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Synthesis and biological evaluation of triazolothienopyrimidine derivatives as novel HIV-1 replication inhibitors

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ABSTRACT

We identified a novel class of triazolothienopyrimidine (TTPM) compounds as potent HIV-1 replication inhibitors during a high-throughput screening campaign that evaluated more than 200,000 compounds using a cell-based full replication assay. Herein, we report the optimization of the antiviral activity in a cell-based assay system leading to the discovery of aryl-substituted TTPM derivatives (**38, 44**, and **45**), which exhibited significant inhibition of HIV-1 replication with acceptable safety margins. These novel and potent TTPMs could serve as leads for further development.

Since it was first reported in 1981, the human immunodeficiency virus (HIV), the causative agent of acquired immunodeficiency syndrome (AIDS), has developed into one of the most serious pandemic health challenges.[1] According to global estimates of the WHO/UNAIDS in 2010, there were more than 34 million people living with HIV, 2.7 million new infections, and 1.8 million AIDS-related deaths.[2] The global HIV/AIDS threats triggered an extensive search for drugs inhibiting HIV-1 replications.[3] Currently, Food and Drug Administration (FDA) has approved 26 drugs belonging to six therapeutic targets for the treatment of HIV infection: nucleoside and nucleotide reverse transcriptase inhibitors (NRTIs/NtRTIs),[4] non-nucleoside reverse transcriptase inhibitors (NNRTIs),[5] protease inhibitors (PIs),[6] integrase inhibitors (INIs),[7] entry (CCR5 co-receptor antagonist),[8] and fusion inhibitors (FIs).[9] The introduction of highly active antiretroviral therapy (HAART)—a combination of NRTIs/NNRTIs and PIs in general—has dramatically reduced the mortality and morbidity among HIV-1 infected patients, transforming HIV/AIDS into a chronic manageable disease.[10] However, there are serious drawbacks of HAART due to the tendency of HIV-1 to rapidly mutate. Long-term HAART treatment leads to the emergence of drug-resistant viral mutants.[11] Also, severe side effects of combination therapy have limited their clinical effectiveness.[12] Therefore, the continuous development of novel anti-HIV agents with acceptable toxicity and an improved resistance profile is undoubtedly needed.

In a high-throughput screening campaign for the discovery of novel antiretrovirals that evaluated more than 200,000 compounds using a cell-based full replication assay based on reporter cells harboring an EGFP expression cassette under the control of the HIV promoter, we identified hit compounds containing a triazolothienopyrimidine scaffold (Fig. 1) that exhibited inhibitory activities against HIV replication at micromolar concentrations. Triazolothienopyrimidine derivatives (TTPM) have been reported only recently to exhibit biological activities as antagonists on serotonin 5-HT_6 receptor,[13,14] and inhibitors of kidney urea transporter,[15]

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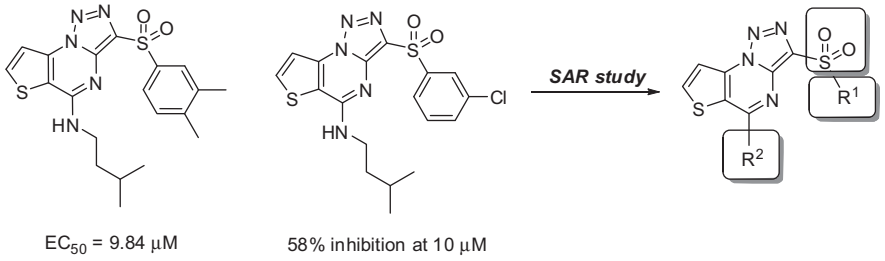
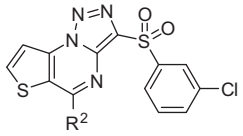
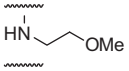
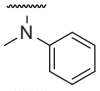
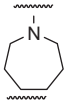
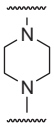
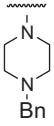


Figure 1. Hit compounds from cell-based HIV-1 replication assay.

