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Article

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Discovery, Structure-Activity Relationship and Binding Mode of Imidazo[1,2-*a*]pyridine Series of Autotaxin Inhibitors

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KEYWORDS Autotaxin, ENPP-2, Lysophosphatidic acid, Lysophosphatidyl choline, FS-3.

ABSTRACT

Autotaxin (ATX) is a secreted enzyme playing a major role in the production of lysophosphatidic acid (LPA) in blood through hydrolysis of lysophosphatidyl choline (LPC). The ATX-LPA signaling axis arouses a high interest in the drug discovery industry as it has been implicated in several diseases including cancer, fibrotic diseases and inflammation among others. An imidazo[1,2-*a*]pyridine series of ATX inhibitors was identified out of a high throughput screening (HTS). A co-crystal structure with one of these compounds and ATX revealed a novel binding mode with occupancy of the hydrophobic pocket and channel of ATX but no interaction with zinc ions of the catalytic site. Exploration of the structure-activity relationship led to compounds displaying high activity in biochemical and plasma assays, exemplified by compound **40**. Compound **40** was also able to decrease the plasma LPA levels upon oral administration to rats.

INTRODUCTION

Autotaxin (ATX) also known as ENPP-2 (ectonucleotide pyrophosphatase / phosphodiesterase 2) or lysophospholipase D is a secreted protein that belongs to the ENPP family of enzymes which is composed of seven members. ATX is the only enzyme of the ENPP family with lysophospholipase D (lysoPLD) activity and is responsible for the hydrolysis of lysophosphatidyl choline (LPC), an abundant plasma phospholipid, towards lysophosphatidic acid (LPA).[1] LPA represents a family of bioactive lipids of varying fatty acyl chain length that signal through at least 6 LPA receptors (LPA_{1-6}) to control a broad range of cellular events (Figure 1).[2]

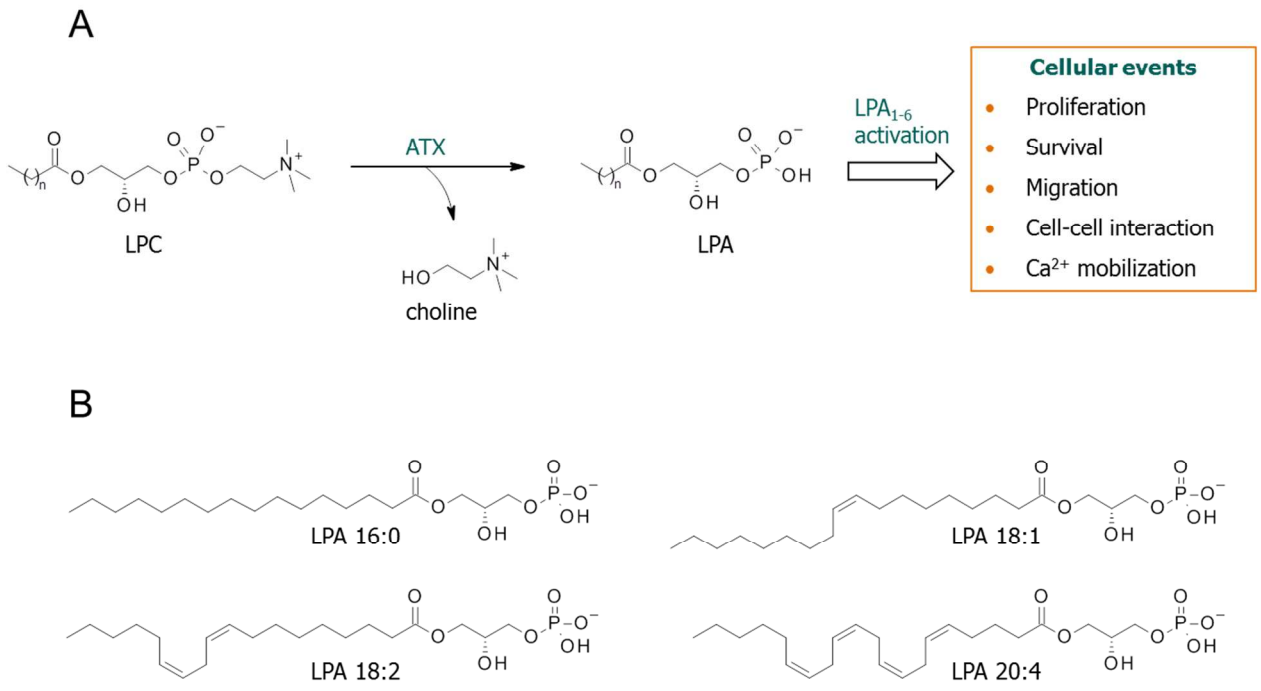


Figure 1. (A) ATX-mediated conversion of LPC into LPA and summary of cellular events triggered by LPA receptor activation. (B) Examples of abundant LPA species in plasma.

In recent years numerous ATX inhibitors were described in the literature and patented with possible application in the treatment of diverse pathologies such as cancer, fibrotic diseases, inflammation, pain and angiogenesis among others.[3] At Galapagos, an ATX drug discovery program was initiated using a classical screening approach, aiming at developing novel therapeutics for fibrotic diseases. The monitoring of ATX-mediated cleavage of LPC requires either LC-MS/MS detection methods for LPA determination or a cascade of biochemical transformations to measure the amount of choline released (Figure 2). As both techniques were not deemed suitable for high-throughput screening (HTS) purposes the use of an alternative fluorogenic substrate was envisaged. A screening campaign was performed on a diverse set of approximately 100,000 compounds using a biochemical assay with the FS-3 fluorogenic substrate as LPC surrogate.[4] Cleavage of FS-3 by ATX leads to the release of a fluorophore,

inhibition of which can be directly measured by fluorescence detection. Several chemical series of ATX inhibitors were identified out of the screening campaign. Optimization and structure activity relationship (SAR) of an imidazo[1,2-*a*]pyridine series to identify lead compound **40** and discussion of the binding modes of ATX inhibitors will be described in this article. Structural modifications of compound **40** leading to the discovery of clinical candidate GLPG1690 (**55**) have recently been disclosed.[5-7]

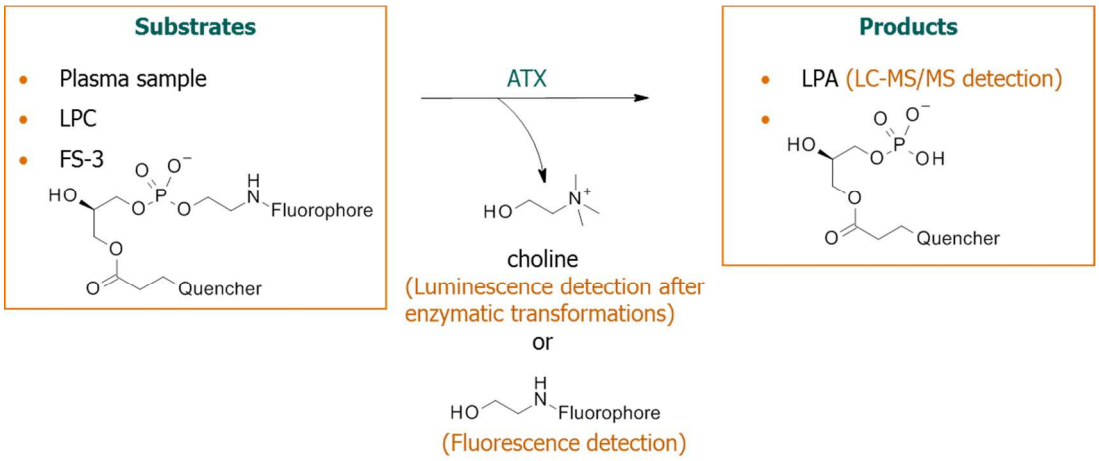


Figure 2. Methods to assess ATX activity *in vitro* and in plasma samples.

RESULTS AND DISCUSSION

Structure-activity relationship

Out of HTS efforts a N-benzyl-2-ethyl-N-methyl-imidazo[1,2-a]pyridin-3-amine series hit series was identified with best compounds displaying an IC_{50} around 30 nM with the FS-3 substrate based assay (FS-3 assay) (Figure 3). Since plasma naturally contains ATX and LPC but not membrane-bound lipid phosphate phosphatases responsible for LPA degradation, incubation of plasma at 37°C leads to the production of LPA that can be measured by LC-MS/MS. Monitoring the inhibition of LPA production by compounds in this plasma assay allowed to evaluate their

inhibitory activity and the impact of plasma proteins on this activity. Best N-benzyl-2-ethyl-N-methyl-imidazo[1,2-a]pyridin-3-amine derivatives were able to show roughly 50% reduction of LPA production at 10 μM. Those compounds suffered from poor metabolic stability which precluded their use in vivo. Compound **1** with improved pharmacokinetic properties was identified as an early lead compound: **1** had a biochemical IC_{50} of 122 nM (FS-3 assay) and displayed 39% bioavailability in rat and low clearance (0.15 L h^{-1} kg^{-1}). However this compound only weakly inhibited the production of LPA in rat plasma, probably due to an insufficient potency.

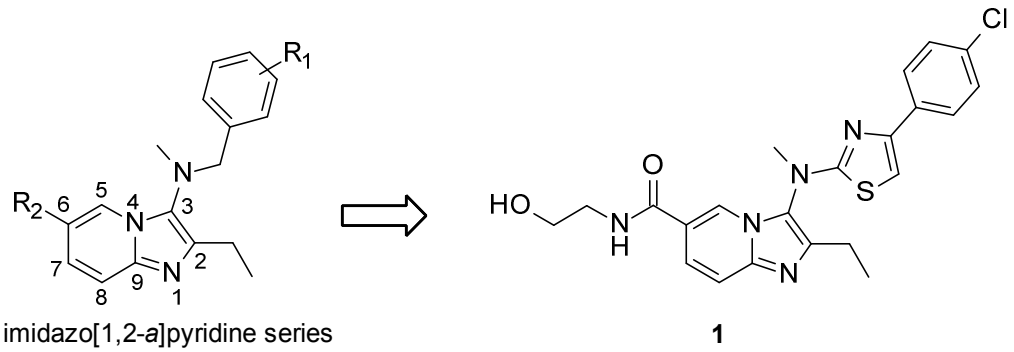
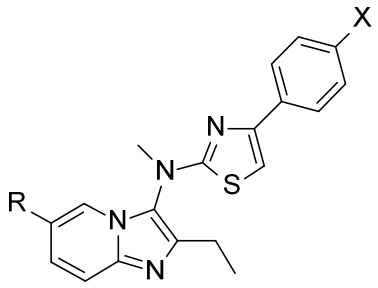


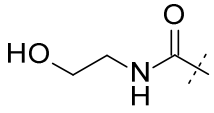
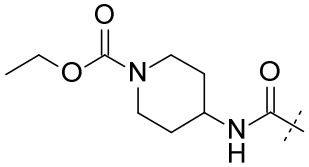
Figure 3. Hit series and compound **1**.

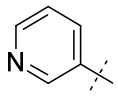
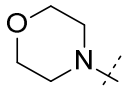
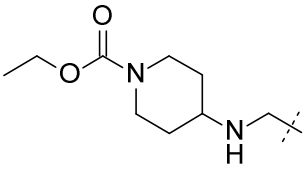
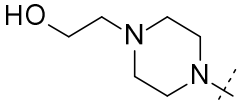
Exploration of the substitution in position 6 of the imidazo[1,2-*a*]pyridine led to the identification of very potent inhibitors **2-6** of ATX mediated FS-3 cleavage (Table 1). Despite nanomolar activity in the FS-3 assay, those compounds displayed only micromolar activity in the biochemical assay using natural substrate LPC. These results clearly indicated divergence in the structure-activity relationships with the natural substrate LPC or with the artificial substrate FS-3. In order to assess the biological relevance of the data returned by biochemical assays, the inhibition of LPA production in plasma was evaluated. Compounds from Table 1 appeared weakly active in this plasma assay, which illustrates the strong disconnect with the results

obtained in the biochemical FS-3 assay. In contrast a better correlation with little to no shift was observed when the potency of compounds for inhibiting the conversion of LPC *in vitro* was compared to the potency in the plasma assay. At this point we hypothesized that the probe substrate used in the FS-3 assay, although mimicking LPC as a substrate, might bind differently to the enzyme owing to the steric bulk associated with the fluorophore and quencher groups. A different binding mode of the substrates used in the different ATX assays might result in differences in the ability of ATX inhibitors to compete with these, possibly explaining discrepancies between the outcome of the FS-3 assay and phenotypic assays.[8] As a consequence further explorations were carried out using LPC as substrate to assess the activity of compounds.

Table 1. Inhibitory activity of compounds 1-6 in biochemical and plasma assays [a]



Compound	R	X	ATX FS-3 (IC_{50}, nM) [b]	ATX LPC (IC_{50}, nM) [b]	Plasma activity (IC_{50}, nM)
1		Cl	122 ± 17	>10,000	> 50,000 [c]
2		Cl	5	6,555	12,555

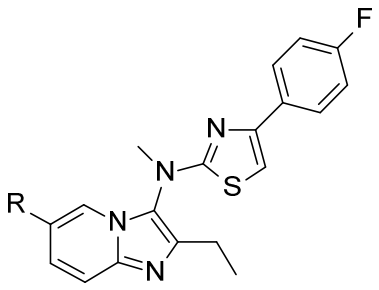
3		F	2	>10,000	> 50,000 [c]
4		F	8	6,800	> 50,000 [c]
5		Cl	9 ± 6	1,210 ± 212	< 5,000 [c, d]
6		F	12 ± 2	2,359 ± 404	6,080

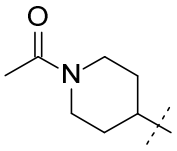
[a] ATX FS-3 and ATX LPC activities were measured using FS-3 and LPC 16:0 as substrate respectively. Plasma activity was evaluated by incubating rat plasma at 37°C for 2 h and comparing LPA 18:2 production in presence or absence of compound. [b] Standard deviations indicated when IC_{50} determination was replicated at least twice. [c] Compounds evaluated at concentrations of 0.5 μM, 5 μM and 50 μM (n = 3). Compounds with IC_{50} indicated > 50,000 nM showed less than 50% reduction of LPA production after incubation at all concentrations. [d] 7% reduction of LPA production at 0.5 μM, 67% at 5 μM, 100% at 50 μM.

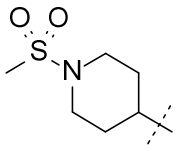
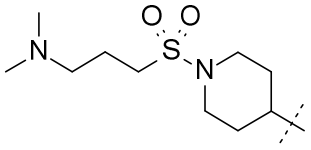
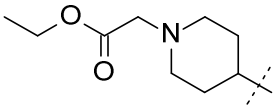
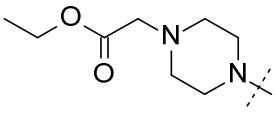
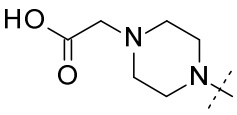
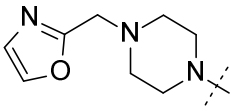
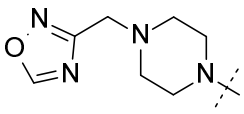
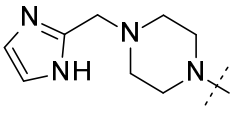
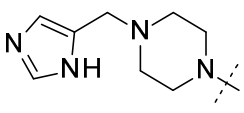
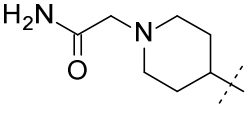
The better potencies in the LPC assay of compounds **5** and **6** *versus* **2** and **4** respectively suggested that a basic amine and an extended substituent at position 6 bearing an H-bond accepting or donating group could impart ATX inhibitory activity. As depicted in Table 2, imidazo[1,2-a]pyridine derivatives **7**-**22** bearing a substituted piperidine or piperazine at position 6 were explored. Compound **8** was identified as a submicromolar inhibitor of ATX, the methyl sulfonamide in compound **8** provided approximately a 2-fold gain of activity versus acetamide group in compound **7**. Compound **8** was also active in rat plasma with an IC_{50} of 1,033 nM. Side-chain extension with a basic group as in compound **9** further improved activity. Alternatively N-substitution with an ethyl acetate group on the piperidine or the piperazine ring provided

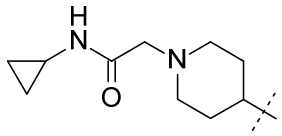
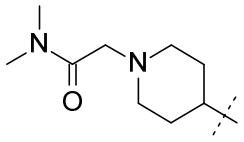
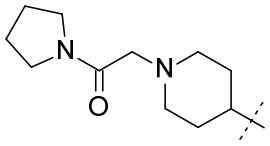
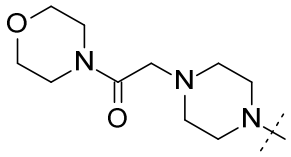
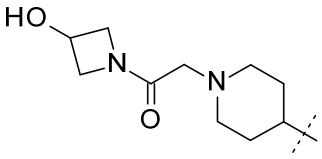
compounds **10** and **11** which displayed notable biochemical potencies. These data suggested that a substituent bearing a basic nitrogen atom and a distal H-bond accepting group was preferred at position 6. However the latter compounds were weakly active in plasma due to plasma instability arising probably from ester cleavage, parent carboxylic acid derivative **12** being poorly active. Investigation of heteroaromatic rings as replacement of the ester function in compounds **13-16** led to a loss of activity in most cases. Only compound **16** retained interesting biochemical potency but the compound had low activity in plasma (IC_{50} = 4,100 nM). Interestingly, whereas unsubstituted and monosubstituted amides were detrimental for activity (compounds **17** and **18**), replacement of the ester function by a disubstituted amide as in compounds **19-22** afforded potent compounds in the biochemical and plasma assays. Compounds **19** and **20** displayed IC_{50} of 476 nM and 115 nM respectively in plasma.

