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Article

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Discovery of a potent α-galactosidase inhibitor by *in situ* analysis of a library of pyrrolizidine-(thio)urea hybrid molecules generated *via* click chemistry

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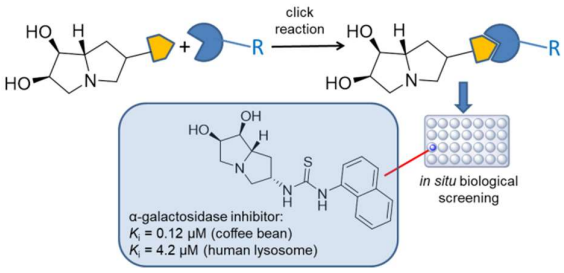
Graphical abstract



Abstract

The parallel synthesis of a 26-membered-library of aromatic/aliphatic-(thio)urea-linked pyrrolizidines followed by *in situ* biological evaluation towards α-galactosidases has been carried out. The combination of the (thio)urea-forming click reaction and the *in situ* screening is pioneer in the search for glycosidase inhibitors and has allowed the discovery of a potent coffee bean α-galactosidase inhibitor (IC_{50} = 0.37 μM, K_i = 0.12 μM) that has also showed inhibition against human lysosomal α-galactosidase (α-Gal A, IC_{50} = 5.3 μM, K_i = 4.2 μM).

Keywords: iminosugars; glycosidase inhibitors; pyrrolizidines; click chemistry; *in situ* screening; α-galactosidases.

Introduction

Glycosidases are enzymes that catalyze the hydrolysis of the glycosidic bond of oligosaccharides and glycoconjugates. They are involved in a wide range of biological processes from metabolism to cell-cell and cell-virus recognition.[1] The inhibition of these enzymes is therefore an important target in order to find new therapeutic agents against diseases such as diabetes, viral infections, lysosomal storage disorders and tumor metastasis.[2] Iminosugars, carbohydrate mimics with the endocyclic oxygen replaced by a nitrogen atom, constitute the most important family of glycosidase inhibitors due to their ability of mimicking the corresponding oxocarbenium ion or the transition state of the enzyme-catalyzed *O*-glycoside hydrolysis, after protonation at physiological pH.[3] Although many natural iminosugars,

including polyhydroxylated piperidines, pyrrolidines, indolizidines, pyrrolizidines and nortropanes (Figure 1a), show potent glycosidase inhibition properties, they often lack of selectivity, which, together with their low membrane permeability, limits their use as pharmacological agents. Thus, numerous efforts have been devoted to the synthesis of more potent and selective inhibitors.[4] Several piperidine derived iminosugars such as Glyset® (*N*-(2-hydroxyethyl)-1-deoxynojirimycin), Zavesca® (*N*-butyl-1-deoxynojirimycin) or more recently Galafold® (1-deoxygalactonojirimycin) (Figure 1b) are commercialized as drugs against type II diabetes, Gaucher and Fabry diseases, respectively.[5]

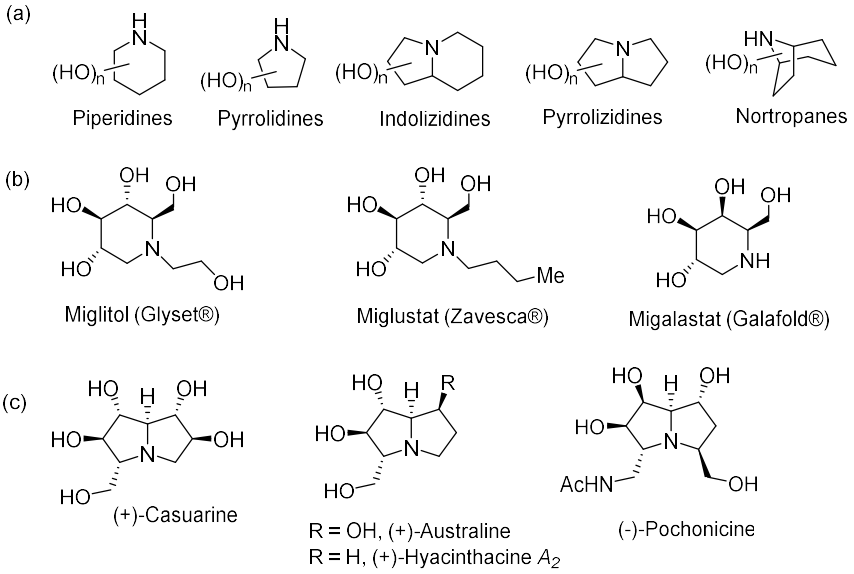


Figure 1. *a) Families of iminosugars; b) Current drugs from iminosugars; c) Examples of bioactive natural pyrrolizidines.*

Pyrrolizidine iminosugars such as casuarine, australine and hyacinthacines (Figure 1c) are good inhibitors of several glucosidases but they often present a limited selectivity, possibly due to the high conformational flexibility of the bicyclic system.[6] With the aim of improving the glycosidase inhibition potency and specificity of these inhibitors, most of the structural modifications made have involved changes in the configuration and number of hydroxyl groups of the pyrrolizidine ring.[7,8] Few examples of other types of modifications in the pyrrolizidine ring (fluorination, *C*-alkylation/arylation and amidation of amino-derivatives) have been described.[9] Recently, the first naturally occurring polyhydroxylated pyrrolizidine containing an acetamidomethyl group (pochonicine) has showed potent inhibition against β-*N*-acetylglucosaminidases (GlcNAcases), being inactive towards other glycosidases. The synthesis of several stereoisomers of pochonicine has been also reported.[10]

Although many of these polyhydroxylated pyrrolizidines present interesting inhibition properties, none of them has been yet approved as a drug. Most polyhydroxylated pyrrolizidines

reported in the bibliography are tetra- or penta-hydroxylated and include an hydroxymethyl substituent on the bicyclic core (Figure 1c). Recent applications of glycosidase inhibitors as chaperones for lysosomal storage diseases (LSDs) require the inhibitors to cross the cell membranes (cell and ER permeability).[5] We and others have demonstrated that the incorporation of (hetero)aromatic moieties into monocyclic iminosugars (*syn*-3,4-dihydroxylated pyrrolidines) affords glycosidase inhibitors with increased potency and selectivity, which may be due to additional non-glycone interactions with the enzyme.[11] Therefore, the attaching of different aliphatic/aromatic moieties into a pyrrolizidine skeleton with a lower degree of hydroxylation seems to be an attractive approach to improve the selectivity and potency of these compounds, increasing, at the same time, the lipophilic character necessary to cross the cell membranes.

Click chemistry[12] represents an efficient synthetic methodology that connects two readily available building blocks. This approach is currently used in drug discovery[13] by providing a mean for the fast preparation of hybrid molecules that facilitate lead optimization by structure-activity relationship (SAR) through the generation of libraries of derivatives. In particular, the use of the copper catalyzed azide alkyne cycloaddition (CuAAC) as click reaction has been broadly exploited for the generation of bioactive molecules in medicinal chemistry.[14] This reaction has been successfully used in the preparation of monomeric and multimeric iminosugar-triazole derivatives in order to improve the glycosidase inhibitory properties of the adequate iminosugar lead.[15] In this sense, we have recently applied a combinatorial strategy based on the generation of libraries of pyrrolidine-triazole derivatives *via* CuAAC that, in combination with their *in situ* biological screening, has allowed us the rapid and efficient discovery of potent glycosidase inhibitors.[11a,16] Part of the success of this strategy relies on the choice of the key reaction for the preparation of the library, as high-yielding reactions that do not generate by-products that could interfere in the further *in situ* biological analysis are needed. Although CuAAC largely fulfills these requirements,[17] the toxicity of copper catalyst may be a limiting factor for its use in the preparation of compounds for pharmaceutical applications,[18] as in many cases copper (I) can coordinate with the compound,[19] being difficult to remove in the purification process. Although other click reactions have been developed and employed in medicinal chemistry,[20] their application for the generation and *in situ* biological screening in the field of glycosidase inhibition is limited.

We report herein the synthesis and biological evaluation of a family of aromatic-linked epimeric pyrrolizidines obtained by different click reactions (urea, thiourea and triazole formation), in order to compare their biological activity. The most potent inhibitor will be chosen as model compound for the generation of a combinatorial library that will be *in situ* screened towards commercial glycosidases (Figure 2).

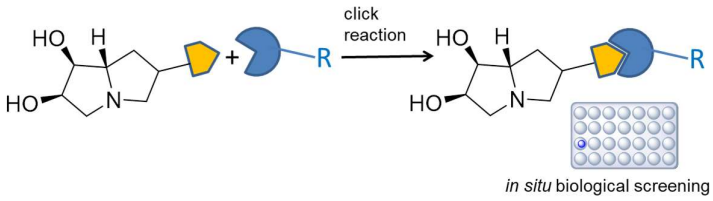
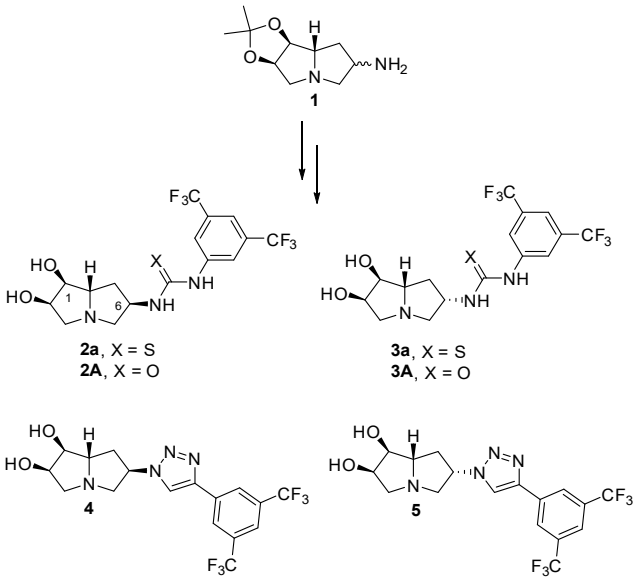


Figure 2. *General strategy developed in this work.*

Results and discussion

Synthesis and selection of the lead compound for the generation of the library.

