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Discovery, Synthesis, and Biological Evaluation of a Novel Group of Selective Inhibitors of Filoviral Entry

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Herein, we report the development of an antifiloviral screening system, based on a pseudotyping strategy, and its application in the discovery of a novel group of small molecules that selectively inhibit the Ebola and Marburg glycoprotein (GP)-mediated infection of human cells. Using Ebola Zaire GP-pseudotyped HIV particles bearing a luciferase reporter gene and 293T cells, a library of 237 small molecules was screened for inhibition of GP-mediated viral entry. From this assay, lead compound **8a** was identified as a selective inhibitor of filoviral entry with an IC_{50} of 30 μM. To analyze functional group requirements for efficacy, a structure−activity relationship analysis of this 3,5-disubstituted isoxazole was then conducted with 56 isoxazole and triazole derivatives prepared using "click" chemistry. This study revealed that while the isoxazole ring can be replaced by a triazole system, the 5-(diethylamino)acetamido substituent found in **8a** is required for inhibition of viral-cell entry. Variation of the 3-aryl substituent provided a number of more potent antiviral agents with IC_{50} values ranging to 2.5 μM. Lead compound **8a** and three of its derivatives were also found to block the Marburg glycoprotein (GP)-mediated infection of human cells.

Introduction

The Ebola and Marburg viruses are enveloped, nonsegmented, single-stranded RNA viruses and among the most dangerous pathogens known. They abruptly emerge in Central Africa, spread rapidly, cause epidemics of severe hemorrhagic fever with unmatched mortality rates (up to 90%) and, as such, are classified as biosafety level 4 agents.[1] Since their respective identification in 1967 and 1976, there have been more than 2300 reported cases of Ebola and Marburg hemorrhagic fever, one-half of which occurred in the past decade. In addition to its effect on humans, there is compelling evidence that Ebola has simultaneously contributed to the catastrophic decline in the western gorilla population in regions of west-central Africa.[2]

Despite recent progress toward the production of vaccines[3] and neutralizing monoclonal antibodies[4] for the prophylaxis of the Ebola and Marburg viruses, it may never be entirely practical, or for that matter, desirable, to inoculate large portions of the population against these emerging pathogens. For that reason and given the growing concern that members of the family Filoviridae might be employed as agents of bioterrorism,[5] the concurrent identification and development of antiviral agents that can substantially mitigate the effects of filoviral infections is a public health priority.

Notwithstanding an increasing understanding of the pathology of filoviruses, only a limited number of low-molecular-weight inhibitors of these systems have been discovered, to date.[6] Existing antifiloviral agents can be characterized by three general modes of action, including (a) impairment of viral mRNA methylation, (b) stimulation of innate antiviral mechanisms, and (c) prevention of virion entry and/or fusion (Figure 1). With regards to the former group, the carbocyclic adenosine analogue 3-deazaadenosine (C-c3Ado, **1**)[7] blocks the cellular enzyme *S*-adenosylhomocysteine hydrolase (SAH[a]) and inhibits the replication of Ebola Zaire in vitro with an IC_{50} of 30 μM.[8] The activity of this antiviral agent has been attributed to diminished methylation of the 5′ cap of viral mRNA by (guanine-7-)methyltransferase, which impairs the translation of viral transcripts.[9] Administration of **1** to Ebola-infected mice has also been found to dramatically increase production of IFN-α,[10] which may serve to counteract the virus's suppression of the innate antiviral response. Unfortunately, compound **1** failed to promote IFN-α production in Ebola-infected monkeys.[11] Glycyrrhizinic acid (**2**), a triterpene glycoside present in licorice extract, has been found to inhibit the replication of a number of viruses, including Marburg and Ebola.[12] In this case, large doses of compound **2** partially protected Ebola-infected mice and slowed the onset of infection but ultimately did not prevent death. Although the precise mode of action has yet to be delineated, there is evidence that **2** may induce IFN-α production as well as

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[a] Abbreviations: EBOV, Ebola virus; EboZ, Ebola Zaire virus; MARV, Marburg virus; VSV, vesicular stomatitis virus; MLV, murine leukemia virus; HIV, human immunodeficiency virus; GP, glycoprotein; IFN-α, interferon alpha; CatB, cathepsin B; CatL, cathepsin L.; *env*, viral envelope glycoprotein; IC_{50}, half maximal inhibitory concentration; SAH, *S*-adenosylhomocysteine hydrolase.

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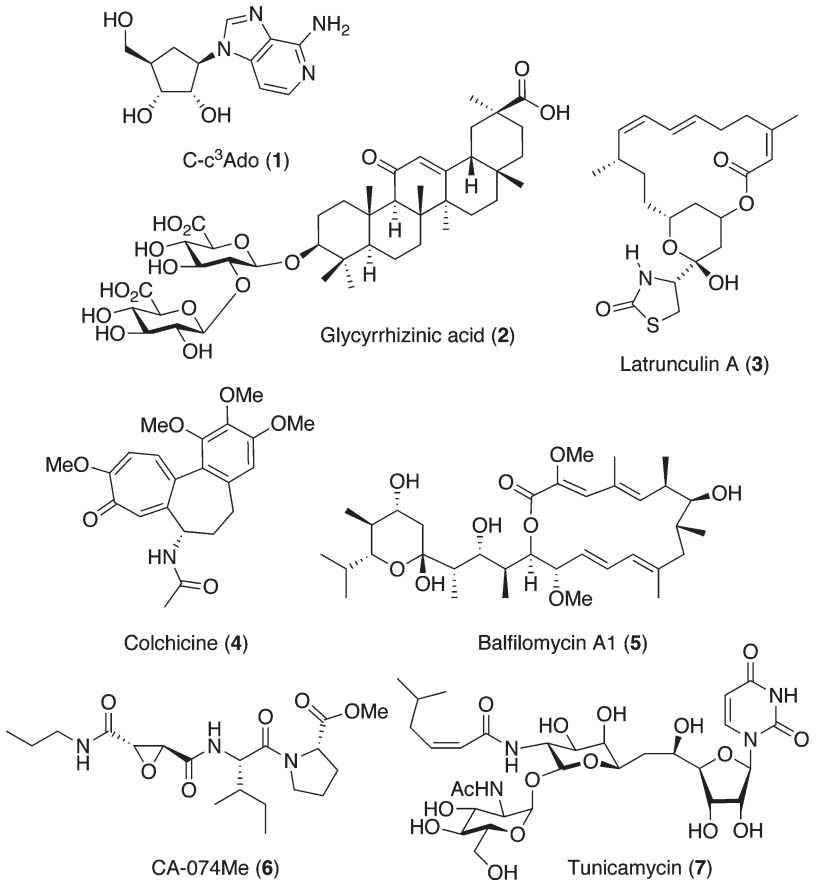


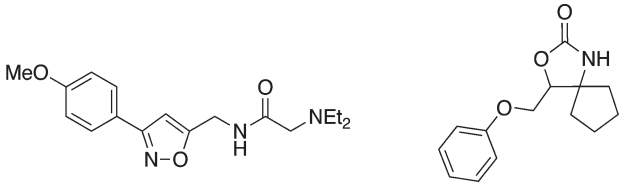
Figure 1. Structure of low-molecular-weight compounds that display antiviral activity against the Ebola and/or Marburg virus: 3-deaza-adenosine (C-c3Ado, **1**), glycyrrhizinic acid (**2**), latrunculin A (**3**), colchicine (**4**), balfilomycin A1 (**5**), CA-074Me (**6**), and tunicamycin (**7**).

inhibit membrane penetration and uncoating of the Ebola virus.[13]

Because EBOV enters target cells by an endocytic pathway,[14] agents that disrupt the efficient trafficking of internalized vesicles, via the various components of the cytoskeleton, hold the potential to abrogate its entry and fusion into cells. Indeed, latrunculin A (**3**) and colchicine (**4**), which respectively impair the formation of microfilaments and microtubules, have been shown to prevent the infection of HeLa cells by Ebola virus GP pseudotypes.[15] Given the importance of low endosome pH during the GP-mediated internalization process, lysosomotropic agents[16,27] which prevent vesicle and endosome acidification are also potential antifiloviral agents. Indeed, HeLa cells pretreated with the bafilomycin A1 (**5**), an inhibitor of vacuolar ATPase, are resistant to infection by pseudotyped HIV-1 virions.[15] This general observation has been touted as evidence for the existence of an acid-dependent, postendocytic conformational change in the virus GP[17] or, as has more recently been suggested, that one or more acid-dependent endosomal proteases are involved the infection process. In this regard, the groups of Cunningham[18] and White[19] have independently demonstrated that the proteases, cathepsin B (CatB) and cathepsin L (CatL), play a key role in viral entry by mediating proteolysis of the EBOV GP. Selective inhibitors of the former enzyme, including **6**, have been shown to reduce the infectivity of VSV pseudotypes bearing Ebola virus GP. The importance of CatB in the EBOV infection process is further underlined by Goldsmith's observation[17] that pretreatment of HeLa cells with the CatB *N*-glycosylation inhibitor tunicamycin (**7**)[20] serves to diminish infection by Ebola GP-pseudotyped HIV virions. Unfortunately, given the demonstrated hypersensitivity of Ebola GP to digestion by other proteases,[21] such as thermolysin, the clinical prospects for antiviral agents that solely target CatB and CatL are not encouraging. More recently, Davey and coworkers have successfully employed siRNA screening to identify cellular gene products, namely calmodulin kinase 2 and phosphatidylinositol-3-kinase, which play critical roles in the infection of cells by the Zaire Ebola virus.[22] Having identified these potential targets for therapeutic intervention, it was found that KN-93[23] and LY294002,[24] known inhibitors of these respective enzymes, effectively block infection by both Ebola GP-pseudotyped HIV particles and live Ebola viruses.

While the inherent difficulties associated with handling, filoviruses have undoubtedly contributed to the slow pace of the development of potential therapeutic agents, the advent of replication-competent pseudotyped or chimeric viruses[25] which utilize the replication machinery of vesicular stomatitis viruses (VSV),[26] murine leukemia viruses (MLV),[27] or human immunodeficiency viruses (HIV)[28] but package the Ebola and Marburg membrane glycoproteins (GP) on the virion surface, offer an opportunity to screen libraries of small molecules for antiviral properties under low level containment (biosafety level 2).[29] Furthermore, because this is a cell-based assay, it has an inherent bias against hit compounds which display cytotoxic properties. While it must be understood that pseudotyped viruses are not the same as wild type filoviruses, the functionality of the surface Ebola glycoprotein in these systems has been shown to closely mimic the native virus with regards to tropism, cell binding, and penetration.[27] Given the

proximal nature of viral fusion and entry during the life cycle of filoviruses, it is this process which appears to merit the greatest attention, vis-à-vis the development of small-molecule antiviral agents.[30] Herein, we report the development of an antifiloviral screening system, based on a pseudotyping strategy, and its application in the discovery of a novel group of small molecules, based on isoxazole **8a** (Figure 2), that selectively inhibit the GP-mediated entry of Ebola and Marburg viruses into human cells.



8a **9**

Figure 2. Using an Ebola Zaire glycoprotein (GP)-pseudotyped HIV virus bearing a luciferase (*luc*) reporter gene and Human 293T, a library of 237 small molecules was assayed for antiviral activity based on prevention of Ebola GP-mediated viral entry. The luciferase activities of the infected cells were determined as a measure of GP-mediated viral infection. From this assay, compounds **8a** and **9** were identified as antiviral agents that inhibit viral cell entry in a dose-dependent manner.

Results and Discussion

1. Chemistry. To confirm the chemical identity of lead hit compound **8a** and also to provide sufficient material for more detailed evaluation of its biological activity, our initial undertaking was to develop an efficient solution-phase synthesis of this small molecule. As shown in Scheme 1, **8a** was conveniently prepared through a Huisgen 1,3-dipolar cycloaddition using the one-pot, copper(I)-catalyzed method reported by Fokin.[31] Thus, *p*-anisaldehyde (**10a**) was first converted to the corresponding aldoxime **11**[32] via reaction with hydroxylamine in the presence of aqueous sodium hydroxide. Without isolation, this substrate was treated with chloramine-T trihydrate[33] to generate the corresponding nitrile oxide **12**, which in the presence of a catalytic amount of copper(II) sulfate and copper powder, underwent [2 + 3] cycloaddition[34] with terminal alkyne **13**,[35] at ambient temperature, to furnish 3,5-disubstituted isoxazole **8a** in good overall yield. Notably, this reaction proceeded with excellent regioselectivity, as evidenced by analysis of the unpurified reaction product mixture by ^{1}H NMR studies, which indicated the absence of the regioisomeric cycloadduct.

Employing the three step, one-pot sequence detailed in Scheme 1, a library of C-5 substituted analogues of compound **8a** were prepared from the corresponding aryl and aliphatic aldehydes and alkyne **13**. In all cases, only a single isoxazole regioisomer was isolated from the reaction mixture (Table 1). To evaluate the impact of changes in both the steric and electronic properties of the arene ring, a variety of substitution patterns were examined as well as heterocyclic analogues, as in the case of **8m**.

To examine the role which the core isoxazole ring of **8a** plays in the antifiloviral activity of this compound, a group of 1,4-disubstituted 1,2,3-triazoles analogues **15** were synthesized through the 1,3-dipolar cycloaddition of aryl and alkyl azides **14** with terminal alkyne **13** (Table 2). In this case, the copper(I) catalyst was generated in situ by the reduction of copper(II) sulfate with sodium ascorbate, as described by Sharpless.[36]

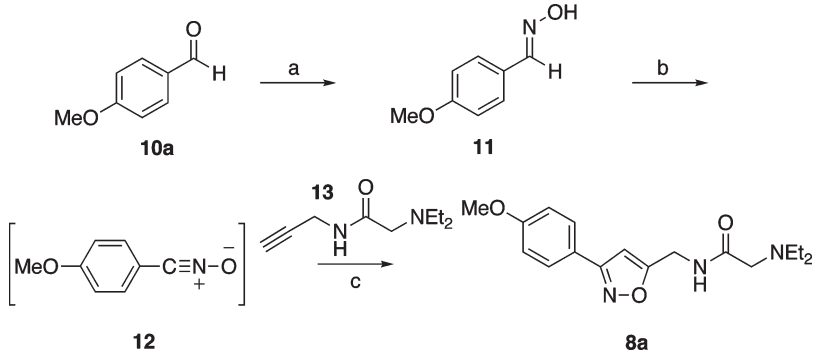
In an effort to study the effect of structural variation at and within the 5-(diethylamino)acetamido substituent in **8a**, two subgroups of derivatives of this system were prepared: those bearing a stereogenic center at the α-position and simple amide derivatives derivatives. The first group of derivatives where conveniently accessed through the coupling of **18** (Scheme 2) with a variety of protected amino acid derivatives **19** (Table 3). Common building block **18** in turned was synthesized from cycloadduct **16** through a sequence of *O*-tosylation, substitution with azide and Staudinger reduction.

Unexpectedly, amide coupling of primary amine **18a** (R = OMe) with carboxylic acids **19** proved to be sluggish when mediated by carbodiimide reagents, such as EDC, and lead to incomplete conversion. In an alternative approach to improve the overall efficiency of this transformation, it was found that activation of the amino acid derivatives as their corresponding mixed isobutyl anhydrides and subsequent treatment with **18a** at low temperature (−40 °C) provided the desired amides **20a**−**g** in good overall yield (Table 3).

While attempts to remove the Cbz protecting groups in **20d** and **20f** under hydrogenolytic conditions, in order to gain the corresponding amine for evaluation and coupling with other amino acids or peptides failed, exposure of these compounds to Pd/C under an atmosphere of H_2 did provide a 1:2 mixture of enaminone **21** and 5-methylisoxazole **22** (Scheme 3). Ultimately, access to free amine **20b** was accomplished by treatment of **20a** with TFA.

The second series of aminomethyl derivatives, **20h**−**n**, incorporated various simple alkyl and aryl chains, including methyl, ethyl, isopropyl, phenyl, and bromomethyl groups.

