials was to evaluate the influence 1,3-thiazolidine-4-one ring and some selected substituents: (i) ortho and meta monohalogeno-substitution at the phenyl ring on C-2 (ii) substituents at the para position different from methoxy, trifluoromethoxy or halogens at the phenyl ring on C-2 (iii) dihalogeno substitution at position 2 and 6 of the phenyl ring on C-2 (iv) trimethyl substituted phenyl ring on C-2 on anti-HIV activity. None of the newly synthesized products inhibits the HIV-1 (III_B) or HIV-2 (ROD) induced cytopathogenic effect in MT-4 cells. Even the corresponding thiazolidine-4-one derivatives-1**6**, **26, 19, 29, 20** and **30** of the acylhydrazone compounds **6**, **9** and **10** [35] were found inactive at subtoxic concentrations against HIV-1 (III_B) (See supplementary materials Table S1 for anti-HIV and cytotoxicity data obtained the using the strains HIV-1 (III_B) and HIV-2 (ROD) in an MT-4/MTT based assay). Our attempt of synthesizing new compounds containing an 1,3-thiazolidine-4-one scaffold to obtain more active and more selective compounds as compared to their starting materials has failed.

The synthesized compounds **14-33** were also evaluated against various types of viruses (*Feline Corona Virus (FIPV), Feline Herpes Virus, HSV-1(KOS), HSV-1(TK-KOS ACV^r), HSV-2(G), Vaccinia virus, Vesicular stomatitis virus, human Cytomegalovirus, Varicella-Zoster virus, Respiratory syncytial virus, Coxsackie B4 virus, Parainfluenza-3 virus, Reovirus-1, Sindbis virus and Punta Toro virus)* in CRFK, HEL, HeLa and Vero cell cultures. None of them showed antiviral activity at subtoxic concentrations (See supplementary materials for Tables S2-S6).

Anti-HCV NS5B RdRp activity of selected compounds from our series **14-33** were also studied. Since the inhibitory potential of compounds were found to vary between 4.1-27%, it can be concluded that no pronounced anti-HCV NS5B RdRp activity was observed at the concentration of 100 µM, which was the highest concentration tested (See supplementary materials for Table S7).

In vitro antibacterial activity of selected compounds **16-23** and **25-32** were evaluated against *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Enterococcus faecalis* ATCC 29212, *Klebsiella pneumoniae* ATCC 4352, *Bacillus subtilis* ATCC 6633, *Staphylococcus epidermidis* ATCC 12228, MRSA and antifungal activity against *Candida albicans* ATCC 10231. Nevertheless, none of the tested compounds were found to show remarkable activity. (See supplementary materials for Table S8).

3. CONCLUSION

Synthesis and characterization of twenty new 1,3-thiazolidine-4-ones **14-33** fell within the context of this paper. Anti-HIV activities of compounds **14-33** against HIV-1 (IIIB) and HIV-2 (ROD) in an MT-4/MTT based assay were evaluated. Compounds **14-33** were also evaluated against various other human pathogenic viruses. None of the compounds showed antiviral activity at subtoxic concentrations. Anti-HCV NS5B RdRp activity of selected compounds from the series **14-33** were found to vary between 4.1-27 % at the concentration of 100 µM.

In vitro antibacterial activity evaluation of selected compounds **16-23** and **25-32**, against a selection of Gram (+) and Gram (-) strains and antifungal activity against *Candida albicans* ATCC 10231 resulted in marginal activity within the tested concentration range.

4. MATERIALS AND METHODS

4.1. Chemicals and instruments

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. Infrared spectra were recorded on a Shimadzu FTIR 8400S and expressed in wavenumber υ (cm^{-1}). NMR spectra were recorded on a Brüker AVANCE-DPX 400 at 400 MHz for ^{1}H-NMR and 100 MHz for ^{13}C-NMR (DEPT and Decoupled), the chemical shifts were expressed in δ (ppm) downfield from tetramethylsilane (TMS) using DMSO-d_6 as solvent. High resolution electron impact and fast atom bombardment mass spectra were recorded on a Jeol JMS-700 instrument. 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. The seperation of compounds **4-23** was performed (by method S_1) at ambient temperature by using a normal phase Waters; µPorasil (3.9×300 mm, 10 µm particle size)

column. All experiments were employed in isocratic mode. The mobil phase was prepared by mixing hexane and ethanol (97:3, v/v) and filtering through a 0.45 µm membrane and degassing 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. The seperation of compounds **4-13** and **24-33** was performed (by method S_2) at ambient temperature by using a reversed phase Nova-Pak C18 (3.9×150 mm, 5 µm particle size) column. All experiments were performed in isocratic mode. The mobil phase was prepared by mixing acetonitrile and bidistilled water (40:60 v/v) followed by subsequent filtering through a 0.45 µm membrane and degassing by ultrasonication, prior to use. Solvent delivery was set at a flow rate of 1 ml.min^{-1}. Detection of the analytes was carried out at 254 and 280 nm. Retention times for compounds **4-33** are listed in Table 1.

Synthesis procedures and structure characterization data for compounds; (2*S*)-2-(benzoylamino)-3-methylbutyric acid (**1**), (2*S*)-2-(benzoylamino)-3-methylbutyric acid methyl ester (**2**), (2*S*)-2-(benzoylamino)-3-methylbutyric acid hydrazide (**3**) and (2*S*)-2-(benzoyl-amino)-3-methyl-*N*'-(arylmethylene)butanohydrazides (**4-13**) have been reported in the literature [34, 35].

