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Design, Synthesis, and Biological Activity of Novel 5-((Arylfuran/1*H*-pyrrol-2-yl)methylene)-2-thioxo-3-(3-(trifluoromethyl)phenyl)thiazolidin-4-ones as HIV-1 Fusion Inhibitors Targeting gp41

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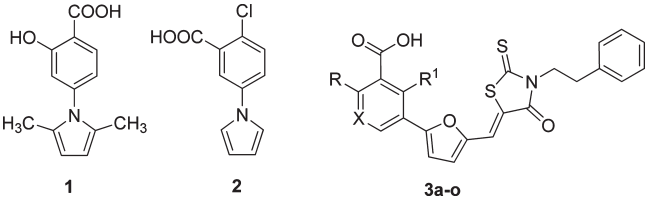
On the basis of our earlier molecular docking analysis, we designed and synthesized 5-((arylfuran/1*H*-pyrrol-2-yl)methylene)-2-thioxo-3-(3-(trifluoromethyl)phenyl)thiazolidin-4-ones (**12a**−**o**) as HIV-1 entry inhibitors. Compounds **12a**−**o** effectively inhibited infection by both laboratory-adapted and primary HIV-1 strains and blocked HIV-1 mediated cell−cell fusion and gp41 six-helix bundle formation. Molecular docking analyses on two highly active inhibitors, **12b**, containing a carboxylic acid group, and **12m**, containing a tetrazole group, indicated that they both fit snugly into the hydrophobic cavity of HIV-1 gp41 from which each has important ionic interactions with lysine 574 (K574). By contrast, molecular docking of **12i**, a less active compound containing a pyrrole instead of a furan ring, indicated a completely different orientation from **12b** and **12m** and missed critical interactions.

Introduction

Multidrug resistance has emerged for many antiretroviral drugs in current use, especially the reverse transcriptase inhibitors (RTIs[a]) and protease inhibitors (PIs). Thus patients with HIV infection/AIDS have increasingly failed to respond to the RTI- and PI-containing anti-HIV regimen, such as the highly active antiretroviral therapy (HAART).[1−3] Therefore, it is essential to develop novel anti-HIV therapeutics targeting different steps of the HIV replication cycle, particularly the HIV fusion and entry events.

The fusion and entry of HIV type 1 (HIV-1) are mediated by the viral envelope glycoprotein (Env) surface subunit gp120 and the transmembrane subunit gp41. The binding of gp120 to the cellular receptor CD4 and to CXCR4 or CCR5 as co-receptor triggers conformation changes in gp41, which result in formation of a six-helix bundle (6-HB) core and promote membrane fusion.[4−6] Three parallel N-terminal heptad repeat (NHR) units often form an inner trimeric coiled-coil, and three C-terminal heptad repeat (CHR) units then pack in an antiparallel manner into the hydrophobic grooves conserved on the central trimeric core.[7−10] A deep hydrophobic cavity in the groove significantly stabilizes the 6-HB and serves as an attractive target for development of small molecule HIV fusion/entry inhibitors.[11]

We previously identified *N*-(4-carboxy-3-hydroxy)phenyl-2,5-dimethylpyrrole **1** (NB-2, MW = 231) and *N*-(3-carboxy-4-chloro)phenylpyrrole **2** (NB-64, MW = 222) as small molecules possessing low micromolar inhibitory activity against HIV-1 infection, HIV-1 Env-mediated cell−cell fusion, and gp41 six-helix bundle formation.[12] Molecular docking analysis showed that both **1** and **2** may bind to the deep hydrophobic cavity on the gp41 NHR-trimer but only fill a part of the space in the cavity.[12] Later, we designed and synthesized a series of 2-aryl-5-(4-oxo-3-phenethyl-2-thioxothiazolidinylidenemethyl)furans **3a**−**o** with molecular weights of ∼500 Da exhibiting anti-HIV-1 activity more potent than **1** and **2**, suggesting that the increased molecular bulk may occupy more space in the hydrophobic cavity, resulting in stronger binding affinity and higher inhibitory activity on 6-HB formation.[13]



1 **2** **3a-o**

In the present study, we have now synthesized new 5-((arylfuran/1*H*-pyrrol-2-yl)methylene)-2-thioxo-3-(3-(trifluoromethyl)phenyl)thiazolidin-4-ones (**12a**−**o**), modifying chemical structures **3a**−**o** by deleting the CH_2CH_2 side chain linker and also in some cases changing the carboxyl group for a tetrazolyl unit and/or the furan ring for pyrrole. Most of the compounds (**12a**−**o**) exhibited improved anti-HIV-1 activity, and two (**12l** and **12m**) showed inhibitory activity against HIV-1_{IIIB} at low nanomolar level and selectivity indexes (SI values) of >2000. These results suggest that

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[a] Abbreviations: CHR, C-terminal heptad repeat; Env, envelope glycoprotein; NHR, N-terminal heptad repeat; XTT, sodium 30-[1-(phenylamino)-carbonyl]-3,4-tetrazolium-bis(4-methoxy-6-nitro)bezenesulfonic acid hydrate; RTI, reverse transcriptase inhibitor; PI, protease inhibitors; mAb, monoclonal antibody; ELISA, enzyme-linked immunosorbent assay; EtOH-DME, ethyl alcohol−ethylene glycol dimethyl ether; 6-HB, six-helix bundle; THF, tetrahydrofuran.