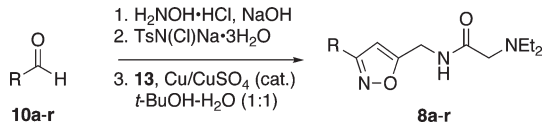
Scheme 1. One-Pot, Three-Step Synthesis of 3,5-Disubstituted Isoxazole **8a**[a]



10a **11** **12** **8a**

[a] Reagents and conditions: (a) $NH_2OH \cdot HCl$, NaOH, *t*-BuOH-H_2O (1:1), rt, 4 h; (b) TsN(Cl)Na·$3H_2O$ (chloramine-T), rt, 3 min; (c) **13**, $CuSO_4 \cdot 5H_2O$ (3 mol %), Cu powder (6 mol %), NaOH aq, rt, 12 h.

Table 1. Preparation of C-5 Substituted Analogues of Lead Compound **8a**



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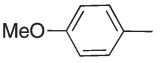
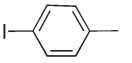
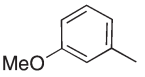
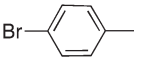
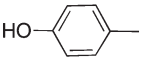
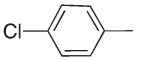
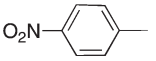
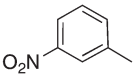
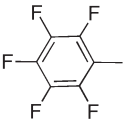
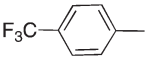
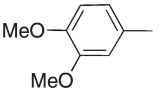
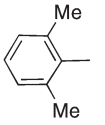
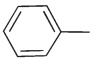
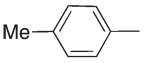
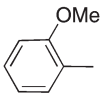
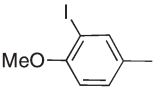
1		**8a**	10		**8j**
2		**8b**	11		**8k**
3		**8c**	12		**8l**
4		**8d**	13		**8m**
5		**8e**	14		**8n**
6		**8f**	15	*n*-Pr	**8o**
7		**8g**	16		**8p**
8		**8h**	17		**8q**
9		**8i**	18		**8r**

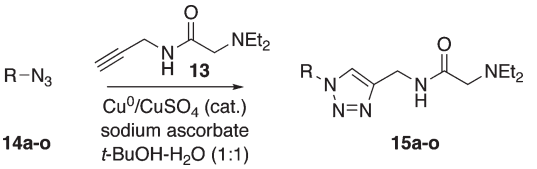
All compounds in this group were prepared via the coupling of **18a** or **18b** and the corresponding carboxylic acids to give the desired C-3/C-5 substituted isoxazole products in good to excellent overall yield (Table 4).

Other analogues of lead compound **8a**, including 4, 5-dihydroisoxazole **27**, ester derivative **28**, and thioamide **29**, were prepared by the dipolar cycloaddition of the nitrile oxide generated from oxime **11** (Scheme 4). The respective dipolarophiles in these reactions, **24**, **25**, and **26**, were readily obtained from *N*,*N*-diethylglycine.

2. Biology. To produce Ebola virus GP pseudotyped HIV virions, 293T cells were cotransfected with the DNA of wild type Ebola Zaire GP (EboZ GP)[28] and the vector pNL4-3. Luc.R-E, which contains the *env* deficient HIV proviral genome and an intact firefly luciferase reporter gene (*luc*) inserted into the pNL4−3 *nef* gene.[37,38] This proviral construct expresses luciferase activity as a marker of viral gene expression, and although it carries a deletion within the *env* coding region, it contains all sequences necessary for reverse transcription, vector integration, and expression of the reporter gene. Upon transfection of cells, the HIV vector and the plasmid encoding the EboZ virus envelope protein are coated and expressed, generating GP pseudotyped HIV virions. These particles are assembled, released by cell lysis, and harvested. To determine the level of incorporation of wt GP protein into the pseudotyped viruses, Western blot analysis of the transfected 293T cell lysates was employed.

To screen for potential Ebola entry inhibitors, individual compounds (30−60 μM, final concentration) were mixed with Ebola GP pseudotyped HIV and the mixture incubated with the target cells (293T). At 24 and 48 h postinfection, cell morphology was examined for signs of toxicity using light microscopy. At 48 h post infection, the cells were lyzed and the level of viral infection analyzed by measuring firefly

Table 2. Preparation of C-1 Substituted, 1,2,3-Triazole Analogues of Compound **8a**



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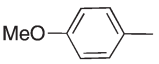
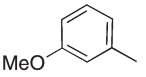
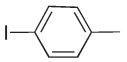
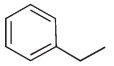
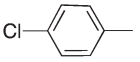
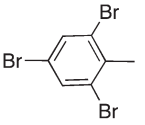
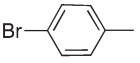
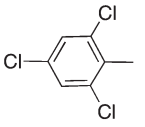
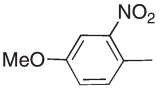
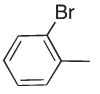
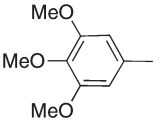
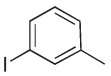
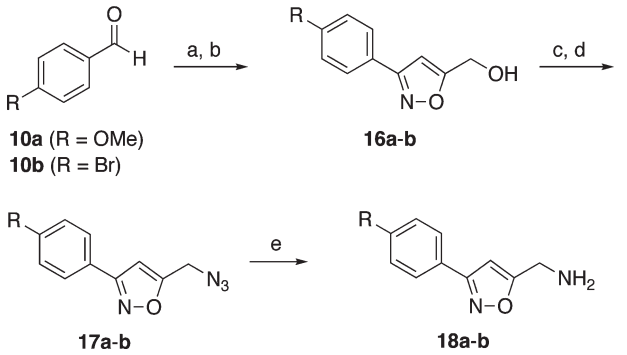
1		**15a**	9		**15i**
2		**15b**	10		**15j**
3		**15c**	11		**15k**
4		**15d**	12		**15l**
5	*n*-heptyl	**15e**	13		**15m**
6		**15f**	14		**15n**
7		**15g**	15		**15o**
8		**15h**			

luciferase enzyme activity using a luminometer. DMSO, the vehicle in which test compounds were dissolved, was used as a background control (final concentration of 0.1−0.2%) and found not to significantly effect infection. Compounds initially identified as viral entry inhibitors were retested in order to confirm their antiviral properties. To confirm that the hit compounds displayed specificity for the function of the Ebola glycoprotein, VSV-G-pseudotyped HIV virions, which carry the envelope protein of the vesicular stomatitis virus (VSV), were produced and the effect of infectivity also examined. Inhibition of Ebola GP-mediated entry but not VSV-G suggests that inhibition is specifically effecting Ebola GP-mediated entry rather than the pseudotyped core: the only difference between Ebola GP and VSV-G pseudotype viruses is the envelope protein and viral entry. In contrast, inhibition of both Ebola and VSV-G entry likely suggests one of two possible situations: (1) the compound in question inhibits postentry replication of the HIV vector, (2) the compound is toxic to cells. To exclude the possibility of cell line bias, compounds were screened against both 293T and HeLa cells for which Ebola GP pseudotyped HIV virions exhibit tropism.[17] Select compounds found to specifically inhibit Ebola cell entry were finally evaluated at a range of concentrations in order to determine the dose-dependent inhibition (IC_{50}).

From an initial screen of an "in-house" library of 237 small molecules, two compounds (**8a** and **9**, Figure 2) were found to decrease infectivity of the Ebola pseudotype virus without apparent side effects on cell morphology and growth. Consequently, these hit compounds were further evaluated for specificity (Figure 3). In comparison to a DMSO control, compound **8a** blocked Ebola GP entry by over 50% at

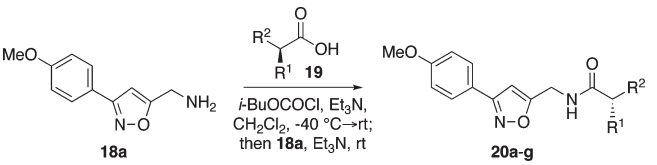
concentrations of 30 μM in both 293T and HeLa cells. In contrast, at this concentration, **8a** did not affect VSV-G mediated cell entry compared to DMSO. Viral infection without added reagents was also examined as a control (NC), and indicated that there was no differences between NC and DMSO (data not shown). These findings suggest that **8a** specifically blocks Ebola GP-mediated viral entry independent of cell type. Titration showed that the IC_{50} of **8a** is approximately 30 μM (Figure 4). Furthermore, **8a** did not affect VSV-G entry even at 120 μM (data not shown), indicating that the inhibition is specific to Ebola entry and not due to cytotoxicity. In contrast to **8a**, similar analysis of hit compound **9** (data not shown) revealed this oxazolidinone derivative to be a nonselective inhibitor of Ebola-GP, Marburg-GP, and VSV-G mediated cell entry. In light of these findings, further studies of this compound were not undertaken.

Scheme 2. Preparation of Building Blocks **18a** and **18b**[a]



[a] Reagents and conditions: (a) $NH_2OH \cdot HCl$, NaOH, *tert*-BuOH, H_2O, rt; (b) chloramine-T, propargyl alcohol, $CuSO_4 \cdot 5H_2O$ (3 mol %), Cu powder (6 mol %), NaOH aq, rt; (c) TsCl, Et_3N, CH_2Cl_2; (d) NaN_3, DMF; (e) PPh_3, H_2O, THF, 0 °C → rt, 3 h.

Table 3. Preparation of C-5 Amidomethyl Analogues of Isoxazole **8a**



entry	R^1	R^2	product
1	NHBoc	Bn	**20a**
2	NH_2	Bn	**20b**[a]
3	NHCbz	*i*-Pr	**20c**
4	NHCbz		**20d**
5	NHAc	CH_2SH	**20e**
6	NHCbz	H	**20f**
7	NHBoc	CH_2COOBn	**20g**

[a] Obtained via Boc deprotection of **20a** with TFA.

To optimize the antiviral properties of **8a**, 55 derivatives of this lead compound were synthesized and evaluated for inhibition of EBOV-GP-mediated viral-cell entry. The results of this investigation are outlined in Tables 5 (isoxazoles) and 6 (triazoles). Most notably, a number of the analogues of **8a** were found to be significantly more potent inhibitors of cell entry, e.g. movement of the methoxy group on the aryl ring from the *para* to *meta* position enhanced the anti-Ebola entry activity (**8b**). Replacement of the methoxy group with halogens, including iodine, bromine, or chlorine, also improved anti-Ebola entry activity (**8k**, **8j**, and **8l**), as did replacement of the *para*-methoxylphenyl group with a 2-furyl substituent (**8m**).

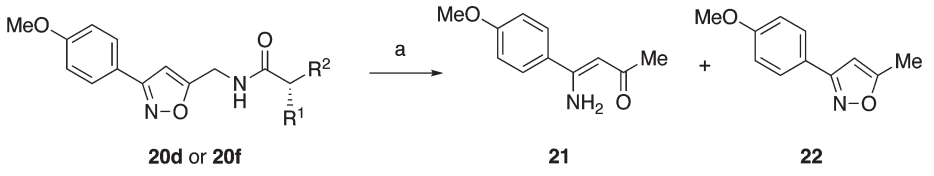
An examination of Table 6 reveals that substitution of the core isoxazole ring in **8a** with a triazole system did not adversely impact antiviral efficacy. Indeed, a number of triazoles, most notably **15b** and **15k**, displayed greater activity than lead compounds **8a**. On the basis of these findings, four of the most active derivatives (**8b**, **8j**, **15b**, and **15k**) were chosen and their IC_{50} values measured using 293T cells (Figure 5). While the IC_{50} of **8b** was improved to 10 μM, compounds **15b** and **15k** displayed higher activity of 5 μM. The highest anti-Ebola activity was shown by **8j** ($IC_{50} = 2.5$ μM). Notably, none of these four compounds displayed inhibition of VSV-G-mediated cell entry (data not shown).

Replacement of the aryl *p*-methoxy substituent with a more polar hydroxyl group led to a decrease in antiviral activity as did removal of the removal of the diethylamino group (**20j**), removal of the ethanamide and diethylamino groups (**22**), and replacement of the amide with an ester (**28**).]

Somewhat unexpectedly, a number of compounds, most notably **8q**, the *p*-methyl analogue of **8a**, and cysteine derivative **20e**, actually enhanced the infectivity of Ebola virus GP pseudotyped HIV virions with 293T cells up to 4-fold. It should be noted, however, that this enhancement of infectivity was found to be cell type-specific because **8q** and **20e** did not enhance Ebola GP-mediated entry in HeLa cells (data not shown). Interestingly, only derivative **20n** displayed appreciable toxicity toward cells: at concentrations of 60 μM and above, this compound killed 293T cells, which all other compounds evaluated did not show any obvious effects on cell morphology and growth. The pronounced cytotoxicity of **20n** may arise from the presence of the α-bromoamide group, which may act as a biological alkylating agents: notable in this regard is the lack of toxicity displayed by compound **20j**, the debrominated derivative of **20n**.

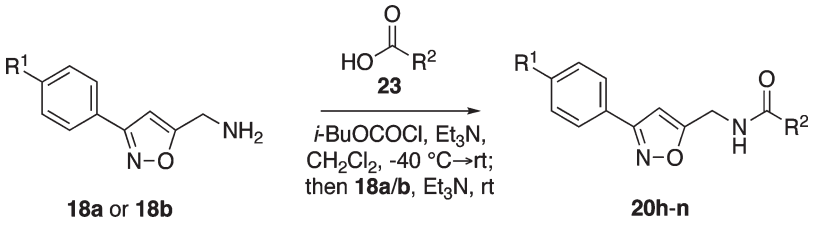
Since an earlier study (LR) suggested that Ebola and Marburg viruses might share a common cellular factor for viral entry,[28] lead compound **8a** and the three derivatives

Scheme 3. Attempted Hydrogenolytic Deprotection of Cbz Derivatives **20d** and **20f**[a]



[a] Reagents and conditions: (a) H_2, Pd/C (10 mol %), EtOAc, 3 h, rt.

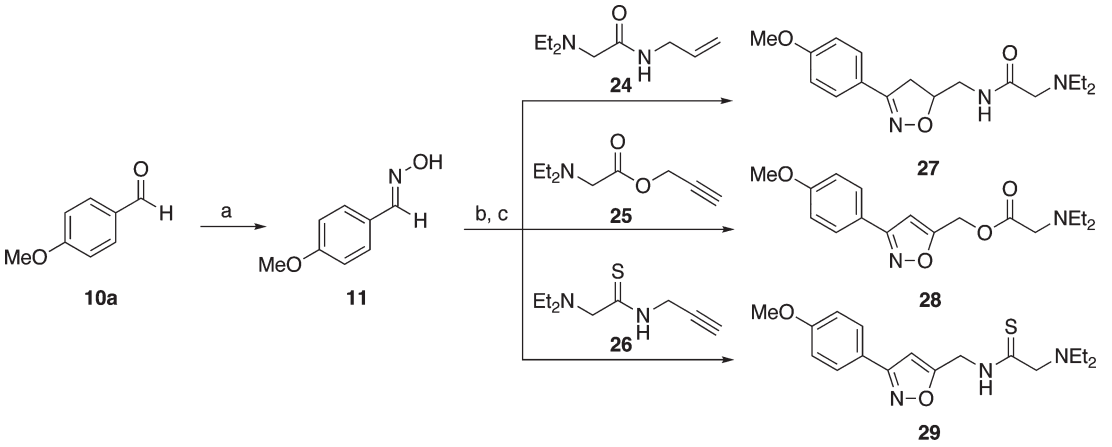
Table 4. Synthesis of C-3/C-5 Substtuted Isoxazoles Derivatives of **8a**



entry	R^1	R^2	product
1	MeO	*I*-BuO	**20h**[a]
2	MeO	CH_2Br	**20i**
3	Br	Me	**20j**
4	Br	Et	**20k**
5	Br	*i*-Pr	**20l**
6	Br	Ph	**20m**
7	Br	CH_2Br	**20n**

[a] Prepared by treatment of **8a** with *i*-BuOCOCl.

