HHS Public Access

Author manuscript

J Med Chem. Author manuscript; available in PMC 2017 July 30.

Published in final edited form as:

J Med Chem. 2016 August 25; 59(16): 7677–7682. doi:10.1021/acs.jmedchem.6b00247.

1,2,3-Triazoles as amide bioisosteres: discovery of a new class of potent HIV-1 Vif antagonists

Idrees Mohammed[a], **Indrasena Reddy Kummetha**[b], **Gatikrushna Singh**[a], **Natalia Sharova**[c], **Gianluigi Lichinchi**[a,b], **Jason Dang**[b], **Mario Stevenson**[c], and **Tariq M. Rana**[*,a,b]

[a]Program for RNA Biology, Sanford Burnham Prebys Medical Discovery Institute, La Jolla, California 92037, USA

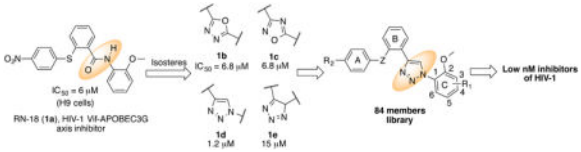
[b]Department of Pediatrics, University of California San Diego School of Medicine, La Jolla, California 92093 USA

[c]Division of Infectious Diseases, Miller School of Medicine, University of Miami, Miami, Florida 33136, USA

Abstract

RN-18–based Viral infectivity factor, Vif antagonists reduce viral infectivity by rescuing APOBEC3G (A3G) expression and enhancing A3G-dependent Vif degradation. Replacement of amide functionality in RN-18 (IC_{50} = 6 μM) by isosteric heterocycles resulted in the discovery of a 1,2,3-trizole, **1d** (IC_{50} = 1.2 μM). We identified several potent HIV-1 inhibitors from a **1d** based library including **5ax** (IC_{50} = 0.01 μM), **5bx** (0.2 μM), **2ey** (0.4 μM), **5ey** (0.6 μM), and **6bx** (0.2 μM).

Graphical abstract



Introduction

Since the start of the AIDS epidemic in 1981, this disease has led to the death of > 30 million people globally. Although the overall growth of the epidemic appears to be slowing, nearly three million new infections and an estimated 1.8 million AIDS-related deaths in 2010 are still very high. Over the past two decades, more than 25 anti-HIV drugs have been developed targeting several different stages of the virus life cycle [1]. Among these inhibitors

*Corresponding Author: Phone: (858) 246 1100, trana@ucsd.edu.

Supporting Information: The Supporting Information is available free of charge on the ACS Publications website at DOI:
The details of general procedures, synthetic schemes, characterization data, antiviral activities, and immunoblotting experiments are given in the S.I. file (PDF)

Author Contributions: The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

of HIV-1 reverse transcriptase and protease, when used in combinations in the highly active antiretroviral therapy (cART), have proven to be highly effective in reducing AIDS-related mortality throughout the world [2]. However, the development of drug resistance and toxic side effects associated with cART have created a need for more potent and less toxic therapies against other viral targets and host-virus interactions [3]. Importantly, in patients on effective cART, plasma viremia can be suppressed to below detectable levels for extended intervals. The ability of cART to sustain this aviremic state has promoted the view that cART is fully suppressive and effectively stops all ongoing viral replication. Since there is rapid recrudescence of plasma viremia upon treatment interruption, regardless of the prior interval of viral suppression, there are long-lived viral reservoirs that maintain viral persistence in the face of cART. Therefore, new antiviral drugs are needed to purge drug resistant viruses from viral reservoirs.

The HIV-1 accessory protein Viral infectivity factor, Vif is essential for in vivo viral replication [4, 5]. HIV-1 Vif protein targets an innate antiviral human DNA-editing enzyme, APOBEC3G (A3G) [6], which inhibits replication of retroviruses [7]. A3G catalyzes critical hypermutations in the viral DNA and acts as an innate weapon against retroviruses.[5] Cells that express A3G are ‘non-permissive’ for viral replication in which HIV-1 must express Vif in order to replicate. In contrast, HIV-1 replication is Vif-independent in host cells that do not express A3G (permissive cells). Since HIV-1 Vif has no known cellular homologs, this protein represents an extremely attractive, yet unrealized, target for antiviral intervention.

The RN-18–based class of small molecule Vif antagonists reduce viral infectivity by enhancing A3G-dependent Vif degradation, increasing A3G incorporation into virions, and enhancing cytidine deamination of the viral genome [8-10]. RN-18 (**1a**) exhibits IC_{50} values of 4.5 μM and 6 μM in CEM cells and H9 cells (non-permissive cells), respectively. RN-18 does not inhibit viral infectivity in MT4 cell line (permissive cells) even at 100 μM demonstrating that these inhibitors are Vif-specific. These findings provided the proof of concept that the HIV-1 Vif-A3G axis is a valid target for developing small molecule-based new therapies for AIDS or for enhancing innate immunity against viruses.

We faced two major challenges for further development of RN-18-based Vif antagonists as clinical candidates: (a) potency; and (b) metabolic stability. To address these questions, we planned to explore isosteric replacement of the amide functionality in RN-18. We reasoned to test a series of conformationally restricted, biocompatible and metabolically stable isosteric hetero-cyclic systems. Next, based on the activity, we would select and develop a suitable bioisosteric[11] series to improve the both activity and pharmacological profiles.

Results and Discussion

In this communication, we describe the successful identification of potent bioisosteric analogues of RN-18. Initially, we designed and synthesized four test molecules by substituting the amide functionality in the lead molecule with isosteric heterocyclic systems such as 1,3,4-oxadiazole[12] **1b**, 1,2,4-oxadiazole[13] **1c**, 1,4-disubstituted-1,2,3-triazole[14] **1d** and 1,5-disubstituted-1,2,3-triazole[15] **1e** (Figure 1).