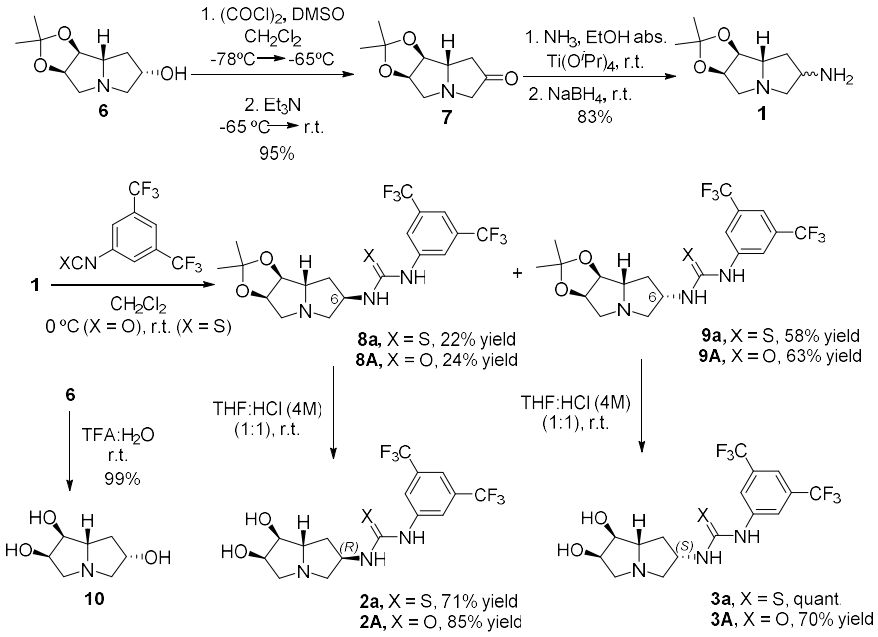
Our goal in this work was the synthesis of several hydroxylated pyrrolizidine derivatives in order to identify a suitable lead compound for the search of new selective glycosidase inhibitors. Thus, we performed a preliminary study of the glycosidase inhibition of several pyrrolizidine derivatives bearing an aromatic moiety through different linkers (Scheme 1). The idea was to prepare these compounds starting from aminopyrrolizidines **1**, and study the effect of the configuration and type of substitution at C-6 on the inhibition of glycosidases by derivatives **2-5**. Two types of click reactions were chosen for this purpose, (a) the Cu(I)-catalyzed azide alkyne cycloaddition (CuAAC) between an azido-pyrrolizidine and an alkyne and (b) the (thio)urea-forming reaction between an amino-pyrrolizidine and an iso(thio)cyanate.



Scheme 1.*Aromatic-(thio)urea/triazole-linked pyrrolizidines for preliminar biological exploration.*

The synthesis of amino-pyrrolizidines **1** started from alcohol **6**,[21] previously described by Wightman and co-workers. Swern oxidation of **6** followed by reductive amination with ammonia in the presence of titanium tetraisopropoxide furnished amines **1** as an inseparable diastereoisomeric mixture. Subsequent reaction between amino-pyrrolizidines **1** and 3,5-

bis(trifluoromethyl)phenylisothiocyanate gave the diastereomeric thioureas **8a** and **9a** which could be separated by column chromatography and were further deprotected to give **2a** and **3a** (Scheme 2). The configuration at C-6 in diastereoisomers **2a** and **3a** was assigned by X-ray crystal structure of the precursors **8a** and **9a** (Figure 3).[22] Ureas **2A** and **3A** were similarly obtained after reaction of **1** with 3,5-bis(trifluoromethyl)phenylisocyanate followed by acidic deprotection. The C6-(*R*)-configuration of diastereoisomer **2A** was assigned by NOESY spectrum which showed a conclusive NOE between H-6 and H-1 (Figure 4). Acidic deprotection of alcohol **6** was also carried out to give known trihydroxylated pyrrolizidine **10**.[21]



Scheme 2. *Synthesis of aromatic-(thio)urea-linked pyrrolizidines.*

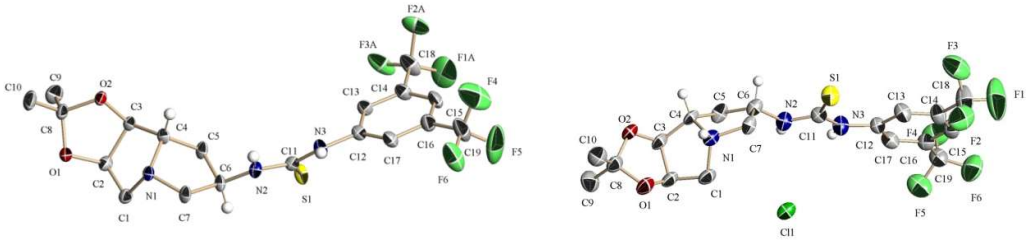
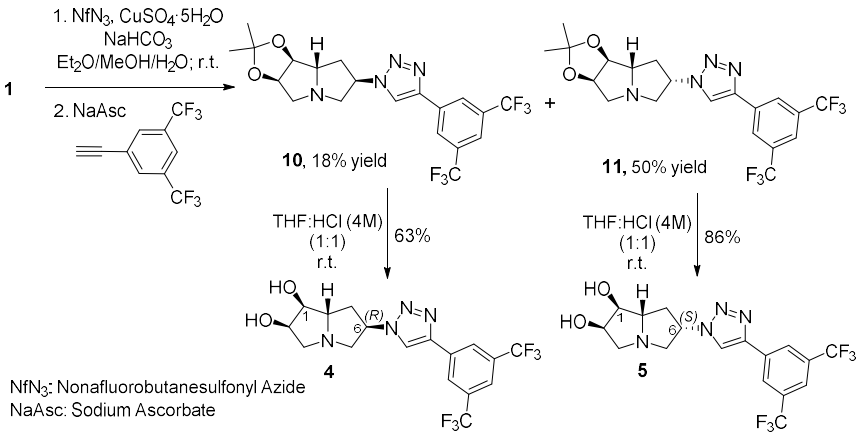


Figure 3. *ORTEP diagrams for* ***8a*** *(left) and* ***9a·HCl*** *(right) with ellipsoids set at 50% probability (most of the hydrogen atoms were omitted for clarity).*

Triazoles **11** and **12** were obtained from amines **1** in a sequential one-pot diazo transfer reaction followed by intermolecular CuAAC (Scheme 3).[23] Subsequent acidic deprotection afforded derivatives **4** and **5**. In the case of **4**, a NOE between H-6 and H-1 confirmed the *R* configuration for C(6) (Figure 4).



Scheme 3. *Synthesis of aromatic-triazole-linked pyrrolizidines.*

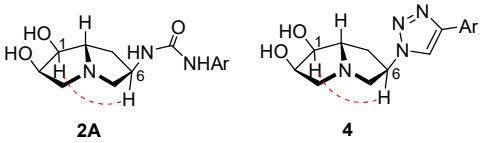
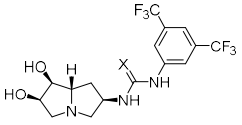
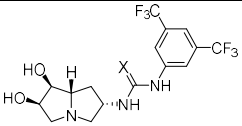
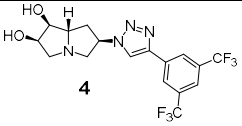
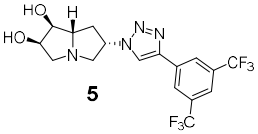
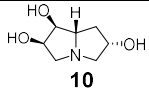
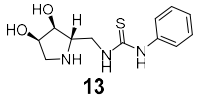


Figure 4. *Confirmation of the C(6) configuration in* ***2A*** *and* ***4*** *by NOE experiments.*

The biological evaluation of aromatic-linked pyrrolizidines **2a**, **2A**, **3a**, **3A**, **4, 5** and alcohol **910** towards eleven commercial glycosidases was carried out (see Table 1). Thiourea **3a** only showed a strong inhibition towards α-galactosidase from coffee beans (IC_{50} = 3.1 μM) and a moderate inhibition against α-mannosidases from Jack bean (IC_{50} = 38 μM). It is noteworthy that the corresponding urea analogue **3A** proved to be a more selective α-galactosidase inhibitor as it lost α-mannosidase inhibition. Triazole derivative **4** showed a moderate inhibition towards α-L-fucosidase (IC_{50} = 46 μM) and α-galactosidase (IC_{50} = 53 μM), and a high inhibition towards α-mannosidase (IC_{50} = 9.1 μM) while its epimer **5** proved to be a moderate but more selective inhibitor of α-galactosidase (IC_{50} = 57 μM). Urea and thiourea pyrrolizidines **2a** and **2A** only showed a weak inhibition towards some of the enzymes, which indicates the importance of the configuration at C6 on the inhibition properties of this type of compounds. Besides, the inhibition data for alcohol **10** in comparison with those of **3a** and **3A**, which have the same configuration at C6, showed that the incorporation of an aromatic moiety through an urea or thiourea bridge considerably improves the enzymatic inhibition. After this preliminar screening, compounds **3a** and **3A** appeared to be interesting leads for the search of new α-galactosidase inhibitors. It is remarkable the difference in the inhibition properties of compound **3A** and our previously described monocycle analogue **13** (see Table 1), despite the slight differences on their aromatic substituents.[16a] While phenyl-thiourea-linked pyrrolidine **13** is a moderate-to-weak β-glucosidase inhibitor, the rigid analogue (pyrrolizidine **3A**) is a potent α-

galactosidase inhibitor, showing the influence of the conformation in the inhibition selectivity profile .

