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Small molecule inhibitors of anthrax lethal factor toxin

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ABSTRACT

This manuscript describes the preparation of new small molecule inhibitors of *Bacillus anthracis* lethal factor. Our starting point was the symmetrical, bis-quinolinyl compound **1** (NSC 12155). Optimization of one half of this molecule led to new LF inhibitors that were desymmetrized to afford more drug-like compounds.

1. Introduction

Aerosolized spores of *Bacillus anthracis* represent one of the most serious bioterrorist threats to the security of the United States. Inhalation of these environmentally stable spores progresses rapidly to a highly fatal systemic infection. In 2001, this category A agent was used in several acts of terrorism in the United States. While ciprofloxacin and doxycycline were effective if administered immediately after suspected contact with *B. anthracis*, there is little doubt that capable terrorists will seek to develop forms of the bacterium resistant to these common antibiotics, especially since mechanisms of resistance are known and resistant alleles may be generated readily. New therapeutic agents based on novel chemical scaffolds are vital to biodefense because they are likely to be effective against both natural and engineered resistant forms of *B. anthracis*, and because there are no pre-existing resistance alleles.

Bacillus anthracis is the cause of the acute and often lethal disease called anthrax.[1] In humans, anthrax is a rare infection acquired by inhalation, ingestion or cutaneous contact with the endospores of *B. anthracis*.[2,3] Anthrax infection has, until recently, been observed mostly in farm animals and in humans who have had direct contact with contaminated animals or animal products. However, for approximately ten years, the Centers for Disease Control (CDC) and public health authorities have been investigating cases of bioterrorism-related anthrax exposure and death in the United States.[4] The CDC recognizes *B. anthracis* as a category A agent of bioterrorism. It is a serious bioterrorism threat because its spores are stable under extreme conditions in the environment, are easily produced and distributed by aerosol (in a powder form), and are highly fatal via inhalation.[5] Penetration of spores via the skin or gastrointestinal tract is generally less dangerous and results in mainly localized disease.

Therapy recommended by the CDC includes the use of ciprofloxacin or doxycycline as well as rifampin, vancomycin, imipenem, clindamycin or chloramphenicol.[6] Beta-lactamases have been found in recent clinical isolates, precluding the use of penicillins for the treatment of anthrax.[6] In addition, researchers have been able to generate ciprofloxacin resistant and doxycycline resistant strains of *B. anthracis* in the laboratory,[7,8] which demonstrates that unscrupulous scientists could engineer drug resistance into strains of the species.

The *B. anthracis* bacterium-secreted endotoxin is comprised of three components: (1) a zinc metallopreotease lethal factor (LF); (2) a calmodulin-activated edema factor adenylate cyclase (EF); and (3) a protective antigen (PA).[9] Although these proteins are independently nontoxic, their concerted action disrupts cell signaling events and can lead to cell death. Importantly, lethal factor combines with protective antigen to form the lethal factor toxin.[10]

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When lethal factor is translocated into the cytoplasm of host target cells, lethal factor cleaves MAP kinase kinases (MAPKKs) and disrupts the signaling pathway mediated by these MAPKKs. NOD-like receptor (NLR) Nlrp1 protein has recently been identified as a physiologically relevant substrate of anthrax lethal toxin,[11–13] and interactions of anthrax lethal factor with protective antigen has been defined by site-directed spin labeling studies.[14]

Lethal factor plays a critical role in all stages of anthrax infection; in the early stages it assists the bacteria by helping evade the host immune system, and in the later stages, when infection is systemic, it targets the epithelial cells to cause vascular barrier dysfunction.[15,16] Zinc metalloprotease enzymes are a well-studied class of enzymes and many are drug targets for which potent inhibitors have been designed.[17] For these reasons, we chose to exploit a lethal factor inhibitor that we discovered in a screening campaign, and to optimize this screening hit for potential use as an antidote for anthrax intoxication. We specifically chose a non-hydroxamic acid inhibitor to increase our chances of finding a drug-like, metabolically stable inhibitor that would allow us to develop a series of compounds with improved pharmacokinetic properties and fewer undesired interactions.

In Figure 1 is shown a cartoon that displays a rudimentary understanding of binding, assembly, uptake and mechanism of action for *B. anthracis*. A variety of approaches have been taken to intervene at different points of this process to prevent anthrax toxin function. Human antibodies against PA that block receptor binding[18] and antibodies specific for lethal factor (LF) that interfere with assembly of the toxin components[19] have been shown to prevent lethal toxin induced death in rodents. Soluble TEM8/ATR and CMG2 proteins have also been shown to be effective in cell culture, preventing anthrax toxin receptor binding.[20,21] Hexa-D-arginine, a furin inhibitor that blocks PA cleavage, has been shown to delay anthrax toxin-mediated cell cytotoxicity in vitro and reduce lethal toxin mediated lethality in rats.[22] Dominant-negative PA mutants have been generated, which co-assemble with the wild-type PA protein, preventing translocation of LF or EF across the cell membrane.[23]

Peptides and peptide-based analogs have also been shown to inhibit anthrax toxin. In one approach, polyvalent peptide inhibitors, which block LF or EF association with the PA pre-pore, have been shown to protect rats from lethal toxin.[24] A second peptide approach targeted the LF active site. LF has a deep and long (40 Å) groove, with an overall negative electrostatic potential, contiguous to the active site, that binds peptide substrates and peptide inhibitors.[25,26] Several peptide inhibitors of LF activity have been identified on the basis of consensus sequences of the MAPKKs.[24,26–29] Incorporation of zinc-binding chemical groups into these peptides greatly enhanced their potency.[30]

The LF target is attractive because it is a zinc-dependent metalloprotease enzyme, and there is a wealth of information that has been assembled for the inhibition of this class of enzyme; a crystal structure for a rhodanine derivative complexed with lethal factor has been reported.[31,32] Several research groups have developed small molecule LF inhibitor programs based on either structural information or high-throughput screening hits; a collection of small molecule inhibitors of anthrax lethal factor is shown in Figure 2. The bis-quinoline **1** is the screening hit[33] around which we began our synthetic efforts of hit and lead optimization. Benzopyranone **2**[34] was shown to be an inhibitor of *Botulinum* neurotoxin A in addition to LF. A class of acylhydrazones represented by compound **3** also blocked the cleavage of MEK1 in 293T cells.[35] The use of a hydroxythiopyranone core as a hydroxamic acid replacement led to the synthesis of compound **4**, which represented a class of inhibitor that was more potent than the corresponding hydroxypyrones.[36,37] Furan **5** was one of several compounds discovered in a HPLC-based high throughput screen in which 'fragment-based focusing' was used to enrich the pool of potential inhibitors.[38] Related compound **6** was designed using an iterative NMR-based binding assay of LF inhibitors and confirmed by X-ray crystallography.[32] Chiral sulfonamide **7** is one of the most potent LF inhibitors reported;[15,16] a synthetic route using asymmetric hydrogenation was also reported for this inhibitor.[39] Hydroxamic acids, which are known zinc-binding agents that have been widely used for the inhibition of a variety of metalloproteases, have also been used

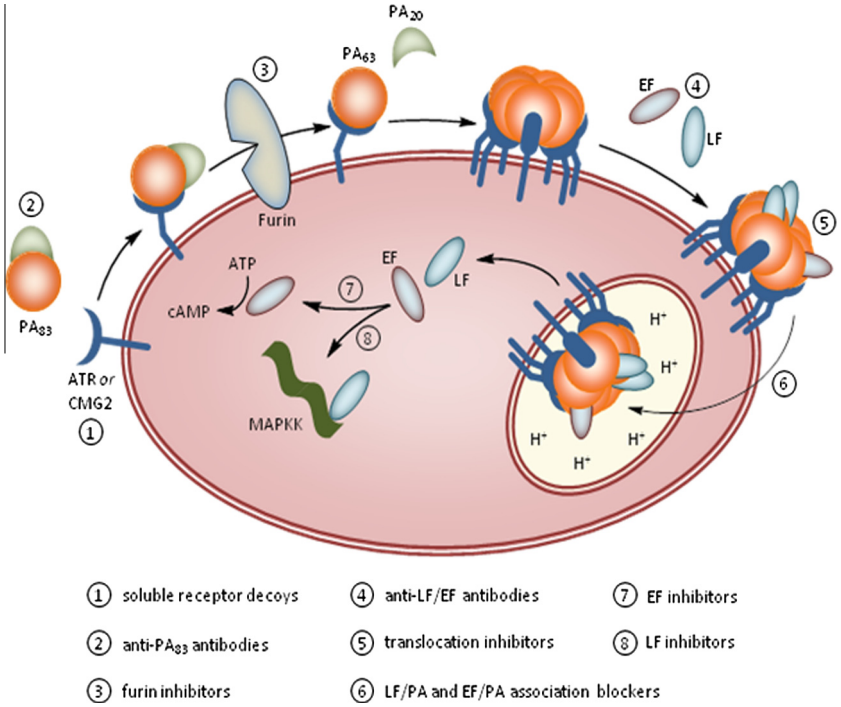


Figure 1. Potential drug targets and points of intervention for the prevention of *Bacillus anthracis* function.

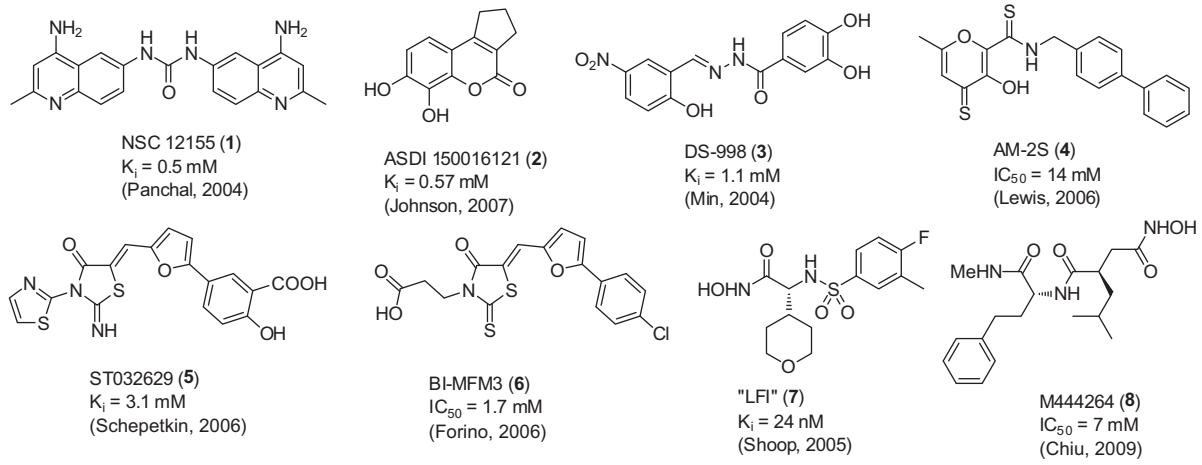


Figure 2. Previously reported anthrax lethal factor inhibitors.

for the inhibition of LF. A representative peptidomimetic hydroxamic acid is compound **8**.[9] Not shown in Figure 2 are miscellaneous additional reported inhibitors of lethal factor protease, including guanidinylated derivatives of neamine[40] and streptamine,[41] aminoglycosides,[42] cationic polyamines,[43] and inhibitors from green tea.[44]

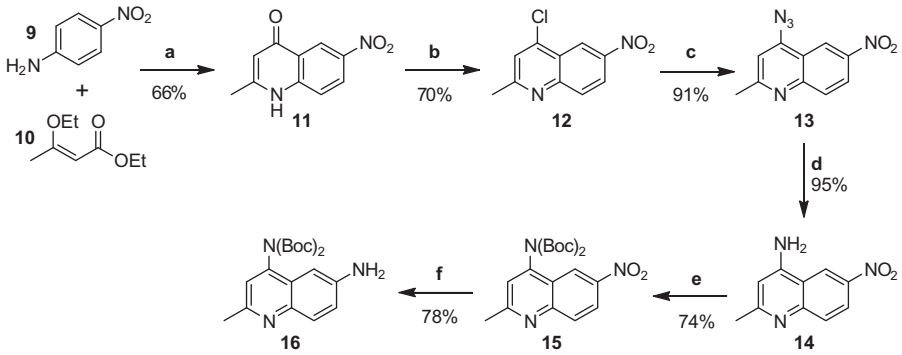
In this report we discuss the design and synthesis of additional quinoline-based inhibitors of LF related to compound **1**. Importantly, we report the synthesis of inhibitor structures that are differentiated from the symmetrical bis-quinoline **1**, which represent an expected improvement in pharmacokinetic properties.

2. Chemistry

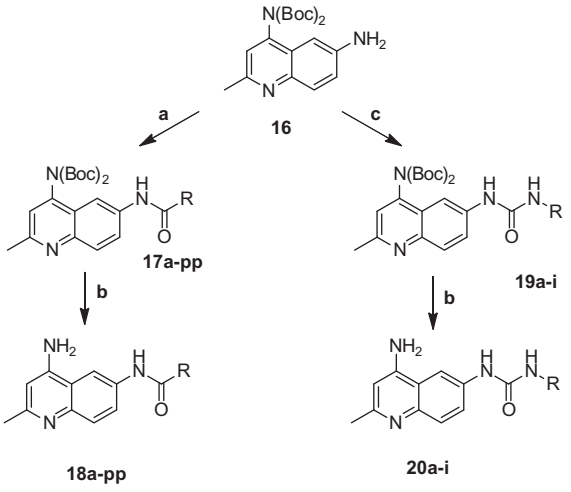
To most efficiently probe the different structural requirements for potent anti-LF activity, we envisioned the preparation of a key intermediate that would be useful for the synthesis of unsymmetrical LF inhibitors. The successful preparation of this versatile diaminoquinoline intermediate, with differentiated amino groups, which we used for the synthesis of many of the compounds in this study, is shown in Scheme 1. Condensation of 4-nitroaniline (**9**) with ethyl 3-ethoxy-2-butenoic acid (**10**) gave 6-nitro-2-methylquinolin-4-one (**11**).[45] Subsequent conversion of quinolinone **11** to the corresponding 4-chloro compound (**12**), followed by displacement of the chloro group with azide gave 6-nitro-4-azidoquinoline (**13**), which was then reduced using a standard Staudinger procedure to provide 4-amino-2-methyl-6-nitroquinoline (**14**). Aminoquinoline **14** was converted to the bis-Boc protected 4-aminoquinoline **15** with Boc anhydride. Reduction of compound **15** using catalytic hydrogenation gave the corresponding 6-amino compound **16**, which we used as a versatile intermediate for the synthesis of non symmetrical functionalized quinolines.