Table 1
Cell-based antiviral activity of TTPM derivatives **5**–**15** with R^2 modifications against HIV-1

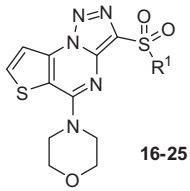


5-15

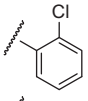
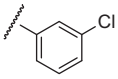
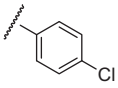
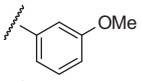
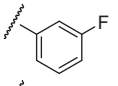
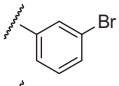
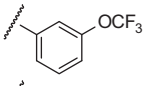
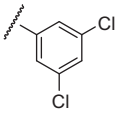
Compd	R^2	EC_{50}[a] (μM)	CC_{50}[b] (μM)	TI[c]
5		1.6	>50	>31
6		2.2	>50	>22
7		1.9	>50	>26
8		2.3	>50	>21
9		6.7	>50	>7.4
10		2.1	>50	>23
11		0.19	75	394
12		4.0	35	8.7
13		0.27	74	274
14		2.1	>50	>23
15		0.71	>50	>70
NVP[d]		0.150	>10	

[a] EC_{50} is the concentration of compound that inhibits HIV-1 replication by 50%. For compounds **5**–**15**, the values are the geometric mean of two determinations; all individual values are within 25% of the mean.
[b] CC_{50} is the cytotoxic concentration of compound that reduces viability of uninfected cells by 50%.
[c] Therapeutic index (TI) is defined by CC_{50}/EC_{50}.
[d] Nevirapine (NVP) was used as a positive control.

Table 2
Cell-based antiviral activity of TTPM derivatives **16**–**25** with R^1 modifications against HIV-1



16-25

Compd	R^1	EC_{50}[a] (μM)	CC_{50}[b] (μM)	TI[c]
16		Inactive	>50	—
17		16	39	2.4
18		Inactive	>50	—
19		1.1	56	50
13		0.27	74	274
20		5.0	70	14
21		1.1	>50	>45
22		0.85	>50	>58
23		3.3	>50	>15
24		10	>50	>5
25		4.0	51	12.7
NVP[d]		0.150	>10	

[a] EC_{50} is the concentration of compound that inhibits HIV-1 replication by 50%. For compounds **16**–**25**, the values are the geometric mean of two determinations; all individual values are within 25% of the mean.
[b] CC_{50} is the cytotoxic concentration of compound that reduces viability of uninfected cells by 50%.
[c] Therapeutic index (TI) is defined by CC_{50}/EC_{50}.
[d] Nevirapine (NVP) was used as a positive control.

whereas anti-HIV activity has not been previously documented. Here we report the preliminary structure–activity relationship (SAR) of TTPMs as a novel class of HIV-1 replication inhibitors.

To explore the structure–activity relationship (SAR) of TTPMs, we synthesized the target compounds (Tables 1–4) as outlined in Schemes 1–3. To first evaluate the effect of R^2 region with various amines and R^1 region in TTPMs, we synthesized the compounds **5**–**25** according to the general routes.[14] The synthetic precursor azide **1** was prepared from 3-amino-thiophene-2-carboxylic acid methyl ester by diazotization, followed by addition of sodium azide at low temperature.[16] The sulfonylacetonitrile building block **2** were synthesized from commercially available sulfonyl chlorides in successive microwave-mediated sulfinate formation, followed by alkylation with chloroacetonitrile.[17] The cyclization/lactamation to construct the tricyclic core was achieved in situ by treating the nitrile enolate with azidothiophene ester **1** under refluxing condition. The lactam **3** was then activated with $POCl_3$ to give key intermediate **4** in good yield.[18] The activated compound **4** was subjected to the substitution with various amines to afford TTPM derivatives **5**–**25**.

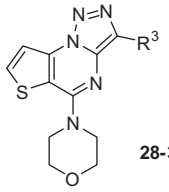
The next series of modifications to be explored was linker replacement in TTPMs. To investigate the effect of linker moiety in TTPMs, the sulfonyl group was modified into its corresponding carbonyl derivatives as shown in Scheme 2. To access the ester/amide analogues **28**–**32**, ethyl 2-cyanoacetate was used in the cyclization/lactamation instead of sulfonylacetonitriles. After the activation of lactam **26**, the R^2 region was first fixed with morpholine, followed by hydrolysis of ester **28** and a typical amide coupling sequence. For the ketone analogue **37**, the α-cyano ketone compound **34** was prepared in two steps via α-bromination of ketone and substitution with sodium cyanide. The precursor **34** was then subjected to the general protocol to give corresponding ketone **37**.

To evaluate the effect of aryl group in R^2 region, the key intermediate **4a** was treated with various aryl boronic acids under microwave-mediated Suzuki coupling condition[19] to give aryl substituted adducts **38–48** as depicted in Scheme 3.

