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Parallel one-pot synthesis and structure–activity relationship study of symmetric formimidoester disulfides as a novel class of potent non-nucleoside HIV-1 reverse transcriptase inhibitors

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ABSTRACT

The molecular duplication of non-nucleoside reverse transcriptase inhibitor (NNRTI) *O*-(2-phthalimidoethyl)–*N*-arylthiocarbamates (C-TCs) led to the identification of symmetric formimidoester disulfides (DSs) as a novel class of potent NNRTIs. The lead compound **1** [dimer of the isothiocarbamic form of TC *O*-(2-phthalimidoethyl)–*N*-phenylthiocarbamate] turned out to prevent the wild-type HIV-1 multiplication in MT-4 cell culture with an EC_{50} value of 0.35 μM. In order to perform a structure–activity relationship (SAR) study, we prepared 40 analogues of **1** by an unprecedented one-pot method of solution-phase parallel synthesis. The SAR strategy was focused on the variation of the *N*-aryl portion (mono-, di- and trisubstitution of the phenyl ring and its replacement with a 1-naphthyl, cyclopropyl or benzyl group) and of the 2-phthalimidoethyl moiety (introduction of a methyl on the phthalimide substructure, replacement of the phthalimide moiety with a phenyl ring and elongation of the ethyl linker). Most DSs proved to inhibit the wild-type HIV-1 replication in cell-based assays and 15 of them were active at nanomolar concentrations. The most potent congeners (**11**, **15**, **16**, **17**, **18**, **19**, **20** and **32**, EC_{50}: 10–70 nM) shared the *N-para*-substituted phenyl moiety. Compound **17** tested in enzyme assay against recombinant wild-type reverse transcriptase displayed an IC_{50} value of 0.74 μM. Compounds **19** and **33** were active at micromolar concentrations against the clinically relevant Y181C and/or K103R resistant mutants.

1. Introduction

Reverse transcriptase (RT) is a key enzyme in the HIV replication cycle and is one of the main targets in the development of drugs for treating HIV infection and AIDS.[1–5] Non-nucleoside RT inhibitors (NNRTIs) bind to an allosteric hydrophobic pocket located at about 10 Å far from the polymerase active site and lock the enzyme into an inactive form by affecting the geometry of the polymerase active site aspartyl residues.[6] In the past 15 years more than 50 structurally diverse NNRTIs have been described.[6–12] Three NNRTIs, nevirapine (Viramune®), delavirdine (Rescriptor®) and efavirenz (Sustiva®) have been approved by FDA for the treatment of HIV infection. Other NNRTIs, like thiocarboxanilide UC-781, capravirine, dapivirine, rilpivirine and etravirine, are currently under clinical investigation.[3] The fact that cross-resistance extends to the whole NNRTI class calls for development of new agents capable of inhibiting clinically relevant NNRTI-resistant mutants.[13,14]

In our previous studies, we reported the discovery of the potent NNRTI classes of *O*-(2-phthalimidoethyl)–*N*-arylthiocarbamates (C-TCs)[15] and structurally related compounds, such as ring-opened analogues (O-TCs),[15] *N*-acylated derivatives (ATCs)[16] and non-phthalimidic congeners (TCs)[17,18] (Figure 1a shows the leads C-TC 12, O-TC 51, ATC 25c, TC 1 and TC 29.) C-TCs and TCs are isosterically related to the NNRTI family of *N*-phenethyl-*N*′-thiazolylthiourea (PETT) derivatives,[19,20] of which Trovirdine is one of the most representative analogues (Fig. 1b).

During chemical studies on the N-sulfonylation of C-TCs, the symmetric formimidoester disulfide[21] (DS) **1** (Fig. 2) was isolated. Compound **1** resulted from the molecular duplication of the isothiocarbamic form of *O*-(2-phthalimidoethyl)–*N*-phenylthiocarbamate (C-TC 12, Fig. 1a). Since examples of anti-HIV symmetric disulfides (e.g., disulfide benzamides,[22,23] Fig. 3) and of symmetric HIV-RT inhibitors (e.g., Suramine and naphthalenedisulfonic acid derivatives,[24] Fig. 3) have been reported in the literature, we wanted to test **1** against wild-type HIV-1 in cell-based assay. The compound showed an EC_{50} value of 0.35 μM, being 3.4-fold more

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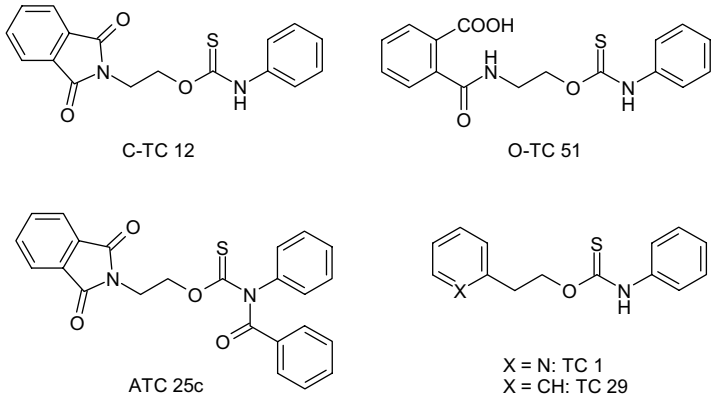
(a) TCs and structurally related compounds



(b) PETT derivatives

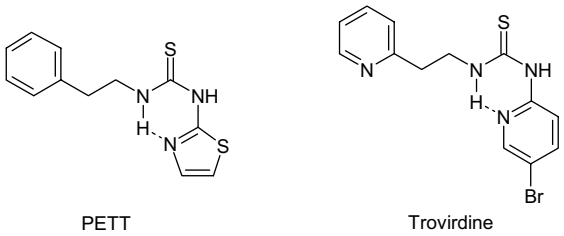


Figure 1. Chemical structure of C-TC 12,[15] O-TC 51,[15] ATC 25c,[16] TC 1,[17] TC 29,[17] PETT and Trovirdine.

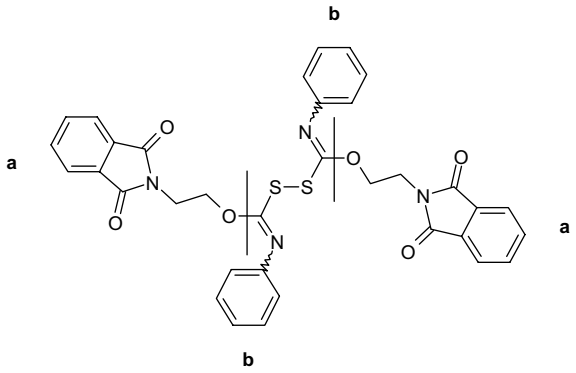


Figure 2. Chemical structure of the lead compound DS **1** segmented in chemofunctional portions **a** and **b**.

potent than its monomer C-TC 12.[15] This encouraged us to further study the dimerization reaction in order to set-up a parallel synthesis method for rapid analoguing of **1**, aimed at the structure–activity relationship (SAR) profiling.

Our SAR strategy was focused on structural modifications of the lead **1** (Fig. 2) by keeping constant the *O*-(2-phthalimidoethyl) substructure (portion **a**) and varying the *N*-phenyl moiety (portion **b**) (**1**–**38**, Tables 1–3). In particular, we investigated the monosubstitution with functional groups with various electronic (inductive and/or mesomeric), steric and lipophilic properties at positions *ortho*, *meta* and *para* (**1**–**20**); the di- and trisubstitution with equal (**21**–**29**, **34**) or different (**30**–**33**, **35**) groups, and the replacement of the phenyl ring with a 1-naphthyl (**36**), cyclopropyl (**37**) or benzyl group (**38**). We also synthesized three disulfides in which the *N*-phenyl ring was *para*-bromo-substituted or unsubstituted, and portion **a** was modified by lengthening the ethyl linker (**39**), by introducing a methyl at position 4 of the phthalimide substructure (**40**) or by replacing the phthalimide moiety with a phenyl ring (**41**) (Table 4).

2. Chemistry

In the modern drug discovery process,[25] and particularly in lead identification and optimization, parallel synthesis plays an important role as it allows to produce a (large) number of compounds in short times by using simple and rapid purification methods. Recently, solution-phase chemistry has largely supplanted solid-phase chemistry as the method of choice for parallel synthesis of small organic molecules.[26]

DS **1**–**41** were prepared in parallel by an unprecedented solution-phase method (Scheme 1), by using ordered arrays of spatially separated reaction vessels (Carousel-6™ reaction station). The parallelization of the procedure was accomplished by varying and optimizing the reaction conditions that had led to the synthesis of DS **1** (data concerning the procedure set-up not shown). The convergent one-pot procedure combined two building blocks: alcohols and isothiocyanates (Fig. 4). Starting alcohols $\mathbf{A}_{1-4}$ (Fig. 4a) were first transformed into their corresponding salts ($\mathbf{S}_{1-4}$) in the presence of sodium hydride in dry polar aprotic solvents (DMF or THF) and then condensed in situ with the suitable isothiocyanate ($\mathbf{I}_{1-38}$, Fig. 4b) to give the corresponding thiocarbamate sodium salts ($\mathbf{B}_{1-41}$). The sequential addition of *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TMEDA) and tosyl chloride resulted in the dimerization of the isothiocarbamic form of salts **B** by the formation of a disulfide bridge. The reaction consisted in the oxidation of **B** thiocarbonyl sulfur caused by tosyl chloride, acting as an atypical oxidizing reagent. The presence of TMEDA greatly increased the yields. The work-up simply required addition of water, followed by filtrations or extractions, and the final products were purified by crystallization. The yields ranged from 8% to 94%.