1,3,4-Oxadiazole **1b** was synthesized with the coupling of hydrazine and 2-iodobenzoic acid (Scheme 1, A). The one pot coupling involves the formation of in situ methyl ester of 2-iodobenzoic acid, which was later refluxed in the presence of hydrazine hydrate to obtain the benzohydrazide derivative **1f** quantitatively. Benzohydrazide **1f** was later reacted with *o*-anisic acid in refluxing phosphoryl chloride leading to the formation of iodo intermediate 1,3,4-oxadiazole **1g**. Intermediate **1g** was reacted with 4-nitrothiophenol under copper (I) catalyzed *S*-arylation conditions[16] leading to the formation of compound **1b**. Synthesis of 1,2,4-oxadiazole 1c was started (Scheme 1, B) with the coupling between the commercially available *N*′-hydroxy-2-methoxybenzimidamide and 2-iodobenzoic acid using dicyclohexyldicarbodiimide[17] leading to the formation of the iodo intermediate 1,2,4-oxadiazole **1h**. *S*-arylation of **1h** with 4-nitrothiophenol under copper (I) catalytic conditions led to the formation of 3,5-disubstituted-1,2,4-oxadiazole, **1c**.

Synthesis of 1,4-disubstituted-1,2,3-triazole analogue **1d** required two synthons; 2-ethynylaniline **1j**, and 1-azido-2-methoxybenzene **1k** (Scheme 1, C). 2-Iodoaniline was reacted with trimethylsilylacetylene under sonogashira reaction conditions catalyzed by bis(triphenylphosphine)palladium chloride in the presence of triethylamine base and copper iodide as co-catalyst[18] leading to the formation of TMS protected ethynylaniline **1i**, which was deprotected using sodium hydroxide affording the required synthon 2-ethynylaniline **1j**. Azide **1k** was synthesized by following a Cham-Lam type of coupling between 2-methoxyphenylboronic acid and sodium azide catalyzed by copper sulfate at room temperature in methanol.[19] Copper-catalyzed click reaction[20] between alkyne **1j** and azide **1k** generated triazole amine **1l** quantitatively in *t*-butanol/water (Scheme 1, D). Triazole amine **1l** was diazotized using sodium nitrite in 5N HCl around -10 °C and concomitantly converted to iodotriazole **1m** by reacting with potassium iodide. Copper (I) catalyzed *S*-arylation of iodotriazole **1m** using 4-nitrothiophenol in DMF solvent and potassium carbonate led to the synthesis of **1d**, IMA-53. 1,5-Disubstituted-1,2,3-triazole **1e** analogue was synthesized initially by reacting alkyne **1j** and azide **1k** under ruthenium catalyzed click chemistry conditions using $Cp^*RuCl(PPh_3)_2$ catalyst in benzene at 80 °C[21] leading to the formation of amine **1n** (Scheme 1, E). Diazotization, iodination (**1o**), and *S*-arylation reaction sequences afforded 1,5-disubstituted-1,2,3-triazole **1e**.

The antiviral activities of the four synthesized RN-18 analogues were measured against wild-type HIV-1 both in non-permissive H9 and permissive MT-4 cells (See details of methods in S.I.). In all the antiviral activity measurements, RN-18 (**1a**) was used as a positive control and the cells cultured without any inhibitor served as negative control. The IC_{50} values of the bioisosteric analogues of RN-18 are presented in Table 1. Both 1,3,4-oxadiazole **1b** (IC_{50} = 6.8 μM) and 1,2,4-oxadiazole **1c** (IC_{50} = 6.8 μM) based analogues exhibited cell-based antiviral activity in the non-permissive H9 cells similar to the lead molecule RN-18 (IC_{50} = 6 μM). Interestingly, 2,5-disubstituted-1,3,4-oxadiazole **1b** showed nonspecific antiviral activity with IC_{50} of 50 μM in permissive MT4 cells. Whereas the 1,4-disubstituted-1,2,3-triazole based analogue **1d** exhibited remarkably better cell-based anti-HIV activity (IC_{50} = 1.2 μM in H9 cells) and specificity (no activity in MT4 cells). On the contrary, 1,5-disubstituted-1,2,3-triazole **1e** analogue exhibited comparatively lesser potency

(IC_{50} = 15 μM in H9 cells) with non-specific activity in the permissive cells (IC_{50} = 25 μM in MT4 cells).

Next, to determine the mechanism of these bioisosteres of RN-18, we analyzed Vif degradation and rescue of A3G levels in the presence of these compounds and compared with RN-18. 293FT cells co-expressing hemagglutinin (HA)-tagged A3G and green fluorescent protein (GFP)-tagged Vif or ΔVif were treated with various compounds (50 μM) for 16 h (see S.I. for methods details). The cell extracts were then analyzed by immunoblotting with anti-HA-A3G, anti-GFP-Vif, and anti-GAPDH antibodies (Figure 2). All the bioisostere analogues of RN-18 resulted in restoring A3G levels in the presence of Vif and down-regulated Vif expression, indicating that these analogues (**1b**, **1c**, **1d** and **1e**) are capable of antagonizing Vif function similar to RN-18. However, analogues **1b**, and **1e** also exhibited some nonspecific activity (Table 1).

These observations were well in-line with the structural similarities in the 3D orientations and planarity except the 1,5-disubstituted-1,2,3-triazole **1e**, which has a twisted structure (see S.I. for the crystallographic data). 1,3,4-Oxadiazole, and 1,2,4-oxadiazole heterocyclic systems have both planarity and dipole moment similar to amide functionality. Similarly, 1,4-disubstituted and 1,5-disubstituted 1,2,3-triazoles possess strong dipole moment beside having better H-bond accepting (N(2) and N(3)), and H-bond donating (triazole C(5)-H) capacity than an amide functionality.[22] However in the present biochemical context, 1,4-disubstituted-1,2,3-triazle **1d** analogue showed both improved antiviral activity (IC_{50} = 1.2 μM) and selectivity (no activity in MT4 cells).

