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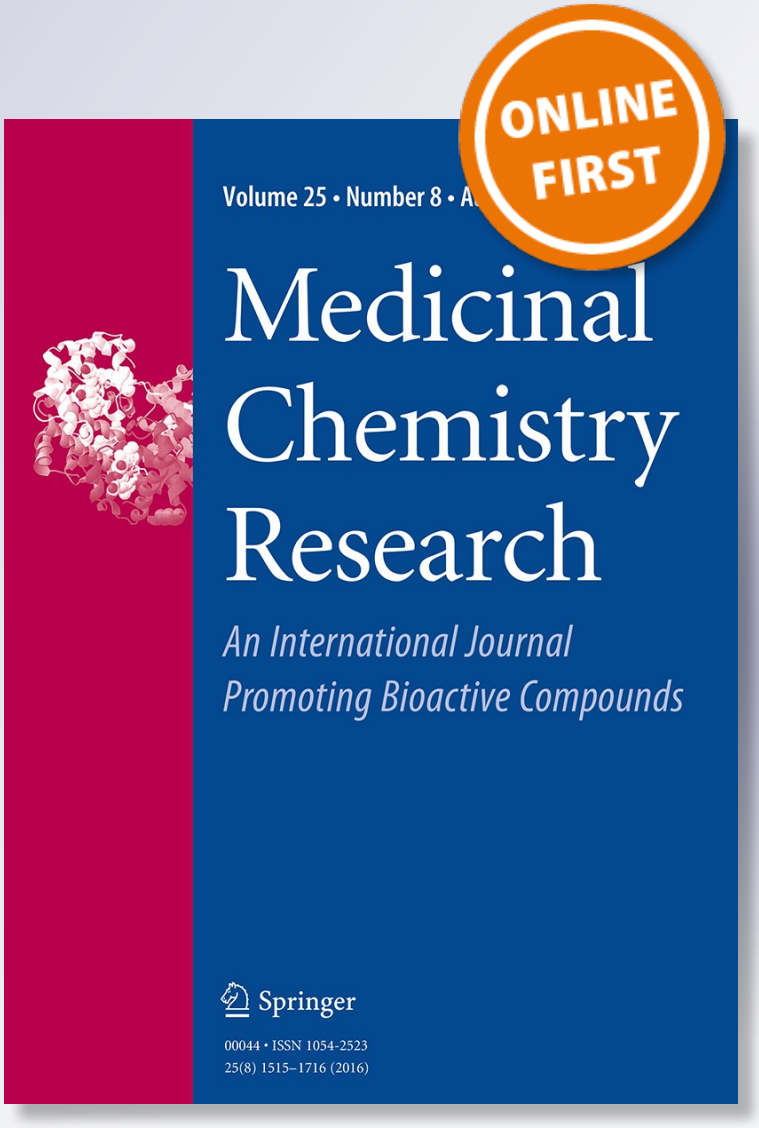

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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Amino acid derivatives. Part 6. Synthesis, in vitro antiviral activity and molecular docking study of new *N*-α-amino acid derivatives conjugated spacer phthalimide backbone

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Abstract A series of phthalimido-(substituted-alkyl-2-thioureido)alkyl carboxylic acid derivatives **5–13** and methyl 2-(4-(phthalimido-2-yl)butanamido)alkyl carboxylates **14–23** were synthesized with the aim to develop newly non-nucleoside reverse transcriptase inhibitors (NNRTIs). The new synthesized compounds were assayed against human immunodeficiency virus-1 and human immunodeficiency virus-2 in MT-4 cells. The results showed that **22** and **23** were the only compounds in the series inhibiting human immunodeficiency virus-1 (III_B strain) replication in cell culture with an EC_{50} value of >2.07 and >4.23 μM (SI = 7 and 5), respectively. In addition, the new analogs were screened against hepatitis virus C genotype 1b in the Huh-5-2 replicon system. Compounds **9** and **12** were the most active analogs of the series and exhibited activity with EC_{50} = 26.7 and 22.0 μM (SI > 1.87 and >2.28, respectively). The molecular docking of **22** with some amino acids of human immunodeficiency virus reverse transcriptase has been studied.

Keywords Antiviral activity · Amino acids · Phthalimide · Molecular docking study

Introduction

Phthalimide derivatives have received considerable attentions in recent years because of their diverse biological activities (Sharma et al., 2010). Various phthalimide derivatives have shown anti-inflammatory (Lima et al., 2002), anticancer (Dredge et al., 2003; Little et al., 2002; Teo et al., 2005; Al-Soud and Al-Masoudi, 2001), anti-human immunodeficiency virus (Moreira et al., 1997; Derpoorten et al., 1997; Al-Masoudi et al., 2010), tumor necrosis factor-α (TNF-α) regulating activity (Miyachi et al., 1997), anticonvulsant (Usifoh et al., 2011; Ragavendran et al., 2007), hypoglycemic (Sou et al., 2001), antiandrogenic (Miyachi et al., 1997), antiangiogenic (Shimazawa et al., 1999), hypolipidimic (Chapman et al., 1984), and antimicrobial (Sosa and Cvetnic, 2005) activities. Thalidomide (**1**) (Fig. 1) is the best-known phthalimide derivative, a hypnotic/sedative drug with teratogenic effect (Hales, 1999), as well as a powerful inhibitor for cytokine TNF-α (Hashimoto, 2002; Noguchi et al., 2002; Klausner et al., 1996), in addition to its effectiveness for the treatment of HIV (Makonkawkeetoon et al., 1993). Hashimoto (2002) have reported the activity of thalidomide and its analogs as cyclooxygenase inhibitors. Furthermore, naphthalimides such as amonafide and mitonafide, exhibited substantial anticancer activities against various animal tumors (Samanta et al., 2001).

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On the other hand, amino acids coupled derivatives are a major of interest, since the amino acid itself had emerged as the key determinant of intracellular phosphate delivery. McGuigan et al. (1998) have described the synthesis of phosphoamidate derivatives of stavudine bearing unusual amino acids as inhibitors for HIV.

Hepatitis C virus (HCV) belongs to a member of *Flaviviridae* and is a worldwide infectious pathogen causing chronic hepatitis that can progress further to hepatocellular carcinoma (Onji and Ohta, 1990). The current therapeutic protocol for HCV infection consists mainly of interferon in combination with ribavirin that usually accompanies with strong side effects and moderate successful rate (Mangia et al., 2005; Lai, 2006). Hence, there is an urgent need to find new regimens to increase the efficacy of anti-HCV therapy.

In view of the various activities of phthalimides, and in continuation of our ongoing research on amino acid derivatives in the synthesis of new analogs of pharmacological interest (Hamad et al., 2010), we described here the development of a new series of amino acids or their ester analogs bearing side-chain phthalimide backbone with evaluation of their HIV and HCV inhibitory activity.