Table 2. SAR in position 6 of the imidazo[1,2-*a*]pyridine ring [a]



Compound	R	ATX LPC (IC_{50}, nM) [b]
7		1,624

8		710 ± 42
9		357 ± 53
10		480 ± 102
11		280 ± 70
12		9,163 ± 4,527
13		1,521 ± 280
14		1,708 ± 611
15		> 4,100
16		771 ± 108
17		2,372 ± 659

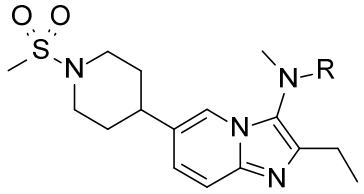
18		1,614 ± 292
19		261 ± 32
20		86 ± 20
21		231 ± 73
22		246 ± 23

[a] ATX LPC activities were measured using LPC 16:0 as substrate. [b] Standard deviations indicated when IC_{50} determination was replicated at least twice.

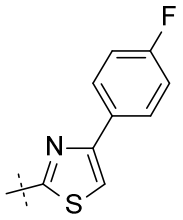
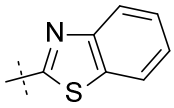
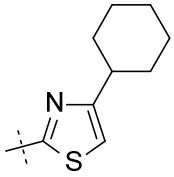
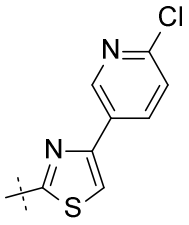
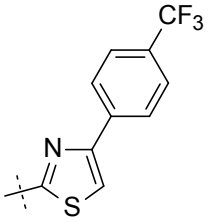
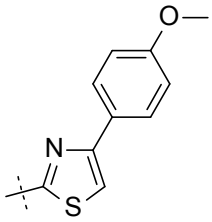
In parallel, exploration was carried out on the substituent in position 3 of the imidazo[1,2-*a*]pyridine scaffold. Early investigations showed that the thiazole ring was important for activity: replacing the thiazole ring by either a phenyl ring or heteroaromatic rings such as 1,2,4-oxadiazole or 1,3,4-oxadiazole rings led to significant loss of activity (data not shown). Therefore investigations focused on the substitution of the thiazole ring as in compounds **23-39** (Table 3). Compound **23** illustrates the loss of activity when the phenyl and thiazole rings were fused. Cyclohexyl replacing 4-fluorophenyl ring in compound **24** maintained activity, albeit with

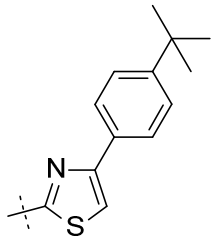
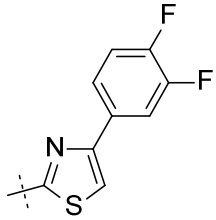
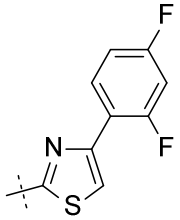
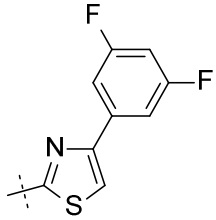
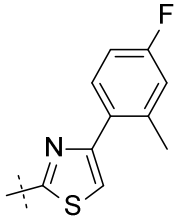
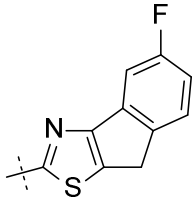
a slight loss of potency. The 4-fluorophenyl ring could also be replaced by a 4-chloro-3-pyridine moiety without any loss of activity in compound **25**. Replacing the *para*-fluorine substituent on the phenyl ring by other halogens, a trifluoromethyl group (**26**) or a methoxy substituent (**27**) was tolerated. However bulky substituents such as *tert*-butyl group (**28**) had a detrimental impact on activity. Di-halogenated compounds **29** and **30** were less potent than the parent compound **8**. The importance of the *para* substitution was well established by the significant loss in potency with the *meta*, *meta*-difluoro compound **31**. Interestingly compound **32** bearing an *ortho*-methyl substituent displayed similar potency compared to analogue **8**. This good result inspired the preparation of rigidified tricyclic analogs **33** and **34**, however the latter compounds only maintained similar level of activity and were not pursued any further. When the *ortho*-methyl substituent of **32** was replaced by a nitrile group in compound **35**, a remarkable improvement of activity was obtained. Although introduction of a methyl group in position 5 of the thiazole decreased potency (**36** *versus* **8**), introduction of a nitrile group in **37** afforded a very potent compound. Replacing the nitrile group by a carboxamide (**38**) or a carboxylic acid (**39**) resulted in a drop of potency.

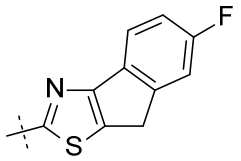
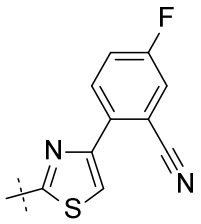
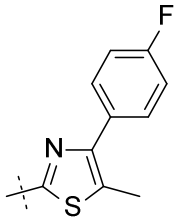
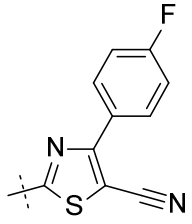
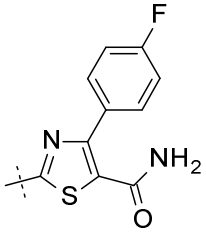
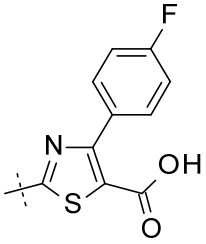
Table 3. SAR on the thiazole ring [a]



Compound	R	ATX LPC (IC_{50}, nM) [b]

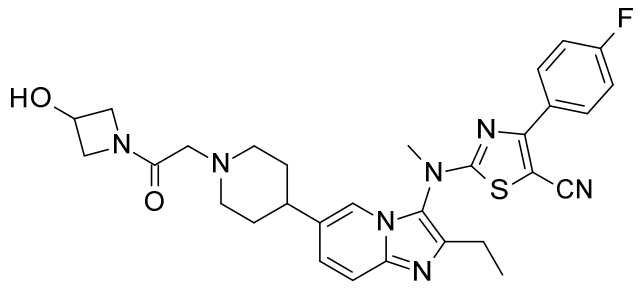
8		710 ± 42
23		> 10,000
24		1,904 ± 359
25		493 ± 11
26		1,001 ± 71
27		1,684 ± 161

28		7,609 ± 707
29		1,899 ± 650
30		2,021 ± 433
31		4,246
32		533 ± 157
33		804 ± 125

34		1,212 ± 25
35		126 ± 37
36		2,266 ± 344
37		138 ± 26
38		1,410 ± 388
39		> 10,000

[a] ATX LPC activities were measured using LPC 16:0 as substrate. b Standard deviations indicated when IC50 determination was replicated at least twice.

Taken together these data indicated that a *para*-substituted 4-phenylthiazole substituent was preferred at position 3 and the introduction of a nitrile group on the *ortho* position of the phenyl ring or on the thiazole ring afforded a significant improvement of activity; our hypothesis to explain this gain of activity will be discussed later in this article. Structural modifications in positions 3 and 6 of the imidazo[1,2-*a*]pyridine scaffold were combined and compound **40**, displaying potencies of 27 nM and 22 nM in the biochemical and rat plasma assays, respectively, was identified as lead compound in the series (Figure 4).



40

Figure 4. Structure of compound **40**.

Co-crystal structure and binding mode discussion

The first experimental structures of ATX (rat ortholog) were concurrently published by two different groups in 2011 revealing important structural features that explain the uncommon biochemical properties of this enzyme within the ENPP family as well as the binding mode of different LPA ligands and inhibitors.[13-15] Based on sequence and structural homology, ATX is composed of 2 somatomedin B-like domains followed by the catalytic ENPP domain and a nuclease-like domain maintained in close contact with the catalytic domain thanks to a peptide

linker (or "lasso") and a disulfide bridge. The catalytic domain reveals 3 pockets arranged in a T-shaped groove: a catalytic pocket that exhibits a nucleophilic threonine residue (Thr^{210} in the human ortholog) and 2 adjacent zinc ions, a hydrophobic pocket that accommodates the hydrophobic tail of a variety of LPC substrates and a so-called hydrophobic channel (or "tunnel") which is proposed to play an important role in the transport and local delivery of LPA products and other hydrophobic substances.[16-18] In order to resolve the binding mode of compound **9**, a dedicated construct of human ATX was produced and purified in house providing a batch of protein suitable for crystallography (see Supporting Information). Co-crystals were obtained and diffracted with a maximum resolution of 2.7 Å, enabling the construction of an experimental model useful to assist and verify drug design hypothesis. Compound **9** binds to the catalytic domain with its imidazo[1,2-a]pyridine core occupying a pivotal spot in the center of the T-shaped groove, orienting each of its 3 substituents towards each of the 3 pockets (Figure 5). The ethyl group in position 2 points in the direction of the catalytic pocket, while substituents in positions 3 and 6 occupy the hydrophobic pocket and hydrophobic channel respectively. In addition to Van der Waals contacts essentially driven here by hydrophobic effects, **9** displays a hydrogen bond between a sulfonamide oxygen and the indole NH of Trp^{261} and potentially a second one, albeit not certain given the electron density, between the basic nitrogen of the C6 substituent and the backbone carbonyl groups of $Trp^{255}$and Gly^{257}.

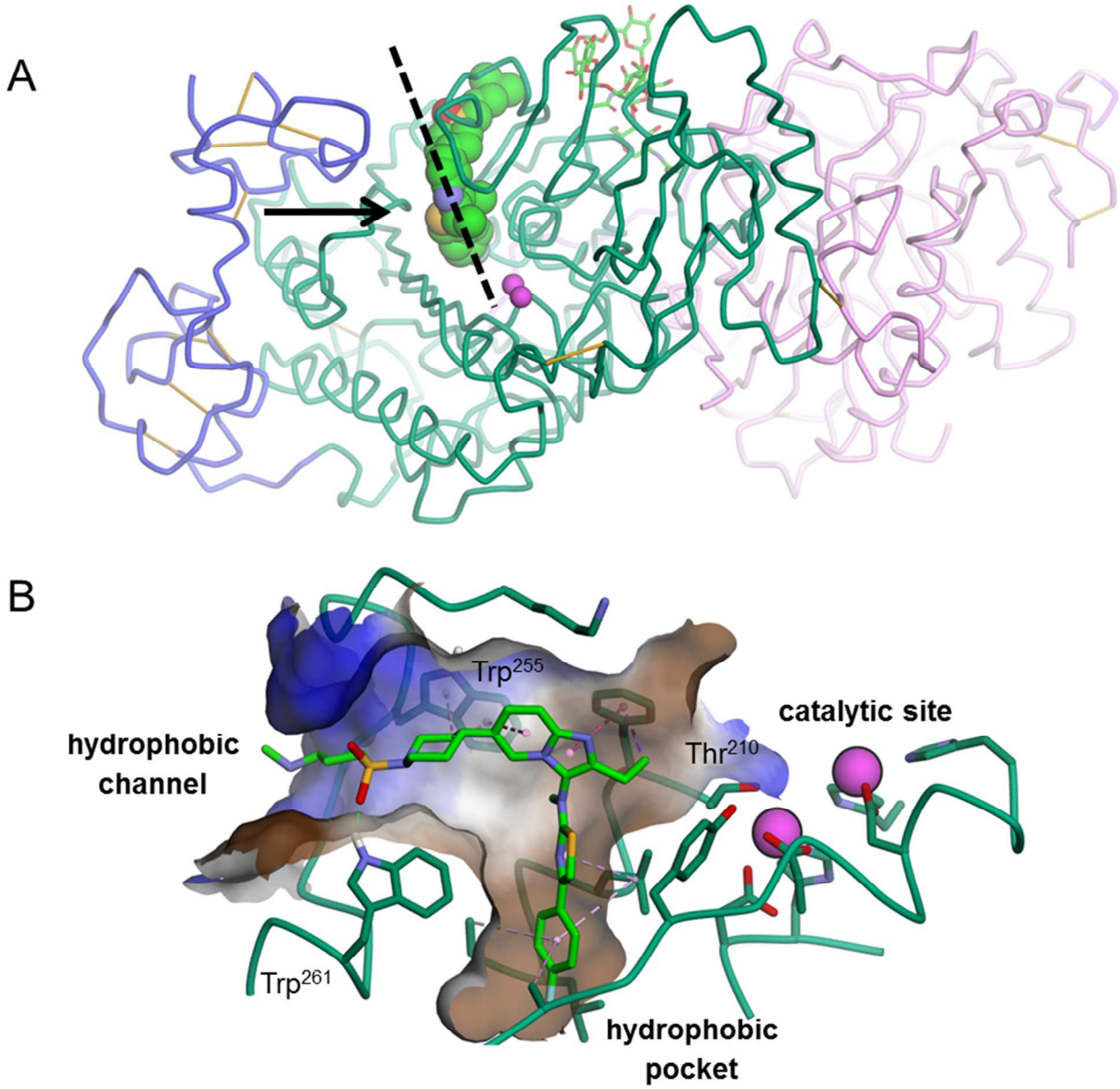


Figure 5. (A) Overall structure of ATX with compound **9** shown in CPK with green carbon atoms. Somatomedin B-like, catalytic and nuclease-like domains are shown in blue, green and pink, respectively. Disulfide bridges are depicted as yellow bars and the two zinc ions of the catalytic site are shown as magenta spheres. The arrow indicates the viewing angle of Figure 5B and the dashed line the clipping plane for the site surface. (B) Focus on the binding pocket showing details of the binding mode of **9**. The surface delineating the site is colored by

hydrophobicity using in a blue-white-brown spectrum (blue for hydrophilic, brown for hydrophobic). For clarity the surface was clipped vertically (see Figure 5A). Dashed lines highlight interactions between compound **9** and ATX, hydrophobic contacts in pink, hydrogen bonding in green.

Since the publication of the first structures of ATX, a variety of endogenous ligands and synthetic inhibitors has been described, examples of which are shown in Figure 6. Interestingly they exhibit a diversity of binding modes which, at this stage, can be classified into 4 types illustrated in Figure 7.