The versatile intermediate **16** was subsequently used for the preparation of amides and ureas as shown in Scheme 2. Target compounds were prepared with a wide variety of substituents to determine important features required for good anti-LF activity. Replacing the second quinoline and forming unsymmetrical target compounds was an important feature of the newly synthesized inhibitors. Amide intermediates **17a–pp** were prepared by treating **16** with different carboxylic acids, using a standard amide coupling procedure, followed by deprotection of the bis-Boc amine with trifluoroacetic acid, to give the amide target compounds **18a–pp**. Likewise, treatment of **16** with isocyanates followed by the same deprotection procedure afforded the bis-Boc protected intermediates **19a–i** and target ureas **20a–i**, respectively.

Our initial assay results (Table 1) showed that two compounds in particular, **18m** and **18t**, were potent inhibitors of LF. Since we determined that both of these compounds were noncompetitive inhibitors of LF, and we did not have any knowledge of where these compounds were binding, we chose to use medicinal chemistry strategies other than molecular modeling to pursue optimization of these structures. To further explore the features of these inhibitors that provide potent anti-LF activity, two additional series of compounds were synthesized; one exploring the nature of the (quinolinyl)phenyl substituent of **18m**, and one exploring the (2-methoxyphenyl)propyl substituent of **18t**.



Scheme 1. Synthesis of versatile diaminoquinoline intermediate **16**. Reagents and conditions: (a) H_2SO_4 (cat.), tetrahydronaphthalene, reflux; (b) $POCl_3$, reflux; (c) NaN_3, DMF/H_2O, 100 °C; (d) PPh_3, THF; (e) Boc_2O, pyridine, 60 °C; (f) ammonium formate, Pd/C, MeOH.



Scheme 2. Synthesis of amide and urea target compounds. Reagents and conditions: (a) HATU, DIPEA, DMF; (b) TFA, CH_2Cl_2; (c) isocyanate, toluene, reflux.

In the former case, a pair of versatile intermediates (**17qq** and **17rr**) were synthesized and subjected to Suzuki coupling reactions with a variety of quinolinylboronic acids (Scheme 3). Thus, coupling of the 3-iodobenzamide **17qq** with quinolinylboronic acids gave cross-coupled products **21a–d**, which were deprotected with trifluoroacetic acid to produce target compounds **22a–d**. In similar fashion, we prepared the 3-position linked isomers **24a–e**, using the 3-iodobenzamide **17rr** in the Suzuki coupling protocol, and by proceeding through the bis-Boc intermediates **23a–e**.

In the case of **18t**, we synthesized a range of analogs designed to test the nature of the linker moiety and the substituents on the phenyl ring. These were synthesized using commercially available 3-phenylpropionic acids using the coupling reactions found in Scheme 2. Additionally, our initial molecular modeling suggested that a halogen on the 4- or 5-position of the 2-methoxyphenyl system could improve the binding affinity of the inhibitors (results not shown). The requisite halogenated 3-phenylpropionic acid starting materials were synthesized using two different methods, as dictated by the availability of starting materials. Thus, the 2-methoxy-5-halo-3-phenylpropionic acids were prepared using the first procedure shown in Scheme 4, starting from 2-methoxy-5-halobenzaldehydes. Wittig homologation of **25a** and **25b** with trimethyl phosphonoacetate gave the corresponding cinnamic esters **26a** and **26b**, which were catalytically hydrogenated to provide the saturated phenylpropionic esters **27a** and **27b**. Hydrolysis of these esters gave the phenylpropionic acids **28a** and **28b**. In the second sequence shown in Scheme 4 2-methoxy-4-substituted benzoic acid methyl esters **29a** and **29b** were reduced with lithium borohydride to give the corresponding benzyl alcohols **30a** and **30b**, which were converted to cinnamic esters **32a** and **32b** in a two-step process. Oxidation of the benzyl alcohols using Swern conditions provided the intermediate corresponding benzaldehydes **31a** and **31b**, which were then converted by Wittig homologation to the cinnamic esters. Reduction of cinnamic esters **32a** and **32b**, using a rhodium catalyst to selectively reduce the double bond and not hydrogenate the aryl halide, gave the 3-phenylpropionic esters **33a** and **33b**, respectively, which were then hydrolyzed to the corresponding phenylpropionic acids **34a** and **34b**.

Based on the activity of the best bis-quinolinyl amide compound (**18m**) and the best simple amide compound (**18t**), we designed a 'hybrid' compound by comparing the structures as shown in Figure 4. In this design, we incorporated amide **18t** in its entirety. We also incorporated the nitrogen atom of the quinoline in the same relative position by appending a pyridine ring to the 4-position of the 3-phenylpropionic acid. Thus, hybrid compound **35** incorporated the functionalities of both compounds **18m** and **18t** that we felt might be necessary for the biological activities of the single molecules.

Synthesis of the hybrid compound **35** is shown in Scheme 5. This preparation allowed us to make good use of previously prepared building blocks. Treatment of phenylpropionic acid **34b**, prepared as shown in Scheme 4, with 3-pyridylboronic acid under Suzuki coupling conditions, afforded the pyridyl-substituted phenylpropionic acid **36**. Coupling of **36** with aminoquinoline **16** (Scheme 1) using HATU provided amide **37**, which was then treated with TFA to remove the amine protecting groups and produce the hybrid compound **35**.

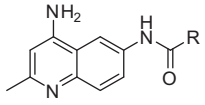
3. Results and discussion

Inhibitory activities of selected compounds against lethal factor (LF), botulinum neurotoxin A (BoNT/A), and matrix metalloproteases (MMPs) 1 and 9, as measured in a FRET-based assay are shown In Table 1. The FRET-bases assay uses a SNAP-based peptide that is cleaved by LF, which can be measured by a change in fluorescence. BoNT/A, MMP-1 and MMP-9 represent important profiling enzymes used as a gauge of target specificity; the inhibition of these enzymes was also measured using the corresponding FRET-tagged peptide substrate. The highly diverse collection of amides selected for synthesis in Table 1 was chosen to provide a variety of structures to survey this particular area of the binding site. Notably, there were two structures that emerged as the highest affinity structures; these were then used as templates for the selection of compounds for Tables 2 and 3. Thus, the (2-methoxyphenyl)propionic acid amide **18t** displayed good affinity (IC_{50} = 3.0 μM) for LF and good selectivity versus BoNT/A (IC_{50} >100 μM) as did the very different 4-(6-quinolinyl)benzoic acid amide **18m**, whose IC_{50} value versus LF was 1.6 μM and whose affinity for BoNT/A was 39 μM. Compound **18m** became the prototype for the compounds that were prepared in Table 2, and compound **18t** was the prototype for the compounds shown in Table 3.

We first turned our attention to prototype compound **18m**, and prepared many isomers of this compound as shown in Table 2. Interestingly, all of the isomers of **18m** did retain some inhibitory activity for LF. The most active isomers were compounds **22a**, **24a** and **24b**, wherein the point of attachment to the quinoline ring is furthest from a bridgehead position. The remaining isomers, all of which demonstrated less activity than **22a**, **24a** and **24b**, (i.e., double digit micromolar inhibitory instead of single digit micromolar activity), have quinoline attachment points that are adjacent to the bridgehead positions. Thus, we believe that the protein pocket that accepts the quinoline ring has a definite preference for the point of attachment, but that the nitrogen atom in the quinoline nucleus can reside in a variety of positions.

Compounds related to **18t** (Table 3) define very distinct and interesting structure activity relationships (SARs). Remarkably, when the 2-methoxy group was moved to the 3-position (**18v**) or the 4-position (**18u**), LF inhibitory activity was lost. Addition of a second methoxy group to the 3-position (**18x**) or the 5-position (**18w**) also led to a complete loss of activity. Addition of bromo (**18ee**) or chloro (**18ff**) to the 5-position also led to inactive compounds, although addition of these substituents to the 4-position (**18hh** and **18gg**, respectively) was tolerated to some extent, since these compounds had reduced affinities (58 μM and 78 μM, respectively). Benzyloxy (**18y**), chloro (**18z**) and methyl (**18aa**) groups instead of methoxy in the 2-position were

Table 1
LF and BoNT/A inhibitory activity of selected amides



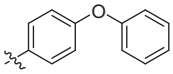
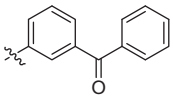
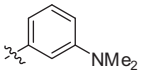
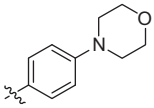
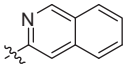
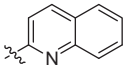
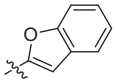
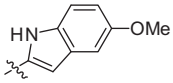
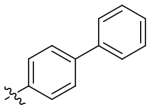
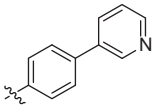
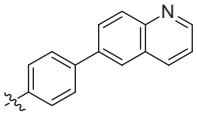
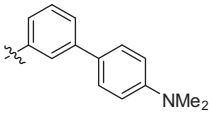
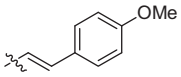
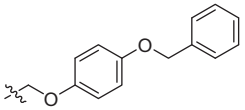
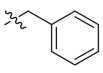
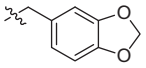
Compd #	R =	IC_{50} (μM)			
		LF[a]	BoNT/A[b]	MMP-1[c]	MMP-9[d]
1 NSC 12155	(see Fig. 1)	3.2	7.2	31	100
18a		70	>100	>100	>100
18b		73	>100	n.d.	n.d.
18c		>100	>100	n.d.	n.d.
18d		>100	47	n.d.	n.d.
18e		>100	>100	n.d.	n.d.
18f		>100	27	n.d.	n.d.
18g		52	>100	n.d.	n.d.
18h		81	>100	n.d.	n.d.
18i		90	>100	n.d.	n.d.
18j		44	35	n.d.	>100
18k		48	>100	n.d.	>100
18l		43	23	n.d.	>100
18m		1.6	38	n.d.	1.7
18n		17	69	n.d.	>100
18o		55	>100	n.d.	n.d.
18p		33	>100	>100	>100
18q		>100	>100	n.d.	n.d.
18r		>100	>100	n.d.	n.d.

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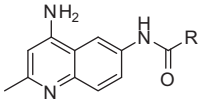
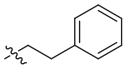
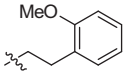
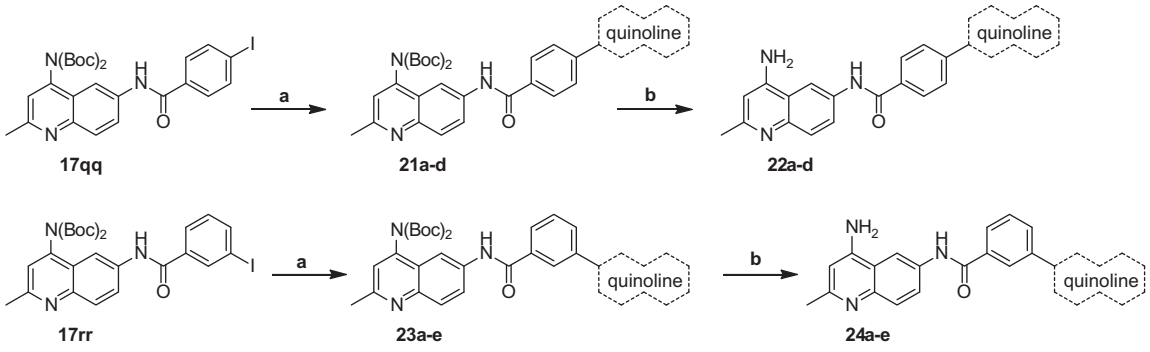
Table 1 (*continued*)

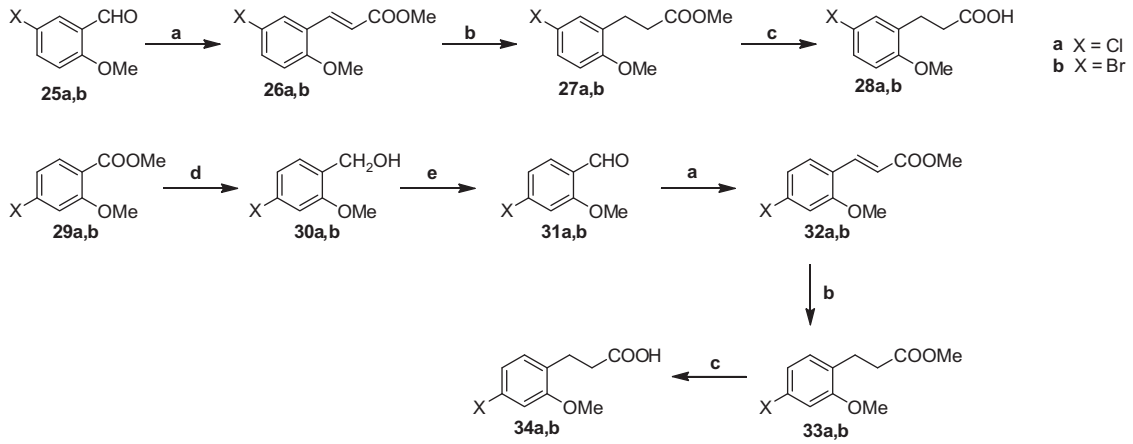


Compd #	R =	IC$_{50}$ (μM) LF[a]	BoNT/A[b]	MMP-1[c]	MMP-9[d]
18s		>100	>100	n.d.	n.d.
18t		3.0	>100	n.d.	>100

Not determined.
[a] *Bacillus anthracis* lethal factor.
[b] *Clostridium bolulinum* neurotoxin A.
[c] Human matrix metalloprotease-1.
[d] Human matrix metalloprotease-9.



Scheme 3. Synthesis of (quinolinyl)phenyl amides. Reagents and conditions: (a) (quinlinyl)boronic acid, Pd(PPh$_3$)$_4$, K$_2$CO$_3$, DMF 60 °C; (b) TFA, CH$_2$Cl$_2$.