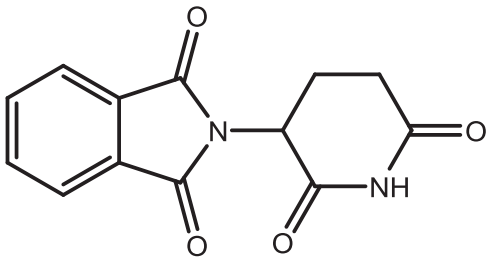


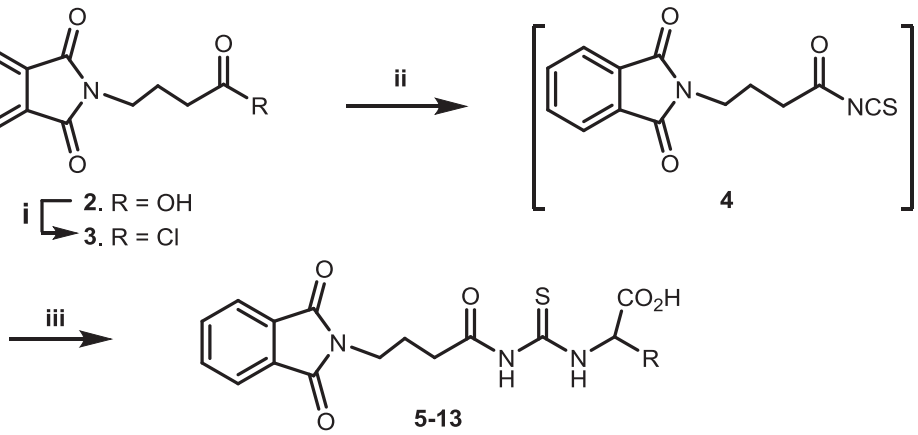
Fig. 1 Chemical structure of thalidomide (**1**)

Results and discussion

Chemistry

Recently, Herrewege et al. (2008) reported that the antiviral activity was positively correlated with the carbon spacer length during the synthesis of new potent HIV agents. In our present work, 4-(phthalimido-2-yl)butyric acid (**2**), having three carbon atoms spacer, has been selected as a key intermediate for the synthesis of new derivatives of phthalimido-(substituted-alkyl-2-thioureido)alkyl carboxylic acid, aiming for evaluation of their in vitro anti-HIV and HCV activity. Thus, treatment of **2** with NH_4SCN in acetone, following Kabbani et al. (2005) approach afforded **5,** which directly treated with the desired amino acid derivatives to give, after purification, the phthalimido thioureido-amino acid derivatives in 80–54 % yield. The synthetic reactions are summarized in Scheme 1.

The structures of **5–13** were determined by their ^{1}H, ^{13}C nuclear magnetic resonance (NMR) and by mass spectra. The phthalimide and its propyl side chain protons showed a similar pattern. Compound **8** was selected for further NMR experiment. From a gradient heteronuclear multiple bond correlation (HMBC) (Willker et al., 1993) NMR spectrum,



	R			R	
5	Me	L-alanine	L-threonine	CH(OH)Me	**10**
6	CH_2OH	L-serine	L-histidine	CH_2-2-imidazole	**11**
7	CH_2SH	L-cysteine	L-arginine	$(CH_2)NHCNHNH_2$	**12**
8	$(CH_2)_2SMe$	L-methionine	L-tyrosine	$(CH_2Ph$	**13**
9	$CHMe_2$	L-leucine			

Scheme 1 *Reagents and conditions*: (i) $SOCl_2$, 1 h, 70 °C; (ii) NH_4NCS, acetone, reflux, 6 h; (iii) R–$CH(NH_2)CO_2H$, acetone, reflux, 6 h

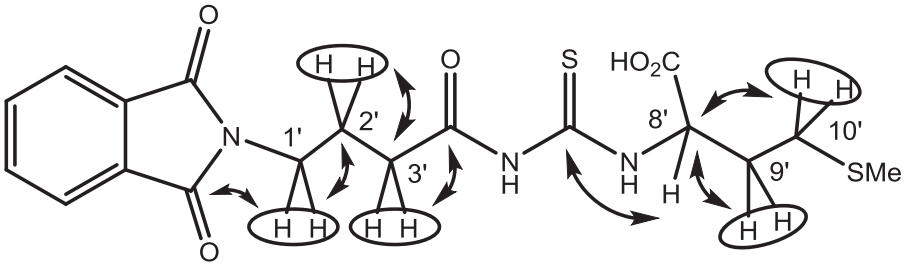
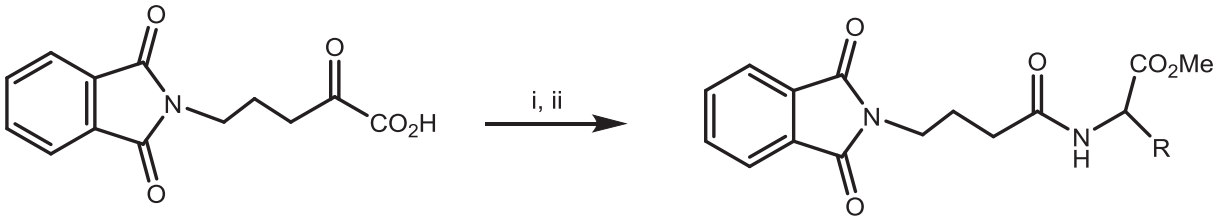
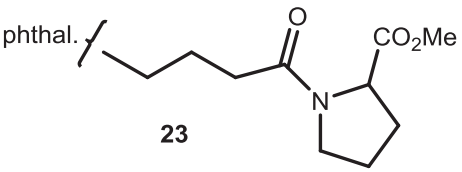


Fig. 2 $J_{C,H}$ correlations in the HMBC NMR spectrum of **8**



	R			R	
14	Me	L-alanine	L-leucine	$CH_2(OH)Me_2$	**19**
15	$CHMe_2$	L-valine	L-threonine	CHOH(Me)	**20**
16	CH_2OH	L-serine	L-phenylalanine	CH_2Ph	**21**
17	$(CH_2SH$	L-cysteine	L-tryptophane	3-CH_2-indole	**22**
18	$(CH_2)_2SMe$	L-methionine			



Scheme 2 *Reagents and conditions*: (i) R–CH(NH_2)CO_2Me, DCC, HOBt,5 °C, MeCN; (ii) then stirring at 0 °C, 1 h, 5 °C, 1 h, 23 °C, 16 h

two $^3J_{C,H}$ couplings between 1′-CH_2 and 2′-CH_2 protons at $\delta = 4.21$ and 1.83 ppm and carbonyl carbon atoms of the phthalimide group and NHCO at $\delta = 168.4$ and 175.7 ppm, respectively, were observed. Further, 8′-H at $\delta = 3.41$ ppm showed a $^3J_{C,H}$ coupling with C=S at $\delta = 187.8$ ppm, whereas 10′-CH_2 protons revealed the same coupling with C-8′ at $\delta = 61.1$ ppm. Similarly, 1′-CH_2 and 2′-CH_2 protons at $\delta = 4.21$ and 1.83 ppm showed two $^2J_{C,H}$ couplings with carbon atoms 2′ and 3′ at $\delta = 24.1$ and 30.7 ppm, respectively. In addition, 3′-CH_2 at $\delta = 2.29$ ppm revealed a $^2J_{C,H}$ coupling with carbonyl carbon atom (NHCO) at $\delta = 175.7$ ppm, while the 9′-CH_2 and 10′-CH_2 protons showed two $^2J_{C,H}$ couplings with C-8′ and C-9′ at $\delta = 61.1$ and 29.6 ppm, respectively (Fig. 2).

A suitable coupling method (Davis and Mohammed, 1981) was employed for the formation of peptides by reaction of the carboxylic acid group with acylated amino acid, using 1-hydroxybenzotriazole (HOBt) (Konig and Geiger, 1970) and *N,N′*-dicyclohexylcarbodiimide (DCC) (Sheeban and Hlavka, 1956) as coupling reagents. HOBt is currently the most frequently used activating agent for the carboxyl group of amino acids. The procedure is fast and suppresses racemization, especially in the presence of DCC (Katritzky et al., 2004).