4.2. Chemistry

4.2.1. General procedure for the synthesis of (2S)-3-[2-(benzoylamino)-3-methylbutanamido]-2-aryl-1,3-thiazolidinones **14-23**

A mixture of compounds **4-13** (0.01 mol) and thioglycolic acid (0.2 mol) was refluxed in dry benzene (100 ml) using a Dean–Stark water separator. Excess benzene was evaporated *in vacuo*. The resulting residue was triturated with saturated $NaHCO_3$ solution until CO_2 evolution ceased. The solid was washed with water, dried and recrystallized from appropriate solvents.

(2*S*)-3-[2-(Benzoylamino)-3-methylbutanamido]-2-(4-chlorophenyl)-1,3-thiazolidinone **(14):** IR (KBr), υ (cm^{-1}): 3365, 3242, 3201 (N-H), 1716 (C=O), 1670 (C=O), 1656 (C=O), 1087 (Ar-Cl). ^{1}H-NMR (DMSO-d$_6$): δ ppm =0.74, 0.81, 0.87, 0.91 (4d, *J: 6.74 Hz,* 6H, >CHCH**(CH$_3$)$_2$**), 1.91-1.96 (m, 1H, >CH**CH(**CH$_3$)$_2$), 3.71, 3.76 (2d, *J: 5.81 Hz,* 1H, S-**CH$_2$**-), 3.87, 3.92 (2d, *J: 5.18 Hz,* 1H, S-**CH$_2$**-), 4.21-4.29 (m, 1H, **>CH**CH-**(**CH$_3$)$_2$), 5.74, 5.82 (2s, 1H, N-**CH**-Ar-S), 7.42-7.49 (m, 6H, Ar-**H**), 7.51-7.56 (m, 1H, Ar-**H**), 7.78, 7.85 (2d, *J: 8.51 Hz*, 2H, Ar-**H**), 8.22, 8.34 (2d, *J: 8.85 Hz*, 1H, Ar-CO**NH-**), 10.30, 10.42 (2s, 1H, -CO-**NH**-N=). HR-MS (FAB$^+$), m/z: 456.0952 (M+Na, $C_{21}H_{22}{}^{37}ClN_3O_3SNa$), 454.0946 (M+Na, $C_{21}H_{22}{}^{35}ClN_3O_3SNa$, base peak), 434.1096 (M+H, $C_{21}H_{23}{}^{37}ClN_3O_3S$), 432.1111 (M+H, $C_{21}H_{23}{}^{35}ClN_3O_3S$).

(2*S*)-3-[2-(Benzoylamino)-3-methylbutanamido]-2-(2,6-dichlorophenyl)-1,3-thiazolidinone **(15):** IR (KBr), υ (cm^{-1}): 3209, 3201 (N-H), 1718 (C=O), 1672 (C=O), 1633 (C=O). ^{1}H-NMR (DMSO-d$_6$): δ ppm =0.69, 0.78, 0.90, 0.96, 1.00, 1.36, 1.77 (7d, *J: 6.74Hz,* 6H, >CHCH**(CH$_3$)$_2$**), 1.84-1.92, 2.02-2.18 (2m, 1H, >CH**CH(**CH$_3$)$_2$), 3.52-3.65, 3.81-3.91 (2m, 2H, -S-**CH$_2$**-), 4.23, 4.36, 4.47 (3t, *J: 8.77Hz,* 1H, **>CH**CH**(**CH$_3$)$_2$), 6.65, 6.71 (2s, 1H, N-**CH**-Ar-S), 7.38-7.58 (m, 6H, Ar-**H**), 7.81, 7.87 (2d, *J=7.1Hz,* 2H, Ar-**H**), 8.23, 8.25, 8.31, 8.33, 8.36, 8.38, 8.39 (7s, 1H, Ar-CO**NH-**), 10.12, 10.21, 10.44, 10.56 (4s, 1H, -CO-NH-N=). HR-MS (FAB$^+$), m/z: 492.0559 (M+Na, $C_{21}H_{21}{}^{37}Cl_2N_3O_3SNa$), 490.0545 (M+Na, $C_{21}H_{21}{}^{37}Cl^{35}ClN_3O_3SNa$), 488.0589 (M+Na, $C_{21}H_{22}{}^{35}Cl_2N_3O_3SNa$, base peak), 468.0759 (M+H, $C_{21}H_{22}{}^{37}Cl^{35}ClN_3O_3S$), 466.0768 (M+H, $C_{21}H_{22}{}^{35}Cl_2N_3O_3S$).

(2*S*)-3-[2-(Benzoylamino)-3-methylbutanamido]-2-(2-fluorophenyl)-1,3-thiazolidinone **(16):** IR (KBr), υ (cm^{-1}): 3254, 3215 (N-H), 1722 (C=O), 1672 (C=O), 1633 (C=O), 1226, 1188 (Ar-F). ^{1}H-NMR (DMSO-d$_6$): δ ppm =0.68, 0.77, 0.86, 0.91 (4d, *J: 6.71Hz,* 6H, >CHCH**(CH$_3$)$_2$**), 1.90-1.95, 2.02-2.08 (2q, 1H, >CH**CH**(CH$_3$)$_2$), 3.75, 3.79, (2d, *J: 8.23 Hz*, 1H, S-**CH$_2$**-), 3.86, 3.90 (2s, 1H, S-**CH$_2$**-), 4.24, 4.38 (2t, *J: 8.41 Hz,* 1H, **>CH**CH**(**CH$_3$)$_2$), 5.96, 6.05 (2s,1H, N-**CH**-Ar-S), 7.15-7.28 (m, 2H, Ar-**H**), 7.35-7.58 (m, 5H, Ar-**H**), 7.79-7.84 (m, 2H, Ar-**H**), 8.22, 8.34 (2d, *J: 8.65Hz,* 1H, Ar-CO**NH-**), 10.41, 10.58 (2s, 1H, -CO-**NH**-N=). HR-MS (FAB$^+$), m/z: 438.1267 (M+Na$^+$, $C_{21}H_{22}FN_3O_3SNa$), 416.1467 (M+H$^+$, $C_{21}H_{23}FN_3O_3S$, base peak).