The reaction sequence for DS preparation represents a new synthetic methodology to afford symmetric formimidoester disulfides

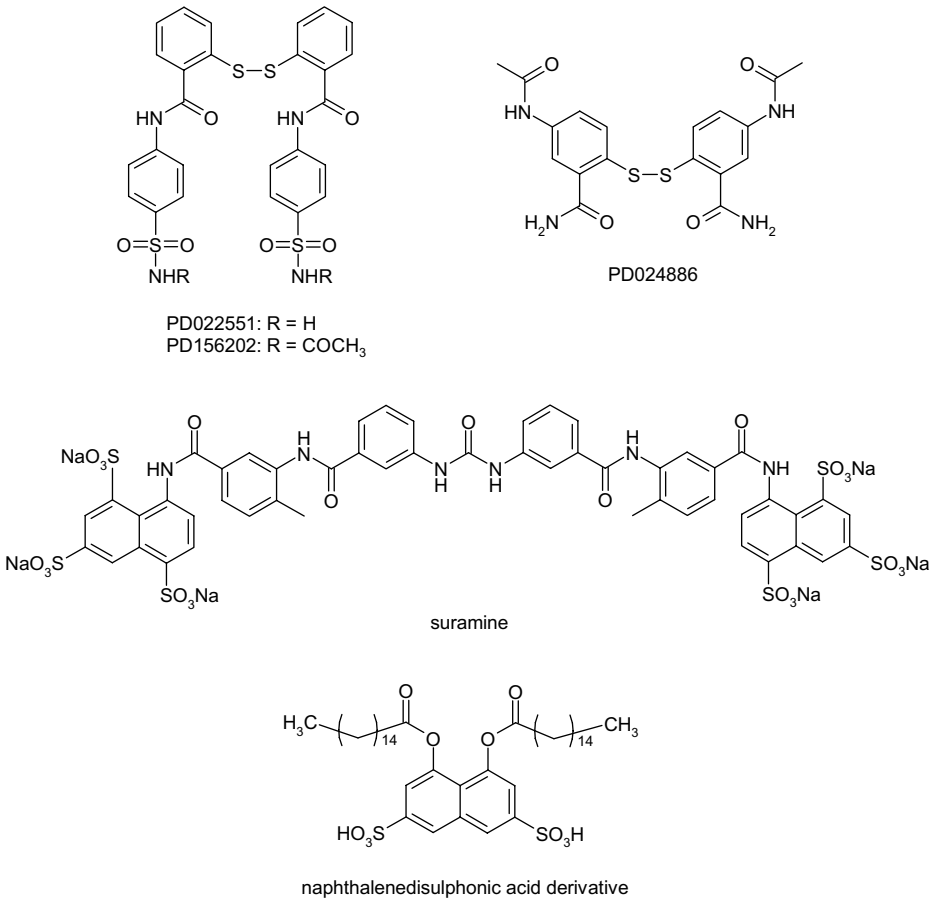


Figure 3. Chemical structure of the anti-HIV symmetric disulfide benzamides PD02251,[22] PD156202[22] and PD024886,[22] and of the symmetric HIV-RT inhibitors Suramine and a naphthalenedisulfonic acid derivative.[24]

from thiocarbamates: in the literature this conversion is reported to be accomplished by using common oxidizing reagents,[27–32] which instead proved to be ineffective for DS synthesis. In fact, when tosyl chloride was replaced by iodine, permanganate, sulfuric anhydride–pyridine complex, pyridinium chlorochromate, oxone or *N*-chlorosuccinimide, the progenitor thiocarbamate or unidentified products were recovered.

The formation of the disulfide bridge has been indirectly confirmed by ^{13}C NMR and ^{1}H NMR spectra (lack of the C=S carbon and NH proton signals, respectively) and by IR spectra (absence of the N–H stretching band and presence of the C=N stretching band at 1625–1662 cm^{-1}).

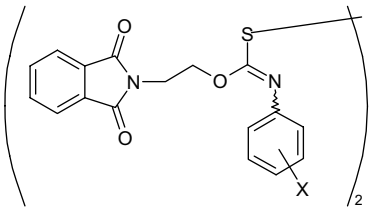
In principle four different DS geometric isomers are possible, but, because of the symmetry of the molecule, their number diminishes to three (*Z*,*Z*; *E*,*Z*; and *E*,*E*, Fig. 5). Computational analysis (see Section 5.3) performed on a simplified derivative of **1** (Fig. 5), selected as a model compound, predicted the *Z*,*Z*-isomer to be, respectively, 1.62 and 1.00 kcal/mol lower in energy than the *E*,*Z*- and *E*,*E*-isomers, and therefore potentially favoured. In the future, X-ray crystallography will be employed to define the stereochemistry of DSs.

3. Biological results and discussion

The antiretroviral activity of DS **1**–**41** was evaluated in MT-4 cell-based assays by assessing the reduction of the HIV-1 induced cytopathogenicity. The results are expressed as EC_{50} values. In parallel with antiretroviral activity, the DS-induced cytotoxicity was evaluated in mock-infected MT-4 cells. The results are expressed as CC_{50} values, which have been used to calculate the selectivity indexes (SI). Trovirdine was employed as the reference molecule (Tables 1–4). To determine whether the title compounds targeted HIV-1 RT, the most potent disulfide was tested also in enzyme assay against the HIV-1 virionic RT (vRT) (Table 5). The most active DSs were screened in cell-based assays against the clinically relevant K103R, Y181C and K103N/Y181C resistant mutants,[33,34] using Efavirenz as reference molecule (Table 6).

The results of Table 1 suggest that, as observed also for C-TCs,[15] the substitution at the *para* position of the *N*-phenyl ring has significant impact on anti-HIV-1 activity: in fact, all the *para*-substituted analogues (**11**–**20**), with the exception of the 4-acetyl **14**, showed nanomolar EC_{50} values (10–100 nM), with a 3.5- to 35-fold increase in potency compared to the lead **1**. The 4-fluoro (**15**), 4-chloro (**16**) and 4-bromo (**17**) derivatives were more or as active as Trovirdine. In particular DS **17**, with an EC_{50} of 10 nM, was the most potent DS of the series. The *ortho* and *meta* positional isomers resulted to be less potent than the corresponding *para* congeners (**15** vs **6** and **3**; **16** vs **7** and **4**; **11** vs **2**; **12** vs **5**; **17** vs **8**; **19** vs **9**; **20** vs **10**). The introduction of a chloro (**7**) or a nitro (**9**) group at position *meta* led to submicromolar inhibitors, even if less potent than the lead **1**, while the other *meta* derivatives (**5**, **6**, **8**, **10**) prevented the viral replication at micromolar concentrations. The *ortho* substitution was detrimental and only the introduction of a fluorine atom (**3**), less bulky than a chlorine (**4**) or a methyl (**2**), was tolerated. The electronic properties of the *N*-phenyl ring substituent did not seem to affect the activity; in fact, the most potent of the *para*-substituted analogues bore either electron-withdrawing groups, such as fluoro (**15**), chloro (**16**), bromo (**17**), iodo (**18**), nitro (**19**), or electron-donating groups, such as methyl (**11**) and methoxy (**20**), and, among the *meta* derivatives,

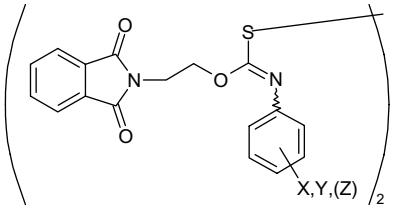
Table 1
Effects of the *N*-phenyl monosubstitution on cytotoxicity and anti-HIV-1 activity of DS **1–20**[a]



Compound	X	CC_{50}[b]	EC_{50}[c]	SI[d]
1	H	80	0.35	229
2	2-CH_3	11	>11	<1
3	2-F	43	1.2	36
4	2-Cl	9	>9	<1
5	3-CF_3	22	2.3	9.6
6	3-F	38	5.0	7.6
7	3-Cl	36	0.4	90
8	3-Br	89	6.0	15
9	3-NO_2	>100	0.7	143
10	3-OCH_3	80	1.3	6.1
11	4-CH_3	21	0.04	525
12	4-CF_3	>100	0.1	>1000
13	4-CN	>100	0.1	>1000
14	4-$COCH_3$	>100	24	>4.2
15	4-F	29	0.02	1450
16	4-Cl	88	0.02	5500
17	4-Br	42	0.01	4200
18	4-I	48	0.03	1600
19	4-NO_2	>100	0.07	>1429
20	4-OCH_3	90	0.06	1500
Trovirdine		60	0.02	3000

[a] Data mean values for three separate experiments. Variation among triplicate samples was less than 10%.
[b] Compound concentration (μM) required to reduce the viability of mock-infected cells by 50%, as determined by the MTT method.
[c] Compound concentration (μM) required to achieve 50% protection of MT-4 cell from HIV-1 induced cytopathogenicity, as determined by the MTT method.
[d] Selectivity index: CC_{50}/EC_{50} ratio.

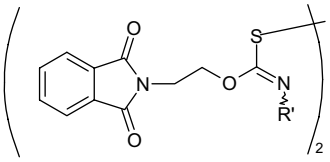
Table 2
Effects of the *N*-phenyl di- and trisubstitution on cytotoxicity and anti-HIV-1 activity of DS **21–35**[a]



Compound	X,Y,(Z)	CC_{50}[b]	EC_{50}[c]	SI[d]
21	3,5-$(CH_3)_2$	>100	3.0	>33
22	2,4-F_2	27	0.5	541
23	2,5-F_2	52	1.3	40
24	3,5-F_2	37	>37	<1
25	2,3-Cl_2	37	18	2.1
26	2,4-Cl_2	>100	4.0	>25
27	2,5-Cl_2	>100	>100	—
28	3,4-Cl_2	>100	7.0	>14
29	3,5-Cl_2	42	>42	<1
30	3-Cl-4-CH_3	>100	0.2	>500
31	4-Cl-3-CF_3	>100	28	3.6
32	4-Cl-3-NO_2	18	0.07	257
33	4-Br-2-CH_3	80	1.8	44
34	2,4,6-F_3	39	1.2	33
35	2,6-$(CH_3)_2$-4-Br	>100	>100	—
Trovirdine		60	0.02	3000

[a,b,c,d] See legend to Table 1.