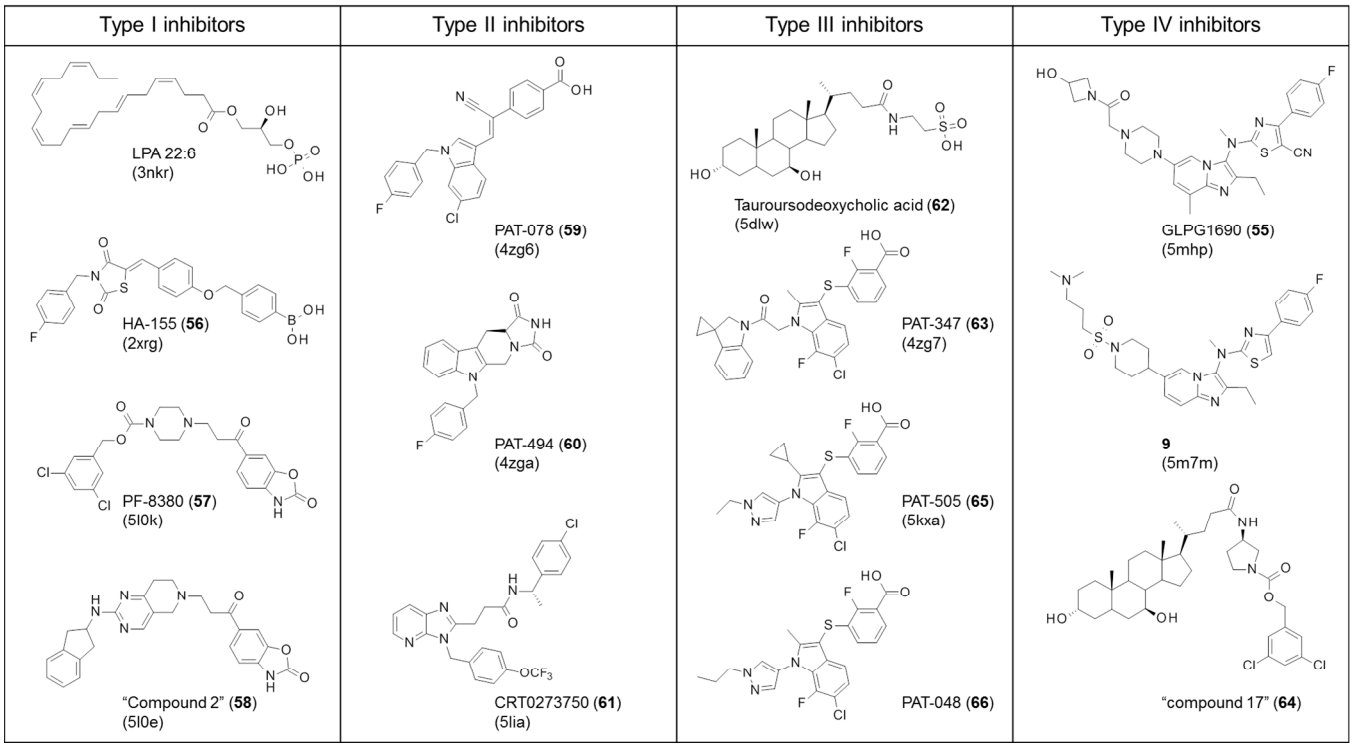


Figure 6. Structures of ATX inhibitors. PDB codes of co-crystal structures with ATX are indicated in brackets (note: reported PDB code for compound **64** (5mom) doesn't match the intended structure).

Type I inhibitors (such as LPA species, HA-155 (**56**), PF-8380 (**57**) or "compound 2" (**58**) in Figure 6) mimic the binding mode of LPC substrate and occupy the catalytic site.[13,14,19] They generally have a zinc binding group and a hydrophobic tail lodged in the hydrophobic pocket (Figure 7A). Such inhibitors leave the hydrophobic channel unoccupied which explains why many structures categorized as Type I inhibitors have a secondary compound occupying the channel, generally a substance from the solution with amphiphilic properties such as LPA or cholesterol derivatives. In fact, all crystallographic structures with type I inhibitors published so far exhibit an electronic density in the channel but not all have a modelled compound in this area, probably because of partial occupancy. Type II inhibitors occupy the hydrophobic pocket by largely exploiting its intrinsic plasticity (Figure 7B). Indeed, in most cases this induced fit significantly restructures the binding pocket involving side-chain conformation variations of residues such as Phe^{214}, Leu^{217}, Phe^{274}, Phe^{275} and Tyr^{307}. Interestingly, type II inhibitors jam the bottleneck between the hydrophobic channel and the catalytic site but do not fully occupy any of them. PAT-078 (**59**), PAT-494 (**60**) and, to a certain extent, CRT0273750 (**61**), represent such Type II inhibitors.[20,21] As for type I inhibitors, the remaining pockets left vacant allow binding of an LPA molecule. By contrast, type III inhibitors bind to the hydrophobic channel leaving the hydrophobic pocket and catalytic site unoccupied (Figure 7C). They comprise PAT-347 (**63**), PAT-505 (**65**), PAT-048 (**66**), and the steroids 7α-hydroxycholesterol and tauroursodeoxycholic acid (TUDCA, **62**).[18,20,22] The binding modes of compounds **55** and **9** differ from that of other inhibitors and can therefore be categorized as type IV inhibitors (Figure 7D). Indeed by occupying both the hydrophobic pocket and the hydrophobic channel, the two sites enabling the binding of LPC, the type IV inhibitors preclude the binding of LPA and bile acid derivatives to ATX and avoid any interactions of substrates with the zinc atoms. Recently another type IV

inhibitor (**64**) has been designed by grafting a "tail" fragment extracted from a type I inhibitor (**57**) on a type III inhibitor (**62**).[23]

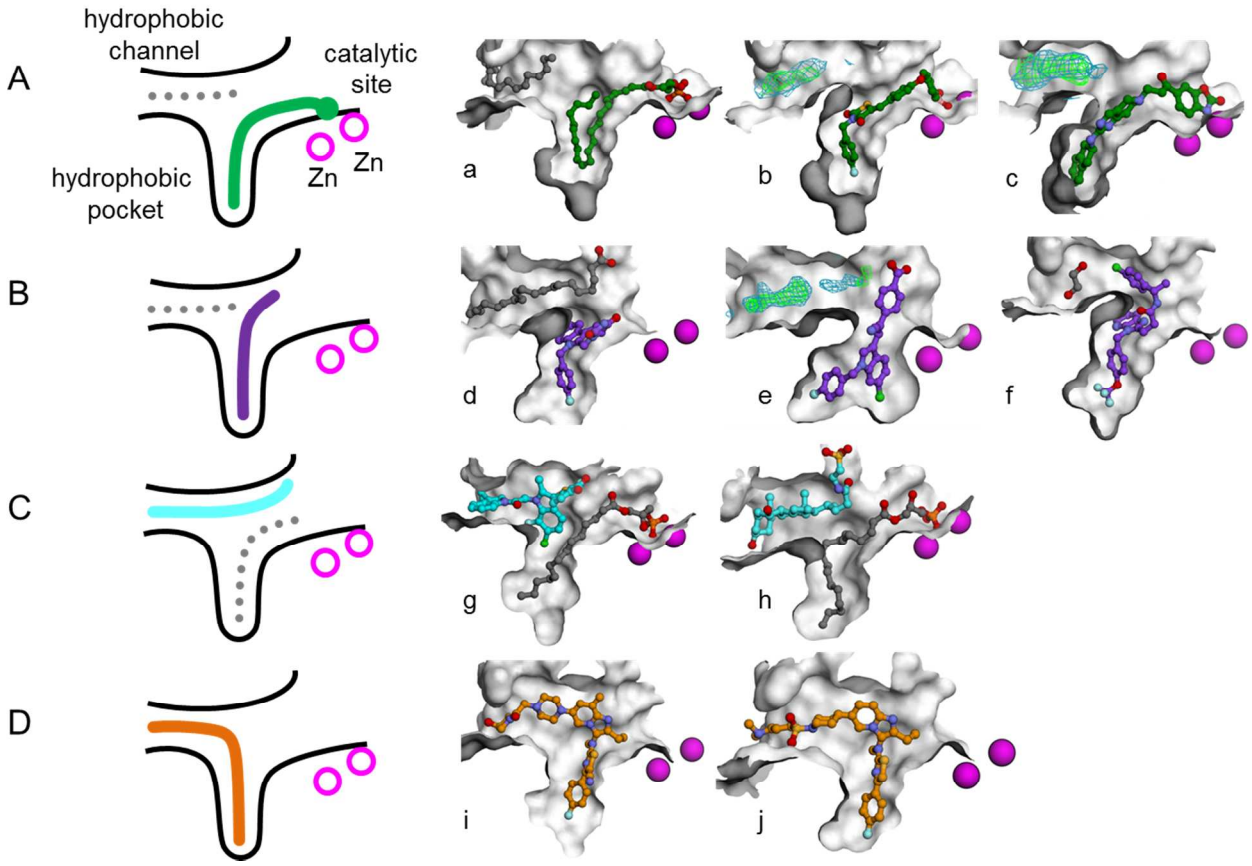


Figure 7. Schematic representation of the autotaxin binding pocket and the different inhibitor binding modes reported to date. Type I, II, III and IV inhibitors are represented in green, purple, cyan and orange respectively on the schemas with matching colors for carbon atoms on the exemplified structures. The grey dotted lines represent secondary ligands modeled next to the inhibitor as shown with grey carbons on the illustrations. Some structures lack secondary ligand models but exhibit a positive electron density in the same region (Fo-Fc map at 3 sigma in green, 2Fo-Fc map at 1 sigma in blue). Zinc ions are depicted in magenta. (A) Binding mode of type I inhibitors LPA 22:6 (a), **56** (b), **58** (c). (B) Binding mode of type II inhibitors **60** (d), **59** (e), **61**

(f). (C) Binding mode of type III inhibitors **63** (g), **62** (h). (D) Binding mode of type IV inhibitors **55** (i), compound **9** (j).

Such diversity of binding modes might impact the properties of ATX inhibitors: recently, on top of LPA formation, a role for LPA transport and delivery to its cognate receptors has been suggested for ATX.[16-18] Whereas all types of ATX inhibitors block the formation of LPA, type IV and possibly some type II inhibitors such as compound **61** could also prevent LPA transport and delivery to LPA receptors through ATX channel occupancy. Consequently, ATX inhibitors could display various modes of action and subsequently different therapeutic effects depending on their binding mode, with some inhibitors allowing while others blocking the binding of fatty acids (LPA and derivatives).[24]

The reported SAR of the series confirms the binding mode determined experimentally. In particular whereas position 6 of the imidazo[1,2-a]pyridine scaffold tolerates a broad range of substituents without significant change of potency, slight structural modification of the substituent at position 3 has a strong impact on activity. Indeed, the former appears to occupy the hydrophobic channel with access to the bulk solvent in contrast to the latter, which is lodged in an enclosed hydrophobic pocket within the structure of the enzyme. An interesting modification which boosted the potency is worth mentioning: by adding a nitrile group on the substituent in position 3, either on the thiazole or on the terminal fluorophenyl moiety, the potency could be systematically increased by a factor 7 on average (matched-molecular pair analysis, data not shown). This effect could be well-explained by analyzing the role of water before and after the binding event using WaterMap.[25] This tool simulates the dynamic behavior of water molecules enabling, among others, the identification of hydration sites that are energetically unfavorable. When the calculation is run without compound **9** in the ATX crystal structure (apo map, Figure

8A), WaterMap locates a high energy water molecule (+7.5 kcal/mol) close to the positions occupied by the hydrogen atoms of the thiazole moiety and the phenyl group of the bound ligand. This indicates that the overall energy of the system should benefit from the binding of a ligand that displaces the otherwise constrained water molecule. Although compounds which lack the cyano group (such as compound **9**) don't seem to allow for a water molecule to occupy the same site (holo map, Figure 8B), a high energy "vacuum bubble" is created instead, penalizing the global free energy of the system. By adding a nitrile group either on the phenyl moiety (compound **35**) or on the thiazole moiety (compounds **37**, **40**), not only the high energy water molecule can be released into the bulk solvent but the pocket is also fully occupied by the ligand.

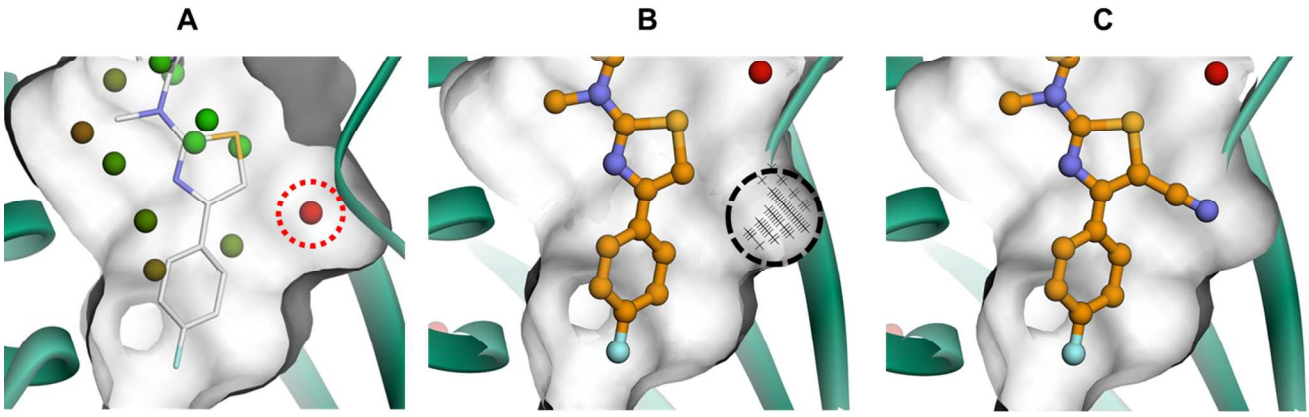


Figure 8. WaterMap results in the hydrophobic pocket. (A) Apo map showing the high energy water molecule highlighted by a dashed circle. Water molecules are colored based on their calculated free energy using a blue (negative, stable)-green (neutral)-red (positive, unstable) spectrum. Albeit absent during the calculation, the ligand is shown in sticks with white carbon atoms. (B) Holo map highlighting the high energy vacuum bubble (crosses circled in black) left by compounds (balls and sticks with orange carbon atoms). (C) Model of a compound with a nitrile group on the thiazole.

In vivo reduction of LPA production by compound 40

As shown in Table 4, compound **40** displays low to moderate clearance in rodents and low oral bioavailability. As conversion of LPC into LPA by ATX is the major source of circulating LPA in blood,[1] in vivo inhibition of this process can be evaluated by quantification of LPA levels in plasma. Compound **40** and other compounds within the chemical series generally displayed little to no potency shift between the biochemical and plasma assays despite a plasma protein binding greater than 99%. The high potency of compound **40** in plasma could therefore compensate for its low oral bioavailability. In order to evaluate in vivo ATX inhibitory activity of compound **40**, reduction of plasma LPA levels upon compound **40** administration to rats was monitored. Rats received a single dose of compound **40** (5 mg/kg) and blood was sampled at 0.5, 1 and 3 hours post-dosing for the determination of plasma levels of both compound **40** and LPA 18:2. Percentage of LPA reduction was calculated by comparing LPA 18:2 levels in animals treated with compound **40** or with vehicle at each time point. As seen in Table 5 compound **40** was able to cause a more than 70% reduction of LPA 18:2 levels over 3 hours, in line with the high potency of the compound in the rat plasma assay (IC_{50} = 22 nM), which is approximately covered by the observed plasma levels for compound **40**. The higher reduction of LPA 18:2 level at 3 h compared to vehicle-treated animals could be due to the presence of an active metabolite which was not investigated at this stage. This result clearly demonstrated in vivo target engagement with compound **40**.

Table 4. In vivo pharmacokinetic properties for compound 40[a]

mouse iv Cl (L h^{-1} kg^{-1})	1.2
mouse iv $t_{1/2}$ (h)	2.0

mouse iv V_{ss} (L kg^{-1})	1.7
mouse po F (%)	12
rat iv Cl (L h^{-1} kg^{-1})	3.1 (6)
rat iv $t_{1/2}$ (h)	2.1 (4)
rat iv V_{ss} (L kg^{-1})	8.3 (11)
rat po F (%)	15 (22)

[a] Data determined from 5 mg/kg po and 1 mg/kg iv doses (n = 3). Between brackets is the coefficient of variation in percentage, CV%. No CV% is available for mice data because of composite blood sampling on a group of six mice.