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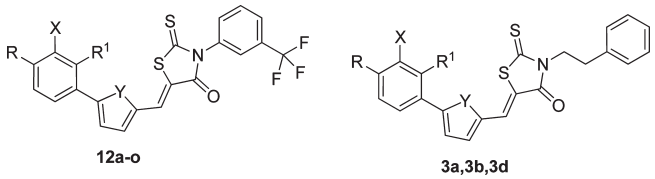
Table 1. Anti-HIV-1 IIIB Activity, Cytotoxicity and Selectivity Indexes of the 5-((Arylfuran/1*H*-pyrrol-2-yl)methylene)-2-thioxo-3-(3-(trifluoromethyl)phenyl)thiazolidin-4-ones **12a**−**o**[a] along with **3a**,**b**,**d**



product	X	Y	R	R^1	MW	EC_{50} (μM)	CC_{50} (μM)	SI[b]
12a	COOH	O	H	H	475.0	0.021 ± 0.003	4.05 ± 0.16	193
12b	COOH	O	H	F	502.5	0.064 ± 0.014	30.96 ± 7.12	484
12c	COOH	O	OH	H	567.9	0.082 ± 0.010	48.75 ± 6.74	550
12d	COOH	O	H	OH	527.5	0.033 ± 0.009	27.60 ± 0.74	836
12e	COOH	O	H	OMe	505.5	0.089 ± 0.040	24.32 ± 4.29	273
12f	COOH	NH	H	H	474.5	1.179 ± 0.101	30.35 ± 2.27	28
12g	COOH	NH	Cl	H	526.9	1.674 ± 0.182	51.92 ± 4.31	31
12h	COOH	NH	F	H	492.5	3.941 ± 0.496	15.67 ± 3.12	4
12i	COOH	NH	H	F	492.5	0.615 ± 0.134	29.81 ± 1.45	48
12j	COOH	NH	H	OMe	505.5	2.498 ± 0.289	42.18 ± 6.30	17
12k	tetrazolyl	O	H	H	517.5	0.037 ± 0.004	7.75 ± 1.26	209
12l	tetrazolyl	O	Cl	H	533.9	0.018 ± 0.002	66.34 ± 11.46	3686
12m	tetrazolyl	O	H	F	517.5	0.014 ± 0.005	27.85 ± 3.79	1989
12n	tetrazolyl	O	F	H	517.5	0.033 ± 0.006	26.88 ± 5.29	815
12o	tetrazolyl	O	OH	H	524.5	0.118 ± 0.008	35.65 ± 6.49	302
3a	COOH	O	H	Cl	435.5	0.074 ± 0.005	30.40 ± 1.95	411
3b	COOH	O	H	F	449.6	0.145 ± 0.021	28.12 ± 3.55	194
3d	COOH	O	H	OH	470.0	0.061 ± 0.003	16.38 ± 4.90	269

[a] Each compound was tested in triplicate; the data are presented as the mean ± SD. [b] SI was calculated on the basis of the CC_{50} for MT-2 cells and EC_{50} for inhibiting infection of $HIV\text{-}1_{IIIB}$.

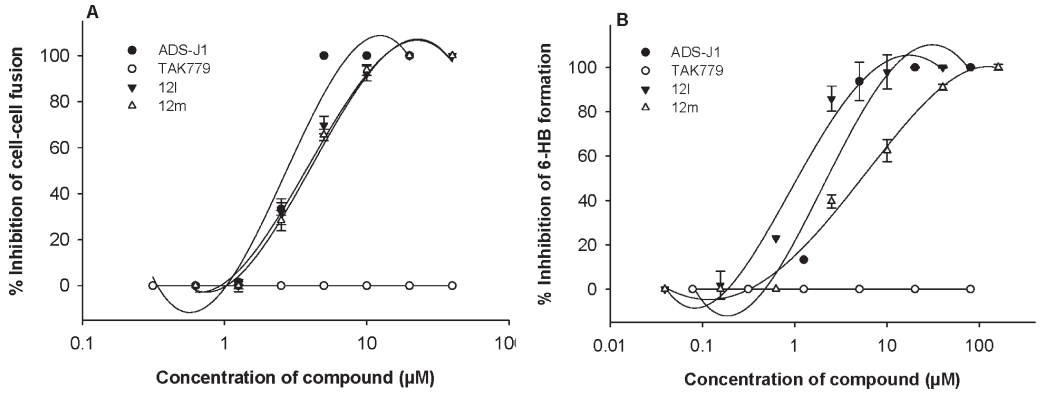
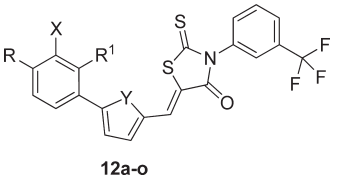


Figure 1. Inhibitory activity of **12l**, **12m**, **13**, and **14** on HIV-1-mediated cell−cell fusion and the gp41 6-HB formation.

compounds **12l** and **12m** could serve as leads for further development of small molecule HIV fusion/entry inhibitors.