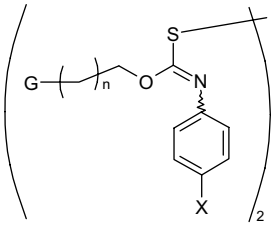
Table 3
Effects of the cycle variation in portion **b** on cytotoxicity and anti-HIV-1 activity of DS **36–38**[a]



Compound	R′	CC_{50}[b]	EC_{50}[c]	SI[d]
36	1-Naphthyl	>100	8.0	13
37	Cyclopropyl	>100	>100	—
38	Benzyl	>100	7.0	14
Trovirdine		60	0.02	3000

[a,b,c,d] See legend to Table 1.

Table 4
Effects of the modifications of portion **a** on cytotoxicity and anti-HIV-1 activity of DS **39–41**[a]

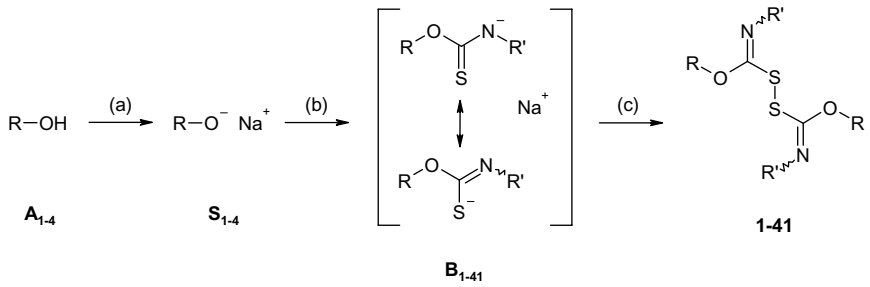


Compound	G	*n*	X	CC_{50}[b]	EC_{50}[c]	SI[d]
39	Phthalimido	2	Br	>100	>100	—
40	4-Methylphthalimido	1	Br	>100	1.3	>77
41	Phenyl	1	H	70	11	6.4
Trovirdine				60	0.02	3,000

[a,b,c,d] See legend to Table 1.

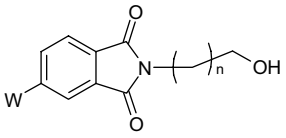
the activity trend was chloro (**7**) > nitro (**9**) > methoxy (**10**) > fluoro (**6**) > bromo (**8**). At the *para* position, a hydrophobic substituent, such as a methyl (**11**) or halogen atom (**15–18**), was preferred to a relatively hydrophilic functionality, such as a nitro- (**19**), cyano- (**13**) or acetyl- (**14**) group (in particular the acetyl group caused a dramatic potency decrease).

The *N*-phenyl di- and trisubstitution led generally to active compounds (Table 2). In particular DS **22** (2,4-difluoro), **30** (3-chloro-4-methyl) and **32** (4-chloro-3-nitro) showed EC_{50} values in the nanomolar concentration range. DS **30** and **32** were, respectively, 1.8- and 5-fold more potent than the lead **1**. The difluoro-derivatives resulted to be more active than the corresponding dichloro-congeners (**22** vs **26** and **23** vs **27**), with the exception of the 3,5-analogues, which in both cases exhibited no antiviral activity (**24**, **29**). In this connection, the replacement of the chlorine atoms with methyl groups (endowed with similar steric and lipophilic properties, but opposite electronic features) was beneficial (**21**). The introduction of a substituent at position *para* confirmed to be effective: the 2,4,6-trifluoro-DS (**34**) and the 2,4- and 3,4-dihalosubstituted derivatives (**22**, **26**, **33** and **28**, **30–32**, respectively) were endowed with higher activity than the corresponding analogues not bearing an halogen atom at the *para* position. Besides, the most potent disubstituted derivatives had a substituent at position 4 (**22**, **30** and **32**). The steric hindrance of the *ortho*-substituent seemed to affect the activity, as the 2-fluoro- (**22**, **23**) and 2,6-difluoro- (**34**) derivatives were more active than the analogues

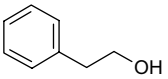


Scheme 1. Reagents and conditions: (a) NaH (1 equiv), dry DMF (THF for **3**, **23**, **37**, **38**, **41**), rt, 30 min; (b) R′NCS (**I**$_{1-38}$, 1 equiv), rt, 2 h; (c) TMEDA (2.1 equiv), TsCl (1.6 equiv), rt, 5 h. For the structure list of alcohols **A**$_{1-4}$ and isothiocyanates **I**$_{1-38}$, see Figure 4.

(a) Alcohols **A**$_{1-4}$



A$_1$: W = H; n= 1
A$_2$: W = CH_3; n= 1
A$_3$: W = H; n= 2



A$_4$

(b) Isothiocyanates R'NCS (**I**$_{1-38}$)

	R'		R'
I$_1$	phenyl	**I**$_{20}$	4-methoxyphenyl
I$_2$	2-tolyl	**I**$_{21}$	3,5-dimethylphenyl
I$_3$	2-fluorophenyl	**I**$_{22}$	2,4-difluorophenyl
I$_4$	2-chlorophenyl	**I**$_{23}$	2,5-difluorophenyl
I$_5$	3-trifluoromethylphenyl	**I**$_{24}$	3,5-difluorophenyl
I$_6$	3-fluorophenyl	**I**$_{25}$	2,3-dichlorophenyl
I$_7$	3-chlorophenyl	**I**$_{26}$	2,4-dichlorophenyl
I$_8$	3-bromophenyl	**I**$_{27}$	2,5-dichlorophenyl
I$_9$	3-nitrophenyl	**I**$_{28}$	3,4-dichlorophenyl
I$_{10}$	3-methoxyphenyl	**I**$_{29}$	3,5-dichlorophenyl
I$_{11}$	4-tolyl	**I**$_{30}$	3-chloro-4-methylphenyl
I$_{12}$	4-trifluoromethylphenyl	**I**$_{31}$	4-chloro-3-trifluoromethylphenyl
I$_{13}$	4-cyanophenyl	**I**$_{32}$	4-chloro-3-nitrophenyl
I$_{14}$	4-acetylphenyl	**I**$_{33}$	4-bromo-2-methylphenyl
I$_{15}$	4-fluorophenyl	**I**$_{34}$	2,4,6-trifluorophenyl
I$_{16}$	4-chlorophenyl	**I**$_{35}$	2,6-dimethyl-4-fluorophenyl
I$_{17}$	4-bromophenyl	**I**$_{36}$	1-naphthyl
I$_{18}$	4-iodophenyl	**I**$_{37}$	cyclopropyl
I$_{19}$	4-nitrophenyl	**I**$_{38}$	benzyl

Figure 4. Building blocks used.

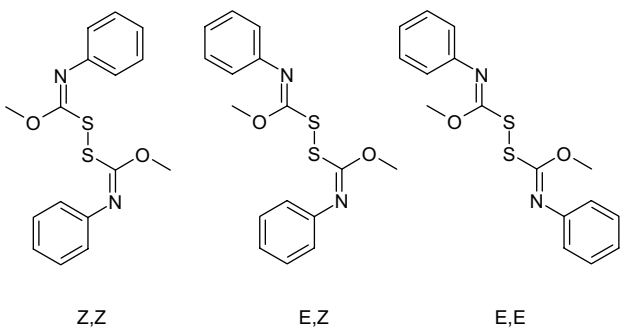


Figure 5. The three possible geometric isomers of the simplified derivative of **1** (2-phthalimidoethyl substituents replaced by methyl groups) used in the computational analysis.

bearing more encumbering groups at those positions (**25**–**27**, **33**, **35**). At the meta position of the 3,4-derivatives, a chlorine atom or a nitro group were favoured in comparison with a trifluoromethyl (compare **28**, **30** and **32** with **31**).

Data of Table 3 show that the replacement of the *N*-phenyl ring with a more sterically demanding 1-naphthyl (**36**) and the insertion of a methylene between the phenyl and the thiocarbamic function (**38**) caused, respectively, a 23- and 20-fold drop in activ-

Table 5
Activity of **17** in enzyme assay against HIV-1 virionic RT (vRT)[a]

Compound	IC_{50}[b] (μM)
17	0.74
Trovirdine	1.06
Efavirenz	0.02

[a] See legend to Table 1.
[b] Compound concentration (μM) required to inhibit the HIV-1 virion-associated RT (vRT) activity by 50%.

Table 6
Anti-HIV-1 activity of **19** and **33** against Y181C and K103R resistant mutants[a]

Compound	EC_{50}[c] (μM)	
	Y181C	K103R
19	57	62
33	35	n.a.[b]
Efavirenz	0.01	0.04

[a,c] See legend to Table 1.
[b] Not active.

ity (**36** and **38** vs **1**), whereas the replacement of the phenyl with a cyclopropyl (**37**) produced activity loss.

Regarding the modifications on portion **a** (Table 4), the introduction of a methyl at position 4 of the phthalimide substructure was not beneficial (**40** vs **17**), differently from TCs where this variation caused potency enhancement.[15] The elongation of the ethyl linker to propyl led to an inactive compound (compare **39** with **17**). Also the omission of the benzofused five-membered ring (**41**) had a negative impact on the inhibitory activity, which nevertheless remained in the micromolar concentration range.