(2*S*)-3-[2-(Benzoylamino)-3-methylbutanamido]-2-(3-fluorophenyl)-1,3-thiazolidinone **(17):** IR (KBr), υ (cm^{-1}): 3371, 3252, 3221 (N-H), 1728, 1716 (C=O), 1674 (C=O), 1654, 1633 (C=O), 1234, 1211 (Ar-F). ^{1}H-NMR (DMSO-d$_6$): δ ppm =0.72, 0.80, 0.88, 0.92 (4d, *J: 6.73 Hz,* 6H, >CHCH**(CH$_3$)$_2$**), 1.91-1.96, 2.02-2.07 (2q, 1H, >CH**CH(**CH$_3$)$_2$), 3.71, 3.75 (2d, *J: 7.11 Hz*, 1H, -S-**CH$_2$**-), 3.89, 3.95 (2d, *J: 6.59 Hz*, 1H, -S-**CH$_2$**-), 4.25, 4.31 (t, *J: 8.35 Hz,* 1H, **>CH**CH(CH$_3$)$_2$), 5.76, 5.86 (2s, 1H, N-**CH**-Ar-S), 7.10-7.55 (m, 7H, Ar-**H**), 7.78, 7.86 (2d, *J: 7.27 Hz*, 2H, Ar- **H**), 8.23, 8.34 (d, *J: 8.88 H*, 1H, Ar-CO**NH-**), 10.35, 10.46 (2s, 1H, -CO-**NH**-N=).^{13}C-NMR-DEPT (DMSO): δ ppm =19.02, 19.07, 19.24, 19.41 (>CHCH**(**CH$_3$)$_2$), 29.57 (4-thiazolidinone **C$_5$**), 30.45 and 30.63 (>CH**CH(**CH$_3$)$_2$),

57.56 and 57.66 (>**C**HCH**(**$CH_3)_2$), 61.15 (4-thiazolidinone **C_2**), 114.58, 114.81 and 115.07, 116.10, 116.19, 116.31 and 116.40, 124.17 and 124.34, 127.97, 128.01, 128.61 and 128.66, 131.81 and 131.84, 134.46, 161.33 (Ar-**C**), 167.00 and 167.05 (**C**ONHN<), 169.15 and 169.22 (Ar-**C**ONH), 170.14 and 170.61 (4-thiazolidinone **C_4**). HR-MS (FAB$^+$), m/z: 438.1240 (M+Na$^+$, $C_{21}H_{22}FN_3O_3SNa$), 416.1444 (M+H$^+$, $C_{21}H_{23}FN_3O_3S$, base peak).

(2*S*)-3-[2-(Benzoylamino)-3-methylbutanamido]-2-(4-fluorophenyl)-1,3-thiazolidinone **(18):** IR (KBr), υ (cm^{-1}): 3367, 3290, 3201 (N-H), 1714 (C=O), 1670 (C=O), 1658, 1633 (C=O), 1224, 1157 (Ar-F). ^{1}H-NMR (DMSO-d_6): δ ppm =0.71, 0.79, 0.86, 0.89 (4d, *J: 6.70 Hz,* 6H, >CHCH**(**$\mathbf{CH_3)_2}$), 1.89-1.94, 2.01-2.06 (2q, 1H, >CH**CH(**$CH_3)_2$), 3.69, 3.73 (2s, 1H, -S-$\mathbf{CH_2}$-), 3.84, 3.90 (2d, *J: 8.05 Hz*, 1H, S-$\mathbf{CH_2}$-), 4.21-4.28 (m, 1H, >**CH**CH**(**$CH_3)_2$), 5.76, 5.88 (2s, 1H, N-**CH**-Ar-S), 7.11, 7.19 (2t, *J: 8.83Hz,* 2H, Ar-**H**), 7.42-7.56 (m, 5H, Ar-**H**), 7.78, 7.86 (2d, *J: 8.51 Hz*, 2H, Ar-**H**), 8.18, 8.31 (2d, *J: 8.42 Hz*, 1H, Ar-CO**NH-**), 10.33 (s, 1H,-CO-**NH**-N=). HR-MS (FAB$^+$), m/z: 438.1278 (M+Na$^+$, $C_{21}H_{22}FN_3O_3SNa$), 416.1424 (M+H$^+$, $C_{21}H_{23}FN_3O_3S$, base peak).