Scheme 4. Synthesis of 4-, and 5-halo-2-methoxyphenylpropionic acids. Reagents and conditions: (a) trimethyl phosphonoacetate, *t*-BuOK, THF; (b) H$_2$, Rh/alumina, MeOH; (c) KOH, MeOH; (d) LiBH$_4$, THF, reflux; (e) oxalyl chloride, DMSO, CH$_2$Cl$_2$, −78 °C.

all inactive; surprisingly, a 2-trifluoromethyl group (**18bb**) afforded moderate activity (IC$_{50}$ = 39 μM). Unsaturation in the propionyl linker (**18dd**) was consistent with moderate affinity (IC$_{50}$ = 48 μM); however, chain length was intolerant to shortening (**18kk**) or lengthening (**18mm**) by even one methylene unit. When the 2-methoxy substituent was replaced with 2-ethoxy (**18ii**) or 2-*n*-propyloxy (**18jj**) substituents, activity was also totally lost. The phenyl ring itself was also not very tolerant to modification, as can be seen from the last three compounds in Table 3; the 3-pyridyl compound **18nn** retained moderate activity (IC$_{50}$ = 41 μM), whereas the 5-pyridyl isomer **18oo** and the thienyl compound **18pp** were both devoid of activity. Thus, in spite of the many variations that were made to the prototype structure **18t** in Table 3, compounds with improved affinity for LF over **18t** were not found, and we concluded that a very narrow but very real range of activity was defined and that we were dealing with a very specific recognition site in the LF protein.

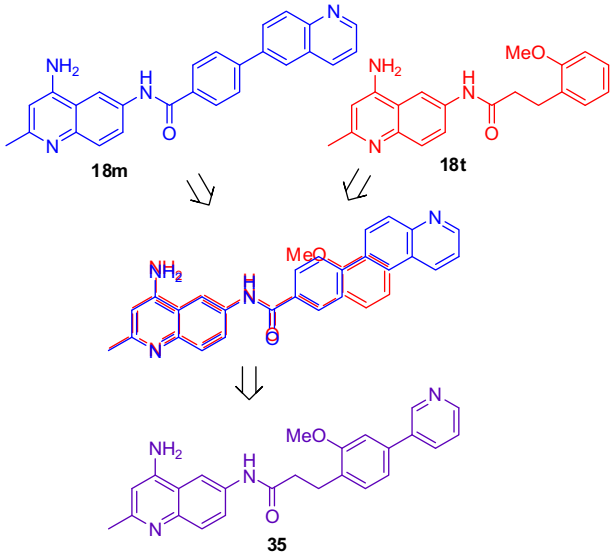
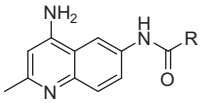


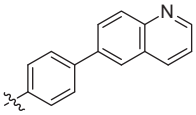
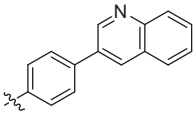
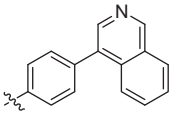
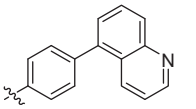
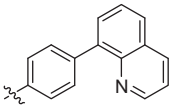
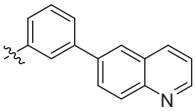
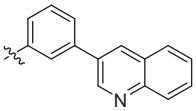
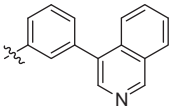
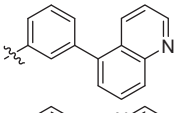
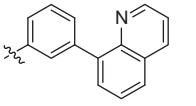
Figure 4. Design of 'hybrid' amide compound **35**.

Table 4 contains a group of diverse ureas that were prepared as described in Scheme 2. Our goal with this series was simply to explore a different linker functionality. Interestingly, compound **20i**, which has the closest relationship to the prototype amide compound **18t**, in that the linker lengths are the same, was devoid of activity. Although this series of compounds retained a modest degree of inhibitory activity for LF, with benzophenone **20g** being the most active (IC_{50} = 25 μM), this series as a whole lost selectivity vs. BoNT/A, and several of the compounds displayed greater affinity for Bont/A than for LF. Thus, we concluded that the propionic amide linker was important for LF affinity and selectivity.

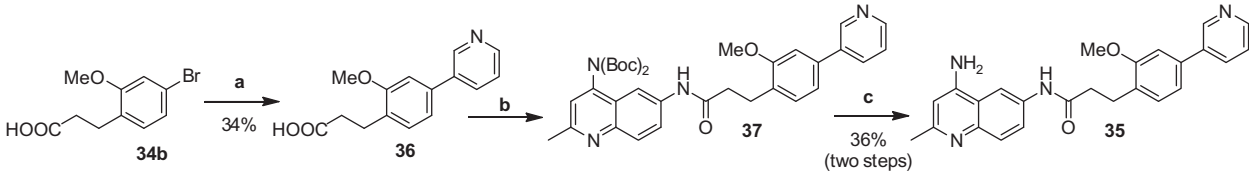
Given the potent anti-LF activities of compounds **18m** and **18t**, we recognized that it might be possible to design a hybrid compound that incorporated elements from both prototype compounds into a single, improved anti-LF agent. In Figure 4 is shown a simple overlay of these two compounds and also the structure of a 'hybrid' pyridyl compound that we envisioned might incorporate the important recognition elements from both **18t** and **18m**. Synthesis of compound **35** was carried out as described above, as shown in Scheme 5. The inhibitory activity of hybrid compound **35** is compared to that of prototype compounds **18t** and **18m** in Figure 3. Although the hybrid compound did display moderate inhibitory activity for LF (IC_{50} = 55 μM), this affinity was, disappointingly, not additive with respect to the two prototype compounds. One possible explanation for the lack of additivity is that the phenyl ring and the pyridyl ring are accessing two separate protein pockets, and that the link between these two aromatic hydrophobes needs further optimization to increase the affinity by combining the two aromatic units.

Table 2
LF and BoNT/A inhibitory activities of quinolinylphenyl amides



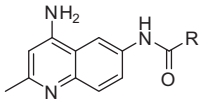
Compd #	R =	IC_{50} (μM) LF[a]	BoNT/A[b]	MMP-1[c]	MMP-9[d]
1 NSC 12155	(see Fig. 1)	3.24	7.2	>100	100
18m		1.6	38	>100	1.7
22a		3.0	>100	78	11
22b		13	65	>100	9.4
22c		10	34	n.d.	n.d.
22d		11	65	>100	17
24a		4.6	26	>100	>100
24b		5.7	45	>100	16
24c		21	>100	50	48
24d		33	68	65	50
24e		36	79	>100	>100

Not determined.
[a] *Bacillus anthracis* lethal factor.
[b] *Clostridium bolulinum* neurotoxin A.
[c] Human matrix metalloprotease-1.
[d] Human matrix metalloprotease-9.



Scheme 5. Synthesis of hybrid amide compound **35**. Reagents and conditions: (a) 3-pyridylboronic acid, $Pd(PPh_3)_4$, K_2CO_3, DMF, 60 °C; (g) **16**, HATU, DIPEA, DMF; (h) TFA, CH_2Cl_2.

Table 3
LF and BoNT/A inhibitory activity of (methoxyphenyl)propionic amides and related compounds



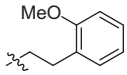
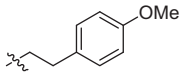
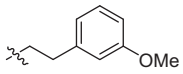
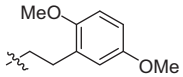
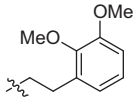
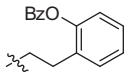
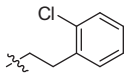
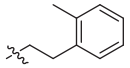
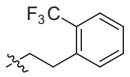
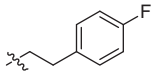
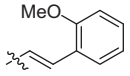
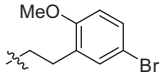
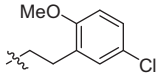
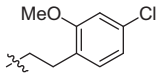
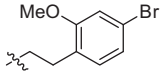
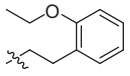
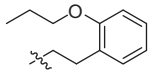
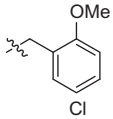
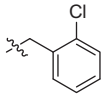
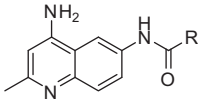
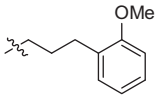
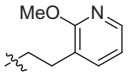
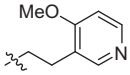
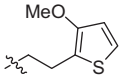
Compd #	R =	IC$_{50}$ (μM) LF[a]	BoNT/A[b]	MMP-1[c]	MMP-9[d]
1 NSC 12155	(see Fig. 1)	3.24	7.2	>100	100
18t		3.0	>100	>100	>100
18u		>100	>100	n.d.	n.d.
18v		>100	>100	n.d.	n.d.
18w		>100	>100	n.d.	n.d.
18x		>100	>100	n.d.	n.d.
18y		>100	>100	n.d.	n.d.
18z		>100	>100	n.d.	n.d.
18aa		>100	>100	n.d.	n.d.
18bb		38	>100	>100	>100
18cc		>100	>100	n.d.	n.d.
18dd		46	>100	>100	>100
18ee		>100	>100	n.d.	n.d.
18ff		>100	>100	n.d.	n.d.
18gg		78	>100	n.d.	n.d.
18hh		58	>100	n.d.	n.d.
18ii		>100	>100	n.d.	n.d.
18jj		>100	>100	n.d.	n.d.
18kk		>100	>100	n.d.	n.d.
18ll		>100	>100	n.d.	n.d.

Table 3 (*continued*)



Compd #	R =	IC$_{50}$ (μM) LF[a]	BoNT/A[b]	MMP-1[c]	MMP-9[d]
18mm		>100	>100	n.d.	n.d.
18nn		41	>100	>100	>100
18oo		>50	>100	n.d.	n.d.
18pp		>100	>100	n.d.	n.d.

Not determined.
[a] *Bacillus anthracis* lethal factor.
[b] *Clostridium bolulinum* neurotoxin A.
[c] Human matrix metalloprotease-1.
[d] Human matrix metalloprotease-9.

4. Conclusion

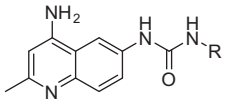
In this study we successfully optimized one half of the bisquinolinyl, symmetrical screening hit **1** and produced desymmetrized compounds that are selective inhibitors of *Bacillus anthracis* lethal factor. Compounds **18m** and **18t** are two of the best compounds in terms of potency and selectivity versus BoNT/A, MMP-1 and MMP-9 that were produced in this synthetic campaign, and provide new starting points with more drug-like features than compound **1**. The present work represents a midpoint in the total optimization of compound **1**, and represents a significant amount of structure activity relationship development that has mapped a portion of a lethal factor binding site that we think resides at or close to the active site of the enzyme.

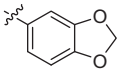
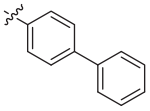
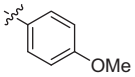
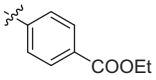
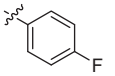
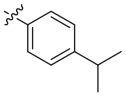
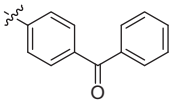
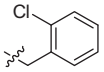
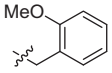
5. Experimental section

5.1. General methods

^{1}H NMR spectra were recorded on a Bruker AV 300 MHz spectrometer in the solvent indicated. Chemical shifts are reported in δ (ppm) units relative to internal standard tetramethylsilane (TMS). Mass spectra were recorded on a Thermo Scientific LCQ Fleet LC/MS instrument. Elemental analyses were performed by Columbia Analytical Services, 19408 Park Row Dr., Suite 320, Houston, TX 19408. Thin-layer chromatography was carried out with silica gel plates (Analtech, Newark, DE) with visualization by UV light (254 nm). Melting points were recorded with an EZ-Melt Apparatus (Stanford Research Systems, Stanford, CA). Column chromatography was carried out on silica gel 60 (Merck 230–400 mesh). All non-aqueous reactions were conducted in oven-dried glassware under a positive pressure of argon, using commercially available dry solvents. The synthesis of protected aminoquinoline **16** is found in the Supplementary data section; all other reagents were obtained from commercial sources.

Table 4
LF and BoNT/A inhibitory activities of ureas



Compd #	R =	IC$_{50}$ (μM) LF[a]	BoNT/A[b]	MMP-1[c]	MMP-9[d]
1 NSC 12155	(see Fig. 1)	3.24	7.2	>100	100
20a		>100	41	n.d.[e]	n.d.
20b		79	79	n.d.	n.d.
20c		>100	41	n.d.	n.d.
20a		87	51	n.d.	n.d.
20e		95	32	n.d.	n.d.
20f		83	62	n.d.	n.d.
20g		26	67	>100	>100
20h		61	>100	n.d.	n.d.
20i		>100	>100	n.d.	n.d.

[a] *Bacillus anthracis* lethal factor.
[b] *Clostridium bolulinum* neurotoxin A.
[c] Human matrix metalloprotease-1.
[d] Human matrix metalloprotease-9.
[e] Not determined.

5.2. Synthesis

5.2.1. *N*-(4-[Bis{*tert*-butoxycarbonyl}amino]-2-methylquinolin-6-yl)-4-iodobenzamide (17qq)

A mixture of 4-(bis[*tert*-butoxycarbonyl]amino)-6-amino-2-methylquinoline (3.00 g, 8.0 mmol), 4-iodobenzoic acid (3.98 g, 16 mmol, 2.0 equiv); 2-(7-aza-1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU; 7.60 g, 20 mmol, 2.5 equiv), DIEA (15 mL, 86 mmol, 10 equiv) in DMF (100 mL) was stirred at room temperature for 48 h. The mixture was poured into 200 mL of 5% aq Na_2CO_3 and extracted with EtOAc (2200 mL). The combined organic extracts were washed with 5% aq Na_2CO_3 (1 × 150 mL), brine (1 × 150 mL), dried ($MgSO_4$), and evaporated to provide a brown solid. The solid was re-crystallized from CH_3CN to provide 2.93 g (61%) of the final product as a fine tan-colored powder: R_f 0.54 (25% EtOAc/Hex); mp 196–256 °C decomp.; ^{1}H NMR (DMSO-d_6) δ 10.63 (s, 1H), 8.38 (d, 1H), 8.13 (dd, 1H), 7.97 (d, 1H), 7.94 (d, 2H), 7.79 (d, 1H), 7.76 (d, 1H), 7.38 (s, 1H), 2.65 (s, 3H), 1.34 (s, 18H).