To determine whether the title compounds targeted HIV-1 RT, the DS **17** was tested in enzyme assay against the HIV-1 virion-associated RT (vRT) (Table 5). The disulfide resulted to be 1.4-fold more potent than Trovirdine and 37-fold less potent than Efavirenz. The considerable difference of potency in enzyme- and cell-based assays (observed also for Trovirdine, C-TCs[15] and ATCs[16] could be ascribed to two factors. The first one is that vRT is a p51–p66 dimer engaged in complex interactions with the viral genome and core proteins that may not become totally disrupted during virion lysis, thus affecting the binding of the NNRTI to the non-nucleoside inhibitor binding site (NNIBS). A second explanation is the possibility that DSs are slow binding kinetics inhibitors.[35]

Interestingly, DS **1**, **3**, **5**, **7**, **10**, **15–17** resulted to be more potent than the corresponding C-TCs endowed with the same substitution pattern, while DS **2**, **9**, **11**–**14**, **18**–**20**, **36** and **41** turned out to be less active.[15,17] The X-ray analysis of C-TC/RT complexes[36] has demonstrated that the hydrogen bond between the C-TC NH group and the Lys101 main chain carbonyl is one of the interactions which contribute the most to stabilize the complexes. Since DSs are unable to establish this hydrogen bond (owing to the dimerization of the C-TC isothiocarbamic form), the differences in activity between the dimers (DSs) and the monomers (C-TCs) might correlate with a different mode of binding to RT of the two classes of inhibitors. In order to elucidate the binding mode of the title compounds, crystallographic studies on RT/DS complexes will be performed.

In cell-based assays, the Y181C, K103R and K103N + Y181C mutated strains proved to be unsusceptible to the DSs, with the exception of **19** and **33** (Table 6), which turned out to be weakly active against Y181C and/or K103R resistant mutants.

All compounds, except the *N*-*ortho*-substituted-phenyl derivatives **2** and **4** and the *N*-3,5-dihalosubstituted-phenyl derivatives **24** and **29**, showed values of CC_{50} higher than EC_{50} (CC_{50} in several cases superior to 100 μM). DS **1**, **11**–**13**, **15**–**20**, **22**, **30** and **32** (Tables 1 and 2) displayed good selectivity indexes.

4. Conclusions

A novel class of potent NNRTIs was discovered and a SAR study has been performed. The scaffold novelty came out from the molecular duplication of the isothiocarbamic form of NNRTI C-TCs. To the best of our knowledge, this is the first example of symmetric disulfides as NNRTIs, and the procedure by which they have been obtained represents a new synthetic method to prepare symmetric formimidoester disulfides from thiocarbamates. The one-pot procedure has been parallelized thus enabling a rapid analoguing. Many DSs were micro- and nanomolar inhibitors and proved to target RT. The *N*-phenyl *para*-substitution was found particularly beneficial and the most potent DS so far synthesized (**17**: *para*-bromo) displayed an EC_{50} value of 10 nM. Two disulfides resulted moderately effective against Y181C and/or K103R mutant strains. Overall, these results encourage us to further study DSs, with the aim at determining their stereochemistry, mode of binding to RT and structural features to improve the resistance profile.

5. Experimental protocols

5.1. Chemistry

5.1.1. General

All chemicals were purchased by Chiminord and Aldrich Chemical, Milan (Italy). Solvents were of reagent grade. THF was distilled in the presence of sodium. DMF was dried on molecular sieves (5 Å 1/16″ inch pellets). Unless otherwise stated, all commercial reagents were used without further purification. Organic solutions were dried over anhydrous sodium sulfate. Thin layer chromatography (TLC) system for routine monitoring the course of reactions and confirming the purity of analytical samples employed aluminium-backed silica gel plates (Merck DC-Alufolien Kieselgel 60 F_{254}): $CHCl_3$ or diethyl ether was used as developing solvents and detection of spots was made by UV light and/or by iodine vapours.

The parallel solution-phase chemistry was performed by using a Carousel-6™ reaction station (Radleys Discovery Technologies, Italian distributor: StepBio, Bologna). The evaporation of solutions in parallel fashion was performed with an Evaposel™ apparatus (Radleys Discovery Technologies, Italian distributor: StepBio, Bologna) operating at reduced pressure of about 15–20 Torr. Yields were not optimized.

Melting points were determined on a Fisher–Johns apparatus and are uncorrected. IR spectra were recorded on a Perkin Elmer 398 spectrometer as KBr discs. ^{1}H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$, DMSO-d_6 or CF_3COOD on a Varian Gemini 200 instrument. Chemical shifts were reported in δ (ppm) units relative to the internal standard tetramethylsilane, and the splitting patterns were described as follows: s (singlet), d (doublet), dd (doublet of doublets), t (triplet), m (multiplet) and br s (broad singlet). The first order values reported for coupling constants *J* were given in Hz. Elemental analyses were performed by an EA1110 Elemental Analyser (Fison-Instruments, Milan) and were within ±0.4% of the theoretical values. The synthesis of Trovirdine and alcohol $\mathbf{A}_2$ was accomplished according to published procedures.[15,19]

5.1.2. Parallel synthesis of disulfides 1–41

Sodium hydride dispersion (60%) in mineral oil (0.40 g, 10 mmol) was added at rt in a single portion into each numbered round-bottomed flask of a Carousel-6™ reaction station, containing a stirred solution of the suitable alcohol $\mathbf{A}_{1-4}$ (10 mmol) in 25 mL of dry DMF (THF for **3**, **23**, **37**, **38** and **41**). After stirring for 30 min, the proper isothiocyanate $\mathbf{I}_{1-38}$ (10 mmol) was added to each reaction mixture, which was then stirred for 2 h at rt (for **38**, the addition of 25 mL of dry DMF was required to allow the stirring of the thick suspension formed). Then, TMEDA (2.44 g, 21 mmol) and tosyl chloride (3.05 g, 16 mmol) were added sequentially, each one in one portion. The resulting mixture was vigorously stirred at rt for 5 h, and then in most cases diluted with 150 mL of water. For **3**, **23**, **37**, **38** and **41**, each reaction mixture was first transferred into an Evaposel™-tube and, after evaporation of THF in vacuo in parallel fashion using an Evaposel™ apparatus, 40 mL of water were added into each tube; the contents of the tubes were then

transferred into a set of beakers and 110 mL of water were added into each beaker.

Two different types of work-up were carried out depending on whether a filterable precipitate was obtained (i) or not (ii). Work-up (i) (**9**, **10**, **14**, **22**, **24**, **26**, **29** and **36**–**38**): the precipitates obtained were filtered off in parallel by an in-house device and dissolved in CH_2Cl_2 (120 mL). The solutions were washed with water (2× 30 mL), dried over anhydrous Na_2SO_4 and filtered in parallel through pads of Florisil (diameter 5 × 2 cm) by an in-house device. Evaporation in parallel under reduced pressure using an Evaposel™ apparatus gave residues, which were purified by crystallization from the suitable solvents or solvent mixtures. Work-up (ii) (**1**–**8**, **11**–**13**, **15**–**21**, **23**, **25**, **27**, **28**, **30**–**35**, and **39**–**41**): the contents of the round-bottomed flasks/beakers were transferred into a set of separating funnels. After parallel extraction with diethyl ether (CH_2Cl_2 for **1**, **12**, **13**, **15**, **19**, **20**, **30** and **32**) (3× 50 mL), the combined extracts of each reaction were washed with water (5× 30 mL), dried over anhydrous Na_2SO_4 and filtered in parallel through pads of Florisil (diameter 5 × 2 cm) by an in-house device. Evaporation in parallel under reduced pressure using an Evaposel™ apparatus gave residues, which were purified by crystallization from the suitable solvents or solvent mixtures.

5.1.2.1. 2-(2-{[({[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)-ethoxy](phenylimino)methyl}dithio)(phenylimino)methyl]oxy}-ethyl)-1,3-dioxoisoindoline 1. Mp 152–154 °C; yield: 44% from CH_2Cl_2/ethanol. IR (KBr) cm^{-1}: 1782, 1719, 1643. ^1H NMR ($CDCl_3$) δ: 3.95 (t, J = 5.4 Hz, 4H, $2CH_2N$), 4.33 (t, J = 5.4 Hz, 4H, $2CH_2O$), 6.71–7.16 (m, 10H, arom. H), 7.57–7.83 (m, 8H, phthal. arom. H). Anal. Calcd for $C_{34}H_{26}N_4O_6S_2$: C, 62.76; H, 4.03; N, 8.61; S, 9.85. Found: C, 62.72; H, 4.13; N, 8.68; S, 9.52.

5.1.2.2. 2-[2-({({[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)-ethoxy][(2-methylphenyl)imino]methyl}dithio)[(2-methylphenyl)-imino]methyl}oxy)ethyl]-1,3-dioxoisoindoline 2. Mp 150–152 °C; yield: 57% from CH_2Cl_2/ethanol. IR (KBr) cm^{-1}: 2950, 1778, 1717, 1642. ^1H NMR ($CDCl_3$) δ: 2.03 (s, 6H, $2CH_3$), 3.91–4.26 (m, 4H, $2CH_2N$), 4.35–4.68 (m, 4H, $2CH_2O$), 6.76–7.18 (m, 8H, arom. H), 7.58–7.95 (m, 8H, phthal. arom. H). Anal. Calcd for $C_{36}H_{30}N_4O_6S_2$: C, 63.70; H, 4.45; N, 8.25; S, 9.45. Found: C, 63.79; H, 4.62; N, 8.01; S, 9.21.

5.1.2.3. 2-[2-({({[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)-ethoxy][(2-fluorophenyl)imino]methyl}dithio)[(2-fluorophenyl)-imino]methyl}oxy)ethyl]-1,3-dioxoisoindoline 3. Mp 113–115 °C; yield: 9% from acetone/ethanol. IR (KBr) cm^{-1}: 1775, 1717, 1635. ^1H NMR ($CDCl_3$) δ: 3.88–4.08 (m, 4H, $2CH_2N$), 4.32–4.53 (m, 4H, $2CH_2O$), 6.73–7.07 (m, 8H, arom. H), 7.57–7.88 (m, 8H, phthal. arom. H). Anal. Calcd for $C_{34}H_{24}F_2N_4O_6S_2$: C, 59.47; H, 3.52; N, 8.16; S, 9.34. Found: C, 59.67; H, 3.79; N, 7.94; S, 9.25.