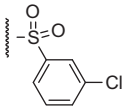
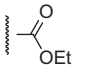
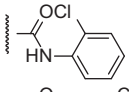
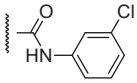
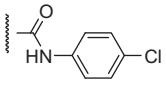
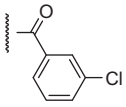
The first series of R^2 modification with various amines was evaluated for their inhibitory activity against HIV-1 replication in a cell-based assay, and Nevirapine was used as a positive control.[20] The assay results of compounds (**5–15**) are summarized in Table 1.

As shown in Table 1, it was apparent that the anti-HIV activities of TTPM compounds are sensitive to structural modifications. Compounds **5–15** with common R^1 moiety exhibited considerable variations in the cellular inhibitory activities from 0.19 μM to 6.7 μM. Compounds containing a free amine and flexible alkyl amines (**5, 6**, and **7**) displayed moderate ranges of activities from EC_{50} = 1.6 μM to 2.2 μM. Compound **10** with *N*-methyl aniline exhibited retained inhibitory activities compared with aniline substituted analogue **8** (EC_{50} = 2.1 and 2.3 μM, respectively). It is clear that the hydrogen bond donor ability of the nitrogen atom is not essential for antiviral activity. Interestingly, the replacement with cyclic amine series such as piperidine (**11**, EC_{50} = 0.19 μM) and morpholine (**13**, EC_{50} = 0.27 μM) showed significantly improved activities. Increasing the ring size from six-membered ring to seven-membered ring led to 20-fold reduction in antiviral activity (compare **11** and **12**), indicating the steric bulkiness of R^2 substitutents plays a pivotal role to achieve enhanced inhibitory activities. The steric effect is

Table 3
Cell-based antiviral activity of TTPM derivatives **28**–**32**, and **37** with R^3 modifications against HIV-1

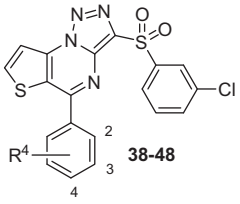


28-32, 37

Compd	R^3	EC_{50}[a] (μM)	CC_{50}[b] (μM)	TI[c]
13		0.27	74	274
28		0.80	>50	>62
29		4.8	42	8.7
30		3.5	>50	>14
31		0.37	>50	>135
32		16	57	3.5
37		0.93	>50	>53
NVP[d]		0.150	>10	

[a] EC_{50} is the concentration of compound that inhibits HIV-1 replication by 50%. For compounds **28**–**32**, **37**, the values are the geometric mean of two determinations; all individual values are within 25% of the mean.
[b] CC_{50} is the cytotoxic concentration of compound that reduces viability of uninfected cells by 50%.
[c] Therapeutic index (TI) is defined by CC_{50}/EC_{50}.
[d] Nevirapine (NVP) was used as a positive control.

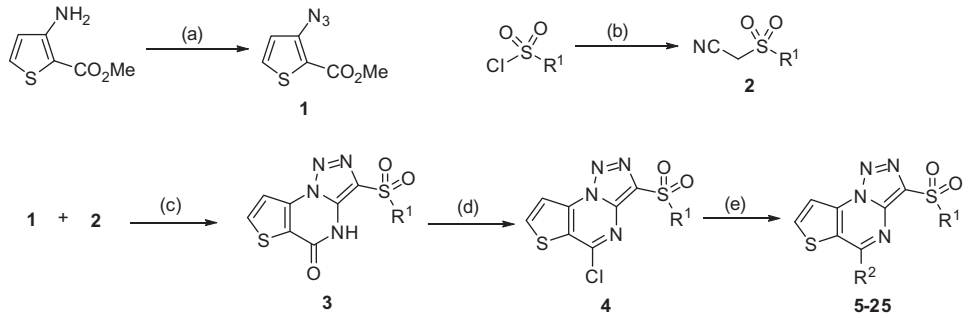
Table 4
Cell-based antiviral activity of TTPM derivatives **38**–**48** with Ar modifications against HIV-1



