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Discovery and SAR of novel and selective inhibitors of urokinase plasminogen activator (uPA) with an imidazo[1,2-*a*]pyridine scaffold

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Abstract

Urokinase plasminogen activator (uPA) is a biomarker and therapeutic target for several cancer types. Its inhibition is regarded as a promising, non-cytotoxic approach in cancer therapy by blocking growth and/or metastasis of solid tumors. Earlier, we reported the Modified Substrate Activity Screening (MSAS) approach and applied it for the identification of fragments with

affinity for uPA's S1 pocket. Here, these fragments are transformed into a novel class of uPA inhibitors with an imidazo[1,2-*a*]pyridine scaffold. The SAR for uPA inhibition around this scaffold is explored and the best compounds in the series have nanomolar uPA affinity and selectivity with respect to the related trypsin-like serine proteases (thrombin, tPA, FXa, plasmin, plasma kallikrein, trypsin, FVIIa). Finally, the approach followed for translating fragments into small molecules with a decorated scaffold architecture is conceptually straightforward and can be expected to be broadly applicable in fragment-based drug design.

Introduction

Urokinase plasminogen activator (uPA, urokinase) is a trypsin-like serine protease and a therapeutical target for many cancer types, including breast, ovarian, and pancreatic cancer.[1,2,3] It is part of an extracellular enzyme system overexpressed in metastasizing solid tumors, comprising the urokinase-type plasminogen activator (uPA), the plasminogen activator inhibitors (PAI's), tissue-type plasminogen activator (tPA) and the uPA receptor (uPAR).[1] The uPAR is an important regulator of extracellular matrix (ECM) proteolysis, cell-ECM interactions and cell signaling.[4] Binding of uPA to its receptor activates the enzyme and triggers a proteolytic cascade through which plasminogen is converted into plasmin. This in turn activates matrix metalloproteases (MMPs) leading to proteolytic degradation of the ECM components.[1,4] As a result, tumor cells degrade the surrounding tissue, invade into healthy tissue and migrate with the blood stream to form metastasized tumors at distant organs. The uPA system also interacts with a number of relevant molecular-biological systems promoting tumor growth: it can activate growth

factors, and interacts with proteins involved in cell adhesion and signal transduction (vitronectin, integrins).[2,4]

Although uPA is a valuable oncology target, clinical development of uPA inhibitors has been problematic. This is most probably related to the doubtful biopharmaceutical performance of compounds developed so far and their insufficient selectivity with respect to other, phylogenetically related trypsin-like proteases. Nonetheless, the field of urokinase inhibitor discovery still produces significant numbers of relevant compounds, mostly small molecules with a competitive, reversible inhibition profile. Wilex's orally available drug candidate WX-671 (**1a, Figure 1**) is currently the most advanced product. This compound is a pro-drug of the corresponding amidine-based, inhibitor WX-UK1 (**1b, Figure 1**). Interestingly, **1a** (K_i = 410 nM) and **1b** are the first inhibitors of uPA in oncology trials worldwide. The latter has successfully completed two clinical phase I trials, and recently showed favorable results in randomized phase II trial in patients with locally advanced non-metastatic pancreatic cancer as well as met its primary objective in phase II trial in patients with HER2 receptor negative metastatic breast cancer (MBC).[5] Next to compounds developed by Wilex (**1a-b**), a significant number of optimized inhibitors of urokinase has been reported, but these have only been investigated in preclinical studies. Pfizer reported 1-isoquinolinylguanidine UK-356,202 (**2**; K_i = 37 nM) and its sulfonamide derivative UK-371,804 (**3**; K_i = 10 nM) as potent and selective inhibitors of uPA.[6] These compounds were selected for preclinical evaluation for the treatment of chronic dermal ulcers, condition characterized by high levels of uPA promoting uncontrolled matrix breakdown and inhibition of wound repair. As a result, compound **3** was demonstrated to inhibit exogenous uPA activity both in human chronic wound fluid *in vitro*, and in the porcine acute excisional wound model.[6c] Besides, according to Barber *et al.* **2** has been selected for

human clinical trials, but apparently these plans have never materialized for unknown reasons.[6a] An amidine based, peptide-derived uPA inhibitor CJ-463 (**4**; K_i = 20 nM) was found to reduce the number of experimental lung metastases in a fibrosarcoma mouse model as well as primary tumor growth and metastasis formation in murine lung carcinoma model.[7] A fragment-based approach by Astex led to the discovery of a mexiletine-derived, low basicity inhibitor of uPA (**5**; IC_{50} = 72 nM) with moderate selectivity against closely related proteases and high oral bioavailability.[8] Abbott Laboratories developed a series of naphthamidine inhibitors of uPA (compound **6** as a representative example; K_i = 263 nM) with improved pharmacokinetic properties, as good oral bioavailability and extended half life in rats.[9] Additionally, the 4-oxazolidinone analogue UK122 (**7**) was reported by Zhu and co-workers as a selective inhibitor of uPA with significant potency to inhibit the migratory and invasive capacity of pancreatic cancer cells *in vitro*.[10]

Other approaches have been focusing on discovery of irreversible uPA ligands, antibodies or peptide-based molecules. Our group described series of highly potent and selective, irreversible diaryl phosphonate inhibitors of uPA (**8a**; IC_{50} = 3.1 ± 0.5 nM, and **8b**; IC_{50} = 3.4 ± 0.4 nM) with significant anti-metastatic activity in a rodent model of breast cancer.[11] Mazar and co-workers invented a novel therapeutic uPAR antibody ATN-658 demonstrating antitumor effects across a variety of tumor models, including inhibition of invasion, metastasis and proliferation as well as induction of apoptosis.[12] Additionally, bicyclic peptide constructs were recently reported as highly potent uPA inhibitors.[13] Nonetheless, given all the preclinical evidence mentioned, it is remarkable that none of the compounds were developed clinically.

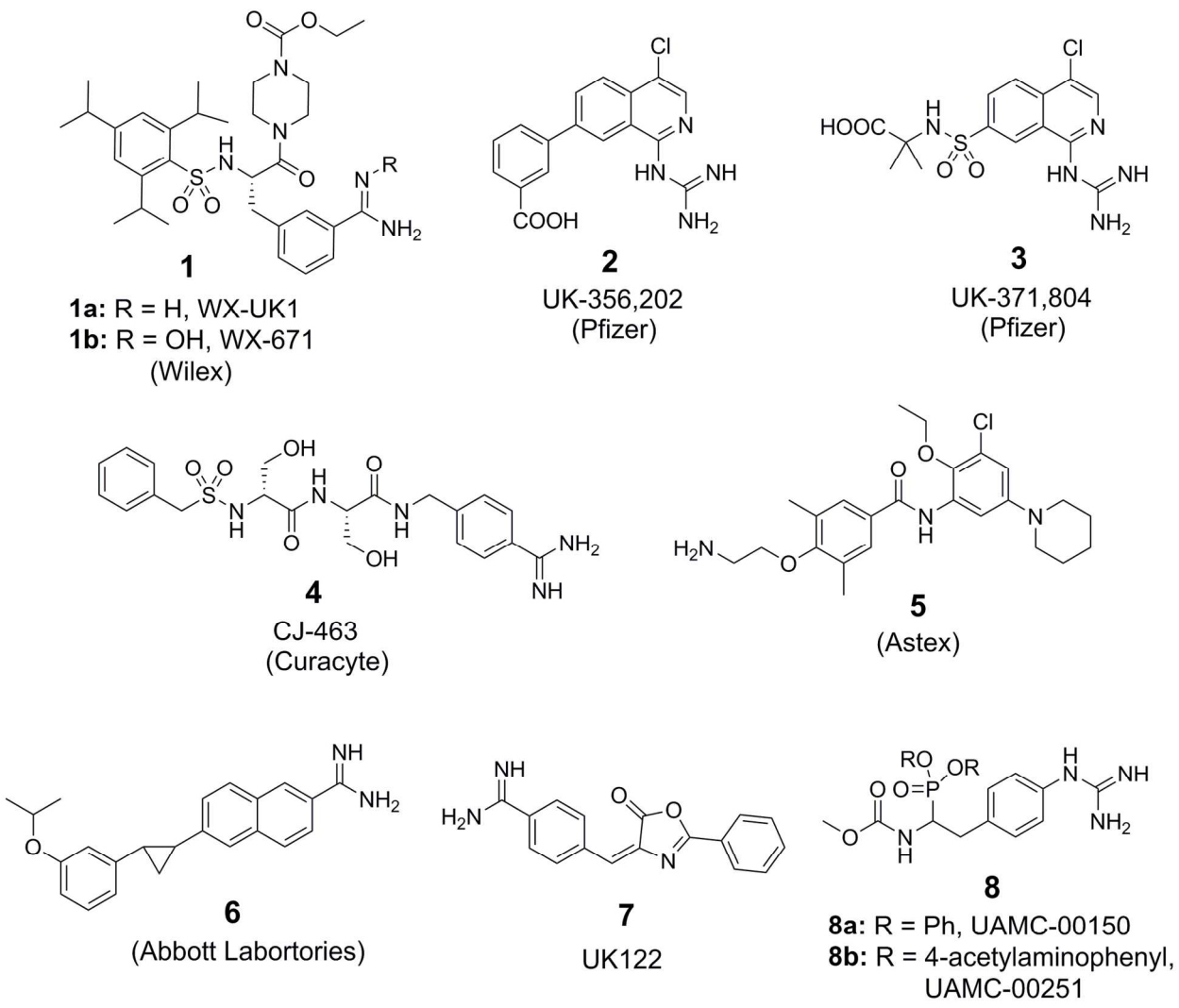


Figure 1. Reported inhibitors of uPA.

This study focuses on the discovery of a novel class of reversible, non-peptide derived uPA inhibitors with an imidazo[1,2-*a*]pyridine scaffold. It directly furthers on an earlier publication in which we reported an optimized version of Substrate Activity Screening (SAS), an approach pioneered by Ellman and co-workers for the discovery of fragments with affinity for the active center of enzymes. Our "Modified Substrate Activity Screening" (MSAS) approach was validated by identifying several fragment-sized ligands of the uPA S1 pocket.[14] As hypothesized

in this earlier report, an S1-binding fragment could be transformed into a druglike uPA inhibitor by grafting it onto a suitable scaffold and by further decorating this scaffold with additional affinity-conferring substituents. This hypothesis will be extensively investigated here and corroborated with the preparation of potent uPA inhibitors. Since, to the best of our knowledge, no earlier examples of imidazopyridine inhibitors of urokinase have been reported, evaluation of the inhibitory potencies under the assay conditions of this manuscript will include the reference compounds **7**, gabexate (**9**) and amiloride (**10**) (**Figure 2**).

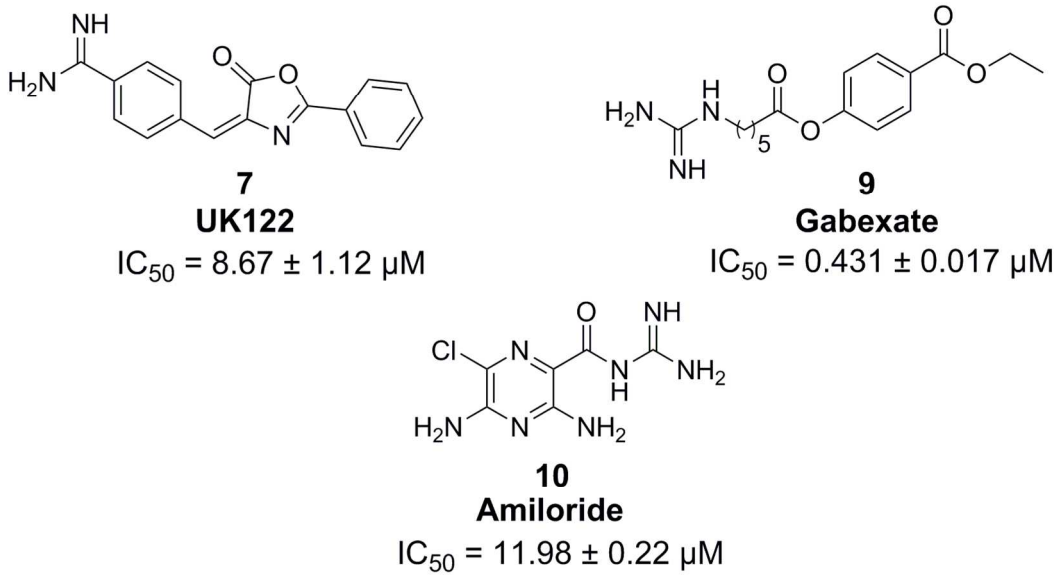


Figure 2. Inhibitory activities of the reference compounds against uPA determined under the assay conditions of this manuscript.

The S1-binding fragments used in this study were demonstrated in our earlier report to possess high micromolar uPA affinity. Their structures are summarized in **Figure 3 (entry A)**. Noteworthy, our earlier publication already included a proof-of-concept example where fragment $\mathbf{R^{1b}}$ was grafted onto the 3-position of an imidazopyridine scaffold. A comparison of the IC_{50}-

values of the separate and the scaffolded fragment indicated the imidazopyridine system to be a novel, potentially useful scaffold for uPA inhibitors.

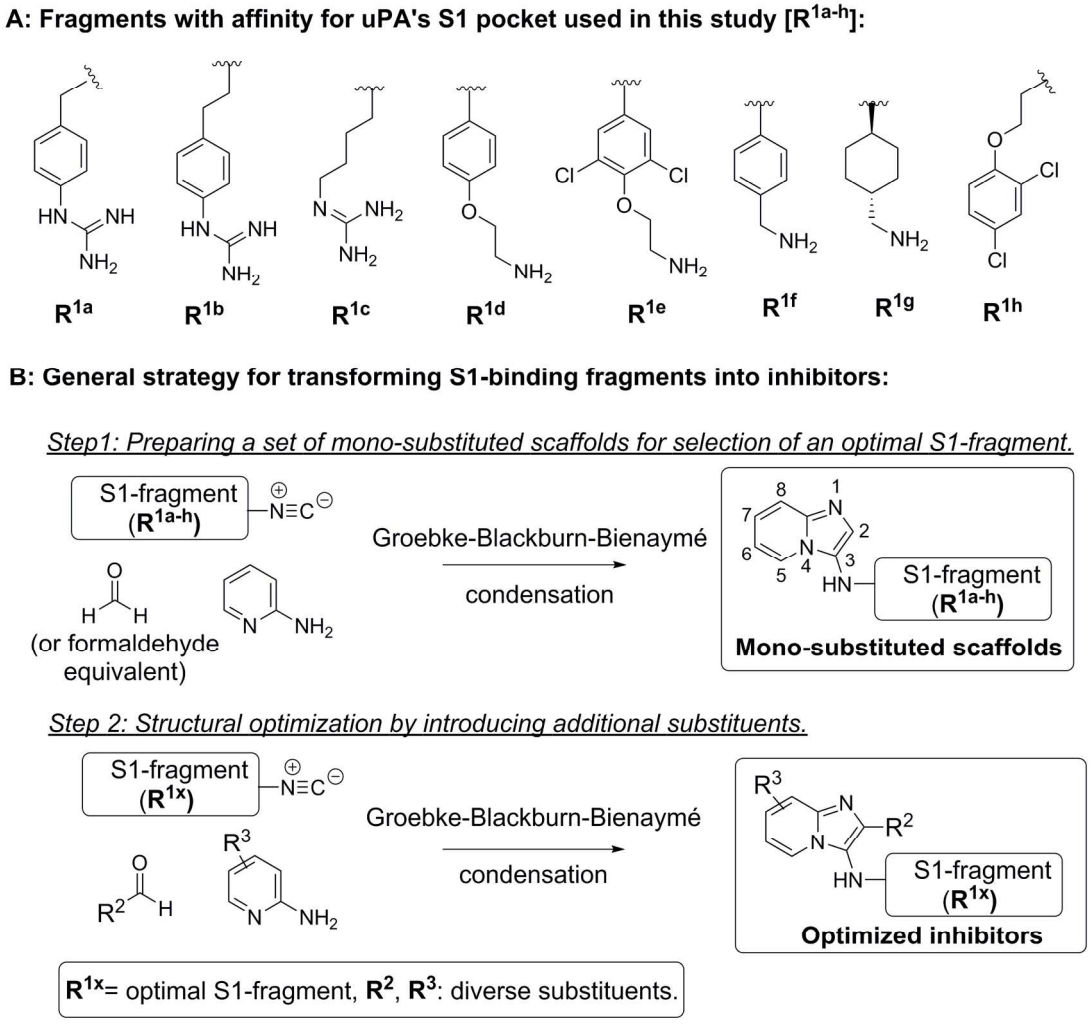


Figure 3. Overview of the strategy followed for the preparation of potent uPA inhibitors with an imidazopyridine scaffold.

The general strategy that was followed in this study for obtaining potent uPA inhibitors, consists of two parts. In the first part (**Figure 3, entry B, Step1**), a set of imidazo[1,2-*a*]pyridines is prepared, each bearing one of the S1-binding fragments (**R^{1a-h}**). The choice to

introduce the S1-binders at the 3-position of the imidazopyridine is mainly governed by practical synthetic considerations (*vide infra*), although our earlier proof-of-concept work already indicated this to be a viable approach for preparing compounds with appreciable uPA-affinity. Comparison of the target potencies of the monosubstituted scaffolds obtained in this manner, is then used to identify the optimal S1-binding substituent. Further optimization (**Figure 3, entry B, Step 2**) is achieved by combining the optimal S1-substituent with additional substituents (R^2 and R^3) on the imidazopyridine ring system. Although we extensively relied on molecular modeling to guide the selection of the R^2 and R^3 substituents, diversification in terms of steric and electronic parameters was an equally important goal.

The key step in the preparation of all target compounds of this study is the so-called Groebke-Blackburn-Bienaymé condensation, a variant of the Ugi reaction, reported for the first time in 1998.[15,16,17] It is based on the three-component coupling of (1) an isocyanide, (2) an aldehyde and (3) a 2-aminoazine in the presence of a suitable catalyst, usually a Lewis acid or Brønsted acid, to generate fused imidazo[1,2-*a*]heterocycles in a one-pot transformation. Taking into account the limited number of commercially available isocyanides and their often non-straightforward synthesis, we considered the latter reaction component the least suited as a source of molecular diversity. As mentioned, this practical consideration was instrumental to reserve the isocyanide-derived R^1 group for the S1-binding fragments identified in the MSAS hits. Conversely, aldehydes are commercially available in abundant numbers. Therefore, the aldehyde-derived R^2-substituent was deemed an appropriate source of steric and electronic diversity. Finally, we also found the commercial availability of decorated 2-aminopyridines to be rather limited. Nonetheless, a significant number of 2-aminopyridines equipped with functional groups (e.g. carboxylates, halides) that can easily be derivatized using standard chemical

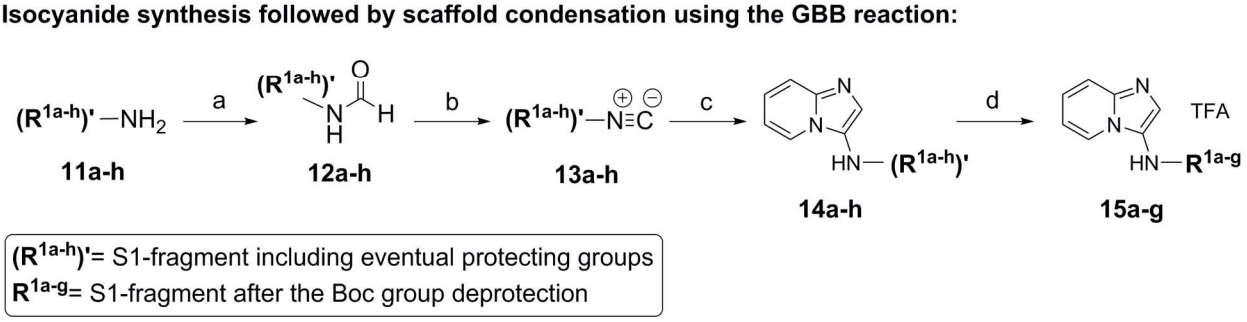
transformations were found to be available. This approach was used to generate sufficient diversity in the produced compound series.

On a general level, imidazo[1,2-*a*]pyridines have already been applied successfully as scaffold moieties in drug discovery, and the Groebke-Blackburn-Bienaymé reaction represents one of the simplest routes for the diversity-oriented synthesis of this pharmacophore. Compounds of this type have been in clinical investigation for various therapeutic targets resulting in several drugs entering the market, as for instance the hypnotic $GABA_A$ receptor ligand zolpidem, the selective phosphodiesterase 3 (PDE3) inhibitor olprinone, or the CXC chemokine receptor 4 (CXCR4) antagonist GSK812397.[18] Our group, to the best of our knowledge, is the first to report the application of an imidazo[1,2-*a*]pyridine scaffold for the construction of uPA inhibitors. Next to producing novel and potent uPA-inhibitors, this study also discloses a significant amount of structure-activity relationship data for this class of compounds. Finally, the present study also successfully exemplifies a novel strategy for transforming low-affinity fragments into potent, druglike compounds with a decorated scaffold architecture. Theoretically, the same strategy (fragment scaffolding, followed by optimization through diversity-oriented introduction of additional substituents) could be applied for all targets where a fragment-based approach to drug discovery is followed.

Results and discussion

First, the set of imidazo[1,2-*a*]pyridines linked with S1-binding fragments $\mathbf{R^{1a-h}}$, was synthesized relying on the Groebke-Blackburn-Bienaymé (GBB) reaction (**Scheme 1**).

Scheme 1. Synthetic steps leading to the monosubstituted scaffold-based inhibitors 14h, 15a-g.[a]



[a]Reagents and conditions: (a) ethyl formate, TEA, 55 °C, 24 h, 55-90%; (b) $POCl_3$, DIPA, DCM, 60-85%; (c) pyridin-2-amine, glyoxylic acid monohydrate, $HClO_4$ (cat), MeOH, rt, 24 h, 38-66%; (d) TFA/DCM (1:1), rt, 1 h, 95-100%.

The isocyanides (**13a-h**) required for this reaction were synthesized from amines **11a-h** by consecutive formylation and dehydration. The formylation reaction was carried out by refluxing the corresponding amines overnight in ethyl formate and in the presence of triethylamine as previously described by Hartman *et al.*,[19] to afford the desired formamides (**12a-h**) in 55-90% yield. Dehydration in the presence of $POCl_3$ and triethylamine led to the formation of the isocyanides (**13a-h**) in low to moderate yields. Careful optimization of the reaction conditions, including the nature of base and the reaction medium, were therefore necessary. Following the protocol described by Ugi *et al.*, we replaced triethylamine by diisopropylamine, which increased not only the average yields (60-85%), but also purity, making chromatographic purification in some cases avoidable.[20] Detailed experimental procedures and characterization data for preparation of amines **11a-h**, formamides **12a-h**, and isocyanides **13a-h** can be found in the Experimental Section.

For obtaining the monosubstituted imidazopyridines (R^2=R^3=H), the GBB-reaction requires formaldehyde as the aldehyde component. However, the scope of this non-concerted [4+1] cycloaddition is rather limited for formaldehyde.[21,22] This is due to formation of unstable imines, resulting in poor conversions. Our initial attempts to use formaldehyde hydrate and paraformaldehyde as potential formaldehyde equivalents did not produce satisfactory results and afforded the desired product in poor yields (< 30%). Following the protocol reported by Kercher *et al.*,[23] we then applied glyoxylic acid as a formaldehyde equivalent, and reacted it with 2-aminopyridine and the set of isocyanides. Glyoxylic acid was indeed found to be an efficient and experimentally more convenient reagent. Further optimization of the original experimental protocol was achieved by using $HClO_4$ as the catalyst and MeOH as the solvent. This resulted in conditions applicable to all isocyanides that were evaluated and yields for the desired products (**14h, 15a-g**) ranging between 38 and 65% yield. All reactions were completed within 24 h.

Evaluation of the uPA inhibitory potency of these monosubstituted imidazo[1,2-*a*]pyridines, revealed that the most potent analogues were guanidinophenyl derivatives **15a** and **15b** (bearing fragments $\mathbf{R^{1a}}$ and $\mathbf{R^{1b}}$) (**Table 1**). These displayed IC_{50}-values of 9.04 ± 0.62 μM and 19.39 ± 1.22 μM, respectively. The hypothetical binding modes of these closely related molecules were investigated by docking studies. Results suggested the guanidine side chain of both compounds to interact through hydrogen bonds with Gly-219, Ser-190 and, as reasonably expectable, with the anionic carboxylate function of uPA's Asp189 (**Figure 4**). In addition, a hydrogen bond between the amine nitrogen of the imidazopyridine ring system and the catalytically important Ser-195 is also predicted both in the case of compounds **15a** and **15b** (**Figure 4**). A helicopter view of the predicted binding of compound **15a** within the active site pocket of uPA is given in **Figure 5a**, showing complementarity between ligand and pocket. The guanidine moiety of the

compound is deeply buried in the active site, while the imidazole nitrogen of the imidazopyridine ring system is pointing into the solvent and not participating in any hydrogen bonding to the protein. The imidazopyridine scaffold in these simulations was found not to contribute to target affinity via directed interactions with the enzyme, although it also does not negatively interfere with binding of **15a** and **15b** to the active center.

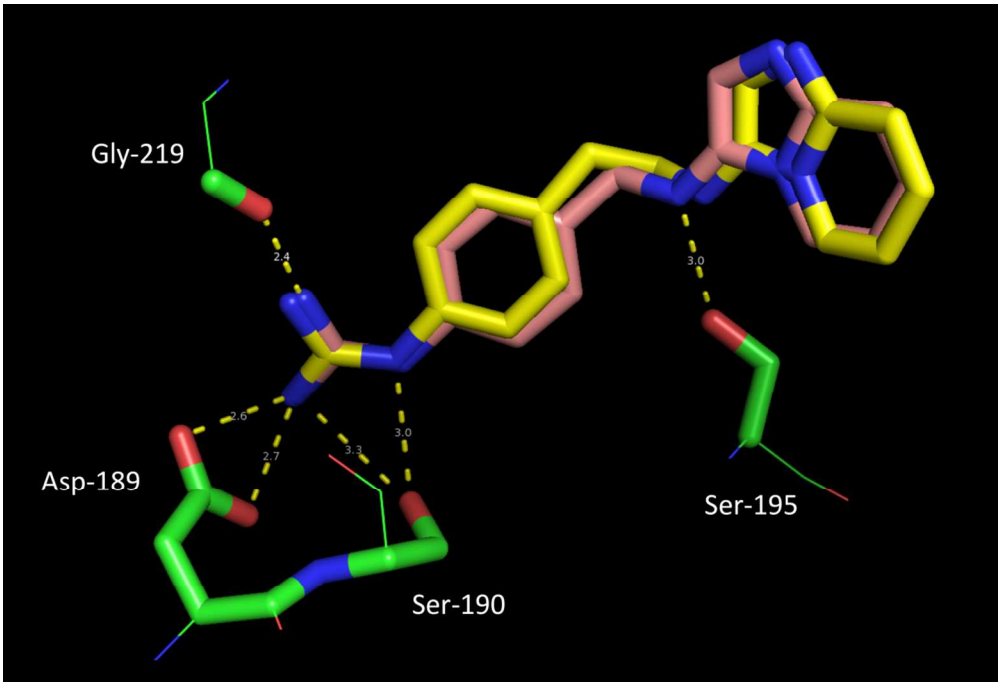
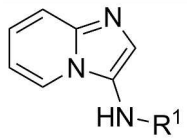


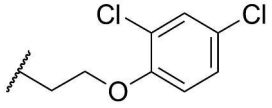
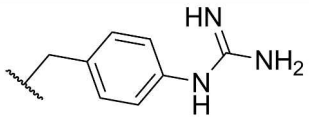
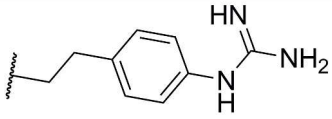
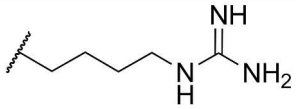
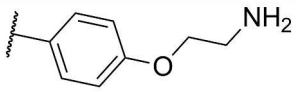
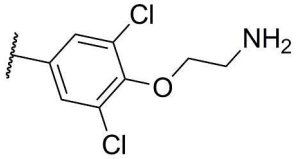
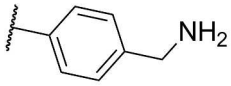
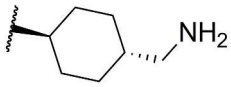
Figure 4. Predicted binding mode of compounds **15a** (light brown) and **15b** (yellow) in the active site of uPA (PDB-code 2O8W). Proposed hydrogen bonds between ligands and labeled protein residues are indicated by yellow dashed lines. Both compounds overlay quite well and show an almost identical binding pattern.