Scheme 4. Synthesis of C-5 Amidomethyl Analogues of **8a**[a]



[a] Reagents and conditions: (a) $NH_2OH \cdot HCl$, NaOH, *tert*-BuOH, H_2O, rt, 4 h; (b) chloramine-T, rt, 3 min; (c) **24**, **25**, or **26**, $CuSO_4 \cdot 5H_2O$ (3 mol %), Cu powder (6 mol %), NaOH aq, rt, 12 h.

that displayed improved anti-Ebola activity (**8j**, **15b**, and **15k**) were also evaluated for their ability to inhibit Marburg GP-mediated viral entry (Figure 6). Each compound (final concentration 60 μM) was used to block Marburg entry into three different cell lines (293T, HeLa and A549). In comparison to a DMSO control, compound **8a** blocked 20−40% of Marburg entry, while **8j**, **15b**, and **15b** displayed more dramatic blocking of Marburg entry, especially in HeLa and A549 (human lung carcinoma) cells (> 90%). Therefore, **8a** and three derivatives not only blocked Ebola entry but also impaired viral entry mediated by Marburg GP independent of cell types. This further supports the hypothesis that Ebola and Marburg viruses share a common cellular factor, and a common entry inhibitor could be developed for both viruses.

Conclusions

In summary, we report the development of an antifiloviral screening system, based on a pseudotyping strategy, and its application in the discovery of a novel group of small molecules that selectively inhibit the Ebola and Marburg glycoprotein (GP)-mediated infection of human cells. Using Ebola Zaire GP-pseudotyped HIV particles bearing a luciferase reporter gene and 293T cells, a library of 237 small molecules was screened for inhibition of GP-mediated viral entry. From this assay, lead compound **8a** was identified as a selective inhibitor of filoviral entry with an IC_{50} of 30 μM. To analyze functional group requirements for efficacy, a structure−activity relationship analysis of this 3,5-disubstituted isoxazole was then conducted with 56 isoxazole and triazole derivatives prepared using "click" chemistry. This study revealed that while the isoxazole ring can be replaced by a triazole system, the 5-(diethylamino)acetamido substituent found in **8a** is required for inhibition of viral cell entry. Variation of the 3-aryl substituent also provided a number of more potent antiviral agents with IC_{50} values ranging to 2.5 μM. Lead compound **8a** and three of its derivatives were also found to block the Marburg glycoprotein (GP)-mediated infection of human cells. This group of compounds are not only potential leads in the search for therapeutic antifiloviral candidates but may also serve as mechanistic probes in the elucidation of the mechanism of filoviral fusion and entry.

Experimental Section

Chemistry. All nonaqueous reactions were carried out in oven- or flame-dried glassware under an atmosphere of nitrogen

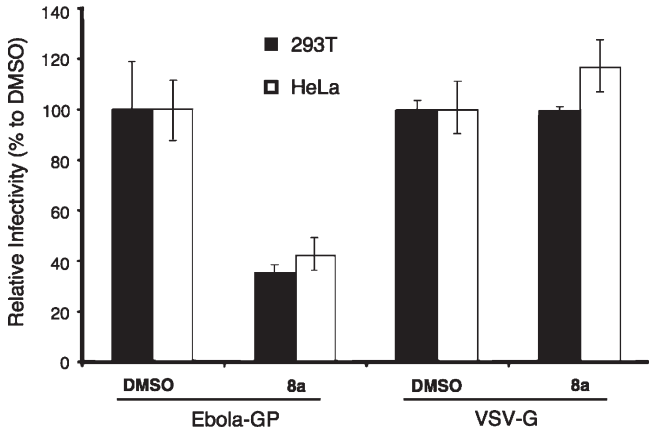


Figure 3. Inhibition of Ebola GP-mediated infection of 293T and HeLa cells. Shown are the effects of lead compound **8a** (final concentration, 30 μM) on the infectivity of Ebola virus GP (Ebola-GP) and VSV-G pseudotyped HIV virions (VSV-G) with 293T (■) and HeLa (□) cell lines. To determine the level of viral infection, 293T and HeLa cells were challenged with pseudotypes in the presence and absence of test compounds. After 48 h, luciferase expression was assessed using the method described in the Experimental Section. Infectivity is expressed as a percentage of luciferase activity, relative to the DMSO control. Values are reported as mean of data from 3−5 independent experiments, ± standard error.

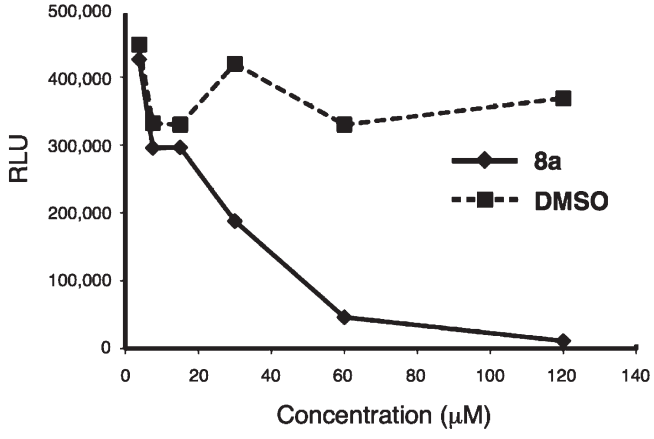


Figure 4. Concentration−response curve for DMSO control (■) and compound **8a** (◆) (range = 3.75−120 μM), which inhibits Ebola GP-mediated viral entry into 293T cells in a dose-dependent manner with an IC_{50} of 30 μM. After 48 h, luciferase expression was assessed using the method described in the Experimental Section and is expressed as relative light units (RLU). Values are reported as mean of data from three independent experiments.

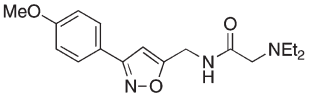
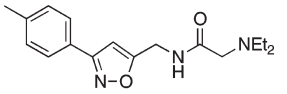
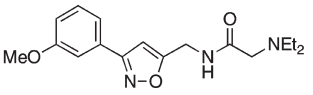
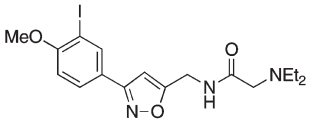
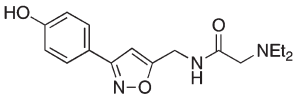
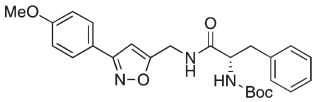
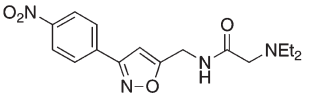
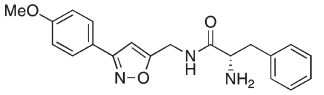
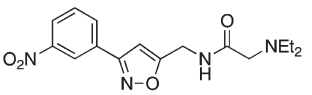
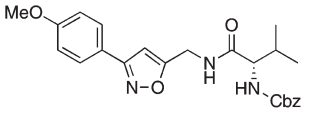
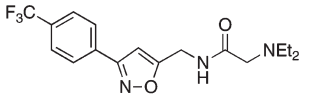
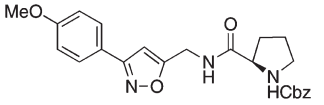
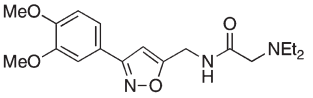
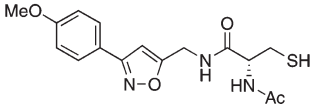
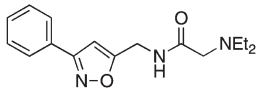
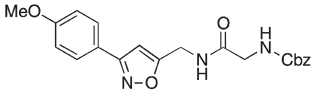
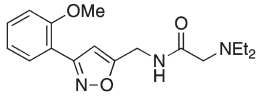
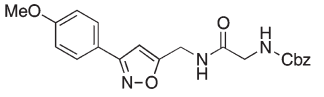
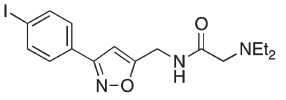
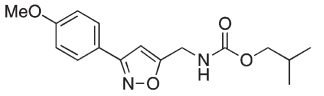
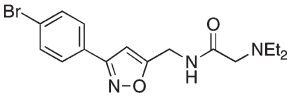
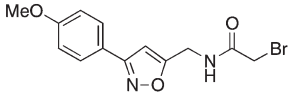
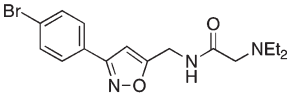
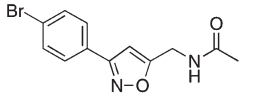
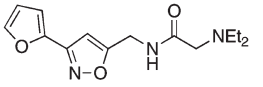
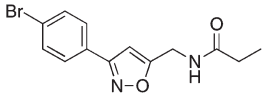
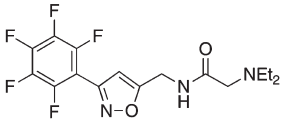
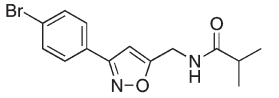
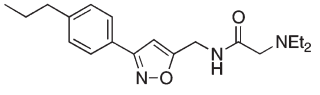
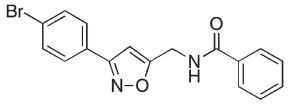
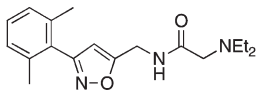
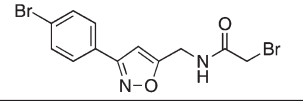
unless otherwise noted. All solvents were reagent grade. Triethylamine was distilled from calcium hydride, under nitrogen, and stored over potassium hydroxide. *N*,*N*-dimethylformamide (DMF) was purchased from a commercial vendor and dried with freshly activated 4 Å molecular sieves prior to use. All products were purified by flash column chromatography using silica gel 60 (mesh 230−400). All other reagents and starting materials, unless otherwise noted, were purchased from commercial vendors and used without further purification. *N*,*N*-Diethylglycine and 3-(4-methoxyphenyl)-5-hydroxymethyl isoxazole (**16a**) were prepared according to procedures reported by Tonellato[39] and Fokin,[31] respectively. All melting points were determined in open Pyrex capillaries with a Thomas−Hoover Unimelt melting point apparatus and are uncorrected. IR spectra were recorded as thin films on sodium chloride plates or as suspensions in compressed potassium bromide discs. ^{1}H and ^{13}C spectra were recorded on a Bruker Avance 400 (400 MHz ^{1}H, 100 MHz ^{13}C) or a Bruker Avance 500 (500 MHz ^{1}H, 125 MHz ^{13}C) spectrometer. DEPT 135 and two-dimensional (COSY, HMQC, HMBC) NMR experiments were employed, where appropriate, to aid in the assignment of signals in the ^{1}H NMR spectra. The purity of the compounds was determined using an analytical HPLC (Agilent 1100 with diode array detector, Supercosil LC 18 (250 mm × 4.6 mm, 5 μm) acetonitrile (unless otherwise noted), 2.00 mL/min) and was confirmed to be ≥95% for all compounds.

General Synthetic Procedure A. Preparation of 3,5-Disubstituted Isoxazoles, as Exemplified by the Preparation of 2-(Diethylamino)-*N*-((3-(4-methoxyphenyl)isoxazol-5-yl)methyl)ethanamide (8a). To a solution of *p*-anisaldehyde (140 mg, 1.03 mmol, 1.0 equiv) and hydroxylamine hydrochloride (75 mg, 1.07 mmol, 1.05 equiv) in a mixture of *t*-BuOH and H_2O (1:1, 4 mL) was added 1 M aqueous NaOH (1.1 mL). The reaction mixture was stirred at rt for 4 h or until thin-layer chromatography indicated consumption of the aldehyde. After oxime formation was complete, chloramine-T (301 mg, 1.07 mmol, 1.05 equiv) was added portionwise over 3 min, followed by $CuSO_4 \cdot 5H_2O$ (5 mg, 3 mol %) and copper powder (4 mg, 6 mol %). *N*′-Propargyl-*N*,*N*-diethylglycine (**13**) (182 mg, 1.07 mmol, 1.05 equiv) was then added, the pH of the reaction medium was adjusted by the addition of 1 M aqueous NaOH (6 drops), and the mixture wasstirred at rt for 12 h. The reaction was then quenched with 1 M aqueous NH_4OH (1 mL), extracted with EtOAc (3 × 15 mL), and the combined organic extracts dried (Na_2SO_4) and concentrated under reduced pressure. The crude product was purified by flash chromatography over silica gel (EtOAc) to provide **8a** (209 mg, 64%); white solid; mp 77−78 °C; R_f 0.15 (EtOAc); t_R − HPLC: 1.46 min (100%). FTIR (KBr pellet) υ_{max} 3336 (br), 2967 (br), 1674, 1612, 1514, 1432, 1254, 1177, 1028, 838 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.94 (s, 1 H), 7.71 (d, J = 8.7 Hz, 2 H), 6.95 (d, J = 8.7 Hz, 2 H), 6.43 (s, 1 H), 4.61 (d, J = 6.0 Hz, 2 H), 3.84 (s, 3 H), 3.09 (s, 2 H), 2.56 (q, J = 7.1 Hz, 4 H), 1.02 (t, J = 7.1 Hz, 6 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.5, 169.3, 162.3, 161.1, 128.3 (2 C), 121.5, 114.4 (2 C), 100.0, 57.4, 55.5, 48.9 (2 C), 34.9, 12.4 (2 C). HRMS-EI calcd for $C_{17}H_{24}N_3O_3$ $[M + H]^+$ 318.1812; found 318.1802.

General Synthetic Procedure B. Preparation of of *N*,*N*-Diethylglycine Derivatives, as Exemplified by the Preparation of *N*′-Propargyl-*N*,*N*-diethylglycine (13). To a stirred solution of *N*,*N*-diethylglycine (312 mg, 2.4 mmol, 1 equiv) and propargylamine (250 μL, 3.8 mmol, 1.6 equiv) in anhydrous CH_2Cl_2 (3.7 mL) at rt was added EDC (818 mg, 4.3 mmol, 1.8 equiv), followed by addition of Et_3N (530 μL, 1.6 equiv). After stirring for 12 h, the reaction was quenched with 1 M aqueous HCl (500 μL) and extracted with EtOAc (3 × 10 mL). The combined organic phases were washed with brine (2 × 10 mL), dried (Na_2SO_4), concentrated under reduced pressure, and the residue purified by flash chromatography over silica gel (EtOAc/hexanes, 1:3) to afford **13** (488 mg, 52%); yellow oil; R_f 0.25 (EtOAc). IR (film) υ_{max} 3308 (br), 2970, 2934, 2823, 1673, 1516, 1456, 1426, 1347, 1260, 1205, 1089 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.53 (br s, 1 H), 4.01 (dd, J = 2.4, 5.4 Hz, 2 H), 2.98 (s, 3 H), 2.50 (q, J = 7.1 Hz, 4 H), 2.18 (t, J = 2.4 Hz, 1 H), 0.98 (t, J = 7.1 Hz, 6 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.0, 79.8, 71.3, 57.4, 48.9 (2 C), 28.6, 12.4 (2 C). HRMS-EI calcd for $C_9H_{16}N_2O$ $[M + H]^+$ 96.1072; found 96.1074.