Table 1. Inhibitory activities of compounds **2**-**5**, **10** and **13** towards glycosidases. % Inhibition at 100 μM of inhibitor and IC_{50} (in parenthesis). Optimal pH for each enzyme, 37 ºC.[a,b]

Compound		α-L-fucosidase (bovine kidney)	α-galactosidase (coffee beans)	α-mannosidase (Jack beans)	β-glucosidase (almonds)
	**2a**, X = S	NI	NI	NI	NI
	2A, X = O	23%	24%	18%	NI
	**3a**, X = S	NI	88% (3.1 μM)	71% (38 μM)	NI
	3A, X = O	NI	82% (9.2 μM)	26%	NI
 **4**		65% (46 μM)	56% (53 μM)	86% (9.1 μM)	NI
 **5**		19%	58% (57 μM)	25%	NI
 **10**		NI	NI	NI	NI
 **13**		NI[d]	NI[d]	NI[d]	76%[c,d]

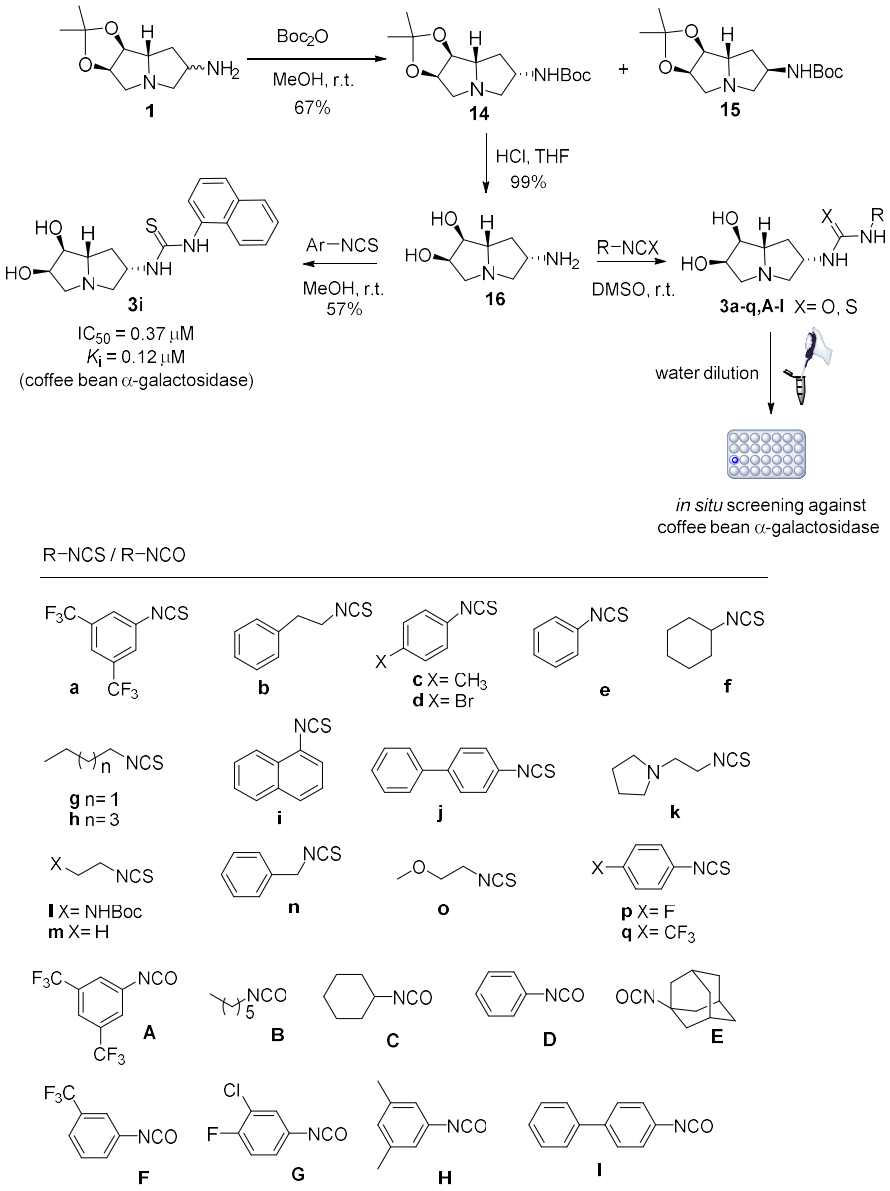
[a] NI = no inhibition detected.
[b] No inhibition was detected towards these other eight enzymes: β-galactosidases (*Aspergillus oryzae* and *Escherichia coli*), α-glucosidases (yeast and rice), β-*N*-acetylglucosaminidase (Jack bean), amyloglucosidase (*Aspergillus niger*), β-mannosidase (snail).
[c] % Inhibition at 1000 μM of inhibitor.
[d] Inhibition data reported in reference 16a.

Generation of a library of aromatic-(thio)urea-linked pyrrolizidines and in situ *screening as α-galactosidase inhibitors*

The (thio)urea-forming click reaction between the unprotected (6*R*)-6-amino-pyrrolizidine **16** and a set of iso(thio)cyanates followed by *in situ* biological evaluation seemed to be ideal for the rapid discovery of potent glycosidase inhibitors. The preparation of diastereomerically pure **16** was achieved after Boc protection of amino-pyrrolizidines **1** followed by chromatographic separation of diastereoisomers **14** and **15** and subsequent acidic deprotection of **14** (Scheme 4).

Parallel (thio)urea-forming reactions between amino-pyrrolizidine **16** (1.2 equiv.) and isothiocyanates **a–q** (1.0 equiv.) and isocyanates **A-I** (1.0 equiv.) were carried out using DMSO

as solvent (Scheme 4). TLC and ESI-MS analysis of the mixtures showed after 6 h at r.t complete conversion and the presence of the desired compounds (See Supporting information for Electrospray mass spectra). After water dilution, the *in situ* screening of the resulting crude pyrrolizidine-(thio)ureas towards α-galactosidase (coffee beans) was carried out in a 96-well microtiter plate containing 5.0 μM of the potential inhibitor in each well, assuming complete reactions (see Experimental section for details).



Scheme 4. *Parallel (thio)urea-forming click reactions followed by* in situ *screening.*

From the preliminary analysis of the (thio)urea-pyrrolizidine library (Figure 5) it is clear that the incorporation of an aromatic/aliphatic moiety to the pyrrolizidine skeleton significantly enhances the enzymatic inhibition compared to amino-pyrrolizidine **16**. Besides, the thiourea

derivatives (**3a-q**) resulted to be better inhibitors than the corresponding urea counterparts (**3A-I**). In particular, thiourea **3i** (93% inhibition at 5.0 μM) stood out among all the derivatives evaluated. In order to carry out a more accurate inhibition study, compound **3i** was prepared in higher scale and purified by column chromatography (Scheme 4). This compound was evaluated towards a collection of commercial glycosidases. At 0.1 mM concentration, **3i** only showed significant inhibition towards α-galactosidase from coffee beans, being the IC_{50} = 0.37 μM and K_i = 0.12 μM (mixed-type of inhibition), and moderate inhibition towards Jack bean α-mannosidase (IC_{50} = 79 μM).

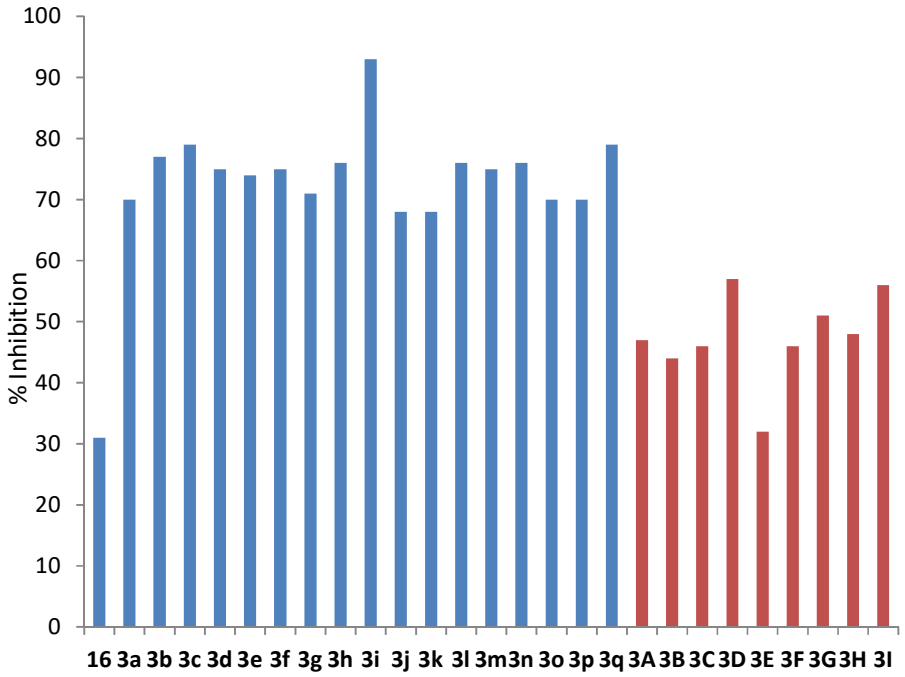


Figure 5. *% Inhibition of α-galactosidase from coffee beans at 5.0 μM of pyrrolizidine-thioureas (in blue) and pyrrolizidine-ureas (in red) (pH 6, 37 ºC).*

Coffee beans α-galactosidase presents 58% homology with human lysosomal α-galactosidase,[24] the enzyme involved in Fabry disease,[25] both belonging to the CAZy family GH27. The X-ray structure of human α-Gal A is known since 2004.[26] The structure is a homodimer with each monomer composed with two domains. Domain 1, which contains an active site, has a higher frequency of point mutations leading to Fabry disease. The previously reported crystal structure of human α-galactosidase in complex with a 1-deoxygalactonojirimycin-arylthiourea shows the ability of the aryl-NH thiourea proton to interact *via* hydrogen bond with the catalytic Asp-231 of α-Gal A.[27] Thus, **3i** was also evaluated against this human enzyme, showing an IC_{50} of 5.3 μM (K_i = 4.2 μM, mixed-type of inhibition). Compound **3a**, which is weaker inhibitor than **3i** towards the plant enzyme, was also evaluated towards the human enzyme, showing also weaker inhibition against the latter (IC_{50} = 68 μM). These data show a correspondence between the

behaviour of our inhibitors against both enzymes, which makes our approach useful for the preliminary selection of compounds as potential human lysosomal α-galactosidase inhibitors.