Results and Discussion

5-((Arylfuran/1*H*-pyrrol-2-yl)methylene)-2-thioxo-3-(3-(trifluoromethyl)phenyl)thiazolidin-4-ones (**12a**−**o**) with molecular weights of 475−568 Da (Table 1) were synthesized by Suzuki−Miyaura cross couplings and subsequent Knoevenagel condensations. The inhibitory activity of **12a**−**o** on $HIV\text{-}1_{IIIB}$ replication in MT-2 cells was assessed using an enzyme-linked immunosorbent assay (ELISA) for p24 measurement as previously described.[14] All 15 compounds (**12a**−**o**) significantly inhibited HIV-1 replication in a dose-dependent manner with the EC_{50} (effective concentration for 50% inhibition) ranging from 0.014 to 2.5 μM. We tested the cytotoxicity of these compounds on MT-2 cells using a colorimetric XTT assay as previously described.[14] Their CC_{50} (concentration causing 50% cytotoxicity) values ranged from 4 to 66 μM, and their selectivity indexes ranged from 4 to 3686 (Table 1). Two of the best compounds against $HIV\text{-}1_{IIIB}$ are **12l** and **12m**, which had EC_{50} of 18 and 14 nM and SI of 3686 and 1989, respectively. The three best compounds that we previously identified,[13] **3a**, **3b**, and **3d** had EC_{50} of 74, 145, and 61 nM and SI of 411, 194, and 269, respectively (Table 1).

We tested the inhibitory activity of **12a**−**o** on infection by primary HIV-1 isolate, 92US657 (clade B, R5), since clade B is the most common subtype in the United States. All 15 compounds (**12a**−**o**) tested in the present study and the three

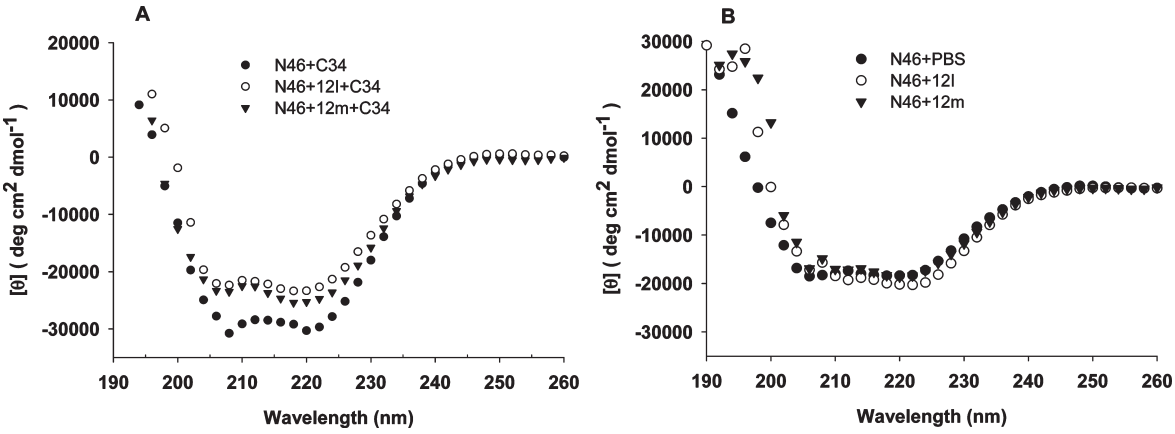
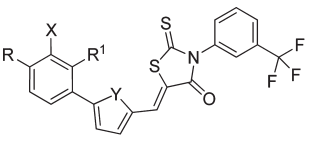


Figure 2. Effect of **12l** and **12m** on the secondary structures of the HIV-1 gp41 6-HB formed by N46 and C34 (A) and the NHR-trimeric coiled coil structure formed by N46 (B).

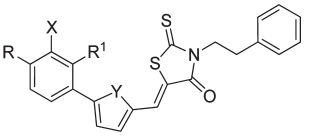
compounds (**3a**, **3b**, and **3d**) identified from our previous study consistently inhibited infection of the HIV-1 92US657 isolate with EC_{50} ranging from 0.3 to 8 μM, demonstrating that 5-((arylfuran/1*H*-pyrrol-2-yl)methylene)-2-thioxo-3-(3-(trifluoromethyl)phenyl)thiazolidin-4-ones **12a**−**o** are effective against both laboratory-adapted and primary HIV-1 strains.