Table 5. In vivo reduction of plasma LPA 18:2 levels in rats after a single po dose of compound 40 at 5 mg/kg[a]

Time point	Compound **40** plasma concentration (nM)	% reduction of plasma LPA 18:2 level
0.5 h	67 ± 15	74 ± 7
1 h	34 ± 5	82 ± 5
3 h	58 ± 16	97 ± 1

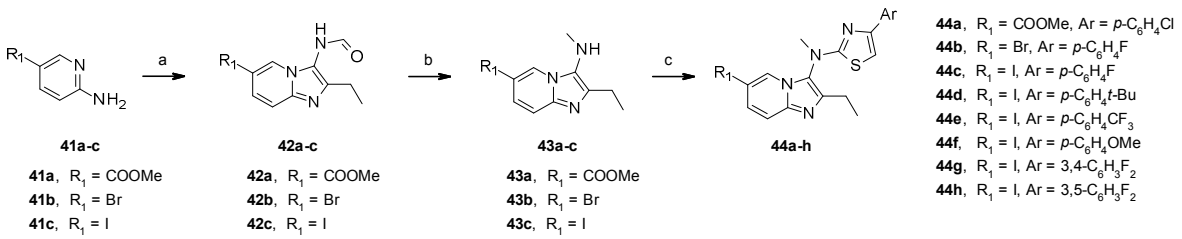
[a] Rats were dosed orally at 5 mg/kg with compound **40** under fasted conditions. Values for plasma concentration and LPA reduction are mean values of 3 animals. Percentage of reduction of LPA 18:2 level was calculated by comparing levels of LPA 18:2 in animals treated with compound **40** or vehicle at each time point.

Chemistry

The general synthesis of imidazo[1,2-*a*]pyridine-3-yl-4-aryl-thiazol-2-amines is depicted in Scheme 1. 3-Formylamino-imidazo[1,2-*a*]pyridine derivatives **42a-c** were prepared by the 3-component Groebke-Blackburn-Bienaymé reaction[9-11] of 5-substituted 2-aminopyridine compounds **41a-c** with propanal and Walborsky's reagent[12] (1,1,3,3-tetramethylbutyl isocyanide)

in the presence of $MgCl_2$. Alkylation and acidic deformylation with methanolic HCl of compounds **42a-c** afforded the 3-alkylamino-imidazo[1,2-*a*]pyridine derivatives **43a-c**. Aminothiazole derivatives **44a-h** were accessed from **43a-c** via the formation of the thiourea intermediate using Fmoc-NCS and piperidine, and subsequent reaction with corresponding bromo- or chloro-acetophenones.

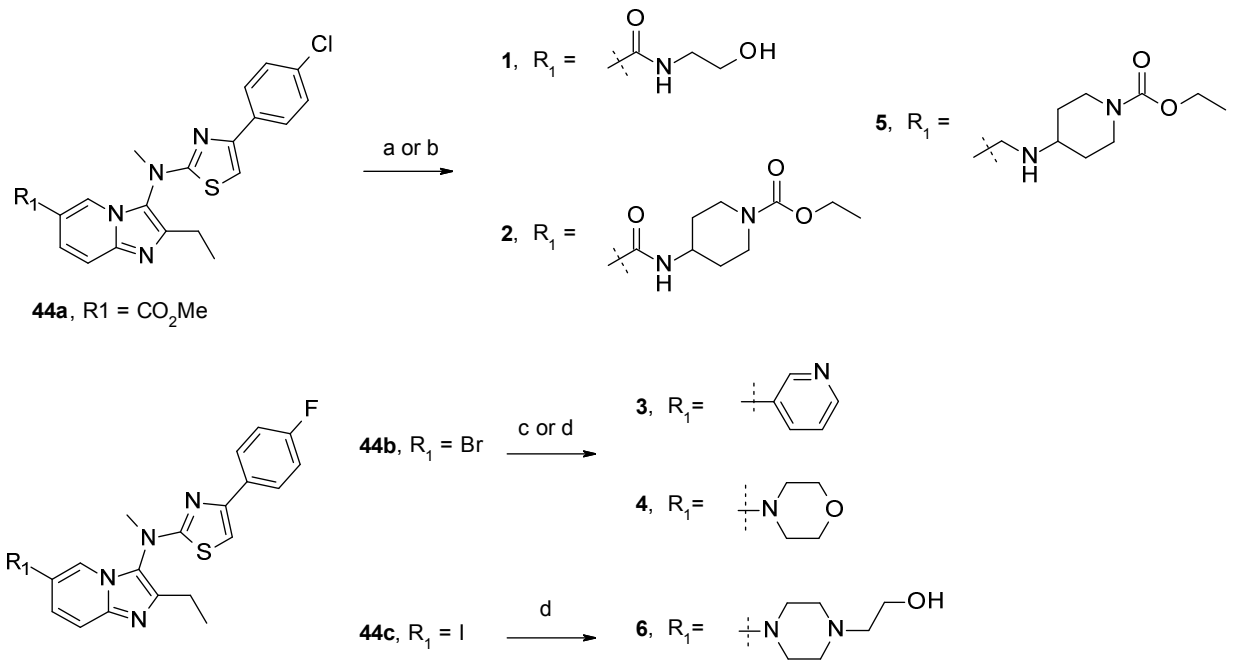
Scheme 1. General synthesis of (2-ethylimidazo[1,2-*a*]pyridine-3-yl)-4-aryl-N-alkylthiazol-2-amine derivatives **44a-h**[a]



[a]Reagents and conditions: (a) (1) 1,1,3,3-tetramethylbutyl isocyanide, EtCHO, $MgCl_2$, *n*-BuOH, 130°C, (2) HCOOH 80°C, 56-78% over 2 steps; (b) (1) MeI, NaH, DMF, rt, (2) HCl, MeOH, rt-reflux 37-80% over 2 steps; (c) (1) Fmoc-NCS, TEA, DCM, rt then piperidine, rt, (2) bromo- or chloroacetophenone, EtOH, reflux, 27-74% over 2 steps.

The synthesis of compounds **1-6** is represented in Scheme 2. Intermediate **44a** was saponified with NaOH or LiOH and subjected to an EDCI-mediated coupling with the corresponding amines to give compounds **1** and **2**. Compound **5** was obtained from **44a** by reduction of the ester to alcohol with LAH followed by a one-pot mesylation/displacement of the mesylate with 4-amino-piperidine-1-carboxylic acid ethyl ester. Buchwald coupling of **44b** and **44c** with morpholine and 2-piperazin-1-yl-ethanol afforded compounds **4** and **6** respectively. Compound **44b** also reacted in a Suzuki coupling with 3-pyridylboronic acid to give **3**.

Scheme 2. Synthesis of compounds from Table 1[a]

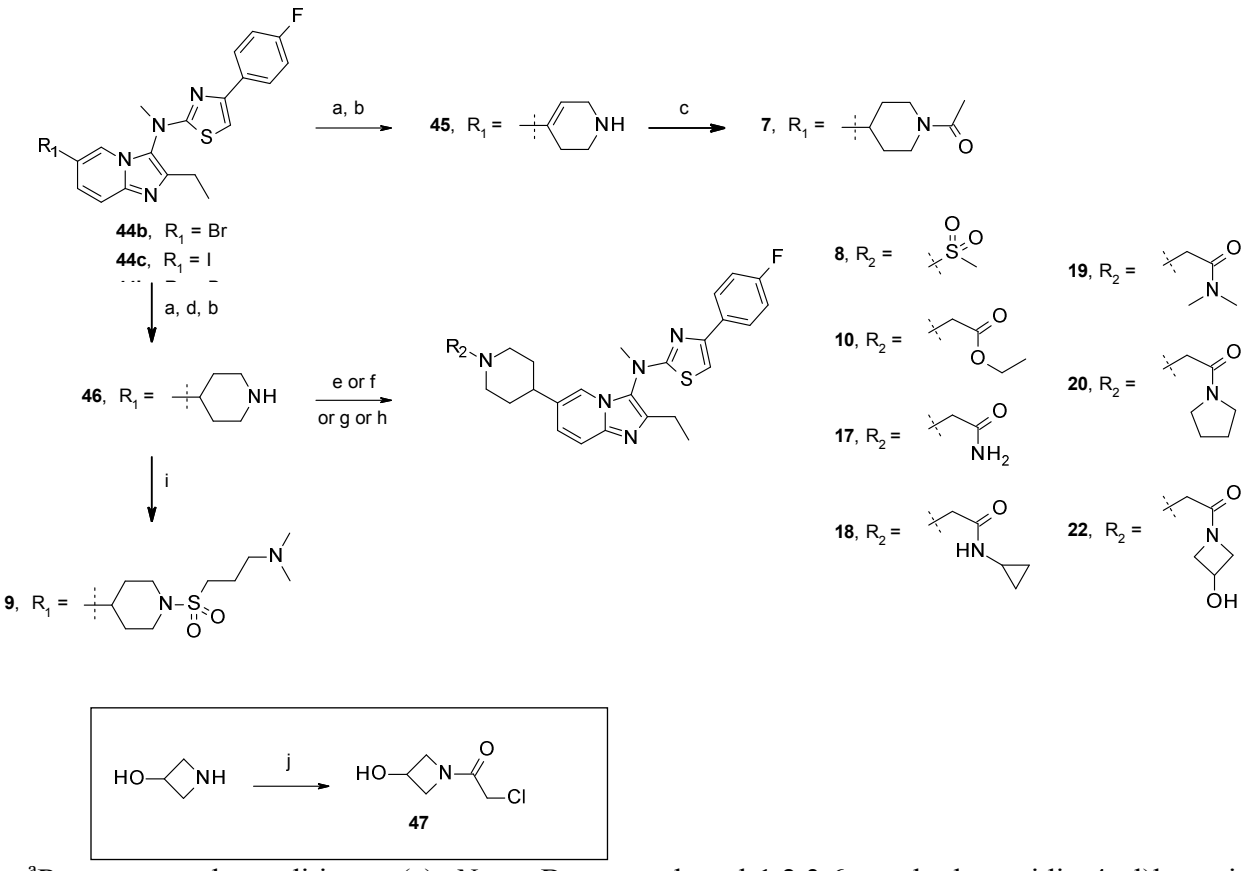


[a]Reagents and conditions: (a) (1) NaOH, MeOH/H_2O or LiOH, THF/H_2O, rt, (2) Amine, EDC.HCl, TEA, HOBt, DMF, rt, 15-74% over 2 steps (for **1** and **2**); (b) (1) $LiAlH_4$, THF, rt, (2) MsCl, TEA, DCM, rt, then amine, rt, 25% over 2 steps (for **5**); (c) pyridine-3-boronic acid, Na_2CO_3, $Pd(PPh_3)_4$, dioxane/H_2O, 90°C, 53% (for **3**); (d) Amine, *t*-BuONa, Pd_2dba_3, Xantphos or DavePhos, toluene or dioxane, 90-120°C, 7-16% (for **4** and **6**).

The syntheses of substituted piperidine derivatives **7**-**10**, **17**-**20** and **22** from intermediate **44c** are depicted in Scheme 3. Suzuki coupling on **44c** with the *N*-*tert*-butoxycarbonyl-1,2,3,6-tetrahydropyridin-4-yl)boronic acid pinacol ester followed by boc deprotection afforded intermediate **45**. Acylation of **45** with acetyl chloride and subsequent reduction of the tetrahydropyridine double bond allowed the synthesis of **7**. Intermediate **46** was synthesized with good yield from **44c** via a Suzuki coupling with *N*-*tert*-butoxycarbonyl-1,2,3,6-tetrahydropyridin-4-yl)boronic acid pinacol ester, reduction of the tetrahydropyridine double bond using $H_2/Pd(OH)_2$ system and final boc deprotection using HCl in MeOH. Intermediate **46** could then react with a wide range of alkylating agents in presence of strong or weak bases to

afford ester compound **10** and amides **17**-**20** and **22**. Chloro acetamide alkylating agents were typically prepared by reaction of chloroacetyl chloride with the corresponding amine as described for the synthesis of intermediate **47**. Reaction of methane sulfonyl chloride with **46** afforded sulfonamide **8**. Compound **9** was obtained from **46** by sulfonylation with 3-chloropropane-1-sulfonyl chloride followed by chlorine displacement with dimethylamine in presence of NaI.

Scheme 3. Synthesis of compounds **7**-**10**, **17**-**20** and **22**[a]

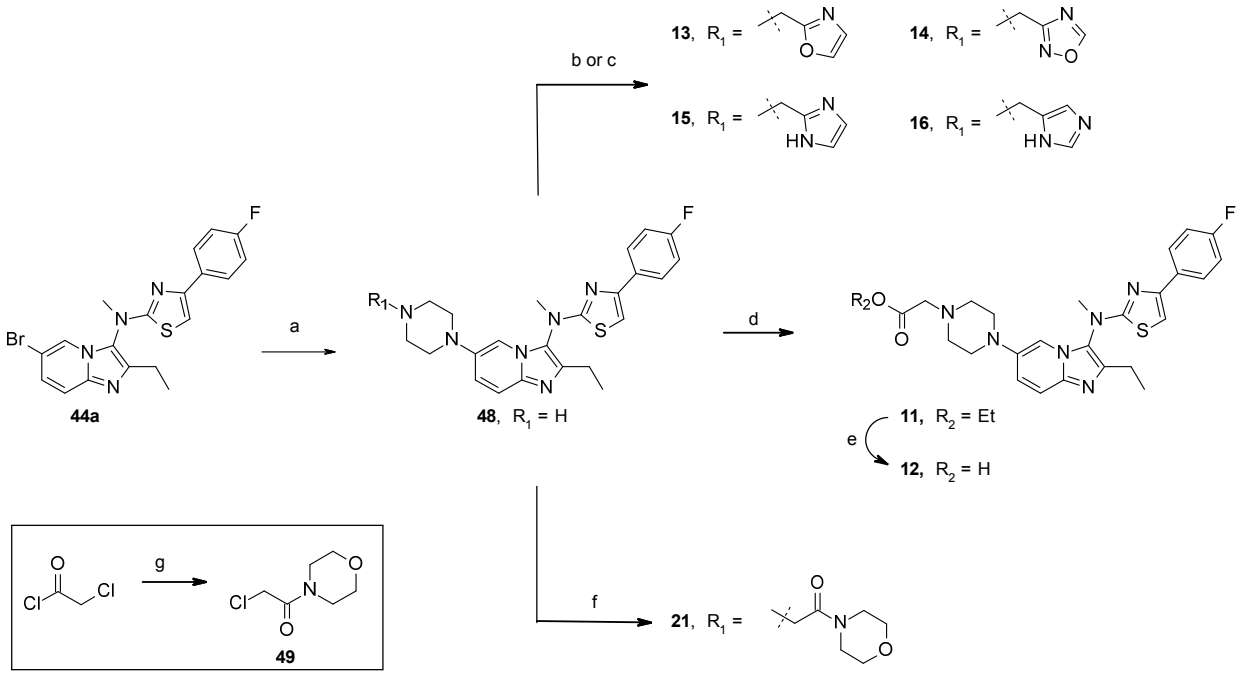


[a]Reagents and conditions: (a) *N*-*tert*-Butoxycarbonyl-1,2,3,6-tetrahydropyridin-4-yl)boronic acid pinacol ester, Na_2CO_3, $Pd(PPh_3)_4$, dioxane/H_2O, 85°C, quant.; (b) HCl, THF/H_2O or dioxane/MeOH or MeOH, 0°C-rt, 71-100%; (c) (1) acetyl chloride, TEA, DCM, rt, 91% (2) H_2, Pd/C, EtOH, 60°C, 34% (for **7**); (d) H_2, $Pd(OH)_2$, DCM/EtOH, rt, 79%; (e) ethyl 2-bromoacetate, NaH, DMF, rt, 56% (for **10**); (f) MsCl, TEA, DCM, 0°C-rt, 17% (for **8**); (g) R_2X, K_2CO_3, MeCN, 70-100°C, 40-100% (for **17, 18, 19, 22**); (h) 2-chloro-1-pyrrolidin-1-yl-

ethanone, TEA, MeCN, 190°C (microwave irradiation), 45% (for **20**); (i) (1) 3-chloropropane-1-sulfonyl chloride, TEA, DCM, rt, 99%, (2) Dimethylamine, NaI, THF, 150°C (microwave irradiation), 59% over 2 steps (for **9**); (j) chloroacetyl chloride, K_2CO_3, H_2O, DCM, 0°C-rt, 70%.