The significant potency of **15a** and **15b** also reflects the ranking of their corresponding S1-fragments $\mathbf{R^{1a}}$ and $\mathbf{R^{1b}}$ during the Modified SAS (MSAS) experiments mentioned earlier, where both displayed among the highest affinities in the collection of „hits". For the other inhibitors in

Table 1 however, the translation of fragment ranking data into potency of the corresponding imidazopyridine inhibitors, seems less straightforward. In general, affinities for compounds **14h, 15c-g** are in the high micromolar range. Noteworthy, the presence of a guanidinobutyl fragment in **15c** did not lead to potent inhibition, despite this fragment's resemblance to the arginine side chain that is used by uPA for recognition of its natural substrates. Likewise, the aminoethoxyphenyl fragments present in **15d** and **15e** displayed satisfactory potency during our earlier MSAS experiments, but did not result in potent imidazopyridine inhibitors. In addition, these fragments also occur in mexiletine analogue and/or its likes, and both of them were confirmed as ligands of uPA's S1 pocket using X-ray crystallography.[8] Comparable discrepancy is present for the dichlorophenoxy fragment in **14h**; while this moiety was ranked close to $\mathbf{R^{1a}}$ and $\mathbf{R^{1b}}$ in the MSAS experiments, grafting it onto an imidazopyridine scaffold leads to a relatively poor inhibitor. Finally, evaluation data for the aminomethyl-substituted **15f** and **15g** also demonstrated these compounds to be weak uPA inhibitors. Taken together, these biochemical evaluation data led to prioritization of **15a** and **15b** in the optimization effort. The slightly higher uPA potency, lower molecular weight and reduced conformational flexibility of **15a** were decisive for focusing on this compound during optimization by introducing additional substituents on different positions of the monosubstituted scaffold.

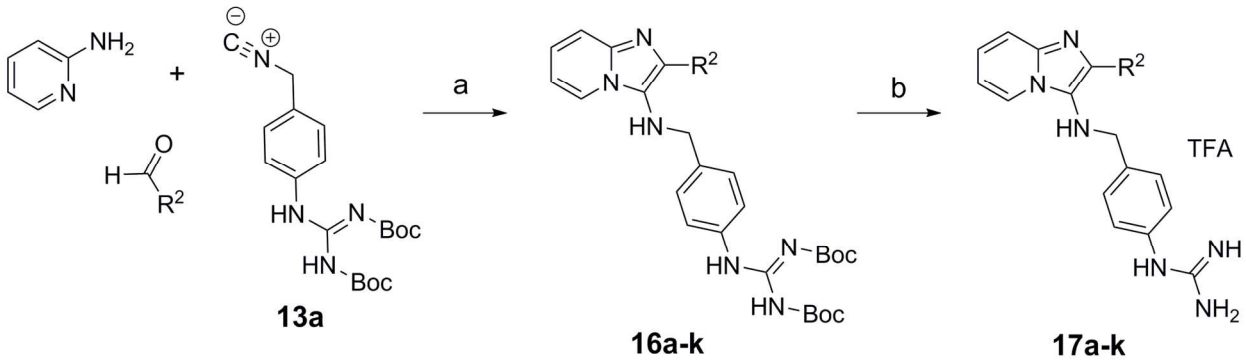
Table 1. Biochemical evaluation of the monosubstituted analogues 14h, 15a-g against uPA.



Cpd	R^1=	IC_{50} (uPA) [μM]
14h		~250
15a		9.04 ± 0.62
15b		19.39 ± 1.22
15c		~200
15d		>1000
15e		500
15f		>500
15g		500

Optimization of the initial hit **15a** began with investigating the influence of an additional substituent at the 2-position of the imidazo[1,2-*a*]pyridine scaffold (compounds **17a-k**, **Table 2**). For preparing these compounds, we reacted 2-aminopyridine and the corresponding isocyanide **13a** with different commercially available aldehydes (**Scheme 2**). Selection of the latter was mainly done in a non-target-biased manner, aiming to cover as much of druglike chemical space as possible by taking steric, electronic and electrostatic parameters into account.

Scheme 2. Synthesis of analogues modified at the 2-position 17a-k.[a]



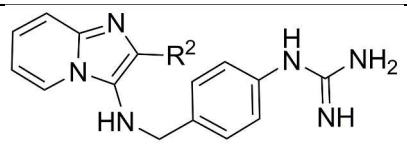
[a]Reagents and conditions: (a) $HClO_4$ (cat), MeOH, rt, 24 h, 22-86%; (b) TFA/DCM (1:1), rt, 1 h, 94-100%.

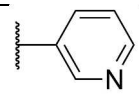
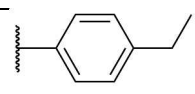
Preparation of the 2-substituted-3-amino-imidazo[1,2-*a*]pyridines followed the classical GBB reaction experimental protocol.[17] Reactions were performed in MeOH, and included pre-formation of the imine intermediate in the presence of a catalytic amount of $HClO_4$ in order to suppress by-products formation. Although the applied experimental protocol in most cases gave satisfactory results (60-86% yield), reaction with some aliphatic aldehydes (e.g.

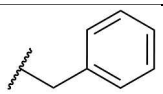
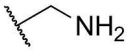
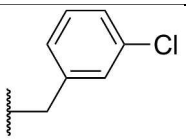
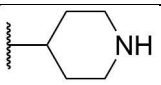
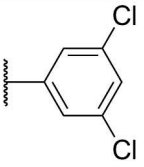
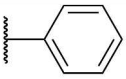
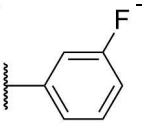
propionaldehyde, 2-aminoacetaldehyde, 3-chlorophenylacetaldehyde) led to poor or moderate yields for compounds **16d-f** (49%, 32%, and 22%, respectively), while 2-methoxyaldehyde and 3-(2,4-dichlorophenyl)propanal failed to react under these conditions. Additionally, two analogues of **15b** (**19a** and **19b)** with a guanidinophenethyl substituent at the 3-position were prepared for comparison of the inhibitory potency (**Table 3**). Compound **19a** has an ethyl substituent at the 2-position of the imidazo[1,2-*a*]pyridine ring, whereas analogue **19b** is comprising of the imidazo[1,2-*a*]pyrazine core and carries 3-chlorophenyl substituent at the 2-position. Preparation of compounds **19a** and **19b** involved the previously used GBB reaction protocol affording intermediates **18a** and **18b** in moderate yields (54 and 49%, respectively).

Most of the performed GBB reactions were complete within 24 h. The final step involved a simple Boc deprotection to afford the desired products in quantitative yields in the form of TFA-salts.

Table 2. Biochemical evaluation of the 2-substituted-3-amino-imidazo[1,2-*a*]pyridines set 17a-k against uPA.



Cpd	R^2=	IC_{50} (uPA) [µM]
17a		250 ± 2.23
17b		63.92 ± 9.42

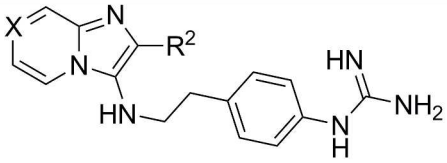
17c		30.25 ± 4.71
17d		48.47 ± 8.47
17e		9.30 ± 2.67
17f		18.03 ± 0.95
17g		~250
17h		99.16 ± 8.69
17i		30.15 ± 1.92
17j		~100
17k		~125

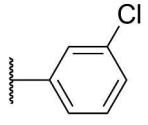
The uPA potency of the prepared set of 2-substituted analogues was then evaluated (**Table 2**). In general, most of the compounds within the 2-substituted imidazo[1,2-*a*]pyridines set displayed reduced uPA affinity. Especially aromatic substituents and sterically demanding aliphatic substituents at the 2-position were not favourable. This detrimental effect is tentatively explained by the computational model used (*vide supra*), suggesting that sterically demanding substituents

at the 2-position increase the internal conformational energy when the compound is in a conformation that is optimal for binding to the uPA S1 pocket. For instance, the 3-pyridyl (**17a**), 3-fluophenyl (**17j**), 4-fluophenyl (**17k**), and 3,5-dichlorophenyl (**17h**) analogues have IC_{50}-values ≥100 μM, indicating at least 10-fold decrease in potency in comparison to the initial hit **15a**. The same is true for the 4-piperidinyl-substituted compound **17g**, displaying an IC_{50} of more than 100 μM. Interestingly, the benzylic substituents in **17c** and **17f** caused a relatively less pronounced affinity decrease in comparison to **15a**, probably due to increased conformational flexibility. Finally, only the aminomethyl-substituted compound **17e** retained the initial binding affinity with an IC_{50} value of 9.30 ± 2.67 μM.

The guanidinophenethyl derivatives **19a**, 1**9b** followed a grossly comparable potency pattern (**Table 3**), although **19a**'s significant drop in potency compared to **15b** cannot be readily rationalized by taking only steric factors into account.

Table 3. Biochemical evaluation of the C3-guanidinophenethyl-substituted analogues 19a, 19b against uPA.

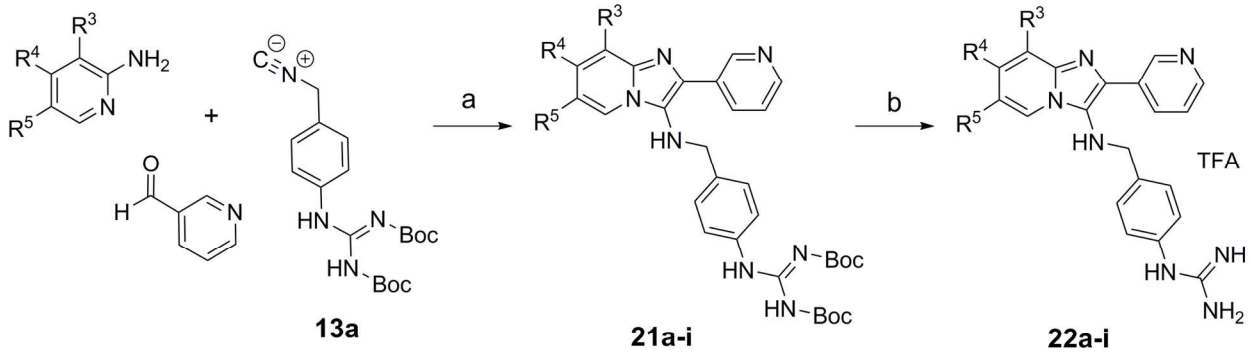


Cpd	R^2=	X=	IC_{50} (uPA) [μM]
19a		C	~150
19b		N	53.78 ± 3.72

Before evaluation data for the 2-substituted imidazo[1,2-*a*]pyridines set were obtained (**Table 2**), we decided to explore substitution at the C6-C8 position conserving the 2-pyridyl substituent (**Scheme 3**). The reason to conserve the latter was inspired by the practically very straightforward synthesis and purification of the compound.

Nine additional 2-(3-pyridyl) analogues (**22a-i**) (**Table 4**) were prepared by varying the aminopyridine reaction partner of the GBB reaction. Aminopyridine building blocks were either commercially available or synthesized separately. Accordingly, 2-amino-*N*-butylisonicotinamide **(20a)** and 6-amino-*N*-butylnicotinamide **(20b)** used for the preparation of compounds **22d** and **22h**, were synthesized by the aminolysis reaction in the presence of an organocatalyst (1,5,7-triazabicyclo[4.4.0]dec-5-ene, TBD), as reported by Kiesewetter *et al.*[24] (**Table 5, entry 2**). Details on the preparation of the latter can be found in the Experimental Section.

Scheme 3. Synthesis of analogues 22a-i.[a]

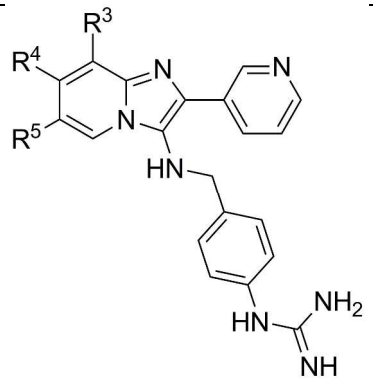


[a]Reagents and conditions: (a) $HClO_4$ (cat), MeOH, rt, 24 h, 25-68% (in case of compound **21d** (i) methyl 2-aminopyridine-4-carboxylate, TBD, DMF, 120 °C, 20 h, 52.5%; (ii) $HClO_4$ (cat), MeOH, rt, 24 h, 60%; compound **21h** (i) methyl 6-aminonicotinate, TBD, DMF, 120 °C, 24 h,

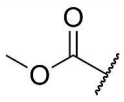
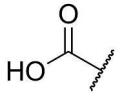
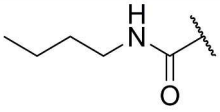
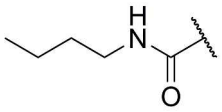
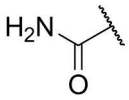
42%; (ii) $HClO_4$ (cat), MeOH, rt, 24 h, 47%); (b) TFA/DCM (1:1), rt, 1 h, 94-100% (in case of compound **22c** (i) NaOH (2 M), DCM/MeOH (9:1), 12 h, rt, 69%; (ii) TFA/DCM (1:1), rt, 1 h, 93%).

Noteworthy, aminopyridines substituted at the 4- or 5-position (e.g. compounds **21d** and **21i**) provided better yields in the GBB reaction (60 and 68%, respectively), whereas 2-aminopyridines substituted at the 3- or 6-position performed worse or failed to react under these conditions. For instance, reaction with 3-methylpyridin-2-amine provided **21a** only in 25% yield, and 3-(trifluoromethyl)pyridin-2-amine was found not to react at all under these conditions. Replacing the reaction solvent (MeOH) by the non-nucleophilic trifluoroethanol, as originally proposed by Bienaymé *et al.,* still did not afford the desired product.[17]

Table 4. Biochemical evaluation of the C2 3-pyridyl substituted analogues 22a-i against uPA.



Cpd	R^3=	R^4=	R^5=	IC_{50} (uPA) [µM]
22a		H	H	27.44 ± 1.34

22b	H		H	14.95 ± 0.81
22c	H		H	26.85 ± 1.57
22d	H		H	6.89 ± 0.80
22e	H	H		23.89 ± 2.69
22f	H	H		~200
22g	H	H		57.55 ± 6.58
22h	H	H		60.84 ± 3.39
22i	H	H		27.26 ± 2.19

The uPA-evaluation results of inhibitors **22a-i** are presented in **Table 4**. These demonstrate that the detrimental effect of the 2-pyridyl substituent on uPA potency, as observed with **17a**, can be significantly reduced by introducing additional affinity-conferring substituents (**Table 4**) on the scaffold. Nonetheless, the position of a substituent was found to have a significant effect on inhibitory activity. Generally, analogues substituted at the 7-position of the imidazopyridine scaffold displayed a higher affinity than compounds bearing a substituent at the 6-position. For instance, the C7 methyl carboxylate moiety in **22b** caused an increase of binding affinity of almost one order of magnitude relative to **17a**. Replacement of the 7-methyl carboxylate group

by an *N*-butylcarboxamide group in **22d** leads to a further increase in affinity. On the other hand, introducing the *N*-butylcarboxamide at the 6-position resulted in analogue **22h** with a significantly reduced uPA affinity (60.84 ± 3.39 μM) compared to **22d**. Also compounds with C6 electron-withdrawing groups like trifluoromethyl- and fluoro-substituted analogues **22f** and **22g** displayed reduced inhibitory potency (IC_{50} ~200 μM and IC_{50} = 57.55 ± 6.58 μM, respectively).

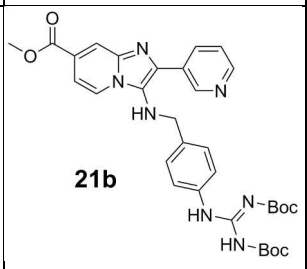
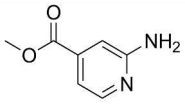
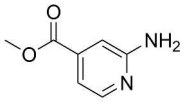
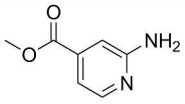
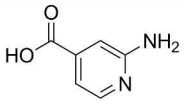
Again, the more favourable results of the 7-substituted congeners can be tentatively explained by a molecular docking study. **Figure 5** clearly indicates that the 6-position of the imidazopyridine scaffold approaches the surface of the pocket and might therefore be compatible with only very small substituent types. On the contrary, the 7-position is more accessible for attaching alternative substituents.

After obtaining the evaluation results, we decided to synthesize a set imidazo[1,2-*a*]pyridines lacking the 2-pyridyl substituent, but with diverse amide groups at the scaffold's 7-position (compounds **25a-f**). The *N*-butylcarboxamide substituent that was already present in **22d** was included in this series.

The synthetic strategy for these molecules involved formation of the required amide substituent via aminolysis of methyl 2-amino-pyridin-4-carboxylate, prior to the GBB reaction with glyoxylic acid and isocyanide **13a** (**Scheme 4**). Although this strategy does not introduce molecular diversity during the final synthetic step, it was deemed preferable for two reasons. First, the aminolysis reaction was found to require harsh reaction conditions (high temperature and pressure, long reaction times) that were not compatible with the labile di-Boc-protecting groups of the guanidine function, resulting in poor yields of the desired product (**Table 5, entry 1**). Second, performing the GBB further on in the synthesis allowed us to reduce the reaction

scale, and therefore consume less of the synthetically more demanding guanidinophenyl-derived isocyanide **13a**. Again, the aminolysis was carried out in the presence of TBD. Optimization of the experimental conditions used for preparation of amides **20a-b** (**Table 5, entry 2**) revealed that by reacting methyl 2-aminopyridine-4-carboxylate with a two-fold excess of the amine component, increasing the catalyst amount (0.3 eq) and performing the reaction in dry toluene at 110 °C, the desired amides (**23a-e)** were obtained in very good to excellent yields and without the need of chromatographic purification (exemplified in **Table 5, entry 4**). The devised protocol does not require protection of the amino group in aminopyridine and was found to be applicable with comparable outcome to methyl 2-aminopyridine-3-carboxylate, methyl 6-aminopyridine-3-carboxylate. Detailed experimental procedures and characterization data for amides **23a-e** can be found in the Experimental Section. Next to aminolysis, direct coupling of 2-aminopyridine-4-carboxylate with the corresponding amine in DMF in the presence of EDC and HOBt was also tried. However, this also returned the desired amide only in 10% yield (**Table 5, entry 5**).

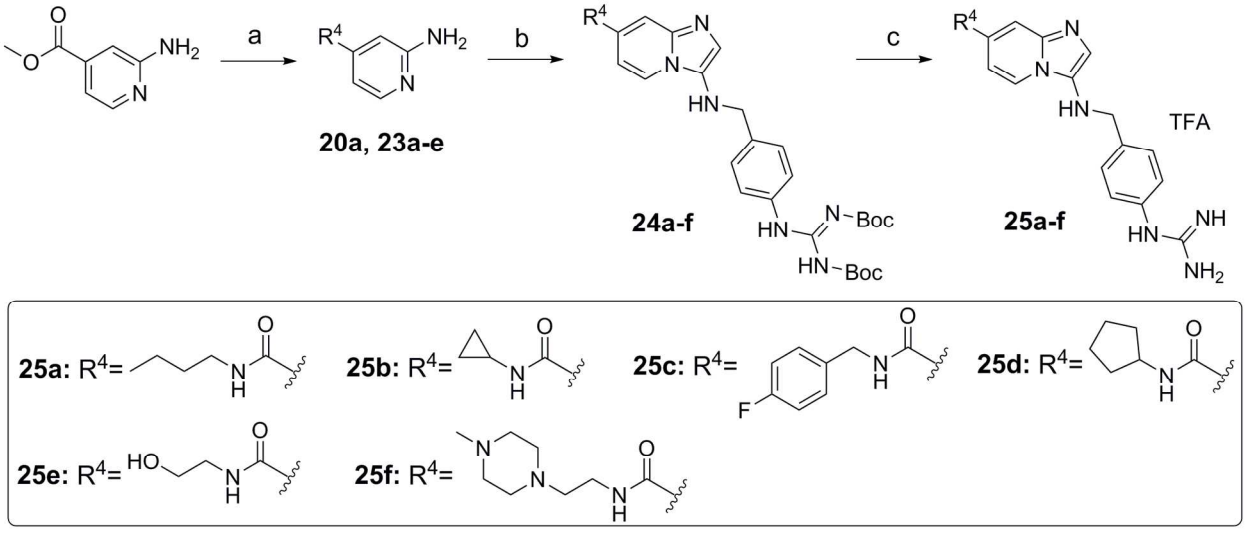
Table 5. Conditions applied to amide bond formation.

Entry	Amine	Carbonyl compound	Catalyst or coupling reagent	Solvent	Conditions	Yield [%]
1	*n*-butylamine (2 eq)	 **21b**	TBD (0.2 eq)	DMF	pressure tube, 120 °C, 24 h	<10
2	*n*-butylamine (2 eq)		TBD (0.2 eq)	DMF	pressure tube, 120°C, 20 h	52.5
3	cyclopropyl amine (2.2 eq)		TBD (0.2 eq)	DMF	pressure tube, 120°C, 21 h	17.2
4	cyclopropyl amine (2.2 eq)		TBD (0.3 eq)	Toluene	pressure tube, 110°C, 17 h	**89**
5	cyclopropyl amine (1.5 eq)		EDC and HOBt	DMF	65 °C, 20 h	10

The prepared amide-substituted aminopyridines were then reacted with glyoxylic acid and isocyanide **13a** following the previously used GBB reaction protocol (**Scheme 4**). Interestingly, reactions involving 2-aminopyridines with 4-substituent proceeded faster and most of them were complete within 6 h. Products of the three-component coupling (3CC) **24a-f** were then subjected to a simple Boc deprotection to afford the desired compounds in the form of TFA-salts, or in

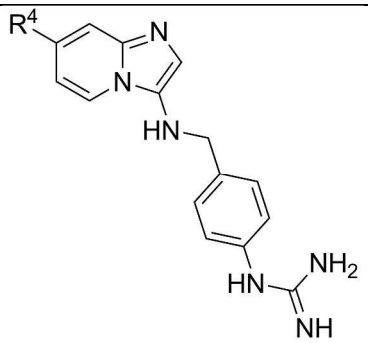
case of compound **25e**, HCl-salt. Finally, six analogues (**25a-f**) were synthesized in moderate yields (42-60%) (**Table 6**).

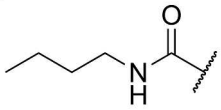
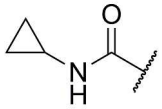
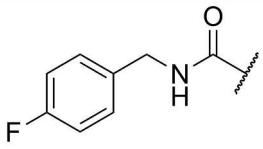
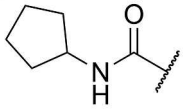
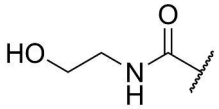
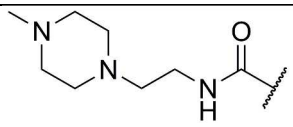
Scheme 4. Synthesis of the amide-substituted analogues 25a-f.[a]



[a]Reagents and conditions: (a) TBD, toluene, 110 °C, 20 h, 86-97% (in case of compound **20a** TBD, DMF, 120 °C, 20 h, 52.5%); (b) glyoxylic acid monohydrate, isocyanide **13a**, $HClO_4$ (cat), MeOH, rt, 6 h, 42-61%; (c) TFA/DCM (1:1), rt, 1 h, 96-100%.

Table 6. Biochemical evaluation of the amide-substituted analogues 25a-f against uPA.