Having discovered compound **1d** as a potent and specific inhibitor of Vif-A3G axis, we decided to optimize the analogue to generate new class of anti-HIV drug candidates for clinical development. We designed and synthesized an 84-membered library using a parallel format exploring various substitution patterns in ring-A, ring-C, and bridge A-B in the **1d** structure (Table 2). In this direction, the synthetic scheme for **1d** (Scheme 1, D) was followed. Synthetic schemes (see Schemes 1S to 6S in S.I.), experimental procedures, and characterization data of all the 84 members of the library are given in the supporting information. Anti-viral activities of the library were determined against wild-type HIV-1 both in non-permissive H9 and permissive MT-4 cells. The IC_{50} values for important compounds are presented in Table 2. Antiviral activities of the complete library is given in the supporting information file as Table 1S. None of the 84 compounds exhibited antiviral activities at 50 μM in non-permissive MT4 cells indicating the requirement of Vif for their function, which is quite remarkable. Further analysis of a selective set of potent compounds showed dose-dependent inhibition of HIV-1 in H9 cells with no significant toxicity at 50 μM as measured by MTS cell viability assays (Fig 1S and 2S).

For few selected compounds (**2dx**, **2ey**, **2gy**, **5ax**, **5bx**, **5gy** and **5ey**) we then determined whether the analogues could upregulate A3G and downregulate Vif in a manner similar to RN-18 and **1d**. Immunoblots for A3G and Vif in the presence of compounds are shown in Figure 3, which clearly showed that the new inhibitors exert the anti-HIV activity via the same mechanism as observed for RN-18 and **1d**. Of the 84 members library, about 30 compounds inhibited HIV-1 with IC_{50} values in the range of 0.01 to 5 μM in the non-

permissive H9 cells. Among them, the compound **5ax** exhibited the most potent activity with IC_{50} of 10 nM, which is about 1000 fold more potent than the original lead molecule, RN-18. Similarly, compounds **2ey**, **5bx**, **5ey**, and **6bx** exhibited IC_{50} values in the range of 0.2 μM to 0.6 μM and compounds **2ax**, **2dx**, **2ex**, **2fx**, **3ax**, **3dx**, **3fx**, **3fy**, **5ay**, **6ex**, **6fx**, **6ey**, **6fy** in the range of 1 μM to 3 μM. Three water soluble choline salts **2gy**, **4gy** and **5gy** exhibited IC_{50} values of 0.2 μM, 0.7 μM, and 0.5 μM, respectively. Overall, the SAR of the library showed striking sensitivity towards the three variables (Z-bridge, R_1 and R_2 substituents) tested in this study. Among various SAR findings few of the noteworthy ones are: in general sulfide (-S-) as bridge Z exhibited overall better activity compared with sulfone (-SO_2-) bridge (in the case of RN-18 sulfone derivative showed better activity)9. However, sulfones (-SO_2-) showed better activities when R_2 substituent was amino group. This study has found replacements such as –$COOCH_3$, -COOH, -CF_3, -NH_2, and –choline carboxylate for the nitro functionality in RN-18.

Conclusion

In summary, this study report three major findings: (a) 1,4-disubstituted-1,2,3-triazole system is a suitable bioisostere in the RN-18 context (b) discovery of a new class of potent Vif antagonists as preclinical candidates for novel AIDS therapy, and (c) generation of potent chemical modulators for perturbing and understanding Vif-A3G cell biology and physiology. Further optimization of 1,4-disubstituted-1,2,4-oxadiazole, **1c** and pre-clinical studies for the selected 1,4-disubstituted-1,2,3-triazole based Vif antagonists are in progress.

Experimental Section

Details of general procedures, and materials are described in the S.I. Parallel synthesis was performed using Carousel 6 (Radleys Discovery Technologies). ^1H and ^{13}C NMR spectra were recorded using a 400 MHz Jeol JNM-ECS spectrometer (equipped with a 5 mm proton/multi-frequency auto-tune and an auto sample changer) with trimethylsilane (TMS) as the internal reference. The spectra are reported in ppm on the δ scale. ESI MS was performed on Waters micromass Model ZQ 4000 using methanol. HRMS was performed on Agilent Technologies 6224A MS-TOF. Purity of the tested compounds was determined using Waters 2695 Module HPLC equipped with Waters 996 photodiode detector at 254 nm. Purity of the final compounds mentioned in the Table-1 and Table-2 was found to be ≥95% in HPLC. X-ray structural determination was performed at UCSD facility using Bruker diffractometer with CCD detectors and low-temperature cryostats.

2-Iodobenzohydrazide (1f)

A suspension of 2-iodobenzoic acid (2.48 g, 10 mmol), and $SOCl_2$ (1.43 g, 12 mmol) in dry benzene 25 mL was refluxed for about 2 hours at 80 °C in the presence of catalytic DMF (2 drops). Benzene and excess $SOCl_2$ were removed under reduced pressure. The residue obtained was slowly treated with methanol (25 mL) at 0 °C and triethylamine (5 mL) was added followed by stirring at room temperature for 2 hours. To the above mixture hydrazine hydrate (1.0 g, 20 mmol) was added drop wise, and the mixture was refluxed at 80 °C for about 3 hours. TLC showed the completion of the reaction. The reaction mixture was dried under reduced pressure and extracted with AcOEt (2 × 25 mL). The organic extract was

sequentially treated with saturated solution of $NaHCO_3$, brine and anhydrous Na_2SO_4. Flash column chromatography using AcOEt: hexane (1:1) afforded a colorless amorphous solid compound **1f** (1.99 g, 76% yield).

2-(2-Iodophenyl)-5-(2-methoxyphenyl)-1,3,4-oxadiazole (1g)

A 50 mL round bottom flask was discharged with *o*-anisic acid (0.30 g, 2 mmol), and benzohydrazide **1f** (0.52 g, 2 mmol) followed by the addition of $POCl_3$ (8 mL). The suspension was refluxed at 110 °C for 8 hours till TLC showed depletion of the starting materials. The reaction mixture was poured into cold saturated solution of K_2CO_3 followed by extraction with AcOEt (2 × 25 mL). The organic extract was treated with saturated solution of $NaHCO_3$, and dried over anhydrous Na_2SO_4. TLC (AcOEt: hexane, 1:1) showed two new spots with almost equal intensity. Flash chromatographic separation of the upper spot using AcOEt: hexane (1:1) afforded the colorless amorphous solid compound **1g** (0.41 g, 55% yield).