Variation of the piperazine substitution (compounds **11**-**16** and **21**) was achieved from intermediate **48** which was obtained from **44b** by a Buchwald coupling with *N*-boc-piperazine using DavePhos as ligand followed by boc cleavage with HCl (Scheme 4). Intermediate **48** could be alkylated with ethyl chloroacetate to give compound **11**, and subsequent ester hydrolysis with LiOH afforded carboxylic acid **12**. Syntheses of compounds **13**, **14** and **21** were performed by alkylation of intermediate **48** with corresponding alkyl chloride reagents. Whereas **13** and **14** could be obtained at room temperature in presence of TEA, heating at 160°C under microwave irradiation was required to get **21** in a moderate yield. Finally, intermediate **48** underwent reductive amination with the corresponding aldehydes to provide compounds **15** and **16**.

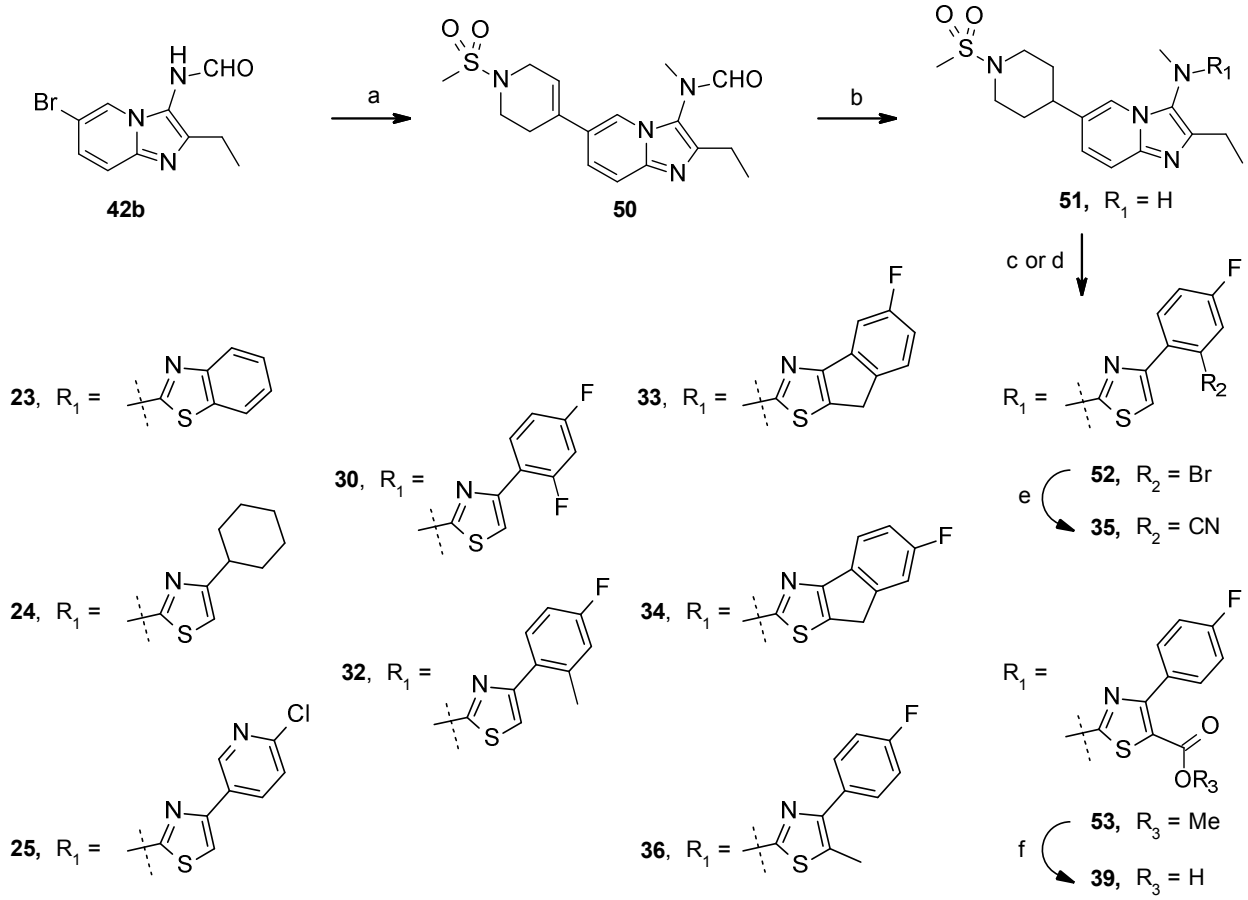
Scheme 4. Synthesis of piperazine derivatives **11**-**16** and **21**[a]



[a]Reagents and conditions: (a) (1) *N*-boc-piperazine, *t*-BuONa, Pd_2dba_3, DavePhos, toluene, 105°C, (2) HCl, dioxane, rt, 79% over 2 steps; (b) R_1Cl, TEA, DCM, rt, 22-24% (for **13**, **14**); (c) R_1CHO, $NaBH_3CN$, TEA, MeOH, rt, 29-49% (for **16**, **15**); (d) ethyl chloroacetate, TEA, MeCN, 0-45°C, 71% (for **11**); (e) LiOH, THF/H_2O, rt, 33% (for **12**); (f) **49**, TEA, MeCN, 160°C (microwave irradiation), 26%; (g) Morpholine, TEA, DCM, 0°C, quant.

Exploration of thiazole substitution could be performed from intermediate **51**, obtained in 4 steps from intermediate **42b** (Scheme 5). Compound **42b** was N-methylated and reacted in a Suzuki coupling with 1-methanesulfonyl-4-(tetramethyl-1,3,2-dioxaborolan-2-yl)-1,2,3,6-tetrahydropyridine to give compound **50**. The tetrahydropyridine double bond was then reduced with H_2/PtO_2 and the obtained intermediate was deformylated to afford **51**. The latter intermediate was converted into thiourea derivative using the FmocNCS/piperidine procedure and then cyclized with the corresponding bromoacetophenone reagents to yield compounds **23-25, 30, 32-34, 36, 52** and **53**. Compound **52** was then transformed into compound **35** by a cyanation reaction using CuCN under microwave irradiation. Carboxylic acid **39** was obtained from ester **53** by saponification with LiOH.

Scheme 5. Synthesis of compounds **23**-**25**, **30**, **32**-**36** and **39**[a]

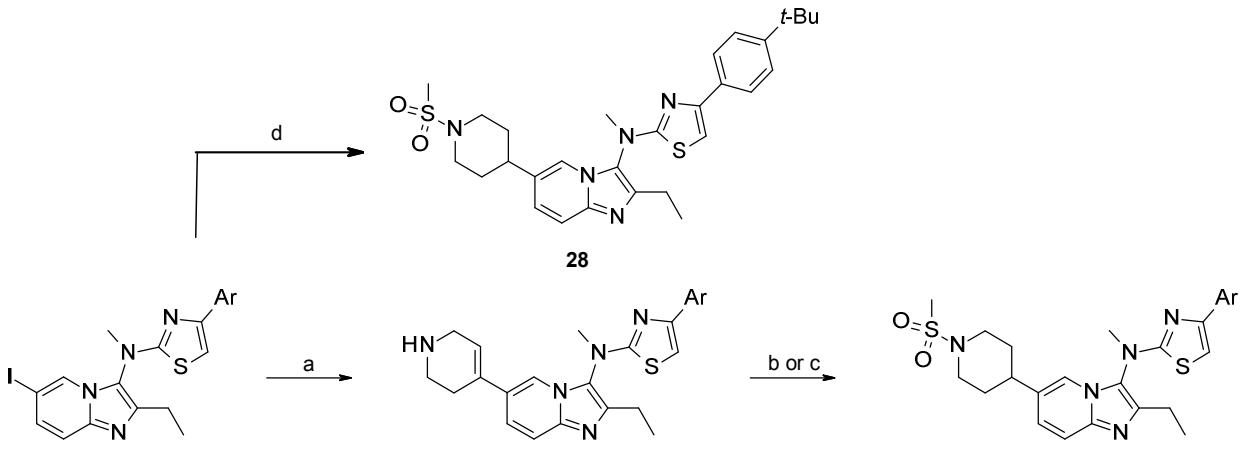


[a]Reagents and conditions: (a) (1) MeI, NaH, DMF, rt, (2) 1-methanesulfonyl-4-(tetramethyl-1,3,2-dioxaborolan-2-yl)-1,2,3,6-tetrahydropyridine, $Pd(PPh_3)_4$, Na_2CO_3, dioxane/H_2O, 85°C, 80% over 2 steps; (b) (1) H_2, PtO_2, THF/MeOH, rt, (2) HCl, dioxane/MeOH, rt, 47% over 2 steps; (c) (1) FmocNCS, TEA, DCM, rt then piperidine, rt, (2) bromoacetophenone derivative, EtOH, reflux, 17-64% over 2 steps (for **24**, **25**, **30**, **32**-**34**, **36**, **52**, **53**); (d) 2-bromobenzo[d]thiazole, $Pd(OAc)_2$, Xantphos, Cs_2CO_3, dioxane, reflux, 36% (for **23**); (e) CuCN, pyridine, 150°C (microwave irradiation), 41% (for **35**); (f) LiOH, THF/H_2O, rt to 50°C, 18% (for **39**).

Other variations of the phenyl part of the molecule were done by functionalization of **44d**-**h** (Scheme 6). Iodo compound **44d** bearing 4-*t*Bu substituent on the phenyl moiety reacted with 1-methanesulfonyl-4-(tetramethyl-1,3,2-dioxaborolan-2-yl)-1,2,3,6-tetrahydropyridine and the

obtained intermediate was hydrogenated over PtO_2 to give compound **28**. **44e-h** reacted in Suzuki coupling with *N*-tert-butoxycarbonyl-1,2,3,6-tetrahydropyridin-4-yl)boronic acid pinacol ester and cleavage of the Boc group with HCl afforded intermediates **54e-h** respectively. The latter intermediates were then submitted to a mesylation/reduction sequence to get compounds **26**, **27**, **29** and **31**.

Scheme 6. Synthesis of compounds **26-29** and **31**[a]

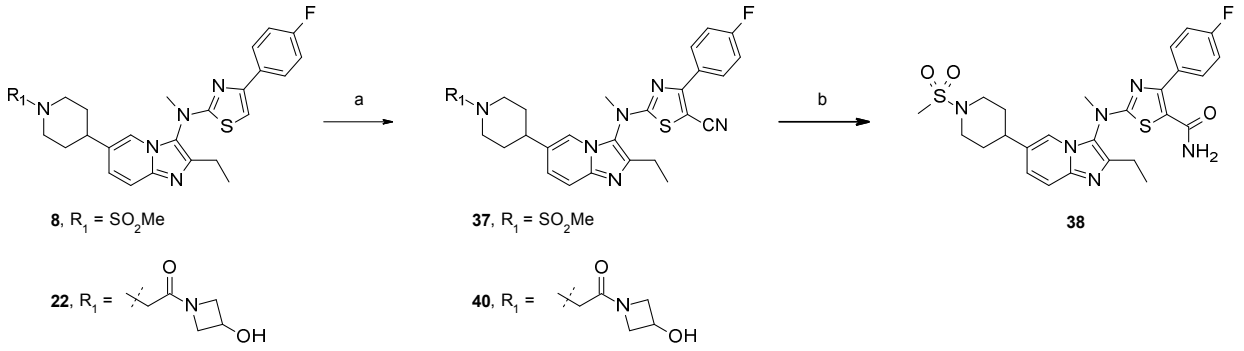


[a]Reagents and conditions: (a) (1) *N*-tert-butoxycarbonyl-1,2,3,6-tetrahydropyridin-4-yl)boronic acid pinacol ester, Na_2CO_3, $Pd(PPh_3)_4$, dioxane/H_2O, 85°C, 79-94%, (2) HCl, dioxane/MeOH, rt, 90-100% (b) (1) MsCl, TEA, DCM, rt, 39-88%, (2) H_2, PtO_2, MeOH/THF, rt, 35-65% (for **26, 27, 29**); (c) (1) H_2, PtO_2, MeOH/THF, rt, 70%, (2) MsCl, TEA, DCM, rt, 40% (for **31**); (d) (1) 1-methanesulfonyl-4-(tetramethyl-1,3,2-dioxaborolan-2-yl)-1,2,3,6-tetrahydropyridine, $Pd(PPh_3)_4$, Na_2CO_3, dioxane/H_2O, 100°C, 99%, (2) H_2, PtO_2, MeOH/THF, rt, 27% for 2 steps.

Finally, as depicted in Scheme 7, bromination of **8** and **22** on the thiazole ring using polymer-supported bromine reagent, followed by cyanation with CuCN afforded compounds **37** and **40**

respectively in low to moderate yields. Compound **37** could react further with concentrated sulfuric acid to provide carboxamide **38**.

Scheme 7. Synthesis of **37, 38** and **40**[a]



[a] Reagents and conditions: (a) (1) bromine on polymer support, DCM, rt, quant., (2) CuCN, pyridine, 125°C (microwave irradiation), 9-41%; (b) conc. H_2SO_4, rt, 29%.

CONCLUSION

A novel imidazo[1,2-*a*]pyridine series of ATX inhibitors was identified out of HTS efforts run with FS-3 as a substrate. Discrepancies in the SAR generated through assays using artificial FS-3 or natural LPC substrates prompted to change the primary assay in order to better correlate with physiological conditions. The co-crystal structure of compound **9** in ATX revealed a novel binding mode in which the compound occupies the hydrophobic pocket which normally accommodates the LPA acyl side chain and the hydrophobic channel thought to play a role in LPA transport and delivery to receptors, but does not bind to any of the zinc atoms of the catalytic site. Considering the possible roles of ATX, such binding mode might offer an advantage over other ATX inhibitors. Indeed, in view of the short half-life of LPA in blood, the hypothesis has been formulated that activation of LPA receptors might require local delivery of

newly synthesized LPA. The ATX hydrophobic channel might play a role as local LPA delivery route. Optimization of ATX inhibitory activity of the series led to compound **40** displaying high potency in biochemical and plasma assays. Compound **40** was also able to decrease LPA levels in rat plasma after oral administration, confirming in vivo target engagement. Further profiling indicated that compound **40** inhibited the hERG channel with an IC_{50} of 2.9 μM and showed time-dependent CYP3A4 inhibition in a human liver microsomes assay. Improvement of these ADMET properties as well as pharmacokinetics properties leading to the identification of clinical candidate **55** has recently been described.[7]

EXPERIMENTAL SECTION

All reagents were of commercial grade and were used as received without further purification, unless otherwise stated. Commercially available anhydrous solvents were used for reactions conducted under inert atmosphere. Reagent grade solvents were used in all other cases, unless otherwise specified. Column chromatography was performed on silica gel 60 (35-70 μm). Thin layer chromatography was carried out using pre-coated silica gel F-254 plates (thickness 0.25 mm). Microwave heating was performed with a Biotage Initiator apparatus. Celpure® P65 is a filtration aid, commercial product (CAS number 61790-53-2). ^{1}H NMR spectra were recorded on a Bruker DPX 400 NMR spectrometer (400 MHz) or a Bruker Advance 300 NMR spectrometer (300 MHz). Chemical shifts (δ) for ^{1}H NMR spectra are reported in parts per million (ppm) relative to tetramethylsilane (δ 0.00) or the appropriate residual solvent peak, i.e. $CHCl_3$ (δ 7.27), as internal reference. Multiplicities are given as singlet (s), doublet (d), triplet (t), quartet (q), quintuplet (quin), multiplet (m) and broad (br). Waters Acquity UPLC with Waters Acquity PDA

detector and SQD mass spectrometer was used to generate UV and MS chromatograms as well as MS spectra. Columns used: UPLC BEH C18 1.7 μm 2.1 x 5 mm VanGuard Pre-column with Acquity UPLC BEH C18 1.7 μm 2.1 x 30 mm column or Acquity UPLC BEH C18 1.7 μm 2.1 x 50 mm column. LC−MS analyses were conducted using the following two methods. Method 1: solvent A, H_2O−0.05% NH_4OH; solvent B, MeCN−0.05% NH_4OH; flow rate, 0.8 mL/min; start 5% B, final 95% B in 1.55 min, linear gradient. Method 2: solvent A, H_2O−0.1% HCO_2H; solvent B, MeCN−0.1% HCO_2H; flow rate, 0.8 mL/min; start 5% B, final 95% B in 1.55 min, linear gradient. All final compounds reported were analyzed using one of these analytical methods and were at least 95% pure. Autopurification system from Waters was used for LC-MS purification. LC-MS columns used: Waters XBridge Prep OBD C18 5 μm 30 × 100 mm (preparative column) and Waters XBridge BEH C18 5 μm 4.6 × 100 mm (analytical column). All the methods used MeCN/H_2O gradients. MeCN and H_2O contained either 0.1% formic acid or 0.1% diethylamine. API5500 QTRAP mass spectrometer from ABSciex was used for the detection and quantification of compound and LPA in plasma.