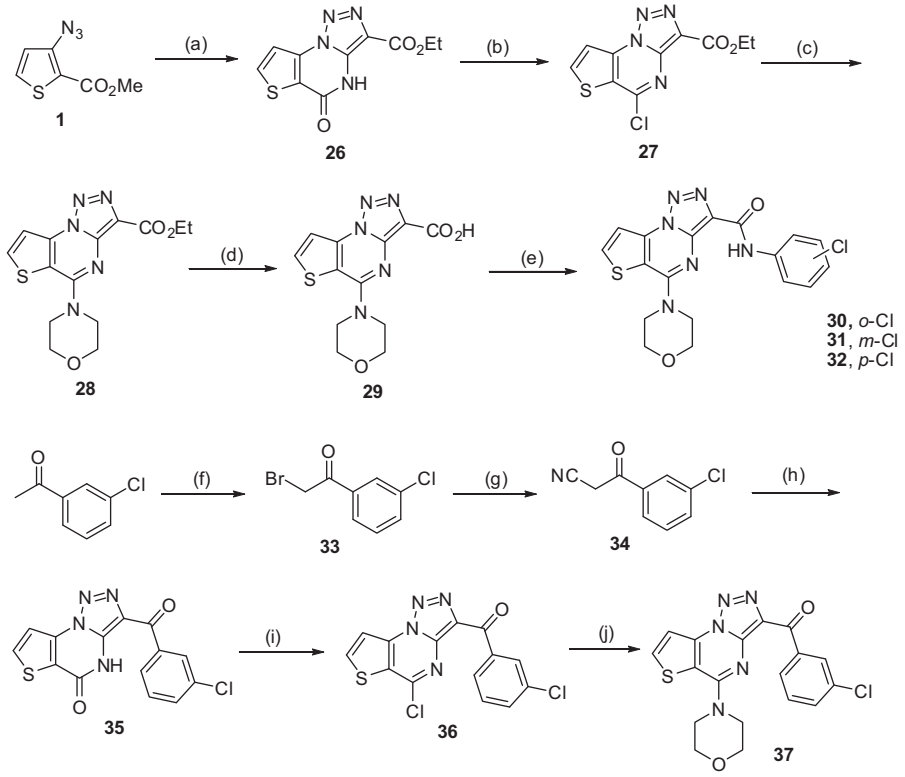
38-48

Compd	R^4	EC_{50}[a] (μM)	CC_{50}[b] (μM)	TI[c]
38	H	0.27	61	225
39	4-Me	0.84	61	72
40	2-Cl	0.67	>50	74
41	3-Cl	0.54	47	87
42	4-Cl	0.53	46	86
43	2-OMe	1.3	45	34
44	3-OMe	0.32	46	143
45	4-OMe	0.06	60	1000
46	4-OEt	0.51	61	119
47	4-OH	0.87	10	11
48	4-NMe_2	3.1	>50	>16
NVP[d]		0.150	>10	

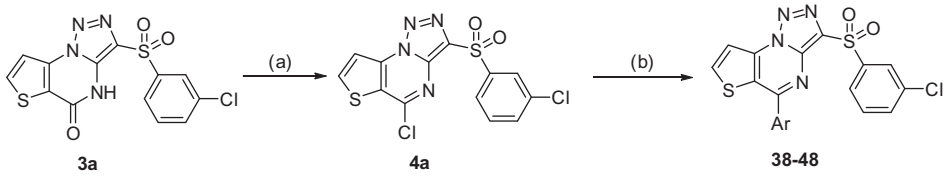
[a] EC_{50} is the concentration of compound that inhibits HIV-1 replication by 50%. For compounds **38**–**48**, the values are the geometric mean of two determinations; all individual values are within 25% of the mean.
[b] CC_{50} is the cytotoxic concentration of compound that reduces viability of uninfected cells by 50%.
[c] Therapeutic index (TI) is defined by CC_{50}/EC_{50}.
[d] Nevirapine (NVP) was used as a positive control.



Scheme 1. Synthesis of triazolothienopyrimidines **5**–**25**. Reagents and conditions: (a) $NaNO_2$, 10% aq HCl, 0 °C, 20 min; then NaN_3, H_2O, 0–25 °C, 1 h; (b) $NaHCO_3$, Na_2SO_3, H_2O, microwave, 100 °C, 30 min; then chloroacetonitrile, microwave, 100 °C, 20 min; (c) NaOEt, EtOH, reflux, overnight; (d) $POCl_3$, pyr, 110 °C, 3 h; (e) amines, Et_3N, DMF, 120 °C, 3 h.



Scheme 2. Synthesis of triazolothienopyrimidines **30**–**32**, and **37.** Reagents and conditions: (a) ethyl 2-cyanoacetate, NaOEt, EtOH, reflux, overnight; (b) $POCl_3$, pyr, 110 °C, 3 h; (c) morpholine, Et_3N, DMF, 120 °C, 3 h; (d) NaOH, EtOH, H_2O, reflux, 2 h; (e) anilines, EDC, HOBt, DMF, 100 °C, 5 h; (f) $CuBr_2$, $EtOAc/CH_2Cl_2$ (1:1), reflux, 10 h; (g) NaCN, EtOH,/H_2O (1:1), 0–25 °C, overnight; (h) **1**, NaOEt, EtOH, reflux, overnight; (i) $POCl_3$, pyr, 110 °C, 3 h; (j) morpholine, Et_3N, DMF, 120 °C, 3 h.