Conclusions

We have prepared a 26-membered-library of aromatic(aliphatic)-(thio)urea-linked pyrrolizidines that after *in situ* screening has allowed the rapid identification of a potent and selective coffee bean α-galactosidase inhibitor (compound **3i**), that also showed inhibition against human lysosomal α-galactosidase. To the best of our knowledge this is the first pyrrolizidine-scaffolded iminosugar showing α-galactosidase inhibition in the nanomolar range. Moreover the high lipophilicity of **3i**, with only two hydroxyl groups and an aromatic substituent, makes this inhibitor of interest for applications requiring the cross of cell membranes (i.e. chaperones for Fabry disease). The combination of the well-known metal-free (thio)urea-forming click reaction and *in situ* biological screening has been successfully used for the first time in the search for new glycosidase inhibitors and could be extended to the discovery of many other biologically relevant molecules.

Experimental part

General methods.

Optical rotations were measured in a 1.0 cm or 1.0 dm tube with a Jasco P-2000 spectropolarimeter. Infrared spectra were recorded with a Jasco FTIR-410 spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded with a Bruker AMX300, AV300 for solutions in $CDCl_3$ and CD_3OD. δ are given in ppm and *J* in Hz. *J* are assigned and not repeated. All the assignments were confirmed by COSY and HSQC experiments. High resolution mass spectra were recorded on a Q-Exactive spectrometer. NMR and mass spectra were registered in CITIUS (University of Seville). TLC was performed on silica gel 60 F_{254} (Merck), with detection by UV light charring with *p*-anisaldehyde, vanillin, ninhydrin or with Pancaldi reagent [$(NH_4)_6MoO_4$, $Ce(SO_4)_2$, H_2SO_4, H_2O]. Silica gel 60 (Merck, 40-60 and 63-200 μm) was used for preparative chromatography.

Generation of the combinatorial library followed by in situ *biological screening.*

To 300 μL of a solution of pyrrolizidine **16** (28 mM in DMSO) in an eppendorf, 100 μL of a solution of the corresponding iso(thio)cyanate (**a**-**q**, **A**-**I**) (70 mM in DMSO) was added. All the eppendorfs containing the resulting mixtures were shaken at room temperature for 6 h and monitored for completion by TLC (AcOEt:Cy, 1:1) and ESI-MS (see Supporting information for mass spectra analysis). Then, the reaction mixtures were diluted with water to the desired concentration and placed in a 96-well microtiter plate in order to perform the enzymatic assays against coffee bean α-galactosidase. The final concentration of (thio)ureas on each well was 5.0

μM (assuming quantitative conversion in the click reaction). The inhibition measures were performed according the general procedure described bellow.

Inhibition studies with commercial enzymes

The % of inhibition towards the corresponding glycosidase was determined in the presence of 0.1 mM (5.0 μM for crude library **3a-q**,**A-I**) of the inhibitor on the well. Each enzymatic assay (final volume 0.12 mL) contains 0.01-0.5 units/mL of the enzyme and 4.2 mM aqueous solution of the appropriate *p*-nitrophenyl glycopyranoside (substrate) buffered to the optimal pH of the enzyme. Enzyme and inhibitor were pre-incubated for 5 min at rt, and the reaction started by addition of the substrate. After 20 min of incubation at 37 °C, the reaction was stopped by addition of 0.1 mL of sodium borate solution (pH 9.8). The *p*-nitrophenolate formed was measured by visible absorption spectroscopy at 405 nm. Under these conditions, the *p*-nitrophenolate released led to optical densities linear with both reaction time and concentration of the enzyme. The IC_{50} value (concentration of inhibitor required for 50% inhibition of enzyme activity) was determined from plots of % inhibition *versus* different inhibitor concentrations. The mode of inhibition for **3i** was determined by the Lineweaver-Burk double-reciprocal plots (1/V versus 1/[S] at different inhibitor concentrations) to give the apparent K_m (the K_m in the presence of the inhibitor). The graph was performed using six substrate concentrations around the K_m of the enzyme for each inhibitor concentration. Four different inhibitor concentrations, bracketing the IC_{50} value, were used for each determination. From the analysis of this graph, a secondary plot was generated from the representation of the slope ($K_m$apparent/V_{max}) *versus* [inhibitor]; K_i was calculated from the negative value of the x-intercept of this plot. In case of mixed-type of inhibition, another secondary plot was generated from the original Lineweaver-Burk graph, plotting the Y-intercepts ($1/V_{max}$apparent) *versus* [inhibitor]; K'_i was calculated from the negative value of the X-intercept of this plot.

Synthesis and characterization of the new compounds.

(1*S*,2*R*,7a*S*)-1,2-*O*-Isopropylidendioxy-pyrrolizidin-6-one (7): Anhydrous DMSO (1.9 mL, 26 mmol) was added dropwise to a stirred solution of oxalyl chloride (1.1 mL, 13 mmol) in anhydrous CH_2Cl_2 (15 mL) at -65 °C. The mixture was stirred at –65 °C for 15 min and then a solution of alcohol **6**[21] (1.28 g, 6.44 mmol) in anhydrous CH_2Cl_2 (20 mL) was added. The mixture was stirred for 5 h at -65 °C and then Et_3N (4.5 mL, 32 mmol) was added. The mixture was allowed to reach r.t. and then evaporated. Chromatographic purification on silica gel (diethyl ether:acetone, 3:1) afforded **7** (1.2 g, 6.1 mmol, 95%) as a pale orange solid. $[\alpha]_D^{27}$ -215.6 (*c* 0.70, CH_2Cl_2). IR ν_{max} 2985, 2938, 1739 (C=O), 1151, 864, 621 cm^{-1}. ^{1}H-NMR (300 MHz, $CDCl_3$, δ ppm, *J* Hz) δ 4.86 (dd, 1H, $J_{2,1}$ = 6.0, $J_{2,3'}$= 4.7, H-2), 4.68 (d, 1H, H-1), 3.86

(dd, 1H, $J_{7a,7'}$ = 11.1, $J_{7a,7}$ = 7.5, H-7a), 3.78-3.31 (m, 2H, H-3, H-5), 3.12 (d, 1H, $J_{5',5}$ = 18.9, H-5'), 2.67 (dd, 1H, $J_{3',3}$ = 11.7, H-3'), 2.39 (dd, 1H, $J_{7,7'}$ = 18.6, H-7), 2.03 (ddd, 1H, $J_{7',5'}$ = 0.9, H-7'), 1.57, 1.34 (2s, 3H each, -C(CH_3)$_2$). ^{13}C-NMR (75.4 MHz, $CDCl_3$, δ ppm) δ 217.7 (C=O), 111.9 (-*C*(CH$_3$)$_2$), 83.9 (C-1), 80.7 (C-2), 68.0 (C-7a), 61.5 (C-3), 59.2 (C-5), 37.2 (C-7), 26.5 (-C(*C*H$_3$)$_2$), 24.7 (-C(*C*H$_3$)$_2$). HRMS (ESI-ORBITRAP) m/z: $[M+H]^+$ calcd. for $C_{10}H_{16}NO_3$ 198.1125; Found 198.1120.**(1*S*,2*R*,6*R*,7a*S*)- and (1*S*,2*R*,6*S*,7a*S*)-6-Amino-1,2-*O*-isopropylidendioxy-pyrrolizidine (1):**

A solution of **7** (239 mg, 1.21 mmol) and Ti(O^{i}Pr)$_4$ (0.71 mL, 2.4 mmol) in abs. EtOH (8 mL) was saturated with ammonia. After 6 h at r.t., $NaBH_4$ (72 mg, 1.8 mmol) was added and the mixture was stirred for 4 h. Then, 2 M NH_4OH (6 mL) was added and the mixture was filtered through celite and washed with EtOH and AcOEt. The filtrate was evaporated and the residue purified through chromatography column on silica gel (CH_2Cl_2:MeOH:NH_4OH, 7:1:0.05→5:1:0.05) to afford **1** (199 mg, 1.01 mmol, 83%) as a mixture of diastereoisomers that was directly used for derivatization.