Cpd	R^4=	IC_{50} (uPA) [µM]
25a		0.097 ± 0.010
25b		0.184 ± 0.007
25c		0.254 ± 0.016
25d		0.174 ± 0.021
25e		0.366 ± 0.017
25f		0.404 ± 0.020

Affinities of compounds within this series were in the nanomolar range, indicating an optimal substitution pattern for the scaffold-based uPA inhibitors. The most potent analogue **25a** (IC_{50} = 97 ± 10 nM) displays an increase in uPA affinity of about two orders of magnitude relative to initial hit **15a**. Similar potency was demonstrated by the cyclopropylamide-substituted analogue **25b** (IC_{50} = 184 ± 7 nM), whereas the 2-(4-methylpiperazine) ethylamide group in compound **25f** (IC_{50} = 404 ± 20 nM) caused a 4-fold affinity decrease in comparison to **25a**. The modeling study of the *N*-butylamide fragment of compound **25a** in the active site of uPA highlights the available space in this region (**Figure 5b**). Besides, formation of an additional hydrogen bond between the amide nitrogen of the *N*-butylamide fragment and the hydroxyl group of Tyr-151 can be observed and could potentially explain the increase in binding affinity that is observed when comparing compound **25a** with compound **15a** (**Figure 5a,b**). The modeling study of compounds **25b-f** revealed similar interactions occurring in the uPA's active site (**Figure 5c**). This additional interaction might also contribute favourably to the compounds' selectivity with respect to related proteases. (*vide infra*)

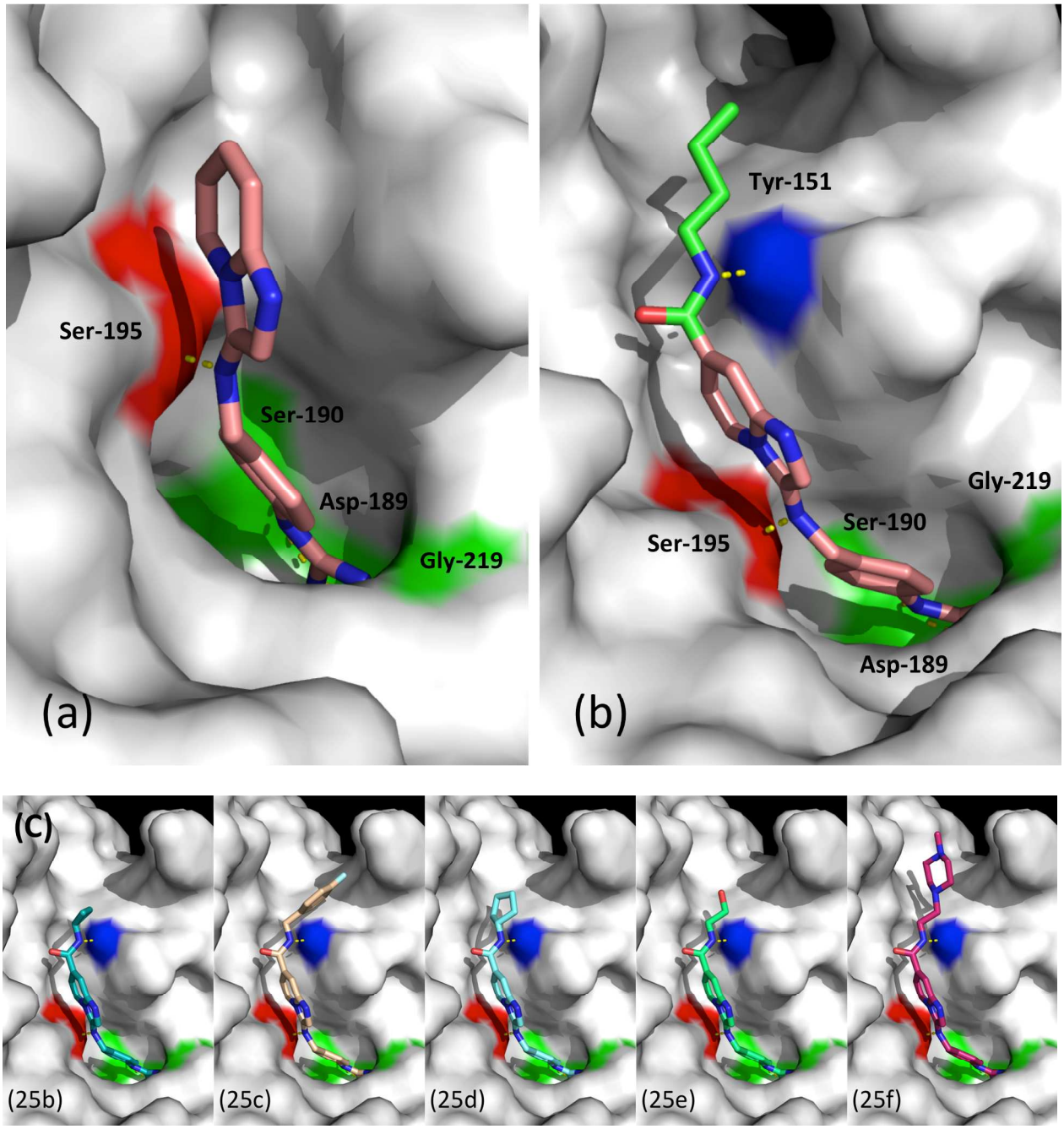


Figure 5. Helicopter view of uPA's active site (PDB-code 2O8W) showing the proposed binding mode of compound **15a** (panel **a**), compound **25a** (panel **b**), compounds **25b-f** (panel **c**). The protein surface is colored white, while the surface generated by residue Ser-195 is colored red and the surface generated by residues Ser-190, Asp-189 and Gly-219 is colored green. Putative hydrogen bonds are shown as dashed yellow lines. Docking of compound **25a** (panel **b**) reveals

an additional hydrogen bond between the ligand and the hydroxyl group of Tyr-151 (colored blue). The R^4 group (**Table 6**) of compound **25a** is colored green and has been docked using idealized geometrical bond and torsion angles.

Kinetic analysis of the identified inhibitors **25a-f** indicated that these are reversible, tight-binding, slow-dissociating inhibitors of uPA. In addition, selectivity of the most potent analogues for uPA was determined with respect to a panel of closely related proteases (tissue-type plasminogen activator (tPA), thrombin, Factor Xa (FXa), plasmin, plasma kallikrein, trypsin, Factor VIIa (FVIIa)). The results are summarized in **Table 7**. Compound **15a** was found to be at least 10-fold more selective for uPA than for the other assayed enzymes, while the amide-substituted compounds **25a-f** were found selective over all specified enzymes. High specificity of these compounds for uPA can probably be related to interacting with Tyr in position 151 of uPA (**Figure 5b, c**). Compound **25a** demonstrated the best affinity as well as selectivity profile among this set: with an $IC_{50} > 100$ μM for all of the related enzymes, this compound has a uPA-selectivity index of more than 10^3.

Besides, since the phenylguanidine (**26**) fragment is an essential part of the identified inhibitors, we decided to check its contribution to selectivity. Given the fact that no inhibition of thrombin, tPA, FXa, plasmin, plasma kallikrein and FVIIa was observed at 100 μM concentration of compound **26**, we can conclude that indeed the phenylguanidine fragment is crucial for selectivity of the identified inhibitors for uPA.

Table 7. Inhibitory activities against uPA and related enzymes (including the reference compounds: 7, gabexate (9), amiloride (10)).

Cpd	IC_{50} [μM] or % of enzyme inhibition at 100 μM concentration[a]							
	uPA	thrombin	tPA	FXa	plasmin	plasma kallikrein	trypsin	FVIIa
7	8.67 ± 1.12	56.1 ± 3.65	68.51 ± 5.05	6.47 ± 0.58	0.34 ± 0.01	1.34 ± 0.19	3.18 ± 0.17	0%
9	0.431 ± 0.017	0.687 ± 0.048	15.36 ± 0.94	4.61 ± 0.54	1.55 ± 0.06	1.35 ± 0.24	7.12 ± 0.13	-
10	11.98 ± 0.22	5.2%	1%	3.5%	0%	0%	31.76 ± 2.88	7.4%
15a	9.04 ± 0.62	23%	33.6%	42%	29.8%	39.4%	114.88 ± 6.78	2.5%
17a	250 ± 2.23	6.6%	9.4%	24.6%	0%	0%	3.3%	0%
25a	0.097 ± 0.010	19.2%	8%	41.8%	13.6%	2.8%	42.7%	1.4%
25b	0.184 ± 0.007	8%	0%	45.4%	3.3%	0%	46%	2.6%

25c	0.254 ± 0.016	19.5%	11.9%	45.7%	8.3%	0%	43.2%	4.2%
25d	0.174 ± 0.021	17.8%	11.2%	47.8%	0%	0%	37.1%	4%
25e	0.366 ± 0.017	4.7%	0%	51.58 ± 2.39	15.6%	0%	43.3%	5%
25f	0.404 ± 0.020	6.6%	2.8%	12.2%	0%	0%	32%	2%
26	57.49 ± 5.20	0%	0%	0%	0%	0%	38.8%	0%

[a] The IC_{50} values and % of enzyme inhibition were determined under the assay conditions of this manuscript.

Conclusions

In conclusion, we have developed a straightforward synthetic strategy for the synthesis of substituted imidazo[1,2-*a*]pyridines as efficient and selective uPA inhibitors. To the best of our knowledge, this is the first reported application of an imidazo[1,2-*a*]pyridine scaffold to the discovery of uPA inhibitors. Compounds **25a-f** have nanomolar uPA potencies and are among the most selective inhibitors reported to date. The presence of a guanidine group in **25a-f** might nonetheless be seen as a liability for further development. If required, a prodrug strategy involving the use of a hydroxyguanidine precursor could offer an effective way to overcome bioavailability issues of this kind. Finally, this study presents a novel and efficient strategy for transforming low-affinity fragments into potent, druglike compounds with a decorated scaffold architecture. The same strategy can be readily applied to inhibitor discovery for other enzyme targets.

Experimental section

Chemistry

Reagents were obtained from Sigma-Aldrich, Acros, Fluorochem or Apollo Scientific and were used without further purification. Synthesized compounds were characterized by ^{1}H-NMR, ^{13}C-NMR and mass spectrometry. ^{1}H-NMR and ^{13}C-NMR spectra were recorded with a Bruker Avance DRX 400 spectrometer, and analyzed by use of MestReNova analytical chemistry software. Chemical shifts are in ppm, and coupling constants are in hertz (Hz). Purities were determined with two diverse UHPLC systems based either on mass detection or on UV detection. All final products have a purity of at least 95%, with the exception of compound **15c**

(purity: 90%). A Waters acquity UPLC system coupled to a Waters TUV detector and a Waters TQD ESI mass spectrometer was used. A Waters Acquity UPLC BEH C18 1.7 μm, 2.1 mm x 50 mm column was used. Solvent A: water with 0.1% formic acid, solvent B: acetonitrile with 0.1% formic acid. Method I: 0.7 ml/min, 0.15 min 95% A, 5% B then in 1.85 min from 95% A, 5% B to 100% B, 0% A, then 0.25 min, 100% B, 0% A, 0.75 min (0.350 ml/min) 95% A, 5% B. Method II (purity method): 0.4 ml/min, 0.15 min 95% A, 5% B then in 4.85 min from 95% A, 5% B to 100% B, 0% A, then 0.25 min, 100% B, 0% A, 0.75 min 95% A, 5% B.The wavelength for UV detection was 254 nm.

Where necessary, flash purification was performed with a Biotage ISOLERA One flash system equipped with an internal variable dual-wavelength diode array detector (200–400 nm). SNAP cartridges were used, for normal phase purifications KP-Sil (10 g, 25 g, 50 g, flow rate of 10-50 mL/min), and for reversed phase purifications KP-C18-HS (12 g, 30 g, flow rate of 10-30 mL/min). Dry sample loading was done by self-packing samplet cartridges using silica and Celite 545, respectively, for normal and reversed phase purifications. Gradients used varied for each purification.

HRMS involved the following: The dry samples were dissolved in 1 mL of methanol to obtain 10 mM stock solutions, and next diluted 100-fold with methanol to a final concentration of 10 μM. Then 10 μl of each sample was injected using the CapLC system (Waters, Manchester, UK) and electrosprayed using a standard electrospray source. Samples were injected with an interval of 3 min. Positive ion mode accurate mass spectra were acquired using a Q-TOF II instrument (Waters, Manchester, UK). The mass spectrometer was calibrated prior to use with a 0.2% H_3PO_4 solution. The spectra were lock-mass- corrected using the known mass of the nearest H_3PO_4 cluster.

Melting points were determined with a Buchi 530 melting point apparatus and are uncorrected.

The following section comprises the synthetic procedures and analytical data for intermediates and all final compounds reported in this manuscript. Most of the final products were obtained in the form of TFA-salts. All TFA-related resonances are omitted in the ^{13}C NMR characterization. Additional data, including tables and schemes for the synthesis of intermediates en route to final compounds, can be found in the Supporting Information. The experimental procedures for all the steps in the synthesis of several final products are summarized here as the General Procedures.

General Prodecure A (Formamide Synthesis). To a round-bottomed flask fitted with a reflux condenser were added the corresponding amine (13.50 mmol) and ethyl formate (20 equiv). The reaction mixture was stirred and heated under reflux while triethylamine (1.5 equiv) was added. After overnight stirring under reflux the reaction was completed. Subsequently, volatiles were evaporated, and the crude product was redissolved in DCM (50 mL). The resulting solution was washed with H_2O (50 mL) and then with brine (50 mL). The organic phase was separated, dried over anhydrous $MgSO_4$, condensed under reduced pressure. In some cases further purification was avoidable, otherwise the corresponding formamide was purified using isolera purification system with a gradient of 20-100% of EtOAc in heptane affording the corresponding formamides **12a-h** in 55-90% yield.

General Prodecure B (Isocyanide Synthesis). To a suspension of formamide (4.08 mmol) in dry DCM (25 mL) was added diisopropylamine (2.7 equiv), and the reaction mixture was stirred at 0 °C under a nitrogen atmosphere. To this solution $POCl_3$ (1.1 equiv) was slowly added through a syringe, and stirring was continued for 1 h at 0 °C, and then for 2 h at rt. After completion of the reaction a solution of Na_2CO_3 in water (2 g/20 mL) was slowly added to maintain 25-30 °C. Stirring was continued for 1 h at rt. More water (20 mL), and DCM (50 mL)

was added. The organic phase was separated, dried over anhydrous $MgSO_4$, and concentrated under reduced pressure. In some cases further purification was avoidable, otherwise the corresponding isocyanide was purified using isolera purification system with a gradient of 0-50% of EtOAc in heptane affording the corresponding isocyanides **13a-h** in 60-85% yield.

General Prodecure C (**TBD-Catalysed Aminolysis Reaction)**. To a solution of methyl 2-aminopyridine-4-carboxylate (0.5 g, 3.29 mmol) in dry toluene (10 mL) were added amine (2.2 equiv) and 1,5,7- triazabicyclo[4.4.0]dec-5-ene (TBD) (0.137 g, 0.986 mmol, 0.3 equiv), and the reaction mixture was stirred in a pressure tube at 110 °C for 20 h. After that time the reaction mixture was cooled down, and stirred at ambient temperature for few hours. The desired product precipitated in the form of white crystals, which were next filtered and washed with cold diethyl ether (3 x 20 mL). The desired amides **23a-e** were obtained in very good to excellent yields (86-97%) and without the need of chromatographic purification.

General Prodecure D (**Preparation of Substituted Imidazo[1,2-*a*]pyridines using the Groebke-Blackburn-Bienaymé (GBB) reaction).** To a solution 2-aminoazine (e.g. 1.104 mmol) in MeOH (10 mL), aldehyde (or formaldehyde equivalent: glyoxylic acid monohydrate, 1.5 equiv)) and $HClO_4$ (cat.) (70% aq solution, 8.48 μL, 0.1 equiv) were added at rt, and the reaction was left stirring for 30 min. After that time the isonitrile component (1.1 equiv) was introduced, and the reaction mixture was stirred at ambient temperature until it was completed, usually for 5-24 h. After that time, the reaction mixture was concentrated under reduced pressure and the crude product was directly purified using isolera purification system with a gradient varying by purification, usually 20-100% of EtOAc in Heptane (1.5% TEA). In some cases reversed phase purification was used, and applied gradient of 10-100% of MeOH in water. The general procedure D was used to prepare compounds **14a-h**, **16a-k, 18a-b, 21a-i, 24a-f.**

General Prodecure E (Standard Procedure for Deprotection of Boc Groups Using TFA/DCM). Product of the GBB reaction (0.195 mmol) containing a basic function protected with the Boc group was dissolved in a mixture of DCM (1.5 mL) and TFA (1.5 mL) in 1:1 ratio, and the reaction mixture was stirred at rt for 1 h. After that time, volatiles were evaporated and the obtained product was washed with diethyl ether (2 x 10 mL) yielding pure final compound in the form of TFA-salt.

1-(4-(aminomethyl)phenyl)-2,3-di-Boc-guanidine (11a). To a solution of 4-(aminomethyl)aniline (0.6 g, 4.91 mmol) in 10% aq acetic acid (40 mL) was added a solution of (Z)-*tert*-butyl(((tert-butoxycarbonyl)amino)(1H-pyrazol-1-yl)methylene)carbamate (1.68 g, 5.40 mmol) in 1,4-dioxane (40 mL). After overnight stirring at rt, water (100 mL) was added and the mixture was washed with diethyl ether (3 x 60 mL). The aqueous phase was basicified with 2 M NaOH to pH 13 and extracted with ethyl acetate (3 x 75 mL). The combined extract was washed with water (2 x 50 mL), dried with anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to give the expected product **11a** in a form of white solid (0.46 g, 80%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.65 (s, 1H), 10.31 (s, 1H), 7.56 (d, J = 8.5 Hz, 2H), 7.27 (d, J = 8.5 Hz, 2H), 3.83 (s, 2H), 1.54 (s, 9H), 1.51 (s, 9H). UPLC/MS: t_r 1.94 min, *m/z* 365.3 $[M+H]^+$.

1-(4-(aminoethyl)phenyl)-2,3-di-Boc-guanidine (11b). The title compound was prepared from 4-(aminoethyl)aniline (0.3 g, 2.203 mmol) using the same synthetic procedure that was applied to the preparation of 1-(4-(aminomethyl)phenyl)- *N,N'*-bis-Boc-guanidine (**11a**), yielding a colorless oil (0.65 g, 78%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.64 (s, 1H), 10.26 (s, 1H), 7.50 (d, J = 8.4 Hz, 2H), 7.15 (d, J = 8.4 Hz, 2H), 3.07 – 2.86 (m, 2H), 2.72 (t, J = 6.0 Hz, 2H), 1.53 (s, 9H), 1.49 (s, 9H). UPLC/MS: t_r 1.60 min, *m/z* 379.3 $[M+H]^+$.

1-(4-aminobutyl)-2,3-di-Boc-guanidine (11c). To a solution of tetramethylenediamine (1.14 mL, 11.34 mmol) in 20:1 mixture of THF (16 mL) and H_2O (0.8 mL) was added dropwise a solution of *N, N′*-bis-Boc-methylisothiourea (1.098 g, 3.78 mmol) in THF (10 mL) at 25 °C. After addition the reaction was heated at 50 °C for 2 h, and then the solvent was evaporated under reduced pressure. The resulting crude was partitioned between DCM and saturated aq $NaHCO_3$. The organic extracts were dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to give the desired product (**11c**) in a form of cloudy oil (1.05 g, 85%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.45 (brs, 1H), 8.30 (s, 1H), 3.44 – 3.31 (m, 2H), 2.69 (t, J = 6.6 Hz, 2H), 1.63 – 1.52 (m, 2H), 1.51 – 1.37 (m, 20H). UPLC/MS: t_r 1.51 min, *m/z* 331.6 $[M+H]^+$.

3,5-dichloro-4-(2-(Boc-amino)ethoxy)aniline (11e). Amine **11e** was obtained in three synthetic steps from the previously prepared starting material. **Step 1: Preparation of *N*-Cbz-4-amino-2,6-dichlorophenol (standard Cbz-protection procedure)**. To a suspension of 4-amino-2,6-dichlorophenol (2.2 g, 12.36 mmol) and $NaHCO_3$ (1.142 g, 13.59 mmol, 1.1 equiv) in THF (20 mL) benzyl chloroformate (1.669 ml, 11.74 mmol) was added dropwise at 0 °C. The reaction was left stirring overnight. After that time EtOAc (70 mL) was added and the organic layer was extracted with 2M HCl (50 mL), washed with brine, dried over anhydrous $MgSO_4$ and evaporated under reduced pressure. The title compound was purified using isolera purification system with a gradient of 0-20% of EtOAc in heptane to afford *N*-Cbz-4-amino-2,6-dichlorophenol (2 g, 52%). **Step 2: Preparation of 3,5-dichloro-*N*-Cbz-4-(2-(Boc-amino)ethoxy)aniline.** To a solution of *N*-Boc-2-bromoethanamine (2.202 g, 8.84 mmol) in DMF (40 mL) *N*-Cbz-4-amino-2,6-dichlorophenol (2.3 g, 7.37 mmol) was added. To this reaction mixture K_2CO_3 (2.037 g, 14.74 mmol) was added in portions. After 4 h stirring at 60 °C, the reaction was completed. The reaction mixture was quenched with water and the aqueous

phase was extracted with EtOAc (3 x 50 mL). The combined organic extract was washed with water (50 mL), then with brine (50 mL), dried over anhydrous $MgSO_4$, and concentrated under reduced pressure to afford a transparent oil. The title compound was purified using isolera purification system with a gradient of 0-25% of EtOAc in heptane (1.7 g, 51%). **Step 3: 3,5-dichloro-4-(2-(Boc-amino)ethoxy)aniline (11e) (standard hydrogenolysis procedure).** First, 3,5-dichloro-*N*-Cbz-4-(2-(Boc-amino)ethoxy)aniline (1.5 g, 3.29 mmol) was dissolved in MeOH (30 mL). After 30 min of bubbling nitrogen gas through the solution, Pd/C (0.631 g, 2.96 mmol) was added, and the reaction was flushed again for 15 min with nitrogen before hydrogen gas was added via a balloon. The reaction was stirred under a hydrogen atmosphere for 50 min. The obtained product was purified using isolera purification system with a gradient of 10-100% of EtOAc in heptane to afford the title amine (0.835 g, 75%). The product was directly used in the next step, formamide synthesis.

(1*R*,4*R*)-4-((Boc-amino)methyl)cyclohexan-1-amine (11g). To a solution of (1*R*,4*R*)-4-((Boc-amino)methyl)cyclohexane-1-carboxylic acid (2.15 g, 8.36 mmol) in anhydrous THF (25 mL) stirred under a nitrogen atmosphere were added triethylamine (2.329 ml, 16.71 mmol, 2 equiv) and diphenylphosphonic azide (2.7 ml, 12.53 mmol, 1.5 equiv). The reaction mixture was stirred under reflux for 1 h for full conversion of the starting material to occur. The reaction mixture was cooled down, solvent was evaporated under vacuum, and the obtained residue was redissolved in a small amount of THF. The obtained solution was poured into a 2M NaOH aq (50 mL) and stirred for 1h at rt. Next EtOAc (75 mL) was added and the aqueous phase was washed thoroughly with EtOAc (3 x 75 mL). The organic phase was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure, washed with a small portion of cold diethyl ether (30 mL) to afford the desired amine as a white solid (1.73 g, 82%). ^{1}H NMR (400 MHz, DMSO) δ 2.81 –

2.67 (m, 3H), 1.91 – 1.78 (m, 2H), 1.70 – 1.58 (m, 2H), 1.37 (s, 9H), 1.30 – 1.08 (m, 3H), 0.94 – 0.78 (m, 2H). ^{13}C NMR (101 MHz, DMSO) δ 155.72, 77.35, 49.51, 45.58, 36.85, 30.67, 28.28, 28.23. UPLC/MS: t_r 1.14 min, *m/z* 229.1 $[M+H]^+$, 457.4 $[2M+H]^+$.

2-(2,4-dichlorophenoxy)ethanamine (11h). To a solution of 2,4-dichlorophenol (0.58 g, 3.56 mmol) in DMF (20 mL) were added *N*-Boc-2-bromoethanamine (0.957 g, 4.27 mmol, 1.2 equiv) and K_2CO_3 (0.984 g, 7.12 mmol, 2 equiv). After overnight stirring at 60 °C, the reaction was quenched with water, and the aqueous phase was extracted with EtOAc (3 x 50 mL). The combined organic extract was washed with water (50 mL), then with brine (50 mL), dried over anhydrous $MgSO_4$, and concentrated under reduced pressure to yield the desired product in a form of transparent oil which rapidly crystallized into white solid material (1.02 g, 65.5%). Next, the obtained *N*-Boc-(2-(2,4-dichlorophenoxy)ethaneamine (1 g, 3.27 mmol) was dissolved in 1:1 mixture of TFA and DCM and the reaction mixture was stirred for 1 h at rt. After that time, volatiles were evaporated under reduced pressure affording the title amine (**11h**) in quantitative yield in the form of TFA-salt (1.493 g, 100%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.97 (brs, 3H), 7.32 (d, *J* = 2.5 Hz, 1H), 7.16 (dd, *J* = 8.8, 2.5 Hz, 1H), 6.82 (d, *J* = 8.8 Hz, 1H), 4.20 (t, *J* = 5 Hz, 2H), 3.48 – 3.37 (m, 2H). UPLC/MS: t_r 1.43 min, *m/z* 206.0 $[M+H]^+$.

***N*-(4-(2,3-di-Boc-guanidino)benzyl)formamide (12a).** The title compound was prepared using the general procedure A to afford a white solid in 68% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.61 (s, 1H), 10.29 (s, 1H), 8.17 (s, 1H), 7.53 (d, *J* = 8.5 Hz, 2H), 7.22 (d, *J* = 8.4 Hz, 2H), 6.07 (s, 1H), 4.39 (d, *J* = 5.8 Hz, 2H), 1.53 (s, 9H), 1.48 (s, 9H). UPLC/MS: t_r 2.00 min, *m/z* 393.3 $[M+H]^+$.

***N*-(4-(2,3-di-Boc-guanidino)phenethyl)formamide (12b).** The title compound was prepared using the general procedure A to afford a white solid in 55% yield. ^{1}H NMR (400 MHz, $CDCl_3$)

δ 11.62 (s, 1H), 10.26 (s, 1H), 8.06 (s, 1H), 7.49 (d, *J* = 8.5 Hz, 2H), 7.14 (d, *J* = 8.5 Hz, 2H), 5.82 (brs, 1H), 3.51 (q, *J* = 6.5 Hz, 2H), 2.79 (t, *J* = 6.7 Hz, 2H), 1.53 (s, 9H), 1.49 (s, 9H). UPLC/MS: t_r 2.16 min, *m/z* 407.5 $[M+H]^+$.

***N*-(4-(2,3-di-Boc-guanidino)butyl)formamide (12c).** The title compound was prepared using the general procedure A to afford a white solid in 56.7% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.49 (s, 1H), 8.29 (t, *J* = 5.5 Hz, 1H), 7.99 (s, 1H), 3.31 – 3.22 (m, 2H), 3.15 – 3.02 (m, 2H), 1.55 – 1.30 (m, 22H). UPLC/MS: t_r 1.83 min, *m/z* 359.6 $[M+H]^+$.

***N*-(4-(2-(Boc-amino)ethoxy)phenyl)formamide (12d).** The title compound was prepared using the general procedure A to afford a white solid in 90% yield. ^{1}H NMR (400 MHz, DMSO) δ 10.01 (s, 1H), 8.19 (d, *J* = 1.9 Hz, 1H), 7.52 – 7.45 (m, 2H), 6.98 (t, *J* = 5.3 Hz, 1H), 6.93 – 6.85 (m, 2H), 3.91 (t, *J* = 5.9 Hz, 2H), 3.26 (q, *J* = 5.8 Hz, 2H), 1.38 (s, 9H). UPLC/MS: t_r 1.54 min, *m/z* 281.2 $[M+H]^+$, 561.4 $[2M+H]^+$.

***N*-(3,5-dichloro-4-(2-(Boc-amino)ethoxy)phenyl)formamide (12e).** The title compound was prepared using the general procedure A to afford a yellow solid in 72% yield. The product was directly used in the next step, isocyanide synthesis.

***N*-(4-((Boc-amino)methyl)phenyl)formamide (12f).** The title compound was prepared using the general procedure A to afford a white solid in 56% yield. ^{1}H NMR (400 MHz, DMSO) δ 10.13 (s, 1H), 8.24 (s, 1H), 7.51 (d, *J* = 8.4 Hz, 2H), 7.32 (brs, 1H), 7.17 (d, *J* = 8.5 Hz, 2H), 4.06 (d, *J* = 6.1 Hz, 2H), 1.38 (s, 9H). UPLC/MS: t_r 1.51 min, *m/z* 251.3 $[M+H]^+$, 501.3 $[2M+H]^+$.

***N*-((1*R*,4*R*)-4-((Boc-amino)methyl)cyclohexyl)formamide (12g).** The title compound was prepared using the general procedure A to afford a white solid in 57% yield. ^{1}H NMR (400 MHz, DMSO) δ 7.91 (m, 1H), 6.80 (t, *J* = 5.6 Hz, 1H), 3.60 – 3.40 (m, 1H), 2.75 (t, *J* = 6.3 Hz, 2H),

1.83 – 1.60 (m, 4H), 1.37 (s, 9H), 1.33 – 1.02 (m, 3H), 0.98 – 0.81 (m, 2H). ^{13}C NMR (101 MHz, DMSO) δ 159.98, 155.70, 77.30, 46.55, 45.77, 37.10, 31.86, 28.95, 28.27. UPLC/MS: t_r 1.45 min, *m/z* 257.1 $[M+H]^+$, 513.4 $[2M+H]^+$.

***N*-(2-(2,4-dichlorophenoxy)ethyl)formamide (12h).** The title compound was prepared using the general procedure A to afford a white solid in 62.2% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.18 (s, 1H), 7.30 (s, 1H), 7.12 (d, *J* = 8.8 Hz, 1H), 6.79 (d, *J* = 8.8 Hz, 1H), 4.03 (t, *J* = 5.1 Hz, 2H), 3.68 (q, *J* = 5.3 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 161.60, 152.73, 130.00, 127.78, 126.41, 123.70, 114.49, 68.19, 37.37. UPLC/MS: t_r 1.98 min, *m/z* 234.0 $[M+H]^+$, 469.1 $[2M+H]^+$.