Amides **14–23** were prepared by coupling **2** with the appropriate methyl ester derivatives of the amino acids (L-alanine, L-valine, L-serine, L-cysteine, L-methionine, L-leucine, L-threonine, L-phenylalanine, L-tryptophane, and L-proline) in the presence of HOBt and DCC as coupling reagents to give **14–23** in 80-59 % yield (Scheme 2).

The structures of **14–23** were determined by their ^{1}H, ^{13}C NMR and mass spectra. Phthalimide and propyl side chain carbon atoms showed a similar pattern and almost identical chemical shifts for those of **5–13**. In addition, all the synthesized compounds were confirmed from their ^{1}H,^{13}C HSQC NMR spectra (Davis et al., 1992).

Biological activities

In vitro anti-HIV activity Compounds **3** and **5–23** were tested for their anti-HIV-1 activity in vitro, using III_B strain in human MT-4 cells, based on a Microculture Tetrazolium (MTT) assay (Pannecouque et al., 2008). Cytotoxicity was

also measured on MT-4 cells, where the results are summarized at Table 1. None of the in vitro tested compounds were found to inhibit HIV-replication at EC_{50} lower than the CC_{50} compared to the antiviral agents navirapine (Hargrave et al., 1991) and azidothymidine (AZT) (Mitsuya et al., 1985). It is interesting to report that compounds **22** and **23** were effected a 50 % reduction of the cytopathogenicity induced by HIV-1 (III_B strain) at a concentration of $EC_{50} > 4.23$ and 20.38 μM, and CC_{50} of 29.61 and 101.90 μM, resulting in SI of 7 and 5, respectively, at non-toxic concentration. However, the anti-HIV activity and selectivity of **22** and **23** are too limited to perform extensive mode of action studies. The synthesis of new analogs of these phthalimide derivatives could lead to the discovery of more potent and selective analogs that will subject for further structural development.

In vitro anti-HCV activity The establishment of stable HCV subgenomic replicon systems (Lohmann et al., 1999; Blight et al., 2000) in the human hepatoblastoma cell line Huh-7 has provided a useful system for the development of new antiviral approaches against HCV (Gao et al., 2001; Llinàs-Brunet et al., 2004; Po et al., 2012). The overriding aims of the new therapeutic strategies are higher efficacy associated with shortened duration of treatment, favorable mode of administration, and thus improved tolerability and adherence. Compounds **3**, **7–9** and **11–13** have been selected for evaluation in vitro for antiviral activity against hepatitis C virus in the Huh-5-2 replicon system (type 1b, Con1 strain). The results are shown in Table 2. Even though for many compounds an EC_{50} was obtained with selectivity index (SI) up to 2.28 and inhibition of 87.3 % (e.g.,: compound **12**), none of the compounds matched the selection criteria of a selective inhibitor of virus replication in this assay (i.e., >70 % inhibition at concentrations that do not elicit an antimetabolic effect on the host cells).

Molecular docking study

Docking study with HIV-1 reverse transcriptase enzyme In the docking study, the X-ray crystal structure of HIV-1 reverse transcriptase (RT) enzyme; PDB ID: 3dlg) was obtained from Protein Data Bank (http://www.rcsb.org) (Ren et al., 2008). The Graphical User Interface program AutoDock Tools and AutoDockTools4 were used to study the interactions and the binding energy of the docked structure (Morris et al., 2009). Polar hydrogens were added followed by computing gasteiger charges and nonpolar hydrogen's were merged into the receptor PDB file. Compound **22** has been selected for the docking modeling study as a best dock pose, used as standard inhibitor with the following energy data (kcal/mol): binding energy = −8.82, competitive inhibition (Ki) = 343.93 nM, intermolecular

Table 1 In vitro anti-HIV-1[a] and HIV-2[b] of new phthalimide derivatives **3, 5–23**

Compd.	Virus strain	EC_{50} (μM)[c]	CC_{50} (μM)[d]	SI[e]
3	III_B	>52.80	52.80	<1
	ROD	>52.80	52.80	<1
5	III_B	>125.0	125.0	<1
	ROD	>125.0	125.0	<1
6	III_B	>89.50	89.50	<1
	ROD	>89.50	89.50	<1
7	III_B	>90.90	90.90	<1
	ROD	>90.90	90.90	<1
8	III_B	>78.22	78.22	<1
	ROD	>78.22	78.22	<1
9	III_B	>26.73	26.73	<1
	ROD	>26.73	26.73	<1
10	III_B	>28.54	28.54	<1
	ROD	>28.54	28.54	<1
11	III_B	>47.26	47.26	<1
	ROD	>47.26	47.26	<1
12	III_B	>12.60	12.60	<1
	ROD	>12.60	12.60	<1
13	III_B	>37.82	37.82	<1
	ROD	>37.82	37.82	<1
14	III_B	>100.76	100.76	<1
	ROD	>100.76	100.76	<1
15	III_B	>125.0	125.0	<1
	ROD	>125.0	125.0	<1
16	III_B	>96.11	96.11	<1
	ROD	>96.11	96.11	<1
17	III_B	>60.33	60.33	<1
	ROD	>60.33	60.33	<1
18	III_B	>18.85	18.85	<1
	ROD	>18.85	18.85	<1
19	III_B	>10.89	10.89	<1
	ROD	>10.89	10.89	<1
20	III_B	>37.82	37.82	<1
	ROD	>37.82	37.82	<1
21	III_B	>37.82	37.82	<1
	ROD	>37.82	37.82	<1
22	III_B	>4.23	29.61	7
	ROD	>29.61	29.61	<1
23	III_B	>20.38	101.90	5
	ROD	>101.90	101.90	<1
AZT	III_B	0.0022	>25	>11363
	ROD	0.00094	>25	>26596
Nevirapine	III_B	0.050	>4.00	>80
	ROD	>4.00	>4.00	<1

[a] Anti-HIV-1 activity measured with strain III_B

[b] anti-HIV-2 activity measured with strain ROD

[c] compound concentration required to achieve 50 % protection of MT-4 cells from the HIV-1 and 2-induced cytopathogenic effect

[d] compound concentration that reduces the viability of mock-infected MT-4 cells by 50 %

[e] *SI* selectivity index (CC_{50}/EC_{50})

energy = −8.45, internal energy = −4.24, torsional energy = 2.68, and unbound extended energy = −1.18. As suggested by the model and visualized in Fig. 3, docking study of **22** has shown satisfactory results. Three hydrogen bonding interactions are shown: Lys395 with O atom of the phthalimide moiety, Thr377 with NH of the indol residue and O atom of methoxy group of ester residue with Lys22 of RT enzyme residues. There are non-bonded amino acid residues such as Trp24, Glu399, and Thr400 are shown the receptor activity.