***N*-[(3,5-Bis(trifluoromethyl)phenyl)]-*N'*-[(1*S*,2*R*,6*R*,7a*S*)-1,2-*O*-isopropylidendioxy-pyrrolizidin-6-yl]thiourea (8a) and *N*-[(3,5-Bis(trifluoromethyl)phenyl)]-*N'*-[(1*S*,2*R*,6*S*,7a*S*)-1,2-*O*-isopropylidendioxy-pyrrolizidin-6-yl]thiourea (9a):** To a solution of diastereomeric amines **1** (196 mg, 0.99 mmol) in CH_2Cl_2 (10 mL), 3,5-bis(trifluoromethyl)phenylisothiocyanate (0.46 mL, 2.5 mmol) was added and the mixture stirred for 2.5 h at r.t. After evaporation to dryness, the crude was purified by column chromatography (CH_2Cl_2:MeOH, 40:1→20:1) to give **8a** (102 mg, 0.22 mmol, 22%) and **9a** (270 mg, 0.58 mmol, 58%) as white solids. Data for **8a**: m.p. 148-150 ºC. $[\alpha]_D^{26}$ -36.5 (*c* 0.54, CH_2Cl_2). IR ν_{max} 3273 (NH), 2990, 1275 (C=S), 1128, 677 cm^{-1}. ^{1}H-NMR (300 MHz, $CDCl_3$, δ ppm, *J* Hz) δ 9.07 (s, 1H, NH), 7.98 (s, 2H, H-arom.), 7.64 (s, 2H, H-arom., NH), 5.17 (brs, 1H, H-6), 4.92-4.89 (m, 1H, H-2), 4.58 (d, 1H, $J_{1,2}$= 6.0, H-1), 3.79-3.73 (m, 1H, H-7a), 3.32 (d, 1H, $J_{3,3'}$ = 11.7, H-3), 3.16-3.06 (m, 2H, H-5, H-5'), 2.85 (dd, 1H, $J_{3',2}$ = 4.8, H-3'), 2.13 (dd, 1H, $J_{7,7'}$ = 13.5, *J* = 6.9, H-7), 2.01-1.90 (m, 1H, H-7'), 1.53 (s, 3H, -C(CH_3)$_2$), 1.35 (s, 3H, -C(CH_3)$_2$). ^{13}C-NMR (75.4 MHz, $CDCl_3$, δ ppm, *J* Hz) δ 181.0 (C=S), 140.6 (C-arom.), 132.2 (q, $^2J_{C,F}$ = 33.5, C-arom.), 123.7-123.3 (m, C-arom.), 123.2 (q, $^1J_{C,F}$ = 272.4, CF_3), 118.5-118.1 (m, C-arom.), 112.1 (-*C*(CH$_3$)$_2$), 83.3 (C-1), 81.1 (C-2), 70.9 (C-7a), 60.0 (C-5), 59.5 (C-3), 55.9 (C-6), 34.9 (C-7), 26.4 (-C(*C*H$_3$)$_2$), 24.4 (-C(*C*H$_3$)$_2$). HRMS (ESI-ORBITRAP) m/z: $[M+H]^+$ calcd. for $C_{19}H_{22}F_6N_3O_2S$ 470.1331; Found 470.1314. Anal. calcd. for $C_{19}H_{21}F_6N_3O_2S$: C, 48.61%; H, 4.51%, N, 8.95%; S, 6.83%, found: C, 48.63%; H, 4.43%; N, 8.97%; S, 6.82%. Data for **9a**: $[\alpha]_D^{27}$ -25.2 (*c* 0.50, CH_2Cl_2). IR ν_{max} 3283 (NH), 2985, 2937, 1275 (C=S), 1125, 681 cm^{-1}. ^{1}H-NMR (300 MHz, $CDCl_3$, δ ppm, *J* Hz) δ 8.75 (s, 1H, NH), 7.88 (s, 2H, H-arom.), 7.66 (s, 1H, H-arom.), 6.87 (brs, 1H, NH), 4.86-4.83 (m, 2H, H-2, H-6), 4.58 (d, 1H, *J* = 5.1, H-1), 3.57-

3.50 (m, 2H, H-5, H-7a), 3.31 (d, 1H, $J_{3,3'}$ = 11.7, H-3), 2.89 (dd, 1H, $J_{3',2}$ = 4.8, H-3'), 2.69 (dd, 1H, $J_{5',5}$ = 12.0, $J_{5',6}$ = 5.7, H-5'), 2.60-2.51 (m, 1H, H-7), 1.51 (s, 3H, -C(CH_3)$_2$), 1.46-1.36 (m, 1H, H-7'), 1.31 (s, 3H, -C(CH_3)$_2$). ^{13}C-NMR (75.4 MHz, $CDCl_3$, δ ppm, *J* Hz) δ 181.3 (C=S), 140.2 (C-arom.), 132.3 (q, $^2J_{C,F}$ = 33.6, C-arom.), 123.5-123.1 (m, C-arom.), 123.1 (q, $^1J_{C,F}$ = 272.4, CF_3), 118.8-118.4 (m, C-arom.), 112.3 (-*C*(CH_3)$_2$), 82.7 (C-1), 80.6 (C-2), 71.4 (C-7a), 59.6 (C-3), 58.8 (C-5), 55.6 (C-6), 33.5 (C-7), 26.3 (-C(*C*H_3)$_2$), 24.2 (-C(*C*H_3)$_2$). HRMS (ESI-ORBITRAP) m/z: $[M+H]^+$ calcd for $C_{19}H_{22}F_6N_3O_2S$ 470.1331; Found 470.1316. Anal. calcd. for $C_{19}H_{21}F_6N_3O_2S$: C, 48.61%; H, 4.51%, N, 8.95%; S, 6.83%, found: C, 48.88%; H, 4.55%; N, 8.54%; S, 6.49%.

General procedure for acidic deprotection:

A solution of the corresponding protected compound (0.5 mmol) in HCl (4M):THF (1:1) (12.0 mL) was stirred at r.t. for 3 h. After evaporation to dryness, the residue was dissolved in THF (10 mL) and NH_4OH (25%) (5 mL) was added. Evaporation of the solvent and purification through silica gel afforded the unprotected derivative.

***N*-[(3,5-Bis(trifluoromethyl)phenyl)]-*N'*-[(1*S*,2*R*,6*R*,7a*S*)-1,2-dihydroxy-pyrrolizidin-6-yl]thiourea (2a):** Acidic deprotection of **8a** (52 mg, 0.11 mmol) followed by chromatographic purification (CH_2Cl_2:MeOH:NH_4OH, 5:1:0.01), afforded **2a** (33 mg, 0.08 mmol, 71%) as a white solid. $[\alpha]_D^{25}$ + 9.0 (*c* 1.1, MeOH). IR ν_{max} 3248 (OH, NH), 1275 (C=S), 1120, 680 cm^{-1}. ^{1}H-NMR (300 MHz, MeOD, δ ppm, *J* Hz) δ 8.27 (s, 2H, H-arom.), 7.65 (s, 1H, H-arom.), 5.08 (quint, 1H, $J_{6,7}$ = $J_{6,7'}$ = $J_{6,5a}$ = $J_{6,5b}$ = 5.7, H-6), 4.38 (q, 1H, $J_{2,1}$ = $J_{2,3'}$ = $J_{2,3}$ = 3.3, H-2), 4.25-4.15 (m, 2H, H-1, H-7a), 3.71-3.62 (m, 2H, H-3, H-5), 3.53 (dd, 1H, $J_{5',5}$ = 12.0, H-5'), 3.33-3.28 (m, 1H, H-3'), 2.43-2.39 (m, 2H, H-7). ^{13}C-NMR (75.4 MHz, MeOD, δ ppm, *J* Hz) δ 183.4 (C=S), 142.9 (C-arom.), 132.7 (q, $^2J_{C,F}$ = 33.2, C-arom.), 124.7 (q, $^1J_{C,F}$ = 271.4, CF_3), 123.8-123.7 (m, C-arom.), 118.3-118.0 (m, C-arom.), 77.4 (C-1), 72.8 (C-2), 70.3 (C-7a), 60.0 (C-3), 59.3 (C-5), 55.5 (C-6), 34.2 (C-7). HRMS (ESI-ORBITRAP) m/z: $[M+H]^+$ calcd. for $C_{16}H_{18}F_6N_3O_2S$ 430.1018; Found 430.1011.

***N*-[(3,5-Bis(trifluoromethyl)phenyl)]-*N'*-[(1*S*,2*R*,6*S*,7a*S*)-1,2-dihydroxy-pyrrolizidin-6-yl]thiourea (3a):** Acidic deprotection of **9a** (52 mg, 0.11 mmol) followed by chromatographic purification (CH_2Cl_2:MeOH:NH_4OH, 5:1:0.01), afforded **3a** (47 mg, 0.11 mmol, cuant.) as a white solid. $[\alpha]_D^{24}$ -38.6 (*c* 0.75, MeOH). IR ν_{max} 3133 (OH, NH), 1275 (C=S), 1121, 681 cm^{-1}. ^{1}H-NMR (300 MHz, MeOD, δ ppm, *J* Hz) δ 8.29 (s, 2H, H-arom.), 7.65 (s, 1H, H-arom.), 5.14-5.04 (m, 1H, H-6), 4.74 (q, 1H, $J_{2,1}$ = $J_{2,3'}$ = $J_{2,3}$ = 3.6, H-2), 4.36 (dd, 1H, $J_{1,7a}$ = 6.0, H-1), 4.14-4.06 (m, 2H, H-7a, H-5), 3.67 (dd, 1H, $J_{3,3'}$ = 12.3, H-3), 3.46 (dd, 1H, H-3'), 3.20 (dd, 1H, $J_{5',5}$ = 11.7, $J_{5',6}$ = 8.7, H-5'), 2.82-2.73 (m, 1H, H-7), 2.21-2.10 (m, 1H, H-7'). ^{13}C-NMR (75.4

MHz, MeOD, δ ppm, *J* Hz) δ 183.5 (C=S), 143.0 (C-arom.), 132.7 (q, $^{2}J_{C,F}$ = 33.3, C-arom.), 124.7 (q, $^{1}J_{C,F}$ = 273.2, CF_3), 122.4-122.1 (m, C-arom.), 118.0-117.8 (m, C-arom.), 77.3 (C-1), 73.3 (C-2), 70.4 (C-7a), 60.1 (C-3), 58.0 (C-5), 55.7 (C-6), 33.9 (C-7). HRMS (ESI-ORBITRAP) m/z: $[M+H]^+$ calcd. for $C_{16}H_{18}F_6N_3O_2S$ 430.1018; Found 430.1016.