5.2.2. *N*-(4-[Bis{*tert*-butoxycarbonyl}amino]-2-methylquinolin-6-yl)-3-iodobenzamide (17rr)

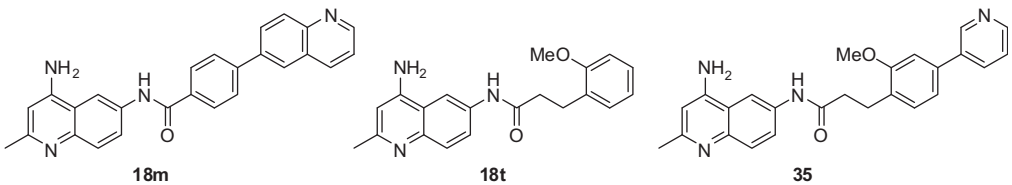
A mixture of 4-(bis[*tert*-butoxycarbonyl]amino)-6-amino-2-methylquinoline (3.00 g, 8.0 mmol), 3-iodobenzoic acid (3.98 g, 16 mmol, 2.0 equiv); 2-(7-aza-1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU; 7.60 g, 20 mmol, 2.5 equiv), DIEA (15 mL, 86 mmol, 10 equiv) in DMF (100 mL) was stirred at room temperature for 48 h. The mixture was poured into 200 mL of 5% aq Na_2CO_3 and extracted with EtOAc (2 × 200 mL). The combined organic extracts were washed with 5% aq Na_2CO_3 (1 × 150 mL), brine (1 × 150 mL), dried ($MgSO_4$), and evaporated to provide a brown solid. The solid was re-crystallized from CH_3CN to provide 2.75 g (58%) of product as a brown granular solid: mp 199–253 °C decomp.; ^{1}H NMR (DMSO-d_6) δ 10.66 (s, 1H), 8.39 (d, 1H), 8.32 (dd, 1H), 8.12 (dd, 1H), 8.01–7.96 (m, 3H), 7.38 (t, 2H), 2.89 (s, 3H), 1.34 (s, 18H).

5.2.3. General synthesis of compounds 18a–pp

Mixtures of 4-(di-*tert*-butoxycarbonylamino)-6-amino-2-methylquinoline (100 mg, 0.27 mmol), the respective carboxylic acid (0.53 mmol, 2 equiv), 2-(7-aza-1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU; 190 mg, 0.500 mmol, 2.0 equiv) and di-*iso*-propylethylamine (0.5 mL, 2.9 mmol, 10 equiv) in DMF (5 mL) were stirred at room temperature for 16 h. The mixtures were poured into 50 mL of 5% aq Na_2CO_3 and extracted with EtOAc (3 × 50 mL). The combined organic layers were washed with 5% aq Na_2CO_3 (1 × 50 mL), brine (1 × 50 mL), dried ($MgSO_4$), and evaporated. The residual materials were co-evaporated with diethyl ether and treated without further purification with 1:1 TFA/DCM (5 mL) for 30 min–2 h. Removal of solvents gave crude materials as solids or oils that were triturated with diethyl ether and recrystallized from CH_3CN/MeOH) to yield the final product as a TFA salt or purified using prep TLC (80:18:2 $CHCl_3$/MeOH/aq CH_3NH_2) to provide the free base. Some of the free bases were further reacted with a slight excess of *p*-toluenesulfonic acid in diethyl ether to provide the corresponding *p*-TsOH salts.

5.2.3.1. *N*-(4-Amino-2-methylquinolin-6-yl)-4-phenoxybenzamide trifluoroacetate (18a).

Compound **18a** was obtained as a light yellow powder: R_f 0.33 (80:18:2 $CHCl_3$/MeOH/aq CH_3NH_2); mp 202–216 °C; ^{1}H NMR (DMSO-d_6) δ 13.02 (b, 1H), 10.60 (b, 1H), 8.80 (b, 1H), 8.60 (b, 1H), 8.06–8.09 (m, 3H), 7.82 (d, 1H), 7.48 (t, 2H), 7.27–7.12 (m, 5H), 6.60 (s, 1H), 2.58 (s, 3H); *m*/*z* expected 369.1, found 370.5 ([M+H]$^+$).



18m IC$_{50}$ = 3.0 μM
18t IC$_{50}$ = 1.5 μM
35 IC$_{50}$ = 55 μM

Figure 3. Activity of hybrid amide compound and corresponding parent compounds.

5.2.3.2. *N*-(4-Amino-2-methylquinolin-6-yl)-3-benzoylbenzamide trifluoroacetate (18b). Compound **18b** was obtained as a light brown powder: R_f 0.37 (80:18:2 $CHCl_3$/MeOH/aq CH_3NH_2); mp 209–218 °C; ^{1}H NMR (DMSO-d_6) δ 13.45 (b, 1H), 10.87 (s, 1H), 8.77 (s, 1H), 8.68 (b, 2H), 8.38–8.31 (m, 2H), 8.06–7.97 (m, 2H), 7.85–7.63 (m, 5H), 7.71–7.58 (m, 2H), 6.59 (s, 1H), 2.58 (s, 3H); *m*/*z* expected 381.1, found 382.6 ([M+H]$^+$).

5.2.3.3. *N*-(4-Amino-6-methylquinolin-6-yl)nicotinamide trifluoroacetate (18c). Compound **18c** was obtained as a yellow powder: R_f 0.19 (80:18:2 $CHCl_3$/MeOH/aq CH_3NH_2); mp 175–180 °C; ^{1}H NMR (DMSO-d_6) δ 13.46 (b, 1H), 10.88 (s, 1H), 9.18 (d, 1H), 8.82–8.78 (m, 3H), 8.70 (b, 2H), 8.38–8.34 (m, 1H), 8.03 (d, 1H), 7.84 (d, 1H), 6.60 (s, 1H), 2.60 (s, 3H); *m*/*z* expected 278.1, found 279.4 ([M+H]$^+$).

5.2.3.4. *N*-(4-Amino-2-methylquinolin-6-yl)furan-2-carboxamide trifluoroacetate (18d). Compound **18d** was obtained as a yellow-orange powder: R_f 0.15 (80:18:2 $CHCl_3$/MeOH/aq CH_3NH_2); mp 192–207 °C; ^{1}H NMR (DMSO-d_6) δ 13.46 (s, 1H), 10.64 (s, 1H), 8.69 (b, 3H), 8.10–7.99 (m, 2H), 7.82 (d, 1H), 7.24 (d, 1H), 6.76–6.72 (m, 1H), 6.59 (s, 1H), 2.58 (s, 3H); *m*/*z* expected 267.1, found 268.2 ([M+H]$^+$).

5.2.3.5. *N*-(4-Amino-2-methylquinolin-6-yl)-3-(dimethylamino) benzamide trifluoroacetate (18e). Compound **18e** was obtained as a tan powder: R_f 0.43 (80:18:2 $CHCl_3$/MeOH/aq CH_3NH_2); mp 210–212 °C; ^{1}H NMR (DMSO-d_6) δ 13.53 (s, 1H), 10.57 (s, 1H), 8.77 (s, 1H), 8.76 (s, 2H), 8.06 (d, 1H), 7.83 (d, 1H), 7.65 (b, 1H), 7.61–7.56 (m, 3H), 6.96 (s, 1H), 6.59 (s, 1H), 2.98 (s, 6H), 2.55 (s, 3H); *m*/*z* expected 320.2, found 321.5 ([M+H]$^+$).

5.2.3.6. *N*-(4-Amino-2-methylquinolin-6-yl)-4-(1-morpholino) benzamide trifluoroacetate (18f). Compound **18f** was obtained as a tan powder: R_f 0.48 (80:18:2 $CHCl_3$/MeOH/aq CH_3NH_2); mp 109–111 °C; ^{1}H NMR (DMSO-d_6) δ 13.48 (s, 1H), 10.42 (s, 1H), 8.77 (s, 1H), 8.76 (b, 2H), 8.07 (m, 1H), 8.04 (d, 2H), 7.81 (d, 1H), 7.06 (d, 2H), 6.58 (s, 1H), 3.76 (t, 4H), 3.28 (t, 4H), 2.58 (s, 3H); *m*/*z* expected 362.2, found 363.5 ([M+H]$^+$).

5.2.3.7. *N*-(4-Amino-2-methylquinolin-6-yl)quinoline-2-carboxamide trifluoroacetate (18g). Compound **18f** was obtained as a pale yellow powder: R_f 0.45 (90:9:1 $CHCl_3$/MeOH/aq CH_3NH_2); mp 224–227 °C; ^{1}H NMR (DMSO-d_6) δ 13.40 (s, 1H), 10.91 (s, 1H) 8.86 (s, 1H), 8.66 (b, 2H), 8.56 (d, 1H), 8.42 (d, 1H), 8.48 (dd, 1H), 8.08–8.03 (m, 2H), 7.92–7.84 (m, 2H), 7.74 (t, 1H), 6.61 (s, 1H), 2.56 (s, 3H); *m*/*z* expected 328.1, found 329.4 ([M+H]$^+$).

5.2.3.8. *N*-(4-Amino-2-methylquinolin-6-yl)isoquinoline-3-carboxamide trifluoroacetate (18h). Compound **18h** was obtained as a yellow powder: R_f 0.40 (80:18:2 $CHCl_3$/MeOH/aq CH_3NH_2); mp 288–290 °C; ^{1}H NMR (DMSO-d_6) δ 14.49 (s, 1H), 11.05 (s, 1H), 9.52 (s, 1H), 8.82 (s, 1H), 8.75 (s, 1H), 8.75 (s, 2H), 8.73–8.72 (m, 3H), 8.12 (d, 1H), 7.94–7.88 (m, 2H), 6.68 (s, 1H), 2.62 (s, 3H); *m*/*z* expected 328.1, found 329.4 ([M+H]$^+$).

5.2.3.9. *N*-(4-Amino-2-methylquinolin-6-yl)benzofuran-2-carboxamide trifluoroacetate (18i). Compound **18i** was obtained as a yellow microcrystalline solid: R_f 0.42 (80:18:2 $CHCl_3$/MeOH/aq CH_3NH_2); mp 218–219 °C; ^{1}H NMR (DMSO-d_6) δ 13.48 (s, 1H), 10.99 (s, 1H), 8.77 (s, 1H), 8.71 (b, 2H), 8.12 (d, 1H), 7.86 (dd, 3H), 7.75 (d, 1H), 7.50 (t, 1H), 7.40 (t, 1H), 6.61 (s, 1H), 2.59 (s, 3H); *m*/*z* expected 317.1, found 318.5 ([M+H]$^+$).

5.2.3.10. *N*-(4-Amino-2-methylquinolin-6-yl)-5-methoxyindole-2-carboxamide trifluoroacetate (18j). Compound **18j** was obtained as a yellow microcrystalline solid: R_f 0.37 (80:18:2 $CHCl_3$/MeOH/aq CH_3NH_2); mp 205–206 °C; ^{1}H NMR (DMSO-d_6) δ 13.53 (s, 1H), 11.63 (s, 1H), 10.62 (s, 1H), 8.94 (s, 3H), 8.13 (d, 1H), 7.97 (d, 1H), 7.40 (s, 2H), 7.16 (s, 1H), 6.91 (d, 1H), 6.60 (s, 1H), 3.83 (s, 3H), 2.56 (s, 3H); *m*/*z* expected 346.1, found 347.4 ([M+H]$^+$).

5.2.3.11. *N*-(4-Amino-2-methylquinolin-6-yl)biphenyl-4-carboxamide trifluoroacetate (18k). Compound **18k** was obtained as a light yellow powder: R_f 0.35 (80:18:2 $CHCl_3$/MeOH/aq CH_3NH_2); mp 263–267; ^{1}H NMR (DMSO-d_6) δ 13.40 (b, 1H), 10.74 (s, 1H), 8.80 (s, 1H), 8.60 (s, 2H), 8.16–8.06 (m, 4H), 7.79–7.77 (m, 4H), 7.55–7.44 (m, 3H), 6.56 (s, 1H), 2.58 (s, 3H); *m*/*z* expected 323.2, found 354.6 ([M+H]$^+$).

5.2.3.12. *N*-(4-Amino-2-methylquinolin-6-yl)-4-(pyridin-3-yl) benzamide trifluoroacetate (18l). Compound **18l** was obtained as a tan powder: R_f 0.27 (80:18:2 $CHCl_3$/MeOH/aq CH_3NH_2); mp 227–228 °C; ^{1}H NMR (DMSO-d_6) δ 13.45 (s, 1H), 10.84 (s, 1H), 9.06 (d, 1H), 8.84 (d, 1H), 8.68 (d, 3H), 8.28 (d, 1H), 8.18 (d, 2H), 8.07 (dd, 1H), 7.99 (d, 2H), 7.85 (d, 1H), 7.64–7.60 (m, 1H), 6.60 (s, 1H), 2.59 (s, 3H); *m*/*z* expected 354.1, found 355.2 ([M+H]$^+$).

5.2.3.13. *N*-(4-Amino-2-methylquinolin-6-yl)-4-(quinoline-6-yl)benzamide trifluoroacetate (18m). Compound **18m** was obtained as a tan powder: R_f 0.29 (80:18:2 $CHCl_3$/MeOH/aq CH_3NH_2); mp 214–216 °C; ^{1}H NMR (DMSO-d_6) δ 13.49 (s, 1H), 10.80 (s, 1H), 8.98 (s, 1H), 8.82 (s, 1H), 8.71 (b, 2H), 8.53 (d, 2H), 8.47 (s, 1H), 8.21 (d, 4H), 8.09 (s, 3H), 7.86 (d, 1H), 7.64 (s, 1H), 6.60 (s, 1H), 2.60 (s, 3H); *m*/*z* expected 404.2, found 405.5 ([M+H]$^+$).

5.2.3.14. *N*-(4-Amino-2-methylquinolin-6-yl)-3-(4-dimethylaminophenyl) benzamide (18n). Compound **18n** was obtained as an off-white powder: R_f 0.28 (80:18:2 $CHCl_3$/MeOH/aq CH_3NH_2); mp 206–213 °C; ^{1}H NMR (DMSO-d_6) δ 10.64 (s, 1H), 8.70 (s, 1H), 8.40 (s, 1H), 8.38 (b, 2H), 8.03 (d, 1H), 7.84 (m, 3H), 7.65–7.51 (m, 3H), 6.84 (d, 2H), 6.57 (s, 1H), 2.89 (s, 6H), 2.56 (s, 3H); *m*/*z* expected 396.2, found 397.5 ([M+H]$^+$).

5.2.3.15. (*E*)-*N*-(4-Amino-2-methylquinolin-6-yl)-4-methoxycinamamide trifluoroacetate (18o). Compound **18o** was obtained as a pink powder: R_f 0.41 (80:18:2 $CHCl_3$/MeOH/aq CH_3NH_2); mp >300 °C; ^{1}H NMR (DMSO-d_6) δ 13.4 (b, 1H), 10.53 (s, 1H), 8.66 (s, 1H), 8.48 (b, 2H), 7.93 (d, 1H), 7.80 (d, 1H), 7.62–7.58 (m, 3H), 7.03 (d, 2H), 6.75 (d, 1H), 6.56 (s, 1H), 3.81 (s, 3H), 2.56 (s, 3H); *m*/*z* expected 333.1, found 334.4 ([M+H]$^+$).