Table 2 In vitro anti-HCV-1b activity, cytotoxicity and inhibition [%] of new phthalimide derivatives **3**, **7**–**9** and **11**–**13**

Compd.	CC_{50} (μM)[a]	EC_{50} (μM)[b]	SI[c]	TI[d]	Inhibition (%)
3	>50	>50	nd	nd	38.7
7	>50	39.9	>1.25	>0.0254	55.2
8	>50	>50	nd	nd	47.5
9	>50	26.7	>1.87	>1.52	88.7
11	>50	43.8	>1.14	>0.0056	53.4
12	>50	22.0	>2.28	>2.44	87.3
13	>50	>50	nd	nd	42.6

[a] *CC_{50}* 50 % cytostatic/cytotoxic concentration (concentration at which 50 % adverse effect is observed on the host cell)

[b] *EC_{50}* 50 % effective concentration (concentration at which 50 % inhibition of virus replication is observed)

[c] *SI* selectivity index (CC_{50}/EC_{50})

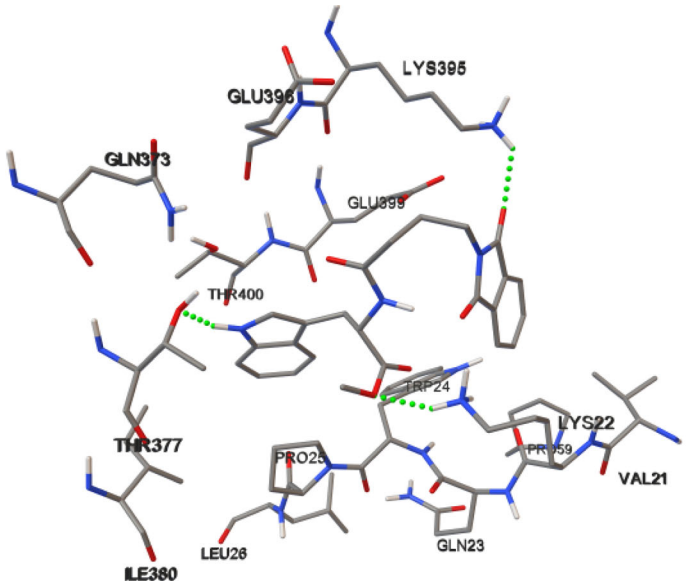
[d] *TI* thepapeutical index (10 logSI)

Experimental section

Chemistry

Melting points are uncorrected and were measured on a Buchi melting point apparatus B-545 (BUCHI Labortechnik AG, Switzerland). Microanalytical data were obtained with a Vario, Elementar apparatus (Shimadzu, Japan). NMR spectra were recorded on 400 MHz and 600 MHz (^{1}H) and 150.91 MHz (^{13}C) spectrometers (Avance III, Bruker, Germany) with TMS as internal standard and on δ scale in ppm. Signal assignments for protons were identified by selective proton decoupling or by correlation spectroscopy spectra. Heteronuclear assignments were verified by heteronuclear single quantum correlation (HSQC), or HMBC experiments. Mass spectra were recorded on 70 eV EI and FAB MAT 8200 spectrometers (FinniganMAT, U.S.A.), using 3-nitrobenzyl alcohol (NBOH) or glycerol as matrix.

General procedure for the synthesis of phthalimido(butanoylthioureido)alkyl carboxylic acid derivatives (**5**–**13**) A solution of 4-(phthalimido-2-yl)butyric acid (**2**) (350 mg, 1.50 mmol) and $SOCl_2$ (5 mL) was heated for 1 h at 70 °C. After cooling, the excess of thionyl chloride was removed under reduced pressure and the residue **3** was dissolved in acetone (10 mL). To a stirred solution of NH_4SCN (174 mg, 2.29 mmol) in acetone (10 mL) was added and the reaction mixture was heated under reflux for 2 h. After cooling and filtration, a suspension of the desired amino acid (1.17 mmol) in dry acetone (10 mL) was added dropwise and the mixture was heated under reflux for 6 h. After cooling, the



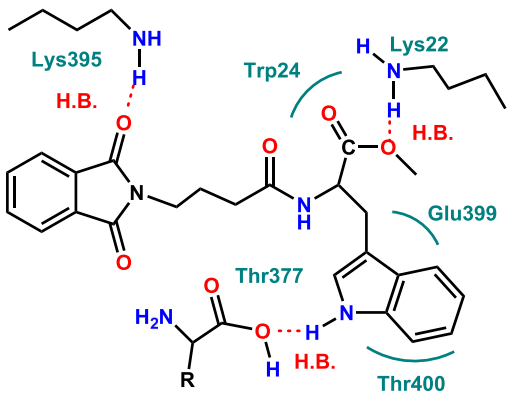


Fig. 3 Docked conformation of **22** showing three hydrogen bonds: Lys395 with O atom of the phthalimide moiety, Thr377 with NH of the indole residue and O atom of OMe ester group and Lys22 of the reverse transcriptase (RT) enzyme residues

solvent was evaporated to dryness and the residue was purified on a column of silica gel (10 g), using, in gradient, MeOH (0–10 %) and $CHCl_3$ as eluent to give the desired thioureido derivative.

2-(3-(4-(Phthalimido-2-yl)butanoyl)thioureido)propanoic acid (**5**) From L-alanine (104 mg). Yield: 365 mg (67 %); M.p. 99–102 °C. ^{1}H NMR (DMSO-d_6): δ = 11.98 (br s., 1H, CO_2H), 8.12 (br s., 1H, NH), 7.86 (m, 4H, H–Ar), 4.25 (m, 2H, 1′-CH_2), 3.39 (m, 1H, 8′-H), 2.26 (m, 2H, 3′-CH_2), 1.81 (m, 2H, 2′-CH_2), 1.24 (t, 3H, J = 7.1 Hz, CH_3). ^{13}C NMR (DMSO-d_6): δ = 185.8 (C=S), 173.8 (CO_2H), 171.9 (NHCO), 167.9 ($C_{phthal.}$=O), 134.3, 131.6, 122.9 ($C_{arom.}$), 61.5 (C8′), 38.9 (C1′), 30.9 (C3′), 23.3(C2′), 16.3 (Me). MS (FAB) *m/z*: 364 $[M+H]^+$. Anal. Calcd. for $C_{16}H_{17}N_3O_5S$ (363.39): C, 52.88; H, 4.72; N, 11.56. Found: C, 52.63; H, 4.59; N, 11.29.

2-(3-(4-(Phthalimido-2-yl)butanoyl)thioureido)-3-hydroxypropanoic acid (**6**) From L-serine (123 mg). Yield: 359 (63 %); M.p. 103–106 °C. ^{1}H NMR (DMSO-d_6): δ = 12.00 (br s., 1H, CO_2H), 8.20 (br s., 1H, NH), 7.85 (m, 4H, H–Ar), 4.31 (t, 2H, J = 6.3 Hz, 1′-CH_2), 4.14 (t, 2H, J = 6.5 Hz, *CH_2*OH), 3.78 (t, 1H, J = 6.7 Hz, 8′-H), 2.27 (t, 2H, J = 6.7 Hz, 3′-CH_2), 1.83 (m, 2H, 2′-CH_2). ^{13}C NMR (DMSO-d_6): δ = 185.6 (C=S), 173.8 (CO_2H), 171.3 (NHCO), 168.0 ($C_{phthal.}$=O), 133.9, 131.4, 123.1 ($C_{arom.}$), 62.7 (C8′), 51.7 (CH_2OH), 39.0 (C1′), 30.5 (C3′), 23.1 (C2′). MS (FAB) *m/z*: 380 $[M+H]^+$. Anal. Calcd. for $C_{16}H_{17}N_3O_6S$ (379.39): C, 50.65; H, 4.52; N, 11.08. Found: C, 50.39; H, 4.40; N, 11.17.