***N*-[(3,5-Bis(trifluoromethyl)phenyl)]-*N'*-[(1*S*,2*R*,6*R*,7a*S*)-1,2-*O*-isopropylidendioxy-pyrrolizidin-6-yl]urea (~~7A~~8A) and *N*-[(3,5-Bis(trifluoromethyl)phenyl)]-*N'*-[(1*S*,2*R*,6*S*,7a*S*)-1,2-*O*-isopropylidendioxy-pyrrolizidin-6-yl]urea (9A):** To a solution of diastereomeric amines **1** (49 mg, 0.25 mmol) in CH_2Cl_2 (2.5 mL), 3,5-bis(trifluoromethyl)phenylisoocyanate ((48 μL, 0.27 mmol) was added and the mixture stirred for 1 h at 0 ºC. After evaporation to dryness, the crude was purified by chromatography column (CH_2Cl_2:MeOH, 30:1→20:1) to give **8A** (28 mg, 0.06 mmol, 24%) and **9A** (71 mg, 0.16 mmol, 63%) as white solids. Data for **8A**: $[\alpha]_D^{23}$ -37.5 (*c* 1.1, CH_2Cl_2). IR ν_{max} 3316 (NH), 2927, 2862, 1692 (C=O), 1124, 704 cm^{-1}. ^{1}H-NMR (300 MHz, $CDCl_3$, δ ppm, *J* Hz) δ 8.32 (s, 1H, NH), 7.94 (s, 2H, H-arom.), 7.43 (s, 1H, H-arom.), 6.91 (d, 1H, $J_{NH,6}$ = 7.8, NH), 4.97 (dd, 1H, $J_{2,1}$ = 6.0, $J_{2,3'}$ = 4.5, H-2), 4.79-4.74 (m, 1H, H-6), 4.66 (d, 1H, H-1), 3.69 (m, 1H, $J_{7a,7'}$ = 11.7, $J_{7a,7'}$ = 6.3, H-7a), 3.42 (d, 1H, $J_{3,3'}$ = 12.3, H-3), 3.08 (dd, 1H, $J_{5,5'}$ = 12.9, $J_{5,6}$ = 6.6, H-5), 2.97 (dd, 1H, $J_{5',6}$ = 2.1, H-5'), 2.76 (dd, 1H, H-3'), 1.87 (dd, 1H, $J_{7,7'}$ = 13.5, $J_{7,7a}$ = 6.6, H-7), 1.75 (td, 1H, $J_{7',6}$ = 6.3, H-7'), 1.53, 1.38 (2s, 3H each, -C(CH_3)$_2$). ^{13}C-NMR (75.4 MHz, $CDCl_3$, δ ppm, *J* Hz) δ 154.7 (C=O), 141.5 (C-arom.), 132.2 (q, $^{2}J_{C,F}$ = 33.0, C-arom.), 123.5 (q, $^{1}J_{C,F}$ = 272.3, CF_3), 118.2-118.0 (m, C-arom.), 115.4-115.3 (m, C-arom.), 111.8 (-*C*$(CH_3)_2$), 82.5 (C-1), 81.4 (C-2), 71.2 (C-7a), 60.6 (C-5), 60.3 (C-3), 51.4 (C-6), 35.6 (C-7), 26.1 (-C(*C*H_3)$_2$), 24.2 (-C(*C*H_3)$_2$). HRMS (ESI-ORBITRAP) m/z: $[M+H]^+$ calcd. for $C_{19}H_{22}F_6N_3O_3$ 454.1560; Found 454.1560. Data for **9A**: $[\alpha]_D^{23}$ -22.1 (*c* 1.0, CH_2Cl_2). IR ν_{max} 3313 (NH), 2982, 2932, 1698 (C=O), 1123, 681 cm^{-1}. ^{1}H-NMR (300 MHz, $CDCl_3$, δ ppm, *J* Hz) δ 8.64 (s, 1H, NH), 7.79 (s, 2H, H-arom.), 7.38 (s, 1H, H-arom.), 6.68 (d, 1H, $J_{NH,6}$ = 6.0, NH), 4.89-4.85 (m, 1H, H-2), 4.60 (d, 1H, $J_{1,2}$ = 5.7, H-1), 4.33-4.21 (m, 1H, H-6), 3.52 (dd, 1H, *J* = 11.4, *J* = 6.9, H-7a), 3.41-3.32 (m, 2H, H-3, H-5), 3.05 (dd, 1H, $J_{3',3}$ = 12.0, $J_{3',2}$ = 4.5, H-3'), 2.66 (dd, 1H, $J_{5',5}$ = 12.0, $J_{5',6}$ = 6.6, H-5'), 2.38-2.29 (m, 1H, H-7), 1.63-1.53 (m, 1H, H-7'), 1.49, 1.31 (2s, 3H each, -C(CH_3)$_2$). ^{13}C-NMR (75.4 MHz, $CDCl_3$, δ ppm, *J* Hz) δ 155.4 (C=O), 140.9 (C-arom.), 132.2 (q, $^{2}J_{C,F}$ = 33.1, C-arom.), 123.3 (q, $^{1}J_{C,F}$ = 272.4, CF_3), 118.4-117.9 (m, C-arom.), 115.8-115.5 (m, C-arom.), 111.9 (-*C*$(CH_3)_2$), 83.2 (C-1), 80.9 (C-2), 71.0 (C-7a), 59.8 (C-5), 59.1 (C-3), 52.4 (C-6), 34.2 (C-7), 26.4 (-C(*C*H_3)$_2$), 24.4 (-C(*C*H_3)$_2$). HRMS (ESI-ORBITRAP) m/z: $[M+H]^+$ calcd. for $C_{19}H_{22}F_6N_3O_3$ 454.1560; Found 454.1560.

***N*-[(3,5-Bis(trifluoromethyl)phenyl)]-*N'*-[(1*S*,2*R*,6*R*,7a*S*)-1,2-dihydroxy-pyrrolizidin-6-yl]urea (2A):** Acidic deprotection of **8A** (32 mg, 0.07 mmol) followed by chromatographic

purification (CH_2Cl_2:MeOH:NH_4OH, 5:1:0.2), afforded **2A** (25 mg, 0.06 mmol, 85%) as a white solid. $[\alpha]_D^{26}$ -15.6 (*c* 0.78, MeOH). IR ν_{max} 3303 (OH, NH), 2937, 1675 (C=O), 1274, 1119, 681 cm^{-1}. ^{1}H-NMR (300 MHz, MeOD, δ ppm, *J* Hz) δ 8.00 (s, 2H, H-arom.), 7.48 (s, 1H, H-arom.), 4.32 (quint, 1H, $J_{6,7} = J_{6,7'} = J_{6,5} = J_{6,5'} = 5.7$, H-6), 4.24-4.20 (m, 1H, H-2), 3.86 (dd, 1H, $J_{1,7a} = 6.3$, H-1), 3.60-3.54 (m, 1H, H-7a), 3.19 (dd, 1H, $J_{3,3'} = 11.4$, H-3), 2.91-2.89 (m, 2H, H-5), 2.85 (dd, 1H, H-3'), 2.11-1.99 (m, 2H, H-7). ^{13}C-NMR (75.4 MHz, MeOD, δ ppm, *J* Hz) δ 157.0 (C=O), 143.3 (C-arom.), 133.1 (q, $^2J_{C,F} = 32.9$, C-arom.), 124.8 (q, $^1J_{C,F} = 271.5$, CF_3), 119.4-118.9 (m, C-arom.), 115.7-115.4 (m, C-arom.), 78.8-78.6 (m, C-1), 73.7-73.6 (m, C-2), 68.2-68.0 (m, C-7a), 61.2 (C-5), 60.4 (C-3), 51.9-51.8 (m, C-6), 36.8 (C-7). HRMS (ESI-ORBITRAP) m/z: $[M+H]^+$ calcd. for $C_{16}H_{18}F_6N_3O_3$ 414.1247; Found 414.1242.

***N*-[(3,5-Bis(trifluoromethyl)phenyl)]-*N'*-[(1*S*,2*R*,6*S*,7a*S*)-1,2-dihydroxy-pyrrolizidin-6-yl]urea (3A):** Acidic deprotection of **9A** (38 mg, 0.08 mmol) followed by chromatographic purification (CH_2Cl_2:MeOH:NH_4OH, 4:1:0.2), afforded **3A** (24 mg, 0.06 mmol, 70%) as a white solid. $[\alpha]_D^{26}$ -25.4 (*c* 0.77, MeOH). IR ν_{max} 3290 (OH, NH), 2924, 1674 (C=O), 1276, 1121, 681 cm^{-1}. ^{1}H-NMR (300 MHz, MeOD, δ ppm, *J* Hz) δ 8.00 (s, 2H, H-arom.), 7.48 (s, 1H, H-arom.), 4.35-4.26 (m, 2H, H-6, H-2), 3.95 (dd, 1H, *J* = 5.7, *J* = 4.5, H-1), 3.57-3.49 (m, 1H, H-7a), 3.38 (dd, 1H, $J_{5,5'} = 9.3$, $J_{5,6} = 6.3$, H-5), 3.15 (dd, 1H, $J_{3,3'} = 11.4$, $J_{3,2} = 4.2$, H-3), 2.91 (dd, 1H, $J_{3',2} = 4.8$, H-3'), 2.53-2.44 (m, 2H, H-7, H-5'), 1.67-1.61 (m, 1H, H-7'). ^{13}C-NMR (75.4 MHz, MeOD, δ ppm, *J* Hz) δ 156.9 (C=O), 143.2 (C-arom.), 133.1 (q, $^2J_{C,F}$ =33.0, C-arom.), 124.8 (q, $^1J_{C,F}$ =271.4, CF_3), 119.4-119.1 (m, C-arom.), 115.7-115.6 (m, C-arom.), 78.9 (C-1), 73.8 (C-2), 68.5 (C-7a), 60.7 (C-5), 59.9 (C-3), 52.8 (C-6), 36.9 (C-7). HRMS (ESI-ORBITRAP) m/z: $[M+H]^+$ calcd. for $C_{16}H_{18}F_6N_3O_3$ 414.1247; Found 414.1241.