5.1.2.4. 2-[2-({({[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)-ethoxy][(2-chlorophenyl)imino]methyl}dithio)[(2-chlorophenyl)imino]methyl}oxy)ethyl]-1,3-dioxoisoindoline 4. Mp 153–155 °C; yield: 45% from DCM/ethanol. IR (KBr) cm^{-1}: 1776, 1718, 1634. ^1H NMR ($CDCl_3$) δ: 4.11 (t, J = 5.2 Hz, 4H, $2CH_2N$), 4.52 (t, J = 5.2 Hz, 4H, $2CH_2O$), 6.93–7.24 (m, 8H, arom. H), 7.68–7.92 (m, 8H, phthal. arom. H). Anal. Calcd for $C_{34}H_{24}Cl_2N_4O_6S_2$: C, 56.75; H, 3.36; N, 7.79; S, 8.91. Found: C, 56.52; H, 3.32; N, 7.67; S, 8.89.

5.1.2.5. 2-[2-({({[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)-ethoxy][(3-trifluoromethylphenyl)imino]methyl}dithio)[(3-trifluoromethylphenyl)imino]methyl}oxy)ethyl]-1,3-dioxoisoindoline 5. Mp 125–126 °C; yield: 68% from diethyl ether/ethanol. IR (KBr) cm^{-1}: 1781, 1717, 1662. ^1H NMR ($CDCl_3$) δ: 3.93 (t, J = 5.2 Hz, 4H, $2CH_2N$), 4.36 (t, J = 5.2 Hz, 4H, $2CH_2O$), 6.88–6.99 (m, 4H, arom. H), 7.14–7.29 (m, 4H, arom. H), 7.56–7.79 (m, 8H, phthal. arom. H). Anal. Calcd for $C_{36}H_{24}F_6N_4O_6S_2$: C, 54.96; H, 3.07; N, 7.12; S, 8.15. Found: C, 55.22; H, 3.25; N, 7.01; S, 8.19.

5.1.2.6. 2-[2-({({[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)-ethoxy][(3-fluorophenyl)imino]methyl}dithio)[(3-fluorophenyl)-imino]methyl}oxy)ethyl]-1,3-dioxoisoindoline 6. Mp 159–161 °C; yield: 18% from diethyl ether. IR (KBr) cm^{-1}: 1781, 1718, 1647. ^1H NMR ($CDCl_3$) δ: 3.86–3.98 (m, 4H, $2CH_2N$), 4.26–4.41 (m, 4H, $2CH_2O$), 6.44–6.75 (m, 6H, arom. H), 6.99–7.13 (m, 2H, arom. H), 7.58–7.83 (m, 8H, phthal. arom. H). Anal. Calcd for $C_{34}H_{24}F_2N_4O_6S_2$: C, 59.47; H, 3.52; N, 8.16; S, 9.34. Found: C, 59.22; H, 3.79; N, 8.16; S, 9.27.

5.1.2.7. 2-[2-({({[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)-ethoxy][(3-chlorophenyl)imino]methyl}dithio)[(3-chlorophenyl)-imino]methyl}oxy)ethyl]-1,3-dioxoisoindoline 7. Mp 140–141 °C; yield: 15% from DCM/ethanol. IR (KBr) cm^{-1}: 1782, 1718, 1642. ^1H NMR ($CDCl_3$) δ: 4.03 (t, J = 5.2 Hz, 4H, $2CH_2N$), 4.45 (t, J = 5.2 Hz, 4H, $2CH_2O$), 6.70–6.88 (m, 4H, arom. H), 6.96–7.22 (m, 4H, arom. H), 7.67–7.96 (m, 8H, phthal. arom. H). Anal. Calcd for $C_{34}H_{24}Cl_2N_4O_6S_2$: C, 56.75; H, 3.36; N, 7.79; S, 8.91. Found: C, 56.66; H, 3.39; N, 7.71; S, 8.88.

5.1.2.8. 2-[2-({({[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)ethoxy]-[(3-bromophenyl)imino]methyl}dithio)[(3-bromophenyl)imino]-methyl}oxy)ethyl]-1,3-dioxoisoindoline 8. Mp 128–130 °C; yield: 84% from DCM/diethyl ether. IR (KBr) cm^{-1}: 1782, 1718, 1652. ^1H NMR ($CDCl_3$) δ: 4.03 (t, J = 5.0 Hz, 4H, $2CH_2N$), 4.45 (t, J = 5.0 Hz, 4H, $2CH_2O$), 6.75–6.84 (m, 2H, arom. H), 6.94–7.24 (m, 6H, arom. H), 7.67–7.94 (m, 8H, phthal. arom. H). Anal. Calcd for $C_{34}H_{24}Br_2N_4O_6S_2$: C, 50.51; H, 2.99; N, 6.93; S, 7.93. Found: C, 50.43; H, 3.31; N, 6.63; S, 7.58.

5.1.2.9. 2-[2-({({[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)-ethoxy][(3-nitrophenyl)imino]methyl}dithio)[(3-nitrophenyl)-imino]methyl}oxy)ethyl]-1,3-dioxoisoindoline 9. Mp 187–189 °C; yield: 44% from DCM/methanol. IR (KBr) cm^{-1}: 1771, 1708, 1629, 1526, 1334. ^1H NMR (DMSO-d_6) δ: 3.85–3.96 (m, 4H, $2CH_2N$), 4.68–4.79 (m, 4H, $2CH_2O$), 7.37–7.99 (m, 16H, arom. H). Anal. Calcd for $C_{34}H_{24}N_6O_{10}S_2$: C, 55.13; H, 3.27; N, 11.35; S, 8.66. Found: C, 55.19; H, 3.48; N, 11.22; S, 8.46.

5.1.2.10. 2-[2-({({[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)-ethoxy][(3-methoxyphenyl)imino]methyl}dithio)[(3-methoxyphenyl)imino]methyl}oxy)ethyl]-1,3-dioxoisoindoline 10. Mp 117–119 °C; yield: 17% from DCM/diethyl ether. IR (KBr) cm^{-1}: 1777, 1721, 1639. ^1H NMR ($CDCl_3$) δ: 3.67 (s, 6H, $2CH_3$), 3.86–3.95 (m, 4H, $2CH_2N$), 4.25–4.33 (m, 4H, $2CH_2O$), 6.28–6.55 (m, 6H, arom. H), 6.94–7.01 (m, 2H, arom. H), 7.55–7.77 (m, 8H, phthal. arom. H). Anal. Calcd for $C_{36}H_{30}N_4O_8S_2$: C, 60.83; H, 4.25; N, 7.88; S, 9.02. Found: C, 60.81; H, 4.32; N, 7.87; S, 9.09.

5.1.2.11. 2-[2-({({[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)-ethoxy][(4-methylphenyl)imino]methyl}dithio)[(4-methylphenyl)-imino]methyl}oxy)ethyl]-1,3-dioxoisoindoline 11. Mp 139–141 °C; yield: 47% from DCM/methanol. IR (KBr) cm^{-1}: 2927, 1780, 1709, 1632. ^1H NMR ($CDCl_3$) δ: 2.22 (s, 6H, $2CH_3$), 3.94 (t, J = 5.4 Hz, 4H, $2CH_2N$), 4.32 (t, J = 5.4 Hz, 4H, $2CH_2O$), 6.58–6.70 (m, 4H, arom. H), 6.88–6.99 (m, 4H, arom. H), 7.59–7.83 (m, 8H, phthal. arom. H). Anal. Calcd for $C_{36}H_{30}N_4O_6S_2$: C, 63.70; H, 4.45; N, 8.25; S, 9.45. Found: C, 63.61; H, 4.42; N, 8.17; S, 9.54.

5.1.2.12. 2-[2-({{[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)-ethoxy][(4-trifluoromethylphenyl)imino]methyl}dithio)[(4-trifluoromethylphenyl)imino]methyl}oxy)ethyl]-1,3-dioxoisoindoline 12. Mp 189–191 °C; yield: 32% from DCM/ethanol. IR (KBr) cm^{-1}: 1781, 1722, 1641. ^1H NMR ($CDCl_3$) δ: 3.98–4.09 (m, 4H, $2CH_2N$), 4.38–4.49 (m, 4H, $2CH_2O$), 6.89–6.99 (m, 4H, arom. H), 7.42–7.51 (m, 4H, arom. H), 7.76–7.88 (m, 8H, phthal. arom. H). Anal. Calcd for $C_{36}H_{24}F_6N_4O_6S_2$: C, 54.96; H, 3.07; N, 7.12; S, 8.15. Found: C, 54.84; H, 3.16; N, 7.05; S, 8.21.

5.1.2.13. 2-[2-({{[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)-ethoxy][(4-cyanophenyl)imino]methyl}dithio)[(4-cyanophenyl)-imino]methyl}oxy)ethyl]-1,3-dioxoisoindoline 13. Mp 222–224 °C; yield: 20% from DCM/petroleum ether. IR (KBr) cm^{-1}: 2224, 1776, 1709, 1634. ^1H NMR ($CDCl_3$) δ:3.93 (t, J = 5.2 Hz, 4H, $2CH_2N$), 4.33 (t, J = 5.2 Hz, 4H, $2CH_2O$), 6.78–6.90 (m, 4H, arom. H), 7.33–7.43 (m, 4H, arom. H), 7.64–7.78 (m, 8H, phthal. arom. H). ^{13}C NMR ($CDCl_3$) δ: 36.43 ($2CH_2N$), 67.29 ($2CH_2O$), 107.77 (2CN), 119.20 (2C), 122.43 (4CH), 123.57 (4 phthal. CH), 131.97 (4 phthal. C), 133.37 (4CH), 134.45 (4 phthal. CH), 149.85 (2C), 153.90 (2C=N), 167.94 (4C=O). Anal. Calcd for $C_{36}H_{24}N_6O_6S_2$: C, 61.71; H, 3.45; N, 11.99; S, 9.15. Found: C, 62.00; H, 3.45; N, 12.08; S, 9.09.