(2*S*)-3-[2-(Benzoylamino)-3-methylbutanamido]-2-(2,6-difluorophenyl)-1,3-thiazolidinone **(19):** IR (KBr), υ (cm^{-1}): 3381, 3336, 3255, 3215, 3205 (N-H), 1716 (C=O), 1676 (C=O), 1656, 1633, 1624 (C=O), 1222, 1190 (Ar-F). ^{1}H-NMR (DMSO-d_6): δ ppm =0.72, 0.78, 0.87, 0.91, 0.99 (5d, *J: 6.70 Hz*, 6H, >CHCH**(**$\mathbf{CH_3)_2}$), 1.91-2.06 (m, 1H, >CH**CH(**$CH_3)_2$), 3.75-3.86 (m, 2H, -S-$\mathbf{CH_2}$-), 4.19-4.49 (m, 1H, >**CH**CH**(**$CH_3)_2$), 6.08, 6.15 (2s, 1H, N-**CH**-Ar-S), 7.07-7.24 (m, 2H, Ar-**H**), 7.31-7.35, 7.37-7.56 (2m, 4H, Ar-**H**), 7.80-7.88 (m, 2H, Ar-**H**), 8.25, 8.37 (2d, *J: 8.58 Hz*, 1H, Ar-CO**NH-**), 10.52 (s, 1H, -CO-**NH**-N=). HR-MS (FAB$^+$), m/z: 456.1193 (M+Na$^+$, $C_{21}H_{21}F_2N_3O_3SNa$, base peak), 434.1382 (M+H$^+$, $C_{21}H_{22}F_2N_3O_3S$).

(2*S*)-3-[2-(Benzoylamino)-3-methylbutanamido]-2-(2-fluoro-6-chlorophenyl)-1,3-thiazolidinone **(20):** IR (KBr), υ (cm^{-1}): 3221 (N-H), 1718 (C=O), 1681 (C=O), 1633 (C=O), 1261, 1247, 1222 (Ar-F). ^{1}H-NMR (DMSO-d_6): δ ppm =0.71, 0.79, 0.88, 0.94 (4d, *J: 6.73 Hz,* 6H, >CHCH**(**$\mathbf{CH_3)_2}$), 1.90-1.95, 2.02-2.07 (2q, 1H, >CH**CH(**$CH_3)_2$), 3.76- 3.85 (m, 2H, -S-$\mathbf{CH_2}$-), 4.24, 4.42 (2t, *J: 8.60 Hz,* 1H, >**CH**CH**(**$CH_3)_2$), 6.31, 6.38 (2s, 1H, N-**CH**-Ar-S), 7.20-7.55 (m, 6H, Ar-**H**), 7.81-7.84 (m, 2H, Ar-**H**), 8.26, 8.36 (2d, *J: 8.59 Hz*, 1H, Ar-CO**NH-**), 10.43, 10.52, 10.58, 10.72 (4s, 1H, -CO-**NH**-N=). HR-MS (FAB$^+$), m/z: 474.0902 (M+Na, $C_{21}H_{21}{}^{37}ClFN_3O_3SNa$,), 472.0894 (M+Na, $C_{21}H_{21}{}^{35}ClFN_3O_3SNa$,), 452.1089 (M+H, $C_{21}H_{22}{}^{37}ClFN_3O_3S$), 450.1093 (M+H, $C_{21}H_{22}{}^{35}ClFN_3O_3S$, base peak).

(2*S*)-3-[2-(Benzoylamino)-3-methylbutanamido]-2-(4-trifluoromethylphenyl)-1,3-thiazolidinone **(21):** IR (KBr), υ (cm^{-1}): 3180, 3142 (N-H), 1705 (C=O), 1670 (C=O), 1396, 1323, 1305, 1159, 1130 (C-F). ^{1}H-NMR (DMSO-d_6): δ ppm =0.73, 0.81, 0.86, 0.91 (4d, *J: 6.72 Hz,* 6H, >CHCH**(**$\mathbf{CH_3)_2}$), 1.84-2.14 (m, 1H, >CH**CH(**$CH_3)_2$), 3.74, 3.78, 3.91, 3.95 (4d, *J: 4.82,* 2H, -S-$\mathbf{CH_2}$-), 4.23-4.27 (m, 1H, >**CH**CH**(**$CH_3)_2$), 5.84, 5.92 (2s, 1H, N-**CH**-Ar-S), 7.41-7.48 (m, 2H, Ar-**H**), 7.52 (t, *J: 7.28 Hz,* 1H, Ar-**H**), 7.65, 7.70 (2s, 4H, Ar-H), 7.73-7.78 (q, 2H, Ar-**H**), 8.21, 8.41 (2d, *J: 8.69 Hz*, 1H, Ar-CO**NH-**), 10.46 (s, 1H, -CO-**NH**-N=).^{13}C-NMR-DEPT (DMSO): δ ppm =19.20 and 19.27 (>CHCH**(**$CH_3)_2$), 29.61 (4-thiazolidinone **C_5**), 30.48 (>CH**C**H**(**$CH_3)_2$), 57.92 (>**C**HCH**(**$CH_3)_2$), 61.14 (4-thiazolidinone **C_2**), 125.76, 127.95, 128.06, 128.53 and 128.99, 131.71, 134.53 (Ar-**C**), 166.86 (**C**ONHN<, Ar-**C**ONH), 170.13 (4-thiazolidinone **C_4**). HR-MS (FAB$^+$), m/z: 488.1195 (M+Na$^+$, $C_{22}H_{22}F_3N_3O_3SNa$, base peak), 466.1408 (M+H$^+$, $C_{22}H_{23}F_3N_3O_3$), 465.11316 (M$^+$, $C_{22}H_{22}F_3N_3O_3S$).