5.2.3.16. *N*-(4-Amino-2-methylquinolin-6-yl)-2-[(4-benzyloxy) phenoxy]acetamide trifluoroacetate (18p). Compound **18p** was obtained as a light yellow powder: R_f 0.23 (80:18:2 $CHCl_3$/MeOH/aq CH_3NH_2); mp 208–210 °C; ^{1}H NMR (DMSO-d_6) δ 13.53 (b, 1H), 10.55 (b, 1H), 8.90 (d, 3H), 7.41 (m, 5H), 6.97 (s, 4H), 6.57 (s, 1H), 5.08 (d, 2H), 4.70 (d, 2H), 2.57 (s, 3H); *m*/*z* expected 413.2, found 414.4 ([M+H]$^+$).

5.2.3.17. *N*-(4-Amino-2-methylquinolin-6-yl)-2-phenylacetamide (18q). Compound **18q** was obtained as a pink crystalline solid: R_f 0.27 (80:18:2 $CHCl_3$/MeOH/aq CH_3NH_2); mp (sublimed 160 °C); ^{1}H NMR (DMSO-d_6) δ 10.67 (s, 1H), 8.61 (d, 1H), 8.55 (s, 2H), 7.90 (dd, 1H), 7.88 (d, 1H), 7.40–7.24 (m, 5H), 6.56 (s, 1H), 3.73 (s, 2H), 2.55 (s, 3H); *m*/*z* expected 291.4, found 292.9 ([M+H]$^+$).

5.2.3.18. *N*-(4-Amino-2-methylquinolin-6-yl)-2-(benzo[*d*][1,3]-dioxol-5-yl)acetamide trifluoroacetate (18r). Compound **18r** was obtained as a brown crystalline solid: R_f 0.30 (90:9:1 $CHCl_3$/MeOH/aq CH_3NH_2); mp >160 °C (subl.); ^{1}H NMR

(DMSO-d_6) δ 13.52 (b, 1H), 10.62 (s, 1H), 8.61 (s, 2H), 8.58 (d, 1H), 7.91 (dd, 1H), 7.77 (d, 1H), 6.95 (d, 1H), 6.89–6.81 (m, 2H), 6.55 (s, 1H), 5.99 (s, 2H), 3.62 (s, 2H), 2.56 (s, 3H); *m*/*z* expected 335.1, found 336.4 ([M+H]$^+$).

5.2.3.19. *N*-(4-Amino-2-methylquinolin-6-yl)-3-phenylpropionamide trifluoroacetate (18s). Compound **18s** was obtained as a yellow powder: R_f 0.18 (80:18:2 $CHCl_3$/MeOH/aq CH_3NH_2); mp 207–209 °C; ^{1}H NMR (DMSO-d_6) δ 13.41 (b, 1H), 10.39 (s, 1H), 8.50 (s, 3H), 7.81 (dd, 2H), 7.33–7.28 (m, 3H), 7.22–7.17 (m, 2H), 6.58 (s, 1H), 2.96 (t, 2H), 2.72 (t, 2H), 2.56 (s, 3H); *m*/*z* expected 305.2, found 306.5 ([M+H]$^+$).

5.2.3.20. *N*-(4-Amino-2-methylquinolin-6-yl)-3-(2-methoxyphenyl)propionamide trifluoroacetate (18t). Compound **18t** was obtained as a tan powder: R_f 0.27 (90:9:1 $CHCl_3$/MeOH/aq CH_3NH_2); mp 240–242 °C; ^{1}H NMR (DMSO-d_6) δ 13.30 (b, 1H), 10.33 (s, 1H), 8.60 (b, 3H), 7.80 (d, 2H), 7.19 (d, 2H), 6.97 (d, 1H), 6.88 (d, 1H), 6.56 (s, 1H), 3.80 (s, 3H), 2.90 (t, 2H), 2.66 (t, 2H), 2.56 (s, 3H); *m*/*z* expected 335.2, found 336.5 ([M+H]$^+$).

5.2.3.21. *N*-(4-Amino-2-methylquinolin-6-yl)-3-(4-methoxyphenyl)propionamide *p*-toluenesulfonate (18u). Compound **18u** was obtained as a white powder: R_f 0.40 (90:9:1 $CHCl_3$/MeOH/aq CH_3NH_2); mp 234–236 °C; ^{1}H NMR (DMSO-d_6) δ 13.33 (s, 1H), 10.33 (s, 1H), 8.6 (b, 3H), 7.78 (q, 2H), 7.48 (d, 2H), 7.14 (dd, 4H), 6.85 (d, 2H), 6.56 (s, 1H), 3.71 (s, 3H), 2.89 (t, 2H), 2.67 (t, 2H), 2.56 (s, 3H), 2.28 (s, 3H).

5.2.3.22. *N*-(4-Amino-2-methylquinolin-6-yl)-3-(3-methoxyphenyl)propionamide trifluoroacetate (18v). Compound **18v** was obtained as a white powder: R_f 0.38 (90:9:1 $CHCl_3$/MeOH/aq CH_3NH_2); mp 234–236 °C; ^{1}H NMR (DMSO-d_6) δ 13.40 (b, 1H), 10.37 (s, 1H), 8.58 (s, 3H), 7.86–7.71 (m, 2H), 7.20 (t, 1H), 6.83 (s, 2H), 6.76 (d, 1H), 6.56 (s, 1H), 3.72 (s, 3H), 2.93 (t, 2H), 2.71 (t, 2H), 2.56 (s, 3H); *m*/*z* expected 335.2, found 336.5 ([M+H]$^+$).

5.2.3.23. *N*-(4-Amino-2-methylquinolin-6-yl)-3-(2,5-dimethoxyphenyl)propionamide *p*-toluenesulfonate (18w). Compound **18w** was obtained as a white powder: R_f 0.43 (90:9:1 $CHCl_3$/MeOH/aq CH_3NH_2); mp 240–244 °C; ^{1}H NMR (DMSO-d_6) δ 13.33 (s, 1H), 10.33 (s, 1H), 8.61 (s, 3H), 7.82 (m, 2H), 7.47 (d, 2H), 7.11 (d, 2H), 6.88 (d, 1H), 6.79–6.76 (m, 2H), 6.56 (s, 1H), 3.75 (s, 3H), 3.66 (s, 3H), 2.88 (t, 2H), 2.65 (t, 2H), 2.56 (s, 3H), 2.28 (s, 3H); *m*/*z* expected 365.2, found 366.4 ([M+H]$^+$).

5.2.3.24. *N*-(4-Amino-2-methylquinolin-6-yl)-3-(2,3-dimethoxyphenyl)propionamide *p*-toluenesulfonate (18x). Compound **18x** was obtained as an off-white powder: R_f 0.48 (90:9:1 $CHCl_3$/MeOH/aq CH_3NH_2); mp 204–206 °C; ^{1}H NMR (DMSO-d_6) δ 13.33 (s, 1H), 10.31 (d, 1H), 8.81 (s, 1H), 8.65 (b, 2H), 7.79 (q, 2H), 7.47 (d, 2H), 7.11 (d, 2H), 6.96–6.77 (m, 3H), 6.55 (s, 1H), 3.81 (s, 3H), 3.75 (s, 3H), 2.93 (t, 2H), 2.66 (t, 2H), 2.55 (s, 3H), 2.28 (s, 3H); *m*/*z* expected 365.2, found 366.5 ([M+H]$^+$).

5.2.3.25. *N*-(4-Amino-2-methylquinolin-6-yl)-3-(2-benzoyloxyphenyl)propionamide trifluoroacetate (18y). Compound **18y** was obtained as a white powder: R_f 0.46 (90:9:1 $CHCl_3$/MeOH/aq CH_3NH_2); mp 176–180 °C; ^{1}H NMR (DMSO-d_6) δ 13.37 (s, 1H), 10.32 (s, 1H), 8.57 (b, 3H), 8.18 (d, 2H), 7.81–7.73 (m, 3H), 7.61 (t, 2H), 7.45–7.43 (m, 1H), 7.36–7.25 (m, 3H), 6.55 (s, 1H), 2.90 (t, 2H), 2.72 (t, 2H), 2.55 (s, 3H); *m*/*z* expected 425.2, found 426.5 ([M+H]$^+$).

5.2.3.26. *N*-(4-Amino-2-methylquinolin-6-yl)-3-(2-chlorophenyl)propionamide trifluoroacetate (18z). Compound **18z** was obtained as a white powder: R_f 0.42 (90:9:1 $CHCl_3$/MeOH/aq CH_3NH_2); mp 214–216 °C; ^{1}H NMR (DMSO-d_6) δ 13.38 (s, 1H), 10.38 (s, 1H), 8.60 (b, 3H), 7.84 (dd, 1H), 7.77 (d, 1H), 7.46–7.39 (m, 2H), 7.32–7.25 (m, 2H), 6.56 (s, 1H), 3.07 (t, 2H), 2.74 (t, 2H), 2.56 (s, 3H); *m*/*z* expected 339.1, found 340.5 ([M+H]$^+$).

5.2.3.27. *N*-(4-Amino-2-methylquinolin-6-yl)-3-(2-methylphenyl)propionamide trifluoroacetate (18aa). Compound **18aa** was obtained as a tan powder: R_f 0.44 (90:9:1 $CHCl_3$/MeOH/aq CH_3NH_2); mp 239–240 °C; ^{1}H NMR (DMSO-d_6) δ 13.40 (b, 1H), 10.39 (s, 1H), 8.59 (s, 3H), 7.85 (dd, 1H), 7.76 (d, 1H), 7.21–7.07 (m, 4H), 6.56 (s, 1H), 2.94 (t, 2H), 2.67 (t, 2H), 2.56 (s, 3H), 2.32 (s, 3H); *m*/*z* expected 319.2, found 320.5 ([M+H]$^+$).

5.2.3.28. *N*-(4-Amino-2-methylquinolin-6-yl)-3-[2-(trifluoromethyl)phenyl]propionamide trifluoroacetate (18bb). Compound **18bb** was obtained as a pale green crystalline solid: R_f 0.29 (90:9:1 $CHCl_3$/MeOH/aq CH_3NH_2); mp 220–225 °C; ^{1}H NMR (DMSO-d_6) δ 13.42 (s, 1H), 10.45 (s, 1H), 8.64 (br s, 3H), 7.82–7.55 (m, 5H), 7.49 (t, 1H), 6.56 (s, 1H), 3.13 (t, 2H), 2.75 (t, 2H), 2.56 (s, 3H); *m*/*z* expected 373.1, found 374.5 ([M+H]$^+$).

5.2.3.29. *N*-(4-Amino-2-methylquinolin-6-yl)-3-(4-fluorophenyl)propionamide *p*-toluenesulfonate (18cc). Compound **18cc** was obtained as a white powder: R_f 0.32 (90:9:1 $CHCl_3$/MeOH/aq CH_3NH_2); mp 222–225 °C; ^{1}H NMR (DMSO-d_6) δ 13.33 (s, 1H), 10.34 (s, 1H), 8.58 (b, 3H), 7.82–7.75 (m, 2H), 7.47 (d, 2H), 7.33–7.28 (m, 2H), 7.14–7.08 (m, 4H), 6.59 (s, 1H), 2.94 (t, 2H), 2.70 (t, 2H), 2.56 (s, 3H), 2.28 (s, 3H); *m*/*z* expected 323.1, found 324.4 ([M+H]$^+$).

5.2.3.30. (*E*)-*N*-(4-Amino-2-methylquinolin-6-yl)-2-methoxycinanamide trifluoroacetate (18dd). Compound **18dd** was obtained as a yellow powder: R_f 0.32 (90:9:1 $CHCl_3$/MeOH/aq CH_3NH_2); mp 218–220 °C; ^{1}H NMR (DMSO-d_6) δ 13.74 (b, 1H), 10.66 (s, 1H), 8.65 (s, 1H), 8.57 (b, 2H), 7.99–7.81 (m, 3H), 7.60 (d, 1H), 7.43 (t, 1H), 7.16 (d, 1H), 7.04 (t, 1H), 6.97–6.92 (m, 1H), 6.58 (s, 1H), 3.91 (s, 3H), 2.57 (s, 3H); *m*/*z* expected 333.1, found 334.4 ([M+H]$^+$).

5.2.3.31. *N*-(4-Amino-2-methylquinolin-6-yl)-3-(5-bromo-2-methoxyphenyl)propionamide trifluoroacetate (18ee). Compound **18ee** was obtained as an off-white crystalline solid: R_f 0.52 (90:9:1 $CHCl_3$/MeOH/aq CH_3NH_2); mp 135–139 °C; ^{1}H NMR (DMSO-d_6) δ 13.42 (s, 1H), 10.35 (s, 1H), 8.61 (b, 3H), 7.82–7.76 (m, 2H), 7.37–7.35 (m, 2H), 6.94 (d, 1H), 6.56 (s, 1H), 3.80 (s, 3H), 2.89 (t, 2H), 2.67 (t, 2H), 2.56 (s, 3H); *m*/*z* expected 413.1, found 414.5 ([M+H]$^+$).

5.2.3.32. *N*-(4-Amino-2-methylquinolin-6-yl)-3-(5-chloro-2-methoxyphenyl)propionamide trifluoroacetate (18ff). Compound **18ff** was obtained as a white powder: R_f 0.33 (90:9:1 $CHCl_3$/MeOH/aq CH_3NH_2); mp 186–189 °C; ^{1}H NMR (DMSO-d_6) δ 13.31 (s, 1H), 10.24 (s, 1H), 8.48 (b, 3H), 7.68 (d, 2H), 7.11 (s, 2H), 6.87 (d, 1H), 6.44 (s, 1H), 3.68 (s, 3H), 2.77 (m, 2H), 2.55 (m, 2H), 2.44 (s, 3H); *m*/*z* expected 369.1, found 370.4 ([M+H]$^+$).

5.2.3.33. *N*-(4-Amino-2-methylquinolin-6-yl)-3-(4-chloro-2-methoxyphenyl)propionamide trifluoroacetate (18gg). Compound **18gg** was obtained as a white powder: R_f 0.31 (90:9:1 $CHCl_3$/MeOH/aq CH_3NH_2); mp 141–143 °C; ^{1}H NMR (DMSO-d_6) δ 13.30 (s, 1H), 10.36 (s, 1H), 8.61 (b, 3H), 7.80 (t, 2H), 7.25 (t, 1H), 7.05 (s, 1H), 6.95 (dd, 1H), 6.55 (s, 1H), 3.87 (s, 3H), 2.70 (t, 2H), 2.86 (t, 2H), 2.56 (s, 3H); *m*/*z* expected 369.1, found 370.4 ([M+H]$^+$).