1-(4-(isocyanomethyl)phenyl)-2,3-di-Boc-guanidine (13a). The title compound was prepared using the general procedure B to afford a white solid in 85% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.63 (s, 1H), 10.39 (s, 1H), 7.66 (d, *J* = 8.6 Hz, 2H), 7.29 (d, *J* = 8.7 Hz, 2H), 4.59 (s, 2H), 1.54 (s, 9H), 1.50 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.53, 153.61, 153.41, 137.17, 128.53, 127.35, 122.61, 84.01, 79.89, 45.23, 28.28, 28.18. UPLC/MS: t_r 2.44 min, *m/z* 375.2 $[M+H]^+$.

1-(4-(2-isocyanoethyl)phenyl)-2,3-di-Boc-guanidine (13b). The title compound was prepared using the general procedure B to afford a white solid in 63% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.63 (s, 1H), 10.33 (s, 1H), 7.57 (d, *J* = 8.5 Hz, 2H), 7.18 (d, *J* = 8.5 Hz, 2H), 3.57 (t, *J* = 7.1 Hz, 2H), 2.95 (t, *J* = 7.1 Hz, 2H), 1.53 (s, 9H), 1.50 (s, 9H). UPLC/MS: t_r 2.43 min, *m/z* 389.2 $[M+H]^+$.

1-(4-isocyanobutyl)-2,3-di-Boc-guanidine (13c). The title compound was prepared using the general procedure B to afford a white solid in 65% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.48 (s,

1H), 8.35 (s, 1H), 3.52 – 3.38 (m, 4H), 1.80 – 1.67 (m, 4H), 1.49 (d, J = 1.9 Hz, 18H). UPLC/MS: t_r 2.22 min, *m/z* 341.6 $[M+H]^+$.

2-(4-isocyanophenoxy)-*N*-Boc-ethanamine (13d). The title compound was prepared using the general procedure B to afford a white solid in 70% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33 – 7.27 (m, 2H), 6.88 – 6.83 (m, 2H), 4.01 (t, J = 5.2 Hz, 2H), 3.53 (q, J = 5.3 Hz, 2H), 1.44 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.92, 158.99, 155.95, 127.93, 119.91, 115.17, 79.85, 67.66, 40.03, 28.49. UPLC/MS: t_r 1.79 min, *m/z* 263.2 $[M+H]^+$.

2-(2,6-dichloro-4-isocyanophenoxy)-*N*-Boc-ethanamine (13e). The title compound was prepared using the general procedure B to afford a white solid in 73% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36 (s, 2H), 4.11 (t, J = 5.0 Hz, 2H), 3.54 (q, J = 5.2 Hz, 2H), 1.45 (s, 9H).

1-(4-isocyanophenyl)-*N*-Boc-methanamine (13f). The title compound was prepared using the general procedure B to afford a white solid in 68% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36 – 7.27 (m, 4H), 4.94 (s, 1H), 4.32 (d, J = 5.7 Hz, 2H), 1.45 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 164.14, 155.97, 140.95, 128.32, 126.69, 80.06, 44.14, 28.49. UPLC/MS: t_r 1.84 min, *m/z* 233.0 $[M+H]^+$, 465.2 $[2M+H]^+$.

1-((1*R*,4*R*)-4-isocyanocyclohexyl)-*N*-Boc-methanamine (13g). The title compound was prepared using the general procedure B to afford a white solid in 80% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.59 (brs, 1H), 3.42 – 3.29 (m, 1H), 2.95 (t, J = 6.5 Hz, 2H), 2.22 – 2.11(m, 2H), 1.87 – 1.71 (m, 2H), 1.63 – 1.31 (m, 12H), 1.02 – 0.85 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 156.12, 154.14, 79.38, 52.10, 45.86, 36.99, 32.83, 28.49, 28.22. UPLC/MS: t_r 1.81 min, *m/z* 239.0 $[M+H]^+$, 477.3 $[2M+H]^+$.

2,4-dichloro-1-(2-isocyanoethoxy)benzene (13h). The title compound was prepared using the general procedure B to afford a white solid in 60% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 (d,

J = 2.5 Hz, 1H), 7.20 (dd, *J* = 8.8, 2.5 Hz, 1H), 6.87 (d, *J* = 8.8 Hz, 1H), 4.21 (t, *J* = 5.6 Hz, 2H), 3.84 (t, *J* = 5.6 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.02, 152.49, 130.52, 127.86, 127.55, 124.79, 115.53, 67.24, 41.28. UPLC/MS: t_r 2.58 min, *m/z* 216.1 $[M+H]^+$.

1-(4-((imidazo[1,2-*a*]pyridin-3-ylamino)methyl)phenyl)-2,3-di-Boc-guanidine (14a). The title compound was prepared according to the general procedure D from pyridin-2-amine, glyoxylic acid monohydrate, isocyanide **13a** (0.35 g, 47.5%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.61 (s, 1H), 10.32 (s, 1H), 8.24 (d, *J* = 6.9 Hz, 1H), 7.56 (d, *J* = 9.1 Hz, 1H), 7.50 – 7.35 (m, 3H), 7.26 (d, *J* = 8.5 Hz, 2H), 7.05 (td, *J* = 6.9, 1.0 Hz, 1H), 6.83 (s, 1H), 4.20 (s, 2H), 1.54 (s, 9H), 1.43 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 154.39, 138.09, 135.93, 135.14, 132.63, 128.42, 128.19, 123.58, 123.50, 114.55, 114.46, 84.35, 80.14, 49.97, 28.24. UPLC/MS: t_r 1.92 min, *m/z* 481.4 $[M+H]^+$.

1-(4-((imidazo[1,2-*a*]pyridin-3-ylamino)ethyl)phenyl)-2,3-di-Boc-guanidine (14b). The title compound was prepared according to the general procedure D from pyridin-2-amine, glyoxylic acid monohydrate, isocyanide **13b** (0.40 g, 64%). UPLC/MS: t_r 2.07 min, *m/z* 495.6 $[M+H]^+$.

1-(4-(imidazo[1,2-*a*]pyridin-3-ylamino)butyl)-2,3-di-Boc-guanidine (14c). The title compound was prepared according to the general procedure D using pyridin-2-amine, glyoxylic acid monohydrate, isocyanide **13c** (0.28 g, 42%). ^{1}H NMR (400 MHz, DMSO) δ 11.50 (s, 1H), 8.33 (t, *J* = 5.4 Hz, 1H), 8.13 (d, *J* = 6.9 Hz, 1H), 7.43 (d, *J* = 9.1 Hz, 1H), 7.10 (ddd, *J* = 9.1, 6.6, 1.1 Hz, 1H), 6.95 (s, 1H), 6.87 (td, *J* = 6.8, 1.0 Hz, 1H), 5.40 (t, *J* = 5.6 Hz, 1H), 3.37 – 3.26 (m, 2H), 3.14 – 3.01 (m, 2H), 1.69 – 1.51 (m, 4H), 1.43 (s, 9H), 1.33 (s, 9H). UPLC/MS: t_r 1.76 min, *m/z* 447.6 $[M+H]^+$.

***N*-(4-(2-(Boc-amino)ethoxy)phenyl)imidazo[1,2-*a*]pyridin-3-amine (14d).** The title compound was prepared according to the general procedure D using pyridin-2-amine, glyoxylic acid monohydrate, isocyanide **13d** (0.42 g, 65%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.87 (dt, *J* = 6.8, 1.2 Hz, 1H), 7.62 (dt, *J* = 9.1, 1.1 Hz, 1H), 7.50 (s, 1H), 7.24-7.18 (m, 1H), 6.83 – 6.70 (m, 3H), 6.53 – 6.46 (m, 2H), 5.44 (s, 1H), 4.99 (brs, 1H), 3.93 (t, *J* = 5.1 Hz, 2H), 3.52 – 3.44 (m, 2H), 1.44 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 156.04, 152.72, 142.96, 139.25, 127.65, 125.34, 124.30, 123.10, 117.73, 115.97, 115.06, 112.76, 79.63, 67.89, 40.30, 28.53. UPLC/MS: t_r 1.37 min, *m/z* 369.2 $[M+H]^+$.

***N*-(3,5-dichloro-4-(2-(Boc-amino)ethoxy)phenyl)imidazo[1,2-*a*]pyridin-3-amine (14e).** The title compound was prepared according to the general procedure D using pyridin-2-amine, glyoxylic acid monohydrate, isocyanide **13e** (0.26 g, 42%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.92 (dt, *J* = 6.8, 1.1 Hz, 1H), 7.69 (d, *J* = 9.1 Hz, 1H), 7.54 (s, 1H), 7.34 – 7.28 (m, 1H), 6.91 (td, *J* = 6.8, 0.9 Hz, 1H), 6.52 (s, 2H), 5.80 (s, 1H), 5.18 (s, 1H), 4.01 (t, *J* = 5.0 Hz, 2H), 3.56 – 3.44 (m, 2H), 1.45 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 156.22, 144.10, 143.91, 142.88, 130.24, 129.99, 125.20, 122.54, 121.73, 118.39, 113.67, 112.94, 79.53, 72.81, 50.92, 28.55. UPLC/MS: t_r 1.55 min, *m/z* 437.1 $[M+H]^+$.

***N*-(4-((Boc-amino)methyl)phenyl)imidazo[1,2-*a*]pyridin-3-amine (14f).** The title compound was prepared according to the general procedure D from pyridin-2-amine, glyoxylic acid monohydrate, isocyanide **13f** (0.40g, 66%). ^{1}H NMR (400 MHz, DMSO) δ 8.00 (s, 1H), 7.95 (d, *J* = 6.8 Hz, 1H), 7.60 (dt, *J* = 9.1, 1.0 Hz, 1H), 7.51 (s, 1H), 7.30 (ddd, *J* = 9.1, 6.7, 1.2 Hz, 1H), 7.23 (t, *J* = 6.0 Hz, 1H), 7.02 (d, *J* = 8.4 Hz, 2H), 6.95 (td, *J* = 6.8, 1.0 Hz, 1H), 6.50 (d, *J* = 8.4 Hz, 2H), 3.98 (d, *J* = 6.1 Hz, 2H), 1.37 (s, 9H). ^{13}C NMR (101 MHz, DMSO) δ 155.68, 144.59,

141.71, 128.28, 126.21, 124.71, 124.10, 123.17, 117.04, 113.08, 112.37, 77.58, 42.91, 28.26. UPLC/MS: t_r 1.38 min, *m/z* 339.1 $[M+H]^+$.

***N*-((1*R*,4*R*)-4-((Boc-amino)methyl)cyclohexyl)imidazo[1,2-*a*]pyridin-3-amine (14g)**. The title compound was prepared according to the general procedure D from pyridin-2-amine, glyoxylic acid monohydrate, isocyanide **13g** (0.26 g, 43%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.19 (d, *J* = 6.9 Hz, 1H), 7.68 (d, *J* = 9.1 Hz, 1H), 7.42 – 7.33 (m, 1H), 7.23 (s, 1H), 7.02 (t, *J* = 6.8 Hz, 1H), 4.62 (brs, 1H), 3.03 – 2.89 (m, 3H), 2.13 – 2.04 (m, 2H), 1.86 – 1.76 (m, 2H), 1.44 (s, 9H), 1.36 – 1.22 (m, 3H), 1.07 – 0.92 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 156.22, 140.85, 130.18, 125.13, 122.87, 116.55, 112.71, 79.26, 56.63, 46.43, 37.95, 33.16, 29.28, 28.54. UPLC/MS: t_r 1.42 min, *m/z* 345.2 $[M+H]^+$.

***N*-(2-(2,4-dichlorophenoxy)ethyl)imidazo[1,2-*a*]pyridin-3-amine (14h).** The title compound was prepared according to the general procedure D from pyridin-2-amine, glyoxylic acid monohydrate, isocyanide **13h**, yielding a yellow solid (0.25 g, 38%), mp 98-100 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03 (d, *J* = 6.9 Hz, 1H), 7.55 (d, *J* = 9.1 Hz, 1H), 7.40 (d, *J* = 2.5 Hz, 1H), 7.23 –7.17 (m, 2H), 7.15 – 7.08 (m, 1H), 6.88 (d, *J* = 8.8 Hz, 1H), 6.80 (td, *J* = 6.8, 0.9 Hz, 1H), 4.21 (t, *J* = 5.1 Hz, 2H), 3.60 – 3.51 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 152.86, 137.39, 132.91, 130.18, 129.52, 127.99, 126.65, 124.04, 123.65, 115.18, 114.59, 114.02, 68.96, 46.18. UPLC/MS: t_r 1.85 min, *m/z* 322.1 $[M+H]^+$, purity: 98%. HRMS: mass calculated for $C_{15}H_{14}N_3OCl_2$: 322.0514; found: 322.0514.

1-(4-((imidazo[1,2-*a*]pyridin-3-ylamino)methyl)phenyl)guanidine (15a). Boc deprotection of compound **14a** was done using the general procedure E to afford the title compound **(15a)** as a yellow oil (0.12 g, 98%). ^{1}H NMR (400 MHz, DMSO) δ 9.98 (s, 1H), 8.63 (d, *J* = 6.9 Hz, 1H), 7.89 – 7.75 (m, 2H), 7.59 – 7.42 (m, 7H), 7.32 (s, 1H), 7.23 (d, *J* = 8.4 Hz, 2H), 4.41 (s, 2H). ^{13}C

NMR (101 MHz, DMSO) δ 155.86, 136.50, 135.53, 134.50, 133.91, 131.08, 128.89, 124.44, 124.39, 115.90, 112.37, 47.46. UPLC/MS: t_r 0.27 min, *m/z* 281.2 $[M+H]^+$, purity: 96%. HRMS: mass calculated for $C_{15}H_{17}N_6$: 281.1515; found: 281.1524.

1-(4-(2-(imidazo[1,2-*a*]pyridin-3-ylamino)ethyl)phenyl)guanidine (15b). Boc deprotection of compound **14b** was done using the general procedure E to afford the title compound **(15b)** as an orange oil (0.09 g, 97%). ^{1}H-NMR (400 MHz, DMSO) δ 9.85 (s, 1H), 8.54 (d, *J* = 6.93, 1H), 7.86 – 7.76 (m, 2H), 7.50 (s, 1H), 7.47 – 7.43 (m, 4H), 7.41 (d, *J* = 8.37, 2H), 7.19 (d, *J* = 8.37, 2H), 6.39 (brs, 1H), 3.39 (t, *J* = 7.3 Hz, 2H), 2.97 (t, *J* = 7.4 Hz, 2H). ^{13}C NMR (101 MHz, DMSO) δ 155.96, 144.01, 137.64, 135.53, 134.14, 133.54, 130.92, 130.11, 124.58, 124.26, 115.80, 112.36, 101.40, 46.18, 34.12. UPLC/MS: t_r 0.28 min, *m/z* 295.3 $[M+H]^+$, purity: 95%. HRMS: mass calculated for $C_{16}H_{19}N_6$: 295.1671; found: 295.1679.

1-(4-(imidazo[1,2-*a*]pyridin-3-ylamino)butyl)guanidine (15c). Boc deprotection of compound **14c** was done using the general procedure E to afford the title compound **(15c)** as an orange oil (0.10 g, 96%). ^{1}H NMR (400 MHz, DMSO) δ 8.57 (d, *J*=6.90 Hz, 1H), 7.96 – 7.67 (m, 4H), 7.44 (td, *J* = 6.9, 1.2 Hz, 1H), 7.41 (s, 1H), 6.26 (s, 1H), 3.21 – 3.04 (m, 5H), 1.72 – 1.53 (m, 4H). ^{13}C NMR (101 MHz, DMSO) δ 156.93, 135.46, 134.44, 130.85, 124.30, 115.71, 112.32, 100.88, 45.72, 44.26, 26.07, 25.59. UPLC/MS: t_r 0.27 min, *m/z* 247.2 $[M+H]^+$, purity: 90%. HRMS: mass calculated for $C_{12}H_{19}N_6$: 247.1671; found: 247.1662.

***N*-(4-(2-aminoethoxy)phenyl)imidazo[1,2-*a*]pyridin-3-amine (15d).** Boc deprotection of compound **14d** was done using the general procedure E to afford the title compound **(15d)** as a yellow oil (0.11 g, 100%). ^{1}H NMR (400 MHz, DMSO) δ 8.43 (d, *J* = 6.8 Hz, 1H), 8.11 (brs, 3H), 8.08 (s, 1H), 8.02 (d, *J* = 9.1 Hz, 1H), 7.97 – 7.89 (m, 1H), 7.48 (t, *J* = 6.4 Hz, 1H), 6.89 (d, *J* = 9.0 Hz, 2H), 6.80 (d, *J* = 9.0 Hz, 2H), 4.08 (t, *J* = 5.1 Hz, 2H), 3.20 (q, *J* = 5.1 Hz, 2H). ^{13}C

NMR (101 MHz, DMSO) δ 152.06, 138.21, 137.50, 132.85, 127.05, 125.06, 116.77, 116.05, 115.84, 114.56, 112.95, 64.95, 30.71. UPLC/MS: t_r 0.18 min, *m/z* 269.0 $[M+H]^+$, purity: 97%. HRMS: mass calculated for $C_{15}H_{17}N_4O$: 269.1402; found: 269.1408.

***N*-(4-(2-aminoethoxy)-3,5-dichlorophenyl)imidazo[1,2-*a*]pyridin-3-amine (15e).** Boc deprotection of compound **14e** was done using the general procedure E to afford the title compound **(15e)** as a white solid (0.12 g, 97%), mp 195-197 °C. ^{1}H NMR (400 MHz, MeOD) δ 8.47 (dt, *J* = 6.9, 1.1 Hz, 1H), 8.07 – 7.95 (m, 3H), 7.52 (td, *J* = 6.7, 1.4 Hz, 1H), 6.87 (s, 2H), 4.18 (t, *J* = 5.1, 2H), 3.38 (t, *J* = 5.0, 2H). ^{13}C NMR (101 MHz, MeOD) δ 144.91, 143.93, 140.06, 135.18, 131.21, 126.57, 126.17, 118.66, 118.58, 115.50, 113.92, 70.17, 40.97. UPLC/MS: t_r 0.24 min, *m/z* 337.0 $[M+H]^+$, purity: 99%. HRMS: mass calculated for $C_{15}H_{15}N_4OCl_2$: 337.0623; found: 337.0623.

***N*-(4-(aminomethyl)phenyl)imidazo[1,2-*a*]pyridin-3-amine (15f).** Boc deprotection of compound **14f** was done using the general procedure E to afford the title compound **(15f)** as a colorless oil (0.26 g, 95%). ^{1}H NMR (400 MHz, DMSO) δ 8.73 (s, 1H), 8.39 (dt, *J* = 6.8, 1.0 Hz, 1H), 8.24 – 8.10 (m, 4H), 8.05 (dt, *J* = 9.1, 1.0 Hz, 1H), 7.97 (ddd, *J* = 9.0, 6.9, 1.1 Hz, 1H), 7.49 (td, *J* = 6.9, 1.1 Hz, 1H), 7.30 (d, *J* = 8.6 Hz, 2H), 6.82 (d, *J* = 8.6 Hz, 2H), 3.93 (q, *J* = 5.5 Hz, 2H). ^{13}C NMR (101 MHz, DMSO) δ 144.75, 137.91, 133.10, 130.49, 125.47, 125.20, 124.99, 116.90, 116.47, 114.08, 113.10, 41.93. UPLC/MS: t_r 0.22 min, *m/z* 239.1 $[M+H]^+$, purity: 97%. HRMS: mass calculated for $C_{14}H_{15}N_4$: 239.1297; found: 239.1292.

***N*-((1*R*,4*R*)-4-(aminomethyl)cyclohexyl)imidazo[1,2-*a*]pyridin-3-amine (15g).** Boc deprotection of compound **14g** was done using the general procedure E to afford the title compound **(15g)** as an orange oil (0.10 g, 98%). ^{1}H NMR (400 MHz, DMSO) δ 8.60 (d, *J* = 6.9 Hz, 1H), 8.04 – 7.82 (m, 5H), 7.81 – 7.74 (m, 1H), 7.52 (s, 1H), 7.43 (t, *J* = 6.8 Hz, 1H), 3.22 –

3.03 (m, 1H), 2.80 – 2.59 (m, 2H), 2.22 – 2.02 (m, 2H), 1.92 – 1.72 (m, 2H), 1.66 – 1.50 (m, 1H), 1.35 – 1.19 (m, 2H), 1.15 – 0.97 (m, 2H). ^{13}C NMR (101 MHz, DMSO) δ 135.49, 133.05, 130.92, 124.43, 115.75, 112.27, 102.65, 53.49, 44.14, 35.17, 31.49, 28.42. UPLC/MS: t_r 0.25 min, *m/z* 245.0 $[M+H]^+$, 489.3 $[2M+H]^+$, purity: 98%. HRMS: mass calculated for $C_{14}H_{21}N_4$: 245.1766; found: 245.1775.

1,2-di-Boc-3-(4-(((2-(pyridin-3-yl)imidazo[1,2-*a*]pyridin-3-yl)amino)methyl)phenyl)guanidine (16a). The title compound was prepared according to the general procedure D from pyridin-2-amine, nicotinaldehyde, isocyanide **13a** (0.67 g, 72%). ^{1}H NMR (400 MHz, DMSO) δ 11.40 (s, 1H), 9.96 (s, 1H), 9.30 (d, *J* = 1.4 Hz, 1H), 8.48 (dd, *J* = 4.7, 1.4 Hz, 1H), 8.40 (d, *J* = 8.0 Hz, 1H), 8.24 (d, *J* = 6.8 Hz, 1H), 7.54 – 7.38 (m, 4H), 7.28 (d, *J* = 8.3 Hz, 2H), 7.19 (t, *J* = 8.1 Hz, 1H), 6.87 (t, *J* = 6.5 Hz, 1H), 5.45 (t, *J* = 6.2 Hz, 1H), 4.09 (d, *J* = 6.1 Hz, 2H), 1.50 (s, 9H), 1.40 (s, 9H). ^{13}C NMR (101 MHz, DMSO) δ 152.80, 147.71, 147.62, 140.94, 136.23, 135.62, 133.47, 131.87, 130.33, 128.49, 127.21, 124.37, 123.56, 123.37, 122.49, 116.91, 111.64, 83.38, 78.83, 50.76, 27.87, 27.69. UPLC/MS: t_r 1.84 min, *m/z* 558.5 $[M+H]^+$.

1,2-di-Boc-3-(4-(((2-(4-ethylphenyl)imidazo[1,2-*a*]pyridin-3-yl)amino)methyl)phenyl)guanidine (16b). The title compound was prepared according to the general procedure D from pyridin-2-amine, 4-ethylbenzaldehyde, isocyanide **13a** (0.50 g, 82%). ^{1}H NMR (400 MHz, DMSO) δ 11.41 (s, 1H), 9.97 (s, 1H), 8.20 (d, *J* = 6.9 Hz, 1H), 8.06 (d, *J* = 8.2 Hz, 2H), 7.55 – 7.37 (m, 3H), 7.38 – 7.21 (m, 4H), 7.18 – 7.09 (m, 1H), 6.82 (td, *J* = 6.8, 1.0 Hz, 1H), 5.27 (t, *J* = 6.2 Hz, 1H), 4.06 (d, *J* = 6.2 Hz, 2H), 2.64 (q, *J* = 7.6 Hz, 2H), 1.51 (s, 9H), 1.40 (s, 9H), 1.22 (t, *J* = 7.6 Hz, 3H). ^{13}C NMR (101 MHz, DMSO) δ 162.69, 152.83, 142.41, 140.38, 136.47, 135.57, 134.53, 132.04, 128.48, 127.75, 126.58, 126.13, 123.70, 123.10, 122.51,

116.65, 111.24, 83.42, 78.82, 50.73, 27.98, 27.92, 27.67, 15.55. UPLC/MS: t_r 2.12 min, *m/z* 585.6 $[M+H]^+$.

1-(4-(((2-benzylimidazo[1,2-*a*]pyridin-3-yl)amino)methyl)phenyl)-2,3-di-Boc-guanidine (16c). The title compound was prepared according to the general procedure D from pyridin-2-amine, 2-phenylacetaldehyde, isocyanide **13a** (0.40 g, 60%). ^{1}H NMR (400 MHz, DMSO) δ 11.41 (s, 1H), 9.97 (s, 1H), 8.14 (d, *J* = 6.8 Hz, 1H), 7.45 (d, *J* = 8.4 Hz, 2H), 7.38 – 7.18 (m, 7H), 7.17 – 7.04 (m, 2H), 6.81 (td, *J* = 6.7, 1.1 Hz, 1H), 5.13 (t, *J* = 6.4 Hz, 1H), 3.99 (d, *J* = 6.5 Hz, 2H), 3.90 (s, 2H), 1.51 (s, 9H), 1.40 (s, 9H). ^{13}C NMR (101 MHz, DMSO) δ 152.82, 140.63, 140.09, 136.35, 135.54, 128.79, 128.55, 128.03, 126.45, 125.62, 122.85, 122.64, 122.47, 116.40, 110.94, 83.39, 78.80, 51.12, 32.70, 27.90, 27.66. UPLC/MS: t_r 1.94 min, *m/z* 571.5 $[M+H]^+$.

1,2-di-Boc-3-(4-(((2-ethylimidazo[1,2-*a*]pyridin-3-yl)amino)methyl)phenyl)guanidine (16d). The title compound was prepared according to the general procedure D from pyridin-2-amine, propionaldehyde, isocyanide **13a** (0.30 g, 49%). ^{1}H NMR (400 MHz, DMSO) δ 11.40 (s, 1H), 9.97 (s, 1H), 8.12 (d, *J* = 6.8 Hz, 1H), 7.46 (d, *J* = 8.2 Hz, 2H), 7.38 – 7.24 (m, 3H), 7.13 – 7.00 (m, 1H), 6.79 (t, *J* = 6.7 Hz, 1H), 5.03 (t, *J* = 6.4 Hz, 1H), 4.03 (d, *J* = 6.2 Hz, 2H), 2.54 (q, *J* = 7.6 Hz, 2H), 1.50 (s, 9H), 1.39 (s, 9H), 1.11 (t, *J* = 7.6 Hz, 3H). UPLC/MS: t_r 1.96 min, *m/z* 509.5 $[M+H]^+$.

1,2-di-Boc-3-(4-(((2-((Boc-amino)methyl)imidazo[1,2-*a*]pyridin-3-yl)amino)methyl)phenyl)guanidine (16e). The title compound was prepared according to the general procedure D from pyridin-2-amine, 2-(Boc-amino)acetaldehyde, isocyanide **13a** (0.21 g, 32%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.62 (s, 1H), 10.34 (s, 1H), 8.00 (d, *J* = 6.8 Hz, 1H), 7.57 (d, *J* = 8.5 Hz, 2H), 7.50 (d, *J* = 9.0 Hz, 1H), 7.25 – 7.14 (m, 3H), 6.82 (t, *J* = 6.7 Hz, 1H), 5.43 –

5.22 (m, 1H), 4.54 – 4.33(m, 1H), 4.17 (d, J = 6.1 Hz, 2H), 4.10 (d, J = 6.5 Hz, 2H), 1.54 (s, 9H), 1.50 (s, 9H), 1.39 (s, 9H). UPLC/MS: t_r 2.02 min, *m/z* 610.7 $[M+H]^+$.