2-(2-Methoxyphenyl)-5-(2-((4-nitrophenyl)thio)phenyl)-1,3,4-oxadiazole (1b)

S-arylation procedure described for the synthesis of **1d** (see below) was followed for the synthesis of compound **1b** using the intermediate **1g** and 4-nitrothiophenol as starting materials. Flash chromatography using AcOEt: hexane (1:3) afforded the compound as a light yellow amorphous solid (0.219 g, 82% yield), which was crystallized using a mixture of DCM and methanol to afford a light yellow crystalline compound **1b**.

5-(2-Iodophenyl)-3-(2-methoxyphenyl)-1,2,4-oxadiazole (1h)

A solution of 2-iodobenzoic acid (0.99 g, 4 mmol) in 15 mL of dry DMF was cooled to 0 °C followed by the addition of dicyclohexylcarbodiimide (1.24 g, 6.0 mmol) under N_2 atmosphere, and the reaction mixture was stirred for an hour at 0 °C. To the above mixture was added commercially available *N*′-hydroxy-2-methoxybenzimidamide (0.664 g, 4 mmol), and stirred for 30 minutes at 0 °C. Then for 3 hours stirring was continued at room temperature. Gradually the reaction mixture was heated up to 110 °C, and kept for 8 hours. The reaction mixture was later poured into ice-cold water, and diluted using AcOEt (20 mL). Dicyclohexylurea crystals formed were separated by filtration. Filtrate organic layer was treated with saturated solution of K_2CO_3, brine and anhydrous Na_2SO_4. Flash chromatography using AcOEt: hexane (1:3) afforded the required compound **1h** as a colorless amorphous solid (1.2 g, 80% yield).

3-(2-Methoxyphenyl)-5-(2-((4-nitrophenyl)thio)phenyl)-1,2,4-oxadiazole (1c)

S-arylation procedure described for the synthesis of **1d** was followed for the synthesis of compound **1c** using the intermediate **1h** and 4-nitrothiophenol as starting materials. Flash chromatography using AcOEt: hexane (1:3) afforded the compound as a light yellow amorphous solid (0.225 g, 84% yield), which was crystallized using a mixture of DCM and methanol to afford a light yellow crystalline compound **1c**.

2-((Trimethylsilyl)ethynyl)aniline (1i)

In a dry 500 mL two-necked round bottom flask 2-iodoaniline (25.0 g, 0.114 mol) was dissolved in 250 mL of deoxygenated triethylamine. To this solution, $PdCl_2(PPh_3)_2$ catalyst (0.8 g, 1.14 mmol, 1 mol%), and copper (I) iodide co-catalyst (0.217 g, 1.14 mmol, 1 mol%) were added. The mixture was stirred for 15 minutes at room temperature under N_2 pressure. To this mixture trimethylacetylene (11.21 g, 0.114 mol) was added and stirred for 12 hours at room temperature. Triethylamine was removed under reduced pressure to get a crude viscous residue. The residue was dissolved in AcOEt (250 mL) treated with saturated brine, Na_2SO_4 and adsorbed on neutral alumina. Flash column chromatography using AcOEt: hexane (1:9) afforded a pale yellow liquid compound **1i** (18.37 g, 85% yield).

2-Ethynylaniline (1j)

A 1 M aqueous solution of NaOH (2.64 g, 65.95 mmol, 1.2 equiv.) was added to a solution of 2-ethynylaniline **1i** (18.0 g, 54.96 mmol, 1 equiv.) dissolved in 200mL of ethanol/THF (1:1). Stirring was continued at room temperature for about 1 hour till TLC showed complete disappearance of the starting material. Organic solvents were evaporated under reduced pressure, and the residue was diluted by adding 50 mL of deionized water and extracted with DCM (2 × 100 mL). Organic extractions were dried over brine and Na_2SO_4 and adsorbed on neutral alumina. Flash column chromatography using AcOEt: hexane (1:4) afforded a colorless pale yellow liquid compound **1j** (6.18 g, 96% yield).

1-Azido-2-methoxybenzene (1k)

To a solution of 2-methoxyphenylboronic acid (1.52 g, 10 mmol) in 20 mL of methanol, sodium azide (0.78 g, 12.0 mmol) was added and stirred. To this mixture $CuSO_4.5H_2O$ (0.249 g, 1 mmol, 10 mol%) was added and stirred at room temperature for about 8 hours till TLC showed completion of the reaction. Methanol was removed under reduced pressure and the residue was treated with saturated solution of sodium bicarbonate followed by extraction with DCM (2 × 20 mL). Organic extractions were dried over anhydrous Na_2SO_4 and adsorbed on silica gel. Flash column chromatography using AcOEt: hexane (1:9) afforded a colorless dark brown liquid compound **1k** (1.34 g, 90% yield).

2-(1-(2-Methoxyphenyl)-1*H*-1,2,3-triazol-4-yl)aniline (1l)

2-Ethynylaniline **1j** (0.234 g, 2 mmol), and 1-azido-2-methoxybenzene **1k** (0.298 g, 2 mmol) were dissolved in 10 ml of a mixture of *tert*-butanol and deionized water (1:1) in a 50 ml round bottom flask. To the stirred solution sodium ascorbate (39.62 mg, 0.2 mmol, 10 mol%), and $CuSO_4.5H_2O$ (24.97 mg, 0.1 mmol, 5 mol%) were added. Stirring was continued overnight till TLC showed t he completion of the reaction. *t*-Butanol was removed under reduced pressure and the viscous residue was extracted with DCM (2 × 10 mL). The combined organic extractions were treated with saturated brine and anhydrous Na_2SO_4 followed by adsorption on neutral alumina. Flash chromatography using AcOEt: hexane (1:3) afforded the triazole amine compound **1l** as a light brown amorphous solid (0.467 g, 88% yield).