General procedure A for the synthesis of amide derivatives. To a solution of ester **44a** (1 eq.) in a mixture of MeOH/water or a mixture of THF/water is added an excess of a 1 N aqueous NaOH solution or LiOH (5 eq.). The reaction mixture is stirred at room temperature overnight, then concentrated *in vacuo* and the residue is dissolved in a mixture of DCM and water. The aqueous layer is extracted with DCM twice. The combined organic layers are washed with a saturated $NaHCO_3$ solution and brine, dried over Na_2SO_4, filtered and concentrated *in vacuo*. The residue is purified by chromatography on silica gel to afford the intermediate carboxylic acid. To a solution of carboxylic acid (1.1 eq.) in DCM are added HOBT (1.2 eq.) and EDC.HCl (1.2 eq.). The reaction mixture is stirred at room temperature for 45 min then a prepared solution of amine

(1 eq.) and TEA (3 eq.) in DCM is added. The reaction mixture is stirred at room temperature until completion and then quenched with water and a 1 N aqueous HCl solution. The aqueous layer is extracted with DCM. The organic layer is washed with a saturated Na_2CO_3 solution and brine, dried over Na_2SO_4, filtered and concentrated *in vacuo*. The residue is purified by chromatography on silica gel to afford the expected compound.

General procedure B for Suzuki-Miyaura coupling. Under inert atmosphere, to a solution of the 6-halo-imidazo[1,2-*a*]pyridin-3-ylamine derivative (1 eq.) in a mixture of dioxane and water (9:1 or 4:1) under argon are successively added sodium carbonate (3 to 4 eq.), boronic acid or 4,4,5,5-tetramethyl-1,3,2-dioxaborolane reagent (1.4 to 1.5 eq.), and $Pd(PPh_3)_4$ or $PdCl_2$dppf (0.05 eq.). The mixture is heated at 85 to 100°C until completion of the reaction. After cooling to room temperature, the reaction mixture is diluted with water and EtOAc and layers are separated. The aqueous phase is extracted 3 times with EtOAc, then the combined organic layers are washed with brine and concentrated *in vacuo*. The residue is purified by chromatography on silica gel to afford the expected compound.

General procedure C for Buchwald coupling. Under inert atmosphere, to a solution of the 6-halo-imidazo[1,2-*a*]pyridin-3-ylamine derivative (1 eq.) in toluene or dioxane under argon are successively added the corresponding amine (1.1 to 2 eq.), sodium *tert*-butoxide or potassium *tert*-butoxide (1.2 to 2 eq.), and then XantPhos or DavePhos (0.015 to 0.075 eq.) and $Pd_2(dba)_3$ (0.007 to 0.05 eq.). The reaction mixture is heated at 85-120°C until completion. After cooling to room temperature, the crude product is filtered on Celpure® P65, the residue is washed with EtOAc and the filtrate concentrated *in vacuo*. The crude product is purified by chromatography on silica gel to afford the expected compound.

General procedure D for the sulfonylation of amine or alcohol derivatives. To a solution of the appropriate amine or alcohol (1 eq.) in DCM at 0°C are added TEA or pyridine (3 to 5 eq.) and the corresponding sulfonyl chloride (1.3 to 2.5 eq.). The reaction mixture is stirred at room temperature until completion. The reaction is quenched with water, diluted with DCM and the aqueous layer is extracted several times with DCM. The combined organic layers are washed with water and brine, dried over Na_2SO_4, filtered and concentrated *in vacuo*. The residue is purified by chromatography on silica gel to afford the expected compound.

General procedure E for nucleophilic substitution. To a solution of nucleophilic reagent (1 to 3 eq.) dissolved in DCM, MeCN or THF are successively added potassium carbonate (2 to 3 eq.) or TEA (4 to 5 eq.), and after a few minutes, the appropriate alkyl halide derivative (1 to 3 eq.). The reaction mixture is stirred at room temperature or heated at 70 to 100°C until completion. Water is added and the reaction mixture is extracted with EtOAc or DCM. The combined organic layers are washed with water and brine, dried over Na_2SO_4, filtered and concentrated *in vacuo*. The residue is purified by chromatography on silica gel, preparative HPLC or by trituration in acetone to afford the expected compound.

General procedure F for reductive amination. To compound **48** (1 eq.) dissolved in MeOH are added TEA (3 eq.) and the aldehyde reagent (2 eq.). The reaction mixture is stirred overnight at room temperature and then sodium cyanoborohydride (3 eq.) is added. The reaction mixture is stirred at room temperature until completion then water is added and the mixture is extracted with DCM. The combined organic layers are washed with a saturated aqueous solution of $NaHCO_3$, brine, dried over Na_2SO_4, filtered and concentrated *in vacuo*. The residue is purified by chromatography on silica gel or preparative HPLC to afford the expected compound.

General procedure G for the synthesis of *N*-(2-ethylimidazo[1,2-*a*]pyridin-3-yl)-*N*-alkyl-4-aryl-thiazol-2-amine derivatives. To a suspension of imidazo[1,2-*a*]pyridin-3-ylamine derivative (1 eq.) in DCM is added TEA (4.5 eq.), the mixture is stirred for 30 min at room temperature and then Fmoc-isothiocyanate (1.3 eq.) is added. The resulting solution is stirred at room temperature for 3 h. Piperidine (3.2 eq.) is then introduced and the reaction mixture is stirred at room temperature overnight. The reaction is quenched with water and the layers are separated. The aqueous layer is extracted with DCM/MeOH. The combined organic layers are washed with brine, dried over Na_2SO_4, filtered and concentrated *in vacuo*. The expected thiourea intermediate is obtained either by chromatography on silica gel or crystallization. The thiourea intermediate (1 eq.) is added to a solution of the corresponding bromo or chloro acetophenone reagent (1.3 eq.) in EtOH. The reaction mixture is stirred at reflux for 3 h then concentrated *in vacuo*. The crude product is triturated in hot EtOAc, stirred for 30 min and allowed to cool to room temperature. The solid is filtered off and rinsed with EtOAc to afford the expected compound.

General procedure H for alkene reduction with PtO_2. The alkene compound is dissolved in a 2/1 MeOH/THF mixture and PtO_2 is added (10%).The mixture is degassed, saturated with H_2 and stirred at room temperature under 1 atm H_2 until completion of the reaction (usually overnight). The mixture is filtered, rinsed with MeOH and concentrated *in vacuo*. The residue is used as such or purified by chromatography on silica gel to afford the hydrogenated compound.

General procedure I: α-bromination of ketones with polymer-supported bromine. The starting ketone (1 eq) is dissolved in DCM and bromine on polymer support (loading 1.2-1.8 mmol/g, 1 to 3 eq.) is added. The resulting suspension is shaken overnight, then the resin is

filtered off and washed with DCM. The filtrate is evaporated to afford the expected brominated derivatives.

General procedure J for the synthesis of (2-ethylimidazo[1,2-*a*]pyridin-3-yl)-*N*-formylamine derivatives. To a solution of 2-aminopyridine derivative (1 eq.) in *n*-BuOH under argon are added successively propionaldehyde (2.5 eq.), $MgCl_2$ (0.04 eq.) and 1,1,3,3-tetramethylbutyl isocyanide (1.15 eq.). The reaction mixture is heated at 130°C for 3.5 h, and then concentrated *in vacuo*. The residue is partitioned between heptane and water, stirred for 15 min and filtered. The solid is collected and dissolved with DCM, dried over Na_2SO_4, filtered and concentrated *in vacuo* to give 2-ethyl-imidazo[1,2-a]pyridin-3-amine intermediate. The latter amine (1 eq.) is dissolved in formic acid and heated at 80°C for 1 h. The reaction mixture is concentrated *in vacuo*. The residue is then triturated in Et_2O. The formed precipitate is filtered, rinsed with Et_2O and dried to afford the corresponding (2-ethylimidazo[1,2-*a*]pyridine-3-yl)-*N*-formylamine derivative.

General procedure K for the synthesis of (2-ethyl-imidazo[1,2-*a*]pyridin-3-yl)-*N*-alkyl-amine derivatives. To a solution of imidazo[1,2-*a*]pyridin-3-ylamine derivative (1 eq.) in DMF is added NaH (1.5 eq.) portionwise, then alkyl iodide (1.4 eq.). The reaction mixture is stirred for 1 h at room temperature then quenched with water and diluted with EtOAc. The aqueous layer is extracted with EtOAc. The combined organic layers are washed with water and brine, dried over Na_2SO_4, filtered and concentrated *in vacuo*. The residue is triturated with DIPE. The solid is filtered, rinsed with DIPE and dried to give intermediate imidazo[1,2-*a*]pyridin-3-yl formamide derivative. A 4 N HCl solution in dioxane or 1.25 N HCl solution in MeOH (9 eq.) is added to a solution of imidazo[1,2-*a*]pyridin-3-yl formamide derivative (1 eq.) in MeOH. The reaction mixture is stirred at a room temperature or refluxed for 3 h. Additional 4 N HCl solution (1.5 eq.)

is added and stirring is continued until completion of the reaction. The reaction mixture is then concentrated *in vacuo* to afford the expected (2-ethyl-imidazo[1,2-*a*]pyridin-3-yl)-*N*-alkyl-amine derivatives as hydrochloride salt.

3-{[4-(4-Chloro-phenyl)-thiazol-2-yl]-methyl-amino}-2-ethyl-imidazo[1,2-*a*]pyridine-6-carboxylic acid (2-hydroxy-ethyl)-amide (1). Compound **44a** was treated with 2-aminoethanol in DMF according to general procedure A to afford the desired product **1** (60 mg, 36% yield over 2 steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.50 (dd, *J* = 1.7, 1.0 Hz, 1 H), 7.81-7.75 (m, 2 H), 7.60-7.56 (m, 1 H), 7.53-7.49 (m, 1 H), 7.40-7.33 (m, 2 H), 6.83 (t, *J* = 5.4 Hz, 1 H), 6.73-6.71 (m, 1 H), 3.81 (t, *J* = 5.0 Hz, 2 H), 3.65-3.54 (m, 5 H), 2.76 (q, *J* = 7.6 Hz, 2 H), 1.35 (t, *J* = 7.6 Hz, 3 H). LC-MS: m/z = 456.0/458.0 [M+H].

4-[(3-{[4-(4-Chloro-phenyl)-thiazol-2-yl]-methyl-amino}-2-ethyl-imidazo[1,2-*a*]pyridine-6-carbonyl)-amino]-piperidine-1-carboxylic acid ethyl ester (2). Compound **44a** was treated with 4-amino-piperidine-1-carboxylic acid ethyl ester in DMF according to general procedure A to afford the desired product **2** (50 mg, 15% yield over 2 steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.53 (d, *J* = 1.5 Hz, 1 H), 7.83-7.73 (m, 2 H), 7.66-7.55 (m, 2 H), 7.40-7.35 (m, 2 H), 6.77-6.66 (m, 1 H), 6.39 (d, *J* = 7.8 Hz, 1 H), 4.21-4.11 (m, 3 H), 4.08 (q, *J* = 7.3 Hz, 2 H), 3.62 (s, 3 H), 2.93-2.84 (m, 2 H), 2.77 (q, *J* = 7.6 Hz, 2 H), 2.05-1.96 (m, 2 H), 1.45-1.38 (m, 2 H), 1.36 (t, *J* = 7.6 Hz, 3 H), 1.23 (t, *J* = 7.3 Hz, 3 H). LC-MS: m/z = 567.0/569.1 [M+H].

(2-Ethyl-6-pyridin-3-yl-imidazo[1,2-*a*]pyridin-3-yl)-[4-(4-fluoro-phenyl)-thiazol-2-yl]-methyl-amine (3). Compound **44b** was treated with pyridine-3-boronic acid according to general procedure B, using $Pd(PPh_3)_4$, to afford the expected compound **3** as an off-white powder (37 mg, 53%). ^{1}H NMR (400 MHz, methanol-d_4) δ ppm 8.83 (d, *J* = 1.9 Hz, 1 H), 8.54 (dd, *J* = 5.0, 1.5 Hz, 1 H), 8.37 (s, 1 H), 8.13 (dt, *J* = 8.0, 2.0 Hz, 1 H), 7.92-7.86 (m, 2 H), 7.67-7.78 (m, 2

H), 7.52 (dd, *J* = 7.9, 4.8 Hz, 1 H), 7.14-7.06 (m, 2 H), 6.97 (s, 1 H), 3.67 (s, 3 H), 2.77 (q, *J* = 7.6 Hz, 2 H), 1.35 (t, *J*=7.6 Hz, 3 H). LC-MS: m/z = 430.0 [M+H].

(2-Ethyl-6-morpholin-4-yl-imidazo[1,2-*a*]pyridin-3-yl)-[4-(4-fluoro-phenyl)-thiazol-2-yl]-methyl-amine (4). Compound **44b** was treated with morpholine according to general procedure C, using Xantphos and sodium tert-butoxide in toluene, to afford the expected compound **4** as a green oil (10 mg, 7%). ^{1}H NMR (400 MHz, methanol-d_4) δ ppm 7.90 (dd, *J* = 8.9, 5.4 Hz, 2 H), 7.47 (d, *J* = 1.0 Hz, 1 H), 7.42-7.29 (m, 2 H), 7.12 (t, *J* = 8.9 Hz, 2 H), 6.95 (s, 1 H), 3.84-3.78 (m, 4 H), 3.61 (s, 3 H), 3.12-2.94 (m, 4 H), 2.70 (q, *J* = 7.6 Hz, 2 H), 1.31 (t, *J* = 7.6 Hz, 3 H). LC-MS: m/z = 438.0 [M+H].

4-[(3-{[4-(4-Chloro-phenyl)-thiazol-2-yl]-methyl-amino}-2-ethyl-imidazo[1,2-*a*]pyridin-6-ylmethyl)-amino]-piperidine-1-carboxylic acid ethyl ester (5). To a solution of compound **44a** (800 mg, 1.87 mmol) in THF (5 mL) under N_2 was added a solution of lithium aluminium hydride in THF (1M, 3.75 mL, 3.75 mmol). The reaction mixture was allowed to stir at room temperature for 1h. The reaction mixture was poured in water and extracted with EtOAc. The combined organic phases were dried over Na_2SO_4, filtered and concentrated *in vacuo*. The residue was purified by chromatography on silica gel to [3-[[4-(4-chlorophenyl)thiazol-2-yl]-methyl-amino]-2-ethyl-imidazo[1,2-a]pyridin-6-yl]methanol. The latter compound was treated according to general procedure D, followed by addition of 4-amino-piperidine-1-carboxylic acid ethyl ester to afford the desired product **5** (37 mg, 25% yield over 2 steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.84-7.79 (m, 2 H), 7.76 (s, 1 H), 7.57 (d, *J* = 9.3 Hz, 1 H), 7.40-7.35 (m, 2 H), 7.29-7.23 (m, 1 H), 6.73 (s, 1 H), 4.11 (q, *J* = 7.1 Hz, 2 H), 4.04 (br. s, 2 H), 3.81 (s, 2 H), 3.62 (s, 3 H), 2.86-2.70 (m, 4 H), 2.64 (tt, *J* = 10.2, 3.8 Hz, 1 H), 1.83 (br. s, 2 H), 1.62 (br. s, 2 H), 1.35 (t, *J* = 7.6 Hz, 3 H), 1.25 (t, *J* = 7.1 Hz, 3 H). LC-MS: m/z = 553.0/555.1 [M+H].