5.1.2.14. 2-[2-({{[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)-ethoxy][(4-acetylphenyl)imino]methyl}dithio)[(4-acetylphenyl)-imino]methyl}oxy)ethyl]-1,3-dioxoisoindoline 14. Mp 214–216 °C; yield: 54% from DMF/ethanol. IR (KBr) cm^{-1}: 1778, 1710, 1681, 1636. ^1H NMR ($CDCl_3$) δ: 2,58 (s, 6H, $2CH_3$), 3.97–4.10 (m, 4H, $2CH_2N$), 4.37–4.50 (m, 4H, $2CH_2O$), 6.83–6.96 (m, 4H, arom. H), 7.67–7.93 (m, 12H, arom. H). ^{13}C NMR ($CDCl_3$) δ: 26.60 ($2CH_3$), 36.51 ($2CH_2N$), 66.97 ($2CH_2O$), 121.59 (4CH), 123.55 (4 phthal. CH), 129.82 (4CH), 132.01 (4 phthal. C), 133.37 (2C), 134.33 (4 phthal. CH), 150.23 (2C), 153.58 (2C=N), 167.95 (4 phthal. C=O), 197.17 (2C=O). Anal. Calcd for $C_{38}H_{30}N_4O_8S_2$: C, 62.11; H, 4.12; N, 7.62; S, 8.73. Found: C, 62.05; H, 4.43; N, 7.55; S, 8.64.

5.1.2.15. 2-[2-({{[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)-ethoxy][(4-fluorophenyl)imino]methyl}dithio)[(4-fluorophenyl)-imino]methyl}oxy)ethyl]-1,3-dioxoisoindoline 15. Mp 153–155 °C; yield: 26% from DCM/ethanol. IR (KBr) cm^{-1}: 1778, 1711, 1630. ^1H NMR ($CDCl_3$) δ: 3.85–3.96 (m, 4H, $2CH_2N$), 4.24–4.36 (m, 4H, $2CH_2O$), 6.57–6.93 (m, 8H, arom. H), 7.56–7.78 (m, 8H, phthal. arom. H). Anal. Calcd for $C_{34}H_{24}F_2N_4O_6S_2$: C, 59.47; H, 3.52; N, 8.16; S, 9.34. Found: C, 59.76; H, 3.60; N, 8.15; S, 9.30.

5.1.2.16. 2-[2-({{[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)-ethoxy][(4-chlorophenyl)imino]methyl}dithio)[(4-chlorophenyl)-imino]methyl}oxy)ethyl]-1,3-dioxoisoindoline 16. Mp 185–187 °C; yield: 31% from diethyl ether. IR (KBr) cm^{-1}: 1779, 1708, 1632. ^1H NMR ($CDCl_3$) δ: 3.84–3.97 (m, 4H, $2CH_2N$), 4.20–4.35 (m, 4H, $2CH_2O$), 6.61–6.73 (m, 4H, arom. H), 6.97–7.12 (m, 4H, arom. H), 7.56–7.80 (m, 8H, phthal. arom. H). ^{13}C NMR ($CDCl_3$) δ: 36.48 ($2CH_2N$), 66.72 ($2CH_2O$), 122.96 (4CH), 123.53 (4 phthal. CH), 129.23 (4CH), 129.66 (2C), 132.02 (4 phthal. C), 134.30 (4 phthal. CH), 144.32 (2C), 153.90 (2C=N), 167.96 (4C=O). Anal. Calcd for $C_{34}H_{24}Cl_2N_4O_6S_2$: C, 56.75; H, 3.36; N, 7.79; S, 8.91. Found: C, 56.71; H, 3.35; N, 7.81; S, 8.51.

5.1.2.17. 2-[2-({{[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)-ethoxy][(4-bromophenyl)imino]methyl}dithio)[(4-bromophenyl)-imino]methyl}oxy)ethyl]-1,3-dioxoisoindoline 17. Mp 146–148 °C; yield: 35% from acetone/diethyl ether. IR (KBr) cm^{-1}: 1780, 1710, 1632. ^1H NMR ($CDCl_3$) δ: 3.95–4.13 (m, 4H, $2CH_2N$), 4.28–4.49 (m, 4H, $2CH_2O$), 6.65–6.90 (m, 4H, arom. H), 7.25–7.45 (m, 4H, arom. H), 7.67–7.91 (m, 8H, phthal. arom. H). Anal. Calcd for $C_{34}H_{24}Br_2N_4O_6S_2$: C, 50.51; H, 2.99; N, 6.93; S, 7.93. Found: C, 50.55; H, 3.13; N, 6.89; S, 7.86.

5.1.2.18. 2-[2-({{[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)-ethoxy][(4-iodophenyl)imino]methyl}dithio)[(4-iodophenyl)-imino]methyl}oxy)ethyl]-1,3-dioxoisoindoline 18. Mp 133–135 °C; yield: 27% from DCM/petroleum ether. IR (KBr) cm^{-1}: 1782, 1719, 1634. ^1H NMR ($CDCl_3$) δ: 3.95–4.14 (m, 4H, $2CH_2N$), 4.29–4.50 (m, 4H, $2CH_2O$), 6.55–6.74 (m, 4H, arom. H), 7.44–7.63 (m, 4H, arom. H), 7.72–7.95 (m, 8H, phthal. arom. H). Anal. Calcd for $C_{34}H_{24}I_2N_4O_6S_2$: C, 45.25; H, 2.68; N, 6.21; S, 7.11. Found: C, 45.55; H, 2.73; N, 5.95; S, 6.86.

5.1.2.19. 2-[2-({{[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)-ethoxy][(4-nitrophenyl)imino] methyl}dithio)[(4-nitrophenyl)-imino]methyl}oxy)ethyl]-1,3-dioxoisoindoline 19. Mp 239–241 °C; yield: 44% from DCM/methanol. IR (KBr) cm^{-1}: 1777, 1720, 1634, 1504, 1346. ^1H NMR (CF_3COOD) δ: 4.25–4.46 (m, 4H, $2CH_2N$), 5.00–5.19 (m, 4H, $2CH_2O$), 7.46–7.72 (m, 4H, arom. H), 7.86–8.05 (m, 8H, phthal. arom. H), 8.18–8.35 (m, 4H, arom. H). Anal. Calcd for $C_{34}H_{24}N_6O_{10}S_2$: C, 55.13; H, 3.27; N, 11.35; S, 8.66. Found: C, 54.81; H, 3.47; N, 11.35; S, 8.72.

5.1.2.20. 2-[2-({{[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)-ethoxy][(4-methoxyphenyl)imino]methyl}dithio)[(4-methoxyphenyl)-imino]methyl}oxy)ethyl]-1,3-dioxoisoindoline 20. Mp 159–161 °C; yield: 8% from DCM/MeOH. IR (KBr) cm^{-1}: 1778, 1710, 1632. ^1H NMR ($CDCl_3$) δ: 3.79 (s, 6H, $2CH_3$), 3.99–4.19 (m, 4H, $2CH_2N$), 4.30–4.51 (m, 4H, $2CH_2O$), 6.70–6.93 (m, 8 H, arom. H), 7.75–7.93 (m, 8H, phthal. arom. H). Anal. Calcd for $C_{36}H_{30}N_4O_8S_2$: C, 60.83; H, 4.25; N, 7.88; S, 9.02. Found: C, 60.71; H, 4.48; N, 7.65; S, 8.63.

5.1.2.21. 2-[2-({{[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)-ethoxy][(3,5-dimethylphenyl)imino]methyl}dithio)[(3,5-dimethylphenyl)imino]methyl}oxy)ethyl]-1,3-dioxoisoindoline 21. Mp 144–146 °C; yield: 39% from DCM/ethanol. IR (KBr) cm^{-1}: 1779, 1722, 1643. ^1H NMR ($CDCl_3$) δ: 2.14 (s, 12H, $4CH_3$), 3.90–4.01 (m, 4H, $2CH_2N$), 4.31–4.43 (m, 4H, $2CH_2O$), 6.30–6.36 (m, 4H, arom. H-2 and H-6), 6.57–6.64 (m, 2H, arom. H-4), 7.58–7.83 (m, 8H, phthal. arom. H). Anal. Calcd for $C_{38}H_{34}N_4O_6S_2$: C, 64.57; H, 4.85; N, 7.93; S, 9.07. Found: C, 64.69; H, 5.07; N, 7.75; S, 8.97.

5.1.2.22. 2-[2-({{[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)-ethoxy][(2,4-difluorophenyl)imino]methyl}dithio)[(2,4-difluorophenyl)imino]methyl}oxy)ethyl]-1,3-dioxoisoindoline 22. Mp 155–157 °C; yield: 39% from diethyl ether/methanol. IR (KBr) cm^{-1}: 1775, 1722, 1639. ^1H NMR ($CDCl_3$) δ: 3.88–4.01 (m, 4H, $2CH_2N$), 4.33–4.43 (m, 4H, $2CH_2O$), 6.51–6.92 (m, 6H, arom. H), 7.58–7.83 (m, 8H, phthal. arom. H). Anal. Calcd for $C_{34}H_{22}F_4N_4O_6S_2$: C, 56.51; H, 3.07; N, 7.75; S, 8.87. Found: C, 56.60; H, 3.08; N, 7.71; S, 8.90.

5.1.2.23. 2-[2-({{[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)-ethoxy][(2,5-difluorophenyl)imino]methyl}dithio)[(2,5-difluorophenyl)imino]methyl}oxy)ethyl]-1,3-dioxoisoindoline 23. Mp 123–125 °C; yield: 26% from diethyl ether. IR (KBr) cm^{-1}: 1780, 1721, 1644. ^1H NMR ($CDCl_3$) δ: 3.97 (t, J = 5.0 Hz, 4H, $2CH_2N$), 4.42 (t, J = 5.0 Hz, 4H, $2CH_2O$), 6.53–6.88 (m, 6H, arom. H), 7.60–7.83 (m, 8H, phthal. arom. H). Anal. Calcd for $C_{34}H_{22}F_4N_4O_6S_2$: C, 56.51; H, 3.07; N, 7.75; S, 8.87. Found: C, 56.45; H, 3.38; N, 7.64; S, 8.69.