4-(2-Iodophenyl)-1-(2-methoxyphenyl)-1*H*-1,2,3-triazole (1m)

In a 50 ml round bottom flask triazole amine **1l** (0.266 g, 1 mmol) was dissolved in 10 mL 5N HCl at 0 °C and stirred for 30 minutes. Sodium nitrite (82.8 mg, 1.2 mmol) dissolved in minimum amount of water was added drop wise to the above mixture at -10 °C. Stirring was continued at -10 °C for a period of 2 hours to get diazozium salt in situ. Urea (~50 mg) was added to the reaction mixture to remove any excess nitrous acid generated in situ. In a separate beaker KI (0.249 g, 1.5 mmol) was dissolved in 5 mL of deionized water and kept at -5 °C. To this solution of KI was added the diazonium hydrochloride solution drop-by-drop using dropping funnel. After addition, stirring was continued for a period of 8 hours at room temperature. The reaction mixture was later diluted with 20 mL of AcOEt and 10 mL of deionized water. Small amount of iodine liberated in the reaction was quenched by the addition of sodium dithionite. Organic layer was separated and was sequentially treated with saturated $NaHCO_3$, saturated brine, and anhydrous Na_2SO_4 followed by adsorption on silica gel. Flash chromatography using AcOEt: hexane (1:3) afforded the compound **1m** as a colorless amorphous solid (0.293 g, 78% yield).

1-(2-Methoxyphenyl)-4-(2-((4-nitrophenyl)thio)phenyl)-1*H*-1,2,3-triazole (1d)

In a dry 25 mL two-neck round bottom flask iodo triazole compound **1m** (0.25 g, 0.66 mmol, 1 equiv.) was dissolved in 5 mL of dry DMF followed by the addition of anhydrous K_2CO_3 (0.110 g, 0.79 mmol, 1.2 equiv.) and catalyst copper iodide (6.31 mg, 0.033 mmol, 5 mol%). The resulting mixture was stirred for 10 minutes under N_2 pressure. 4-Nitrothiophenol (0.123 g, 0.79 mmol, 1.2 equiv.) dissolved in 2 ml of anhydrous DMF was added to the above reaction mixture, and stirred at 110 °C for 8 hours. The reaction mixture was poured then into ice-cold water followed by extraction with AcOEt (2 × 10 mL). Organic extractions were treated sequentially with saturated K_2CO_3 solution, and anhydrous Na_2SO_4. The dried organic extract was adsorbed on silica gel and flash chromatography using AcOEt: hexane (1:3) afforded the compound **1d** as a light yellow amorphous solid (0.219 g, 82% yield). The amorphous solid was crystallized using a mixture of DCM and methanol to afford a light yellow crystalline compound **1d**.

2-(1-(2-Methoxyphenyl)-1*H*-1,2,3-triazol-5-yl)aniline (1n)

2-Ethynylaniline **1j** (0.234 g, 2 mmol), and 1-azido-2-methoxybenzene **1k** (0.298 g, 2 mmol) were dissolved in 10 mL of anhydrous benzene. To the above stirred solution, $Cp^*RuCl(PPh_3)_2$ (15.90 mg, 0.02 mmol, 1 mol%) catalyst was added and the reaction mixture was refluxed at 80 °C under N_2 pressure for 3 hours till TLC showed the completion of the reaction. Benzene was removed under reduced pressure and the viscous residue was extracted with DCM (2 × 10 mL). The combined extractions were dried over Na_2SO_4 followed by adsorption on neutral alumina. Flash chromatography using AcOEt: hexane (2:3) afforded compound **1n** as a brown amorphous solid (0.488 g, 92% yield).

5-(2-Iodophenyl)-1-(2-methoxyphenyl)-1*H*-1,2,3-triazole (1o)

Procedure described for the synthesis of intermediate **1m** was followed using **1n** as starting material to afford a colorless amorphous solid compound **1o** (0.282 g, 75% yield).

1-(2-Methoxyphenyl)-5-(2-((4-nitrophenyl)thio)phenyl)-1*H*-1,2,3-triazole (1e)

S-arylation procedure described for the synthesis of compound **1d** was followed for the synthesis of compound **1e** using the intermediate **1o** and 4-nitrothiophenol as starting materials. Flash chromatography using AcOEt: hexane (1:3) afforded the compound as a light yellow amorphous solid (0.227 g, 85% yield), which was crystallized using a mixture of DCM and methanol to afford a light yellow crystalline compound **1e**.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported in part by grants from the NIH MH 100942 and DA 039562.

References

1. Mehellou Y, Clercq ED. Twenty-six years of anti-HIV drug discovery: where do we stand and where do we go? J Med Chem. 2010; 53:521–538. [PubMed: 19785437]
2. Thompson MA, Aberg JA, Cahn P, Montaner JSG, Rizzardini G, Telenti A, Gatell JM, Günthard HF, Hammer SM, Hirsch MS, Jacobsen DM, Reiss P, Richman DD, Volberding PA, Yeni P, Schooley RT. Antiretroviral treatment of adult HIV infection: 2010 recommendations of the International AIDS Society-USA panel. JAMA. 2010; 304:321–333. [PubMed: 20639566]
3. Volderbing PA, Deeks SG. Antiretroviral therapy and management of HIV injection. Lancet. 2010; 376:49–62. [PubMed: 20609987]
4. Gabuzda DH, Lawrence K, Langhoff E, Terwillliger E, Dorfman T, Haseltine WA, Sodroski J. Role of vif in replication of human immunodeficiency virus type 1 in CD4+ T lymphocytes. J Virol. 1992; 66:6489–6495. [PubMed: 1357189]
5. Strebel K, Daugherty D, Clouse K, Cohen D, Folks T, Martin MA. The HIV *A* (*sor*) gene product is essential for virus infectivity. Nature. 1987; 328:728–730. [PubMed: 2441266]
6. Sheehy AM, Gaddis NC, Choi JD, Malim MH. Isolation of a human gene that inhibits HIV-1 infection and is suppressed by the viral Vif protein. Nature. 2002; 418:646–650. [PubMed: 12167863]
7. Malim MH, Bieniasz PD. HIV restriction factors and mechanisms of evasion. Cold Spring Harb Perspect Med. 2012; 2:a006940. [PubMed: 22553496]
8. Ali A, Wang J, Nathans RS, Cao H, Sharova N, Stevenson M, Rana TM. Synthesis and structure-activity relationship studies of HIV-1 virion infectivity factor (Vif) inhibitors that block viral replication. ChemMedChem. 2012; 7:1217–1229. [PubMed: 22555953]
9. Mohammed I, Parai MK, Jiang X, Sharova N, Singh G, Stevenson M, Rana TM. SAR and lead optimization of an HIV-1 Vif-APOBEC3G axis inhibitor. ACS Med Chem Lett. 2012; 3:465–469. [PubMed: 24533175]
10. Nathans R, Cao H, Sharova N, Ali A, Sharkey M, Stranska R, Stevenson M, Rana TM. Small-molecule inhibition of HIV-1 Vif. Nat Biotechol. 2008; 26:1187–1192.
11. Meanwell NA. Synopsis of some recent tactical application of bioisosterism in drug design. J Med Chem. 2011; 54:2529–2591. [PubMed: 21413808]
12. Ko E, Liu J, Perez LM, Lu G, Schaefer A, Burgess K. Universal peptidomimetics. J Am Chem Soc. 2011; 133:462–477. [PubMed: 21182254]
13. Borg S, Vollinga RC, Labarre M, Payza K, Terenius L, Luthman K. Design, synthesis, and evaluation of Phe-Gly mimetics: heterocyclic building blocks of pseudopeptides. J Med Chem. 1999; 42:4331–4342. [PubMed: 10543877]