Scheme 3. Synthesis of triazolothienopyrimidines **38**–**48**. Reagents and conditions: (a) $POCl_3$, pyr, 110 °C, 3 h; (b) boronic acids, $Pd(dppf)Cl_2$, Na_2CO_3, DME/H_2O (3:1), microwave, 110 °C, 5 min.

further exemplified by compound **9**, which showed over 35-fold reduced antiviral activity as compared to compound **11**. However, analogue **15** containing *N*-benzyl piperazine showed 3-fold improved potency compared to that with *N*-methyl analogue **14**, suggesting that additional favorable interactions in the form of π–π or hydrophobic interactions could be further utilized to increase its inhibitory activity.

We next investigated the effect of R^1 moiety in TTPM derivatives (**16–25**) with morpholine in R^2 region (Table 2). Compounds (**16**, **18**) with simple alkyl or cyclohexyl R^1 group showed complete

loss of activity. It indicated that an aryl group at R^1 region is vital to exhibit antiviral activity. Significant variations in potency were observed depending on the substitution patterns on the phenyl ring. *Meta*-position on the phenyl ring with an electron-withdrawing group is the most influential compared to *ortho*- and *para*-positions. Compound **13** with *meta*-Cl exhibited 4-fold and 18-fold enhanced potency compared to *ortho*-Cl and *para*-Cl analogues (**19**, **20**). The antiviral activities among halides at *meta*-position are chloro- > fluoro- > bromo-analogues in order. Interestingly, the introduction of an additional chlorine atom at *meta*-position suffered from 14-fold reduced antiviral activity (compare **13** and **25**). Alkoxy analogues (**21, 24**) were less potent than chloro compound **13**. From these results, the optimal substitution for R^1 phenyl ring is the mono-substitution with a chlorine atom at *meta*-position (**13**, EC_{50} = 0.27 μM).

The effect of linker moiety in TTPMs was evaluated by replacing the sulfonyl group with its corresponding carbonyl derivatives and the results were summarized in Table 3. Ester analogue **28** displayed moderate inhibitory activity and its corresponding acid **29** showed 6-fold reduced potency, which was presumably due to its low cell membrane permeability in our cell-based assay system. Similar preference for *meta*-chloro substitution on phenyl ring was also observed with amide analogues. Amide analogue **31** with *m*-Cl showed 9-fold and 43-fold higher potency than other amide analogues with *o*-Cl **30** and *p*-Cl **32**, respectively. Ketone derivative **37** showed comparable activity. Based on these results, other linker moieties could be further utilized to improve antiviral activities and drug-like properties of TTPM derivatives.

After the preliminary SAR with R^1, R^2 and linker modification, we further investigated the R^2 region with substituted phenyl derivatives **38**–**48** as shown in Table 4. Unsubstituted phenyl analogue **38** exhibited equipotent antiviral activity compared to compound **13** with morpholine. Although no significant variations in activities were observed on the analogues containing electron-withdrawing group (**40**, **41**, and **42**), the derivatives with electron-donating group (**43**, **44**, and **45**), clearly displayed the impact of substitutions on the phenyl ring. *para*-Methoxy analogue **45** exhibited 21-fold and 5-fold enhanced potency compared to the corresponding *ortho*- and *meta*-methoxy analogues (**43**, **44**), respectively. Increasing the size of alkyl group from methoxy **45** to ethoxy **46** led to 8-fold reduction in activity. In case of hydroxy analogue **47**, it suffered from increased cytotoxicity. Surprisingly, compound **48** with *para*-dimethylamino group showed greatly reduced inhibitory activity, indicating *para*-methoxy substitution is optimal for phenyl ring to obtain significant activity with acceptable therapeutic index.

In summary, a series of TTPM derivatives was synthesized and evaluated as HIV-1 replication inhibitors in vitro. From preliminary SAR in a cell-based full replication assay, we discovered aryl-substituted TTPM derivatives (**38, 44**, and **45**), which exhibited significant inhibitory activity along with acceptable safety margins. Mode of action and further optimization of this novel class of anti-HIV agents is currently under investigation.

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References and notes

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