We evaluated the inhibitory activity of **12l** and **12m** on the HIV-1-mediated cell−cell fusion and the gp41 6-HB formation using a small molecule HIV-1 fusion inhibitor 7-[6-phenylamino-4-[4-[(3,5-disulfo-8-hydroxynaphthyl)azo]-2-methoxy-5-methylphenylamino]-1,3,5-triazin-2-yl]-4-hydroxy-3-[(2-methoxy-5-sulfophenyl)azo]-2-naphthalenesulfonic acid (ADS-J1, **13**), targeting gp41 and interfering with the 6-HB core formation,[14,18] and *N*,*N*-dimethyl-*N*-[4-[[[2-(4-methylphenyl)-6,7-dihydro-5*H*-benzocyclohepten-8-yl]carbonyl]amino]benzyl]tetrahydro-2*H*-pyran-4-aminium chloride (TAK-779, **14**), which is a CCR5 antagonist,[19] as a positive and negative control, respectively. Figure 1 shows that **13** was effective in blocking the fusion between the H9 cells infected by HIV-1 IIIB (X4 virus) with the uninfected target (MT-2) cells and also inhibits gp41 6-HB core formation between the gp41 NHR-peptide N36 and CHR-peptide C34 as measured by ELISA using a 6-HB-spepcific mAb NC-1,[20] whereas **14** exhibits no inhibition in both assays. Like **13**, compounds **12l** and **12m** significantly inhibit HIV-1-mediated cell−cell fusion with EC_{50} of 3.93 ± 0.06 and 3.95 ± 0.14 μM, respectively. Similarly, **12l** and **12m** also inhibited the gp41 6-HB core formation in a dose-dependent manner with EC_{50} of 1.28 ± 0.39 and 4.38 ± 0.39 μM, respectively. As detected by CD spectroscopy, the mixture of the N46 and C34 peptides exhibited typical helical structure with α-helicity of 90%. However, in the presence of the **12l** (2 μM) or **12m** (2 μM), the α-helicity of the N46/C34 complex was reduced to 69% and 75%, respectively (Figure 2A), further confirming that **12l** and **12m** are able to disrupt the gp41 6-HB core formation. At the same time, we also investigated whether or not the active compounds **12l** and **12m** could distort the gp41 NHR-trimeric coiled coil using an NHR-peptide N46, which was shown to form automatically trimeric coiled coil structure in physiological solution.[21] Consistently, N46 peptide alone in PBS formed typical coiled coil structure with α-helicity of 52%. Addition of **12l** or **12m** to N46 did not significantly affect the CD spectra of N46 (Figure 2B). These results suggest that these compounds inhibited HIV-1 fusion by targeting the HIV-1 gp41 and blocking gp41 6-HB core formation but having no disrupting effect on the NHR-trimeric coiled coil structure.

Table 2. Inhibitory Activity of the 5-((Arylfuran/1*H*-pyrrol-2-yl)-methylene)-2-thioxo-3-(3-(trifluoromethyl)phenyl)thiazolidin-4-ones **12a**−**o**[a] along with **3a**,**b**,**d** on Infection by a Primary HIV-1 Isolate, 92US657 (R5, Clade B)



12a-o



3a,3b,3d

product	X	Y	R	R^1	EC_{50} (μM)
12a	COOH	O	H	H	2.38 ± 0.28
12b	COOH	O	H	F	2.17 ± 0.23
12c	COOH	O	OH	H	8.06 ± 2.94
12d	COOH	O	H	OH	5.89 ± 0.26
12e	COOH	O	H	OMe	4.16 ± 0.13
12f	COOH	NH	H	H	20.83 ± 3.24
12g	COOH	NH	Cl	H	4.71 ± 0.09
12h	COOH	NH	F	H	2.78 ± 0.57
12i	COOH	NH	H	F	3.43 ± 0.00
12j	COOH	NH	H	OMe	2.94 ± 0.47
12k	tetrazolyl	O	H	H	1.50 ± 0.42
12l	tetrazolyl	O	Cl	H	0.32 ± 0.06
12m	tetrazolyl	O	H	F	0.99 ± 0.23
12n	tetrazolyl	O	F	H	0.87 ± 0.35
12o	tetrazolyl	O	OH	H	0.32 ± 0.02
3a	COOH	O	H	Cl	0.521 ± 0.039
3b	COOH	O	H	F	1.075 ± 0.111
3d	COOH	O	H	OH	0.477 ± 0.050

[a] Each compound was tested in triplicate, and the data are presented as the mean ± SD.

It is evident from the results that the introduction of tetrazolyl moiety as a replacement isostere of carboxylic acid may not have substantially improved their anti-HIV-1 activity but resulted in significant reduction of cytotoxicity, thereby increasing their selectivity indexes. Notably, introduction of a pyrrole ring in place of furan reduced the activity substantially (**12g**−**j**). Structure−activity relationship (SAR) analysis indicates that all the furans (**12a**−**e** and **12k**−**o**) are more potent (about 40-fold) than the pyrroles (**12f**−**i**) against HIV-1 IIIB infection (Table 1), suggesting that oxygen (O) is preferred over the amino (NH) group at the Y position. Interestingly, the compounds **12k**−**o** are more effective than **12a**−**e** against