14. Valverde IE, Bauman A, Kluba CA, Vomstein S, Walter MA, Mindt TL. 1,2,3-Triazoles as amide bond mimics: triazole scan yields protease-resistant peptidomimetics for tumor targeting. Angew Chem Int Ed. 2013; 52:8957–8960.
15. Tam A, Arnold U, Soellner MB, Raines RT. Protein prosthesis: 1,5-disubstituted[1,2,3]triazoles as cis-peptide bond surrogates. J Am Chem Soc. 2007; 129:12670–12671. [PubMed: 17914828]
16. Sperotto E, van Klink GPM, de Vries JG, van Koten G. Ligand-free copper-catalyzed C-S coupling of aryl iodides and thiols. J Org Chem. 2008; 73:5625–5628. [PubMed: 18570479]
17. (a) Liang GB, Feng DD. An improved oxadiazole synthesis using peptide coupling reagents. Tetrahedron Lett. 1996; 37:6627–6630.(b) Kumar D, Patel G, Johnson EO, Shah K. Synthesis and anticancer activities of novel 3,5-disubstituted-1,2,4-oxadiazoles. Bioorg Med Chem Lett. 2009; 19:2739–2741. [PubMed: 19376704]
18. Sonogashira K, Tohda Y, Hagihara N. A convenient synthesis of acetylenes: catalytic substitutions of acetylenic hydrogen with bromoalkenes, iodoarenes and bromopyridines. Tetrahedron Lett. 1975; 16:4467–4470.
19. Tao CZ, Cui X, Li J, Liu AX, Liu L, Guo QX. Copper-catalyzed synthesis of aryl azides and 1-aryl-1,2,3-triazoles from boronic acids. Tetrahedron Lett. 2007; 48:3525–3529.
20. Rostovtsev VV, Green LG, Fokin VV, Sharpless KB. A stepwise Huisgen cycloaddition process: copper(I)-catalyzed regioselective “ligation” of azides and terminal alkynes. Angew Chem Int Ed. 2002; 41:2596–2599.
21. Zhang L, Chen X, Xue P, Sun HHY, Williams ID, Sharpless KB, Fokin VV, Jia G. Ruthenium-catalyzed cycloaddition of alkynes and organic azides. J Am Chem Soc. 2005; 127:15998–15999. [PubMed: 16287266]
22. Tron GC, Pirali T, Billington RA, Canonico PL, Sorba G, Genazzani AA. Click chemistry reactions in medicinal chemistry: applications of the 1,3-dipolar cycloaddition between azides and alkynes. Med Res Rev. 2008; 28:278–308. [PubMed: 17763363]

Abbreviations

APOBEC3G (A3G)	Apolipoprotein B mRNA editing enzyme catalytic polypeptide like 3G
C. C	choline carboxylate
DCM	dichloromethane
DMF	dimethylformamide
TMS	trimethylsilyl
TLC	thin-layer chromatography
THF	tetra-hydrofuran
Vif	Viral infectivity factor