2-(3-(4-(Phthalimido-2-yl)butanoyl)thioureido)-3-mercaptopropanoic acid (**7**) From L-cysteine (142 mg). Yield: 454 mg (75 %); M.p. 95–98 °C. ^{1}H NMR (DMSO-d_6):δ = 11.06 (br s., 1H, CO_2H), 7.89 (m, 4H, H–Ar),4.24 (m, 2H, 1′-CH_2′), 3.58 (m, 2H, 8′-H), 3.32 (m, 2H, CH_2SH), 2.27 (m, 2H, 3′-CH_2′), 1.78 (m, 2H, 2′-CH_2). ^{13}C NMR (DMSO-d_6): δ = 186.6 (C=S), 179.6 (CO_2H), 172.0 (NHCO), 167.9 ($C_{phthal.}$=O), 134.1, 129.7, 128.6, 122.9 ($C_{arom.}$), 61.3 (C-8′), 38.8 (C1′), 30.1 (C3′), 23.8 (C2′), 23.1 (CH_2SH). MS (FAB) *m/z*: 418 $[M+Na]^+$. Anal. Calcd. for $C_{16}H_{17}N_3O_6S_2$ (395.45): C, 48.60; H, 4.30; N, 10.63. Found: C, 48.41; H, 4.25; N, 10.44.

2-(3-(4-(Phthalimido-2-yl)butanoyl)thioureido)-4-(methylthio)butanoic acid (**8**) From L-methionine (174 mg). Yield: 464 mg (73 %); M.p. 134–137 °C. ^{1}H NMR (DMSO-d_6): δ = 11.52 (br s., 1H, CO_2H), 7.87 (m, 4H, H–Ar), 4.21 (m, 2H, 1′-CH_2), 3.41 (m, 1H, 8′-H), 2.56 (m, 2H, CH_2CH_2SMe), 2.29 (m, 2H, 3′-CH_2), 2.11 (CH_2CH_2SMe), 2.07 (SMe), 1.83 (m, 2H, 2′-CH_2). ^{13}C NMR (DMSO-d_6): δ = 187.8 (C=S), 176.0 (CO_2H), 175.7 (NHCO), 168.4 ($C_{phthal.}$=O), 133.3, 130.7, 129.1, 122.3 ($C_{arom.}$), 61.1 (C-8′), 38.9 (C1′), 30.7 (C3′), 29.6 (CH_2CH_2SMe), 29.0 (CH_2*CH_2*SMe), 24.1 (C2′), 13.9 (SMe). MS (FAB) *m/z*: 446 $[M+Na]^+$. Anal. Calcd. for $C_{18}H_{21}N_3O_5S_2$ (423.51): C, 51.05; H, 5.00; N, 9.92. Found: C, 50.88; H, 4.89; N, 9.75.

2-(3-(4-(Phthalimido-2-yl)butanoyl)thioureido)-4-methylpentanoic acid (**9**) From L-leucine (154 mg). Yield: 480 mg (79 %); M.p. 72–75 °C. ^{1}H NMR (DMSO-d_6):δ = 11.72 (br s., 1H, CO_2H), 8.12 (br s., 1H, NH), 7.83 (m, 4H, H–Ar), 4.29 (m, 2H, 1′-CH_2), 3.42 (m, 1H, 8′-H), 2.30 (m, 2H, 3′-CH_2), 1.90 (m, 2H, 2′-CH_2), 1.56 (m, 1H, $CHMe_2$), 0.88, 0.87 (2xs, 6H, $CHMe_2$). ^{13}C NMR (DMSO-d_6): δ = 187.4 (C=S), 174.1 (CO_2H), 170.9 (NHCO), 166.9 ($C_{phthal.}$=O), 132.4, 129.9, 122.8 ($C_{arom.}$), 67.4 (C8′), 38.6 (C1′), 31.8 (C3′), 29.5 ($CHMe_2$), 23.8 (C2′), 21.4 ($CHMe_2$). MS (FAB) *m/z*: 406 $[M+H]^+$. Anal. Calcd. for $C_{19}H_{23}N_3O_5S$ (405.47): C, 56.28; H, 5.72; N, 10.36. Found: C, 56.02; H, 5.81; N,10.52.

2-(3-(4-(Phthalimido-2-yl)butanoyl)thioureido)-3-hydroxybutanoic acid (**10**) From L-threonine (139 mg). Yield: 401 mg (68 %); M.p. 69–72 °C. ^{1}H NMR (DMSO-d_6): δ = 12.16 (br s., 1H, CO_2H), 7.97 (m, 4H, H–Ar),4.30 (m, 2H, 1′-CH_2), 4.12 (m, 1H, CH_2OH), 3.61 (br s., 1H, OH), 3.43 (m, 2H, 8′-CH_2), 2.29 (m, 2H, 3′-CH_2), 1.90 (m, 2H, 2′-CH_2), 1.25 (d, 3H, J = 9.9 Hz, CHMe). ^{13}C NMR (DMSO-d_6): δ = 186.7 (C=S), 173.8 (CO_2H), 172.6 (NHCO), 167.9 ($C_{phthal.}$=O), 134.3, 131.6, 122.9 ($C_{arom.}$), 70.5 (C8′), 67.3 (CHOH), 39.0 (C1′), 31.0 (C3′), 23.4 (C2′), 18.1 (Me). MS (FAB): *m/z* 394 $[M+H]^+$. Anal. Calcd. for $C_{17}H_{19}N_3O_5S$ (393.41): C, 51.90; H, 4.87; N, 10.68. Found: C, 51.76; H, 4.72; N, 10. 24.

2-(3-(4-(Phthalimido-2-yl)butanoyl)thioureido)-3-(imidazole-4-yl)propanoic acid (**11**) From L-histidine (183 mg). Yield: 348 mg (54 %); M.p. 65–69 °C. ^{1}H NMR (DMSO-d_6): δ = 12.10 (br s., 1H, CO_2H), 11.83 (br s., 1H, $NH_{imidazole}$), 8.08 (br s., 1H, $NH_{imidazole}$), 7.97 (m, 4H, H–Ar), 7.82 (m, 4H, H–Ar), 7.69 (d, 1H, J = 5.2 Hz, 5-$H_{imidazole}$), 4.29 (t, 2H, J = 6.5 Hz, 1′-CH_2), 3.65 (m, 2H, 8′-H), 3.16 (s, 2H, CH_2), 2.25 (t, 2H, J = 6.6 Hz, 3′-CH_2), 1.80 (t, 2H, J = 6.6 Hz, 2′-CH_2). ^{13}C NMR (DMSO-d_6): δ = 188.2 (C=S), 173.0 (CO_2H), 172.7 (NHCO), 167.9 ($C_{phthal.}$=O), 134.3 ($C(2)_{imidazole}$), 131.6, 122.9, ($C_{arom.}$), 63.8 (C8′), 39.5 (C1′), 30.8 (C3′), 25.2 (CH_2), 23.2 (C2′). MS (FAB) *m/z*: 430 $[M+H]^+$. Anal. Calcd. for $C_{19}H_{19}N_5O_5S$ (429.45): C, 53.14; H, 4.46; N, 16.31. Found: C, 52.88; H, 4.32; N, 16.04.

2-(3-(4-(Phthalimido-2-yl)butanoyl)thioureido)-5-guanidinopentanoic acid (**12**) From L-arginine (203 mg). Yield: 404 mg (60 %); M.p. 129–132 °C. ^{1}H NMR (DMSO-d_6): δ = 11.72 (s, 1H, CO_2H), 8.32 (br s., 1H, NH),7.83 (m, 4H, H–Ar), 4.27 (m, 2H, 1′-CH_2), 3.48 (m, 1H, 8′-H), 2.59 (m, 2H, $CH_2CH_2CH_2N$), 2.29 (m, 2H, 3′-CH_2), 1.79 (m, 2H, 2′-CH_2), 1.69 (m, 2H, $CH_2CH_2CH_2N$), 1.48 (m, 2H,

$CH_2CH_2CH_2N$). ^{13}C NMR (DMSO-d_6): δ = 188.3 (C=S), 174.6 (CO_2H), 172.1 (NHCO), 167.3 ($C_{phthal.}$=O), 158.4 (NC=NH), 132.3, 125.6, 122.5 ($C_{arom.}$), 63.8 (C8′), 42.5 ($CH_2CH_2CH_2N$), 38.8 (C1′), 34.9 ($CH_2CH_2CH_2N$), 29.8 (C3′), 24.2 (C2′+ $CH_2CH_2CH_2N$).MS (FAB) *m/z*: 452 $[M+Na]^+$. Anal. Calcd. for $C_{19}H_{24}N_6O_5S$ (448.50): C, 50.88; H, 5.39; N, 18.74. Found: C, 50.65; H, 5.35; N, 18.51.