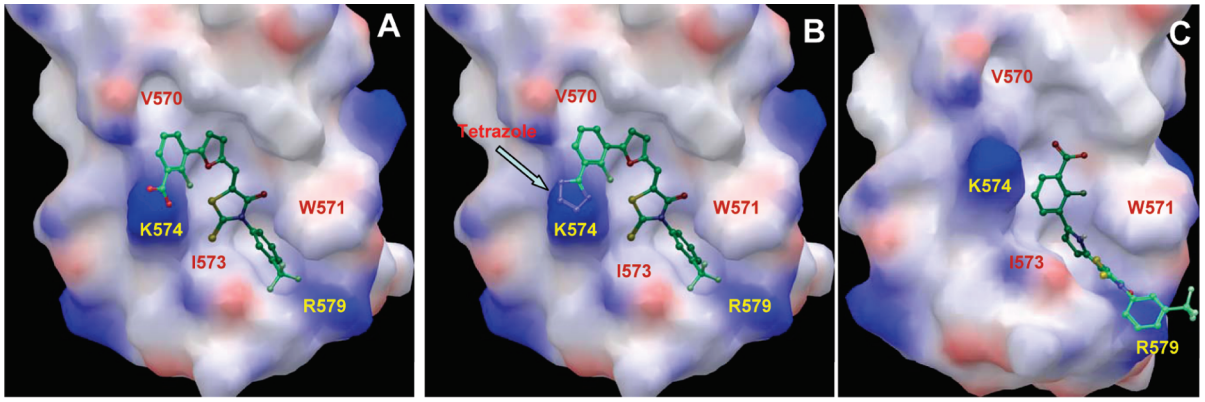
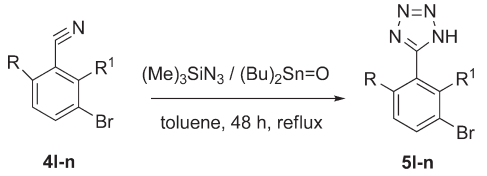


Figure 3. Docking of two highly active compounds, **12b** and **12m**, and one less active compound, **12i**, in the HIV-1 gp41 hydrophobic cavity. (A) **12b** docked in the hydrophobic cavity, showing interaction with K574. (B) The tetrazole-containing compound, **12m**, docked in the HIV-1 gp41 hydrophobic cavity, showing similar interactions as in A. (C) Poorly active compound **12i** docked in the same cavity but lacking ionic interactions with K574 (or R579) and with only a small hydrophobic part inserted in the cavity.

Scheme 1. Synthesis of 3-Tetrazolylbromobenzenes **5l−n**



4l-n **5l-n**

infection by the primary HIV-1 isolate 92US657 (Table 2), indicating that the tetrazolyl group is preferred over the COOH group at the X position in the 5-((arylfuran-2-yl)-methylene)-2-thioxo-3-(3-(trifluoromethyl)phenyl)thiazolidin-4-ones. This information is important for optimization of the lead compounds with improved antiviral activity.

A molecular docking study containing one each of the active carboxylic acid (**12b**) and tetrazolyl (**12m**) compounds with one of the lesser active pyrrole compounds (**12i**) indicated that **12b** and **12m** dock into the hydrophobic cavity almost identically (Figure 3A and Figure 3B), whereas poorly active **12i** docks in a very different orientation (Figure 3C). As previously reported,[12,15−17] a negatively charged group in the inhibitor forms a salt bridge with lysine 574 (K574) in the periphery of the hydrophobic cavity of HIV-1 gp41, which is essential for antiviral activity. The hydrophobic portions of **12b** and **12m** penetrate deeply into the cavity with good interaction with the hydrophobic residues (few shown in the figures for clarity). Poorly active inhibitor **12i** docked in a very different orientation with the carboxylic acid group far from any positively charged residues such as K574 or R579, thus without significant charge−charge interaction. Although a portion of the hydrophobic part of **12i** occupies the cavity, the ionic interaction seems critical for these inhibitors to show potent activity. These interactions, identified by docking simulations, rationalized the activity of **12b** and **12m** and relatively poor activity of **12i**.

Chemistry

3-Tetrazolylbromobenzenes **5l−n** were synthesized in 56−83% yield from the corresponding bromocyanobenzenes **4l−n** by slight modification of a literature method (Scheme 1).[22]

2-Thioxo-3-(3-(trifluoromethyl)phenyl)thiazolidin-4-one **6**,[23] 3-bromo-2-hydroxybenzoic acid **5d**,[24] 3-bromo-2-methoxybenzoic acid **5e**,[24] 5-bromo-1*H*-pyrrole-2-carboxaldehyde **7**,[25] and 3-borono-2-methoxybenzoic acid **5j**[26] were synthesized by the literature methods as indicated.

Synthesis of 2-Aryl-5-formylfurans 9a−e,k−n and Pyrroles 9f−j. 2-Aryl-5-formylfurans **9a−e,k−n** were obtained in 29−60% yields by palladium-catalyzed Suzuki−Miyaura cross-coupling[27,28] of 5-formyl-2-furylboronic acid **8** with the corresponding aryl bromides **5a−e,k−n** by modification of a literature method.[29] Reactions of 5-bromopyrrole-2-carboxaldehyde **7** with 3-carboxyarylboronic acids **5f−j** used a modification of Bumagin's method[30] to give 2-aryl-5-formylpyrroles **9f−j** (65−86%) (Scheme 2, Table 3).