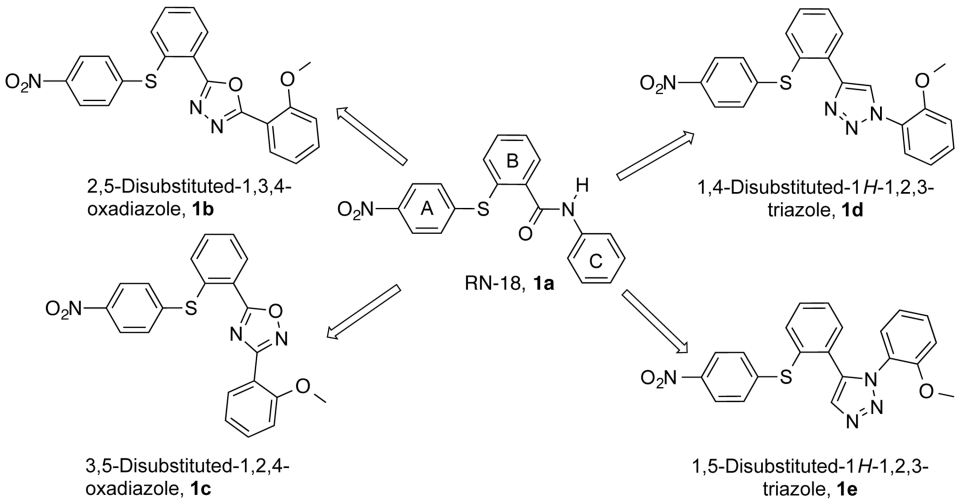


Figure 1.
Amide bioisosteres of **1a**, RN-18

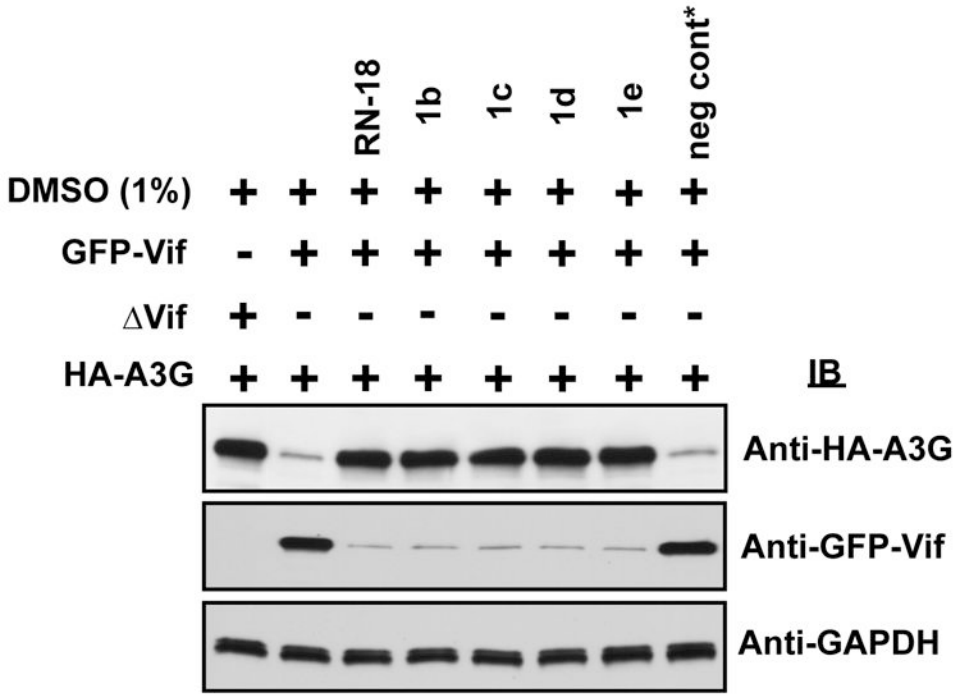


Figure 2.

Bioisosteric analogues of RN-18 enhance A3G levels and reduce Vif expression. 293FT cells co-expressing HA-tagged A3G and GFP-tagged Vif or ΔVif were incubated in the presence (50 μM) or in the absence of the compounds for 16 h. *See S.I. for the structure of negative control (compound 8s). Anti-HA-A3G, anti-GFP-Vif, and anti-GAPDH antibodies were used for immunoblotting (see S.I. for details).

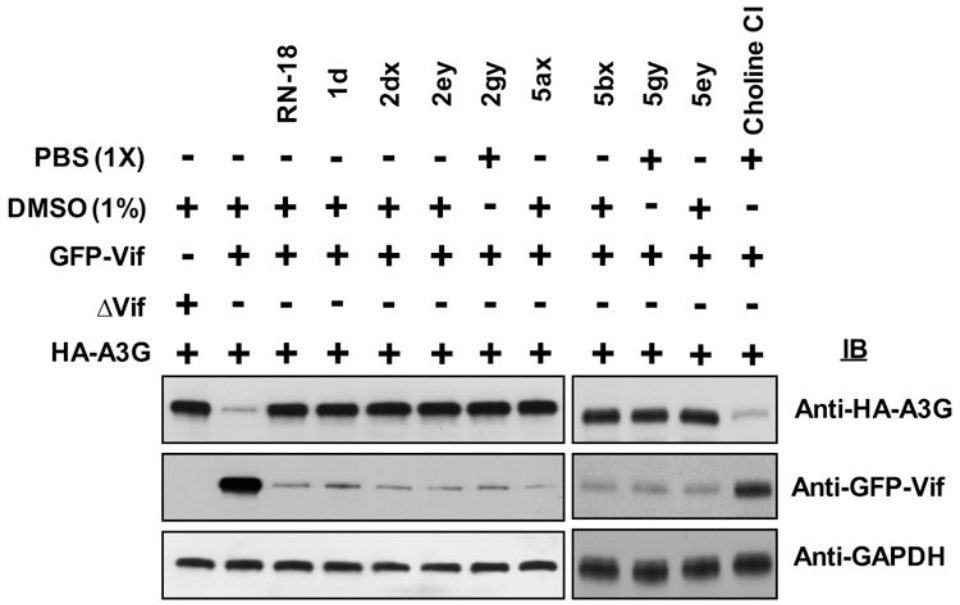
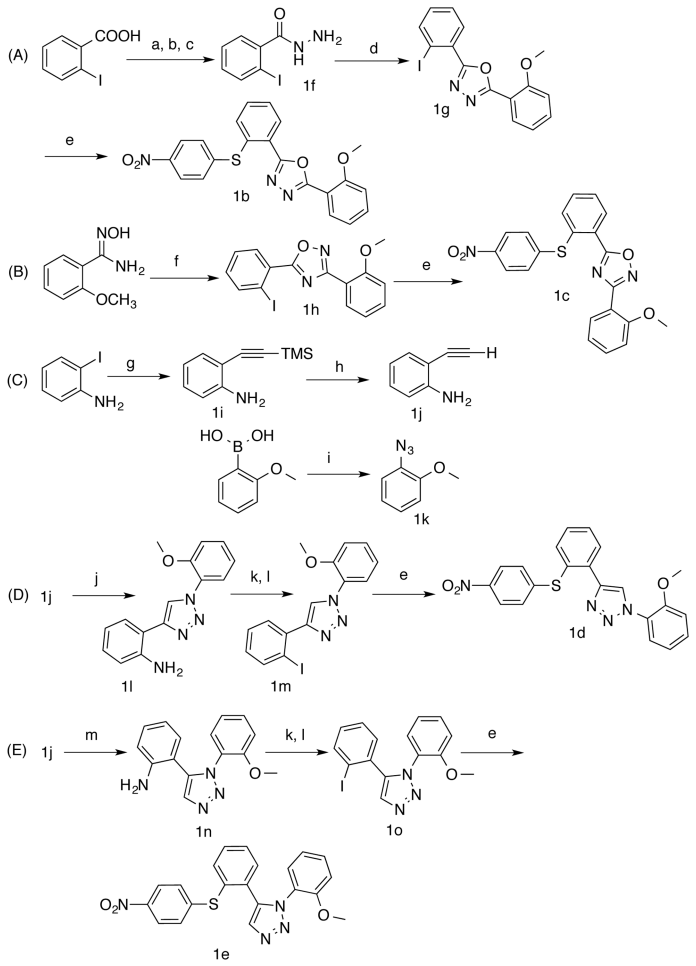


Figure 3.