(2*S*)-3-[2-(Benzoylamino)-3-methylbutanamido]-2-(4-methoxyphenyl)-1,3-thiazolidinone **(22):** Yield 38%; mp 235-238^{o}C (Ethanol). IR (KBr), υ (cm^{-1}): 3254, 3207 (N-H), 1726 (C=O), 1691, 1676 (C=O), 1633 (C=O). ^{1}H-NMR (DMSO-d_6): δ ppm =0.75, 0.81, 0.86, 0.91 (4d, *J: 6.74 Hz,* 6H, >CHCH**(**$\mathbf{CH_3)_2}$), 1.92-2.04 (q, 1H, >CH**CH(**$CH_3)_2$), 3.72, 3.83, 3.87 (3d, *J: 1.91 Hz,* 5H, -S-$\mathbf{CH_2}$- and Ar-$\mathbf{OCH_3}$), 4.25-4.34 (m,1H, >**CH**CH**(**$CH_3)_2$), 5.69, 6.76 (2s, 1H, N-**CH**-Ar-S), 6.83-6.93 (m, 2H, Ar-**H**), 7.31-7.56 (m, 5H, Ar-**H**), 7.78, 7.85 (2d, *J: 8.51Hz*, 2H, Ar-**H**), 8.16, 8.31 (2d, *J: 8.71 Hz*, 1H, Ar-CO**NH-**), 10.24, 10.32 (2s, 1H, -CO-**NH**-N=). HR-MS (FAB$^+$), m/z: 450.1460 (M+Na$^+$, $C_{22}H_{25}N_3O_4SNa$), 428.1640 (M+H$^+$, $C_{22}H_{26}N_3O_4S$).

(2*S*)-3-[2-(Benzoylamino)-3-methylbutanamido]-2-(2,4,6-trimethylphenyl)-1,3-thiazolidinone **(23):** Yield 31%; mp 109-110^{o}C (Diethylether). IR (KBr), υ (cm^{-1}): 3188 (N-H), 1716 (C=O), 1674 (C=O), 1635 (C=O). ^{1}H-NMR (DMSO-d_6): δ ppm =0.57, 0.67, 0.72, 0.76, 1.14 (5d, *J: 6.74 Hz,* 6H, >CHCH**(**$\mathbf{CH_3)_2}$), 2.04-2.14 (q, 1H, >CH**CH(**$CH_3)_2$), 2.15, 2.26, 2.43, 2.47 (4s, 9H, Ar-$\mathbf{CH_3}$(2,4,6)), 3.64-3.91 (m, 2H, -S-$\mathbf{CH_2}$-), 4.15, 4.34, 5.14 (3t, *J: 8.63 Hz* 1H, >**CH**CH**(**$CH_3)_2$), 5.73, 5.83, 6.31, 6.51, 6.75, 6.90 (6s, 1H, N-**CH**-Ar-S), 7.31-7.56 (m, 5H, Ar-**H**), 7.78-7.89 (m, 2H, Ar-**H**), 8.19, 8.42 (2d, *J: 9.09 Hz*, 1H, Ar-CO**NH-**), 10.31, 10.34, 10.40, 10.43 (4s, 1H, -CO-**NH**-N=). HR-MS (FAB$^+$), m/z: 462.1847 (M+Na$^+$, $C_{24}H_{29}N_3O_3SNa$), 440.2000 (M+H$^+$, $C_{24}H_{30}N_3O_3S$, base peak).

4.2.2. General procedure for the synthesis of (2S)-3-[2-(benzoylamino)-3-methylbutanamido]-2-aryl-5-methyl-1,3-thiazolidinones **24-33**

A mixture of compounds **4-13** (0.01 mol) and thiolactic acid (0.2 mol) was refluxed in dry benzene (100 ml) using a Dean–Stark water separator. Excess benzene was evaporated *in vacuo*. The resulting residue was triturated with saturated $NaHCO_3$ solution until CO_2 evolution ceased. The solid was washed with water, dried and recrystallized from appropriate solvents.

(2*S*)-3-[2-(Benzoylamino)-3-methylbutanamido]-2-(4-chlorophenyl)-5-methyl-1,3-thiazolidine-4-one **(24):** IR (KBr), υ (cm^{-1}): 3273, 3215 (N-H), 1718 (C=O), 1674 (C=O), 1635 (C=O). ^{1}H-NMR (DMSO-d_6): δ ppm =0.71-0.95, 1.47-1.51 (2m, 9H, >CHCH**(CH$_3$)$_2$**, -S-CH-**CH$_3$**), 1.91-1.98 (m, 1H, >CH**CH(**CH$_3$)$_2$), 4.02-4.26 (m, 2H, -S-**CH**-CH$_3$, >**CH**CH**(**CH$_3$)$_2$), 5.73, 5.82 (2s, 1H, N-**CH**-Ar-S), 7.33-7.54 (m, 7H, Ar-**H**), 7.75-7.87 (m, 2H, Ar-**H**), 8.23, 8.26, 8.28 (3s, 1H, Ar-CO**NH-**), 10.89 (s, 1H, -CO-**NH**-N=). HR-MS (FAB$^+$), m/z: 470.1081 (M+Na, $C_{22}H_{24}{}^{37}ClN_3O_3SNa$,), 468.1097 (M+Na, $C_{22}H_{24}{}^{35}ClN_3O_3SNa$, base peak), 448.1294 (M+H, $C_{22}H_{25}{}^{37}ClN_3O_3S$), 446.1317 (M+H, $C_{22}H_{25}{}^{35}ClN_3O_3S$), 430.1165 ($C_{20}H_{24}{}^{37}ClN_3O_2SNa$), 428.1178 ($C_{20}H_{24}{}^{35}ClN_3O_2SNa$).