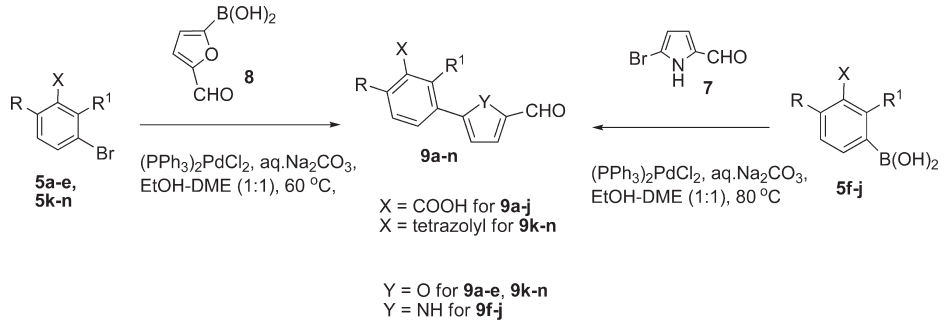
Synthesis of 5(4-Hydroxy-3-(1*H*-tetrazol-5-yl)phenyl)furan-2-carbaldehyde 9o. 5-Bromo-2-hydroxybenzonitrile **10** was treated with 5-formylfuran-2-ylboronic acid **8** in the presence of 3 equiv of sodium carbonate and 0.05 mol equiv of dichlorobis(triphenylphosphine)palladium(II) as catalyst in a mixture of DME/ethanol at 65 °C for 18 h to afford 5-(5-formylfuran-2-yl)-2-hydroxybenzonitrile **11** in 52% yield. 5-(5-Formylfuran-2-yl)-2-hydroxybenzonitrile **11** was treated with 1.3 equiv of sodium azide in the presence of triethylamine hydrochloride in DMF for 30 h at 120 °C to afford 5(4-hydroxy-3-(1*H*-tetrazol-5-yl)phenyl)furan-2-carbaldehyde **9o** in 42% yield (Scheme 3).

Synthesis of 5-((Arylfuran/1*H*-pyrrol-2-yl)methylene)-2-thioxo-3-(3-(trifluoromethyl)phenyl)thiazolidin-4-ones 12a−o. Knoevenagel condensation[32−34] of 2-thioxo-3-(3-(trifluoromethyl)phenyl)thiazolidin-4-one **6** with aldehydes **9a−o** gave 5-((aryl-furan/1*H*-pyrrol-2-yl)methylene)-2-thioxo-3-(3-(trifluoromethyl)phenyl)thiazolidin-4-ones **12a−o** (11−69%) (Scheme 4, Table 4), characterized by ^{1}H and ^{13}C NMR spectroscopy and elemental analyses. For aldehydes **9a−e,k,n** the reaction was carried out in ethanol, catalyzed by 2,2,6,6-tetramethylpiperidine at 80 °C, and for aldehydes **9f−j,l,m,o** reaction was carried out in toluene, containing 10 mol % of diisopropylethylamine.

Conclusion

We report syntheses of 5-((arylfuran/1*H*-pyrrol-2-yl)-methylene)-2-thioxo-3-(3-(trifluoromethyl)phenyl)thiazolidin-4-ones **12a−o** by Suzuki−Miyaura cross-coupling, followed by Knoevenagel condensation. Compounds **12a−o** all exhibit anti-HIV-1 activity, with **12l** and **12m** showing high potency against infection by laboratory-adapted and primary HIV-1 strains with EC_{50} at low nanomolar level and inhibiting

Scheme 2. Synthesis of 2-Aryl-5-formylfurans **9a**−**e**,**k**−**n** and Pyrroles **9f**−**j**[a]



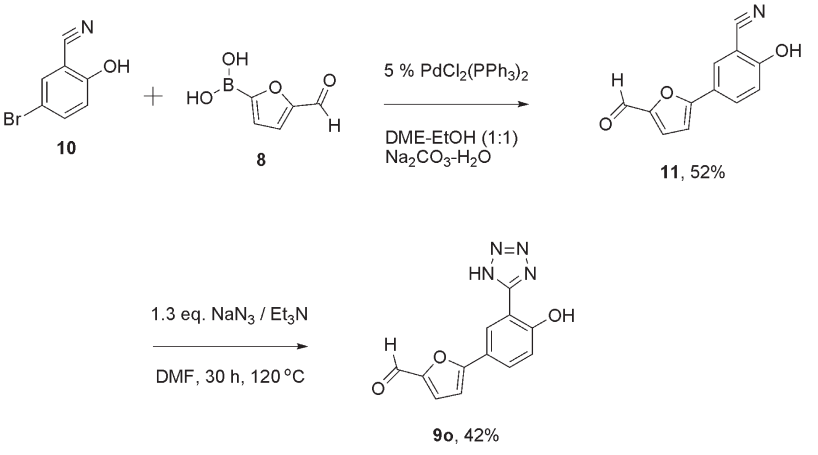
[a] For designation of X, Y, R, and R^1 in **5** and **9**, see Table 3.

Table 3. Preparation of 2-Aryl 5-formylfurans **9a**−**e**,**k**−**n** and 2-Aryl-5-formylpyrroles **9f**−**j**

product **9**	X of **5** and **9**	Y of **5** and **9**	R of **5** and **9**	R1 of **5** and **9**	reaction time (h)	yield (%) of **9**[a]
9a	COOH	O	H	Cl	16	49[b]
9b	COOH	O	H	F	8	60[b]
9c	COOH	O	OH	H	8	47[b]
9d	COOH	O	H	OH	15	51[c]
9e	COOH	O	H	OMe	18	52
9f	COOH	NH	H	H	18	86
9g	COOH	NH	Cl	H	18	61
9h	COOH	NH	F	H	18	65
9i	COOH	NH	H	F	18	67[d]
9j	COOH	NH	H	OMe	18	76
9k	tetrazolyl	O	H	H	16	49[d]
9l	tetrazolyl	O	Cl	H	20	56
9m	tetrazolyl	O	H	F	20	39[d]
9n	tetrazolyl	O	F	H	18	29[d]

[a] Isolated yields. [b] Reference 13. [c] Reference 31. [d] Crude yield; 9i, 9m, and 9n were used without further purification.