2-(3-(4-(Phthalimido-2-yl)butanoyl)thioureido)-3-(4-hydroxyphenyl)propanoic acid (**13**) From L-tyrosine (212 mg). Yield: 526 mg (77 %); M.p. 112–115 °C. ^1H NMR (DMSO-d_6): δ = 11.21 (br s., 1H, CO_2H), 7.95 (br s., 1H, NH), 7.79 (m, 4H, H–Ar), 7.14–6.92 (m, 4H, H–Ar), 5.12 (s, 1H, OH), 4.31 (m, 2H, 1′-CH_2), 3.69 (m, 1H, 8′-H), 2.93 (d, 2H, *J* = 6.3 Hz, CH_2), 2.29 (m, 2H, 3′-CH_2), 1.92 (m, 2H, 2′-CH_2). ^{13}C NMR (DMSO-d_6): δ = 187.1 (C=S), 175.8 (CO_2H), 173.4 (NHCO), 167.9 ($C_{phthal.}$=O), 155.3 ($C_{arom.}$-OH),131.1, 129.4, 129.1, 128.8, 128.5, 115.7 ($C_{arom.}$), 66.5 (C-8′), 38.9 (C1′), 34.4 (CH_2Ar), 30.3 (C3′), 22.4 (C2′). MS (FAB) *m/z*: 456 $[M+H]^+$. Anal. Calcd. for $C_{22}H_{21}N_3O_6S$ (455.48): C, 58.01; H, 4.65; N, 9.23. Found: C, 57.83; H, 4.54; N, 8.08.

General procedure for the synthesis of methyl 2-(4-(phthalimido-2-yl)butanamido)alkyl carboxylates (**14**–**23**) To a cold solution of the amino acid ester (1.50 mmol), at - 5 °C, in MeCN (15 mL), 4-(phthalimido-2-yl)butyric acid (**2**) (350 mg, 1.50 mmol), HOBt (203 mg, 1.50 mmol) and *N,N′*-dicyclohexylcarbodiimide (DCC) (309 mg, 1.5 mmol) were added successively. The reaction mixture was stirred at 0 °C for 1 h, at 5 °C for 1 h, and at 23 °C for 16 h. Dicyclohexylurea (DCU) was filtered, and the filtrate was evaporated to dryness and the residue was dissolved in ethyl acetate, filtered, washed successively with saturated NaCl solution, 5 % $NaHCO_3$ solution, 1 M HCl, followed by washing with saturated NaCl solution and finally with water. The residue was dried (Na_2SO_4), filtered, evaporated to dryness and purified on a SiO_2 column (10 g). Elution with MeOH (0–10) and $CHCl_3$ as an eluent afforded the pure amide derivative.

Methyl 2-(4-(phthalimido-2-yl)butanamido)propanoate (**14**) From L-alanine methyl ester (155 mg). Yield: 382 mg (80 %); M.p. 79–82 °C. ^1H NMR (DMSO-d_6): δ = 8.21 (br s., 1H, NH), 8.01 (m, 4H, H–Ar), 4.26 (m, 2H, 1′-CH_2), 4.12 (d, 1H, *J* = 5.1 Hz, NCH), 3.55 (s, 3H, OAc), 2.23 (m, 2H, 3′-CH_2), 1.76 (m, 2H, 2′-CH_2), 1.39 (d, 3H, *J* = 5.4 Hz, NCHMe). ^{13}C NMR (DMSO-d_6): δ = 73.7 (NHCO), 171.1 (CO_2Me), 167.4 ($C_{phthal.}$=O), 134.0, 130.3, 122.5 ($C_{arom.}$), 51.8 ($CHCO_2Me$), 51.5 (CO_2Me), 39.8 (C1′), 32.9 (C3′), 23.2 (C2′), 18.1 (Me). MS (FAB) *m/z*: 319 $[M+H]^+$. Anal. Calcd. for $C_{16}H_{18}N_2O_5$ (318.32): C, 60.37; H, 5.70; N, 8.80. Found: C, 60.03; H, 5.62; N, 9.09.

Methyl 2-(4-(phthalimido-2-yl)butanamido)-3-methylbutanoate (**15**) From L-valine methyl ester (197 mg). Yield: 384 mg (74 %); M.p. 65–69 °C. ^1H NMR (DMSO-d_6): δ = 8.05 (m, 4H, H–Ar), 7.88 (d, 1H, *J* = 7.8 Hz, NH), 4.29 (m, 2H, 2′-CH_2), 4.11 (t, 1H, *J* = 6.0 Hz, NCH), 3.54 (s, 3H, OAc), 2.25 (m, 3H, 3′-CH_2+$CHMe_2$), 1.82 (d, 2H, *J* = 6.6 Hz, 2′-CH_2), 0.90, 0.88 (2xs, 6H, Me_2). ^{13}C NMR (DMSO-d_6): δ = 173.8 (NHCO), 170.2 (CO_2Me), 167.9 ($C_{phthal.}$=O), 134.2, 131.6, 122.9 ($C_{arom.}$), 57.3 ($CHCO_2Me$), 51.6 (CO_2Me), 39.2 (C1′), 33.3 (C3′), 29.8 ($CHMe_2$), 24.4 (C2′), 18.9, 18.3 ($CHMe_2$). MS (FAB) *m/z*: 347 $[M+H]^+$. Anal. Calcd. for $C_{18}H_{22}N_2O_5$ (346.38): C, 62.42; H, 6.40; N, 8.09. Found: C, 62.18; H, 6.48; N, 7.89.

Methyl 2-(4-(phthalimido-2-yl)butanamido)-3-hydroxypropanoate (**16**) From L-serine methyl ester (179 mg). Yield: 366 mg (73 %); M.p. 143–145 °C. ^1H NMR (DMSO-d_6): δ = 7.92 (m, 4H, H–Ar), 5.02 (t, 1H, *J* = 5.1 Hz, OH), 4.31 (m, 2H, 1′-CH2), 4.15 (m, 2H, CH_2OH), 3.80 (m, 1H, $CHCH_2OH$), 3.54 (s, 3H, OAc),2.25 (m, 2H, 3′-CH_2), 1.85 (m, 2H, 2′-CH_2). ^{13}C NMR (DMSO-d_6): δ = 173.4 (NHCO), 170.8(CO_2Me), 167.2 ($C_{phthal.}$=O), 133.8, 129.8, 122.7 ($C_{arom.}$), 60.2 (CH_2OH), 53.8 ($CHCO_2Me$), 51.8 (CO_2Me), 39.6 (C1′), 32.6 (C3′), 23.8 (C2′). MS (FAB) *m/z*: 357 $[M+Na]^+$. Anal. Calcd. for $C_{16}H18N_2O_6$ (334.32): C, 57.48; H, 5.43; N, 8.38. Found: C, 57.26; H, 5.38; N, 7.87.