5.1.2.24. 2-[2-({({[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)-ethoxy][(3,5-difluorophenyl) imino]methyl}dithio)[(3,5-difluorophenyl)imino]methyl}oxy)ethyl]-1,3-dioxoisoindoline 24. Mp 145–147 °C; yield: 36% from diethyl ether. IR (KBr) cm^{-1}: 1781, 1717, 1647. ^1H NMR ($CDCl_3$) δ: 3.87–3.97 (m, 4H, $2CH_2N$), 4.28–4.39 (m, 4H, $2CH_2O$), 4.15–4.49 (m, 6H, arom. H), 7.58–7.82 (m, 8H, phthal. arom. H). Anal. Calcd for $C_{34}H_{22}F_4N_4O_6S_2$: C, 56.51; H, 3.07; N, 7.75; S, 8.87. Found: C, 56.76; H, 3.42; N, 7.36; S, 8.88.

5.1.2.25. 2-[2-({({[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)-ethoxy][(2,3-dichlorophenyl)imino]methyl}dithio)[(2,3-dichlorophenyl)imino]methyl}oxy)ethyl]-1,3-dioxoisoindoline 25. Mp 134–135 °C; yield: 59% from diethyl ether/ethanol. IR (KBr) cm^{-1}: 1776, 1721, 1639. ^1H NMR ($CDCl_3$) δ: 3.88–4.05 (m, 4H, $2CH_2N$), 4.33–4.51 (m, 4H, $2CH_2O$), 6.71–7.08 (m, 6H, arom. H), 7.53–7.88 (m, 8H, phthal. arom. H). Anal. Calcd for $C_{34}H_{22}Cl_4N_4O_6S_2$: C, 51.79; H, 2.81; N, 7.11; S, 8.13. Found: C, 51.79; H, 3.18; N, 6.94; S, 8.27.

5.1.2.26. 2-[2-({({[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)-ethoxy][(2,4-dichlorophenyl)imino]methyl}dithio)[(2,4-dichlorophenyl)imino]methyl}oxy)ethyl]-1,3-dioxoisoindoline 26. Mp 130–132 °C; yield: 55% from diethyl ether. IR (KBr) cm^{-1}: 1775, 1718, 1643. ^1H NMR ($CDCl_3$) δ: 3.92–4.08 (m, 4H, $2CH_2N$), 4.36–4.47 (m, 4H, $2CH_2O$), 6.78–7.11 (m, 6H, arom. H), 7.60–7.82 (m, 8H, phthal. arom. H). Anal. Calcd for $C_{34}H_{22}Cl_4N_4O_6S_2$: C, 51.79; H, 2.81; N, 7.11; S, 8.13. Found: C, 51.66; H, 3.14; N, 7.00; S, 8.10.

5.1.2.27. 2-[2-({({[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)-ethoxy][(2,5-dichlorophenyl)imino]methyl}dithio)[(2,5-dichlorophenyl)imino]methyl}oxy)ethyl]-1,3-dioxoisoindoline 27. Mp 173–175 °C; yield: 49% from DCM/ethanol. IR (KBr) cm^{-1}: 1776, 1715, 1639. ^1H NMR ($CDCl_3$) δ: 4.01–4.25 (m, 4H, $2CH_2N$), 4.47–4.67 (m, 4H, $2CH_2O$), 6.91–7.28 (m, 6H, arom. H), 7.70–7.91 (m, 8H, phthal. arom. H). Anal. Calcd for $C_{34}H_{22}Cl_4N_4O_6S_2$: C, 51.79; H, 2.81; N, 7.11; S, 8.13. Found: C, 51.85; H, 3.15; N, 6.80; S, 8.11.

5.1.2.28. 2-[2-({({[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)-ethoxy][(3,4-dichlorophenyl)imino]methyl}dithio)[(3,4-dichlorophenyl)imino]methyl}oxy)ethyl]-1,3-dioxoisoindoline 28. Mp 195–197 °C; yield: 57% from DCM/ethanol. IR (KBr) cm^{-1}: 1781, 1721, 1634. ^1H NMR ($CDCl_3$) δ: 3.96–4.08 (m, 4H, $2CH_2N$), 4.38–4.50 (m, 4H, $2CH_2O$), 6.73 (dd, J = 8.6 Hz, J = 2.4 Hz, 2H, arom. H-6), 6.97 (d, J = 2.4 Hz, 2H, arom. H-2), 7.26 (d, J = 8.4 Hz, 2H, arom. H-5), 7.70–7.93 (m, 8H, phthal. arom. H). Anal. Calcd for $C_{34}H_{22}Cl_4N_4O_6S_2$: C, 51.79; H, 2.81; N, 7.11; S, 8.13. Found: C, 51.57; H, 2.99; N, 7.15; S, 8.12.

5.1.2.29. 2-[2-({({[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)-ethoxy][(3,5-dichlorophenyl)imino]methyl}dithio)[(3,5-dichlorophenyl)imino]methyl}oxy)ethyl]-1,3-dioxoisoindoline 29. Mp 184–186 °C; yield: 72% from diethyl ether. IR (KBr) cm^{-1}: 1783, 1719, 1650. ^1H NMR ($CDCl_3$) δ: 3.90–3.98 (m, 4H, $2CH_2N$), 4.35–4.44 (m, 4H, $2CH_2O$), 6.64–6.70 (m, 4H, arom. H-2 and H-6), 6.94–6.98 (m, 2H, arom. H-4), 7.61–7.83 (m, 8H, phthal. arom. H). Anal. Calcd for $C_{34}H_{22}Cl_4N_4O_6S_2$: C, 51.79; H, 2.81; N, 7.11; S, 8.13. Found: C, 51.51; H, 3.18; N, 7.05; S, 8.09.

5.1.2.30. 2-[2-({({[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)-ethoxy][(3-chloro-4-methylphenyl)imino]methyl}dithio)[(4-methyl-3-chlorophenyl)imino]methyl}oxy)ethyl]-1,3-dioxoisoindoline 30. Mp 123–125 °C; yield: 15% from petroleum ether/methanol. IR (KBr) cm^{-1}: 1777, 1710, 1635. ^1H NMR ($CDCl_3$) δ: 2.32 (s, 6H, $2CH_3$), 3.99–4.18 (m, 4H, $2CH_2N$), 4.35–4.58 (m, 4H, $2CH_2O$), 6.68–7.22 (m, 6H, arom. H), 7.73–7.96 (m, 8H, phthal. arom. H). Anal. Calcd for $C_{36}H_{28}Cl_2N_4O_6S_2$: C, 57.83; H, 3.77; N, 7.49; S, 8.58. Found: C, 58.02; H, 4.02; N, 7.32; S, 8.57.

5.1.2.31. 2-[2-({({[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)-ethoxy][(4-chloro-3-trifluoromethylphenyl)imino]methyl}dithio)-[(4-chloro-3-trifluoromethylphenyl)imino]methyl}oxy)ethyl]-1,3-dioxoisoindoline 31. Mp 163–164 °C; yield: 54% from diethyl ether/ethanol. IR (KBr) cm^{-1}: 1777, 1711, 1640. ^1H NMR ($CDCl_3$) δ: 3.91 (t, J = 5.0 Hz, 4H, $2CH_2N$), 4.33 (t, J = 5.0 Hz, 4H, $2CH_2O$), 6.87 (dd, J = 8.4 Hz, J = 2.4 Hz, 2H, arom. H-6), 7.09 (d, J = 2.4 Hz, 2H, arom. H-2), 7.21 (d, J = 8.4 Hz, 2H, arom. H-5), 7.55–7.77 (m, 8H, phthal. arom. H). Anal. Calcd for $C_{36}H_{22}F_6Cl_2N_4O_6S_2$: C, 50.54; H, 2.59; N, 6.55; S, 7.49. Found: C, 50.43; H, 2.69; N, 6.54; S, 7.30.

5.1.2.32. 2-[2-({({[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)-ethoxy][(4-chloro-3-nitrophenyl)imino]methyl}dithio)[(4-chloro-3-nitrophenyl)imino]methyl}oxy)ethyl]-1,3-dioxoisoindoline 32. Mp 169–170 °C; yield: 57% from DCM. IR (KBr) cm^{-1}: 1771, 1724, 1634. ^1H NMR ($CDCl_3$) δ: 3.87–3.98 (m, 4H, $2CH_2N$), 4.33–4.43 (m, 4H, $2CH_2O$), 6.97 (dd, J = 8.6 Hz, J = 2.4 Hz, 2H, arom. H-6), 7.24–7.37 (m, 4H, arom. H-2 and H-5), 7.62–7.79 (m, 8H, phthal. arom. H). Anal. Calcd for $C_{34}H_{22}Cl_2N_6O_{10}S_2$: C, 50.54; H, 2.74; N, 10.38; S, 7.92. Found: C, 50.41; H, 2.90; N, 10.20; S, 8.20.

5.1.2.33. 2-[2-({({[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)-ethoxy][(4-bromo-2-methylphenyl)imino]methyl}dithio)[(4-bromo-2-methylphenyl)imino]methyl}oxy)ethyl]-1,3-dioxoisoindoline 33. Mp 125–127 °C; yield: 57% from diethyl ether. IR (KBr) cm^{-1}: 1771, 1708, 1634. ^1H NMR ($CDCl_3$) δ: 1.91 (s, 6H, $2CH_3$), 3.91–4.02 (m, 4H, $2CH_2N$), 4.33–4.43 (m, 4H, $2CH_2O$), 6.50–6.58 (m, 2H, arom. H), 6.95–7.04 (m, 4H, arom. H), 7.61–7.78 (m, 8H, phthal. arom. H). Anal. Calcd for $C_{36}H_{28}Br_2N_4O_6S_2$: C, 51.69; H, 3.37; N, 6.70; S, 7.67. Found: C, 51.94; H, 3.40; N, 6.31; S, 7.60.