5.2.3.34. *N*-(4-Amino-2-methylquinolin-6-yl)-3-(4-bromo-2-methoxyphenyl)propionamide trifluoroacetate (18hh). Compound **18hh** was obtained as a white powder: R_f 0.34 (90:9:1 $CHCl_3$/MeOH/aq CH_3NH_2); mp 122–126 °C; ^{1}H NMR (DMSO-d_6) δ 13.44 (s, 1H), 10.36 (s, 1H), 8.60 (b, 3H), 7.79 (m, 2H), 7.15–7.05 (m, 3H), 6.56 (s, 1H), 3.83 (s, 3H), 2.87 (t, 2H), 2.65 (t, 2H), 2.56 (s, 3H); *m*/*z* expected 413.1, found 414.5 ([M+H]$^+$).

5.2.3.35. *N*-(4-Amino-2-methylquinolin-6-yl)-3-(2-ethoxyphenyl)propionamide trifluoroacetate (18ii). Compound **18ii** was obtained as a white powder: R_f 0.34 (90:9:1 $CHCl_3$/MeOH/aq CH_3NH_2); mp 122–126 °C; ^{1}H NMR (DMSO-d_6) δ 13.38 (s, 1H), 10.33 (s, 1H), 8.62 (b, 3H), 7.84–7.75 (m, 2H), 7.20–7.15 (m, 2H), 6.95 (d, 1H), 6.87–6.83 (m, 1H), 6.56 (s, 1H), 4.05 (q, 2H), 2.92 (t, 2H), 2.67 (t, 2H), 2.56 (s, 3H), 1.36 (t, 3H); *m*/*z* expected 349.2, found 350.4 ([M+H]$^+$).

5.2.3.36. *N*-(4-Amino-2-methylquinolin-6-yl)-3-[2-(1-propoxy)phenyl]propionamide trifluoroacetate (18jj). Compound **18jj** was obtained as a white powder: R_f 0.47 (90:9:1 $CHCl_3$/MeOH/aq CH_3NH_2); mp 125–127 °C; ^{1}H NMR (DMSO-d_6) δ 13.36 (b, 1H), 10.32 (s, 1H), 8.62 (s, 1H), 8.57 (b, 2H), 7.84–7.75 (m, 2H), 7.20–7.15 (m, 2H), 6.94 (d, 1H), 6.85 (t, 1H), 6.56 (s, 1H), 3.95 (t, 2H), 2.93 (t, 2H), 2.70 (t, 2H), 2.56 (s, 3H), 1.76 (q, 2H), 1.02 (t,3H); *m*/*z* expected 363.2, found 364.4 ([M+H]$^+$).

5.2.3.37. *N*-(4-Amino-2-methylquinolin-6-yl)-2-(2-methoxyphenyl)acetamide trifluoroacetate (18kk). Compound **18kk** was obtained as a white powder: R_f 0.48 (80:18:2 $CHCl_3$/MeOH/aq CH_3NH_2); mp 178–180 °C; ^{1}H NMR (DMSO-d_6) δ 13.45 (s, 1H), 10.51 (s, 1H), 8.65 (d, 1H), 8.60 (b, 2H), 7.87 (dd, 1H), 7.80 (d, 1H), 7.28–7.24 (m, 2H), 6.99 (d, 1H), 6.92 (t, 1H), 6.55 (s, 1H), 3.77 (s, 3H), 3.71 (s, 2H), 2.56 (s, 3H); *m*/*z* expected 321.1, found 322.4 ([M+H]$^+$).

5.2.3.38. *N*-(4-Amino-2-methylquinolin-6-yl)-2-(2-chlorophenyl)acetamide trifluoroacetate (18ll). Compound **18ll** was obtained as a white powder: R_f 0.18 (90:9:1 $CHCl_3$/MeOH/aq CH_3NH_2); mp 215–216 °C; ^{1}H NMR (DMSO-d_6) δ 13.42 (s, 1H), 10.69 (s, 1H), 8.63 (b, 3H), 7.86 (dd, 2H), 7.48–7.45 (m, 2H), 7.33–7.29 (m, 2H), 6.55 (s, 1H), 3.93 (s, 2H), 2.56 (s, 3H); *m*/*z* expected 325.1, found 326.5 ([M+H]$^+$).

5.2.3.39. *N*-(4-Amimo-2-methylquinolin-6-yl)-4-(4-methoxyphenyl)butyramide trifluoroacetate (18mm). Compound **18mm** was obtained as a white powder: R_f 0.38 (90:9:1 $CHCl_3$/MeOH/aq CH_3NH_2); mp 199–210 °C; ^{1}H NMR (DMSO-d_6) δ 13.34 (b, 1H), 10.29 (s, 1H), 8.65 (b, 3H), 7.83–7.72 (m, 2H), 7.14 (d, 2H), 6.86 (d, 2H), 6.56 (s, 1H), 3.77 (s, 3H), 2.69–2.60 (m, 5H), 2.41 (t, 2H), 1.93 (t, 2H); *m*/*z* expected 349.2, found 350.5 ([M+H]$^+$).

5.2.3.40. *N*-(4-Amino-2-methylquinoline-6-yl)-3-(2-methoxypyridin-3-yl)propionamide trifluoroacetate (18nn). Compound **18nn** was obtained as a white powder: R_f 0.29 (90:9:1 $CHCl_3$/MeOH/aq CH_3NH_2); mp 224–226 °C; ^{1}H NMR (DMSO-d_6) δ 13.83 (b, 1H), 10.49 (s, 1H), 8.59 (b, 3H), 8.02 (t, 1H), 7.85 (dd, 2H), 7.58 (d, 1H), 6.92 (dd, 1H), 6.58 (s, 1H), 3.90 (s, 3H), 2.89 (t, 2H), 2.71 (t, 2H), 2.57 (s, 3H); *m*/*z* expected 336.2, found 337.4 ([M+H]$^+$).

5.2.3.41. *N*-(4-Amino-2-methylquinolin-6-yl)-3-(4-methoxypyridin-3-yl)propionamide (18oo). Compound **18oo** was obtained as a tan powder: R_f 0.21 (90:9:1 $CHCl_3$/MeOH/aq CH_3NH_2); mp 205–207 °C; ^{1}H NMR (DMSO-d_6) δ 10.25 (s, 1H), 8.49 (s, 1H), 8.33 (d, 1H), 8.26 (s, 1H), 8.03 (b, 2H), 7.74 (s, 2H), 7.02 (d, 1H), 6.52 (s, 1H), 3.87 (s, 3H), 2.90 (t, 2H), 2.67 (t, 2H), 2.51 (s, 3H); *m*/*z* expected 336.2, found 337.4 ([M+H]$^+$).

5.2.3.42. *N*-(4-Amino-2-methylquinolin-6-yl)-3-(3-methoxythiophene-2-yl)propionamide trifluoroacetate (18pp). Compound **18pp** was obtained as a cream-colored powder: R_f 0.29 (90:9:1 $CHCl_3$/MeOH/aq CH_3NH_2); mp 229–231 °C; ^{1}H NMR (DMSO-d_6) δ 13.43 (b, 1H), 10.40 (s, 1H), 8.61 (b, 3H), 7.87–7.70 (m, 2H), 7.24 (d, 1H), 6.97 (d, 1H), 6.56 (s, 1H), 3.78 (s, 3H), 2.98 (t, 2H), 2.66 (t, 2H), 2.56 (s, 3H); *m*/*z* expected 341.1, found 342.3 ([M+H]$^+$).

5.2.4. General synthesis of compounds 20a–i

Mixtures of 4-(di-*tert*-butoxycarbonylamino)-6-amino-2-methylquinoline (100 mg, 0.27 mmol) and the corresponding isocyanates (0.29 mmol, 1.1 equiv) in dichloromethane (3 mL) were stirred at room temperature for 16 h. The residues obtained after removing the solvents were treated with 1:1 TFA/DCM until deprotection was complete (4 h). Removal of the TFA solutions gave essentially pure solids that were washed with ether and dried to give the final compounds as TFA salts.

5.2.4.1. 1-(4-Amino-2-methylquinolin-6-yl)-3-(benzo[*d*][1,3]dioxol-5-yl)urea trifluoroacetate (20a). Compound **20a** was obtained as a yellow microcrystalline solid: R_f 0.30 (90:9:1 $CHCl_3$/MeOH/aq CH_3NH_2); mp 209–210 °C; ^{1}H NMR (DMSO-d_6) δ 13.36 (s, 1H), 9.18 (s, 1H), 8.98 (s, 1H), 8.59 (b, 2H), 8.33 (d, 1H), 7.89 (dd, 1H), 7.78 (d, 1H), 7.25 (d, 1H), 6.83 (m, 2H), 6.55 (s, 1H), 5.98 (s, 2H), 2.56 (s, 3H); *m*/*z* expected 336.1, found 337.3 ([M+H]$^+$).

5.2.4.2. 1-(4-Amino-2-methylquinolin-6-yl)-3-(biphenyl-4-yl)urea trifluoroacetate (20b). Compound **20b** was obtained as a cream-colored powder: R_f 0.30 (90:9:1 $CHCl_3$/MeOH/aq CH_3NH_2); mp 211–212 °C; ^{1}H NMR (DMSO-d_6) δ 13.37 (s, 1H), 9.32 (s, 1H), 9.24 (s, 1H), 8.55 (b, 2H), 8.39 (d, 1H), 7.92 (dd, 1H), 7.76 (d, 1H), 7.63 (m, 6H), 7.45 (t, 2H), 7.32 (t, 1H), 6.56 (s, 1H), 2.57 (s, 3H); *m*/*z* expected 368.2, found 369.4 ([M+H]$^+$).

5.2.4.3. 1-(4-Amino-2-methylquinolin-6-yl)-3-(4-methoxyphenyl)urea trifluoroacetate (20c). Compound **20c** was obtained as a cream-colored powder: R_f 0.29 (90:9:1 $CHCl_3$/MeOH/aq CH_3NH_2); mp 170–171 °C; ^{1}H NMR (DMSO-d_6) δ 13.37 (s, 1H), 9.19 (s, 1H), 8.93 (s, 1H), 8.58 (b, 2H), 8.35 (d, 1H), 7.89 (dd, 1H), 7.76 (d, 1H), 7.41 (d, 2H), 6.89 (d, 2H), 6.55 (s, 1H), 3.72 (s, 3H), 2.56 (s, 3H); *m*/*z* expected 322.1, found 323.3 ([M+H]$^+$).

5.2.4.4. 1-(4-Amino-2-methylquinolin-6-yl)-3-(4-ethoxycarbonylphenyl)urea trifluoroacetate (20d). Compound **20d** was obtained as a light gray powder: R_f 0.30 (90:9:1 $CHCl_3$/MeOH/aq CH_3NH_2); mp 202–204 °C; ^{1}H NMR (DMSO-d_6) δ 13.42 (s, 1H), 9.76 (s, 1H), 9.65 (s, 1H), 8.62 (b, 2H), 8.43 (s, 1H), 7.90 (t, 3H), 7.79 (d, 1H), 7.66 (d, 2H), 6.56 (s, 1H), 4.28 (q, 2H), 2.56 (s, 3H), 1.31 (t, 3H); *m*/*z* expected 364.2, found 365.4 ([M+H]$^+$).

5.2.4.5. 1-(4-Amino-2-methylquinolin-6-yl)-3-(4-fluorophenyl)urea trifluoroacetate (20e). Compound **20e** was obtained as a light yellow powder: R_f 0.32 (90:9:1 $CHCl_3$/MeOH/aq CH_3NH_2); mp 206–207 °C; ^{1}H NMR (DMSO-d_6) δ 13.39 (s, 1H), 9.38 (s, 1H), 9.26 (s, 1H), 8.37 (b, 2H), 8.15 (s, 1H), 7.90 (d, 1H), 7.77 (d, 1H), 7.52 (dd, 2H), 7.15 (t, 2H), 6.55 (s, 1H), 2.56 (s, 3H); *m*/*z* expected 310.1, found 311.3 ([M+H]$^+$).

5.2.4.6. 1-(4-Amino-2-methylquinolin-6-yl)-3-(4-isopropylphenyl)urea trifluoroacetate (20f). Compound **20f** was obtained as a cream-colored powder: R_f 0.27 (90:9:1 $CHCl_3$/MeOH/aq CH_3-

NH_2); mp 203–204 °C; ^{1}H NMR (DMSO-d_6) δ 13.37 (b, 1H), 9.21 (s, 1H), 9.00 (s, 1H), 8.60 (b, 2H), 8.35 (s, 1H), 7.91 (d, 1H), 7.77 (d, 1H), 7.42 (d, 2H), 7.17 (d, 2H), 6.56 (s, 1H), 2.84 (t, 1H), 2.56 (s, 3H), 1.18 (d, 6H); *m*/*z* expected 334.2, found 335.4 ([M+H]$^+$).

5.2.4.7. 1-(4-Amino-2-methylquinolin-6-yl)-3-(4-benzoylphenyl)urea trifluoroacetate (20g). Compound **20g** was obtained as a cream-colored powder: R_f 0.25 (90:9:1 $CHCl_3$/MeOH/aq CH_3NH_2); mp 193–195 °C; ^{1}H NMR (DMSO-d_6) δ 13.39 (s, 1H), 9.65 (s, 1H), 9.47 (s, 1H), 4.64 (b, 2H), 8.42 (s, 1H), 7.92 (d, 1H), 7.81–7.60 (m, 8H), 7.55 (d, 2H), 6.57 (s, 1H), 2.57 (s, 3H); *m*/*z* expected 396.2, found 397.4 ([M+H]$^+$).

5.2.4.8. 1-(4-Amino-2-methylquinolin-6-yl)-3-(2-chlorobenzyl) urea trifluoroacetate (20h). Compound **20h** was obtained as a white solid: R_f 0.19 (90:9:1 $CHCl_3$/MeOH/aq CH_3NH_2); mp 194–198 °C; ^{1}H NMR (DMSO-d_6) δ 13.34 (s, 1H), 9.18 (s, 1H), 8.51 (br s, 2H), 8.30 (s, 1H), 7.85 (d, 1H), 7.73 (d, 1H), 7.44–7.33 (m, 4H), 7.06 (s, 1H), 6.52 (s, 1H), 4.40 (br s, 2H), 2.54 (s, 3H); *m*/*z* expected 340.1, found 341.2 ([M+H]$^+$).