1-(4-(((2-(3-chlorobenzyl)imidazo[1,2-*a*]pyridin-3-yl)amino)methyl)phenyl)-2,3-di-Boc-guanidine (16f). The title compound was prepared according to the general procedure D from pyridin-2-amine, 2-(3-chlorophenyl)acetaldehyde, isocyanide **13a** (0.14 g, 22%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.61 (s, 1H), 10.33 (s, 1H), 8.09 (d, J = 7.0 Hz, 1H), 7.63 – 7.50 (m, 3H), 7.37 – 7.30 (m, 1H), 7.23 – 7.06 (m, 7H), 6.96 (t, J = 6.7 Hz, 1H), 4.04 – 3.91 (m, 4H), 3.31 (s, 1H), 1.54 (s, 9H), 1.49 (s, 9H). UPLC/MS: t_r 2.15 min, *m/z* 605.7 $[M+H]^+$.

1-(4-(((2-(1-Boc-piperidin-4-yl)imidazo[1,2-*a*]pyridin-3-yl)amino)methyl)phenyl)-2,3-di-Boc-guanidine (16g). The title compound was prepared according to the general procedure D from pyridin-2-amine, 1-Boc-piperidine-4-carbaldehyde, isocyanide **13a** (0.35 g, 72%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.62 (s, 1H), 10.34 (s, 1H), 8.05 (d, J = 6.4 Hz, 1H), 7.82 – 7.66 (m, 1H), 7.63 – 7.55 (m, 2H), 7.29 – 7.20 (m, 3H), 6.97 – 6.82 (m, 1H), 4.10 (d, J = 6.4 Hz, 2H), 3.42 – 3.18 (m, 1H), 2.93 – 2.62 (m, 2H), 2.02 – 1.82 (m, 2H), 1.76 – 1.65 (m, 2H), 1.61 – 1.41 (m, 29H). UPLC/MS: t_r 2.16 min, *m/z* 664.8 $[M+H]^+$.

1-(4-(((2-(3,5-dichlorophenyl)imidazo[1,2-*a*]pyridin-3-yl)amino)methyl)phenyl)-2,3-di-Boc-guanidine (16h). The title compound was prepared according to the general procedure D from pyridin-2-amine, 3,5-dichlorobenzaldehyde, isocyanide **13a** (0.42 g, 86%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.61 (s, 1H), 10.32 (s, 1H), 8.12 – 8.02 (m, 1H), 7.96 (d, J = 1.9 Hz, 2H), 7.66 – 7.59 (m, 1H), 7.55 – 7.48 (m, 2H), 7.30 – 7.18 (m, 4H), 6.87 (t, J = 6.5 Hz, 1H), 4.15 – 4.08 (m, 2H), 1.54 (s, 9H), 1.50 (s, 9H). UPLC/MS: t_r 2.29 min, *m/z* 625.6 $[M+H]^+$.

1,2-di-Boc-3-(4-(((2-phenylimidazo[1,2-*a*]pyridin-3-yl)amino)methyl)phenyl)guanidine (16i). The title compound was prepared according to the general procedure D from pyridin-2-

amine, benzaldehyde, isocyanide **13a** (0.14 g, 34%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.61 (s, 1H), 10.30 (s, 1H), 8.13 (d, *J* = 6.6 Hz, 1H), 8.06 – 8.00 (m, 2H), 7.94 (brs, 1H), 7.86 – 7.73 (m, 1H), 7.55 – 7.41 (m, 4H), 7.40 – 7.32 (m, 2H), 7.20 (d, *J* = 8.2 Hz, 2H), 7.01 – 6.92 (m, 1H), 4.15 (d, *J* = 5.2 Hz, 2H), 1.54 (s, 9H), 1.50 (s, 9H). UPLC/MS: t_r 2.08 min, *m/z* 557.5 $[M+H]^+$.

1-(4-(((2-(3-fluorophenyl)imidazo[1,2-*a*]pyridin-3-yl)amino)methyl)phenyl)-2,3-di-Boc-guanidine (16j). The title compound was prepared according to the general procedure D from pyridin-2-amine, 3-fluorobenzaldehyde, isocyanide **13a** (0.36 g, 75%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.63 (s, 1H), 10.32 (s, 1H), 8.01 (d, *J* = 6.8 Hz, 1H), 7.83 – 7.72 (m, 2H), 7.65 – 7.51(m, 3H), 7.45 – 7.36 (m, 1H), 7.28 (d, *J* = 8.4 Hz, 2H), 7.18 (ddd, *J* = 8.9, 6.7, 1.1 Hz, 1H), 7.02 (tdd, *J* = 8.4, 2.6, 0.9 Hz, 1H), 6.80 (td, *J* = 6.8, 0.9 Hz, 1H), 4.15 (d, *J* = 6.1 Hz, 2H), 3.57 (s, 1H), 1.54 (s, 9H), 1.51 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.63, 163.3 (d, *J* = 242.4 Hz), 153.68, 153.46, 136.54, 135.17, 130.38 (d, *J* = 8.5 Hz), 128.83, 126.13, 122.70 (d, *J* = 6.3 Hz), 122.64 (d, *J* = 6.1 Hz), 122.61, 117.24, 114.67 (d, *J* = 21.5 Hz), 114.02 (d, *J* = 23.1 Hz), 112.62, 83.97, 79.86, 52.01, 28.34, 28.23. UPLC/MS: t_r 1.97 min, *m/z* 575.6 $[M+H]^+$.

1-(4-(((2-(4-fluorophenyl)imidazo[1,2-*a*]pyridin-3-yl)amino)methyl)phenyl)-2,3-di-Boc-guanidine (16k). The title compound was prepared according to the general procedure D from pyridin-2-amine, 4-fluorobenzaldehyde, isocyanide **13a** (0.27 g, 63%). ^{1}H NMR (400 MHz, DMSO) δ 11.41 (s, 1H), 9.96 (s, 1H), 8.25 – 8.12 (m, 3H), 7.50 – 7.40 (m, 3H), 7.34 – 7.22 (m, 4H), 7.16 (ddd, *J* = 9.0, 6.6, 1.2 Hz, 1H), 6.84 (td, *J* = 6.8, 1.1 Hz, 1H), 5.32 (t, *J* = 6.2 Hz, 1H), 4.06 (d, *J* = 6.2 Hz, 2H), 1.51 (s, 9H), 1.40 (s, 9H). ^{13}C NMR (101 MHz, DMSO) δ 161.30 (d, *J* = 243.9 Hz), 152.83, 152.18, 140.46, 136.35, 135.59, 133.61, 131.07 (d, *J* = 3.0 Hz), 128.50, 128.46 (d, *J* = 9.1 Hz), 126.19, 123.98, 123.25, 122.55, 116.72, 115.17 (d, *J* = 21.1 Hz), 111.37, 83.39, 78.80, 50.69, 27.90, 27.66. UPLC/MS: t_r 1.97 min, *m/z* 575.6 $[M+H]^+$.

1-(4-(((2-(pyridin-3-yl)imidazo[1,2-*a*]pyridin-3-yl)amino)methyl)phenyl)guanidine (17a). Boc deprotection of compound **16a** was done using the general procedure E to afford the title compound **(17a)** as a yellow solid (0.30 g, 95%), mp 146 °C. ^{1}H NMR (400 MHz, DMSO) δ 10.01 (s, 1H), 9.07 (d, *J* = 1.8 Hz, 1H), 8.68 (dd, *J* = 5.0, 1.5 Hz, 1H), 8.64 (d, *J* = 6.8 Hz, 1H), 8.39 (d, *J* = 8.1 Hz, 1H), 7.91 – 7.73 (m, 2H), 7.72 – 7.65 (m, 1H), 7.57 (brs, 4H), 7.39 (t, *J* = 6.8 Hz, 1H), 7.29 (d, *J* = 8.4 Hz, 2H), 7.08 (d, *J* = 8.3 Hz, 2H), 4.17 (s, 2H). ^{13}C NMR (101 MHz, DMSO) δ 155.85, 147.87, 146.29, 138.05, 137.09, 136.25, 134.51, 131.44, 129.46, 128.29, 125.03, 124.52, 124.15, 115.65, 113.47, 50.10. UPLC/MS: t_r 0.23 min, *m/z* 358.4 $[M+H]^+$, purity: 99%. HRMS: mass calculated for $C_{20}H_{20}N_7$: 358.1780; found: 358.1778.

1-(4-(((2-(4-ethylphenyl)imidazo[1,2-*a*]pyridin-3-yl)amino)methyl)phenyl)guanidine (17b). Boc deprotection of compound **16b** was done using the general procedure E to afford the title compound **(17b)** as a yellow solid (0.10 g, 98%), mp 165-166 °C. ^{1}H NMR (400 MHz, DMSO) δ 10.01 (s, 1H), 8.68 (d, *J* = 6.8 Hz, 1H), 7.92 – 7.82 (m, 4H), 7.54 (brs, 4H), 7.49 – 7.40 (m, 3H), 7.33 (d, *J* = 8.4 Hz, 2H), 7.12 (d, *J* = 8.4 Hz, 2H), 5.98 (brs, 1H), 4.16 (s, 2H), 2.70 (q, *J* = 7.6 Hz, 2H), 1.24 (t, *J* = 7.6 Hz, 3H). ^{13}C NMR (101 MHz, DMSO) δ 155.85, 145.61, 137.07, 136.54, 134.55, 132.63, 129.37, 128.51, 127.35, 127.11, 125.58, 125.19, 124.50, 124.10, 116.44, 112.13, 49.96, 28.03, 15.39. UPLC/MS: t_r 1.12 min, *m/z* 385.5 $[M+H]^+$, 769.8 $[2M+H]^+$, purity > 99%. HRMS: mass calculated for $C_{23}H_{25}N_6$: 385.2141; found: 385.2139.

1-(4-(((2-benzylimidazo[1,2-*a*]pyridin-3-yl)amino)methyl)phenyl)guanidine (17c). Boc deprotection of compound **16c** was done using the general procedure E to afford the title compound **(17c)** as a white solid (0.31 g, 100%), mp 110 °C. ^{1}H NMR (400 MHz, DMSO) δ 10.15 (s, 1H), 8.64 (d, *J* = 6.8 Hz, 1H), 7.92 – 7.79 (m, 2H), 7.64 (brs, 4H), 7.47 (td, *J* = 6.8, 1.4 Hz, 1H), 7.41 (d, *J* = 8.4 Hz, 2H), 7.37 – 7.12 (m, 7H), 4.18 (s, 2H), 4.07 (s, 2H). ^{13}C NMR (101

MHz, DMSO) δ 155.90, 137.38, 137.15, 136.28, 134.64, 132.44, 129.51, 128.67, 128.61, 128.34, 126.89, 125.71, 125.25, 124.19, 116.48, 111.97, 50.30, 29.11. UPLC/MS: t_r 0.97 min, *m/z* 371.4 $[M+H]^+$, purity > 99%. HRMS: mass calculated for $C_{22}H_{23}N_6$: 371.1984; found: 371.1978.

1-(4-(((2-ethylimidazo[1,2-*a*]pyridin-3-yl)amino)methyl)phenyl)guanidine (17d). Boc deprotection of compound **16d** was done using the general procedure E, affording the title compound **(17d)** as a colorless oil (0.10 g, 98%). ^{1}H NMR (400 MHz, DMSO) δ 10.01 (s, 1H), 8.62 (d, *J* = 6.8 Hz, 1H), 7.90 – 7.82 (m, 2H), 7.54 (brs, 4H), 7.49 – 7.43 (m, 1H), 7.40 (d, *J* = 8.4 Hz, 2H), 7.19 (d, *J* = 8.4 Hz, 2H), 5.78 (brs, 1H), 4.17 (s, 2H), 2.64 (q, *J* = 7.6 Hz, 2H), 1.12 (t, *J* = 7.6 Hz, 3H). ^{13}C NMR (101 MHz, DMSO) δ 155.88, 137.50, 135.97, 134.59, 132.08, 129.56, 128.97, 126.98, 125.01, 124.14, 116.39, 111.84, 50.37, 16.82, 12.93. UPLC/MS: t_r 0.24 min, *m/z* 309.4 $[M+H]^+$, purity: 96%. HRMS: mass calculated for $C_{17}H_{21}N_6$: 309.1828; found: 309.1812.

1-(4-(((2-(aminomethyl)imidazo[1,2-*a*]pyridin-3-yl)amino)methyl)phenyl)guanidine (17e). Boc deprotection of compound **16e** was done using the general procedure E, affording the title compound **(17e)** as an orange oil (0.08 g, 94%). ^{1}H NMR (400 MHz, MeOD) δ 8.18 (dt, *J* = 6.9, 1.2 Hz, 1H), 7.48 (dt, *J* = 9.1, 1.1 Hz, 1H), 7.44 (d, *J* = 8.5 Hz, 2H), 7.31 (ddd, *J* = 9.1, 6.7, 1.3 Hz, 1H), 7.24 (d, *J* = 8.5 Hz, 2H), 6.93 (td, *J* = 6.8, 1.1 Hz, 1H), 4.23 (s, 2H), 4.06 (s, 2H). ^{13}C NMR (101 MHz, DMSO) δ 155.97, 137.57, 137.26, 134.71, 131.03, 130.08, 129.50, 124.85, 124.23, 115.51, 113.54, 50.18, 48.62. UPLC/MS: t_r 0.16 min, *m/z* 310.3 $[M+H]^+$, purity: 97%. HRMS: mass calculated for $C_{16}H_{20}N_7$: 310.1780; found: 310.1775.

1-(4-(((2-(3-chlorobenzyl)imidazo[1,2-*a*]pyridin-3-yl)amino)methyl)phenyl)guanidine (17f). Boc deprotection of compound **16f** was done using the general procedure E, affording the title compound **(17f)** as an orange oil (0.09 g, 97%). ^{1}H NMR (400 MHz, MeOD) δ 8.61 (d, *J* =

6.8 Hz, 1H), 7.93 – 7.83 (m, 1H), 7.74 (d, *J* = 9.0 Hz, 1H), 7.51 – 7.39 (m, 3H), 7.36 – 7.07 (m, 6H), 4.27 (s, 2H), 4.09 (s, 2H). ^{13}C NMR (101 MHz, MeOD) δ 158.01, 140.11, 139.93, 138.63, 135.75, 135.67, 134.05, 131.47, 131.01, 129.90, 129.73, 128.45, 128.17, 128.05, 126.43, 126.27, 117.80, 112.98, 51.99, 30.33. UPLC/MS: t_r 1.24 min, *m/z* 405.4 $[M+H]^+$, purity: 98%. HRMS: mass calculated for $C_{22}H_{22}N_6Cl$: 405.1594; found: 405.1602.

1-(4-(((2-(piperidin-4-yl)imidazo[1,2-*a*]pyridin-3-yl)amino)methyl)phenyl)guanidine (17g). Boc deprotection of compound **16g** was done using the general procedure E, affording the title compound **(17g)** as a white solid (0.25 g, 94%), mp 108 °C. ^{1}H NMR (400 MHz, DMSO) δ 10.28 (s, 1H), 9.13 – 8.94 (m, 1H), 8.80 – 8.63 (m, 1H), 8.60 (d, *J* = 6.8 Hz, 1H), 7.94 – 7.79 (m, 2H), 7.67 (brs, 4H), 7.50 – 7.35 (m, 3H), 7.19 (d, *J* = 8.2 Hz, 2H), 4.18 (s, 2H), 3.46 – 3.29 (m, 2H), 3.17 – 3.02 (m, 1H), 3.00 – 2.84 (m, 2H), 1.99 – 1.79 (m, 2H), 1.75 – 1.59 (m, 2H). ^{13}C NMR (101 MHz, DMSO) δ 155.95, 137.42, 136.72, 134.63, 129.77, 126.92, 125.01, 124.17, 116.22, 112.38, 50.41, 43.03, 29.91, 27.41. UPLC/MS: t_r 0.23 min, *m/z* 364.4 $[M+H]^+$, purity > 99%. HRMS: mass calculated for $C_{20}H_{26}N_7$: 364.2250; found: 364.2242.

1-(4-(((2-(3,5-dichlorophenyl)imidazo[1,2-*a*]pyridin-3-yl)amino)methyl)phenyl)guanidine (17h). Boc deprotection of compound **16h** was done using the general procedure E, affording the title compound **(17h)** as a yellow solid (0.19 g, 100%), mp 151-153 °C. ^{1}H NMR (400 MHz, DMSO) δ 10.03 (s, 1H), 8.58 (d, *J* = 6.8 Hz, 1H), 8.01 (d, *J* = 1.9 Hz, 2H), 7.80 – 7.73 (m, 1H), 7.71 – 7.62 (m, 2H), 7.57 (brs, 4H), 7.35 (d, *J* = 8.4 Hz, 2H), 7.27 (t, *J* = 6.8 Hz, 1H), 7.14 (d, *J* = 8.4 Hz, 2H), 4.16 (s, 2H). ^{13}C NMR (101 MHz, DMSO) δ 155.83, 138.41, 137.07, 134.53, 133.27, 130.19, 129.45, 128.22, 127.68, 125.24, 124.77, 123.99, 114.81, 114.15, 50.25. UPLC/MS: t_r 1.29 min, *m/z* 425.4 $[M+H]^+$, purity > 99%. HRMS: mass calculated for $C_{21}H_{19}N_6Cl_2$: 425.1048; found: 425.1059.

1-(4-(((2-phenylimidazo[1,2-*a*]pyridin-3-yl)amino)methyl)phenyl)guanidine (17i). Boc deprotection of compound **16i** was done using the general procedure E, affording the title compound **(17i)** as an orange oil (0.09 g, 97%). ^{1}H NMR (400 MHz, MeOD) δ 8.69 (dt, *J* = 6.9, 1.0 Hz, 1H), 7.96 – 7.89 (m, 1H), 7.87 – 7.80 (m, 3H), 7.62 – 7.53 (m, 3H), 7.48 (td, *J* = 6.9, 1.1 Hz, 1H), 7.28 (d, *J* = 8.4 Hz, 2H), 7.10 (d, *J* = 8.4 Hz, 2H), 4.24 (s, 2H). ^{13}C NMR (101 MHz, MeOD) δ 157.96, 139.42, 138.31, 135.49, 134.47, 131.17, 130.91, 130.36, 128.93, 128.04, 126.28, 118.01, 112.87, 51.34. UPLC/MS: t_r 1.02 min, *m/z* 357.4 $[M+H]^+$, purity: 96%. HRMS: mass calculated for $C_{21}H_{21}N_6$: 357.1828; found: 357.1810.

1-(4-(((2-(3-fluorophenyl)imidazo[1,2-*a*]pyridin-3-yl)amino)methyl)phenyl)guanidine (17j). Boc deprotection of compound **16j** was done using the general procedure E, affording the title compound **(17j)** as a white solid (0.16 g, 96%), mp 153 °C. ^{1}H NMR (400 MHz, DMSO) δ 10.04 (s, 1H), 8.65 (d, *J* = 6.8 Hz, 1H), 7.91 – 7.76 (m, 4H), 7.65 – 7.59 (m, 1H), 7.57 (brs, 4H), 7.41 (td, *J* = 6.7, 1.3 Hz, 1H), 7.37 – 7.29 (m, 3H), 7.11 (d, *J* = 8.4 Hz, 2H), 6.03 (s, 1H), 4.17 (s, 2H). ^{13}C NMR (101 MHz, DMSO) δ, 162.30 (d, *J* = 243.8 Hz), 155.83, 137.25, 137.05, 134.54, 132.07, 131.13 (d, *J* = 8.5 Hz), 129.39, 127.92, 125.12, 124.09, 123.34 (d, *J* = 2.6 Hz), 116.04 (d, *J* = 20.6 Hz), 113.92 (d, *J* = 23.8 Hz), 112.86, 50.04. UPLC/MS: t_r 0.99 min, *m/z* 375.5 $[M+H]^+$, purity > 99%. HRMS: mass calculated for $C_{21}H_{20}N_6F$: 375.1733; found: 375.1721.

1-(4-(((2-(4-fluorophenyl)imidazo[1,2-*a*]pyridin-3-yl)amino)methyl)phenyl)guanidine (17k). Boc deprotection of compound **16k** was done using the general procedure E, affording the title compound **(17k)** as a light-yellow oil (0.15 g, 95%). ^{1}H NMR (400 MHz, DMSO) δ 10.09 (s, 1H), 8.69 – 8.62 (m, 1H), 8.03 – 7.93 (m, 2H), 7.90 – 7.78 (m, 2H), 7.73 – 7.50 (m, 4H), 7.46 – 7.37 (m, 3H), 7.31 (d, *J* = 8.4 Hz, 2H), 7.11 (d, *J* = 8.4 Hz, 2H), 5.96 (brs, 1H), 4.15 (s, 2H). ^{13}C NMR (101 MHz, DMSO) δ 162.54 (d, *J* = 247.8 Hz), 155.92, 137.06, 136.92, 134.59,

132.19, 129.79 (d, *J* = 8.6 Hz), 129.40, 127.30, 125.32, 125.16, 124.24, 124.09, 116.16, 116.11 (d, *J* = 21.9 Hz), 112.57, 49.98. UPLC/MS: t_r 0.97 min, *m/z* 375.3 $[M+H]^+$, purity: 99%. HRMS: mass calculated for $C_{21}H_{20}N_6F$: 375.1733; found: 375.1751.

1-(4-(2-((2-ethylimidazo[1,2-*a*]pyridin-3-yl)amino)ethyl)phenyl)-2,3-di-Boc-guanidine (18a). The title compound was prepared according to the general procedure D from pyridin-2-amine, 4- propionaldehyde, isocyanide **13b** (0.35 g, 54%). ^{1}H NMR (400 MHz, DMSO) δ 11.43 (s, 1H), 9.94 (s, 1H), 7.96 (d, *J* = 6.8 Hz, 1H), 7.43 (d, *J* = 8.4 Hz, 2H), 7.34 (dt, *J* = 9.0, 1.0 Hz, 1H), 7.20 (d, *J* = 8.5 Hz, 2H), 7.05 (ddd, *J* = 9.0, 6.6, 1.3 Hz, 1H), 6.77 (td, *J* = 6.7, 1.1 Hz, 1H), 4.61 (t, *J* = 6.2 Hz, 1H), 3.16 – 3.07 (m, 2H), 2.76 (t, *J* = 7.5 Hz, 2H), 2.66 (q, *J* = 7.5 Hz, 2H), 1.50 (s, 9H), 1.39 (s, 9H), 1.21 (t, *J* = 7.5 Hz, 3H). ^{13}C NMR (101 MHz, DMSO) δ 152.93, 139.90, 138.73, 136.56, 134.58, 128.86, 125.62, 122.85, 122.52, 122.19, 116.24, 110.73, 83.36, 49.75, 36.02, 27.87, 27.67, 19.88, 14.22. UPLC/MS: t_r 2.03 min, *m/z* 523.6 $[M+H]^+$.

1-(4-(2-((2-(3-chlorophenyl)imidazo[1,2-*a*]pyrazin-3-yl)amino)ethyl)phenyl)-2,3-di-Boc-guanidine (18b). The title compound was prepared according to the general procedure D from pyrazin-2-amine, 3-chlorobenzaldehyde, isocyanide **13b** (0.18 g, 49%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.62 (s, 1H), 8.85 (s, 1H), 8.16 – 8.07 (m, 1H), 7.96 – 7.90 (m, 1H), 7.79 – 7.69 (m, 2H), 7.46 – 7.34 (m, 4H), 7.10 (d, *J* = 8.3 Hz, 2H), 4.31 (brs, 1H), 3.42 – 3.32 (m, 2H), 2.83 (t, *J* = 6.4 Hz, 2H), 1.55 (s, 9H), 1.49 (s, 9H). UPLC/MS: t_r 1.44 min, *m/z* 606.5 $[M+H]^+$.

1-(4-(2-((2-ethylimidazo[1,2-*a*]pyridin-3-yl)amino)ethyl)phenyl)guanidine (19a). Boc deprotection of compound **18a** was done using the general procedure E, affording the title compound **(19a)** as a colorless oil (0.24 g, 98%). ^{1}H NMR (400 MHz, DMSO) δ 10.01 (s, 1H), 8.47 (d, *J* = 6.8 Hz, 1H), 7.90 – 7.80 (m, 2H), 7.53 (brs, 4H), 7.43 (ddd, *J* = 6.8, 5.9, 2.2 Hz, 1H), 7.31 (d, *J* = 8.4 Hz, 2H), 7.15 (d, *J* = 8.4 Hz, 2H), 3.29 – 3.16 (m, 2H), 2.92 – 2.76 (m, 4H), 1.28

(t, J = 7.6 Hz, 3H). ^{13}C NMR (101 MHz, DMSO) δ 156.06, 137.67, 135.91, 133.50, 131.91, 129.95, 128.44, 127.39, 124.90, 124.50, 116.27, 111.82, 48.95, 35.77, 16.93, 13.16. UPLC/MS: t_r 0.28 min, *m/z* 323.4 $[M+H]^+$, purity: 97%. HRMS: mass calculated for $C_{18}H_{23}N_6$: 323.1984; found: 323.1986.

1-(4-(2-((2-(3-chlorophenyl)imidazo[1,2-*a*]pyrazin-3-yl)amino)ethyl)phenyl)guanidine (19b). Boc deprotection of compound **18b** was done using the general procedure E, affording the title compound **(19b)** as a yellow oil (0.12 g, 96%). ^{1}H NMR (400 MHz, DMSO) δ 9.64 (s, 1H), 8.97 (d, J = 1.3 Hz, 1H), 8.31 (dd, J = 4.7, 1.4 Hz, 1H), 8.10 (t, J = 1.7 Hz, 1H), 8.00 (dt, J = 7.7, 1.3 Hz, 1H), 7.87 (d, J = 4.7 Hz, 1H), 7.48 (t, J = 7.8 Hz, 1H), 7.45 – 7.40 (m, 1H), 7.34 (brs, 4H), 7.18 (d, J = 8.4 Hz, 2H), 7.07 (d, J = 8.4 Hz, 2H), 5.51 (s, 1H), 3.25 (t, J = 7.1 Hz, 2H), 2.81 (t, J = 7.4 Hz, 2H). ^{13}C NMR (101 MHz, DMSO) δ 155.97, 140.46, 137.52, 135.38, 135.19, 134.91, 133.48, 133.31, 130.64, 130.54, 129.77, 127.93, 126.54, 125.58, 124.58, 116.87, 48.17, 35.92. UPLC/MS: t_r 1.38 min, *m/z* 406.4 $[M+H]^+$, purity: 99%. HRMS: mass calculated for $C_{21}H_{21}N_7Cl$: 406.1547; found: 406.1548.

2-amino-*N*-butylisonicotinamide (20a). The title compound was prepared according to the TBD-catalysed aminolysis reaction. To a solution of methyl 2-aminopyridine-4-carboxylate (0.3 g, 1.972 mmol) in dry DMF (10 mL) were added butan-1-amine (0.391 mL, 3.94 mmol, 2 equiv) and 1,5,7- triazabicyclo[4.4.0]dec-5-ene (TBD) (54.9 mg, 0.394 mmol, 0.2 equiv), and the reaction mixture was stirred in a pressure tube at 120 °C for 20 h. The crude product was purified by isolera, using normal phase chromatography and by applying gradient from 0-10% of MeOH in EtOAc, to afford a white solid (0.20 g, 52.5%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.10 (dd, J = 5.3, 0.5 Hz, 1H), 6.93 – 6.89 (s, 1H), 6.84 (dd, J = 5.4, 1.4 Hz, 1H), 6.20 (brs, 1H), 3.48 – 3.38 (m, 2H), 1.64 – 1.54 (m, 2H), 1.46 – 1.34 (m, 2H), 0.95 (t, J = 7.3 Hz, 3H). ^{13}C NMR (101 MHz,

$CDCl_3$) δ 165.97, 158.91, 148.07, 144.47, 110.69, 107.11, 40.02, 31.70, 20.27, 13.89. UPLC/MS: t_r 0.31 min, *m/z* 194.3 $[M+H]^+$.