(2*S*)-3-[2-(Benzoylamino)-3-methylbutanamido]-2-(2,6-dichlorophenyl)-5-methyl-1,3-thiazolidine-4-one **(25):** IR (KBr), υ (cm^{-1}): 3333, 3238, 3171 (N-H), 1718 (C=O), 1668 (C=O), 1643 (C=O). ^{1}H-NMR (DMSO-d_6): δ ppm =0.63-1.03 (m, 6H, >CHCH**(CH$_3$)$_2$**)), 1.48-1.61 (d, *J: 7,0 Hz*, 3H, -S-CH-**CH$_3$**), 1.85-1.94, 2.03-2.08 (2m, 1H, >CH**CH(**CH$_3$)$_2$), 4.12-4.25, 4.45-4.53 (2m, 2H, -S-**CH**-CH$_3$, >**CH**CH**(**CH$_3$)$_2$), 6.59, 6.63, 6.67, 6.71 (4s, 1H, N-**CH**-Ar-S), 7.38-7.57 (m, 6H, Ar-**H**), 7.78-7.90 (m, 2H, Ar-**H**), 8.24, 8.36 (2t, *J: 5.65 Hz,* 1H, Ar-CO**NH-**), 10.43, 10.47, 10.59, 10.61 (4s, 1H, -CO-**NH**-N=). HR-MS (FAB$^+$), m/z: 504.0705 (M+Na, $C_{22}H_{23}{}^{35}Cl^{37}ClN_3O_3SNa$), 502.0710 (M+Na, $C_{22}H_{23}{}^{35}Cl_2N_3O_3SNa$), 484.0873 (M+H, $C_{22}H_{24}{}^{37}Cl_2N_3O_3S$), 482.0894 (M+H, $C_{22}H_{25}{}^{35}Cl^{37}ClN_3O_3S$), 480.0904 ($C_{22}H_{24}{}^{35}Cl_2N_3O_2SNa$, base peak).

(2*S*)-3-[2-(Benzoylamino)-3-methylbutanamido]-2-(2-fluorophenyl)-5-methyl-1,3-thiazolidine-4-one **(26):** Yield 23%; mp 86°C. IR (KBr), υ (cm^{-1}): 3325, 3242, 3182, 3171 (N-H), 1720, 1705 (C=O), 1670 (C=O), 1643 (C=O). ^{1}H-NMR (DMSO-d_6): δ ppm =0.66-0.93 (m, 6H, >CHCH**(CH$_3$)$_2$**), 1.49-1.52 (q, 3H, -S-CH-**CH$_3$**), 1.91-2.07 (m, 1H, >CH**CH(**CH$_3$)$_2$, 4.06-4.37 (m, 2H, -S-**CH**-CH$_3$, >**CH**CH**(**CH$_3$)$_2$), 5.94, 5.99, 6.04, 6.08 (4s, 1H, N-**CH**-Ar-S), 7.14-7.59 (m, 7H, Ar-**H**), 7.78-7.85 (m, 2H, Ar-**H**), 8.23, 8.25, 8.27, 8.33, 8.35 (5s, 1H, Ar-CO**NH-**), 10.48, 10.65 (2s, 1H, -CO-**NH**-N=). HR-MS (FAB$^+$), m/z: 452.1443 (M+Na, $C_{22}H_{24}FN_3O_3SNa$, base peak), 430.1607 (M+H, $C_{22}H_{25}FN_3O_3S$).

(2*S*)-3-[2-(Benzoylamino)-3-methylbutanamido]-2-(3-fluorophenyl)-5-methyl-1,3-thiazolidine-4-one **(27):** IR (KBr), υ (cm^{-1}): 3244, 3205 (N-H), 1716 (C=O), 1670 (C=O), 1635 (C=O), 1234 (Ar-F). ^{1}H-NMR (DMSO-d_6): δ ppm =0.69-1.01 (m, 6H, >CHCH**(CH$_3$)$_2$**), 1.49-1.55 (m, 3H, -S-CH-**CH$_3$**), 1.92-1.95, 2.03-2.07 (2q, 1H, >CH**CH(**CH$_3$)$_2$), 4.02-4.33 (m, 2H, -S-**CH**-CH$_3$, >**CH**CH**(**CH$_3$)$_2$), 5.75, 5.86 (2s, 1H, N-**CH**-Ar-S), 7.10-7.55 (m, 7H, Ar-**H**), 7.76-7.87 (m, 2H, Ar-**H**), 8.25, 8.34 (2t, *J: 8.83 Hz,* 1H, Ar-CO**NH-**), 10.40, 10.53 (2s, 1H, -CO-**NH**-N=). HR-MS (FAB$^+$), m/z: 452.1432 (M+Na, $C_{22}H_{24}FN_3O_3SNa$, base peak), 430.1594 (M+H, $C_{22}H_{25}FN_3O_3S$).