(1*S*,2*R*,6*R*,7a*S*)-1,2-*O*-Isopropylidendioxy-6-[(4-(3,5-bis(trifluoromethyl)phenyl))-1*H*-1,2,3-triazole-1-yl)]pyrrolizidine (11) and (1*S*,2*R*,6*S*,7a*S*)-1,2-*O*-isopropylidendioxy-6-[(4-(3,5-bis(trifluoromethyl)phenyl))-1*H*-1,2,3-triazole-1-yl)]pyrrolidizidine (12): To a solution of diastereomeric amines **1** (100 mg, 0.50 mmol) in MeOH:H_2O (2:1) (2.1 mL), $NaHCO_3$ (189 mg, 2.00 mmol), $CuSO_4 \cdot 5H_2O$ (13 mg, 0.05 mmol) and a solution of NfN_3[23] (326 mg, 1.00 mmol) in Et_2O (1.4 mL) were added. After stirring at r.t for 6 h, 1-ethynyl-3,5-bis(trifluoromethyl)benzene (120 µL, 0.55 mmol) and sodium ascorbate (150 mg, 0.75 mmol) were added and the mixture was stirred at r.t. overnight. Then, solvent was evaporated and the residue dissolved in CH_2Cl_2 and washed with sat. aq. soln. of $NaHCO_3$. The organic phase was dried (Na_2SO_4), filtered and evaporated and the crude product was purified through chromatography column on silica gel (Et_2O:acetone, 15:1→ 6:1) to give **11** (41 mg, 0.09 mmol, 18%) and **12** (115 mg, 0.25 mmol, 50%) as yellowish oils. Data for **11**: $[\alpha]_D^{25}$ -14.6 (*c* 0.87, CH_2Cl_2). IR ν_{max} 2927, 1277, 1126, 682 cm^{-1}. ^{1}H-NMR (300 MHz, $CDCl_3$, δ ppm, *J* Hz) δ 8.28

(s, 2H, H-arom.), 8.11 (s, 1H, H-5(triazole)), 7.82 (s, 1H, H-arom.), 5.47-5.40 (m, 1H, H-6), 4.92 (td, 1H, $J_{2,3'}$ = 6.0, J = 2.1, H-2), 4.60 (dd, 1H, J = 6.0, J = 1.8, H-1), 3.92-3.86 (m, 1H, H-7a), 3.42-3.37 (m, 3H, H-5, H-5', H-3), 2.92 (dd, 1H, $J_{3,3'}$ = 12.0, H-3'), 2.46 (ddd, 1H, $J_{7,7'}$ = 14.4, J = 7.5, J = 2.4, H-7), 2.30-2.19 (m, 1H, H-7'), 1.56, 1.35 (2s, 3H each, -C(CH_3)$_2$). ^{13}C-NMR (75.4 MHz, $CDCl_3$, δ ppm, J Hz) δ 145.7 (C-4(triazole)), 133.0 (C-arom.), 132.5 (q, $^2J_{C,F}$ = 33.4, C-arom.), 126.0-125.7 (m, C-arom.), 123.5 (q, $^1J_{C,F}$= 272.4, CF_3), 121.9-121.7 (m, C-arom.), 119.5 (C-5(triazole)), 112.6 (-C(CH$_3$)$_2$), 84.8 (C-1), 81.4 (C-2), 70.5 (C-7a), 61.4 (C-6), 60.5 (C-5), 59.2 (C-3), 36.2 (C-7), 27.1 (-C(CH$_3$)$_2$), 25.1 (-C(CH$_3$)$_2$). HRMS (ESI-ORBITRAP) m/z: $[M+H]^+$ calcd. for $C_{20}H_{21}F_6N_4O_2$ 463.1563; Found 463.1559. Data for **12**: $[\alpha]_D^{25}$ -37.5 (*c* 0.57, CH_2Cl_2). IR ν_{max} 2925, 1277, 1127, 682 cm^{-1}. ^{1}H-NMR (300 MHz, $CDCl_3$, δ ppm, J Hz) δ 8.26 (s, 2H, H-arom.), 7.94 (s, 1H, H-5(triazole)), 7.83 (s, 1H, H-arom.), 5.19-5.09 (m, 1H, H-6), 4.94-4.91 (m, 1H, H-2), 4.69 (brd, 1H, J = 6, H-1), 3.76-3.69 (m, 2H, H-5, H-7a), 3.40-3.21 (m, 3H, H-5', H-3, H-3'), 2.70-2.61 (m, 1H, H-7), 2.36-2.25 (m, 1H, H-7'), 1.56, 1.34 (2s, 3H each, -C(CH_3)$_2$). ^{13}C-NMR (75.4 MHz, $CDCl_3$, δ ppm, J Hz) δ 145.4 (C-4(triazole)), 132.7 (C-arom.), 132.5 (q, $^2J_{C,F}$ = 33.6, C-arom.), 125.9-125.6 (m, C-arom.), 123.5 (q, $^1J_{C,F}$ = 272.5, CF_3), 122.0-121.7 (m, C-arom.), 120.9-120.7 (C-5(triazole)), 112.0 (-C(CH$_3$)$_2$), 83.7 (C-1), 81.3 (C-2), 70.4 (C-7a), 62.6 (C-6), 59.2 (C-5), 59.1 (C-3), 35.3 (C-7), 26.8 (-C(CH$_3$)$_2$), 24.9 (-C(CH$_3$)$_2$). HRMS (ESI-ORBITRAP) m/z: $[M+H]^+$ calcd. for $C_{20}H_{21}F_6N_4O_2$ 463.1563; Found 463.1574.

(1*S*,2*R*,6*R*,7a*S*)-1,2-Dihydroxy-6-[(4-(3,5-bis(trifluoromethyl)phenyl))-1*H*-1,2,3-triazole-1-yl)]pyrrolizidine (4): Acidic deprotection of **11** (28.3 mg, 0.06 mmol) followed by chromatographic purification (CH_2Cl_2:MeOH:NH_4OH, 8:1:0.01), afforded **4** (16.3 mg, 0.04 mmol, 63%) as a white solid. $[\alpha]_D^{26}$ -0.5 (*c* 1.17, MeOH). IR ν_{max} 3324 (OH), 2935, 2851, 1277, 1123, 702 cm^{-1}. ^{1}H-NMR (300 MHz, MeOD, δ ppm, J Hz) δ 8.74 (s, 1H, H-5(triazole)), 8.44 (s, 2H, H-arom.), 7.93 (s, 1H, H-arom.), 5.42-5.35 (m, 1H, H-6), 4.32-4.27 (m, 1H, H-2), 3.96 (dd, 1H, $J_{1,7a}$ = 6.9, H-1), 3.74 (q, 1H, $J_{7a,7}$ = $J_{7a,7'}$ = 6.9, H-7a), 3.44 (dd, 1H, $J_{5,5'}$ = 11.7, $J_{5,6}$ = 4.2, H-5), 3.27-3.19 (m, 2H, H-3, H-5'), 2.92 (dd, 1H, $J_{3',3}$ = 11.7, H-3'), 2.69-2.60 (m, 1H, H-7), 2.43-2.34 (m, 1H, H-7'). ^{13}C-NMR (75.4 MHz, MeOD, δ ppm, J Hz) δ 146.0 (C-4(triazole)), 134.7 (C-arom.), 133.5 (q, $^2J_{C,F}$ = 33.4, C-arom.), 126.7-126.6 (m, C-arom.), 124.8 (q, $^1J_{C,F}$ = 271.7, CF_3), 122.8 (C-5(triazole)), 122.4-122.3 (m, C-arom.), 78.8 (C-1), 74.2 (C-2), 68.5 (C-7a), 63.1 (C-6), 61.2 (C-5), 60.3 (C-3), 36.8 (C-7). HRMS (ESI-ORBITRAP) m/z: $[M+H]^+$ calcd. for $C_{17}H_{17}F_6N_4O_2$ 423.1250; Found 423.1244.