2-[4-(2-Ethyl-3-{[4-(4-fluoro-phenyl)-thiazol-2-yl]-methyl-amino}-imidazo[1,2-*a*]pyridin-6-yl)-piperazin-1-yl]-ethanol (6). Compound **44c** was treated with 2-piperazin-1-yl-ethanol according to general procedure C, using Davephos and potassium *tert*-butoxide in toluene, to afford the expected compound **6** (161 mg, 16%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.88-7.83 (m, 2 H), 7.50 (d, *J* = 9.7 Hz, 1 H), 7.18 (s, 1 H), 7.17-7.06 (m, 3 H), 6.67 (s, 1 H), 3.67-3.62 (t, *J* = 5.3 Hz, 2 H), 3.61 (s, 3 H), 3.09-3.05 (m, 4 H), 2.72 (q, *J* = 7.6 Hz, 2 H), 2.70-2.66 (m, 4 H), 2.61 (t, *J* = 5.3 Hz, 2 H), 1.33 (t, *J* = 7.6 Hz, 3 H). LC-MS: m/z = 481.3 [M+H].

1-[4-(2-Ethyl-3-{[4-(4-fluoro-phenyl)-thiazol-2-yl]-methyl-amino}-imidazo[1,2-*a*]pyridin-6-yl)-piperidin-1-yl]-propan-1-one (7). To compound **45** (100 mg, 0.21 mmol) dissolved in DCM (2 mL) and TEA (89 µL, 0.64 mmol) was added propionyl chloride (22 µL, 0.26 mmol). After 15 min of stirring at room temperature, the reaction mixture was quenched with water and extracted with DCM. The organic layer was washed with water and brine, dried over Na_2SO_4, filtered and concentrated *in vacuo*. The crude product was purified by chromatography on silica gel to afford the expected amide as a yellow oil. The latter intermediate (55 mg, 0.11 mmol) was dissolved in EtOH (2 mL) and reacted in a continuous-flow hydrogenation reactor (55°C, 50 bar hydrogen pressure) using a 10% Pd/C cartridge. The reaction mixture was then concentrated to dryness and purified by chromatography on silica gel to afford the expected compound **7** as a colorless oil (16 mg, 24% yield over 2 steps). ^{1}H NMR (400 MHz, methanol-d_4) δ ppm 7.93-7.78 (m, 3 H), 7.52 (d, *J* = 9.3 Hz, 1 H), 7.38 (dd, *J* = 9.3, 1.7 Hz, 1 H), 7.17-7.05 (m, 2 H), 6.94 (s, 1 H), 4.66 (d, 1 H, *J* = 13.2 Hz), 4.05 (d, *J* = 12.6 Hz, 1 H), 3.62 (s, 3 H), 3.17 (t, *J* = 12.4 Hz, 1 H), 2.89 (tt, *J* = 12.2, 3.4 Hz, 1 H), 2.78-2.63 (m, 3 H), 2.42 (q, *J* = 7.6 Hz, 2 H), 1.95-1.82 (m, 2 H), 1.77-1.51 (m, 2 H), 1.31 (t, *J* = 7.6 Hz, 3 H), 1.11 (t, *J* = 7.6 Hz, 3 H). LC-MS: m/z = 492.6 [M+H].

[2-Ethyl-6-(1-methanesulfonyl-piperidin-4-yl)-imidazo[1,2-*a*]pyridin-3-yl]-[4-(4-fluoro-phenyl)-thiazol-2-yl]-methyl-amine (8). Compound **46** was treated with methane sulfonyl chloride according to general procedure D to afford the expected compound **8** as a colorless oil (11 mg, 17%). ^{1}H NMR (400 MHz, methanol-d_4) δ ppm 7.93-7.86 (m, 3 H), 7.53 (d, J = 9.3 Hz, 1 H), 7.41 (dd, J = 9.3, 1.7 Hz, 1 H), 7.15-7.09 (m, 2 H), 6.95 (s, 1 H), 3.86-3.79 (m, 2 H), 3.63 (s, 3 H), 2.87-2.61 (m, 8 H), 1.97-1.90 (m, 2 H), 1.87-1.74 (m, 2 H), 1.32 (t, J = 7.6 Hz, 3 H). LC-MS: m/z = 514.0 [M+H].

{6-[1-(3-Dimethylamino-propane-1-sulfonyl)-piperidin-4-yl]-2-ethyl-imidazo[1,2-*a*]pyridin-3-yl}-[4-(4-fluoro-phenyl)-thiazol-2-yl]-methyl-amine (9). Compound **46** was treated with 3-chloropropanesulfonyl chloride according to general procedure D to afford the expected sulfonamide as a yellow foam (50 mg, 99%). The latter intermediate was treated with a 2 M solution of dimethylamine in THF according to general procedure E to afford compound **9** as a colorless oil (30 mg, 59%). ^{1}H NMR (400 MHz, methanol-d_4) δ ppm 7.93-7.85 (m, 3 H), 7.53 (d, J = 9.3 Hz, 1 H), 7.39 (dd, J = 9.3, 1.7 Hz, 1 H), 7.11 (t, J = 8.9 Hz, 2 H), 6.93 (s, 1 H), 3.84 (d, J = 12.0 Hz, 2 H), 3.62 (s, 3 H), 3.09-3.01 (m, 2 H), 2.94-2.88 (m, 2 H), 2.81-2.73 (m, 1 H), 2.72 (q, J = 7.6 Hz, 2 H), 2.47-2.41 (m, 2 H), 2.24 (s, 6 H), 1.98-1.87 (m, 4 H), 1.82-1.69 (m, 2 H), 1.31 (t, J = 7.6 Hz, 3 H). LC-MS: m/z = 585.1 [M+H].

[4-(2-Ethyl-3-{[4-(4-fluoro-phenyl)-thiazol-2-yl]-methyl-amino}-imidazo[1,2-*a*]pyridin-6-yl)-piperidin-1-yl]-acetic acid ethyl ester (10). To compound **46** (200 mg, 0.46 mmol) dissolved in DMF (3 mL) was added NaH (60% in oil, 28 mg, 0.69 mmol) and the mixture was stirred at room temperature for 1.3 h. Ethyl bromoacetate (61 µL, 0.55 mmol) was added and the reaction mixture was stirred at room temperature for 1 h. The reaction was quenched with AcOH (3 drops), diluted with EtOAc and water, then basified with a saturated aqueous solution of

Na_2CO_3 and extracted with EtOAc. The combined organic layers were washed with water and brine, dried over Na_2SO_4, filtered and concentrated *in vacuo*. The residue was purified by chromatography on silica gel to afford the expected compound **10** as a yellow oil (134 mg, 56%). ^{1}H NMR (400 MHz, methanol-d_4) δ ppm 7.98-7.92 (m, 2 H), 7.90 (s, 1 H), 7.58 (dd, *J* = 9.3, 0.7 Hz, 1 H), 7.46 (dd, *J* = 9.3, 1.7 Hz, 1 H), 7.20-7.14 (m, 2 H), 6.99 (s, 1 H), 4.24 (q, *J* = 7.3 Hz, 2 H), 3.68 (s, 3 H), 3.31 (s, 2 H), 3.12-3.08 (m, 2 H), 2.78 (q, *J* = 7.6 Hz, 2 H), 2.73-2.63 (m, 1 H), 2.41-2.32 (m, 2 H), 1.92-1.85 (m, 4 H), 1.38 (t, *J* = 7.6 Hz, 3 H), 1.32 (t, *J*=7.3 Hz, 3 H). LC-MS: m/z = 522.2 [M+H].

[4-(2-Ethyl-3-{[4-(4-fluoro-phenyl)-thiazol-2-yl]-methyl-amino}-imidazo[1,2-*a*]pyridin-6-yl)-piperazin-1-yl]-acetic acid ethyl ester (11). To compound **48** (127 mg, 0.29 mmol) dissolved in MeCN (2 mL) were slowly added ethyl chloroacetate (47µL, 0.44 mmol) and TEA (160 µL, 1.17 mmol). The reaction mixture was allowed to stir at 45°C for 18 h, then quenched with a saturated aqueous solution of NH_4Cl and extracted with Et_2O and EtOAc. The combined organic layers were dried over Na_2SO_4, filtered and concentrated *in vacuo*. The residue was purified by chromatography on silica gel to afford the expected compound **11** as a viscous oil (108 mg, 71%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.89-7.84 (m, 2 H), 7.50 (d, *J* = 9.7 Hz, 1 H), 7.20-7.04 (m, 4 H), 6.67 (s, 1 H), 4.19 (q, *J* = 7.3 Hz, 2 H), 3.61 (s, 3 H), 3.27 (s, 2 H), 3.12-3.07 (m, 4 H), 2.79-2.68 (m, 6 H), 1.33 (t, *J* = 7.6 Hz, 3 H), 1.28 (t, *J* = 7.3 Hz, 3 H). LC-MS: m/z = 523.0 [M+H].

[4-(2-Ethyl-3-{[4-(4-fluoro-phenyl)-thiazol-2-yl]-methyl-amino}-imidazo[1,2-*a*]pyridin-6-yl)-piperazin-1-yl]-acetic acid (12). Compound **11** (104 mg, 0.12 mmol) and LiOH (24 mg, 0.995 mmol) were stirred in a mixture of THF (1 mL) and water (1 mL) at room temperature for 48 h. A 1 N aqueous solution of HCl was added and the reaction mixture was extracted twice

with DCM. The aqueous phase was concentrated *in vacuo* and the expected compound **12** (33 mg, 33%) was obtained after LC-MS purification of the residue. ^{1}H NMR (400 MHz, methanol-d_4) δ ppm 7.93-7.86 (m, 2 H), 7.45 (dd, *J* = 9.4, 1.2 Hz, 1 H), 7.41-7.35 (m, 2 H), 7.11 (t, *J* = 8.9 Hz, 2 H), 6.95 (s, 1 H), 3.61 (s, 3 H), 3.25-3.13 (m, 6 H), 2.95-2.90 (m, 4 H), 2.70 (q, *J* = 7.6 Hz, 2 H), 1.31 (t, *J* = 7.6 Hz, 3 H). LC-MS: m/z = 495.0 [M+H].

[2-Ethyl-6-(4-oxazol-2-ylmethyl-piperazin-1-yl)-imidazo[1,2-*a*]pyridin-3-yl]-[4-(4-fluoro-phenyl)-thiazol-2-yl]-methyl-amine (13). Compound **48** was treated with 2-chloromethyl-oxazole according to general procedure E to afford compound **13** as a yellow oil (17 mg, 22%). ^{1}H NMR (400 MHz, methanol-d_4) δ ppm 7.92-7.85 (m, 3 H), 7.44 (dd, *J* = 9.6, 0.8 Hz, 1 H), 7.38-7.29 (m, 2 H), 7.15 (d, *J* = 0.8 Hz, 1 H), 7.11 (t, *J* = 8.9 Hz, 2 H), 6.93 (s, 1 H), 3.77 (s, 2 H), 3.59 (s, 3 H), 3.16-3.02 (m, 4 H), 2.73-2.63 (m, 6 H), 1.30 (t, *J* = 7.6 Hz, 3 H). LC-MS: m/z = 518.1 [M+H].

[2-Ethyl-6-(4-[1,2,4]oxadiazol-3-ylmethyl-piperazin-1-yl)-imidazo[1,2-*a*]pyridin-3-yl]-[4-(4-fluoro-phenyl)-thiazol-2-yl]-methyl-amine (14). Compound **48** was treated with 3-chloromethyl-[1,2,4]oxadiazole according to general procedure E to afford compound **14** (13 mg, 24%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.70 (br. s, 1 H), 7.86-7.81 (m, 2 H), 7.67 (d, *J* = 9.8 Hz, 1 H), 7.23 (dd, *J* = 9.8, 2.0 Hz, 1 H), 7.17 (d, *J* = 1.7 Hz, 1 H), 7.10 (t, *J* = 8.7 Hz, 2 H), 6.69 (s, 1 H), 3.83 (s, 2 H), 3.59 (s, 3 H), 3.15-3.10 (m, 4 H), 2.79-2.71 (6 H, m), 1.35 (t, *J* = 7.6 Hz, 3 H). LC-MS: m/z = 519.1 [M+H].

{2-Ethyl-6-[4-(1H-imidazol-2-ylmethyl)-piperazin-1-yl]-imidazo[1,2-*a*]pyridin-3-yl}-[4-(4-fluoro-phenyl)-thiazol-2-yl]-methyl-amine (15). Compound **48** was reacted with 1H-imidazole-2-carbaldehyde according to general procedure F to afford compound **15** (22 mg, 29%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.88-7.83 (m, 2 H), 7.49 (d, *J* = 9.7 Hz, 1 H), 7.16 (d,

J = 2.0 Hz, 1 H), 7.14-7.06 (m, 3 H), 7.01 (s, 2 H), 6.66 (s, 1 H), 3.72 (s, 2 H), 3.60 (s, 3 H), 3.08-3.01 (m, 4 H), 2.72 (q, *J* = 7.6 Hz, 2 H), 2.68-2.64 (m, 4 H), 1.33 (t, *J* = 7.6 Hz, 3 H). LC-MS: m/z = 517.3 [M+H].

{2-Ethyl-6-[4-(3H-imidazol-4-ylmethyl)-piperazin-1-yl]-imidazo[1,2-*a*]pyridin-3-yl}-[4-(4-fluoro-phenyl)-thiazol-2-yl]-methyl-amine (16). Compound **48** was reacted with 3H-imidazole-4-carbaldehyde according to general procedure F to afford compound **16** (37 mg, 49%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.92-7.80 (m, 2 H), 7.64 (s, 1 H), 7.47 (d, *J* = 9.7 Hz, 1 H), 7.17 (s, 1 H), 7.13-7.05 (m, 3 H), 7.01 (s, 1 H), 6.66 (s, 1 H), 3.66 (s, 2 H), 3.59 (s, 3 H), 3.12-3.08 (m, 4 H), 2.77-2.65 (m, 6 H), 1.32 (t, *J* = 7.6 Hz, 3 H). LC-MS: m/z = 517.3 [M+H].

2-[4-(2-Ethyl-3-{[4-(4-fluoro-phenyl)-thiazol-2-yl]-methyl-amino}-imidazo[1,2-*a*]pyridin-6-yl)-piperidin-1-yl]-acetamide (17). Compound **46** was treated with 2-bromoacetamide according to general procedure E to afford compound **17** as a colorless oil (120 mg, quantitative yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.90-7.84 (m, 2 H), 7.62 (s, 1 H), 7.56 (d, *J* = 9.2 Hz, 1 H), 7.17 (dd, *J* = 9.3, 1.7 Hz, 1 H), 7.14-7.07 (m, 2 H), 7.08 (br. s, 1 H), 6.68 (s, 1 H), 5.42 (br. s, 1 H), 3.63 (s, 3 H), 3.06-2.96 (m, 4 H), 2.76 (q, *J* = 7.6 Hz, 2 H), 2.57-2.47 (m, 1 H), 2.31-2.25 (m, 2 H), 1.92-1.84 (m, 2 H), 1.81-1.69 (m, 2 H), 1.35 (t, *J* = 7.6 Hz, 3 H). LC-MS: m/z = 493.3 [M+H].

***N*-Cyclopropyl-2-[4-(2-ethyl-3-{[4-(4-fluoro-phenyl)-thiazol-2-yl]-methyl-amino}-imidazo[1,2-*a*]pyridin-6-yl)-piperidin-1-yl]-acetamide (18).** Compound **46** was treated with 2-chloro-N-cyclopropyl-acetamide according to general procedure E to afford compound **18** as an orange foam (114 mg, 58%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.87 (dd, *J* = 8.9, 5.4 Hz, 2 H), 7.62 (s, 1 H), 7.56 (d, *J* = 9.3 Hz, 1 H), 7.18 (dd, *J* = 9.3, 1.7 Hz, 1 H), 7.11 (t, *J* = 8.7 Hz, 2 H), 6.69 (s, 1 H), 3.63 (s, 3 H), 2.99 (s, 2 H), 2.94-2.88 (m, 2 H), 2.76 (q, *J* = 7.6 Hz, 2 H), 2.74-2.69

(m, 1 H), 2.57-2.45 (m, 1 H), 2.30-2.20 (m, 2 H), 1.91-1.81 (m, 2 H), 1.77-1.66 (m, 2 H), 1.35 (t, *J* = 7.6 Hz, 3 H), 0.85-0.74 (m, 2 H), 0.57-0.48 (m, 2 H). LC-MS: m/z = 533.3 [M+H].