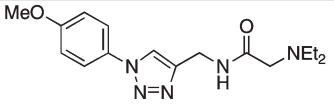
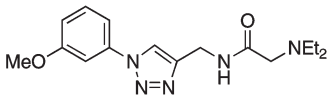
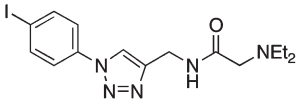
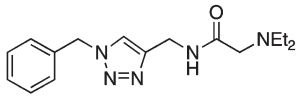
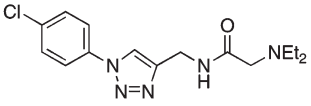
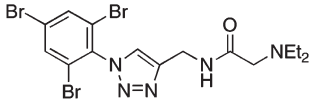
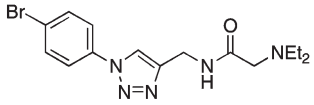
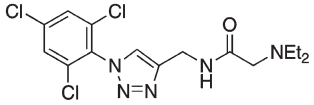
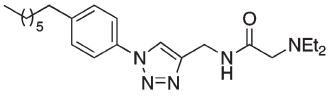
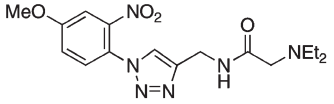
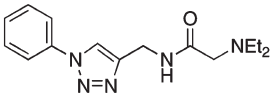
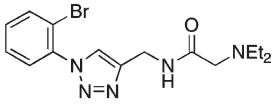
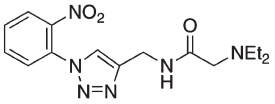
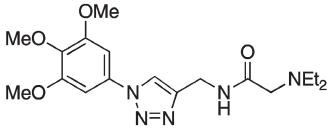
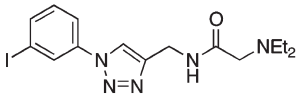
General Synthetic Procedure C. Preparation of 3,5-Disubstituted Triazoles, as Exemplified by the Preparation of 2-(Diethylamino)-*N*-((1-(4-methoxyphenyl)-1*H*-1,2,3-triazol-4-yl)methyl)ethanamide (15a). To a solution of 1-azido-4-methoxybenzene (108 mg, 0.72 mmol, 1.0 equiv) in a mixture of *t*-BuOH and H_2O (1:1, 3 mL) was added *N*′-propargyl-*N*,*N*-diethylglycine (**13**) (109 mg, 0.72 mmol, 1.0 equiv), followed by addition of 1 M aqueous sodium ascorbate (72 μL, 0.1 mmol) and $CuSO_4 \cdot 5H_2O$ (2.5 mg, 0.02 mmol, 6 mol %). The heterogeneous reaction mixture was stirred vigorously at rt for 12 h. After completion of the reaction, as indicated by thin-layer chromatography, it was diluted with water (15 mL) and extracted with EtOAc (3 × 15 mL). The combined organic extracts were dried (Na_2SO_4), filtered, and concentrated under reduced pressure.

Table 5. Anti-Ebola Activity Data for Lead Compound **8a** and its Isoxazole Analogues as Inhibitors of Ebola GP-Mediated Cell Entry[a]

Compound	*Structure*	*Infectivity*[b]	*Compound*	*Structure*	*Infectivity*
8a		56	**8q**		381
8b		4	**8r**		4
8c		132	**20a**		188
8d		30	**20b**		106
8e		37	**20c**		220
8f		24	**20d**		254
8g		160	**20e**		421
8h		181	**20f**		148
8i		87	**20g**		118
8j		6	**20h**		118
8k		30	**20i**		121
8l		17	**20j**		105
8m		77	**20k**		99
8n		52	**20l**		116
8o		102	**20m**		82
8p		59	**20n**		Kills cells at 60 μM

[a] Ebola virus GP pseudotyped HIV virions were mixed with compounds prior to infecting 293T cells (final concentration of compounds was 60 μM) After 48 h, luciferase expression was assessed using the method described in the Experimental Section. [b] Infectivity is expressed as a percentage of luciferase activity, relative to the DMSO control.

Table 6. Anti-Ebola Activity Data for Lead Compound **8a** and its Triazole Analogues as Inhibitors of Ebola GP-Mediated Cell Entry[a]

Compound	Structure	Infectivity[b]	Compound	Structure	Infectivity
15a		58	**15i**		90
15b		4	**15j**		35
15c		105	**15k**		3
15d		18	**15l**		33
15e		60	**15m**		92
15f		46	**15n**		19
15g		66	**15o**		69
15h		111			

[a] Ebola virus GP pseudotyped HIV virions were mixed with compounds prior to infecting 293T cells (final concentration of compounds was 60 μM) After 48 h, luciferase expression was assessed using the method described in the Experimental Section. [b] Infectivity is expressed as a percentage of luciferase activity, relative to the DMSO control.

The crude 1,2,3-triazole was purified by flash chromatography over silica gel (EtOAc) to provide **15a** (174 mg, 82%); off-white solid; mp 109−110 °C; R_f 0.10 (EtOAc); t_R −. HPLC: 5.4 min (MeOH, 99.6%). FTIR (KBr pellet) υ_{max} 3349 (br), 2966 (br), 1672, 1518, 1255, 1043, 833 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.05 (br s, 1 H), 7.89 (s, 1 H), 7.60 (dd, J = 1.9, 5.0 Hz, 1 H), 7.00 (dd, J = 1.9, 5.0 Hz, 1 H), 4.60 (d, J = 6.2 Hz, 2 H), 3.86 (s, 3 H), 3.05 (s, 2 H), 2.53 (q, J = 7.2 Hz, 4 H), 0.99 (t, J = 7.2 Hz, 6 H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.6, 160.0, 145.6, 130.7, 122.3 (2 C), 120.7, 114.9 (2 C), 57.7, 55.8, 48.9 (2 C), 34.59, 12.4 (2 C). HRMS-EI calcd for $C_{16}H_{23}N_5O_2$ $[M + H]^+$ 318.1930; found 318.1942.

General Synthetic Procedure D. Preparation of 3,5-Disubstituted Isoxazoles via Amide Coupling, as Exemplified by the Preparation of Benzyl 2-((3-(4-Methoxyphenyl)isoxazol-5-yl)methylamino)-2-oxoethylcarbamate (20f). To a stirred solution of carbobenxyloxyglycine (200 mg, 0.618 mmol, 1 equiv) in anhydrous CH_2Cl_2 (5 mL) under an atmosphere of nitrogen, at −40 °C ($MeCN/CO_2$), was added isobutyl chloroformate (105 μL, 0.624 mmol, 1.05 equiv) and triethylamine (86 μL, 0.618 mmol, 1 equiv). After stirring for 10 min, the cold bath was removed and reaction mixture was allowed to warm to rt and stirred for 12 h. 3(4-Methoxyphenyl)isoxazol-5-yl)methanamine (**18b**) (100 mg, 0.618 mmol, 1 equiv) and triethylamine (86 μL, 0.618 mmol, 1 equiv) were then added sequentually. The reaction mixture was stirred for 12 h at rt and then quenched with 1 M aqueous HCl, and the aqueous portion extracted with EtOAc (3 × 20 mL). The combined organic extracts were dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The residue was purified by flash chromatography over silica gel (EtOAc/hexanes, 1:4) to provide **20f** (332 mg, 88%); white solid; mp 117−119 °C; R_f 0.28 (EtOAc/hexanes, 1:3); t_R −. HPLC: 1.46 min (100%). FTIR (KBr pellet) υ_{max} 3323, 3285, 1690, 1658, 1548, 1293, 1247, 1165, 1074 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.41 (d, J = 8.7 Hz, 2 H), 8.03 (m, 5 H), 7.65 (d, J = 8.7 Hz, 2 H), 7.27 (s, 1 H), 6.08 (br s, 1 H), 5.96 (s, 2 H), 5.81 (s, 2 H), 4.69 (d, J = 5.9 Hz, 2 H), 4.54 (s, 3 H), 2.86 (s, 1 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.7, 166.2, 162.4, 161.3, 156.5, 129.7, 128.7 (2 C), 128.3 (5 C), 121.1, 114.5 (2 C), 102.5, 67.4, 57.2, 55.5, 42.7. HRMS-EI calcd for $C_{21}H_{22}N_3O_5$ $[M + H]^+$ 396.4165; found 396.4168.

2-(Diethylamino)-*N*-((3-(3-methoxyphenyl)isoxazol-5-yl)methyl)-ethanamide (8b). Yield 36%; white solid; mp 90−92 °C; R_f 0.10 (EtOAc); t_R − HPLC: 1.47 min (97.6%). FTIR (KBr pellet) υ_{max} 3308 (br), 2966, 2926, 1669, 1608, 1506, 1456, 1254, 1158, 1042, 1000 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.05 (s, 1 H), 7.35 (m, 3 H), 7.00 (m, 1 H), 6.43 (m, 1 H), 6.48 (s, 2 H), 4.64 (d, J = 6.2 Hz, 2 H), 3.86 (s, 3 H), 3.12 (s, 2 H), 2.60 (q, J = 7.1 Hz, 4 H), 1.05 (t, J = 7.1 Hz, 6 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.3, 169.6, 162.7, 160.1, 130.2, 130.1, 119.5, 116.4, 111.8, 100.5, 57.4, 48.9 (2 C), 35.0, 29.9, 12.3 (2 C). HRMS-ESI calcd for $C_{17}H_{24}N_3O_3$ $[M + H]^+$ 318.38122; found 318.18129.

2-(Diethylamino)-*N*-((3-(4-hydroxyphenyl)isoxazol-5-yl)methyl)-ethanamide (8c). Yield 62%; colorless oil; R_f 0.12 (EtOAc); t_R − HPLC: 1.45 min (99.2%). IR (film) υ_{max} 3349 (br), 2967, 2811, 1671, 1517, 1487, 1373, 1203, 1036, 856 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.17 (s, 1 H), 7.56 (d, J = 8.7 Hz, 2 H), 6.87 (d, J = 8.7 Hz, 2 H), 6.31 (s, 1H), 4.59 (d, J = 6.2 Hz, 1 H), 3.12 (s, 2 H), 2.59 (q, J = 7.1 Hz, 4 H), 0.91 (t, J = 7.1 Hz, 6 H).

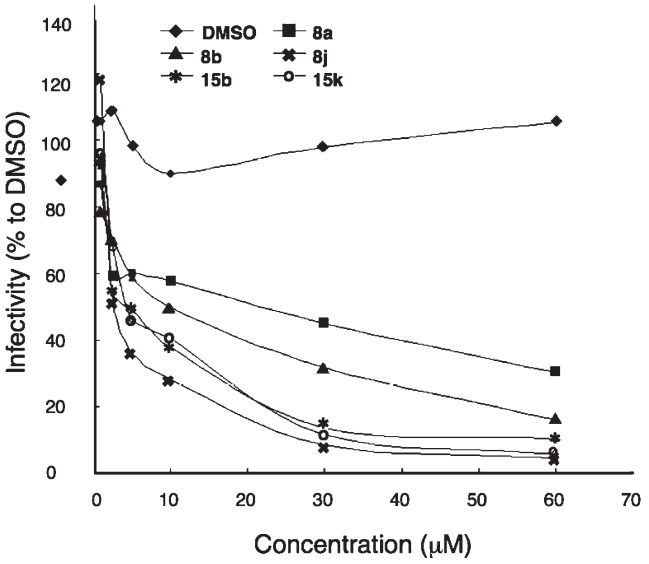


Figure 5. Concentration−response curve for DMSO control (◆) and compounds **8a** (■), **8b** (▲), **8j** (×), **15b** (∗) and **15k** (○) (range = 3.75−60 μM), which inhibit Ebola GP-mediated viral entry into 293T cells in a dose dependent manner with respective IC_{50} values of 30, 10, 2.5, and 5 μM (see also Tables 5 and 6). To determine the level of infection, 293T cells were challenged with pseudotypes in the presence and absence of test compounds. After 48 h, luciferase expression was assessed using the method described in the Experimental Section. Infectivity is expressed as a percentage of luciferase activity relative to the DMSO control. Values are reported as mean of data from 3−5 independent experiments, ± standard error.

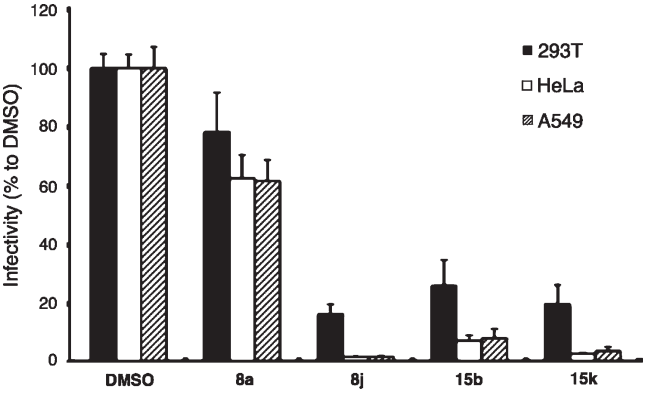


Figure 6. Inhibition of Marburg GP-mediated infection. Shown are the effects of compounds **8a**, **8j**, **15b**, and **15k** (final concentrations, 60 μM) on the infectivity of Marburg GP-pseudotyped HIV virions with 293T (black bars), HeLa (white bars), and A549 (gray bars) cell lines. To determine the level of infection by pseudotype viruses, cells were challenged with pseudotypes in the presence and absence of test compounds. After 48 h, luciferase expression was assessed using the method described in the Experimental Section. Infectivity is expressed as a percentage of luciferase activity relative to the DMSO control. Values are reported as mean of data from three independent experiments, ± standard error.

^{13}C NMR (100 MHz, $CDCl_3$) δ 173.5, 168.8, 162.4, 158.5, 128.5 (2 C), 121.0, 116.2 (2 C), 100.5, 57.4, 49.0 (2 C), 35.1, 12.4 (2 C). HRMS-EI calcd for $C_{16}H_{21}N_3O_3$ $[M + H]^+$ 303.3562; found 303.3568.

2-(Diethylamino)-*N*-((3-(4-nitrophenyl)isoxazol-5-yl)methyl)-ethanamide (8d). Yield 17%; colorless oil; R_f 0.12 (EtOAc); t_R − HPLC: 1.45 min (100%). IR (film) υ_{max} 3338 (br), 2967, 2966, 2853, 1673, 1610, 1522, 1456, 1434, 1229, 1157, 1074, 914, 842 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.32 (d, J = 8.8 Hz, 2H), 8.12 (br s, 1 H), 7.98 (d, J = 8.8 Hz, 2 H), 6.58 (s, 1 H), 4.66 (d, J = 6.3 Hz, 2 H), 3.11 (s, 2 H), 2.58 (q, J = 7.1 Hz, 4 H), 1.04 (t, J = 7.1 Hz, 6 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.8, 170.9, 161.0, 149.0, 135.2, 127.9 (2C), 124.4 (2 C), 100.6, 57.5, 49.0 (2 C), 34.9, 12.5 (2 C). HRMS-EI calcd for $C_{16}H_{21}N_4O_4$ $[M + H]^+$ 333.3623; found 333.3628.

2-(Diethylamino)-*N*-((3-(3-nitrophenyl)isoxazol-5-yl)methyl)-ethanamide (8e). Yield 15%; off-white solid; mp 90−91 °C; R_f 0.11 (EtOAc); t_R − HPLC: 1.45 min (98.8%). FTIR (KBr pellet) υ_{max} 3291, 3152, 3054, 2967, 2932, 2805, 1654, 1525, 1498, 1348, 1224, 1045, 984 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.62 (m, 1 H), 8.31 (dd, J = 2.1, 8.2 Hz, 1 H), 8.15 (d, J = 7.9 Hz, 1 H), 8.04 (br s, 1H), 7.66 (t, J = 8.0 Hz, 1 H), 6.59 (s, 1 H), 4.67 (d, J = 6.3 Hz, 2 H), 3.12 (s, 2 H), 2.60 (q, J = 7.1 Hz, 4 H), 1.05 (t, J = 7.1 Hz, 6 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.7, 170.9, 160.9, 148.8, 132.7, 130.9, 130.3, 124.9, 122.04, 100.4, 57.5, 49.00 (2 C), 35.02, 12.5 (2 C). HRMS-EI calcd for $C_{16}H_{21}N_4O_4$ $[M + H]^+$ 333.3623; found 333.3629.

2-(Diethylamino)-*N*-((3-(4-(trifluoromethyl)phenyl)isoxazol-5-yl)-methyl)ethanamide (8f). Yield 58%; colorless oil; R_f 0.11 (EtOAc); t_R − HPLC: 1.47 min (96.8%). IR (film) υ_{max} 3308, 2967, 2927, 1672, 1608, 1515, 1354, 1252, 1176, 898, 832 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.19 (s, 1 H), 7.91 (d, J = 8.1 Hz, 2 H), 7.72 (d, J = 8.1 Hz, 2 H), 6.55 (s, 1 H), 4.65 (d, J = 6.2 Hz, 2 H), 3.16 (s, 2 H), 2.63 (q, J = 7.2 Hz, 4 H), 1.07 (t, J = 7.2 Hz, 6 H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.3, 161.6, 132.5, 131.9, 128.7, 127.3 (2 C), 126.1 (2 C), 100.4, 57.3, 48.8 (2 C), 35.0, 29.9, 12.15 (2 C). HRMS-ESI calcd for $C_{17}H_{21}F_3N_3O_3[M + H]^+$ 356.15804; found 356.15787.