Triaozle based Vif antagonist small molecules enhance A3G levels and reduce Vif expression. 293FT cells co-expressing HA-tagged A3G and GFP-tagged Vif or ΔVif were incubated in the presence (50 μM) or in the absence of the compounds for 16 h. Choline chloride was used as a negative control.



Scheme 1. Synthesis of isosteric analogues of RN-18[a]

[a]Reagents and conditions: (a) $SOCl_2$, cat. DMF, benzene, 80 °C, 2h; (b) CH_3OH, TEA, o °C-rt., 2h; (c) $NH_2NH_2.H_2O$, 80 °C, 3h; (d) *o*-anisic acid, $POCl_3$, 110 °C, 8h; (e) 4-nitrothiophenol, K_2CO_3, 5 mol%, Cul, DMF, 110 °C, 8h; (f) 2-iodobenzoic acid, DCC, DMF, rt to 100 °C, 8h; (g) Trime-thylsilyl acetylene, 1 mol% $PdCl_2(PPh_3)_2$, 1 mol% Cul, NEt_3, rt, 12h; (h) NaOH (aq), ethanol/THF (1:1), rt, 1h; (i) NaN_3, 10 mol% $CUSO_4.5H_2O$, CH_3OH, rt, 8h; (j) **1k**, 5 mol% $CuS0_4$ $5H_2O$, 10 mol% Na ascorbate, *t*-BuOH/H_2O (1:1), rt, overnight; (k) $NaNO_2$, 5N HCl, −10 to −5 °C, 2h; (l) KI, −10 to −5 °C, 8h; (m) **1k**, 1 mol% $Cp^*RuCl(PPh_3)_2$, benzene, 80 °C, 3h.

Table 1

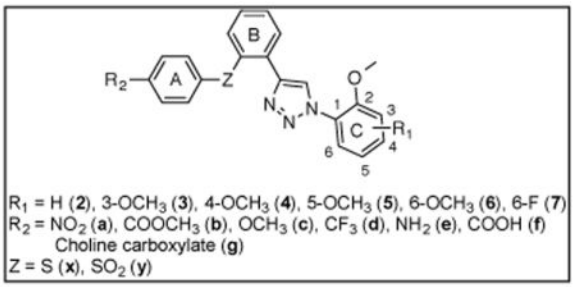
IC_{50} values of the isosteric analogues of RN-18

Compd.	Antiviral activity (IC_{50} μM)	
	H_9 cells	MT_4 cells
1a, RN-18	6	N.A.
1b	6.8	50
1c	6.8	N.A.
1d	1.2	N.A.
1e	15	25

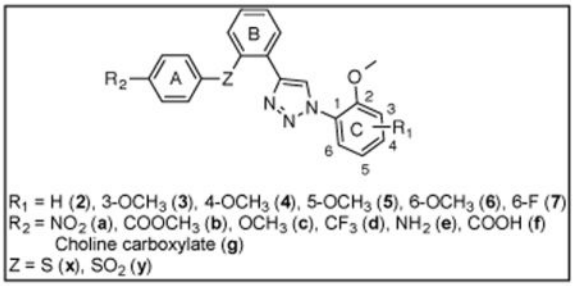
N.A. = no activity even at 50 μM conc.

Table 2

IC_{50} values of the library



Compd.	Z	R_1	R_2	Antiviral activity (IC_{50} μM) H9 Cells
2ax (1d)	S	H	NO_2	1.2
2dx	S	H	CF_3	2.6
2ex	S	H	NH_2	2.5
2fx	S	H	COOH	1.0
2ay	SO_2	H	NO_2	13.8
2cy	SO_2	H	OCH_3	4.3
2dy	SO_2	H	CF_3	4.8
2ey	SO_2	H	NH_2	0.4
2fy	SO_2	H	COOH	8.2
2gy	SO_2	H	C.C.†	0.2
3ax	S	3-OCH_3	NO_2	1.1
3bx	S	3-OCH_3	$COOCH_3$	8
3cx	S	3-OCH_3	OCH_3	4.7
3dx	S	3-OCH_3	CF_3	1.9
3fx	S	3-OCH_3	COOH	2.8
3gx	S	3-OCH_3	C.C.†	4.3
3by	SO_2	3-OCH_3	$COOCH_3$	4.7
3ey	SO_2	3-OCH_3	NH_2	12.4
3fy	SO_2	3-OCH_3	COOH	1.4
4fx	S	4-OCH_3	COOH	7.1
4dy	SO_2	4-OCH_3	CF_3	12
4gy	SO_2	4-OCH_3	C.C.†	0.7
5ax	S	5-OCH_3	NO_2	0.01
5bx	S	5-OCH_3	$COOCH_3$	0.2
5cx	S	5-OCH_3	OCH_3	15.7
5fx	S	5-OCH_3	COOH	4.5



R_1 = H (**2**), 3-OCH_3 (**3**), 4-OCH_3 (**4**), 5-OCH_3 (**5**), 6-OCH_3 (**6**), 6-F (**7**)
R_2 = NO_2 (**a**), $COOCH_3$ (**b**), OCH_3 (**c**), CF_3 (**d**), NH_2 (**e**), COOH (**f**)
Choline carboxylate (**g**)
Z = S (**x**), SO_2 (**y**)

Compd.	Z	R_1	R_2	Antiviral activity (IC_{50} μM) H9 Cells
5ay	SO_2	5-OCH_3	NO_2	1.0
5by	SO_2	5-OCH_3	$COOCH_3$	4.6
5ey	SO_2	5-OCH_3	NH_2	0.6
5gy	SO_2	5-OCH_3	C.C.[†]	0.5
6bx	S	6-OCH_3	$COOCH_3$	0.2
6ex	S	6-OCH_3	NH_2	1.5
6fx	S	6-OCH_3	COOH	1.9
6ey	SO_2	6-OCH_3	NH_2	1.5
6fy	SO_2	6-OCH_3	COOH	1.2
7ax	S	6-F	NO_2	3.9
7bx	S	6-F	$COOCH_3$	7.8
7fx	S	6-F	COOH	4.9
7ey	SO_2	6-F	NH_2	15

[†]Choline carboxylate