5.2.4.9. 1-(4-Amino-2-methylquinolin-6-yl)-3-(2-methoxybenzyl)urea trifluoroacetate (20i). Compound **20i** was obtained as a white solid: R_f 0.15 (90:9:1 $CHCl_3$/MeOH/aq CH_3NH_2); mp 128–131 °C; ^{1}H NMR (DMSO-d_6) δ 13.36 (s, 1H), 10.33 (s, 1H), 8.47 (br s, 3H), 7.85–7.72 (m, 2H), 7.25–7.13 (m, 2H), 6.99 (d, 1H), 6.56 (s, 1H), 3.8 (s, 3H), 2.9 (t, 2H), 2.69 (t, 2H), 2.56 (s, 3H); *m*/*z* expected 336.2, found 337.3 ([M+H]$^+$).

5.2.5. General synthesis of compounds 22a–d

Mixtures of *N*-(4-amino-2-methylquinolin-6-yl)-4-iodobenzamide **(17qq)** (100 mg, 0.17 mmol), the corresponding quinolinyl boronic acid (0.17 mmol, 1.1 equiv), $Pd(OAc)_2$ (1.1 mg, 0.005 mmol), PPh_3 (3.9 mg, 0.015 mmol) and Na_2CO_3 (21.0 mg, 0.198 mmol) in 2:1 *i*-PrOH/H_2O (10 mL) were heated to 85 °C in a sealed tube under an argon atmosphere. After 45 min the mixtures were cooled to room temperature and filtered through a plug of celite. The filtrates were dissolved in EtOAc (150 mL), washed with brine (3 × 30 mL), dried ($MgSO_4$), and concentrated to yield crude product that was co-evaporated with diethyl ether. The solids thus obtained were dissolved in 1:1 TFA/DCM (20 mL) and stirred at room temperature for 4 h. The solutions were evaporated in vacuo to give crude products which were purified by recrystallization from MeOH/$CHCl_3$.

5.2.5.1. *N*-(4-Amino-2-methylquinolin-6-yl)-4-(quinolin-3-yl) benzamide trifluoroacetate (22a). Compound **22a** was obtained as a cream-colored powder: R_f 0.2–0.4 (80:18:2 $CHCl_3$/MeOH/aq CH_3NH_2); mp 268–270 °C; ^{1}H NMR (DMSO-d_6) δ 10.81 (s, 1H), 9.36 (s, 1H), 8.79 (s, 2H), 8.70 (b, 2H), 8.22 (d, 2H), 8.12 (t, 5H), 7.85 (dd, 2H), 7.70 (t, 1H), 6.62 (s, 1H), 2.59 (s, 3H); *m*/*z* expected 404.2, found 405.6 ([M+H]$^+$).

5.2.5.2. *N*-(4-Amino-2-methylquinolin-6-yl)-4-(isoquinolin-4-yl)benzamide trifluoroacetate (22b). Compound **22b** was obtained as a tan powder: R_f 0.2–0.4 (80:18:2 $CHCl_3$/MeOH/aq CH_3NH_2); mp 205–207 °C; ^{1}H NMR (DMSO-d_6) δ 13.53 (s, 1H), 10.84 (s, 1H), 9.41 (s, 1H), 8.85 (s, 1H), 8.73 (b, 2H), 8.53 (s, 1H), 8.29–8.17 (m, 3H), 8.11 (d, 1H), 7.86–7.77 (m, 6H), 6.62 (s, 1H), 2.60 (s, 3H); *m*/*z* expected 404.2, found 405.5 ([M+H]$^+$).

5.2.5.3. *N*-(4-Amino-2-methylquinolin-6-yl)-4-(quinolin-5-yl) benzamide trifluoroacetate (22c). Compound **22c** was obtained as a cream-colored powder: R_f 0.2–0.4 (80:18:2 $CHCl_3$/MeOH/aq CH_3NH_2); mp 220–223 °C; ^{1}H NMR (DMSO-d_6) δ 13.47 (s, 1H), 10.81 (s, 1H), 8.99 (d, 1H), 8.85 (s, 1H), 8.72 (b, 2H), 8.26–8.21 (m, 3H), 8.15–8.07 (m, 2H), 7.92 (d, 1H), 7.86 (d, 1H), 7.73 (d, 2H), 7.66 (d, 1H), 7.60 (dd, 1H), 6.61 (s, 1H), 2.60 (s, 3H); *m*/*z* expected 404.2, found 405.6 ([M+H]$^+$).

5.2.5.4. *N*-(4-Amino-2-methylquinolin-6-yl)-4-(quinolin-8-yl) benzamide trifluoroacetate (22d). Compound **22d** was obtained as a cream-colored powder: R_f 0.2–0.4 (80:18:2 $CHCl_3$/MeOH/aq CH_3NH_2); mp 230–232 °C; ^{1}H NMR (DMSO-d_6) δ 13.43 (s, 1H), 10.76 (s, 1H), 8.93 (dd, 1H), 8.85 (d, 1H), 8.68 (b, 2H), 8.46 (dd, 1H), 8.14–8.06 (m, 4H), 7.88–7.84 (m, 4H), 7.74 (t, 1H), 7.62 (dd, 1H), 6.61 (s, 1H), 2.59 (s, 3H); *m*/*z* expected 404.2, found 405.5 ([M+H]$^+$).

5.2.6. General synthesis of compounds 24a–e

Mixtures of *N*-(4-amino-2-methylquinolin-6-yl)-4-iodobenzamide **(17rr**; 100 mg, 0.17 mmol), the corresponding quinolinyl boronic acid (0.17 mmol, 1.1 equiv), $Pd(OAc)_2$ (1.0 mg, 0.005 mmol), PPh_3 (3.9 mg, 0.015 mmol) and Na_2CO_3 (21.0 mg, 0.198 mmol) in 2:1 *i*-PrOH/H_2O (10 mL) were heated to 85 °C in a sealed tube under an argon atmosphere. After 45 min the mixtures were cooled to room temperature and filtered through a plug of celite. The filtrates were dissolved in EtOAc (150 mL), washed with brine (3 × 30 mL), dried ($MgSO_4$), and concentrated to yield crude product that was co-evaporated with diethyl ether. The solids thus obtained were dissolved in 1:1 TFA/DCM (20 mL) and stirred at room temperature for 4 h. The solutions were evaporated in vacuo to give crude products which were purified by recrystallization from MeOH/$CHCl_3$.

5.2.6.1. *N*-(4-Amino-2-methylquinolin-6-yl)-3-(quinoline-6-yl) benzamide trifluoroacetate (24a). Compound **24a** was obtained as a cream-colored powder: R_f 0.38 (80:18:2 $CHCl_3$/MeOH/aq CH_3NH_2); mp 212–214 °C; ^{1}H NMR (DMSO-d_6) δ 13.46 (s, 1H), 10.85 (s, 1H), 8.97 (d, 1H), 8.80 (s, 1H), 8.71 (b, 2H), 8.50 (dd, 3H), 8.22 (q, 3H), 8.09 (q, 3H), 7.85 (d, 1H), 7.75 (t, 1H), 7.67–7.72 (m, 1H), 6.60 (s, 1H), 2.56 (s, 3H); *m*/*z* expected 404.2, found 405.5 ([M+H]$^+$).

5.2.6.2. *N*-(4-Amino-2-methylquinolin-6-yl)-3-(quinolin-3-yl) benzamide trifluoroacetate (24b). Compound **24b** was obtained as a cream-colored powder: R_f 0.2–0.4 (80:18:2 $CHCl_3$/MeOH/aq CH_3NH_2); mp 238–239 °C; ^{1}H NMR (DMSO-d_6) δ 13.49 (s, 1H), 10.85 (s, 1H), 9.39 (d, 1H), 8.79 (s, 2H), 8.72 (b, 2H), 8.54 (s, 1H), 8.18 (d, 1H), 8.10 (d, 4H), 7.88–7.69 (m, 4H), 6.61 (s, 1H), 2.59 (s, 3H); *m*/*z* expected 404.2, found 405.5 ([M+H]$^+$).

5.2.6.3. *N*-(4-Amino-2-methylquinolin-6-yl)-3-(isoquinolin-4-yl)benzamide trifluoroacetate (24c). Compound **24c** was obtained as a white powder: R_f 0.2–0.4 (80:18:2 $CHCl_3$/MeOH/aq CH_3NH_2); mp 213–215 °C; ^{1}H NMR (DMSO-d_6) δ 13.47 (s, 1H), 10.77 (s, 1H), 9.42 (s, 1H), 8.78 (s, 1H), 8.69 (b, 2H), 8.56 (s, 1H), 8.29–8.17 (m, 3H), 8.06 (d, 1H), 7.87–7.79 (m, 6H), 6.59 (s, 1H), 2.59 (s, 3H); *m*/*z* expected 404.2, found 405.5 ([M+H]$^+$).

5.2.6.4. *N*-(4-Amino-2-methylquinolin-6-yl)-3-(quinolin-5-yl) benzamide trifluoroacetate (24d). Compound **24d** was obtained as a beige powder: R_f 0.2–0.4 (80:18:2 $CHCl_3$/MeOH/aq CH_3NH_2); mp 244–247 °C; ^{1}H NMR (DMSO-d_6) δ 13.47 (s, 1H), 10.76 (s, 1H), 8.99 (d, 1H), 8.78 (s, 1H), 8.68 (b, 2H), 8.24 (d, 1H), 8.18–8.13 (m, 3H), 8.06 (d, 1H), 7.91 (t, 1H), 7.85–7.79 (m, 3H), 7.69 (d, 1H), 7.59 (dd, 1H), 6.59 (s, 1H), 2.58 (s, 3H); *m*/*z* expected 404.2, found 405.5 ([M+H]$^+$).

5.2.6.5. *N*-(4-Amino-2-methylquinolin-6-yl)-3-(quinolin-8-yl)benzamide trifluoroacetate (24e). Compound **22e** was obtained as an off-white powder: R_f 0.38 (80:18:2 $CHCl_3$/MeOH/aq CH_3NH_2); mp 247–248 °C; ^{1}H NMR (DMSO-d_6) δ 13.46 (s, 1H), 10.73 (s, 1H), 8.93 (dd, 1H), 8.80 (s, 1H), 8.70 (b, 2H), 8.48 (dd, 1H), 8.32 (s, 1H), 8.09–8.05 (m, 3H), 7.95–7.66 (m, 5H), 7.61 (dd, 1H), 6.59 (s, 1H), 2.58 (s, 3H); *m*/*z* expected 404.2, found 405.5 ($[M+H]^+$).

5.2.7. (*E*)-Methyl 3-(5-chloro-2-methoxyphenyl)acrylate (26a)

To a suspension of trimethyl phosphonoacetate (6.66 g, 36.6 mmol, 1.2 equiv) and potassium *tert*-butoxide (5.13 g, 45.7 mmol, 1.5 equiv) in anhydrous THF (150 mL) at 0 °C was added a solution of 5-chlorobenzaldehyde (5.20 g, 30.5 mmol) in THF (10 mL) over 10–15 min. The resulting suspension was stirred from 0 °C to room temperature overnight. The solvent was evaporated under reduced pressure, and the residue was diluted with water (50 mL). The aqueous layer was extracted with EtOAc (2 × 100 mL), and the combined organic layers were washed with brine (1 × 30 mL), dried ($MgSO_4$) and concentrated to yield a crude product as an oil. Recrystallization from 10% EtOAc/hexanes gave 3.77 g (55%) of a yellow crystalline solid: R_f 0.17 (5% EtOAc/hexane; mp 56–58 °C; ^{1}H NMR ($CDCl_3$) δ 7.9 (d, 1H), 7.45 (s, 1H), 7.3–7.26 (m, 1H), 6.84 (d, 1H), 6.49 (d, 1H), 3.87 (s, 3H), 3.80 (s, 3H).

5.2.8. (*E*)-Methyl 3-(5-bromo-2-methoxyphenyl)acrylate (26b)

To a suspension of trimethyl phosphonoacetate (11.6 g, 28 mmol, 1.2 equiv) and potassium *tert*-butoxide (3.91 g, 34.9 mmol, 1.5 equiv) in anhydrous THF (100 mL) at 0 °C was added a solution of 5-bromobenzaldehyde (5.0 g, 23.3 mmol) in THF (100 mL) over 15 min. The resulting suspension was stirred from 0 °C to room temperature overnight. The solvent was evaporated under reduced pressure, and the residue was diluted with water (50 mL). The aqueous layer was extracted with EtOAc (2 × 100 mL), and the combined organic layers were washed with brine (1 × 30 mL), dried ($MgSO_4$) and concentrated to yield a crude product as an oil. Recrystallization from 10% EtOAc/hexanes gave 1.55 g (25%) of a yellow crystalline solid: R_f 0.50 (2:1 hexane/EtOAc); ^{1}H NMR ($CDCl_3$) δ 7.89 (d, Hz, 1H), 7.59 (d, 1H), 7.42 (dd, 2 H), 6.78 (d, 1H), 6.49 (d, 1H), 3.87 (s, 3H), 3.80 (s, 3H).

5.2.9. Methyl 3-(5-chloro-2-methoxyphenyl)propionate (27a)

To a solution of (*E*)-methyl 3-(5-chloro-2-methoxyphenyl)acrylate (**26a**; 3.70 g, 16 mmol) in MeOH (100 mL) was added 5% Rh/alumina (0.2 g). The reaction flask was evacuated, flushed with hydrogen (3×), and the mixture was stirred under 1 atm of hydrogen for 6 h. The mixture was filtered through celite, and the filtrate was concentrated under vacuum to give a clear oil that was dried under high vacuum to give 3.45 g (93%) of the product as a clear oil; ^{1}H NMR ($CDCl_3$) δ 7.15–7.1 (m, 1H), 6.74 (d, 2H), 3.79 (s, 3H), 3.66 (s, 3H), 2.89 (t, 2H), 2.58 (t, 2H).

5.2.10. Methyl 3-(5-bromo-2-methoxyphenyl)propionate (27b)

To a solution of (*E*)-methyl 3-(5-chloro-2-methoxyphenyl)acrylate (**26b**; 1.42 g, 5.2 mmol) in MeOH (40 mL) was added 5% Rh/alumina (70 mg). The reaction flask was evacuated, flushed with hydrogen (3×), and the mixture was stirred under a 1 atm of hydrogen for 6 h. The mixture was filtered through celite, and the filtrate was concentrated under vacuum to give a clear oil that was dried under high vacuum to give 1.08 g (76%) of the product as; ^{1}H NMR ($CDCl_3$) δ 7.3–7.24 (m, 2H), 6.69 (d, 1H), 3.79 (s, 3H), 3.67 (s, 3H), 2.88 (t, 2H), 2.58 (t, 2H).