Scheme 3. Synthesis of 5-(4-Hydroxy-3-(1*H*-tetrazol-5-yl)phenyl)furan-2-carbaldehyde **9o**



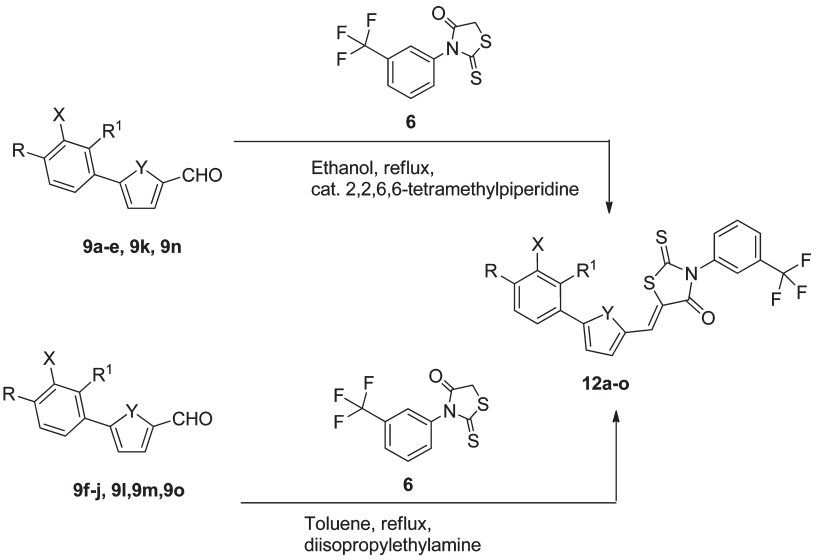
HIV-1-mediated cell−cell fusion and the gp41 six-helix bundle formation. The docking study was able to rationalize the antiviral activity of those two active compounds along with a lesser active compound, **12i**. The improved selectivity of **12l** and **12m** suggests that these molecules can be used as leads for developing novel small molecule HIV fusion inhibitors for treatment of HIV/AIDS patients who fail to respond to other antiretroviral therapeutics.

Experimental Section

Melting points were determined using a capillary melting point apparatus equipped with a digital thermometer and are uncorrected. ^{1}H (300 MHz) and ^{13}C (75 MHz) NMR spectra were recorded in DMSO-d_6 (with tetramethylsilanes as the internal standard), unless otherwise stated. Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, br s = broad singlet, m = multiplet), coupling constants (J values) expressed in Hz. Elemental analyses were performed on a Carlo Erba EA-1108 instrument. All the reactions were performed in flame-dried glassware. The solvents (ethylene glycol dimethyl ether (DME) and THF) were dried by the usual methods and distilled before use. Purity of compounds **12a**−**o** was determined by elemental analyses and/or HPLC; purity of target compounds was ≥95% except otherwise noted. Analytical HPLC analyses were performed on Shimadzu SPD-20-A using the following: column, Zorbax Rx-C18 with detection at 254 nm; solvent, MeOH (100%); flow rate of 0.5 mL/min.

Docking of Selected Inhibitors onto the HIV-1 gp41 Hydrophobic Cavity. We used the automated docking software Glide

Scheme 4. Synthesis of 5-((Arylfuran/1*H*-pyrrol-2-yl)methylene)-2-thioxo-3-(3-(trifluoromethyl)phenyl)thiazolidin-4-ones **12a**−**o**[a]



[a] For designation of X, Y, R, and R^1 in **9** and **12**, see Tables 3 and 4.

Table 4. Preparation of 5-((Arylfuran/1*H*-pyrrol-2-yl)methylene)-2-thioxo-3-(3-(trifluoromethyl)phenyl)thiazolidin-4-ones **12a**−**o**

product **12**	X of **12**	Y of **12**	R of **12**	R^1 of **12**	reaction time (h)	yield (%) of **12**[a]
12a	COOH	O	H	Cl	8	11
12b	COOH	O	H	F	8	44
12c	COOH	O	OH	H	6	31
12d	COOH	O	H	OH	8	24
12e	COOH	O	H	OMe	8	59
12f	COOH	NH	H	H	16	48
12g	COOH	NH	Cl	H	16	63
12h	COOH	NH	F	H	16	50
12i	COOH	NH	H	F	16	69
12j	COOH	NH	H	OMe	12	49
12k	tetrazolyl	O	H	H	8	31
12l	tetrazolyl	O	Cl	H	16	58
12m	tetrazolyl	O	H	F	16	45
12n	tetrazolyl	O	F	H	8	22
12o	tetrazolyl	O	OH	H	10	40

[a] Isolated yields.