Methyl 2-(4-(phthalimido-2-yl)butanamido)-3-mercaptopronoate (**17**) From L-cysteine methyl ester (203 mg). Yield: 363 mg (69 %); M.p. 112–115 °C. ^1H NMR (DMSO-d_6): δ = 7.92 (m, 4H, H–Ar), 4.56 (m, 1H, NCH), 4.24 (m, 2H, 1′-CH_2), 3.56 (s, 3H, OAc), 3.30 (m, 2H, CH_2SH), 2.22 (m, 2H, 3′-CH_2), 1.85 (m, 2H, 2′-CH_2). ^{13}C NMR (DMSO-d_6): δ = 173.2 (NHCO), 171.1 (CO_2Me), 168.0 ($C_{phthal.}$=O), 132.9, 130.1, 122.5 ($C_{arom.}$), 56.2 ($CHCO_2Me$), 51.4 (CO_2Me), 39.8 (C1′), 33.4 (C3′), 25.4 (CH_2SH), 23.4 (C2′). MS (FAB) *m/z*: 373 $[M+Na]^+$. Anal. Calcd. for $C_{16}H_{18}N_2O_5S$ (350.39): C, 54.85; H, 5.18; N, 7.99. Found: C, 54.67; H, 5.02; N, 8.41.

Methyl 2-(4-(phthalimido-2-yl)butanamido)-4-(methythio) butanoate (**18**) From L-methionine methyl ester (245 mg). Yield: 444 mg (78 %); M.p. 123–125 °C. ^1H NMR (DMSO-d_6): δ = 7.86 (br s., 4H, H–Ar), 4.40 (m, 1H, NCH),4.19 (m, 2H, 1′-CH_2), 3.56 (s, 3H, OAc), 2.52 (m, 2H, CH_2CH_2SMe), 2.22 (m, 2H, 3′-CH_2), 2.14 (br s., 2H, CH_2CH_2SMe), 2.10 (SMe), 1.88 (m, 2H, 2′-CH_2). ^{13}C NMR (DMSO-d_6): δ = 173.6 (NHCO),171.5 (CO_2Me), 166.9 ($C_{phthal.}$=O), 133.8, 129.9, 123.1 ($C_{arom.}$), 54.2 ($CHCO_2Me$), 51.8 (CO_2Me), 38.9 (C1′), 33.2 (C3′), 29.5 (CH_2CH_2SMe), 28.8 (CH_2CH_2SMe), 23.2 (C2′), 14.2 (SMe). MS (FAB) *m/z*: 380 $[M+H]^+$. Anal. Calcd. for $C_{18}H_{22}N_2O_5S$ (379.44): C, 57.13; H, 5.86; N, 7.40. Found: C, 57.24; H, 5.77; N, 7.26.

Methyl 2-(4-(phthalimido-2-yl)butanamido)-4-methylpentanoate (**19**) From L-leucine methyl ester (218 mg). Yield: 405 mg (75 %); M.p. 71–74 °C. ^{1}H NMR (DMSO-d_6): δ = 7.89 (br s., 4H, H–Ar), 4.47 (m, 1H, NCH),4.22 (m, 2H, 1′-CH_2), 3.52 (s, 3H, OAc), 2.13 (m, 2H, 3′-CH_2), 1.82 (m, 2H, 2′-CH_2+CH_2CHMe_2),1.52 (m, 1H, $CHMe_2$), 1.06, 1.04 (2xs, 6H, Me_2). ^{13}C NMR (DMSO-d_6): δ = 174.0 (NHCO), 170.0(CO_2Me), 168.0 ($C_{phthal.}$=O), 134.3, 131.6, 123.0 ($C_{arom.}$), 53.8 ($CHCO_2Me$), 52.1 (CO_2Me), 39.1 (C1′+ CH_2CHMe_2), 32.0 (C3′), 24.4 ($CHMe_2$), 23.9 (C2′), 21.8 ($CHMe_2$). MS (FAB) *m/z*: 361 $[M+H]^+$. Anal. Calcd. for $C_{19}H_{24}N_2O_5$ (360.40): C, 63.32; H, 6.71; N, 7.77. Found: C, 63.07; H, 6.60; N, 7.49.

Methyl 2-(4-(phthalimido-2-yl)butanamido)-3-hydroxybutanoate (**20**) From L-threonine methyl ester (200 mg). Yield: 418 mg (80 %); M.p. 91–93 °C. ^{1}H NMR (DMSO-d_6): δ = 7.89 (br s., 4H, H–Ar), 4.47 (m, 1H, NCH), 4.22 (m, 2H, 1′-CH_2), 3.52 (s, 3H, OAc), 2.13 (m, 2H, 3′-CH_2), 1.82 (m, 2H, 2′-CH_2+CH_2CHMe_2), 1.52 (m, 1H, $CHMe_2$), 1.06, 1.04 (2xs, 6H, Me_2). ^{13}C NMR (DMSO-d_6): δ = 173.6 (NHCO), 170.3(CO_2Me), 167.8 ($C_{phthal.}$=O), 133.8, 131.0, 123.1 ($C_{arom.}$), 66.3 (CHOHMe), 60.8 ($CHCO_2$Me), 51.6(CO2Me), 39.8 (C1′), 32.2 (C3′), 23.8 (C2′), 18.6 (Me). MS (FAB) *m/z*: 349 $[M+H]^+$. Anal. Calcd. for $C_{17}H_{20}N_2O_6$ (348.35): C, 58.61; H, 5.79; N, 8.04. Found: C, 58.43; H, 5.68; N, 8.18.

Methyl 2-(4-(phthalimido-2-yl)butanamido)-3-phenylpropanoate (**21**) From L-phenylalanine methyl ester (269 mg). Yield: 444 mg (75 %); M.p. 85–88 °C. ^{1}H NMR (DMSO-d_6): δ = 7.74-7.61 (m, 4H, H–Ar), 7.46-7.16 (m, 5H, H–Ar), 4.46 (m, 1H, NCH), 4.27 (m, 2H, 1′-CH_2), 3.56 (s, 3H, OAc), 3.18 (d, 2H, J = 7.6 Hz, CH_2Ph), 2.29 (m, 2H, 3′-CH_2), 1.87 (m, 2H, 2′-CH_2). ^{13}C NMR (DMSO-d_6): δ = 173.8 (NHCO), 170.6 (CO_2Me), 167.2 ($C_{phthal.}$=O), 135.8, 132.2, 131.8, 128.3, 128.1, 124.6 ($_{Carom.}$), 52.7 ($CHCO_2$Me), 52.2 (CO_2Me), 39.1 (C-1′), 35.3 (CH_2Ph), 31.7 (C3′), 23.8 (C2′). MS (FAB) *m/z*: 395 $[M+H]^+$. Anal. Calcd. for $C_{22}H_{22}N_2O_5$ (394.42): C, 66.99; H, 5.62; N, 7.10. Found: C, 66.78; H, 5.57; N, 6.93.