(2*S*)-3-[2-(Benzoylamino)-3-methylbutanamido]-2-(4-fluorophenyl)-5-methyl-1,3-thiazolidine-4-one **(28):** Yield 8%; mp 179-182°C (Petroleum ether). IR (KBr), υ (cm^{-1}): 3240 (N-H), 1718 (C=O), 1672 (C=O), 1633 (C=O), 1224 (Ar-F). ^{1}H-NMR (DMSO-d_6): δ ppm = 0.71-1.14 (m, 6H, >CHCH**(CH$_3$)$_2$**), 1.50, 1.52 (2d, J*: 2.17 Hz,* 3H, -S-CH-**CH$_3$**), 1.71-2.09 (m, 1H, >CH**CH(**CH$_3$)$_2$), 4.00-4.32 (m, 2H, -S-**CH**-CH$_3$, >**CH**CH**(**CH$_3$)$_2$), 5.73, 5.83 (2s, 1H, N-**CH**-Ar-S), 7.09-7.33 (m, 2H, Ar-**H**), 7.39-7.56 (m, 5H, Ar-**H**), 7.76-7.94 (m, 2H, Ar-**H**), 8.21, 8.33 (2t, *J: 9.07Hz*, 1H, Ar-CO**NH-**), 10.34, 10.46 (2s, 1H, -CO-**NH**-N=).^{13}C-NMR-DEPT (DMSO): δ ppm =19.13, 19.29, 19.34, 19.40, 19.50, 19.82, 19.88 (>CHCH**(C**H$_3$)$_2$), 20.28, 20.43 (4-thiazolidinoneC_5-**CH$_3$**), 30.46, 30.55, 30.63 (>CH**C**H**(**CH$_3$)$_2$), 38.52 (4-thiazolidinone **C$_5$**), 57.61, 57.91 (>**C**HCH**(**CH$_3$)$_2$), 60.14, 60.45 (4-thiazolidinone **C$_2$**), 115.64, 115.76, 115.86 and 115.97, 127.92 and 127.99. 128.02, 128.069, 128.53, 128.55 and 128,61, 129.23, 130.33, 130.42, 130.56, 130.68 and 130.94, 131.03, 131.69 and 131,75, 134.53 and 134.57, 161.5 and 161.7 (Ar-**C**), 166.84 and 166.87 (**C**ONHN<), 170.02, 170.08, 170.51 (Ar-**C**ONH), 171.89, 172.02 (4-thiazolidinone **C$_4$**). HR-MS (FAB$^+$), m/z: 452.1396 (M+Na, $C_{22}H_{24}FN_3O_3SNa$, base peak), 430.1569 (M+H, $C_{22}H_{25}FN_3O_3S$).

(2*S*)-3-[2-(Benzoylamino)-3-methylbutanamido]-2-(2,6-difluorophenyl)-5-methyl-1,3-thiazolidine-4-one **(29):** IR (KBr), υ (cm^{-1}): 3217, 3203 (N-H), 1726 (C=O), 1678 (C=O), 1635 (C=O), 1188 (Ar-F). ^{1}H-NMR (DMSO-d_6): δ ppm =0.68-0.94 (m, 6H, >CHCH**(CH$_3$)$_2$**), 1.48-1.54 (m, 3H, -S-CH-**CH$_3$**), 1.92-1.96, 2.01-2.05 (2m, 1H, >CH**CH-(**CH$_3$)$_2$), 4.11-4.24, 4.35-4.43 (2m, 2H, -S-**CH**-CH$_3$, >**CH**CH**(**CH$_3$)$_2$), 6.03, 6.09, 6.17 (3s, 1H, N-**CH**-Ar-S), 7.07-7.15 (m, 2H, Ar-**H**)**,** 7.43-7.56 (m, 4H, Ar-**H**), 7.79-7.85 (m, 2H, Ar-**H**), 8.25, 8.33, 8.36 (3t, *J: 8.96 Hz,*

1H, Ar-CO**NH-**), 10.55, 10.76 (2d, *J: 4.57 Hz*,1H, -CO-**NH**-N=). HR-MS (FAB$^+$), m/z: 470.1320 (M+Na, $C_{22}H_{23}F_2N_3O_3SNa$,), 448.1501 (M+H, $C_{22}H_{23}F_2N_3O_3S$, base peak).

(2*S*)-3-[2-(Benzoylamino)-3-methylbutanamido]-2-(2-chloro-6-fluorophenyl)-5-methyl-1,3-thiazolidine-4-one **(30):** IR (KBr), υ (cm^{-1}): 3227 (N-H), 1718 (C=O), 1681 (C=O), 1633 (C=O), 1253 (Ar-F). ^{1}H-NMR (DMSO-d_6): δ ppm =0.65-0.95 (m, 6H, >CHCH**(CH$_3$)$_2$**), 1.51 (t, *J: 7.78 Hz*, 3H, -S-CH-**CH$_3$**), 2.04-2.09 (m, 1H, >CH**CH(**CH$_3$)$_2$), 4.11-4.42 (m, 2H, -S-**CH**-CH$_3$, >**CH**CH**(**CH$_3$)$_2$), 6.03, 6.09, 6.17 (3s, 1H, N-**CH**-Ar-S), 7.28-7.56 (m, 6H, Ar-**H**), 7.79-7.84 (q, 2H, Ar-**H**), 8.25, 8.34, 8.36 (3s, 1H, Ar-CO**NH-**), 10.36, 10.92 (2d, *J: 4.57 Hz*, 1H, -CO-**NH**-N=).^{13}C-NMR-DEPT (DMSO): δ ppm =18.87, 18.95 (4-thiazolidinoneC_5-**CH$_3$**), 19.02, 19.12, 19.30, 19.36, 19.43, 19.52, 19.79, 19.91 (>CHCH**(**CH$_3$)$_2$), 30.32, 30.78 (>CH**CH(**CH$_3$)$_2$), 38.79, 38.96 (4-thiazolidinone**C$_5$**), 53.87, 54.17, 54.43 (4-thiazolidinone **C$_2$**), 57.18, 57.27, 57.76 (>**C**HCH(CH$_3$)$_2$), 116.76, 126.08, 128.03, 128.59, 131.70 and 131.75, 132.03, 134.51 and 134.66, 160.19 (Ar-**C**), 166.82, 166.93 (**C**ONHN<), 170.51, 170.58 (Ar-**C**ONH), 171.07 (4-thiazolidinone **C$_4$**). HR-MS (FAB$^+$), m/z: 488.1020 (M+Na, $C_{22}H_{23}{}^{37}ClFN_3O_3SNa$), 486.1030 (M+Na, $C_{22}H_{23}{}^{35}ClFN_3O_3SNa$, base peak), 466.1205 (M+H, $C_{22}H_{24}{}^{37}ClFN_3O_3S$), 464.1223 (M+H, $C_{22}H_{24}{}^{35}ClFN_3O_3S$).