***N*-(3-(3,4-Dimethoxyphenyl)isoxazol-5-yl)methyl)-2-(diethyl-amino)ethanamide (8g).** Yield 12%; colorless oil; R_f 0.11 (EtOAc); t_R − HPLC: 1.44 min (96.6%). IR (film) υ_{max} 3324 (br), 2965, 2933, 1674, 1605, 1516, 1426, 1262, 1206, 1134, 1074, 809 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.02 (br s, 1 H), 7.39 (d, J = 1.9 Hz, 1 H), 7.27 (d, J = 1.9 Hz, 1 H), 6.92 (s, 1H), 6.91 (s, 1 H), 6.46 (s, 1 H), 5.30 (s, 1 H), 4.61 (d, J = 6.2 Hz, 2 H), 3.91 (s, 3 H), 3.90 (s, 3 H), 3.11 (s, 2 H), 2.58 (q, J = 7.0 Hz, 4 H), 2.17 (s, 2 H), 1.04 (t, J = 7.0 Hz, 6 H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.2, 161.2, 151.6, 150.6, 129.1, 127.4, 119.9, 110.9, 109.2, 107.7, 100.0, 57.2, 56.0, 48.7 (2 C), 34.8, 12.2 (2 C). HRMS-EI calcd for $C_{18}H_{26}N_3O_4$ $[M + H]^+$ 348.4167; found 348.4162.

2-(Diethylamino)-*N*-((3-phenylisoxazol-5-yl)methyl)ethanamide (8h). Yield 66%; white solid; mp 118−120 °C; R_f 0.37 (EtOAc); t_R − HPLC: 1.42 min (100%). FTIR (KBr pellet) υ_{max} 3331 (br), 2965, 2933, 1709, 1612, 1529, 1432, 1366, 1253, 1175, 1029, 837 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.10 (s, 1 H), 7.78 (m, 2 H), 7.45 (m, 3 H), 6.50 (s, 1 H), 4.67 (d, J = 6.2 Hz, 2 H), 3.16 (s, 2 H), 2.63 (q, J = 7.1 Hz, 4 H), 1.07 (t, J = 7.1 Hz, 6 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.6, 162.8, 136.5, 132.2, 130.3, 129.1 (2 C), 127.0 (2 C), 100.4, 57.3, 49.0 (2 C), 35.0, 12.3 (2 C). HRMS-EI calcd for $C_{16}H_{22}N_3O_2[M + H]^+$ 288.3648; found 288.3651.

***N*-((3-(2-Methoxyphenyl)isoxazol-5-yl)methyl)-2-(diethylamino)-ethanamide (8i).** Yield 60%; colorless oil; R_f 0.12 (EtOAc); t_R − HPLC: 1.43 min (100%). IR film υ_{max} 3308 (br), 2962, 2926, 1669, 1603, 1506, 1456, 1254, 1158, 1042 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.97 (br s, 1 H), 7.85 (dd, J = 1.7, 7.7 Hz, 1 H), 7.40 (dt, J = 1.7, 9.0 Hz, 1 H), 7.02 (t, J = 7.5 Hz, 1 H), 7.0 (d, J = 8.4 Hz, 1 H), 4.63 (d, J = 6.1 Hz, 2 H), 3.86 (s, 3 H), 3.09 (s, 2 H), 2.56 (q, J = 7.1 Hz, 4 H), 1.03 (t, J = 7.1 Hz, 6 H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.4, 168.3, 160.3, 157.3, 131.4, 129.5, 121.3, 121.0, 117.9, 111.6, 103.6, 57.4, 49.0 (2 C), 35.0, 12.4 (2 C). HRMS-ESI calcd for $C_{17}H_{24}N_3O_3$ $[M + H]^+$ 318.18122; found 318.18129.

***N*-((3-(4-Iodophenyl)isoxazol-5-yl)methyl)-2-(diethylamino)-ethanamide (8j).** Yield 68%;hite solid; mp 104−105 °C; R_f 0.21 (EtOAc); t_R − HPLC: 1.45 min (95.4%). FTIR (KBr pellet) υ_{max} 3287 (br), 3123, 2968, 2935, 2870, 2819, 1666, 1521, 1425, 1216, 1067, 1027 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.99 (br s, 1H), 7.79 (d, J = 6.5 Hz, 2 H), 7.51 (d, J = 6.5 Hz, 2 H), 6.45 (s, 1 H), 4.63 (d, J = 6.2 Hz, 2 H), 3.08 (s, 2 H), 2.55

(q, J = 7.3 Hz, 4 H), 1.02 (t, J = 7.3 Hz, 6 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.6, 170.1, 162.0, 138, 129.5 (2 C), 128.6 (2 C), 100.2, 96.5, 57.5, 49.0 (2 C), 35.0, 12.5 (2 C). HRMS-ESI calcd for $C_{16}H_{21}IN_3O_2$ $[M + H]^+$ 414.06730; found 414.06654.

***N*-((3-(4-Bromophenyl)isoxazol-5-yl)methyl)-2-(diethylamino)-ethanamide (8k).** Yield 64%; yellow oil; R_f 0.11 (EtOAc); t_R − HPLC: 1.47 min (98.2%). IR (film) υ_{max} 3307 (br), 2966, 2923, 1670, 1508, 1427, 1275, 1072, 830 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.04 (s, 1 H), 7.64 (d, J = 8.4 Hz, 2 H), 7.58 (d, J = 8.4 Hz, 2 H), 6.47 (s, 1 H), 4.63 (d, J = 6.1 Hz, 2 H), 4.29 (s, 2 H), 3.16 (s, 2 H), 2.61 (q, J = 6.9 Hz, 4 H), 0.88 (t, J = 6.9 Hz, 6 H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.5, 170.0, 161.8, 136.3, 129.5 (2 C), 128.3 (2 C), 127.6, 100.3, 57.4, 49.0 (2 C), 35.0, 12.5 (2 C). HRMS-ESI calcd for $C_{16}H_{21}BrN_3O_2$ $[M + H]^+$ 366.08117; found 366.08056.

***N*-(3-(4-Chlorophenyl)isoxazol-5-yl)methyl)-2-(diethylamino)-ethanamide (8l).** Yield 42%; white solid; mp 81−83 °C; R_f 0.11 (EtOAc); t_R − HPLC: 1.46 min (97.1%). FTIR (KBr pellet) υ_{max} 3286 (br), 2969, 1659, 1521, 1424, 1090, 1028, 847, 805 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.01 (s, 1 H), (d, J = 8.4 Hz, 2 H), (d, J = 8.4 Hz, 2 H), 6.47 (s, 2 H), 4.63 (d, J = 6.2 Hz, 2 H), 3.10 (s, 2 H), 2.57 (q, J = 7.1 Hz, 4 H), 1.03 (t, J = 7.1 Hz, 6 H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.6, 170.0, 161.8, 136.3, 129.4 (2 C), 128.3 (2 C), 127.6, 100.3, 57.5, 49.0 (2 C), 35.0, 12.5 (2 C). HRMS-ESI calcd for $C_{16}H_{21}ClN_3O_2$ $[M + H]^+$ 322.13168; found 322.13131.

2-(Diethylamino)-*N*-((3-(2-furyl)isoxazol-5-yl)methyl)ethanamide (8m). Yield 56%; colorless oil; R_f 0.16 (EtOAc); t_R − HPLC: 1.46 min (96.8%). IR (film) υ_{max} 3342 (br), 2966 (br), 2928, 1669, 1517, 1455, 1334, 1259, 1207, 1049 (br) cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.08 (br s, 1 H), 7.54 (dd, J = 0.6, 1.2 Hz, 1 H), 6.87 (dd, J = 0.5, 2.8 Hz, 1 H), 6.51 (dd, J = 1.6, 1.8 Hz, 1 H), 6.43 (s, 1 H), 4.61 (d, J = 6.2 Hz, 2 H), 3.15 (s, 2 H), 2.62 (d, J = 7.1 Hz, 4 H), 1.05 (t, J = 7.1 Hz, 6 H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.4, 155.1, 144.1, 111.9, 110.6, 99.9, 57.01, 49.0 (2 C), 34.9, 29.9, 28.8, 12.3 (2 C). HRMS-EI calcd for $C_{14}H_{20}N_3O_3$ $[M + H]^+$ 278.3269; found 278.3265.

2-(Diethylamino)-*N*-((3-(pentafluorophenyl)isoxazol-5-yl)methyl)-ethanamide (8n). Yield 68%: white solid; mp 100−102 °C; R_f 0.39 (EtOAc); t_R − HPLC: 1.42 min (100%). FTIR (KBr pellet) υ_{max} 3316, 2973, 2939, 2877, 2823, 1670, 1508, 1423, 1388, 1361, 1211, 1079, 995, 817 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02 (br s, 1 H), 6.51 (s, 1 H), 4.70 (d, J = 6.2 Hz, 2H), 3.11 (s, 2 H), 2.58 (q, J = 7.0 Hz, 4 H), 1.04 (t, J = 7.0 Hz, 6 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.4, 170.2, 161.0, 151.7, 145.8, 143.8, 139.0, 137.0, 103.6, 103.1, 57.2, 48.8 (2 C), 34.7, 12.2 (2 C). HRMS-EI calcd for $C_{16}H_{17}F_5N_3O_3$ $[M + H]^+$ 378.3171; found 378.3176.

2-(Diethylamino)-*N*-((3-propylisoxazol-5-yl)methyl)ethanamide (8o). Yield 46%; colorless oil; R_f 0.11 (EtOAc); t_R − HPLC: 1.47 min (100%). IR (film) υ_{max} 3324 (br), 2965, 2933, 1674, 1605, 1516, 1426, 1262, 1206, 1134, 1074, 809 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.82 (br s, 1 H), 6.00 (s, 1 H), 4.55 (d, J = 6.2 Hz, 1 H), 3.10 (s, 2 H), 2.61 (t, J = 7.5 Hz, 2 H), 2.57 (q, J = 7.1 Hz, 4 H), 1.67 (s, 2 H), 1.03 (t, J = 7.1 Hz, 6 H), 0.97 (t, J = 7.4 Hz, 3 H), 1.05 (t, J = 7.1 Hz, 6 H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 168.7, 164.3, 125.5, 101.8, 57.4, 49.0 (2 C), 35.0, 28.2, 21.8, 13.9, 12.4 (2 C). HRMS-EI calcd for $C_{13}H_{24}N_3O_2$ $[M + H]^+$ 254.3486; found 254.3489.

2-(Diethylamino)-*N*-((3-(2,6-dimethylphenyl)isoxazol-5-yl)-methyl)ethanamide (8p). Yield 69%; colorless oil; R_f 0.12 (EtOAc); t_R − HPLC: 1.44 min (96.1%). IR (film) υ_{max} 3319 (br), 2969 (br), 2932, 2871, 2823, 1676, 1601, 1513, 1456, 1423, 1388, 1355, 1207, 1162 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.00 (br s, 1 H), 7.20 (t, J = 7.9 Hz, 1 H), 7.08 (d, J = 7.9 Hz, 2 H), 6.09 (s, 1 H), 4.66 (d, J = 6.3 Hz, 2 H), 3.08 (s, 2 H), 2.54 (q, J = 7.1 Hz, 4 H), 2.12 (d, J = 2.7 Hz, 6 H), 1.01 (t, J = 7.1 Hz, 6 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.4, 169.3, 162.3, 137.4 (2 C), 129.2, 128.9 (2 C), 127.6, 103.1 (2 C), 57.4, 49.04 (2 C), 34.9, 20.4, 12.4 (2 C). HRMS-EI calcd for $C_{18}H_{26}N_3O_2$ $[M + H]^+$ 316.2019; found 316.2010

2-(Diethylamino)-*N*-((3-(4-methylphenyl)isoxazol-5-yl)methyl)-ethanamide (8q). Yield 50%; yellow oil; R_f 0.17 (EtOAc); t_R − HPLC: 1.43 min (100%). IR (film) υ_{max} 3330 (br), 2962, 1674, 1612, 1516, 1432, 1254, 1168, 1026 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.15 (br s, 1H), 7.66 (d, J = 7.9 Hz, 2H), 7.24 (d, J = 7.9 Hz, 2H), 6.48 (s, 1H), 4.61 (br s, 1H), 4.11 (q, J = 7.2 Hz, 4H), 2.39 (s, 3H), 1.25 (t, J = 7.2 Hz, 6H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.3, 169.3, 162.3, 161.3, 137.4 (2 C), 129.2, 128.9 (2 C), 127.6, 102.1, 57.4, 49.04 (2 C), 34.9, 12.4 (2 C). HRMS-EI calcd for $C_{17}H_{24}N_3O_2$ $[M + H]^+$ 302.3914; found 302.3926.

***N*-((3-(3-Iodo-4-methoxyphenyl)isoxazol-5-yl)methyl)-2-(diethyl-amino)ethanamide (8r).** Yield 62%; colorless oil; R_f 0.22 (EtOAc); t_R − HPLC: 1.43 min (99.6%). IR film υ_{max} 3324 (br), 2960, 2928, 1668, 1605, 1516, 1426, 1262, 1134, 1071 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.20 (d, J = 2.1 Hz, 1 H), 7.99 (s, 1 H), 7.73 (dd, J = 2.0, 8.4 Hz, 1 H), 6.86 (d, J = 8.5 Hz, 1 H), 6.45 (s, 1 H), 4.61 (d, J = 6.2 Hz, 2 H), 3.93 (s, 3 H), 3.11 (s, 2 H), 2.57 (q, J = 7.1 Hz, 4 H), 1.03 (t, J = 7.1 Hz, 6 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.6, 169.7, 161.1, 159.6, 138.0, 128.3, 119.5, 111.0, 100.1, 86.4, 57.5, 56.7, 49.0 (2 C), 35.0, 12.5 (2 C). HRMS-EI calcd for $C_{17}H_{23}IN_3O_3$ $[M + H]^+$ 444.0784; found 444.0789.

2-(Diethylamino)-*N*-((1-(4-iodophenyl)-1*H*-1,2,3-triazol-4-yl)-methyl)ethanamide (15b). Yield 90%; white solid; mp 127−129 °C; R_f 0.19 (MeOH/EtOAc, 1%); t_R − HPLC: 5.3 min (MeOH, 97.2%). FTIR (KBr pellet) υ_{max} 3291, 3152, 3054, 2967, 2932, 2805, 1654, 1525, 1498, 1348, 1224, 1045 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02 (br s, 1 H), 7.97 (s, 1 H), 7.81 (d, J = 8.7 Hz, 2 H), 7.47 (d, J = 8.7 Hz, 2 H), 4.58 (d, J = 6.1 Hz, 2 H), 3.04 (s, 2 H), 2.52 (q, J = 7.1 Hz, 4 H), 0.96 (t, J = 7.1 Hz, 6 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.6, 146.1, 139.0 (2 C), 136.7, 122.1 (2 C), 120.3, 93.7, 57.5, 48.8 (2 C), 34.5, 12.3 (2 C). HRMS-EI calcd for $C_{15}H_{21}IN_5O$ $[M + H]^+$ 414.2646; found 414.2648

***N*-((1-(4-Chlorophenyl)-1*H*-1,2,3-triazol-4-yl)methyl)-2-(diethyl-amino)ethanamide (15c).** Yield 55%; white solid; mp 100 °C; R_f 0.61 (EtOAc/hexanes, 1:3); t_R − HPLC: 1.47 min (97.6%). FTIR (KBr pellet) υ_{max} 3286 (br), 2972, 1660, 1521, 1424, 1090, 1028 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02 (br s, 1 H), 7.96 (s, 1 H), 7.66 (d, J = 8.8 Hz, 1 H), 7.49 (d, J = 8.8 Hz, 2 H), 4.59 (d, J = 6.2 Hz, 2 H), 3.04 (s, 1 H), 2.52 (q, J = 7.1 Hz, 4 H), 0.99 (t, J = 7.1 Hz, 6 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.8, 146.2, 135.7, 134.8, 130.1 (2 C), 121.8 (2 C), 120.6, 57.6, 48.9 (2 C), 34.5, 12.4 (2 C). HRMS-EI calcd for $C_{15}H_{21}ClN_5O$ $[M + H]^+$ 322.1435; found 322.1437.