6-amino-*N*-butylnicotinamide (20b). The title compound was prepared according to the TBD-catalysed aminolysis reaction. To a solution of methyl 6-aminonicotinate (0.3 g, 1.972 mmol) in dry DMF (9 mL) were added butan-1-amine (0.391 mL, 3.94 mmol, 2 equiv) and 1,5,7-triazabicyclo[4.4.0]dec-5-ene (TBD) (54.9 mg, 0.394 mmol, 0.2 equiv), and the reaction mixture was stirred in a pressure tube at 120 °C for 24 h. The crude product was purified by isolera, using normal phase chromatography and by applying gradient from 10-100% of EtOAc in heptane to 0-15% of MeOH in EtOAc, to afford a white solid (0.16 g, 42%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.45 (d, *J* = 2.3 Hz, 1H), 7.88 (dd, *J* = 8.6, 2.3 Hz, 1H), 6.50 (dd, *J* = 8.6, 0.7 Hz, 1H), 5.94 (brs, 1H), 3.48 – 3.39 (m, 2H), 1.63 – 1.53 (m, 2H), 1.47 – 1.35 (m, 2H), 0.95 (t, *J* = 7.3 Hz, 3H). UPLC/MS: t_r 0.25 min, *m/z* 194.2 $[M+H]^+$.

1-(4-(((8-methyl-2-(pyridin-3-yl)imidazo[1,2-*a*]pyridin-3-yl)amino)methyl)phenyl)-2,3-di-Boc-guanidine (21a). The title compound was prepared according to the general procedure D from 3-methylpyridin-2-amine, nicotinaldehyde, isocyanide **13a** (0.09 g, 25%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.62 (s, 1H), 10.30 (s, 1H), 9.22 (d, *J* = 2.0 Hz, 1H), 8.53 (dd, *J* = 4.8, 1.4 Hz, 1H), 8.32 (d, *J* = 7.9 Hz, 1H), 7.90 (d, *J* = 6.8 Hz, 1H), 7.52 (d, *J* = 8.4 Hz, 2H), 7.37 (ddd, *J* = 7.9, 4.8, 0.8 Hz, 1H), 7.23 (d, *J* = 8.3 Hz, 2H), 6.98 (d, *J* = 6.8 Hz, 1H), 6.73 (t, *J* = 6.8 Hz, 1H), 4.12 (s, 2H), 2.63 (s, 3H), 1.53 (s, 9H), 1.50 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 153.61, 147.97, 147.91, 142.03, 136.47, 135.30, 135.09, 130.15, 129.42, 128.85, 127.37, 126.70, 124.29, 123.84, 122.52, 120.62, 112.76, 83.93, 79.83, 52.07, 28.24, 16.81. UPLC/MS: t_r 1.95 min, *m/z* 572.6 $[M+H]^+$.

methyl 3-((4-(2,3-di-Boc-guanidino)benzyl)amino)-2-(pyridin-3-yl)imidazo[1,2-*a*]pyridine-7-carboxylate (21b). The title compound was prepared according to the general procedure D from methyl 2-aminoisonicotinate, nicotinaldehyde, isocyanide **13a** (0.40 g, 49%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.61 (brs, 1H), 9.12 (d, *J* = 1.6 Hz, 1H), 8.52 (dd, *J* = 4.9, 1.5 Hz, 1H), 8.32 (dt, *J* = 8.0, 1.9 Hz, 1H), 8.28 (dd, *J* = 1.5, 0.9 Hz, 1H), 8.03 (dd, *J* = 7.2, 0.9 Hz, 1H), 7.48 (d, *J* = 8.5 Hz, 2H), 7.45 – 7.36 (m, 2H), 7.22 (d, *J* = 8.5 Hz, 2H), 4.16 (d, *J* = 5.9 Hz, 2H), 3.96 (s, 3H), 1.59 – 1.45 (m, 18H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.90, 154.42, 153.86, 148.25, 147.68, 140.89, 136.44, 135.97, 135.28, 135.19, 130.26, 128.96, 127.90, 125.90, 124.09, 122.86, 122.16, 120.54, 111.62, 84.08, 80.14, 52.70, 50.96, 28.24. UPLC/MS: t_r 2.08 min, *m/z* 616.6 $[M+H]^+$.

3-((4-(2,3-di-Boc-guanidino)benzyl)amino)-2-(pyridin-3-yl)imidazo[1,2-*a*]pyridine-7-carboxylic acid (21c). The title compound was prepared by base hydrolysis of an ester function in compound **21b**. To a solution of compound **21b** (0.10 g, 0.194 mmol) in a 9:1 mixture of DCM (1.8 mL) and MeOH (0.2 mL), was added a 2 M solution of sodium hydroxide (0.145 mL, 0.291 mmol, 1.5 equiv) and the reaction was left stirring for 12 h at rt. The crude product was purified by isolera, using reversed phase chromatography and by applying gradient of 10-100% of MeOH in water, to afford a yellow solid (0.08 g, 69%). ^{1}H NMR (400 MHz, MeOD) δ 9.03 (d, *J* = 1.7 Hz, 1H), 8.57 (dd, *J* = 5.0, 1.1 Hz, 1H), 8.52 – 8.46 (m, 1H), 8.37 (dd, *J* = 7.2, 0.9 Hz, 1H), 8.21 (dd, *J* = 1.4, 1.0 Hz, 1H), 7.68 (dd, *J* = 7.9, 5.0 Hz, 1H), 7.53 (dd, *J* = 7.2, 1.5 Hz, 1H), 7.33 (d, *J* = 8.5 Hz, 2H), 7.10 (d, *J* = 8.5 Hz, 2H), 4.19 (s, 2H), 1.57 – 1.45 (m, 18H). UPLC/MS: t_r 1.89 min, *m/z* 602.7 $[M+H]^+$.

***N*-butyl-3-((4-(2,3-di-Boc-guanidino)benzyl)amino)-2-(pyridin-3-yl)imidazo[1,2-*a*]pyridine-7-carboxamide (21d).** The title compound was prepared according to the general

procedure D from aminopyridine **20a**, nicotinaldehyde, isocyanide **13a** to afford a light-yellow solid (0.41 g, 60%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.61 (s, 1H), 10.29 (s, 1H), 9.26 – 9.19 (m, 1H), 8.56 (dd, *J* = 4.9, 1.6 Hz, 1H), 8.36 – 8.29 (m, 1H), 8.05 (dd, *J* = 7.1, 0.8 Hz, 1H), 7.98 – 7.94 (m, 1H), 7.48 (d, *J* = 8.5 Hz, 2H), 7.42 (ddd, *J* = 8.0, 4.9, 0.7 Hz, 1H), 7.30 (dd, *J* = 7.1, 1.6 Hz, 1H), 7.16 (d, *J* = 8.5 Hz, 2H), 6.67 (t, *J* = 5.5 Hz, 1H), 4.16 – 4.06 (m, 2H), 3.98 – 3.83 (m, 1H), 3.52 – 3.43 (m, 2H), 1.69 – 1.58 (m, 2H), 1.54 (s, 9H), 1.50 (s, 9H), 1.46 – 1.36 (m, 2H), 0.97 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.57, 153.76, 148.17, 147.50, 140.49, 136.59, 135.21, 134.80, 129.70, 128.88, 127.49, 124.15, 122.77, 122.74, 115.47, 111.56, 84.05, 79.85, 52.00, 40.20, 31.77, 28.24, 20.33, 13.94. UPLC/MS: t_r 2.02 min, *m/z* 657.7 $[M+H]^+$.

1,2-di-Boc-3-(4-(((6-methyl-2-(pyridin-3-yl)imidazo[1,2-*a*]pyridin-3-yl)amino)methyl)phenyl)guanidine (21e). The title compound was prepared according to the general procedure D from 5-methylpyridin-2-amine, nicotinaldehyde, isocyanide **13a** to afford a yellow solid (0.17 g, 46%). UPLC/MS: t_r 1.98 min, *m/z* 572.6 $[M+H]^+$.

1,2-di-Boc-3-(4-(((2-(pyridin-3-yl)-6-(trifluoromethyl)imidazo[1,2-*a*]pyridin-3-yl)amino)methyl)phenyl)guanidine (21f). The title compound was prepared according to the general procedure D from 5-(trifluoromethyl)pyridin-2-amine, nicotinaldehyde, isocyanide **13a** to afford a yellow solid (0.13 g, 37%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.61 (s, 1H), 10.30 (brs, 1H), 9.26 (s, 1H), 8.57 (d, *J* = 4.0 Hz, 1H), 8.38 – 8.28 (m, 2H), 7.62 (d, *J* = 9.4 Hz, 1H), 7.53 – 7.47 (m, 2H), 7.47 – 7.41 (m, 1H), 7.29 (dd, *J* = 9.4, 1.8 Hz, 1H), 7.21 – 7.14 (m, 2H), 4.20 – 4.13 (m, 2H), 3.77 (t, *J* = 5.3 Hz, 1H), 1.53 (s, 9H), 1.50 (s, 9H). UPLC/MS: t_r 2.18 min, *m/z* 626.7 $[M+H]^+$.

1-Boc-3-(4-(((6-fluoro-2-(pyridin-3-yl)imidazo[1,2-*a*]pyridin-3-yl)amino)methyl)phenyl)guanidine (21g). The title compound was prepared according to the

general procedure D from 5-fluoropyridin-2-amine, nicotinaldehyde, isocyanide **13a** and using 2,2,2-trifluoroethanol (8 mL) as a solvent, to afford a yellow solid (0.25 g, 65%). ^{1}H NMR (400 MHz, MeOD) δ 9.06 (dd, *J* = 2.2, 0.8 Hz, 1H), 8.46 (dd, *J* = 4.9, 1.6 Hz, 1H), 8.32 (ddd, *J* = 8.0, 2.2, 1.7 Hz, 1H), 8.21 (ddd, *J* = 4.2, 2.4, 0.7 Hz, 1H), 7.54 – 7.45 (m, 2H), 7.31 –7.23 (m, 1H), 7.14 (d, *J* = 8.5 Hz, 2H), 7.04 (d, *J* = 8.5 Hz, 2H), 4.13 (s, 2H), 1.47 (s, 9H). ^{13}C NMR (101 MHz, MeOD) δ 153.73, 148.64 (d, *J* = 3.1 Hz), 140.94, 137.64, 136.39, 135.16, 131.77, 130.68, 129.86, 125.04 (d, *J* = 32.7 Hz),124.87, 118.64, 118.42 (d, *J* = 8.9 Hz), 111.04 (d, *J* = 42.1 Hz), 79.98, 52.05, 28.64. UPLC/MS: t_r 1.28 min, *m/z* 476.5 $[M+H]^+$.

***N*-butyl-3-((4-(2,3-di-Boc-guanidino)benzyl)amino)-2-(pyridin-3-yl)imidazo[1,2-*a*]pyridine-6-carboxamide (21h).** The title compound was prepared according to the general procedure D from aminopyridine **20b**, nicotinaldehyde, isocyanide **13a** to afford a yellow solid (0.23 g, 47%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.67 (s, 1H), 9.43 (d, *J* = 1.4 Hz, 1H), 9.24 (s, 1H), 8.55 (dd, *J* = 4.8, 1.5 Hz, 1H), 8.50 – 8.42 (m, 1H), 7.65 (d, *J* = 9.5 Hz, 1H), 7.54 (d, *J* = 9.4 Hz, 1H), 7.41 (dd, *J* = 7.9, 4.8 Hz, 1H), 7.30 (d, *J* = 8.3 Hz, 2H), 7.18 (d, *J* = 8.3 Hz, 2H), 7.04 – 6.88 (m, 1H), 4.59 – 4.40 (m, 1H), 4.08 (d, *J* = 5.6 Hz, 2H), 3.31 – 3.16 (m, 2H), 1.62 – 1.47 (m, 11H), 1.45 (s, 9H), 1.41 – 1.30 (m, 2H), 0.92 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 164.95, 155.02, 153.40, 148.22, 148.00, 141.87, 136.22, 135.73, 134.69, 130.10, 129.13, 128.29, 125.24, 124.69, 123.92, 123.56, 120.76, 116.29, 84.18, 80.26, 52.47, 40.31, 31.58, 28.29, 28.25, 20.42, 13.97. UPLC/MS: t_r 2.00 min, *m/z* 657.7 $[M+H]^+$.

3-((4-(2,3-di-Boc-guanidino)benzyl)amino)-2-(pyridin-3-yl)imidazo[1,2-*a*]pyridine-6-carboxamide (21i). The title compound was prepared according to the general procedure D from 6-aminonicotinamide, nicotinaldehyde, isocyanide **13a**. The crude product was purified by

isolera, using reversed phase chromatography and by applying gradient of 10-100% of MeOH in water, to afford a yellow solid (0.30 g, 68%). UPLC/MS: t_r 1.76 min, *m/z* 601.7 $[M+H]^+$.

1-(4-(((8-methyl-2-(pyridin-3-yl)imidazo[1,2-*a*]pyridin-3-yl)amino)methyl)phenyl)guanidine (22a). Boc deprotection of compound **21a** was done using the general procedure E, affording the title compound **(22a)** as an orange oil (0.04 g, 99%). ^{1}H NMR (400 MHz, MeOD) δ 8.77 (brs, 1H), 8.65 (dd, *J* = 4.9, 1.4 Hz, 1H), 8.57 (d, *J* = 6.8 Hz, 1H), 8.28 – 8.23 (m, 1H), 7.73 (d, *J* = 7.2 Hz, 1H), 7.63 (dd, *J* = 8.0, 5.0 Hz, 1H), 7.40 (t, *J* = 7.0 Hz, 1H), 7.21 (d, *J* = 8.4 Hz, 2H), 7.09 (d, *J* = 8.4 Hz, 2H), 4.24 (s, 2H), 2.67 (s, 3H). ^{13}C NMR (101 MHz, MeOD) δ 158.03, 149.97, 148.98, 139.47, 139.41, 138.74, 135.56, 133.41, 130.91, 130.05, 126.35, 125.61, 124.96, 124.79, 123.87, 117.88, 51.42, 15.99. UPLC/MS: t_r 0.26 min, *m/z* 372.5 $[M+H]^+$, purity: 98%. HRMS: mass calculated for $C_{21}H_{22}N_7$: 372.1937; found: 372.1935.

methyl 3-((4-guanidinobenzyl)amino)-2-(pyridin-3-yl)imidazo[1,2-*a*]pyridine-7-carboxylate (22b). Boc deprotection of compound **21b** was done using the general procedure E, affording the title compound **(22b)** as an orange oil (0.09 g, 94%). ^{1}H NMR (400 MHz, MeOD) δ 9.13 (brs, 1H), 8.73 – 8.59 (m, 2H), 8.39 (dd, *J* = 7.2, 0.7 Hz, 1H), 8.25 – 8.20 (m, 1H), 7.84 – 7.73 (m, 1H), 7.53 (dd, *J* = 7.2, 1.5 Hz, 1H), 7.26 (d, *J* = 8.4 Hz, 2H), 7.10 (d, *J* = 8.4 Hz, 2H), 4.26 (s, 2H), 3.98 (s, 3H). UPLC/MS: t_r 1.09 min, *m/z* 416.5 $[M+H]^+$, purity: 97%. HRMS: mass calculated for $C_{22}H_{22}N_7O_2$: 416.1835; found: 416.1846.

3-((4-guanidinobenzyl)amino)-2-(pyridin-3-yl)imidazo[1,2-*a*]pyridine-7-carboxylic acid (22c). Boc deprotection of compound **21c** was done using the general procedure E, affording the title compound **(22c)** as a yellow solid (0.05 g, 93%). ^{1}H NMR (400 MHz, MeOD) δ 9.12 (brs, 1H), 8.72 – 8.58 (m, 2H), 8.42 (dd, *J* = 7.2, 0.7 Hz, 1H), 8.28 – 8.21 (m, 1H), 7.85 – 7.75 (m,

1H), 7.58 (dd, *J* = 7.2, 1.5 Hz, 1H), 7.26 (d, *J* = 8.4 Hz, 2H), 7.11 (d, *J* = 8.4 Hz, 2H), 4.27 (s, 2H). ^{13}C NMR (101 MHz, MeOD) δ 158.01, 157.23, 146.21, 145.55, 140.02, 139.75, 137.59, 137.31, 135.57, 131.09, 126.35, 124.95, 121.19, 118.91, 114.16, 51.83. UPLC/MS: t_r 0.17 min, *m/z* 402.3 $[M+H]^+$, purity: 98%. HRMS: mass calculated for $C_{21}H_{20}N_7O_2$: 402.1678; found: 402.1673.

***N*-butyl-3-((4-guanidinobenzyl)amino)-2-(pyridin-3-yl)imidazo[1,2-*a*]pyridine-7-carboxamide (22d).** Boc deprotection of compound **21d** was done using the general procedure E, affording the title compound **(22d)** as a light-yellow solid (0.08 g, 100%), mp 148-150 °C. ^{1}H NMR (400 MHz, DMSO) δ 9.84 (s, 1H), 9.26 – 9.17 (m, 1H), 8.75 (t, *J* = 5.5 Hz, 1H), 8.68 (d, *J* = 4.3 Hz, 1H), 8.61 (d, *J* = 8.0 Hz, 1H), 8.45 (d, *J* = 7.1 Hz, 1H), 8.11 (s, 1H), 7.76 (dd, *J* = 8.0, 5.2 Hz, 1H), 7.55 – 7.38 (m, 5H), 7.30 (d, *J* = 8.3 Hz, 2H), 7.09 (d, *J* = 8.3 Hz, 2H), 5.90 (s, 1H), 4.17 (s, 2H), 3.37 – 3.23 (m, 2H), 1.60 – 1.47 (m, 2H), 1.42 – 1.28 (m, 2H), 0.92 (t, *J* = 7.3 Hz, 3H). UPLC/MS: t_r 1.13 min, *m/z* 457.5 $[M+H]^+$, purity > 99%. HRMS: mass calculated for $C_{25}H_{29}N_8O$: 457.2464; found: 457.2446.

1-(4-(((6-methyl-2-(pyridin-3-yl)imidazo[1,2-*a*]pyridin-3-yl)amino)methyl)phenyl)guanidine (22e). Boc deprotection of compound **21e** was done using the general procedure E, affording the title compound **(22e)** as an orange oil (0.07 g, 95%). ^{1}H NMR (400 MHz, MeOD) δ 8.82 (s, 1H), 8.64 (d, *J* = 4.5 Hz, 1H), 8.52 – 8.46 (m, 1H), 8.28 – 8.20 (m, 1H), 7.87 – 7.74 (m, 2H), 7.63 (dd, *J* = 8.0, 5.0 Hz, 1H), 7.25 (d, *J* = 8.5 Hz, 2H), 7.10 (d, *J* = 8.5 Hz, 2H), 4.26 (s, 2H), 2.52 (d, *J* = 0.9 Hz, 3H). ^{13}C NMR (101 MHz, MeOD) δ 158.03, 149.95, 148.35, 139.43, 138.13, 137.99, 137.33, 135.62, 131.05, 129.50, 128.95, 126.33, 125.81, 124.74, 123.98, 112.80, 51.54, 18.15. UPLC/MS: t_r 0.28 min, *m/z* 372.4 $[M+H]^+$, purity: 98%. HRMS: mass calculated for $C_{21}H_{22}N_7$: 372.1937; found: 372.1938.

1-(4-(((2-(pyridin-3-yl)-6-(trifluoromethyl)imidazo[1,2-*a*]pyridin-3-yl)amino)methyl)phenyl)guanidine (22f). Boc deprotection of compound **21f** was done using the general procedure E, affording the title compound **(22f)** as a colorless oil (0.08 g, 95%). ^{1}H NMR (400 MHz, MeOD) δ 9.17 (s, 1H), 8.66 (d, *J* = 8.2 Hz, 1H), 8.64 – 8.56 (m, 2H), 7.74 (dd, *J* = 8.0, 5.2 Hz, 1H), 7.69 (d, *J* = 9.5 Hz, 1H), 7.52 (dd, *J* = 9.5, 1.7 Hz, 1H), 7.26 (d, *J* = 8.4 Hz, 2H), 7.10 (d, *J* = 8.4 Hz, 2H), 4.24 (s, 2H). UPLC/MS: t_r 1.24 min, *m/z* 426.5 $[M+H]^+$, purity > 99%.

1-(4-(((6-fluoro-2-(pyridin-3-yl)imidazo[1,2-*a*]pyridin-3-yl)amino)methyl)phenyl)guanidine (22g). Boc deprotection of compound **21g** was done using the general procedure E, affording the title compound **(22g)** as a yellow solid (0.17 g, 96%), mp 141 °C. ^{1}H NMR (400 MHz, MeOD) δ 9.03 (d, *J* = 1.7 Hz, 1H), 8.70 (dd, *J* = 5.3, 1.4 Hz, 1H), 8.68 – 8.64 (m, 1H), 8.60 – 8.54 (m, 1H), 7.88 – 7.72 (m, 3H), 7.26 (d, *J* = 8.4 Hz, 2H), 7.10 (d, *J* = 8.4 Hz, 2H), 4.25 (s, 2H). ^{13}C NMR (101 MHz, MeOD) δ 157.96, 157.35, 154.95, 146.61, 145.43, 140.42, 139.40, 138.42, 135.65, 131.16, 131.01 (d, *J* = 2.3 Hz), 129.26, 128.06, 126.84, 126.27, 123.85 (d, *J* = 26.4 Hz), 116.36 (d, *J* = 8.7 Hz), 113.06 (d, *J* = 42.6 Hz), 51.58. UPLC/MS: t_r 0.26 min, *m/z* 376.3 $[M+H]^+$, purity > 99%. HRMS: mass calculated for $C_{20}H_{19}N_7F$: 376.1686; found: 376.1685.

***N*-butyl-3-((4-guanidinobenzyl)amino)-2-(pyridin-3-yl)imidazo[1,2-*a*]pyridine-6-carboxamide (22h).** Boc deprotection of compound **21h** was done using the general procedure E, affording the title compound **(22h)** as a yellow oil (0.13 g, 96%). ^{1}H NMR (400 MHz, DMSO) δ 9.91 (s, 1H), 9.21 (d, *J* = 1.7 Hz, 1H), 8.82 (s, 1H), 8.75 (t, *J* = 5.5 Hz, 1H), 8.65 (dd, *J* = 5.0, 1.3 Hz, 1H), 8.58 (d, *J* = 8.0 Hz, 1H), 7.85 (d, *J* = 9.4 Hz, 1H), 7.75 – 7.66 (m, 2H), 7.53 (brs, 4H), 7.31 (d, *J* = 8.3 Hz, 2H), 7.10 (d, *J* = 8.3 Hz, 2H), 4.18 (s, 2H), 3.36 – 3.25 (m, 2H),

1.59 – 1.49 (m, 2H), 1.42 – 1.30 (m, 2H), 0.92 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (101 MHz, DMSO) δ 163.53, 155.85, 145.69, 144.78, 140.28, 137.58, 136.99, 134.43, 129.55, 128.91, 125.42, 124.94, 124.19, 120.83, 115.08, 50.71, 40.15, 31.22, 19.69, 13.76. UPLC/MS: t_r 1.12 min, *m/z* 457.5 $[M+H]^+$, purity > 99%. HRMS: mass calculated for $C_{25}H_{29}N_8O$: 457.2464; found: 457.2477.

3-((4-guanidinobenzyl)amino)-2-(pyridin-3-yl)imidazo[1,2-*a*]pyridine-6-carboxamide (22i). Boc deprotection of compound **21i** was done using the general procedure E, affording the title compound **(22i)** as an orange oil (0.11 g, 100%). ^{1}H NMR (400 MHz, MeOD) δ 9.17 (brs, 1H), 8.95 (dd, *J* = 1.6, 1.0 Hz, 1H), 8.77 – 8.70 (m, 2H), 8.10 (dd, *J* = 9.4, 1.7 Hz, 1H), 7.96 – 7.88 (m, 1H), 7.79 (dd, *J* = 9.4, 0.9 Hz, 1H), 7.28 (d, *J* = 8.4 Hz, 2H), 7.11 (d, *J* = 8.4 Hz, 2H), 4.28 (s, 2H). ^{13}C NMR (101 MHz, MeOD) δ 167.86, 157.93, 145.44, 144.35, 141.52, 141.47, 139.61, 135.72, 131.22, 131.16, 130.22, 130.01, 127.65, 127.58, 127.35, 126.34, 123.81, 114.99, 52.23. UPLC/MS: t_r 0.17 min, *m/z* 401.4 $[M+H]^+$, purity: 98%. HRMS: mass calculated for $C_{21}H_{21}N_8O$: 401.1838; found: 401.1838.

2-amino-*N*-cyclopropylisonicotinamide (23a). The title compound was prepared according to the general procedure C using cyclopropanamine to afford a white solid (0.51 g, 89%). ^{1}H NMR (400 MHz, MeOD) δ 7.96 (dd, *J* = 5.4, 0.8 Hz, 1H), 6.87 (dd, *J* = 1.5, 0.8 Hz, 1H), 6.84 (dd, *J* = 5.4, 1.5 Hz, 1H), 2.87 – 2.79 (m, 1H), 0.84 – 0.77 (m, 2H), 0.66 – 0.59 (m, 2H). ^{13}C NMR (101 MHz, MeOD) δ 170.31, 161.43, 148.75, 145.18, 110.95, 108.14, 23.97, 6.48. UPLC/MS: t_r 0.18 min, *m/z* 178.2 $[M+H]^+$.

2-amino-*N*-(4-fluorobenzyl)isonicotinamide (23b). The title compound was prepared according to the general procedure C using (4-fluorophenyl)methanamine to afford a white solid (0.70 g, 87%). ^{1}H NMR (400 MHz, DMSO) δ 9.08 (brs, 1H), 8.00 (d, *J* = 5.1 Hz, 1H), 7.39 –

7.28 (m, 2H), 7.21 – 7.09 (m, 2H), 6.89 – 6.80 (m, 2H), 6.14 (s, 2H), 4.42 (s, 2H). ^{13}C NMR (101 MHz, DMSO) δ 165.61, 161.18 (d, *J* = 242.1 Hz), 160.31, 148.35, 142.70, 135.59 (d, *J* = 3.0 Hz), 129.21 (d, *J* = 8.1 Hz), 115.01 (d, *J* = 21.3 Hz), 109.04, 106.08, 41.86. UPLC/MS: t_r 1.09 min, *m/z* 246.3 $[M+H]^+$.

2-amino-*N*-cyclopentylisonicotinamide (23c). The title compound was prepared according to the general procedure C using cyclopentanamine to afford a white solid (0.61 g, 90%). ^{1}H NMR (400 MHz, MeOD) δ 7.96 (d, *J* = 5.0, 1H), 6.89 – 6.83 (m, 2H), 4.28 (p, *J* = 6.9 Hz, 1H), 2.08 – 1.95 (m, 2H), 1.83 – 1.71 (m, 2H), 1.69 – 1.50 (m, 4H). ^{13}C NMR (101 MHz, MeOD) δ 168.67, 161.36, 148.66, 145.75, 111.14, 108.23, 53.04, 33.32, 24.93. UPLC/MS: t_r 0.18 min, *m/z* 206.3 $[M+H]^+$.