5.2.11. 3-(5-Chloro-2-methoxyphenyl)propionic acid (28a)

Methyl 3-(5-chloro-2-methoxyphenyl)propionate (**27a**; 3.40 g, 15 mmol) was dissolved in a 1.0 M solution of KOH in MeOH (45 mL, 45 mmol, 5 equiv), and the combined solution was heated at reflux overnight. The solvent was evaporated, and the resulting solid was dissolved in water (15 mL). The pH was adjusted to 3 with 10% aq HCl, and the precipitate formed was filtered and washed with water. The solid was dissolved in diethyl ether and the solution was dried ($MgSO_4$), filtered, and evaporated to give 2.70 g (79%) of product as a white powder: R_f 0.36 (25% EtOAc/hexane); mp 92–94 °C; ^{1}H NMR (DMSO-d_6) δ 12.14 (s, 1H), 7.25–7.20 (m, 2 H), 6.96 (d, 1H), 3.78 (s, 3 H), 2.75 (t, 2H), 2.47 (t, 2H).

5.2.12. 3-(5-Bromo-2-methoxyphenyl)propionic acid (28b)

Methyl 3-(5-bromo-2-methoxyphenyl)propionate (**27a**; 1.19 g, 4.0 mmol) was dissolved in a 1.0 M solution of KOH in MeOH (20 mL, 20 mmol, 5 equiv), and the combined solution was heated at reflux overnight. The solvent was evaporated, and the resulting solid was dissolved in water (15 mL). The pH was adjusted to 3 with 10% aq HCl, and the precipitate formed was filtered and washed with water. The solid was dissolved in diethyl ether and the solution was dried ($MgSO_4$), filtered, and evaporated to give 0.99 g (95%) of product as a white powder: R_f 0.33 (1:1 hexane/EtOAc); mp 114–116 °C; ^{1}H NMR (DMSO-d_6) δ 12.12 (br s, 1H), 7.35 (dd, $J_1 = J_2 = 9$ Hz, 1H), 6.92 (d, 1H), 3.78 (s, 3H), 2.75 (t, 2H), 2.46 (t, 2H).

5.2.13. 4-Bromo-2-methoxybenzyl alcohol (30b)

To a solution of methyl 4-bromo-2-methoxybenzoate (7.14 g, 29 mmol) in THF (35 mL) was added a 2 M solution of $LiBH_4$ in THF (35 mL, 70 mmol, 2.4 equiv). The solution was heated at 60 °C for 16 h. The reaction was then cooled to room temperature, and the solvent was removed under vacuum. The residue was stirred in a 5% aqueous acetic acid solution (pH 6) for 15 min. The aqueous layer was extracted with EtOAc (2 × 50 mL), then the combined organic extracts were washed with brine, dried ($MgSO_4$), filtered, and concentrated to yield the crude product as a tan oil. The oil was purified by flash chromatography using 5% EtOAc in hexanes to provide 5.96 g (94%) of the product as a white solid: R_f 0.30 (10% EtOAc/hexane); ^{1}H NMR ($CDCl_3$) δ 7.30 (d, 1H), 7.14 (d, 1H), 7.11 (s, 1H), 5.09 (t, 1H), 4.42 (d, 2H,), 3.77 (s, 3H).

5.2.14. 4-Bromo-2-methoxybenzaldehyde (31b)

A solution of anhydrous DMSO (5.1 mL, 73 mmol, 3.0 equiv) in anhydrous dichloromethane (50 mL) was cooled to −78 °C in a dry ice/*i*-PrOH bath. Oxalyl chloride (6.4 mL, 73 mmol, 3.0 equiv) was added dropwise over 45 min. The mixture was stirred for 15 min and a solution of 4-bromo-2-methoxybenzyl alcohol (**30b**; 5.30 g, 24 mmol) in dichloromethane (10 mL) was added dropwise. The resulting mixture was stirred for 30 min at −78 °C. Triethylamine (12.4 g, 120 mmol, 5.0 equiv) was then added, and the mixture was allowed to warm to rt over 16 h. The mixture was quenched with water (100 mL) and extracted with chloroform (3 × 200 mL). The combined organic extracts were washed with water (1 × 150 mL), brine (1 × 150 mL), dried ($MgSO_4$), filtered, and evaporated to yield the crude product as dark residue. The residue was purified by flash chromatography using chloroform to obtain an off-white solid that was recrystallized from hexanes to yield 3.73 g (71%) of the product as a white solid: R_f 0.18 (5% EtOAc/hexane); mp 65–67 °C; ^{1}H NMR ($CDCl_3$) δ 10.39 (s, 1H), 7.68 (d, 1H), 7.2–7.16 (m, 2H), 3.94 (s, 3H).

5.2.15. (*E*)-Methyl 3-(4-bromo-2-methoxyphenyl)acrylate (32b)

To a suspension of potassium *tert*-butoxide (7.83 g, 70 mmol, 3.0 equiv) in THF (300 mL) at 0 °C was added trimethyl phosphonoacetate (10.6 g, 58 mmol, 2.5 equiv). The resulting clear yellow solution was stirred for 15 min at 0 °C, then a solution of (4-bromo-2-methoxy)benzaldehyde (**31b**; 5.00 g, 23 mmol) in THF (20 mL) was added dropwise. The resulting mixture was warmed

from 0 °C to room temperature over 16 h. The solvent was removed under vacuum, then the crude mixture was diluted with water (50 mL) and extracted with EtOAc (2 × 100 mL). The combined organic extracts were washed with water (1 × 50 mL), brine (1 × 50 mL), dried ($MgSO_4$), filtered, and concentrated to provide a dark residue. The residue was purified by flash chromatography using 5% EtOAc in hexanes to obtain 3.53 g (56%) of product as a white solid: R_f 0.19 (5% EtOAc/hexane; ^{1}H NMR ($CDCl_3$) δ 8.02 (d, 1H), 7.47 (dd, 1H), 7.26–7.22 (m, 1H), 6.93 (t, 1H), 6.84 (d, 1H), 6.62 (d, 1H), 6.2 (s, 1H), 3.83 (s, 3H).

5.2.16. Methyl 3-(4-bromo-2-methoxyphenyl)propionate (33b)

To a solution of (*E*)-methyl 3-(4-bromo-2-methoxyphenyl)acrylate (**32b**; 1.43 g, 5.6 mmol) in MeOH (20 mL) was added 5% Rh/alumina (200 mg). The reaction flask was evacuated, flushed with hydrogen (3×), and the mixture was shaken under 20 psi of hydrogen for 3 h. The mixture was filtered through celite, and the filtrate was concentrated under vacuum to give a clear oil that was dried under high vacuum to give 1.38 g (97%) of the product as a colorless oil; ^{1}H NMR ($CDCl_3$) δ 7.0–6.96 (m, 3H), 3.82 (s, 3H), 3.66 (s, 3H), 2.87 (t, 2H), 2.57 (t, 2H).

5.2.17. 3-(4-Bromo-2-methoxyphenyl)propionic acid (34b)

Methyl 3-(4-bromo-2-methoxyphenyl)propionate (1.38 g, 5.1 mmol) was dissolved in a 1.0 M solution of KOH in MeOH (25 mL, 25 mmol, 5 equiv), and the combined solution was heated at 60 °C overnight. The solvent was evaporated, and the resulting solid was dissolved in water (15 mL). The aqueous solution was washed with $CHCl_3$ (2 × 20 mL), and the pH of the aqueous portion was adjusted to 3 with 3 M aqueous HCl. The precipitate that formed was filtered and washed with water. The solid was dissolved in diethyl ether and the solution was dried ($MgSO_4$), filtered, and evaporated to give 1.12 g (86%) of product as a white powder: R_f 0.31 (25% EtOAc/hexane; mp 112 °C (subl.); ^{1}H NMR (DMSO-d_6) δ 12.1 (b, 1H), 6.9–6.83 (m, 3H), 3.58 (s, 3H), 2.51 (t, 2H), 2.22 (t, 2H).

5.2.18. 3-(2-Methoxy-4-(pyridin-3-yl)phenyl)propionic acid (36)

A mixture of 3-(4-bromo-2-methoxyphenyl)propionic acid (**34**; 520 mg, 2.0 mmol), pyridine-3-boronic acid pinacol ester (470 mg, 2.11 mmol, 1.05 equiv), palladium acetate (14 mg, 0.06 mmol, 3%), triphenylphosphine (48 mg, 0.18 mmol, 9%), and sodium carbonate (254 mg, 2.40 mmol, 1.2 equiv) in 10% *i*-PrOH/water (20 mL) was heated to 80 °C in a sealed vessel under argon for 4 h. The mixture was then cooled and filtered through celite. The filtrate was diluted with EtOAc (100 mL) and washed with water (45 mL). The organic layer was dried ($MgSO_4$), filtered, and evaporated. The crude solid was purified by preparative TLC using 1% AcOH in EtOAc to provide 117 mg (34%) of product as a white solid: R_f 0.11 (25% EtOAc/hexane; mp >300 °C; ^{1}H NMR (DMSO-d_6) δ 12.12 (br s, 1H), 8.91 (d, 1H), 8.55 (d, 1H), 8.1–8.06 (m, 1H), 7.47 (dd, 1H), 7.28–7.20 (m, 3H), 3.90 (s, 3H), 2.82 (t, 2H), 2.52 (t, 2H, partially under DMSO).

5.2.19. *N*-(4-Amino-2-methylquinolin-6-yl)-3-(2-methoxy-4-(pyridin-3-yl)phenyl)propanamide (35)

A mixture of 4-(di-*tert*-butoxycarbonylamino)-6-amino-2-methylquinoline (**16**; 128 mg, 0.342 mmol), 3-(2-methoxy-4-(pyridin-3-yl)phenyl)propionic acid (**36**; 176 mg, 0.680 mmol, 2.0 equiv), 2-(7-aza-1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU; 258 mg, 0.68 mmol, 2.0 equiv), and *N*,*N*-diisopropylethylamine (0.7 mL, 4.0 mmol, 10 equiv) in DMF (5 mL) was stirred at room temperature for 20 h. The mixture was then poured into 5% aqueous Na_2CO_3 (50 mL), and extracted with EtOAc (3 × 50 mL). The combined organic extracts were washed with 5% aqueous Na_2CO_3 (50 mL), brine (50 mL), dried ($MgSO_4$), and evaporated produce a tan-colored residue. The reside was co-evaporated with diethyl ether and treated without further purification with 1:1 TFA/DCM (5 mL) for 2 h. The solvent was evaporated and the crude product was purified by preparative TLC using 80:18:2 $CHCl_3$/MeOH/aq CH_3NH_2. The solids thus obtained were recrystallized from CH_3CN/MeOH to yield 61 mg (36%) of product as an off-white powder: R_f 0.19 (80:18:2 $CHCl_3$/MeOH/aq CH_3NH_2); mp 219–221 °C; ^{1}H NMR (DMSO-d_6) δ 13.30 (b, 1H), 10.36 (s, 1H), 8.93 (s, 1H), 8.61 (s, 2H), 8.57 (b, 2H), 8.11–8.08 (d, 1H), 7.86–7.76 (m, 2H), 7.50–7.46 (m, 1H), 7.30 (m, 1H), 7.23 (d, 2H), 6.56 (s, 1H), 3.92 (s, 3H), 2.97 (t, 2H), 2.71 (t, 2H), 2.56 (s, 3H); *m*/*z* expected 412.2, found 413.3 ($[M+H]^+$).

5.3. Biological assays

5.3.1. BoNT/A LC FRET assay: 96-well format for IC_{50} determinations

Hit confirmations, IC_{50} determinations, and kinetic analysis for inhibitor mechanism of action were carried out utilizing a 96-well FRET assay microplate format. For hit confirmation, compound (25 μM final), 20 μM SNAP-25 substrate (MOCAc-Lys-Lys-Val-Tyr-Pro-Tyr-Pro-Met-Glu-Lys(Dnp)-NH_2),[46] and 2 nM BoNT/A LC) were incubated at 37 °C for 40 min, which was the initial linear rate phase of catalytic activity, in the presence of buffer (50 mM HEPES 0.05% Tween [pH 7.4]) in a volume of 100μL. The reactions were stopped by the addition of acetic acid to 0.5% prior to measuring the fluorescence of the cleaved substrate at 485 nm following excitation at 398 nm in a Molecular Devices (Sunnyvale, CA) plate reader.

5.3.2. *Bacillus anthracis* lethal factor (BaLF) FRET assay

A BaLF FRET assay was performed using 20 μM peptide substrate (MCA-KKVYPYPME[dnp]K amide), 20 mM HEPES–0.05% Tween [pH 8.2] and 5.55 nM BaLF and incubating at 37 °C for 30 min as described previously.[33] Inactivation of the enzyme was achieved by addition of acetic acid to 0.5% and the fluorescent signal of the cleaved substrate was measured at 395 nm after excitation at 324 nm.

5.3.3. Human matrix metalloproteinase (MMP) 1 and 9 assays

MMP 1 and 9 FRET assays were performed to assess specificity of the compounds. An assay mixture consisting of 0.05 % NP-40, 50 mM MOPS (pH 6.0), 50 μM fluorogenic substrate (Enzo Life Sciences Catalog Number P-128), and either MMP-1 (38 nM) or MMP-9 (13 nM) was incubated at 37 °C for 60 min (assay linear range 90 min). The reactions were then stopped with the addition of acetic acid (0.5% [final]) and fluorescence read at 440 nm after excitation at 340 nm.

5.3.4. Kinetic analysis of BaLF inhibition

The kinetics of BaLF inhibition by compounds **18t** and **18m** were examined in detail utilizing the FRET assay results. Eadie-Hofstee transformations of the assay results (Atkins and Nimmo, 1975) indicate these compounds exert a noncompetitive mechanism of inhibition on BaLF activity (see Supplementary data) as evidenced by a decrease in V_{max} (*y* intercept) and no change in K_m (line slope) with increasing inhibitor concentrations. The kinetic assay data was also analyzed utilizing a Michaelis-Menten plot (GraphPad Prism® Software). The analyzed data revealed that the inhibitor IC_{50} value did not change significantly as a function of substrate concentration and, the substrate K_m value did not change significantly as a function of inhibitor concentration; both observations are indicative of a noncompetitive inhibition mechanism. Compound **18t** had a calculated K_i value of 2.9 μM while Compound **18m** had a calculated K_i value of 1.1 μM.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2013.11.009.

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