***N*-((1-(4-Bromophenyl)-1*H*-1,2,3-triazol-4-yl)methyl)-2-(diethyl-amino)ethanamide (15d).** Yield 86%; yellow solid; mp 116−118 °C; R_f 0.17 (EtOAc); t_R − HPLC: 1.45 min (100%). FTIR (KBr pellet) υ_{max} 3288 (br), 3056, 2970, 2817, 1663, 1500, 1349, 1265, 1045 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.03 (br s, 1 H), 7.97 (s, 1 H), 7.62 (m, 4 H), 4.59 (d, J = 6.1 Hz, 2 H), 3.04 (s, 3 H), 2.52 (q, J = 7.1 Hz, 4 H), 0.98 (t, J = 7.1 Hz, 6 H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.7, 146.2, 136.1, 133.1 (2 C), 122.6, 122.0 (2 C), 120.5, 57.6, 48.9 (2 C), 34.5, 12.4 (2 C). HRMS-EI calcd for $C_{15}H_{21}BrN_5O$ $[M + H]^+$ 367.2641; found 367.2643.

2-(Diethylamino)-*N*-((1-heptyl-1*H*-1,2,3-triazol-4-yl)methyl)-ethanamide (15e). Yield 59%; white solid; mp 120 °C; R_f 0.10 (EtOAc); t_R − HPLC: 1.47 min (98.8%). FTIR (KBr pellet) υ_{max} 3297, 1646, 1544, 1420, 1264, 1221, 1026 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.95 (br s, 1 H), 7.51 (s, 1 H), 4.53−4.56 (m, 1 H), 4.31−4.33 (m, 1 H), 3.04 (s, 2H), 2.55 (q, J = 7.1 Hz, 4 H), 1.88 (m, 2H), 1.33 (m, 9H), 0.99 (t, J = 7.1 Hz, 6 H), 0.87 (t, J = 5.1 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.6, 145.1, 122.1, 57.6, 50.6, 48.9 (2 C), 34.6, 31.8, 29.9, 28.8, 26.6, 22.7, 14.2, 12.5 (2 C). HRMS-EI calcd for $C_{16}H_{32}N_5O$ $[M + H]^+$ 310.2610; found 310.2604.

2-(Diethylamino)-*N*-((1-phenyl-1*H*-1,2,3-triazol-4-yl)methyl)-ethanamide (15f). Yield 50%; white solid; mp 92 °C; R_f 0.29 (MeOH/EtOAc, 1%); t_R − HPLC: 1.47 min (99.7%). FTIR (KBr pellet) υ_{max} 3288 (br), 3153, 3056, 2970, 2933, 2871, 2817, 1663, 1524, 1500, 1349, 1265, 1224 cm^{-1}. ^{1}H NMR (500 MHz,

$CDCl_3$) δ 8.03 (br s, 1 H), 7.97 (s, 1 H), 7.60−7.65 (m, 4 H), 4.59 (d, J = 6.1 Hz, 2 H), 3.04 (s, 2 H), 2.52 (q, J = 7.1 Hz, 4 H), 0.99 (t, J = 7.1 Hz, 6 H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.7, 146.2, 136.1, 133.1 (2 C), 122.6, 122.0 (2 C), 120.5, 57.6, 48.9 (2 C), 34.5, 12.5 (2 C). HRMS-ESI calcd for $C_{15}H_{21}BrN_5O$ $[M + H]^+$ 366.09240; found 366.09183.

***N*-((1-(2-Nitrophenyl)-1*H*-1,2,3-triazol-4-yl)methyl)-2-(diethylamino)ethanamide (15g).** Yield 42%; yellow oil; R_f 0.13 (MeOH/EtOAc, 1%); t_R − HPLC: 1.46 min (100%). IR υ_{max} 3343, 2970, 29.34, 2874, 2821, 1668, 1536, 1355, 1039 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.05 (dd, J = 1.4, 8.1 Hz, 2 H), 7.85 (s, 1 H), 7.78 (dt, J = 1.4, 7.7 Hz, 1 H), 7.68 (dt, J = 1.4, 8.1 Hz, 1 H), 7.59 (dd, J = 1.4, 8.0 Hz, 1 H), 4.63 (d, J = 6.1 Hz, 1 H), 3.05 (s, 2 H), 2.52 (q, J = 7.1 Hz, 4 H), 0.99 (t, J = 7.1 Hz, 6 H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.7, 145.8, 144.6, 134.0, 131.0, 130.3, 127.9, 125.7, 124.0, 57.5, 48.9 (2 C), 34.4, 12.4 (2 C). HRMS-EI calcd for $C_{15}H_{21}N_6O_3$ $[M + H]^+$ 333.1675; found 333.16758.

***N*-((1-(3-Iodophenyl)-1*H*-1,2,3-triazol-4-yl)methyl)-2-(diethylamino)ethanamide (15h).** Yield 87%; yellow solid; mp 77 °C; R_f 0.29 (EtOAc); t_R − HPLC: 1.47 min (100%). FTIR (KBr pellet) υ_{max} 3343, 2967, 2933, 1668, 1587, 1519, 1486, 1043 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.1 (t, J = 1.7 Hz, 1 H), 8.03 (br s, 1 H), 7.99 (s, 1 H), 7.75 (d, J = 8.0 Hz, 2 H), 7.70 (dd, J = 1.8, 8.0 Hz, 1 H), 7.24 (t, J = 8.0 Hz, 1 H), 4.60 (d, J = 6.2 Hz, 2 H), 3.05 (s, 2 H), 2.53 (q, J = 7.1 Hz, 4 H), 0.99 (t, J = 7.1 Hz, 6 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.7, 146.1, 138.2, 137.9, 131.3, 129.4, 120.6, 119.7, 94.6, 57.6, 48.9 (2 C), 34.5, 12.4 (2 C). HRMS-EI calcd for $C_{15}H_{21}N_5OI$ $[M + H]^+$ 414.0791; found 414.0788.

2-(Diethylamino)-*N*-((1-(3-methoxyphenyl)-1*H*-1,2,3-triazol-4-yl)methyl)ethanamide (15i). Yield 78%; yellow oil; R_f 0.12 (MeOH/EtOAc, 1%); t_R − HPLC: 1.46 min (97.2%). IR (film) υ_{max} 3346, 2967, 2932, 1670, 1609, 1506, 1254, 1157, 1043 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03 (br s, 1 H), 7.97 (s, 1 H), 7.41 (t, J = 8.2 Hz, 1 H), 7.33 (t, J = 2.2 Hz, 1 H), 7.24 (d, J = 1.4 Hz, 1 H), 6.97 (dd, J = 2.7, 8.2 Hz, 1 H), 4.62 (d, J = 6.2 Hz, 2 H), 3.82 (s, 3 H), 3.06 (d, J = 4.4 Hz, 2H), 2.54 (q, J = 7.1 Hz, 4 H), 1.02 (t, J = 7.1 Hz, 6 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.6, 160.8, 145.8, 138.2, 130.7, 120.5, 114.9, 112.5, 106.4, 57.6, 55.9, 48.9 (2C), 34.5, 12.5 (2 C). HRMS-EI calcd for $C_{16}H_{24}N_5O_2$[M + H]$^+$ 318.3941; found 318.3943.

***N*-((1-Benzyl-1*H*-1,2,3-triazol-4-yl)methyl)-2-(diethylamino)-ethanamide (15j).** Yield 80%; white solid; mp 67−69 °C; R_f 0.15 (EtOAc); t_R − HPLC: 1.47 min (99.2%). FTIR (KBr pellet) υ_{max} 3342 (br), 2966 (br), 2928, 1669, 1517, 1455, 1334, 1259, 1207, 1049 (br) cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.85 (br s, 1 H), 7.36 (s, 1 H), 7.27 (m, 2 H), 7.18 (m, 1 H), 5.42 (s, 2 H), 4.43 (d, J = 6.1 Hz, 2 H), 2.92 (s, 2 H), 2.41 (q, J = 7.1 Hz, 4 H), 0.87 (t, J = 7.1 Hz, 6 H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.4, 145.5, 134.7, 129.2 (2 C), 128.9, 128.2 (2 C), 122.0, 57.5, 48.8 (2C), 34.4, 29.8, 12.3 (2 C). HRMS-EI calcd for $C_{16}H_{23}N_5O$ [M + H]$^+$ 302.1981; found 302.1983.

2-(Diethylamino)-*N*-((1-(2,4,6-tribromophenyl)-1*H*-1,2,3-triazol-4-yl)methyl)ethanamide (15k). Yield 67%; white solid; mp 129−130 °C; R_f 0.22 (EtOAc/hexanes, 1:1); t_R − HPLC: 1.46 min (97.1%). FTIR (KBr pellet) υ_{max} 3349 (br), 2967, 2811, 1671, 1517, 1487, 1373, 1203, 1036 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.87 (br s, 1 H), 7.87 (s, 1 H), 7.72 (s, 1 H), 7.53 (s, 1 H), 4.67 (d, J = 1.1 Hz, 1 H), 3.04 (s, 1 H), 2.52 (q, J = 7.1 Hz, 4 H), 0.97 (t, J = 7.1 Hz, 6 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.6, 145.5 135.4 (2 C), 132.6, 129.1, 125.6, 123.7, 123.4, 57.6, 49.2 (2C), 34.4, 12.6 (2 C). HRMS-ESI calcd for $C_{15}H_{19}Br_3N_5O$ $[M + H]^+$ 521.91342; found 521.91266.

2-(Diethylamino)-*N*-((1-(2,4,6-trichlorophenyl)-1*H*-1,2,3-triazol-4-yl)methyl)ethanamide (15l). Yield 23%; yellow solid; mp 102−103 °C; R_f 0.74 (EtOAc/hexanes, 1:3); t_R − HPLC: 1.46 min (96.0%). FTIR (KBr pellet) υ_{max} 3349 (br), 2967, 2822, 1671, 1521, 1487, 1392, 1203, 1036 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.04 (br s, 1 H), 7.70 (s, 1 H), 7.53 (s, 2 H), 4.67 (d, J = 6.2 Hz, 2 H), 2.99 (s, 2 H), 2.52 (q, J = 7.1 Hz, 4 H), 0.99 (t, J = 7.1 Hz, 6 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.7, 145.4 (2 C), 137.5, 134,6 (2 C), 129.1, 124.6, 57.7, 49.2 (2 C), 34.4, 29.9, 12.6 (2 C). HRMS-EI calcd for $C_{15}H_{19}Cl_3N_5O$ $[M + H]^+$ 390.0655; found 390.0656.

2-(Diethylamino)-*N*-((1-(4-methoxy-2-nitrophenyl)-1*H*-1,2,3-triazol-4-yl)methyl)ethanamide (15m). Yield 54%; dark-red oil; R_f 0.10 (EtOAc); t_R − HPLC: 1.45 min (99.2%). IR υ_{max} 3347, 2967, 2935, 1670, 1540, 1519, 1351, 1284, 1245, 1039 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03 (br s, 1 H), 7.78 (s, 1 H), 7.56 (d, J = 2.4 Hz, 1 H), 7.46 (d, J = 8.0 Hz, 1 H), 7.25 (m, 1 H), 7.23 (d, J = 2.7 Hz, 1 H), 4.62 (d, J = 6.1 Hz, 2 H), 3.95 (s, 3 H), 3.04 (s, 2 H), 2.53 (q, J = 7.1 Hz, 4 H), 0.99 (t, J = 7.1 Hz, 6 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.7, 161.0, 145.6, 129.5, 124.4, 123.2, 119.5, 110.8, 57.6, 56.6, 49.0 (2 C), 34.5, 29.9, 12.5 (2 C). HRMS-EI calcd for $C_{16}H_{23}N_6O_4$ $[M + H]^+$ 363.1781; found 363.1783.

***N*-((1-(2-Bromophenyl)-1*H*-1,2,3-triazol-4-yl)methyl)-2-(diethylamino)ethanamide (15n).** Yield 78%; yellow oil; R_f 0.77 (EtOAc/hexanes, 1:5); t_R − HPLC: 1.46 min (99.5%). IR υ_{max} 3336, 2967, 2931, 2825, 1670, 1519, 1492, 1041 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.05 (br s, 1 H), 7.91 (s, 1 H), 7.73 (dd, J = 1.0, 8.1, 1 H), 7.45−7.53 (app dtd, J = 1.8, 7.8, 16.0 Hz, 2 H), 7.38 (dt, J = 1.8, 7.8 Hz, 1 H), 4.65 (d, J = 6.1 Hz, 2 H), 3.1 (s, 2 H), 2.54 (q, J = 7.1 Hz, 4 H), 0.99 (t, J = 7.1 Hz, 6 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.47, 144.9, 136.7, 134.1, 131.4, 128.6, 128.3, 124.4, 118.7, 57.5, 49.0 (2 C), 34.5, 12.4 (2 C). HRMS-EI calcd for $C_{15}H_{21}BrN_5O$ $[M + H]^+$ 366.0929; found 366.0934.

2-(Diethylamino)-*N*-((1-(3,4,5-trimethoxyphenyl)-1*H*-1,2,3-triazol-4-yl)methyl)ethanamide (15o). Yield 32%; white solid; mp 125−127 °C; R_f 0.11 (EtOAc); t_R − HPLC: 1.47 min (98.8%). FTIR (KBr pellet) υ_{max} 2968, 2931, 1682, 1605, 1509, 1473, 1419, 1231, 1128, 1043, 1006 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.84 (br s, 1 H), 7.95 (s, 2 H), 6.92 (s, 1 H), 4.59 (d, J = 6.2 Hz, 2 H), 3.91 (s, 6 H), 3.87 (s, 3 H), 3.05 (s, 2 H), 2.53 (q, J = 7.1 Hz, 4 H), 0.99 (t, J = 7.1 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.7, 154.1, 145.8, 138.4, 133.0 (2 C), 120.8, 98.4 (2 C), 61.23, 57.6, 56.6 (2 C), 48.8 (2 C), 34.5, 12.4 (2 C). HRMS-ESI calcd for $C_{18}H_{28}N_5O_4$ $[M + H]^+$ 378.21358; found 378.21378.

5-(Azidomethyl)-3-(4-methoxyphenyl)isoxazole (17a). To a solution of 3-(4-methoxyphenyl)isoxazol-5-yl)methanol (**16a**) (728 mg, 3.55 mmol, 1.0 equiv) and *p*-toluenesulfonyl chloride (682 mg, 3.58 mmol, 1.01 equiv) in dry CH_2Cl_2 (9.5 mL) was added Et_3N (0.560 mL, 4.28 mmol, 1.21 equiv), and reaction mixture was stirred at rt for 12 h until thin-layer chromatography indicated consumption of the alcohol. The reaction was then quenched with brine (15 mL), extracted with CH_2Cl_2 (3 × 15 mL), and the combined organic extracts dried (Na_2SO_4) and concentrated under reduced pressure to provide the corresponding tosylate ester (96%). A mixture of this material (1.23 g, 3.43 mmol, 1.0 equiv) and NaN_3 (0.42 g, 6.51 mmol, 1.9 equiv) in DMF (14 mL) was then stirred at rt for 3 h, quenched with brine (30 mL), and extracted with EtOAc (3 × 15 mL). The combined organic layers were washed with brine (2 × 15 mL), dried (Na_2SO_4), and concentrated under reduced pressure to provide **17a** (829 mg, 65%); colorless oil; R_f 0.31 (EtOAc/hexanes, 1:3). IR (film) υ_{max} 3129, 2103, 1613, 1431, 1253, 1177, 1028, 837 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (d, J = 8.8 Hz, 2 H), 6.99 (d, J = 8.8 Hz, 2 H), 6.56 (s, 1 H), 4.50 (s, 2 H), 3.87 (s, 3 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.0, 162.5, 161.4, 128.5 (2 C), 114.6 (2 C), 101.3, 55.6, 45.8. MS-EI calcd for $C_{11}H_{11}N_4O_2$ [M + H]$^+$ 230.2248; found 231.0882.

1-(3-(4-Methoxyphenyl)isoxazol-5-yl)methaneamine (18a). To a solution of 5-(azidomethyl)-3-(4-methoxyphenyl)isoxazole **17a** (160 mg, 0.695 mmol, 1.0 equiv) in dry THF (2 mL) at 0 °C was added PPh_3 (192 mg, 0.730 mmol, 1.1 equiv) portionwise over 5 min. After stirring at rt for 12 h, reaction mixture was quenched with H_2O (5 mL) and extracted with Et_2O (3 × 15 mL).