2-amino-*N*-(2-hydroxyethyl)isonicotinamide (23d). The title compound was prepared according to the general procedure C using 2-aminoethan-1-ol to afford a white solid (0.49 g, 86%). ^{1}H NMR (400 MHz, DMSO) δ 8.41 (t, *J* = 5.5 Hz, 1H), 7.97 (d, *J* = 5.2 Hz, 1H), 6.84 – 6.79 (m, 2H), 6.10 (s, 2H), 4.72 (t, *J* = 5.6 Hz, 1H), 3.49 (q, *J* = 6.0 Hz, 2H), 3.29 (q, *J* = 6.1 Hz, 2H). ^{13}C NMR (101 MHz, DMSO) δ 165.65, 160.27, 148.22, 142.95, 109.10, 106.07, 59.59, 42.09. UPLC/MS: t_r 0.17 min, *m/z* 182.3 $[M+H]^+$.

2-amino-*N*-(2-(4-methylpiperazin-1-yl)ethyl)isonicotinamide (23e). The title compound was prepared according to the general procedure C using 2-(4-methylpiperazin-1-yl)ethan-1-amine to afford a white solid (0.45 g, 97%). ^{1}H NMR (400 MHz, MeOD) δ 7.98 (dd, *J* = 5.4, 0.7 Hz, 1H), 6.90 (dd, *J* = 1.5, 0.7 Hz, 1H), 6.87 (dd, *J* = 5.4, 1.5 Hz, 1H), 3.51 (t, *J* = 6.8 Hz, 2H), 2.60 (t, *J* = 6.8 Hz, 2H), 2.57 – 2.34 (m, 8H), 2.28 (s, 3H). ^{13}C NMR (101 MHz, MeOD) δ 168.70, 161.47, 148.81, 145.28, 110.89, 108.14, 57.90, 55.65, 53.67, 45.98, 37.95. UPLC/MS: t_r 0.16 min, *m/z* 264.3 $[M+H]^+$.

***N*-butyl-3-((4-(2,3-di-Boc-guanidino)benzyl)amino)imidazo[1,2-*a*]pyridine-7-carboxamide (24a).** The title compound was prepared according to the general procedure D from aminopyridine **20a**, glyoxylic acid monohydrate, isocyanide **13a** to afford a light-yellow solid (0.24 g, 42%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.61 (s, 1H), 10.27 (brs, 1H), 8.29 (d, *J* = 6.7 Hz, 1H), 7.98 (s, 1H), 7.58 (brs, 1H), 7.51 (d, *J* = 6.8 Hz, 1H), 7.37 (d, *J* = 8.3 Hz, 2H), 7.21 (d, *J* = 8.3 Hz, 2H), 6.88 (s, 1H), 5.09 (brs, 1H), 4.12 (s, 2H), 3.52 – 3.34 (m, 2H), 1.68 – 1.59 (m, 2H), 1.54 (s, 9H), 1.46 – 1.32 (m, 11H), 0.92 (t, *J* = 7.3 Hz, 3H). UPLC/MS: t_r 1.94 min, *m/z* 580.6 $[M+H]^+$.

***N*-cyclopropyl-3-((4-(2,3-di-Boc-guanidino)benzyl)amino)imidazo[1,2-*a*]pyridine-7-carboxamide (24b).** The title compound was prepared according to the general procedure D from aminopyridine **23a**, glyoxylic acid monohydrate, isocyanide **13a** to afford a light-yellow solid (0.30 g, 48%). UPLC/MS: t_r 1.85 min, *m/z* 564.6 $[M+H]^+$.

3-((4-(2,3-di-Boc-guanidino)benzyl)amino)-*N*-(4-fluorobenzyl)imidazo[1,2-*a*]pyridine-7-carboxamide (24c). The title compound was prepared according to the general procedure D from aminopyridine **23b**, glyoxylic acid monohydrate, isocyanide **13a** to afford a light-yellow solid (0.32 g, 50%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.61 (s, 1H), 10.28 (s, 1H), 8.09 – 7.98 (m, 2H), 7.50 (d, *J* = 8.4 Hz, 2H), 7.43 – 7.30 (m, 5H), 7.03 – 6.95 (m, 2H), 6.91 (s, 1H), 4.57 (d, *J* = 5.6 Hz, 2H), 4.23 (s, 2H), 1.54 (s, 9H), 1.39 (s, 9H). UPLC/MS: t_r 1.97 min, *m/z* 632.6 $[M+H]^+$.

***N*-cyclopentyl-3-((4-(2,3-di-Boc-guanidino)benzyl)amino)imidazo[1,2-*a*]pyridine-7-carboxamide (24d).** The title compound was prepared according to the general procedure D from aminopyridine **23c**, glyoxylic acid monohydrate, isocyanide **13a** to afford a light-yellow solid (0.26 g, 43%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.96 (d, *J* = 7.2 Hz, 1H), 7.84 (brs, 1H), 7.53 (d, *J* = 8.5 Hz, 2H), 7.29 (d, *J* = 8.5 Hz, 2H), 7.23 (dd, *J* = 7.2, 1.4 Hz, 1H), 7.04 (s, 1H), 4.42 –

4.30 (m, 1H), 4.22 (s, 2H), 2.14 – 1.98 (m, 2H), 1.81 – 1.60 (m, 4H), 1.58 – 1.49 (m, 11H), 1.46 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.68, 163.44, 153.56, 139.62, 136.15, 135.05, 132.75, 129.24, 128.55, 122.83, 122.72, 121.81, 115.79, 110.64, 83.99, 79.88, 51.99, 50.36, 33.17, 33.14, 28.28, 28.20, 24.00. UPLC/MS: t_r 1.91 min, *m/z* 592.6 $[M+H]^+$.

3-((4-(2,3-di-Boc-guanidino)benzyl)amino)-*N*-(2-hydroxyethyl)imidazo[1,2-*a*]pyridine-7-carboxamide (24e). The title compound was prepared according to the general procedure D from aminopyridine **23d**, glyoxylic acid monohydrate, isocyanide **13a** to afford a light-yellow solid (0.38 g, 61%). UPLC/MS: t_r 1.74 min, *m/z* 568.6 $[M+H]^+$.

3-((4-(2,3-di-Boc-guanidino)benzyl)amino)-*N*-(2-(4-methylpiperazin-1-yl)ethyl)imidazo[1,2-*a*]pyridine-7-carboxamide (24f). The title compound was prepared according to the general procedure D from aminopyridine **23e**, glyoxylic acid monohydrate, isocyanide **13a** to afford a light-yellow solid (0.40 g, 58%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.63 (s, 1H), 10.33 (s, 1H), 7.98 (d, *J* = 5.6 Hz, 1H), 7.89 (brs, 1H), 7.58 (d, *J* = 8.4 Hz, 2H), 7.36 (d, *J* = 8.4 Hz, 2H), 7.23 – 7.16 (m, 1H), 7.10 (s, 1H), 4.32 (d, *J* = 4.0 Hz, 2H), 3.67 – 3.50 (m, 2H), 2.96 – 2.48 (m, 10H), 2.33 (s, 3H), 1.53 (s, 9H), 1.49 (s, 9H). UPLC/MS: t_r 1.58 min, *m/z* 650.9 $[M+H]^+$.

***N*-butyl-3-((4-guanidinobenzyl)amino)imidazo[1,2-*a*]pyridine-7-carboxamide (25a).** Boc deprotection of compound **24a** was done using the general procedure E, affording the title compound **(25a)** as a yellow oil (0.08 mg, 100%). ^{1}H NMR (400 MHz, MeOD) δ 8.61 (dd, *J* = 7.2, 0.7 Hz, 1H), 8.24 – 8.20 (m, 1H), 7.77 (dd, *J* = 7.2, 1.6 Hz, 1H), 7.58 (d, *J* = 8.5 Hz, 2H), 7.32 – 7.27 (m, 3H), 4.52 (s, 2H), 3.43 (t, *J* = 7.2 Hz, 2H), 1.69 – 1.58 (m, 2H), 1.50 – 1.37 (m, 2H), 0.98 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (101 MHz, MeOD) δ 165.84, 158.08, 138.72, 137.64, 136.82, 136.31, 135.56, 130.33, 126.66, 125.32, 115.16, 112.36, 105.46, 49.35, 41.16, 32.40,

21.17, 14.10. UPLC/MS: t_r 0.62 min, *m/z* 380.4 $[M+H]^+$, purity > 99%. HRMS: mass calculated for $C_{20}H_{26}N_7O$: 380.2199; found: 380.2214.

***N*-cyclopropyl-3-((4-guanidinobenzyl)amino)imidazo[1,2-*a*]pyridine-7-carboxamide (25b).** Boc deprotection of compound **24b** was done using the general procedure E, affording the title compound **(25b)** as a yellow solid (0.11 mg, 96%), mp 80 °C. ^{1}H NMR (400 MHz, MeOD) δ 8.61 (d, *J* = 7.3 Hz, 1H), 8.25 – 8.18 (m, 1H), 7.76 (dd, *J* = 7.3, 1.6 Hz, 1H), 7.58 (d, *J* = 8.5 Hz , 2H), 7.34 – 7.25 (m, 3H), 4.52 (s, 2H), 2.96 – 2.87 (m, 1H), 0.89 – 0.82 (m, 2H), 0.73 – 0.66 (m, 2H). ^{13}C NMR (101 MHz, MeOD) δ 167.35, 158.09, 138.70, 137.38, 136.71, 136.37, 135.59, 130.33, 126.67, 125.31, 115.23, 112.34, 105.28, 49.34, 24.38, 6.53. UPLC/MS: t_r 0.26 min, *m/z* 364.3 $[M+H]^+$, purity > 99%. HRMS: mass calculated for $C_{19}H_{22}N_7O$: 364.1886; found: 364.1879.

***N*-(4-fluorobenzyl)-3-((4-guanidinobenzyl)amino)imidazo[1,2-*a*]pyridine-7-carboxamide (25c).** Boc deprotection of compound **24c** was done using the general procedure E, affording the title compound **(25c)** as a yellow oil (0.08 mg, 97%). ^{1}H NMR (400 MHz, MeOD) δ 8.62 (dd, *J* = 7.3, 0.8 Hz, 1H), 8.30 – 8.21 (m, 1H), 7.80 (dd, *J* = 7.3, 1.6 Hz, 1H), 7.58 (d, *J* = 8.4 Hz, 2H), 7.45 – 7.36 (m, 2H), 7.35 – 7.24 (m, 3H), 7.12 – 7.00 (m, 2H), 4.59 (s, 2H), 4.52 (s, 2H). ^{13}C NMR (101 MHz, MeOD) δ 165.78, 163.58 (d, *J* = 244.2 Hz), 158.09, 138.70, 137.42, 136.73, 136.40, 135.62 (d, *J* = 3.4 Hz), 135.59, 130.79 (d, *J* = 8.2 Hz), 130.33, 126.68, 125.40, 116.23 (d, *J* = 21.7 Hz), 115.23, 112.49, 105.31, 49.34, 44.24. UPLC/MS: t_r 1.75 min, *m/z* 432.4 $[M+H]^+$, purity > 99%. HRMS: mass calculated for $C_{23}H_{23}N_7OF$: 432.1948; found: 432.1956.

***N*-cyclopentyl-3-((4-guanidinobenzyl)amino)imidazo[1,2-*a*]pyridine-7-carboxamide (25d).** Boc deprotection of compound **24d** was done using the general procedure E, affording the title compound **(25d)** as a yellow oil (0.09 mg, 100%). ^{1}H NMR (400 MHz, MeOD) δ 8.61 (dd, *J* =

7.2, 0.9 Hz, 1H), 8.22 (dd, *J* = 1.6, 1.0 Hz, 1H), 7.79 (dd, *J* = 7.2, 1.6 Hz, 1H), 7.59 (d, *J* = 8.5 Hz, 2H), 7.33 – 7.26 (m, 3H), 4.52 (s, 2H), 4.40 – 4.28 (m, 1H), 2.13 – 2.00 (m, 2H), 1.88 – 1.74 (m, 2H), 1.72 – 1.57 (m, 4H). ^{13}C NMR (101 MHz, MeOD) δ 165.69, 158.09, 138.73, 137.93, 136.76, 136.33, 135.59, 130.34, 126.68, 125.26, 115.43, 112.34, 105.22, 53.56, 49.36, 33.32, 24.96. UPLC/MS: t_r 0.60 min, *m/z* 392.6 $[M+H]^+$, purity > 99%). HRMS: mass calculated for $C_{21}H_{26}N_7O$: 392.2199; found: 392.2180.

3-((4-guanidinobenzyl)amino)-*N*-(2-hydroxyethyl)imidazo[1,2-*a*]pyridine-7-carboxamide (25e). Boc deprotection of compound **24e** was done following the standard procedure for deprotection of Boc groups using HCl/Dioxane. A solution of HCl/Dioxane (4M, 2 mL) was cooled by an ice-water bath under nitrogen gas. Compound **24e** (65 mg, 0.115 mmol) was added in one portion to this solution, and the reaction mixture was stirred for 2 h at rt. After that time, volatiles were evaporated and the obtained product was washed with diethyl ether (2 x 5 mL) yielding HCl-salt of the title compound **25e** as a yellow solid (0.05 g, 98%). ^{1}H NMR (400 MHz, MeOD) δ 8.67 (dd, *J* = 7.3, 0.8 Hz, 1H), 8.27 (dd, *J* = 1.5, 0.9 Hz, 1H), 7.81 (dd, *J* = 7.2, 1.6 Hz, 1H), 7.61 (d, *J* = 8.5 Hz, 2H), 7.33 (s, 1H), 7.30 (d, *J* = 8.5 Hz, 2H), 4.53 (s, 2H), 3.75 (t, *J* = 5.7 Hz, 2H), 3.56 (t, *J* = 5.7 Hz, 2H). ^{13}C NMR (101 MHz, DMSO) δ 163.21, 156.09, 136.46, 134.71, 134.44, 134.35, 128.94, 124.47, 124.23, 113.67, 111.30, 102.78, 59.41, 47.11, 42.71. UPLC/MS: t_r 0.25 min, *m/z* 368.4 $[M+H]^+$, purity: 96%. HRMS: mass calculated for $C_{18}H_{22}N_7O_2$: 368.1835; found: 368.1823.

3-((4-guanidinobenzyl)amino)-*N*-(2-(4-methylpiperazin-1-yl)ethyl)imidazo[1,2-*a*]pyridine-7-carboxamide (25f). Boc deprotection of compound **24f** was done using the general procedure E, affording the title compound **(25f)** as an orange oil (0.06, 97%). ^{1}H NMR (400 MHz, MeOD) δ 8.63 (dd, *J* = 7.3, 0.9 Hz, 1H), 8.29 – 8.25 (m, 1H), 7.80 (dd, *J* = 7.3, 1.6 Hz,

1H), 7.59 (d, J = 8.5 Hz, 2H), 7.32 (s, 1H), 7.30 (d, J = 8.5 Hz, 2H), 4.52 (s, 2H), 3.73 (t, J = 5.9 Hz, 2H), 3.54 –3.26 (m, 8H), 3.12 (t, J = 5.9 Hz, 2H), 2.92 (s, 3H). ^{13}C NMR (101 MHz, MeOD) δ 166.40, 158.08, 138.69, 137.06, 136.67, 136.42, 135.60, 130.33, 126.68, 125.30, 115.27, 112.64, 105.18, 57.27, 53.15, 50.70, 49.31, 43.46, 37.11. UPLC/MS: t_r 0.25 min, *m/z* 450.6 $[M+H]^+$, purity: 97%.

Biochemistry

Enzymatic assays were performed with use of BioTek Microplate Reader (Synergy MX). Data collection and analysis were performed using Gen5 Microplate Software and Microsoft Excel.

The synthesized imidazo[1,2-*a*]pyridine derivatives **14h, 15a-g, 17a-k, 19a-b, 22a-i, 25a-f** were evaluated for their inhibitory activity against uPA. Human enzyme, the urokinase plasminogen activator (uPA) was obtained from HYPHEN BioMed. Inhibitor kinetic assays were using urokinase chromogenic substrate BIOPHEN CS-61(44) (pyro-Glu-Gly-Arg-pNA, K_M= 80 μM) purchased from HYPHEN BioMed. The IC_{50} values were determined using a spectrophotometric assay. All the experiments were conducted in duplicate in a 50 mM HEPES buffer (Sigma-Aldrich) at pH 8.2 as described in our previous work.[14] The readout consisted of the evaluation of uPA-mediated para-nitroaniline release from the chromogenic substrate pyro-Glu-Gly-Arg-pNA at 100 μM concentration.[25] Enzymatic activity was measured during 5 min at 37 °C. Absorbance was monitored at λ=405 nM. All compounds were initially screened at three concentrations (100 μM, 10 μM and 1 μM) in order to estimate the range of the IC_{50} value. Those which were able to reduce uPA activity by at least 50% at 100 μM concentration were submitted to an exact IC_{50} determination. Final IC_{50} values of the most potent inhibitors were the

average of three independent experimental results. Additionally, control experiments using commercial inhibitors were included, and they involved: guanidinophenyl fragment (**26**), uPA Inhibitor II: UK122 (**7**, Santa Cruz Biotechnology), gabexate mesylate (**9**, Enzo Life Sciences), amiloride (**10**, Selleckchem).[10,26,27,28]

Determination of the selectivity for uPA

The inhibitor kinetic assays and determination of the IC_{50} values for thrombin, tPA, FXa, plasmin, plasma kallikrein, trypsin and FVIIa were performed in the same manner as for uPA, using HEPES buffer at pH 8.2 for thrombin, tPA, and FXa, at pH 7.0 for plasmin, at pH 7.4 for plasma kallikrein, trypsin, and HEPES buffer at pH 7.5 (50 mM HEPES, 100 mM NaCl, 5 mM $CaCl_2$, 0.1% BSA) for FVIIa. In case of the latter one, enzymatic assay was performed in the presence of tissue factor Innovin (Dade Behring). For each of the enzymes a specific chromogenic substrate was used. The substrates were obtained from HYPHEN BioMed or Sigma-Aldrich. In case of thrombin (from human plasma, Sigma), the activated protein C chromogenic substrate Biophen CS-21(66) (pyroGlu-Pro-Arg-pNA-HCl, K_m = 400 μM) was used at 415 μM concentration in assay. In case of tPA (recombinant human tPA, HYPHEN BioMed), the tPA and broad spectrum chromogenic substrate Biophen CS-05(88) (H-D-Ile-Pro-L-Arg-pNA-2HCl, K_m = 1000 μM) was used at 1000 μM concentration in assay. As for Factor Xa (purified human Factor Xa, HYPHEN BioMed), the Factor Xa chromogenic substrate Biophen CS-11(32) (Suc-Ile-Glu(γPip)-Gly-Arg-pNa-HCl) was used at 411 μM concentration in assay. In case of plasmin (from human plasma, Sigma), the activated protein C chromogenic substrate, Biophen CS-21(66) (pyroGlu-Pro-Arg-pNA-HCl, K_m = 400 μM) was used at 400 μM concentration in assay. In case of plasma kallikrein (from human plasma, Sigma), the kallikrein

chromogenic substrate, Biophen CS-31(02) (D-Pro-Phe-Arg-pNA-2HCl, K_m = 269 μM) was used at 269 μM concentration in assay. In case of trypsin (from bovine pancreas, Sigma), the trypsin chromogenic substrate, BAPNA (*N*α-benzoyl-D,L-Arg-pNA-HCl, K_m = 1 mM) was used at 425 μM concentration in assay. As for FVIIa (from human plasma, purified, Enzo), the Factor VIIa chromogenic substrate ($MeSO_2$-Cha-Abu-Arg-pNA) was used at 800 μM concentration in assay.

Selectivity assays included the guanidinophenyl fragment (**26**), as well as previously reported uPA inhibitors UK-122 (**7**), gabexate (**9**), amiloride (**10**) as positive controls.[10,26,27,28] Selectivity data are summarized in **Table 7**.

Molecular modeling

Proposed binding conformations of compounds **15a, 15b** and **25a-f** in the binding pocket of uPA were generated starting from the protein crystal structure of human uPA in complex with 1-phenylguanidine (PDB-code 2O8W[29,30]). Visualization of the crystal structure was done with **PyMol**[31] and compounds **15a** and **15b** where docked in the active site using **AutoDock 4.2**[32] with standard parameters and after manually removing the 1-phenylguanidine ligand from its crystal structure. Compounds **25a-f** were generated from the docked structure of compound **15a** by manually building the *N*-substituted amide fragment onto the imidazopyridine ring system of compound **15a** using optimal bond lengths, bond angles and torsion angles.

ASSOCIATED CONTENT

Supporting Information is available that contains detailed synthetic procedures and analytical data for the synthesized compounds together with extended inhibitory screening data. This material is available free of charge via the Internet at http://pubs.acs.org.

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ABBREVIATIONS

uPA, urokinase plasminogen activator; tPA, tissue-type plasminogen activator; FXa, Factor Xa; PAI's, plasminogen activator inhibitors; uPAR, urokinase plasminogen activator receptor; ECM, extracellular matrix; MMPs, matrix metallopreoteases; SAS, substrate activity screening; MSAS, modified substrate activity screening; GBB, Groebke-Blackburn-Bienaymé reaction; 3CC, three-component coupling reaction; PDE3; phosphodiesterase 3; CXCR4, CXC chemokine receptor 4.

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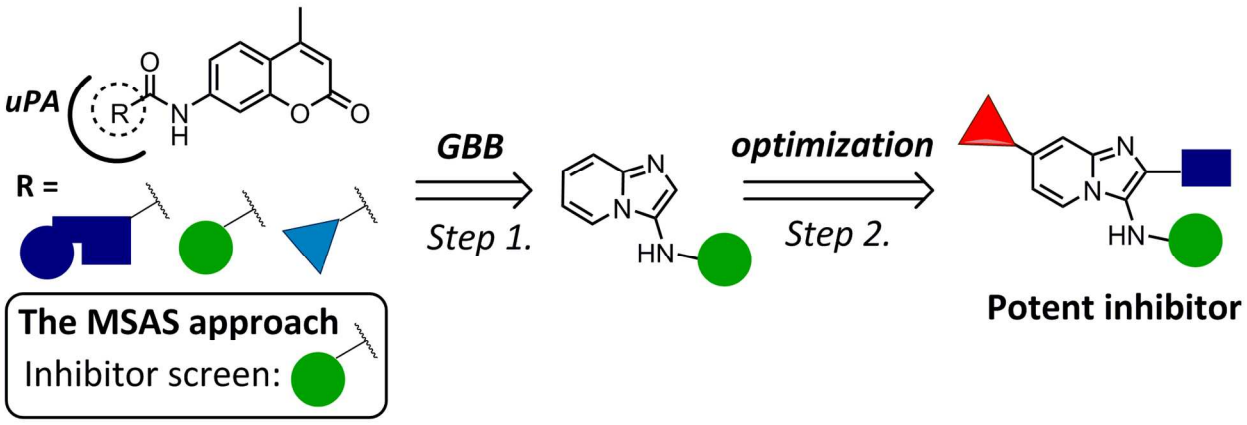
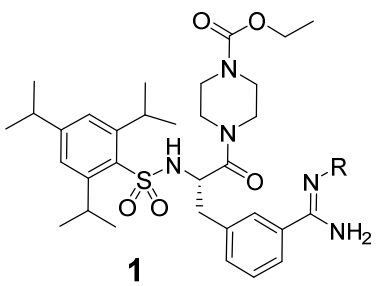
Table of Contents Graphic



FIGURES:

Figure 1. Reported inhibitors of uPA.



1

1a: R = H, WX-UK1
1b: R = OH, WX-671
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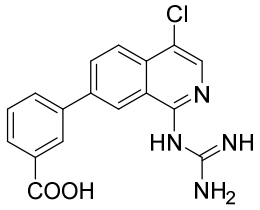

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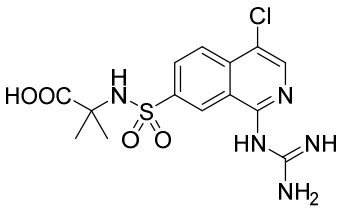

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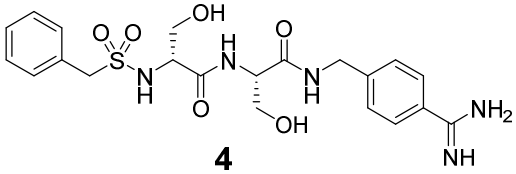

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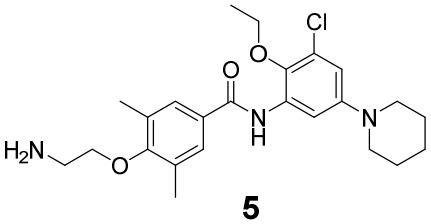

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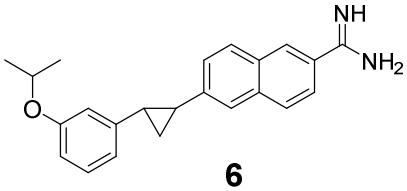

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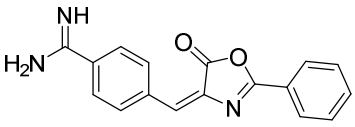
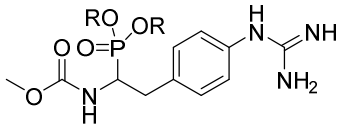


7

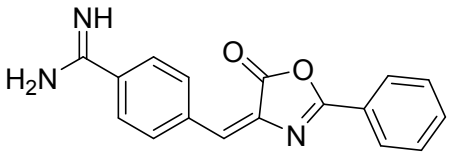
UK122



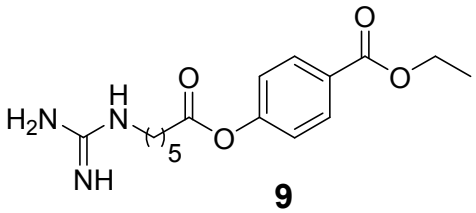
8

8a: R = Ph, UAMC-00150
8b: R = 4-acetylaminophenyl, UAMC-00251

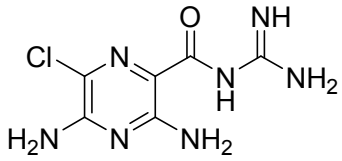
Figure 2. Inhibitory activities of the reference compounds against uPA determined under the assay conditions of this manuscript.



7
UK122
IC_{50} = 8.67 ± 1.12 μM



9
Gabexate
IC_{50} = 0.431 ± 0.017 μM



10
Amiloride
IC_{50} = 11.98 ± 0.22 μM

Figure 3. Overview of the strategy followed for the preparation of potent uPA inhibitors with an imidazopyridine scaffold.