(2*S*)-3-[2-(Benzoylamino)-3-methylbutanamido]-2-(4-trifluoromethylphenyl)-5-methyl-1,3-thiazolidine-4-one **(31):** Yield 44%; mp 95 & 115^{o}C (Petroleum ether). IR (KBr), υ (cm^{-1}): 3282, 3265, 3198, 3153 (N-H), 1720 (C=O), 1674 (C=O), 1635 (C=O). ^{1}H-NMR (DMSO-d_6): δ ppm =0.70-1.05 (m, 6H, >CHCH**(CH$_3$)$_2$**), 1.16-1.54 (m, 3H, -S-CH-**CH$_3$**), 1.94-2.04 (m, 1H, >CH**CH(**CH$_3$)$_2$), 4.05-4.28 (m, 2H, -S-**CH**-CH$_3$, >**CH**CH**(**CH$_3$)$_2$), 5.82, 5.89 (2s, 1H, N-**CH**-Ar-S), 7.39-7.55, 7.62-7.86 (2m, 9H, Ar-**H**), 8.31, 8.36, 8.39 (3t, *J: 6.37 Hz*, 1H, Ar-CO**NH-**), 10.37, 10.79 (2s, 1H, -CO-**NH**-N=). HR-MS (FAB$^+$), m/z: 502.1427 (M+Na, $C_{23}H_{24}F_3N_3O_3SNa$, base peak), 480.1533 (M+H, $C_{23}H_{25}F_3N_3O_3S$).

(2*S*)-3-[2-(Benzoylamino)-3-methylbutanamido]-2-(4-methoxylphenyl)-5-methyl-1,3-thiazolidine-4-one **(32):** IR (KBr), υ (cm^{-1}): 3257, 3230 (N-H), 1722 (C=O), 1676 (C=O), 1633 (C=O), 1244 (C-O). ^{1}H-NMR (DMSO-d_6): δ ppm =0.72-0.92 (m, 6H, >CHCH**(CH$_3$)$_2$**), 1.49 (d, *J: 6.97 Hz*, 3H, -S-CH-**CH$_3$**), 1.78-2.16 (m, 1H, >CH**CH(**CH$_3$)$_2$), 3.68, 3.75 (2s, 3H, Ar-OCH$_3$), 4.01 (t, 1H, *J: 7.19 Hz*, -S-**CH**-CH$_3$), 4.28 (d, *J: 8.25 Hz*, 1H, >**CH**CH**(**CH$_3$)$_2$), 5.68, 5.74 (2d, *J: 7.94 Hz*, 1H, N-**CH**-Ar-S), 6.83-6.93 (m, 2H, Ar-**H**), 7.30-7.55 (m, 5H, Ar-**H**), 7.76-7.87 (m, 2H, Ar-**H**), 8.06, 8.19, 8.23, 8.39 (4s, 1H, Ar-CO**NH-**), 10.17, 10.49 (2s, 1H,-CO-**NH**-N=). HR-MS (FAB$^+$), m/z: 464.1596 (M+Na, $C_{23}H_{27}N_3O_4SNa$, base peak), 442.1798 (M+H, $C_{23}H_{28}N_3O_4S$).

(2*S*)-3-[2-(Benzoylamino)-3-methylbutanamido]-2-(2,4,6-trimethylphenyl)-5-methyl-1,3-thiazolidine-4-one **(33):** IR (KBr), υ (cm^{-1}): 3252, 3246 (N-H g.t.), 1716 (C=O), 1668 (C=O), 1635 (C=O). ^{1}H-NMR (DMSO-d_6): δ ppm =0.55-1.07 (m, 6H, >CHCH**(CH$_3$)$_2$**), 1.49 (d, *J:6.97 Hz*, 3H, -S-CH-**CH$_3$**), 2.04-2.29, 2.31-2.52 (2m, 10H, >CH**CH(**CH$_3$)$_2$, Ar-(C**H$_3$**)$_3$), 4.04-4.37 (m, 2H, -S-**CH**-CH$_3$, >**CH**CH**(**CH$_3$)$_2$), 6.28, 6.46, 6.48 (3s, 1H, N-**CH**-Ar-S), 6.75-6.88 (m, 2H, Ar-**H**), 7.31-7.55 (m, 3H, Ar-**H**), 7.73-7.89 (m, 2H, Ar-**H**), 8.24, 8.26, 8.40, 8.41, 8.42 (5s, 1H, Ar-CO**NH-**), 10.45 (s, 1H, -CO-**NH**-N=). HR-MS (FAB$^+$), m/z: 476.1978 (M+Na, $C_{25}H_{31}N_3O_3SNa$, base peak), 454.2203 (M+H, $C_{25}H_{32}N_3O_3S$).

4.3. Antiviral and antibacterial assays

For experimental details on the inhibition of HIV-induced cytopathicity in MT-4 cells, the antiviral assays other than HIV-1, NS5B inhibition assay and antibacterial acivity evaluation, please see supplementary material.

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Appendix A. Supplementary Material

Supplementary material related to this article can be accessed at https://dx.doi.org/10.29228/jrp.41.

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