The combined organic layers ware washed with brine (2 × 10 mL), dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The crude product was purified by flash chromatography over silica gel (EtOAc) to provide **18a** (158 mg, 77%); white solid; mp 184 °C, R_f 0.10 (EtOAc); t_R − HPLC: 20.8 min (THF, 97.9%). IR (film) υ_{max} 3240 (br), 3058, 1591, 1437, 1333, 1265, 1158, 909, 814 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 7.70 (d, J = 8.8 Hz, 2 H), 6.94 (d, J = 8.8 Hz, 2 H), 6.37 (s, 1 H), 3.97 (s, 2 H), 3.82 (s, 3 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.3, 162.1, 161.0, 128.2 (2 C), 121.7, 114.4 (2 C), 98.5, 55.4, 38.5. MS-EI calcd for $C_{11}H_{12}N_2O_2$ $[M + H]^+$ 205.0972; found 205.0970.

1-(3-(4-Bromophenyl)isoxazol-5-yl)methaneamine (18b). To a solution of 5-(azidomethyl)-3-(4-bromophenyl)isoxazole (393 mg, 0.154 mmol, 1.0 equiv) in dry THF (2 mL) at 0 °C was added PPh_3 (125 mg, 0.476 mmol, 1.1 equiv) portionwise over 3 min. After stirring at rt for 12 h, reaction mixture was quenched with H_2O (5 mL) and extracted with Et_2O (3 × 15 mL). The combined organic layers ware washed with brine (2 × 10 mL), dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The crude product was purified by flash chromatography over silica gel (EtOAc) to provide **18b** (218 mg, 86%); colorless liquid; R_f 0.43 (EtOAc/hexanes, 1:1); t_R − HPLC: 20.9 min (THF, 20.8%). IR (film) υ_{max} 3236 (br), 3018, 1601, 1402, 1333, 1265, 1158, 886 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 7.67 (d, J = 8.8 Hz, 2 H), 7.53 (d, J = 8.8 Hz, 2 H), 6.55 (s, 1 H), 4.92 (br s, 2 H), 4.83 (s, 2 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.3, 148.1, 144.3, 129.9 (2 C), 128.2, 126.2 (2C), 101.1, 57.2. MS-EI calcd for $C_{10}H_9BrN_2O_2$ $[M + H]^+$ 253.1800; found 253.1809.

***tert*-Butyl(*S*)-1-((3-(4-methoxyphenyl)isoxazol-5-yl)methylcarbamoyl)-2-phenyl Ethylcarbamate (20a).** Yield 84%; white solid; mp 146−148 °C; R_f 0.51 (EtOAc/hexanes, 1:1); t_R − HPLC: 1.44 min (95.6%). IR (film) υ_{max} 3296 (br), 2923, 1662, 1612, 1529, 1432, 1366, 1253, 1174, 1029 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ 7.71 (d, J = 8.8 Hz, 2 H), 7.27 (m, 4 H), 7.17 (m, 2 H), 6.97 (d, J = 8.8 Hz, 2 H), 6.45 (s, 1 H), 6.32 (s, 1 H), 4.99 (s, 1 H), 4.52 (d, J = 6.0 Hz, 1 H), 3.87 (s, 3 H), 3.10 (m, 2 H), 1.41 (s, 9 H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.6, 168.6, 162.3, 161.2, 136.5 (3 C), 129.0 (2 C), 129.1 (2 C), 128.4 (2 C), 127.3, 121.5, 114.5 (2 C), 100.4, 56.1, 55.6, 38.4, 35.4, 28.5 (3 C). HRMS-ESI calcd for $C_{25}H_{29}N_3O_5$ $[M + H]^+$ 474.19997; found 474.20001.

(*S*)-2-Amino-*N*-((3-(4-methoxyphenyl)isoxazol-5-yl)methyl)-3-phenylpropaneamide (20b). To a *tert*-butyl (*S*)-1-((3-(4-methoxyphenyl)isoxazol-5-yl)methylcarbamoyl)-2-phenyl ethylcarbamate (**20a**) (53 mg, 0.117 mmol) was added mixture of TFA and H_2O (9:1, 1.5 mL). After 10 min, EtOH (3 mL) was added and reaction was concentrated under reduced pressure to provide **20b** (36 mg, 88%); white solid; $[\alpha]_D$ −26.1 (c = 0.52, MeOH); mp 166−170 °C; R_f 0.11 (EtOAc); t_R − HPLC: 1.46 min (100%). FTIR (KBr pellet) υ_{max} 2920, 2851, 1583, 1652, 1558, 1456, 1134 cm^{-1}. ^1H NMR (400 MHz, CD_3OD) δ 7.71 (d, J = 8.9 Hz, 2 H), 7.26 (m, 5 H), 7.02 (d, J = 8.9 Hz, 2H), 6.49 (s, 1 H), 4.58 (d, J = 16.0 Hz, 1 H), 4.45 (d, J = 16.0 Hz, 1 H), 4.08 (t, J = 7.3 Hz, 1 H), 3.84 (s, 3 H), 3.17 (dd, J = 7.4, 13.8 Hz, 1 H), 3.08 (dd, J = 7.4, 13.8 Hz, H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.6, 169.8, 164.5, 163.8, 163.2, 135.6, 130.6 (2 C), 130.2 (2 C), 129.4 (2 C), 129.0, 122.5, 115.6 (2 C), 101.6, 56.0, 38.8, 36.0. HRMS-EI calcd for $C_{20}H_{22}N_3O_3$ $[M + H]^+$ 352.4070; found 352.4073.

Benzyl (*S*)-1-((3-(4-Methoxyphenyl)isoxazol-5-yl)methylcarbamoyl)-2-methylpropyl carbamate (20c). Yield 48% as a colorless oil; R_f 0.26 (EtOAc/hexanes, 1:3); t_R − HPLC: 1.60 min (99.8%). IR (film) υ_{max} 3348 (br), 2965, 1722, 1613, 1529, 1432, 1253, 1028, 837 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ 7.73 (d, J = 8.8 Hz, 2 H), 7.36 (m, 3 H), 7.32 (m, 2 H), 6.97 (d, J = 8.8 Hz, 2 H), 6.58 (s, 1 H), 5.32 (d, J = 13.7 Hz, 2 H), 5.24 (d J = 13.7 Hz, 2 H), 5.12 (s, 2 H), 4.37 (m, 1 H), 3.86 (s, 3 H), 2.20 (m, 1 H), 1.56 (s, 1 H), 0.97 (d, J = 6.85 Hz, 3 H), 0.88 (d, J = 6.85 Hz, 3 H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.7, 166.4, 162.4, 161.3, 156.4, 128.8 (2 C), 128.4 (5 C), 121.2, 114.5 (2 C), 102.4, 67.4, 59.2, 57.1, 55.6, 31.4, 19.2, 17.7. HRMS-ESI calcd for $C_{24}H_{28}N_3O_5$ $[M + H]^+$ 438.4962; found 438.4960.

(*S*)-Benzyl 2-((3-(4-Methoxyphenyl)isoxazol-5-yl)methylcarbamoyl)pyrrolidine-1-carboxylate (20d). Yield 55% colorless oil; $[\alpha]_D$ −38.1 (c = 0.40, MeOH); R_f 0.13 (EtOAc/hexanes, 1:1); t_R − HPLC: 1.60 min (95.9%). IR (film) υ_{max} 3315 (br), 2953 (br), 1700, 1612, 1529, 1431, 1356, 1253, 1176, 1118, 1028 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 7.70 (d, J = 6.9 Hz, 2 H), 7.34 (m, 5 H), 6.94 (d, J = 6.9 Hz, 2 H), 6.44 (s, 1 H), 5.17 (s, 2 H), 4.57 (m, 2 H), 4.40 (m, 1 H), 3.85 (s, 3 H), 3.53 (m, 2 H), 3.46 (m, 1 H), 2.19 (s, 1 H), 1.83−1.86 (m, 4 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.0, 169.4, 162.4, 161.2. 156.7, 136.4, 128.8 (2 C), 128.4 (2 C), 128.2 (2 C), 121.7, 114.5 (2 C), 100.2, 67.8, 60.8, 55.6, 47.4, 41.2, 35.7, 28.3, 25.8. HRMS-EI calcd for $C_{24}H_{26}N_3O_5$ $[M + H]^+$ 436.1867; found 436.1856.

(*S*)-2-(Acetylamino)-3-mercapto-*N*-((3-4(methoxyphenyl)-isoxazol-5-yl)methyl)) Propanamide (20e). Yield 37%; white solid; mp 112 °C; R_f 0.51 (EtOAc/hexanes, 1:1); t_R − HPLC: 1.60 min (95.4%). IR (film) υ_{max} 2922, 1671, 1612, 1528, 1431, 1253, 1177, 1028 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ 7.91 (m, 1 H), 7.73 (d, J = 8.8 Hz, 2 H), 6.97 (d, J = 8.8 Hz, 2 H), 6.45 (s, 1 H), 6.41 (s, 1 H), 4.68 (s, 2 H), 4.02 (s, 2 H), 3.86 (s, 3 H), 3.84 (s, 3 H), 2.07 (d, J = 4.9 Hz, 2 H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.3, 170.6, 165.6, 162.3, 161.1, 128.4 (2 C), 121.8, 114.5 (2 C), 100.2, 98.7, 56.2, 55.6, 38.6, 22.7. HRMS-EI calcd for $C_{25}H_{29}N_3O_5$ $[M + H]^+$ 350.1117; found 350.1108.

***tert*-Butyl(*S*)-1-((3-(4-methoxyphenyl)isoxazol-5-yl)methylcarbamoyl)-2-((benzyloxy)carbonyl)ethylcarbamate (20g).** Yield 57%; white solid; $[\alpha]_D$ −5.0 (c = 0.46, MeOH); mp 86 °C; R_f 0.26 (EtOAc/hexanes, 1:3); t_R − HPLC: 1.61 min (97.6%). IR (film) υ_{max} 3375 (br), 2975, 1717, 1614, 1498, 1253, 1163, 1028 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ 7.71 (d, J = 8.8 Hz, 2 H), 7.32 (m, 5 H), 6.95 (d, J = 8.8 Hz, 4 H), 6.55 (s, 1 H), 5.55 (d, J = 8.7 Hz, 1 H), 5.21 (d, J = 2.2 Hz, 2 H), 5.10 (s, 2 H), 4.67 (m, 1 H), 3.84 (s, 3 H), 3.09 (dd, J = 4.6, 17.2 Hz, 1 H), 2.90 (dd, J = 4.6, 17.2 Hz, 1 H), 1.43 (s, 9 H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.8, 170.6, 166.3, 162.3, 161.2, 155.5, 135.4, 128.7 (5 C), 128.5 (2 C), 114.5 (2 C), 102.3, 80.5, 67.1, 60.5, 57.6, 55.5, 50.1, 36.9, 28.4 (3 C). HRMS-EI calcd for $C_{27}H_{31}N_3O_7$ $[M + H]^+$ 510.5589; found 510.5598.

Isobutyl((3-(4-methoxyphenyl)isoxazol-5-yl)methyl)carbamate (20h). To a stirred solution of isobutyl chloroformate (31 μL, 0.183 mmol, 1.01 equiv) in anhydrous CH_2Cl_2 (2 mL) under an atmosphere of N_2 at −40 °C ($MeCN/CO_2$) and Et_3N (17 μL, 0.182 mmol, 1 equiv) was added 5-aminomethyl-3(4-methoxyphenyl)isoxazole (**18b**) (159 mg, 0.182 mmol, 1 equiv). After stirring for 10 min, the cold bath was removed, and reaction mixture was warming up to rt and stirred for 12 h. Reaction was quenched with 1 M HCl, and the aqueous portion was extracted with EtOAc (3 × 20 mL). The combined organic extracts were dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The residue was purified by flash chromatography over silica gel (EtOAc/hexanes, 1:5) to provide **20h** (52 mg, 93%); colorless oil; R_f 0.42 (EtOAc/hexanes, 1:5); t_R − HPLC: 1.61 min (97.2%). IR (film) υ_{max} 2962, 1751, 1614, 1432, 1253, 1178, 970 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 7.73 (d, J = 8.8 Hz, 2 H), 6.96 (d, J = 8.8 Hz, 2 H), 6.62 (s, 1 H), 5.26 (s, 2 H), 3.97 (d, J = 6.6 Hz, 2 H), 3.86 (s, 3 H), 2.17 (s, 2 H), 1.99 (m, 1 H), 1.59 (s, 6 H), 0.95 (d, J = 6.9 Hz, 6 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.3, 161.3, 154.8, 129.8, 128.5 (2 C), 121.1, 114.6 (2 C), 102.4, 75.0, 59.7, 55.6, 28.0, 19.1 (2 C). HRMS-EI calcd for $C_{16}H_{21}N_2O_4$ $[M + H]^+$ 305.3489; found 305.3493.

2-Bromo-*N*-((3-(4-methoxyphenyl)isoxazol-5-yl)methyl)-ethaneamide (20i). Yield 36%; colorless oil; R_f 0.20 (EtOAc/hexanes, 1:5); t_R − HPLC: 1.62 min (99.2%). IR (film) υ_{max} 2961, 1760, 1614, 1431, 1257, 1023, 802 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 7.73 (d, J = 8.6 Hz, 2 H), 6.97 (d, J = 8.6 Hz, 2 H), 6.61 (s, 1 H), 5.31 (s, 2 H), 4.15 (s, 2 H), 3.86 (s, 3 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.9, 165.9, 162.4, 161.3, 128.4

(2 C), 121.1, 114.5 (2 C), 102.8, 57.8, 55.5, 40.7. HRMS-EI calcd for $C_{13}H_{14}BrN_2O_3$ $[M + H]^+$ 326.1659; found 326.1661.

***N*-((3-(4-Bromophenyl)isoxazol-5-yl)methyl)ethanamide (20j).** Yield 88% as a white solid; mp 168−169 °C; R_f 0.41 (EtOAc); t_R − HPLC: 1.61 min (98.5%). FTIR (KBr pellet) υ_{max} 3271, 1651, 1558, 1423, 1295, 1224, 1101, 1028, 825 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 7.65 (d, J = 8.5 Hz, 2 H), 7.58 (d, J = 8.5 Hz, 2 H), 7.27 (s, 1 H), 6.50 (br s, 1 H), 4.60 (d, J = 6.0 Hz, 2 H), 2.07 (s, 3 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.3, 169.8, 162.0, 132.4(2 C), 128.5(2 C), 127.9, 124.7, 100.6, 34.5, 23.3. MS-EI calcd for $C_{12}H_{10}Br_2N_2O_2$ $[M + H]^+$ 375.0359; found 375.0364.

***N*-((3-(4-Bromophenyl)isoxazol-5-yl)methyl)propanamide (20k).** Yield 86% as a white solid; mp 171−172 °C, R_f 0.19 (EtOAc); t_R − HPLC: 1.60 min (98.9%). FTIR (KBr pellet) υ_{max} 3207, 1657, 1524, 1360, 828, 725 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 7.64 (d, J = 8.5 Hz, 2 H), 7.57 (d, J = 8.5 Hz, 2 H), 6.48 (s, 1 H), 6.17 (br s, 1 H), 4.59 (d, J = 5.9 Hz, 2 H), 2.28 (q, J = 7.1 Hz, 2 H), 1.84 (t, J = 7.1 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.0, 169.9, 161.9, 132.4, 128.5, 127.9, 124.6, 100.5, 35.3, 29.6, 9.8. MS-EI calcd for $C_{13}H_{13}BrN_2O_2$ $[M + H]^+$ 310.1665; found 310.1658.

***N*-((3-(4-Bromophenyl)isoxazol-5-yl)methyl)-2-methylpropanamide (20l).** Yield 86%; yellow oil; R_f 0.43 (EtOAc/hexanes, 1:1); t_R − HPLC: 1.46 min (99,2%). IR (film) υ_{max} 3287, 1648, 1548, 1424, 1222, 826 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 7.65 (d, J = 8.5 Hz, 2 H), 7.59 (d, J = 8.5 Hz, 2 H), 6.48 (s, 1 H), 6.48 (br s, 1 H), 4.60 (d, J = 6.0 Hz, 2 H), 2.43 (m, 1 H), 2.19 (d, J = 6.9 Hz, 6 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.2, 170.0, 161.7, 132.4 (2 C), 128.5 (2 C), 127.9, 124.6, 100.5, 35.7, 35.4, 19.7 (2 C). HRMS-EI calcd for $C_{14}H_{15}BrN_2O_2$ $[M + H]^+$ 323.1851; found 323.1895.