Methyl 2-(4-(phthalimido-2-yl)butanamido)-3-(indol-3-yl) propanoate (**22**) From L-tryptophane methyl ester (327 mg). Yield: 507 mg (78 %); M.p. 132–135 °C. ^{1}H NMR (DMSO-d_6): δ = 7.88 (br s., 4H, H–Ar), 7.56–7.13 (m, 5H, H–Ar+2-H_{indole}), 4.32 (m, 2H, 1′-CH_2), 4.23 (m, 1H, NCH), 3.60 (s, 3H, OAc), 3.29 (d, 2H, J = 7.1 Hz, CH_{2indol}), 2.23 (m, 2H, 3′-CH2), 1.88 (m, 2H, 2′-CH_2). ^{13}C NMR (DMSO-d_6): δ = 172.7(NHCO), 171.3 (CO_2Me), 167.8 ($C_{phthal.}$=O), 136.0, 132.0, 130.7, 128.5, 126.4, 124.1, 120.5, 118.9, 111.0, 108.9 ($C_{arom.}$), 53.9 ($CHCO_2$Me), 51.2 (CO_2Me), 39.3 (C1′), 31.7 (C3′), 27.8 ($CH_{2indole}$), 23.5 (C2′). MS (FAB) *m/z*: 456 $[M+Na]^+$. Anal. Calcd. for $C_{24}H_{23}N_3O_5$ (433.46): C, 66.50; H, 5.35; N, 9.69. Found: C, 66.37; H, 5.42; N, 9.53.

Methyl 1-(4-(phthalimido-2-yl)butanoyl)pyrrolidine-2-carboxylate (**23**) From L-proline methyl ester (194 mg). Yield: 305 mg (59 %); Mp 103–106 °C. ^{1}H NMR (DMSO-d_6): δ = 7.79 (br s, 4H, H-Ar), 4.30 (m, 2H, 1′-CH_2), 3.97 (m, 1H, 2-$CH_{2pyrrol.}$), 3.55 (s, 3H, OAc), 3.40 (m, 2H, 5-$CH_{2pyrrol.}$), 2.32 (m, 2H, 3-$CH_{2pyrrol.}$), 2.25 (m, 2H, 3′-CH_2), 1.90 (m, 4H, 2′-CH_2+4-$CH_{2pyrrol.}$). ^{13}C NMR (DMSO-d_6): δ = 173.8 (NHCO), 170.7 (CO_2Me), 167.4 ($C_{phthal.}$=O), 131.8, 129.9, 123.6 ($C_{arom.}$), 63.9 ($CHCO_2$Me), 51.0 (CO_2Me), 48.3 ($C(5)_{pyrrol.}$), 39.6 (C1′), 31.4 (C3′), 27.8 ($C(3)_{pyrrol.}$), 24.2 ($C(4)_{pyrrol.}$), 23.7 (C2′). MS (FAB) *m/z*: 345 $[M+H]^+$. Anal. Calcd. for $C_{18}H_{20}N_2O_5$ (344.36): C, 62.78; H, 5.85; N, 8.13. Found: C, 62.57, H, 5.74; N, 7.95.

In vitro HIV assay

Evaluation of the antiviral activity of compounds **5–14** against the HIV-1 strain (III_B) and the HIV-2 strain (ROD) in MT-4 cells was performed using an MTT assay as described previously (Pannecouque et al., 2008). In brief, stock solutions (10 times final concentration) of test compounds were added in 25 µL volumes to two series of triplicate wells 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). Untreated control, HIV and mock-infected cell samples, were included for each sample. HIV-1 (III_B) (Popovic et al., 1984) or HIV-2 (ROD) (Barré-Sinoussi et al., 1983) stock (50 µL) at 100–300 $CCID_{50}$ (50 % cell culture infectious dose) or culture medium was added to either of 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 compounds. Exponentially growing MT-4 cells (Witvrouw et al., 1999) were centrifuged for 5 min at 1000 rpm, and the supernatant was discarded. The MT-4 cells were resuspended at 6 × 105 cells per mL, and 50-µL volumes were transferred to the microtiter tray wells. Five days after infection, the viability of the mock-infected cells and HIV-infected cells was examined spectrophotometrically.

In vitro HCV assay

Huh-5-2 cells, containing the HCV genotype 1b I389luc-ubi-neo/NS3-3′/5.1 replicon (Vrolijk et al., 2003) were subcultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10 % Fetal calf serum (FCS),

1 % non-essential amino acids, 1 % penicillin/streptomycin, and 2 % Geneticin at a ratio of 1:3–1:4, and grown for 3–4 days in 75 cm^2 tissue culture flasks. One day before the addition of the compound, cells were harvested and seeded in assay medium (DMEM, 10 % FCS, 1 % non-essential amino acids, 1 % penicillin/ streptomycin) at a density of 6500 cells/well (100 µL/well) in 96-well tissue culture microtiter plates for the evaluation of anti-metabolic effect and cultur plate (Perkin Elmer) for the evaluation of the antiviral effect. The microtiter plates were incubated overnight (37 °C, 5 % CO_2, 95–99 % relative humidity), yielding a non-confluent cell monolayer.

The evaluation of the anti-metabolic as well as antiviral effect of each compound was performed in parallel. Four-step, 1-to-5 compound dilution series were prepared for the first screen, to collect data for a more detailed dose-response curve, an eight-step, 1-to-2 dilution series was used. Following assay setup, the microtiter plates were incubated for 72 h. (37 °C, 5 % CO_2, 95–99 % relative humidity). For the evaluation of anti-metabolic effects, the assay medium was aspirated, replaced with 75 µL of a 5 % MTS solution in phenol red-free medium and incubated 45 for 1.5 h. (37 °C, 5 % CO_2, 95–99 % relative humidity). Absorbance was measured at a wavelength of 498 nm (Safire², Tecan), and optical densities (OD values) were converted to percentage of untreated controls. For the evaluation of antiviral effects, the assay medium was aspirated and the cell monolayers were washed with phosphate buffered saline. The wash buffer was aspirated, and 25 µL of Glo Lysis Buffer (Promega) was added allowing for cell lysis to proceed for 5 min at room temperature. Subsequently, 50 µL of Luciferase Assay System (Promega) was added, and the luciferase luminescence signal was quantified immediately (1,000 ms integration time/well, Safire², Tecan). Relative luminescence units were converted into percentage of untreated controls. The EC_{50} (values calculated from the dose-response curve) represent the concentrations at which 50 % inhibition, respectively, of viral replication is achieved. The CC_{50} (value calculated from the dose-response curve) represents the concentration at which the metabolic activity of the cells is reduced by 50 % as compared to untreated cells. A concentration of a compound is considered to elicit a genuine antiviral effect in the HCV replicon system when the anti-replicon effect is well above the 70 % threshold at concentrations where no significant anti-metabolic activity is observed (Vrolijk et al., 2003).

Cells and HCV viruses

The Huh-5-2 and Huh-9-13 HCV subgenomic replicon-containing cells were provided by Prof R Bartenschlager (University of Heidelberg, Heidelberg, Germany).

Conclusions

A series of phthalimido-(substituted-alkyl-2-thioureido) alkyl carboxylic acid derivatives **5–13** and methyl 2-(4-(phthalimido-2-yl)butanamido)alkyl carboxylates **14–23** were synthesized. The new synthesized compounds were assayed against HIV-1 and HIV-2 in MT-4 cells. Compounds **22** and **23** showed significant inhibition of HIV-1 with EC_{50} value of >2.07 and >4.23 µM (SI = 7 and 5), respectively. The anti-HIV activity results suggest that compounds **22** and **23** might act as new candidates for non-nucleoside reverse transcriptase inhibitors. Furthermore, the new analogs were screened against HCV genotype 1b in the Huh-5-2 replicon system. Compounds **9** and **12** showed a moderate activity with EC_{50} = 26.7 and 22.0 µM (SI > 1.87 and >2.28, respectively). These analogs could be promising agents in HCV inhibition studies after further structural modification. The molecular docking study of **22** showed hydrogen bond interactions with some amino acids of HIV reverse transcriptase enzyme.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

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