(1*S*,2*R*,6*S*,7a*S*)-1,2-Dihydroxy-6-[(4-(3,5-bis(trifluoromethyl)phenyl))-1*H*-1,2,3-triazole-1-yl)]pyrrolidizidine (5): Acidic deprotection of **12** (83 mg, 0.18 mmol) followed by chromatographic purification (CH_2Cl_2:MeOH:NH_4OH, 5:1:0.02), afforded **5** (65 mg, 0.16 mmol, 86%) as a white solid. $[\alpha]_D^{26}$ -25.6 (*c* 0.98, MeOH). IR ν_{max} 3319 (OH), 2925, 1277,

1121, 682 cm^{-1}. ^{1}H-NMR (300 MHz, MeOD, δ ppm, *J* Hz) δ 8.77 (s, 1H, H-5(triazole)), 8.44 (s, 2H, H-arom.), 7.94 (s, 1H, H-arom.), 5.31-5.21 (m, 1H, H-6), 4.34 (q, 1H, $J_{2,1} = J_{2,3} = J_{2,3'} =$ 4.2, H-2), 4.05 (dd, 1H, $J_{1,7a} = 5.7$, H-1), 3.75-3.67 (m, 2H, H-5, H-7a), 3.25-3.20 (m, 2H, H-3', H-5'), 3.05 (dd, 1H, $J_{3,3'} = 11.4$, H-3), 2.88-2.79 (m, 1H, H-7), 2.43-2.32 (m, 1H, H-7'). ^{13}C-NMR (75.4 MHz, MeOD, δ ppm, *J* Hz) δ 146.0 (C-4(triazole)), 134.5 (C-arom.), 133.7 (q, $^2J_{C,F}$ = 33.4, C-arom.), 126.8-126.6 (m, C-arom.), 124.7 (q, $^1J_{C,F} = 271.6$, CF_3), 123.4 (C-5(triazole)), 122.6-122.3 (m, C-arom.), 78.6 (C-1), 74.0 (C-2), 68.7 (C-7a), 62.4 (C-6), 60.3 (C-5), 60.0 (C-3), 36.8 (C-7). HRMS (ESI-ORBITRAP) m/z: $[M+H]^+$ calcd. for $C_{17}H_{17}F_6N_4O_2$ 423.1250; Found 423.1239.

(1*S*,2*R*,6*S*,7a*S*)-6-Amino-1,2-*O*-dihydroxy-pyrrolizidine (16): Boc_2O (509 mg, 2.33 mmol) was added to a solution of amines **1** (272.6 mg, 1.37 mmol) in MeOH (6 mL) and the mixture was stirred at r.t. for 8 h. Evaporation of the solvent and chromatographic purification on silica gel (CH_2Cl_2:MeOH, 30:1) afforded **14** (199 mg, 0.67 mmol, 49%) and **15** (76 mg, 0.25 mmol, 18%) as white solids. Compound **14** (90.9 mg, 0.3 mmol) was dissolved in THF (1.5 mL) and 4M HCl (1.5 mL) was added. The solution was stirred at r.t. for 6 h. Evaporation of the solvent and chromatographic purification on Dowex 50WX8 eluting with MeOH, H_2O and NH_4OH 25%, afforded **16** (47.1 mg, 0.29 mmol, 99%) as a brown solid. $[\alpha]_D^{26}$ -21.1 (*c* 0.74, MeOH). ^{1}H-NMR (300MHz, MeOD, δ ppm, *J* Hz) δ 4.28 (ap q, 1H, $J_{2,3b}$= 5.1, $J_{2,3a}$= 4.8, $J_{2,1}$= 4.5, H-2), 3.90 (ap t, 1H, $J_{1,7a}$= 5.1, H-1), 3.51-3.39 (m, 2H, H-6, H-7a), 3.21 (dd, 1H, $J_{5a,6}$= 6.0, $J_{5a,5b}$= 9.0, H-5a), 3.08 (dd, 1H, $J_{3a,3b}$= 11.4, H-3a), 2.86 (dd, 1H, H-3b), 2.38-2.30 (m, 1H, H-7), 2.28 (t, 1H, $J_{5b,6}$= 9.5, H-5b), 1.45-1.35 (m, 1H, H-7'). ^{13}C-NMR (75.4 MHz, MeOD, δ ppm, *J* Hz) 78.9 (C-1), 73.6 (C-2), 69.1 (C-6), 63.7 (C-5), 59.6 (C-3), 54.4 (C-7a), 39.8 (C-7). HRMS (ESI-ORBITRAP) m/z: $[M+H]^+$ calcd. for $C_7H_{15}N_2O_2$ 159.1128; Found 159.1123.

***N*-(2-Naphthyl)-*N'*-[(1*S*,2*R*,6*S*,7a*S*)-1,2-dihydroxy-pyrrolizidin-6-yl]thiourea (3i):** To a solution of amine **16** (41 mg, 0.26 mmol) in MeOH (2.0 mL), 2-naphthyl isocyanate (55.6 mg, 0.3 mmol) was added and the mixture stirred for 5 h at r.t. After evaporation to dryness, the crude was purified by chromatography column (CH_2Cl_2:MeOH, 1:1) to give **3i** (51 mg, 0.15 mmol, 57%) as a white solid. $[\alpha]_D^{26}$ -16.8 (*c* 0.67, $CDCl_3$:MeOH 1:1). ^{1}H-NMR (300MHz, MeOD:$CDCl_3$ 1:1, δ ppm, *J* Hz) δ 7.92-7.87 (m, 3H, Arom), 7.56-7.50 (m, 3H, Arom), 7.43 (dd, 1H, $J_{H,H}$= 7.5, $J_{H,H}$= 1.2, Arom) 5.06 (m, 1H, H-6) 4.13 (q, 1H, $J_{2,1} = J_{2,3a} = J_{2,3b} = 4.6$, H-2), 3.72 (t. 1H, $J_{1,7a}$= 4.6, H-1), 3.45-3.35 (m, 2H, H-5a, H-7a), 2.99 (dd, 1H, $J_{3a,3b}$=11.7, H-3a), 2.69 (dd, 1H, H-3b) 2.46-2.37 (m, 1H, H-7), 2.25 (t, 1H, $J_{5b,5a} = J_{5b,6}$= 9.4, H-5b), 1.42 (q, 1H, $J_{7',7} = J_{7',6} = J_{7',7a} = 10.6$, H-7'). ^{13}C-NMR (75.4 MHz, $CDCl_3$:MeOH 1:1, δ ppm) δ 182.4 (C=S), 135.3, 130.7, 129.0, 128.9, 127.5, 127.2, 126.2, 125.9, 123.1 (Arom), 78.1 (C-1), 72.9 (C-2), 67.7 (C-

7a), 59.5 (C-5), 59.2 (C-3), 56.7 (C-6), 35.8 (C-7). HRMS (ESI-ORBITRAP) m/z: $[M+H]^+$ calcd. for $C_{18}H_{22}N_3O_2S$ 344.1427; Found 344.1423.

X-ray Structural Analysis of compounds 8a and 9a·HCl.

Crystals of suitable size for X-ray diffraction analysis of **8a** and **9a·HCl** were coated with dry perfluoropolyether and mounted on glass fibres and fixed in a cold nitrogen stream (T = 213 K) to the goniometer head. Data collection was performed on a Bruker-Nonius X8Apex-II CCD diffractometer, using monochromatic radiation λ(Mo K_α) = 0.71073 Å, by means of ω and φ scans with a width of 0.50 degree. The data were reduced (SAINT)[28] and corrected for absorption effects by the multi-scan method (SADABS).[29] The structures were solved by direct methods (SIR-2002)[30] and refined against all F^2 data by full-matrix least-squares techniques (SHELXL-2016/6)[31] minimizing $w[F_o^2-F_c^2]^2$. All non-hydrogen atoms were refined anisotropically. The hydrogen atoms were included from calculated positions and refined riding on their respective carbon atoms with isotropic displacement parameters. One CF_3- group of the compound **8a** was found to be clearly disordered and was modelled in two positions with the same occupation coefficient. A search for solvent accessible voids in the crystal structure for both compounds using PLATON[32], showed a potential solvent volume, impossible to model even with the most severe restraints. The corresponding CIF data represent SQUEEZE[33] treated structures, with the undefined solvent excluded from the structural model. The SQUEEZE results were appended to the CIF. A summary of cell parameters, data collection, structure solution, and refinement for these two crystal structures are given bellow. The corresponding crystallographic data were deposited with the Cambridge Crystallographic Data Centre as supplementary publications. The data can be obtained free of charge *via* www.ccdc.ac.uk/data.request/cif.

Crystal data for 8a: $C_{19}H_{21}F_6N_3O_2S•CH_2Cl_2$, M = 554.37, a = 9.9180(4) Å, b = 9.8167(4) Å, c = 12.7446(5) Å, α = 90°, β = 99.023(2)°, γ = 90°, V = 1225.49(9) Å^3, T = 193(2) K, space group $P2_1$, Z = 2, 18120 reflections measured, 4446 independent reflections (R_{int} = 0.0289). The final R_1 values were 0.0494 ($I > 2\sigma(I)$). The final $wR(F^2)$ values were 0.1448 ($I > 2\sigma(I)$). The final R_1 values were 0.0514 (all data). The final $wR(F^2)$ values were 0.1465 (all data). The goodness of fit on F^2 was 1.047. Flack parameter = 0.04(2). CCDC 1834757.

Crystal data for 9a.HCl: $C_{19}H_{22}F_6N_3O_2S•Cl$, M = 505.90, a = 12.9162(4) Å, b = 31.0699(10) Å, c = 5.8565(2) Å, α = 90°, β = 90°, γ = 90°, V = 2350.24(13) Å^3, T = 193(2) K, space group $P2_12_12$, Z = 4, 26691 reflections measured, 4121 independent reflections (R_{int} = 0.0363). The final R_1 values were 0.0554 ($I > 2\sigma(I)$). The final $wR(F^2)$ values were 0.1344 ($I > 2\sigma(I)$). The final R_1 values were 0.0599 (all data). The final $wR(F^2)$ values were 0.1369 (all data). The goodness of fit on F^2 was 1.150. Flack parameter = -0.03(2). CCDC 1834758.

Supporting information

^{1}H- and ^{13}C-NMR spectra for all the new compounds. NOESY spectra for **2A** and **4**. Analysis of the library by ESI-MS spectra. Lineweaver-Burk plots and secondary representations for inhibitor **3i**. X-ray crystallography data for **8a** and **9a**.

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