***N*-3-(4-Bromophenyl)isoxazol-5-yl)benzamide (20m).** Yield 90%; white solid; mp 166−168 °C; R_f 0.51 (EtOAc/hexanes, 1:1); t_R − HPLC: 1.60 min (95.6%). FTIR (KBr pellet) υ_{max} 3357, 3262, 1644, 1532, 1306, 1159, 816 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 7.81 (m, 3 H), 7.65 (m, 2 H), 7.58 (m, 2 H), 7.50 (d, J = 8.2 Hz, 2 H), 7.46 (m, 2 H), 7.31 (d, J = 8.2 Hz, 2 H), 6.77 (br s, 1 H), 6.57 (s, 1 H), 4.82 (d, J = 6.0, 2 H), 2.44 (s, 2 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.2, 170.0, 161.7, 132.4 (5H), 128.8 (2C), 128.5 (2C), 127.9, 124.6, 100.5, 57.7, 35.4. HRMS-EI calcd for $C_{17}H_{14}BrN_2O_2$ $[M + H]^+$ 358.2093; found 358.2090.

2-Bromo-*N*-((3-(4-bromophenyl)isoxazol-5-yl)methyl)ethanamide (20n). Yield 73%; yellow oil; R_f 0.11 (EtOAc/hexanes, 1:5); t_R − HPLC: 1.52 min (100%). IR (film) υ_{max} 3296 (br), 1647, 1544, 1420, 1263, 1221, 1027 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ 7.65 (d, J = 8.5 Hz, 2H), 7.59 (d, J = 8.5 Hz, 2H), 7.13 (br s, 1H), 6.53 (s, 1H), 4.67 (d, J = 6.1 Hz, 2H), 4.13 (s, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 168.8, 132.4 (2 C), 128.5 (2 C), 127.8, 100.9, 42.6, 35.7. HRMS-EI calcd for $C_{12}H_{11}Br_2N_2O_2$ $[M + H]^+$ 372.9187; found 372.9190.

(3*Z*)-4-Amino-4-(4-methoxyphenyl)but-3-en-2-one (21) and 3-(4-Methoxyphenyl)-5-methylisoxazole (22). A round-bottom flask was flashed with N_2 and charged with benzyl 2-((3-(4-methoxyphenyl)isoxazol-5-yl)methylamino)-2-oxoethylcarbamate (**20f**) (21 mg, 0.055 mmol), 5% Pd/C (4 mg, 0.03 mmol), and EtOAc (2.0 mL) and then flushed with N_2 and placed under an atmosphere of H_2 (1 atm). After stirring for 3 h at rt, the flask was flashed with N_2 and the reaction mixure filtered through a plug of Celite 521. After thoroughly washing the filter cake with MeOH (25 mL), the combined filtrates were concentrated under reduced pressure and the residue purified by flash chromatography over silica gel (EtOAc/hexanes, 1:1) to afford a mixture of (3*Z*)-4-amino-4-(4-methoxyphenyl)but-3-en-2-one (**21**) (2.2 mg, 21%) and 3-(4-methoxyphemyl)-5-methylisoxazole (**22**) (4.8 mg, 48%). Analytical data for **21**: white solid; mp 81−83 °C (lit[40] 81−84 °C); R_f 0.66 (EtOAc); t_R − HPLC: 1.48 min (100%). ^1H NMR (400 MHz, $CDCl_3$) δ 7.51 (d, J = 6.8 Hz, 2 H), 6.94 (d, J = 6.8 Hz, 2 H), 5.43 (s, 1 H), 3.85 (s, 3 H), 2.15 (s, 2 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 197.4, 161.8, 160.8, 129.4, 127.9 (2 C), 114.5 (2 C), 94.7, 55.6, 30.0. Analytical data for **22**: white solid; mp 92 °C (lit[41] 92−93 °C); R_f 0.84 (EtOAc); t_R − HPLC: 1.47 min (97.6%). ^1H NMR (400 MHz, $CDCl_3$) δ 7.73 (d, J = 8.8 Hz, 2 H), 6.97 (d, J = 8.8 Hz, 2 H), 6.24 (s, 1 H), 3.86 (s, 3 H), 2.47 (s, 2 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.8, 162.4 161.1, 128.4 (2 C), 122.1, 114.5 (2 C), 99.7, 55.6, 12.6.

***N*-Allyl-2-(diethylamino)acetamide (24).** Yield 80% colorless oil; R_f 0.34 (EtOAc). IR (film) υ_{max} 3353, 2969, 2934, 2874, 2821, 1677, 1515, 1455, 1205, 1163, 1088, 990, 916 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 7.48 (br s, 1 H), 5.80 (m, 1 H), 5.12 (dq, J = 1.5, 17 Hz, 3 H), 5.07 (dq, J = 1.3, 10.4 Hz, 1 H), 3.85 (m, 2 H), 3.00 (s, 2 H), 2.51 (q, J = 7.1 Hz, 4 H), 0.98 (t, J = 7.1 Hz, 6 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.9, 134.5, 115.9, 57.5, 48.8 (2 C), 41.2, 12.4 (2 C). HRMS-EI calcd for $C_9H_{18}N_2O$ $[M + H]^+$ 170.1419; found 170.1415.

Prop-2-yn-1-yl(diethylamino) Acetate (25). Yield 58% colorless oil; R_f 0.23 (EtOAc); IR (film) υ_{max} 3291, 2971, 2844, 1750, 1652, 1456, 1386, 1157, 1008 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 4.70 (d, J = 2.5 Hz, 2 H), 3.37 (s, 3 H), 2.65 (q, J = 7.2 Hz, 4 H), 2.47 (t, J = 2.4 Hz, 1 H), 1.05 (t, J = 7.2 Hz, 6 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.8, 75.1, 60.6, 54.0, 51.9, 47.9 (2 C), 14.4 (2 C). HRMS-EI calcd for $C_9H_{16}NO_2$ $[M + H]^+$ 170.2288; found 170.2292.

2-(Diethylamino)-*N*-prop-2-yn-1-ylethanthioamide (26). A solution of *N*′-propargyl-*N*,*N*-diethylglycine (**13**) (222 mg, 1.6 mmol, 1.6 equiv) and Lawesson's reagent (320 mg, 1.0 mmol, 1 equiv) in 6.5 mL of anhydrous THF was heated at reflux for 12 h. The resulting reaction mixture was cooled down to rt and concentrated under reduced pressure. Crude product was purified by flash chromatography over silica gel (EtOAc/hexanes, 1:1) give **26** (126 mg, 87%) as yellow oil; R_f 0.60 (EtOAc). IR (film) υ_{max} 3208, 2960, 2836, 1683, 1595, 1499, 1461, 1293, 1251, 1135, 1027, 925, 831, 733 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 9.60 (br s, 1 H), 4.47 (s, 2 H), 3.50 (s, 3 H), 2.56 (q, J = 7.2 Hz, 4 H), 2.31 (t, J = 2.6 Hz, 1 H), 1.01 (t, J = 7.2 Hz, 6 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 201.5, 77.9, 72.8, 65.7, 47.8 (2 C), 34.4, 12.6 (2 C). HRMS-EI calcd for $C_9H_{17}N_2S$ $[M + H]^+$ 185.3097; found 185.3096.

2-(Diethylamino)-*N*-(3-(4-methoxyphenyl)4,5-dihydroisoxazol-5-yl)methyl)ethanamide (27). Yield 75% colorless oil; R_f 0.14 (EtOAc); t_R − HPLC: 1.45 min (98.7%). IR (film) υ_{max} 2967, 2927, 1672, 1608, 1515, 1354, 1252, 1176, 898, 832 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ 7.84 (br s, 1 H), 7.57 (d, J = 3.2 Hz, 2 H), 7.26 (d, J = 3.2 Hz, 2 H), 4.85 (m, 1 H), 3.84 (s, 3 H), 3.60 (m, 2 H), 3.37 (dd, J = 10.7, 16.8 Hz, 1 H), 3.10 (dd, J = 10.7, 16.8 Hz, 1 H), 3.03 (d, J = 17.1 Hz, 1 H), 2.96 (d, J = 17.1 Hz, 1 H), 2.97 (q, J = 7.2 Hz, 4 H), 0.94 (t, J = 7.2 Hz, 6 H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.2, 161.3, 156.5, 128.4 (2 C), 122.0, 114.3 (2 C), 79.8, 57.5, 55.6, 49.0 (2 C), 42.0, 37.9, 12.5 (2 C). HRMS-EI calcd for $C_{17}H_{26}N_3O_3$ $[M + H]^+$ 320.1974; found 320.1976.

(3-(4-Methoxyphenyl)isoxazol-5-yl)methyl(diethylamino) Acetate (28). Yield 79% colorless oil; R_f 0.44 (EtOAc); t_R − HPLC: 1.46 min (98.2%). IR (film) υ_{max} 3370 (br), 2967, 2934, 1752, 1613, 1529, 1431, 1253, 1177, 1028, 837 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 7.73 (d, J = 8.6 Hz, 2 H), 6.97 (d, J = 8.6 Hz, 2 H), 6.58 (s, 1 H), 5.25 (s, 2 H), 3.86 (s, 3 H), 3.42 (s, 2 H), 2.67 (q, J = 7.2 Hz, 4 H), 1.07 (t, J = 7.2 Hz, 6 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.9, 166.9, 162.4, 161.3, 129.8, 128.4 (2 C), 114.5 (2 C), 102.4, 99.9, 55.6, 54.1, 47.9 (2 C), 12.4 (2 C). HRMS-EI calcd for $C_{17}H_{23}N_2O_4$ $[M + H]^+$ 319.1658; found 319.1657.

2-(Diethylamino)-*N*-((3-(4-methoxyphenyl)isoxazol-5-yl)-ethanethioamide (29). Yield 54% yellow oil; R_f 0.67 (EtOAc); t_R − HPLC: 1.45 min (97.1%). IR (film) υ_{max} 2918, 2849, 1611, 1528, 1431, 1383, 1254, 1177, 1114, 1028 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 10.05 (br s, 1 H), 7.73 (d, J = 8.8 Hz, 2 H), 6.97 (d, J = 8.8 Hz, 2 H), 6.55 (s, 1 H), 5.10 (s, 2 H), 3.86 (s, 3 H), 3.60 (s, 2 H), 2.62 (q, J = 7.1 Hz, 4 H), 1.04 (t, J = 7.1 Hz, 6 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.5, 167.1, 162.5, 161.3, 128.4 (2 C), 121.4, 14.6 (2 C), 101.2, 55.6, 48.8 (2 C), 40.2, 29.9, 12.5 (2 C). HRMS-EI calcd for $C_{17}H_{23}N_3O_2S$ $[M + H]^+$ 334.4564; found 334.4568.

Biology. Cell Lines and Antibodies. 293T Human embryonic kidney cells were maintained in Dulbecco's Modified Eagle's

Medium (MEM) supplemented with 10% fetal calf serum with penicillin and streptomycin plus 300 g of Geneticin per mL. HeLa cells were maintained in the same medium without Geneticin. A549 Human alveolar adenocarcinoma cells were maintained in Dulbecco's Modified Eagle's Medium (MEM) supplemented with 10% fetal calf serum. The mouse monoclonal antibody 12B5-1-1, which recognizes the GP1 of Ebola virus Zaire (EBOZ) GP, was kindly provided by Mary K. Hart (U.S. Army Medical Research Institute of Infectious Diseases).[42] The mouse anti-HIV p24 monoclonal antibody was obtained from the National Institutes of Health AIDS Research and Reference Reagent program.[43]

Preparation of Pseudotyped Viruses. To produce Ebola virus GP pseudotyped HIV virions, 293T producer cells were cotransfected with HIV vector pNL4-3-Luc-R-E- (National Institutes of Health AIDS Research and Reference Reagent program; catalogue no, 3418)[37,38] and the DNA of wild-type (wt) Ebola virus Zaire glycoprotein (wt Ebola-GP), which was synthesized by multiple rounds of overlapping PCR based on the EBOZ genome sequence (GenBank accession no. L11365). In the pseudotyping experiments, 2 μg DNA of wt Ebola-GP and 2 μg of pNL4-3-Luc-R-E were used to transfect 293T cells (90% confluent) in six-well plates by Lipofectamine 2000 according to the protocol of the supplier (Invitrogen). The supernatants containing the pseudotyped viruses were collected twice (at 24 and 48 h posttransfection), combined, clarified from floating cells and cells debris by low-speed centrifugation, and filtered through a 0.45 μm pore size filter (Nalgene). Then, 1 mL of supernatant was used to infect cells, and the rest stored at -80 °C for future use.

The plasmid vector expressing the GP of Marburg (Lake Victoria strain) was prepared as previously described.[44] Marburg virus GP (Lake Victoria strain) and VSV-G pseudotyped HIV virions where produced in a similar manner, i.e. through cotransfection with pNL4-3-Luc-R-E-, according to previous published procedures.[44]

Western Blotting. To evaluate EBOZ GP expression, the 293T producer cells were lysed in 0.2 mL of Triton X-100 lysis buffer (50 mM Tris-HCl [pH 7.5], 150 mM NaCl, 5 mM EDTA, 1% Triton X-100, and a protease inhibitors cocktail consisting of 10 g of leupeptin per mL, 5 g of aprotinin per mL, and 2 mM phenylmethylsulfonyl fluoride) at 48 h after cotransfection. The protein samples were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to a polyvinylidene difluoride membrane. The membrane was first incubated with anti-EBOZ GP1 monoclonal antibody 12B5-1-1(1:5000 dilution) for 1 h and then probed with peroxidase-conjugated goat antimouse antiserum (Pierce) for 1 h. The bands were visualized by the chemiluminescence method according to the protocol of the supplier (Pierce).

Detection of GP Incorporation in Pseudotyped Viruses. To evaluate the incorporation of wt GP protein into the pseudotyped viruses, 2 mL of pseudotyped virus was layered onto a 3 mL cushion of 20% (wt/vol) sucrose in phosphate-buffered saline and centrifuged at 55000 rpm for 30 min in a Beckman SW41 rotor. The pelleted pseudotyped viruses were lysed in 50 μL of Triton X-100 lysis buffer, and a 25 μL sample was subjected to SDS-PAGE. Expression of the EBOZ GP protein was detected by Western blotting as described above. A mouse anti-HIV p24 monoclonal antibody (1:5,000 dilution) was used as the primary antibody to detect the HIV p24 protein.

Assay of Infectivity of Pseudotyped Viruses. Human 293T, A549, or HeLa cells (3×10^5 cells) were seeded in six-well plates one day prior to infection. These targeted cells were incubated with 1 mL of the pseudotyped viruses for 5 h. The cells were then lysed in 200 μL of cell culture lysis reagent (Promega) at 48 h postinfection. The luciferase activity was measured using a luciferase assay kit (Promega) and an FB12 luminometer (Berthold detection system) according to the supplier's protocol. Each experiment was done in triplicate and repeated at least three times.

Compound Blocking Assay. Pseudotype viruses were incubated with individual compounds at different concentration (for initial screening, concentrations or 30−60 μM were employed) briefly at room temperature. This mixture was then added to 293T, A549, or HeLa cells, which were seeded one day prior to the assay in poly-L-lysine-treated 24-well plates. After overnight incubation, the supernatant was replaced with fresh media. The cells were lysed in 100 μL of cell culture lysis reagent (Promega) 48 h postinfection. The luciferase activity was measured using a luciferase assay kit (Promega) and an FB12 luminometer (Berthold detection system), according to the supplier's protocol.

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Supporting Information Available: A schematic representation of the production of Ebola GP-pseudotyped HIV viruses and the transduction mediated by them. ^{1}H and ^{13}C NMR spectra for all new compounds described in the work. This material is available free of charge via the Internet at http://pubs.acs.org.

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