5.1.2.34. 2-[2-({({[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)-ethoxy][(2,4,6-trifluorophenyl)imino]methyl}dithio)[(2,4,6-trifluorophenyl)imino]methyl}oxy)ethyl]-1,3-dioxoisoindoline 34. Mp 167–169 °C; yield: 30% from diethyl ether. IR (KBr) cm^{-1}: 1772, 1714, 1639. ^1H NMR ($CDCl_3$) δ: 3.97–4.08 (m, 4H, $2CH_2N$), 4.53–4.63 (m, 4H, $2CH_2O$), 6.65–6.77 (m, 4H, arom. H), 7.63–7.84 (m, 8H, phthal. arom. H). Anal. Calcd for $C_{34}H_{20}F_6N_4O_6S_2$: C, 53.83; H, 2.66; N, 7.38; S, 8.45. Found: C, 53.63; H, 3.01; N, 7.07; S, 8.80.

5.1.2.35. 2-[2-({({[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)-ethoxy][(4-bromo-2,6-dimethylphenyl)imino]methyl}dithio)-[(4-bromo-2,6-dimethylphenyl)imino]methyl}oxy)ethyl]-1,3-dioxoisoindoline 35. Mp 185–187 °C; yield: 49% from ethyl acetate. IR (KBr) cm^{-1}: 1777, 1713, 1638. ^1H NMR ($CDCl_3$) δ: 1.86 (s, 12H, $4CH_3$), 3.97–4.08 (m, 4H, $2CH_2N$), 4.55–4.65 (m, 4H, $2CH_2O$), 6.82 (br s, 4H, arom. H-3 and H-5), 7.69–7.84 (m, 8H, phthal. arom. H). Anal. Calcd for $C_{38}H_{32}Br_2N_4O_6S_2$: C, 52.79; H, 3.73; N, 6.48; S, 7.42. Found: C, 52.49; H, 3.76; N, 6.55; S, 7.24.

5.1.2.36. 2-[2-({({[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)-ethoxy][(1-naphthyl)imino]methyl}dithio)[(1-naphthyl)imino]-methyl}oxy)ethyl]-1,3-dioxoisoindoline 36. Mp 179–180 °C; yield: 49% from DCM/methanol. IR (KBr) cm^{-1}: 1776, 1719, 1625. ^1H NMR ($CDCl_3$) δ: 3.95–4.13 (m, 4H, $2CH_2N$), 4.46–4.63 (m, 4H, $2CH_2O$), 6.76–6.85 (m, 2H, arom. H), 7.09–7.90 (m, 20H, arom. H). Anal. Calcd for $C_{42}H_{30}N_4O_6S_2$: C, 67.19; H, 4.03; N, 7.46; S, 8.54. Found: C, 66.95; H, 4.12; N, 7.31; S, 8.94.

5.1.2.37. 2-(2-{[({[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)-ethoxy](cyclopropylimino)methyl}dithio)(cyclopropylimino)-methyl]oxy}ethyl)-1,3-dioxoisoindoline 37. Mp 164–165 °C; yield: 34% from diethyl ether/methanol. IR (KBr) cm^{-1}: 1780, 1714, 1629. ^1H NMR ($CDCl_3$) δ: 0.28–0.58 (m, 8H, 4 cycloprop. CH_2), 2.50–2.63 (m, 2H, 2 cycloprop. CH), 3.81 (t, J = 5.4 Hz, 4H, $2CH_2N$), 4.10 (t, J = 5.4 Hz, 4H, $2CH_2O$), 7.55–7.81 (m, 8H, phthal. arom. H). ^{13}C NMR ($CDCl_3$) δ: 7.82 (4 cycloprop. CH_2), 30.74 (2 cycloprop. CH), 36.74 ($2CH_2N$), 65.16 ($2CH_2O$), 123.42 (4CH), 132.21 (4C), 134.00 (4CH), 153.09 (2C=N), 167.99 (4C=O). Anal. Calcd for $C_{28}H_{26}N_4O_6S_2$: C, 58.12; H, 4.53; N, 9.68; S, 11.08. Found: C, 58.19; H, 4.63; N, 9.31; S, 10.96.

5.1.2.38. 2-(2-{[({[2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)-ethoxy](benzylimino)methyl}dithio) (benzylimino)methyl]oxy}-ethyl)-1,3-dioxoisoindoline 38. Mp 113–115 °C; yield: 41% from diethyl ether. IR (KBr) cm^{-1}: 1782, 1714, 1633. ^1H NMR ($CDCl_3$) δ: 3.89–4.03 (m, 4H, $2CH_2N$), 4.31–4.48 (m, 4H, $2CH_2O$), 4.60–4.71 (m, 4H, $2CH_2Bn$), 6.95–7.28 (m, 10H, arom. H), 7.57–7.81 (m, 8H, phthal. arom. H). Anal. Calcd for $C_{36}H_{30}N_4O_6S_2$: C, 63.70; H, 4.45; N, 8.25; S, 9.45. Found: C, 63.35; H, 4.53; N, 8.17; S, 9.21.

5.1.2.39. 2-[3-({({[3-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)-propoxy][(4-bromophenyl)imino]methyl}dithio)[(4-bromophenyl)-imino]methyl}oxy)propyl]-1,3-dioxoisoindoline 39. Mp 193–195 °C; yield: 57% from diethyl ether/ethanol. IR (KBr) cm^{-1}: 1780, 1727, 1637. ^1H NMR ($CDCl_3$) δ: 2.13–2.33 (m, 4H, $2CH_2$), 3.81–3.99 (m, 4H, $2CH_2N$), 4.38–4.57 (m, 4H, $2CH_2O$), 6.73–6.92 (m, 4H, arom. H), 7.36–7.55 (m, 4H, arom. H), 7.68–7.92 (m, 8H, phthal. arom. H). Anal. Calcd for $C_{36}H_{28}Br_2N_4O_6S_2$: C, 51.69; H, 3.37; N, 6.70; S, 7.66. Found: C, 51.63; H, 3.65; N, 6.62; S, 7.33.

5.1.2.40. 2-[2-({({[2-(1,3-Dioxo-1,3-dihydro-5-methyl-2*H*-isoindol-2-yl)ethoxy][(4-bromophenyl)imino]methyl}dithio)[(4-bromophenyl)imino]methyl}oxy)ethyl]-1,3-dioxo-5-methyl-isoindoline 40. Mp 193–195 °C; yield: 94% from DCM/ethanol. IR (KBr) cm^{-1}: 1781, 1724, 1638. ^1H NMR ($CDCl_3$) δ: 2.54 (s, 6H, $2CH_3$), 3.97–4.05 (m, 4H, $2CH_2N$), 4.37–4.45 (m, 4H, $2CH_2O$), 6.68–6.78 (m, 4H, arom. H), 7.25–7.36 (m, 4H, arom. H), 7.51–7.77 (m, 6H, phthal. arom. H). Anal. Calcd for $C_{36}H_{28}Br_2N_4O_6S_2$: C, 51.69; H, 3.37; N, 6.70; S, 7.66. Found: C, 51.73; H, 3.52; N, 6.68; S, 7.65.

5.1.2.41. {[(2-Phenylethoxy){[(2-phenylethoxy)(phenylimino)-methyl]dithio}methylene]amino}benzene 41. Mp 60–62 °C; yield: 23% from acetone/methanol. IR (KBr) cm^{-1}: 1629. ^1H NMR ($CDCl_3$) δ: 2.98 (t, J = 6.6 Hz,, 4H, $2CH_2Ph$), 4.44 (m, 4H, $2CH_2O$), 6.75–6.86 (m, 4H, arom. H), 7.02–7.12 (m, 4H, arom. H), 7.17–7.34 (m, 12H, arom. H). ^{13}C NMR ($CDCl_3$) δ: 35.28 ($2CH_2$), 70.70 ($2CH_2O$), 121.89 (4CH), 124.57 (2CH), 126.81 (2CH), 128.78 (4CH), 129.31 (4CH), 129.43 (4CH), 138.28 (2C), 146.27 (2C), 153.98 (2C=N). Anal. Calcd for $C_{30}H_{28}N_2O_2S_2$: C, 70.28; H, 5.50; N, 5.46; S, 12.51. Found: C, 70.34; H, 5.69; N, 5.42; S, 12.48.

5.2. Virology: materials and methods

The biological evaluation of DS **1**–**41** was performed according to previously reported procedures.[15,16]

5.3. Computational analysis

[(Methoxy{[methoxy(phenylimino)methyl]dithio}methylene)amino]benzene (i.e., the simplified derivative of **1**, with the two 2-phthalimidoethyl substituents replaced by two methyl groups) has been selected as template molecule for the study of formimidoester disulfides stereochemistry. The structure of the reference molecule (*Z*,*Z* isomer) was generated and energy minimized using the MM2 force field included in MacroModel.[37] A Drive analysis (Macromodel) was performed on the torsion angles defined by atoms S–C=N–C(Ar). The following parameters were set: starting angle: 0; angle increment: 30°; total rotation: 360°. The *E*,*E* and *Z*,*E* isomers obtained by the Drive analysis were superimposed to the original *Z*,*Z* isomer to assess that the conformation of the S–S bond was retained. For energy comparison, the three isomers were energy minimized using the Hamiltonian AM1 as implemented in MOPAC package version 6.0. All the calculations were performed in vacuo on Silicon Graphic O2 workstation. Predicted isomer energies: *Z*,*Z* (77.29 kcal/mol); *E*,*E* (78.29 kcal/mol); *E*,*Z* (79.91 kcal/mol).

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