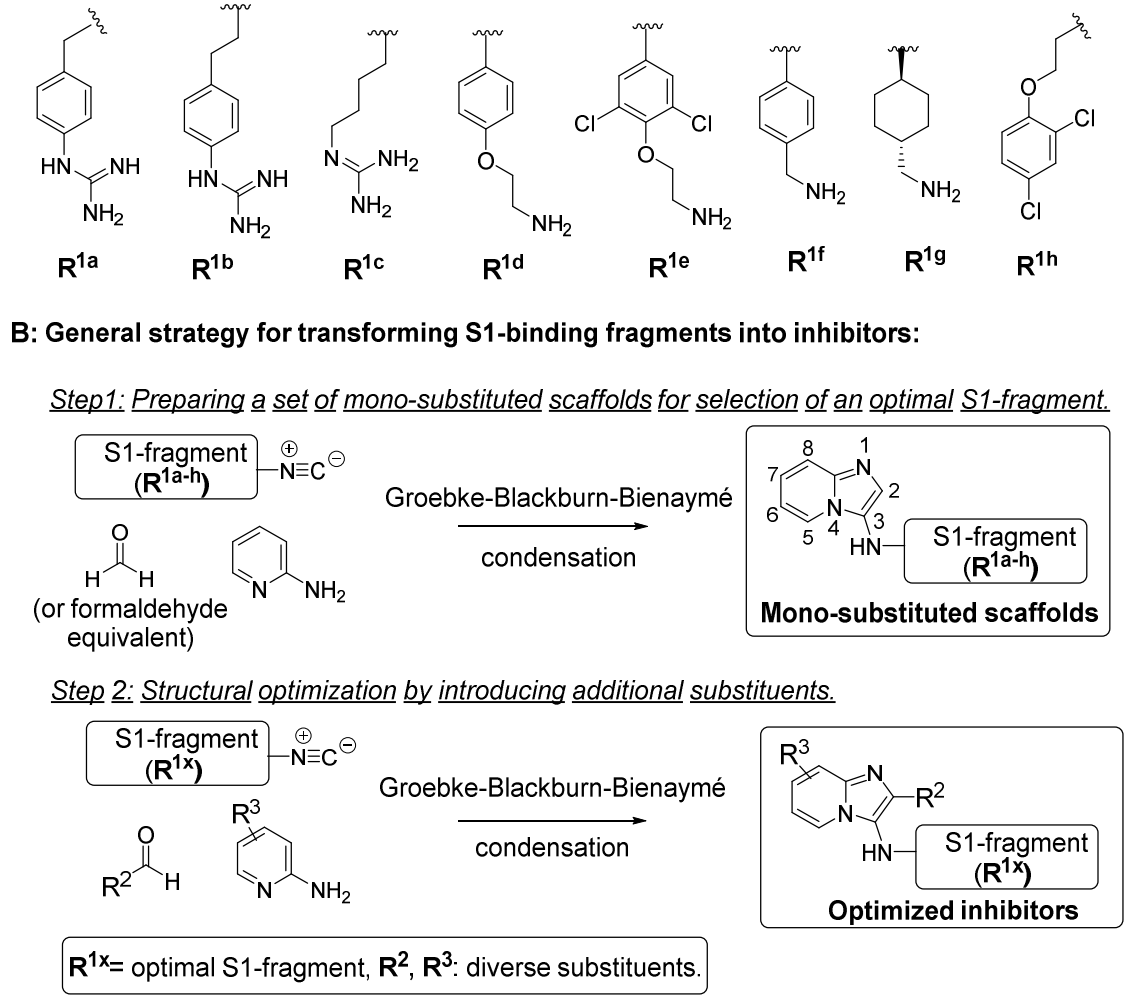


Figure 4. Predicted binding mode of compounds **15a** (light brown) and **15b** (yellow) in the active site of uPA (PDB-code 2O8W). Proposed hydrogen bonds between ligands and labeled protein residues are indicated by yellow dashed lines. Both compounds overlay quite well and show an almost identical binding pattern.

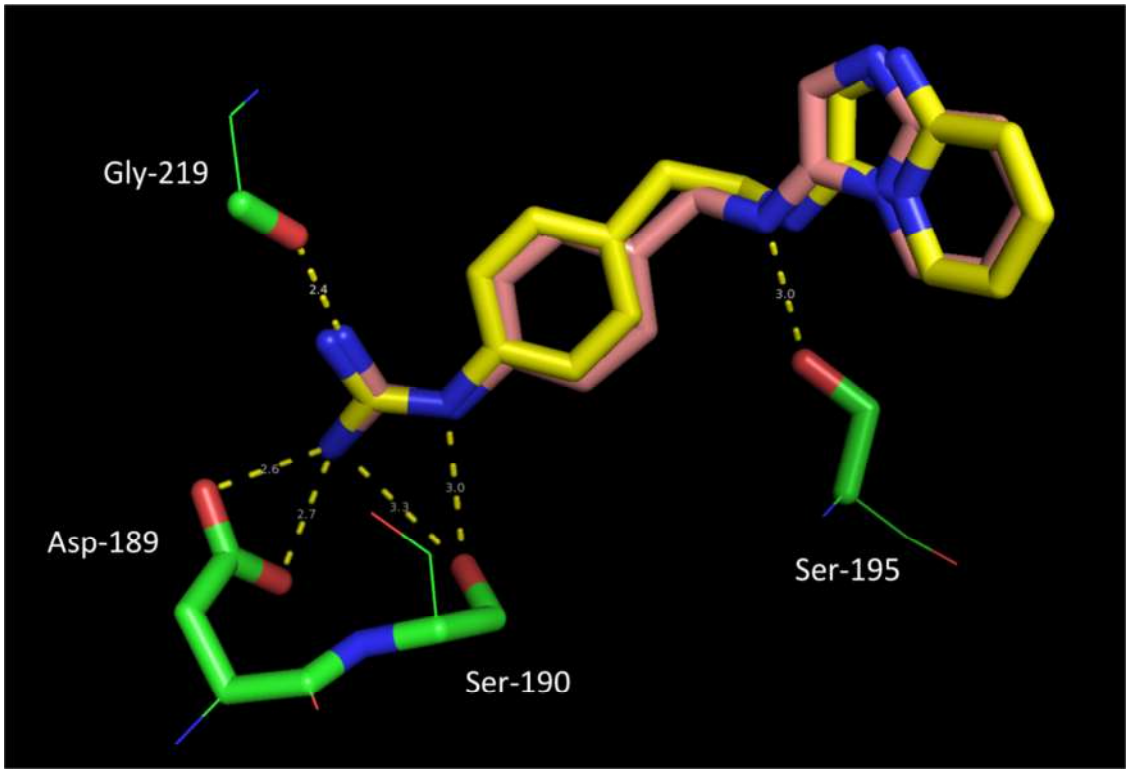
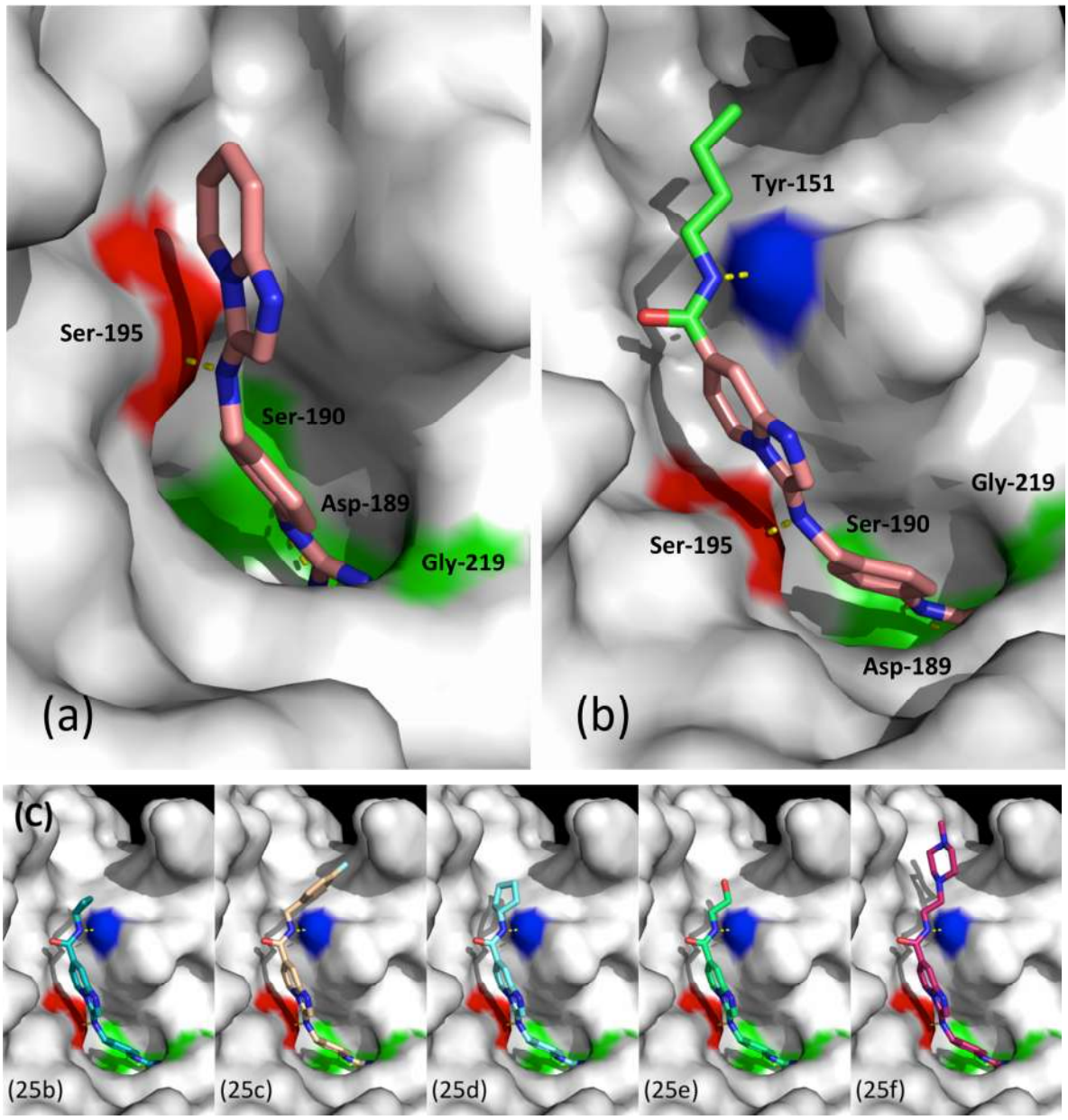


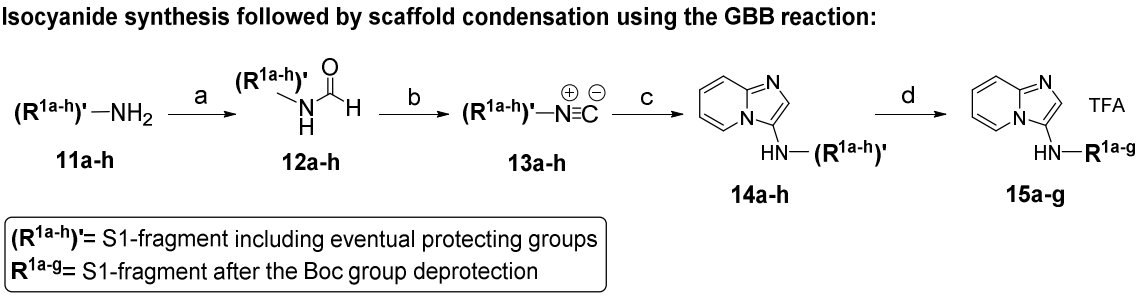
Figure 5. Helicopter view of uPA's active site (PDB-code 2O8W) showing the proposed binding mode of compound **15a** (panel **a**), compound **25a** (panel **b**), compounds **25b-f** (panel **c**). The protein surface is colored white, while the surface generated by residue Ser-195 is colored red and the surface generated by residues Ser-190, Asp-189 and Gly-219 is colored green. Putative hydrogen bonds are shown as dashed yellow lines. Docking of compound **25a** (panel **b**) reveals an additional hydrogen bond between the ligand and the hydroxyl group of

Tyr-151 (colored blue). The R^4 group (**Table 6**) of compound **25a** is colored green and has been docked using idealized geometrical bond and torsion angles.



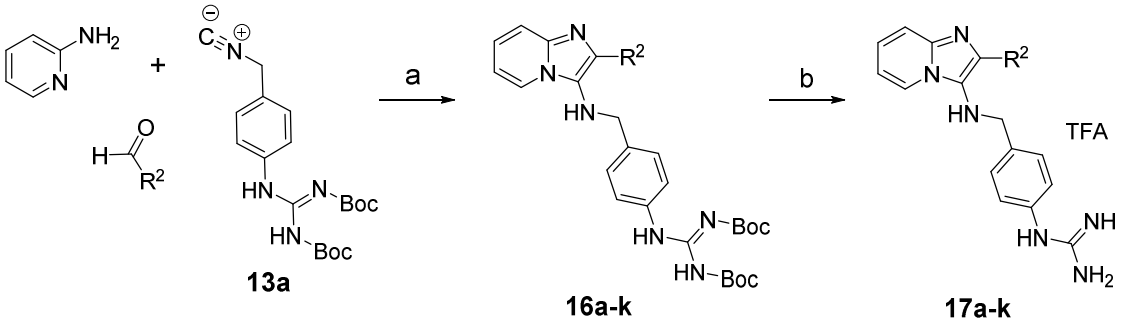
SCHEMES:

Scheme 1. Synthetic steps leading to the mono-substituted scaffold-based inhibitors 14h, 15a-g.[a]



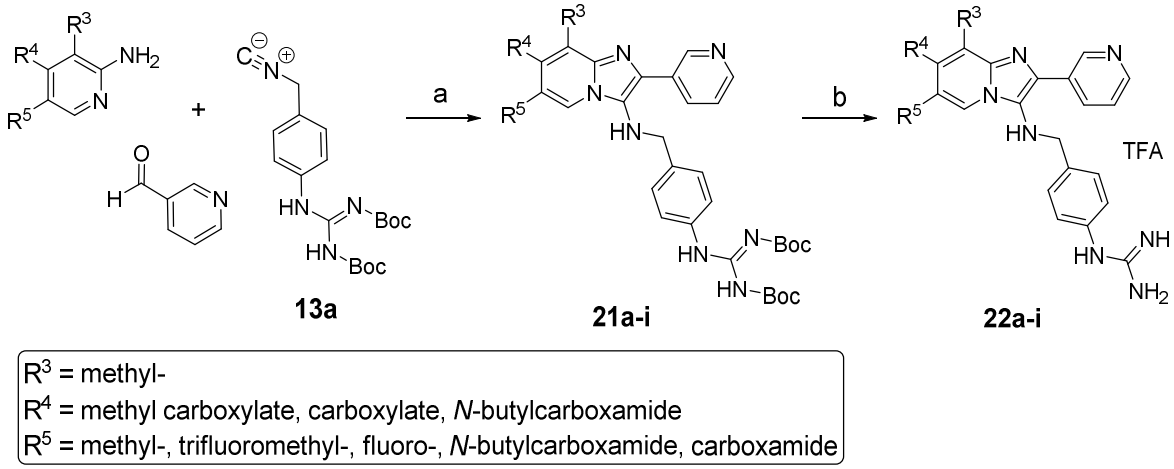
[a]Reagents and conditions: (a) ethyl formate, TEA, 55 °C, 24 h, 55-90%; (b) $POCl_3$, DIPA, DCM, 60-85%; (c) pyridin-2-amine, glyoxylic acid monohydrate, $HClO_4$ (cat), MeOH, rt, 24 h, 38-66%; (d) TFA/DCM (1:1), rt, 1 h, 95-100%.

Scheme 2. Synthesis of analogues modified at the 2-position 17a-k.[a]



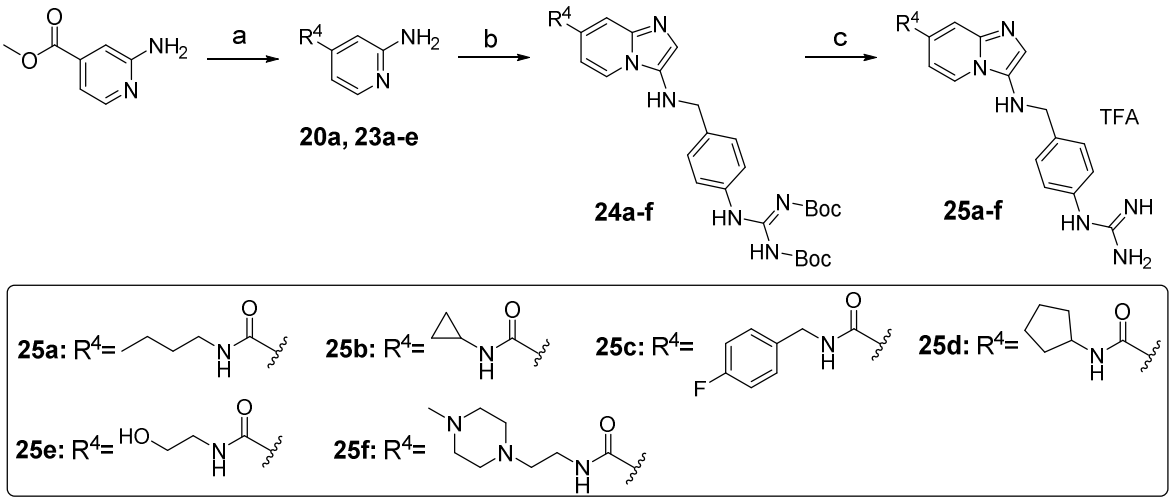
[a]Reagents and conditions: (a) $HClO_4$ (cat), MeOH, rt, 24 h, 22-86%; (b) TFA/DCM (1:1), rt, 1 h, 94-100%.

Scheme 3. Synthesis of analogues 22a-i.[a]



[a]Reagents and conditions: (a) $HClO_4$ (cat), MeOH, rt, 24 h, 25-68% (in case of compound **21d** (i) methyl 2-aminopyridine-4-carboxylate, TBD, DMF, 120 °C, 20 h, 52.5%; (ii) $HClO_4$ (cat), MeOH, rt, 24 h, 60%; compound **21h** (i) methyl 6-aminonicotinate, TBD, DMF, 120 °C, 24 h, 42%; (ii) $HClO_4$ (cat), MeOH, rt, 24 h, 47%); (b) TFA/DCM (1:1), rt, 1 h, 94-100% (in case of compound **22c** (i) NaOH (2 M), DCM/MeOH (9:1), 12 h, rt, 69%; (ii) TFA/DCM (1:1), rt, 1 h, 93%).

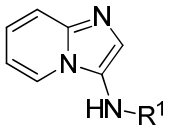
Scheme 4. Synthesis of the amide-substituted analogues 25a-f.[a]



[a]Reagents and conditions: (a) TBD, toluene, 110 °C, 20 h, 86-97% (in case of compound **20a** TBD, DMF, 120 °C, 20 h, 52.5%); (b) glyoxylic acid monohydrate, isocyanide **13a**, $HClO_4$ (cat), MeOH, rt, 6 h, 42-61%; (c) TFA/DCM (1:1), rt, 1 h, 96-100%.

TABLES:

Table 1. Biochemical evaluation of the monosubstituted analogues 14h, 15a-g against uPA.



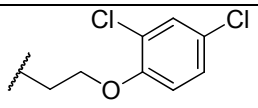
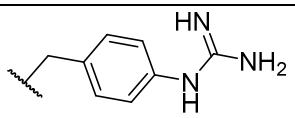
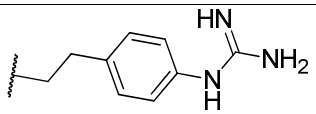
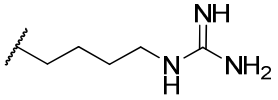
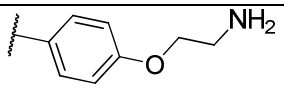
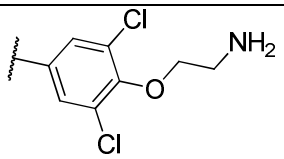
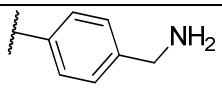
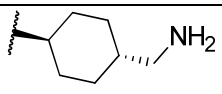
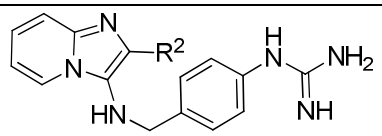
Cpd	R^1=	IC_{50} (uPA) [µM]
14h		~250
15a		9.04 ± 0.62
15b		19.39 ± 1.22
15c		~200
15d		>1000
15e		500
15f		>500
15g		500

Table 2. Biochemical evaluation of the 2-substituted-3-amino-imidazo[1,2-*a*]pyridines set 17a-k against uPA.



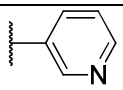
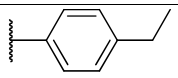
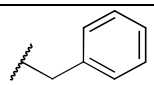
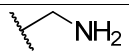
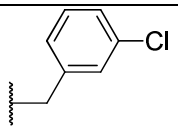
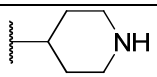
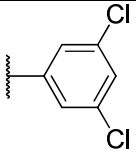
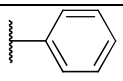
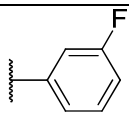
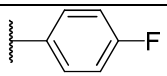
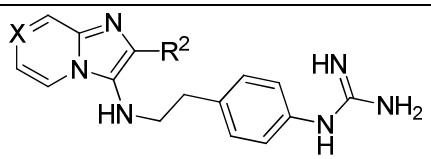
Cpd	R^2=	IC_{50} (uPA) [µM]
17a		250 ± 2.23
17b		63.92 ± 9.42
17c		30.25 ± 4.71
17d		48.47 ± 8.47
17e		9.30 ± 2.67
17f		18.03 ± 0.95
17g		~250
17h		99.16 ± 8.69
17i		30.15 ± 1.92
17j		~100
17k		~125

Table 3. Biochemical evaluation of the C3-guanidinophenethyl-substituted analogues 19a, 19b against uPA.



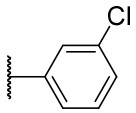
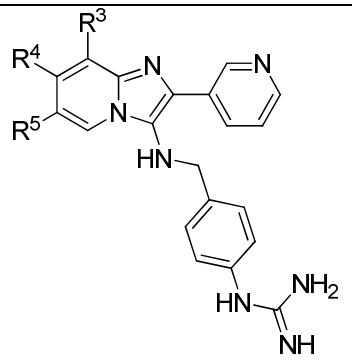
Cpd	R^2=	X=	IC_{50} (uPA) [µM]
19a		C	~150
19b		N	53.78 ± 3.72

Table 4. Biochemical evaluation of the C2 3-pyridyl substituted analogues 22a-i against uPA.



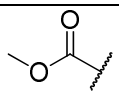
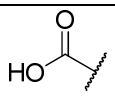
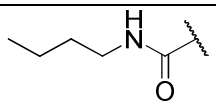
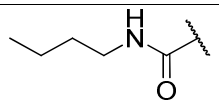
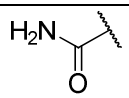
Cpd	R^3=	R^4=	R^5=	IC_{50} (uPA) [μM]
22a		H	H	27.44 ± 1.34
22b	H		H	14.95 ± 0.81
22c	H		H	26.85 ± 1.57
22d	H		H	6.89 ± 0.80
22e	H	H		23.89 ± 2.69
22f	H	H		~200
22g	H	H		57.55 ± 6.58
22h	H	H		60.84 ± 3.39
22i	H	H		27.26 ± 2.19

Table 5. Conditions applied to amide bond formation.

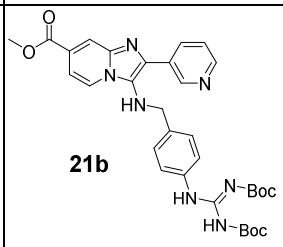
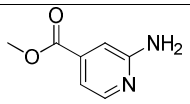
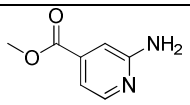
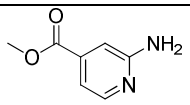
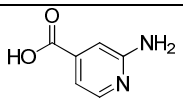
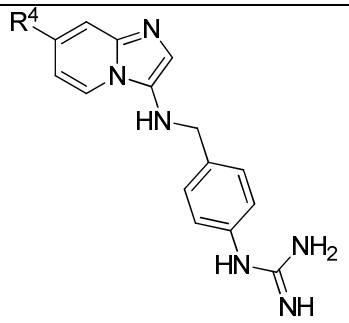
Entry	Amine	Carbonyl compound	Catalyst or coupling reagent	Solvent	Conditions	Yield [%]
1	*n*-butylamine (2 eq)	 **21b**	TBD (0.2 eq)	DMF	pressure tube, 120 °C, 24 h	<10
2	*n*-butylamine (2 eq)		TBD (0.2 eq)	DMF	pressure tube, 120°C, 20 h	52.5
3	cyclopropyl amine (2.2 eq)		TBD (0.2 eq)	DMF	pressure tube, 120°C, 21 h	17.2
4	cyclopropyl amine (2.2 eq)		TBD (0.3 eq)	Toluene	pressure tube, 110°C, 17 h	**89**
5	cyclopropyl amine (1.5 eq)		EDC and HOBt	DMF	65 °C, 20 h	10

Table 6. Biochemical evaluation of the amide-substituted analogues 25a-f against uPA.



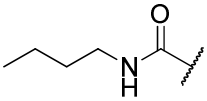
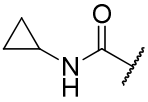
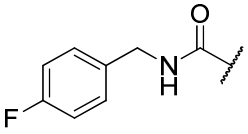
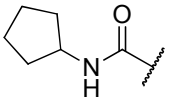
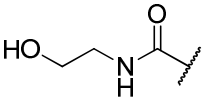
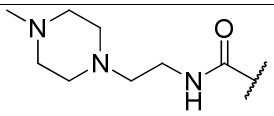
Cpd	R^4=	IC_{50} (uPA) [μM]
25a		0.097 ± 0.010
25b		0.184 ± 0.007
25c		0.254 ± 0.016
25d		0.174 ± 0.021
25e		0.366 ± 0.017
25f		0.404 ± 0.020

Table 7. Inhibitory activities against uPA and related enzymes (including the reference compounds: UK122 (7), gabexate (9), amiloride (10)).

Cpd	IC_{50} [μM] or % of enzyme inhibition at 100 μM concentration[a]							
	uPA	thrombin	tPA	FXa	plasmin	plasma kallikrein	trypsin	FVIIa
7	8.67 ± 1.12	56.1 ± 3.65	68.51 ± 5.05	6.47 ± 0.58	0.34 ± 0.01	1.34 ± 0.19	3.18 ± 0.17	0%
9	0.431 ± 0.017	0.687 ± 0.048	15.36 ± 0.94	4.61 ± 0.54	1.55 ± 0.06	1.35 ± 0.24	7.12 ± 0.13	-
10	11.98 ± 0.22	5.2%	1%	3.5%	0%	0%	31.76 ± 2.88	7.4%
15a	9.04 ± 0.62	23%	33.6%	42%	29.8%	39.4%	114.88 ± 6.78	2.5%
17a	250 ± 2.23	6.6%	9.4%	24.6%	0%	0%	3.3%	0%
25a	0.097 ± 0.010	19.2%	8%	41.8%	13.6%	2.8%	42.7%	1.4%
25b	0.184 ± 0.007	8%	0%	45.4%	3.3%	0%	46%	2.6%
25c	0.254 ± 0.016	19.5%	11.9%	45.7%	8.3%	0%	43.2%	4.2%
25d	0.174 ± 0.021	17.8%	11.2%	47.8%	0%	0%	37.1%	4%
25e	0.366 ± 0.017	4.7%	0%	51.58 ± 2.39	15.6%	0%	43.3%	5%

25f	0.404 ± 0.020	6.6%	2.8%	12.2%	0%	0%	32%	2%
26	57.49 ± 5.20	0%	0%	0%	0%	0%	38.8%	0%

[a] The IC_{50} values and % of enzyme inhibition were determined under the assay conditions of this manuscript.

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