5.6 within Schrodinger Suite 2010 (Schrödinger, Portland, OR), which applies a hierarchical series of filters to identify the best possible orientation (poses) of the ligand in the binding site of the receptor. We selected the X-ray structure of the HIV-1 gp41 core (1AIK), available from the Protein Data Bank (PDB) at the Research Collaboratory for Structural Bioinformatics (RCSB), as the target receptor because this structure is one of the highest resolution gp41 core structures available and has been extensively used in our research program. Three-dimensional coordinates of the ligands, their isomeric, ionization, and tautomeric states were generated using the LigPrep tool available within the Schrödinger software. The protein was prepared using the protein preparation tool available in the software. A grid file encompassing the area in the cavity that contains information on the properties of the associated receptor was created. Conformational flexibility of the ligands was handled via an exhaustive conformational search. We used Schrödinger's proprietary GlideScore scoring function in extra precision (XP) mode to score the optimized poses. The poses were selected on the basis of the salt-bridge and other hydrophobic and hydrogen bond interactions.

Assessment of the Anti-HIV-1 Activity. The inhibitory activity of compounds on $HIV\text{-}1_{IIIB}$ replication in MT-2 cells was determined as previously described.[12] Briefly, MT-2 cells (1×10^5/mL) were infected with an $HIV\text{-}1_{IIIB}$ strain (100 $TCID_{50}$) in 200 μL of RPMI 1640 medium containing 10% FBS in the presence or absence of a test compound at graded concentrations overnight, followed by replacement of the culture supernatants with fresh media containing no test compounds. After incubation at 37 °C for 4 days, an amount of 100 μL of culture supernatants was collected from each well, mixed with equal volumes of 5% Triton X-100, and assayed by ELISA for p24 production as previously described.[12] The percentage inhibition of p24 production was calculated as previously described.[35] EC_{50} values were calculated using a computer program, designated CalcuSyn,[36] kindly provided by Dr. T. C. Chou (Sloan-Kettering Cancer Center, NY).

ELISA for Screening for Compounds That Inhibit the gp41 6-HB Core Formation. A sandwich ELISA as previously described[37] was used to determine the inhibitory activity of a compound on the gp41 6-HB core formation. Briefly, NHR-peptide N36 (2 μM) was preincubated with a test compound at the indicated concentrations at 37 °C for 30 min, followed by addition of CHR-peptide C34 (2 μM). After incubation at 37 °C for 30 min, the mixture was added to wells of a 96-well polystyrene plate (Costar, Corning Inc., Corning, NY) which were precoated with IgG (2 μg/mL) purified from rabbit antisera directed against the N36/C34 mixture. Then the mAb NC-1, which is specific for the gp41 core,[20] biotin-labeled goat-anti-mouse IgG (Sigma Chemical Co., St. Louis, MO), streptavidin-labeled horseradish peroxidase (Zymed, S. San Francisco, CA), and the substrate

3,3′,5,5′-tetramethylbenzidine (Sigma) were added sequentially. Absorbance at 450 nm was measured using an ELISA reader (Ultra 384, Tecan, Research Triangle Park, NC). The percent inhibition by the compounds and their EC_{50} values were calculated using the software Calcusyn as described above.

Circular Dichroism Analysis. The effect of the compounds on the coiled coil structure of the gp41 NHR-trimer formed by N46 peptide (we used N46 here rather than N36, since N36 cannot automatically form NHR-trimer in physiological buffer) and the 6-HB formed between N46 and C34 peptides was determined by CD spectroscopy as previously described.[21] Briefly, N46 and C34 peptides were diluted in doubly distilled H_2O (pH7.0) and 50 mM sodium phosphate and 150 mM NaCl (PBS, pH 7.2), respectively, to a final concentration of 10 μM. For investigation of the effect of a compound on N-trimer, the mixture N46 and the compound (or PBS as control) was incubated in a 37 °C water bath for 0.5 h before testing. For investigation of the effect of a compound on 6-HB formation, the mixture N46 and the compound (or PBS as control) was incubated in a 37 °C water bath for 0.5 h, followed by addition of C34 peptide and an additional incubation of 30 min. The spectra of each sample were acquired on Jasco spectropolarimeter (model J-715, Jasco Inc., Japan) at room temperature, using a 5.0 nm bandwidth, 0.1 nm resolution, 0.1 cm path length, 4.0 s response time, and a 50 nm/min scanning speed and were corrected by subtraction of the background corresponding to the solvent. The α-helicity was calculated from the CD signal by dividing the mean residue ellipticity at 222 nm by the value expected for 100% helix formation (i.e., 33000 deg cm^2 $dmol^{-1}$) according to previous studies.[38,39]

Detection of in Vitro Cytotoxicity. The in vitro cytotoxicity of compounds on MT-2 cells was measured by XTT assay as previously described.[14] Briefly, an amount of 100 μL of the test compound at graded concentrations was added to equal volumes of cells (5×10^5/mL) in wells of 96-well plates. After incubation at 37 °C for 4 days, 50 μL of XTT solution (1 mg/mL) containing 0.02 μM of phenazine methosulphate was added. After incubation at 37 °C for 4 h, the absorbance at 450 nm was measured with an ELISA reader. The CC_{50} (concentration for 50% cytotoxicity) values were calculated using the CalcuSyn program.[36]

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Supporting Information Available: Synthetic procedures and characterization data of compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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