2-[4-(2-Ethyl-3-{[4-(4-fluoro-phenyl)-thiazol-2-yl]-methyl-amino}-imidazo[1,2-*a*]pyridin-6-yl)-piperidin-1-yl]-N,N-dimethyl-acetamide (19). Compound **46** was treated with 2-chloro-N,N-dimethyl-acetamide according to general procedure E to afford compound **19** (82 mg, 40%). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 7.87 (dd, *J* = 8.9, 5.5 Hz, 2 H), 7.61 (s, 1 H), 7.54 (d, *J* = 9.2 Hz, 1 H), 7.18 (dd, *J*=9.2, 1.6 Hz, 1 H), 7.10 (t, *J*=8.7 Hz, 2 H), 6.67 (s, 1 H), 3.61 (s, 3 H), 3.19 (s, 2 H), 3.07 (s, 3 H), 3.05-2.99 (m, 2 H), 2.95 (s, 3 H), 2.74 (q, *J* = 7.6 Hz, 2 H), 2.57-2.42 (m, 1 H), 2.29-2.11 (m, 2 H), 1.87-1.74 (m, 4 H), 1.34 (t, *J* = 7.6 Hz, 3 H). LC-MS: m/z = 521.4 [M+H].

2-[4-(2-Ethyl-3-{[4-(4-fluoro-phenyl)-thiazol-2-yl]-methyl-amino}-imidazo[1,2-*a*]pyridin-6-yl)-piperidin-1-yl]-1-pyrrolidin-1-yl-ethanone (20). To a solution of compound **46** (30 mg, 0.069 mmol) in MeCN (2 mL) in a sealed-cap tube were added TEA (38 µL, 0.276 mmol) and 2-chloro-1-pyrrolidin-1-yl-ethanone (20 mg, 0.138 mmol). The vial was sealed and the reaction mixture was heated at 190°C under microwave irradiation for 20 min. The reaction was quenched with water and the mixture was extracted with DCM. The aqueous phase was then made acidic with a 1N aqueous HCl solution (2 mL) and extracted twice with DCM. The aqueous phase was made basic with a saturated aqueous solution of NaHCO3 and extracted twice with DCM. The combined organic layers were washed with a saturated aqueous solution of $NaHCO_3$ and with brine, dried over Na_2SO_4, filtered and concentrated *in vacuo*. The residue was purified by chromatography on silica gel to afford the expected compound **20** as a colorless oil (17 mg, 45%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.87 (dd, *J* = 8.7, 5.5 Hz, 2 H), 7.61 (br. s, 1 H), 7.54 (d, *J* = 9.3 Hz, 1 H), 7.18 (dd, *J* = 9.3, 1.7 Hz, 1 H), 7.10 (t, *J* = 8.7 Hz, 2 H), 6.67 (s, 1

H), 3.61 (s, 3 H), 3.49 (q, *J* = 6.6 Hz, 4 H), 3.15 (s, 2 H), 3.10-3.05 (m, 2 H), 2.75 (q, *J* = 7.6 Hz, 2 H), 2.55-2.44 (m, 1 H), 2.28-2.17 (m, 2 H), 1.94 (q, *J* = 6.6 Hz, 2 H), 1.89-1.77 (m, 6 H), 1.34 (t, *J* = 7.6 Hz, 3 H). LC-MS: m/z = 547.4 [M+H].

2-[4-[2-ethyl-3-[[4-(4-fluorophenyl)thiazol-2-yl]-methyl-amino]imidazo[1,2-*a*]pyridin-6-yl]piperazin-1-yl]-1-morpholino-ethanone (21). Compound **48** (150 mg, 0.32 mmol) was dissolved in MeCN (5 mL) and TEA (132 µL, 0.95 mmol) before addition of **49** (104 mg, 0.63 mmol). The reaction was heated under microwave irradiation at 160 °C for 40 min, then diluted with water and DCM and the aqueous phase was extracted 2 times with DCM. The combined organic layers were washed with water and brine, dried over Na_2SO_4, filtered and concentrated *in vacuo*. The residue was purified by chromatography on silica gel to afford the expected compound **21** (46 mg, 26%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.91-7.87 (m, 2 H), 7.57 (d, *J* = 9.7 Hz, 1 H), 7.21-7.11 (m, 4 H), 6.72 (s, 1 H), 3.70-3.63 (m, 11 H), 3.27 (s, 2 H), 3.13-3.06 (m, 4 H), 2.80-2.70 (m, 6H), 1.37 (t, *J* = 7.6 Hz, 3 H). LC-MS: m/z = 564.3 [M+H].

2-[4-(2-Ethyl-3-{[4-(4-fluoro-phenyl)-thiazol-2-yl]-methyl-amino}-imidazo[1,2-*a*]pyridin-6-yl)-piperidin-1-yl]-1-(3-hydroxy-azetidin-1-yl)-ethanone (22). Compound **46** was reacted with **47** according to general procedure E to afford compound **22** as an off-white powder (1.85 g, 76%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.86 (dd, *J* = 8.8, 5.4 Hz, 2 H), 7.61 (br. s, 1 H), 7.55 (d, *J* = 9.3 Hz, 1 H), 7.20 (dd, *J* = 9.3, 1.7 Hz, 1 H), 7.10 (t, *J* = 8.7 Hz, 2 H), 6.67 (s, 1 H), 4.72-4.65 (m, 1 H), 4.46 (dd, *J* = 9.2, 7.2 Hz, 1 H), 4.28 (dd, *J* = 10.4, 7.0 Hz, 1 H), 4.14-4.09 (m, 1 H), 3.90 (dd, *J*=10.8, 4.4 Hz, 1 H), 3.61 (s, 3 H), 3.10-2.99 (m, 4 H), 2.73 (q, *J* = 7.6 Hz, 2 H), 2.56-2.45 (m, 1 H), 2.28-2.17 (m, 2 H), 1.94-1.76 (m, 4 H), 1.34 (t, *J* = 7.6 Hz, 3 H). LC-MS: m/z = 549.3 [M+H].

Benzothiazol-2-yl-[2-ethyl-6-(1-methanesulfonyl-piperidin-4-yl)-imidazo[1,2-*a*]pyridin-3-yl]-methyl-amine (23). Compound **51** (150 mg, 0.446 mmol), cesium carbonate (436 mg, 1.337 mmol), XantPhos (39 mg, 0.067 mmol), 2-bromobenzo[*d*]thiazole (105 mg, 0.490 mmol) and dioxane (6 mL) were stirred for 10 min with argon bubbling. $Pd(OAc)_2$ (20 mg, 0.089 mmol) was added and the reaction mixture was refluxed overnight, then cooled to room temperature and diluted with EtOAc. The reaction mixture was filtered, the filtrate wad diluted with water and extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered and concentrated *in vacuo*. The residue was purified by chromatography on silica gel to afford the expected compound **23** (75 mg, 36%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.71 (d, *J* = 8.0 Hz, 1 H), 7.63-7.55 (m, 2 H), 7.50 (d, *J* = 7.2 Hz, 1 H), 7.36 (t, *J* = 7.2 Hz, 1 H), 7.21-7.08 (m, 2 H), 3.96-3.90 (m, 2 H), 3.65 (s, 3 H), 2.81 (s, 3 H), 2.80-2.65 (m, 4 H), 2.59 (t, *J* = 15.6 Hz, 1 H), 1.96-1.93 (m, 2 H), 1.91-1.58 (m, 2 H), 1.34 (t, *J* = 7.6 Hz, 3 H). LC-MS: m/z = 470.3 [M+H].

(4-Cyclohexyl-thiazol-2-yl)-[2-ethyl-6-(1-methanesulfonyl-piperidin-4-yl)-imidazo[1,2-*a*]pyridin-3-yl]-methyl-amine (24). *Step 1: 2-bromo-1-cyclohexyl-ethanone.* To LDA (2 M in THF / heptane / ethylbenzene, 7.70 mmol) in THF (20 mL) at -78°C was added dropwise cyclohexyl-ethanone (810 mg, 6.42 mmol) in THF (10 mL) and the mixture was stirred at -78°C for 1 h. Then chlorotrimethylsilane (1.62 mL, 12.84 mmol) was added dropwise and the reaction was stirred at -78°C for 3.5 h. The mixture was poured into saturated aqueous $NaHCO_3$ solution, the aqueous layer was then extracted with Et_2O twice and combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated. The residue was taken up in THF (25 mL), at 0°C NBS (1.14 g, 6.42 mmol) and $NaHCO_3$ (809 mg, 9.63 mmol) were added and the mixture was stirred at 0°C for 10 min and then at rt for 1 h. The mixture was quenched with saturated

aqueous $NaHCO_3$ solution. The aqueous phase was then extracted with Et_2O twice and combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated to afford the expected compound (1.3 g, quantitative yield) that was used without further purification in the next step. *Step 2: (4-Cyclohexyl-thiazol-2-yl)-[2-ethyl-6-(1-methanesulfonyl-piperidin-4-yl)-imidazo[1,2-a]pyridin-3-yl]-methyl-amine* (**24**). Compound **51** was treated with 2-bromo-1-cyclohexyl-ethanone according to general procedure G to afford the desired product **24** (28 mg, 20% yield over 2 steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.60 (s, 1 H), 7.55 (d, *J* = 9.7 Hz, 1 H), 7.12 (dd, *J* = 9.3, 1.7 Hz, 1 H), 6.07 (d, *J* = 0.8 Hz, 1 H), 3.97-3.94 (m, 2 H), 3.52 (s, 3 H), 2.83 (s, 3 H), 2.81-2.56 (m, 6 H), 2.12-2.09 (m, 2 H), 1.99-1.92 (m, 2 H), 1.90-1.68 (m, 6 H), 1.45-1.35 (m, 4 H), 1.33 (t, *J* = 7.6 Hz, 3 H). LC-MS: m/z = 502.4 [M+H].

[4-(6-Chloro-pyridin-3-yl)-thiazol-2-yl]-[2-ethyl-6-(1-methanesulfonyl-piperidin-4-yl)-imidazo[1,2-*a*]pyridin-3-yl]-methyl-amine (25). *Step 1: 2-bromo-1-(6-chloro-3-pyridyl)ethanone.* 1-(6-Chloro-3-pyridyl)ethanone (150 mg, 0.96 mmol) was dissolved in THF (5 mL), pyridinium tribromide (320 mg, 1.02 mmol) and the mixture was stirred at rt overnight. The reaction was quenched with saturated aqueous $NaHCO_3$ solution and the aqueous phase was then extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated. The crude mixture was purified by chromatography on silica gel to obtain 2-bromo-1-(6-chloro-3-pyridyl)ethanone. *Step 2: [4-(6-Chloro-pyridin-3-yl)-thiazol-2-yl]-[2-ethyl-6-(1-methanesulfonyl-piperidin-4-yl)-imidazo[1,2-a]pyridin-3-yl]-methyl-amine* (**25**). Compound **51** was treated with 2-bromo-1-(6-chloro-pyridin-3-yl)-ethanone according to general procedure G to afford the desired product **25** (39 mg, 60% yield over 2 steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.84-8.77 (m, 1 H), 8.36 (d, *J* = 9.3 Hz, 1 H), 8.03 (dd, *J* = 8.3, 2.4 Hz, 1 H), 7.83 (s, 1 H), 7.75 (dd, *J* = 9.3, 1.5 Hz, 1 H), 7.36 (d, *J* = 8.3 Hz, 1 H), 7.05-6.90 (m, 1 H),

4.00-3.96 (m, 2 H), 3.67 (s, 3 H), 3.05-2.91 (m, 2 H), 2.86-2.75 (m, 6 H), 2.07-1.96 (m, 2 H), 1.91-1.80 (m, 2 H), 1.33 (t, *J* = 7.6 Hz, 3 H). LC-MS: m/z = 531.1/533.3 [M+H].

***N*-[2-Ethyl-6-(1-methylsulfonyl-4-piperidyl)imidazo[1,2-*a*]pyridin-3-yl]-*N*-methyl-4-[4-(trifluoromethyl)phenyl]thiazol-2-amine (26).** Compound **54e** (95 mg, 0.16 mmol) was submitted to general procedure D using TEA and methane sulfonyl chloride to afford *N*-[2-ethyl-6-(1-methylsulfonyl-3,6-dihydro-2H-pyridin-4-yl)imidazo[1,2-*a*]pyridin-3-yl]-N-methyl-4-[4-(trifluoromethyl)phenyl]thiazol-2-amine a colorlesss a yellow oil (43 mg, 39%). The latter compound (35 mg, 0.067 mmol) was reduced following general procedure H and compound **26** was obtained as a colorless oil after purification by chromatography on silica gel (15 mg, 43%).^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.03 (d, *J*=8.1 Hz, 2 H), 7.71-7.61 (m, 4 H), 7.19 (dd, *J* = 9.3, 1.8 Hz, 1 H), 6.91 (s, 1 H), 4.01-3.96 (m, 2 H), 3.67 (s, 3 H), 2.84 (s, 3 H), 2.72-2.75 (m, 4 H), 2.70-2.62 (m, 1 H), 2.02-1.98 (m, 2 H), 1.92-1.82 (2 H, m), 1.43 (t, *J* = 7.6 Hz, 3 H). LC-MS: m/z = 564.1 [M+H].

[2-Ethyl-6-(1-methanesulfonyl-piperidin-4-yl)-imidazo[1,2-*a*]pyridin-3-yl]-[4-(4-methoxy-phenyl)-thiazol-2-yl]-methyl-amine (27). Compound **54f** (75 mg, 0.16 mmol) was treated with TEA and methane sulfonyl chloride according to general procedure B to afford *N*-[2-ethyl-6-(1-methylsulfonyl-3,6-dihydro-2H-pyridin-4-yl)imidazo[1,2-*a*]pyridin-3-yl]-4-(4-methoxyphenyl)-*N*-methyl-thiazol-2-amine as a yellow oil (72 mg, 88%). The latter intermediate (66 mg, 0.120 mmol) underwent hydrogenation following general procedure H to give compound **27** as a colorless oil after purification on silica gel (22 mg, 35%). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 7.85-7.79 (m, 2 H), 7.63 (s, 1 H), 7.60 (d, *J* = 9.1 Hz, 1 H), 7.16 (dd, *J* = 9.1, 1.8 Hz, 1 H), 6.98-6.91 (m, 2 H), 6.62 (s, 1 H), 3.96-3.92 (m, 2 H), 3.86 (s, 3 H), 3.63 (s, 3 H), 2.84-2.69 (m, 7 H),

2.68-2.58 (m, 1 H), 2.02-1.92 (m, 2 H), 1.92-1.74 (m, 2 H), 1.35 (t, *J* = 7.6 Hz, 3 H). LC-MS: m/z = 526.3 [M+H].

[4-(4-*tert*-Butyl-phenyl)-thiazol-2-yl]-[2-ethyl-6-(1-methanesulfonyl-piperidin-4-yl)-imidazo[1,2-*a*]pyridin-3-yl]-methyl-amine (28). Compound **44d** (70 mg, 0.14 mmol) was submitted to general procedure B with 1-methanesulfonyl-4-(tetramethyl-1,3,2-dioxaborolan-2-yl)-1,2,3,6-tetrahydropyridine to afford 4-(4-*tert*-butylphenyl)-*N*-[2-ethyl-6-(1-methylsulfonyl-3,6-dihydro-2H-pyridin-4-yl)imidazo[1,2-*a*]pyridin-3-yl]-N-methyl-thiazol-2-amine as a yellow oil (74 mg, 99%). The latter compound (66 mg, 0.120 mmol) was submitted to general procedure H to afford compound **28** as a colorless oil (18 mg, 27%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.83-7.78 (m, 2 H), 7.63 (br. s, 1 H), 7.58 (d, *J* = 9.3 Hz, 1 H,), 7.46-7.41 (m, 2 H), 7.15 (dd, *J* = 9.3, 1.7 Hz, 1 H), 6.70 (s, 1 H), 3.99-3.90 (m, 2 H), 3.62 (s, 3 H), 2.81 (s, 3 H), 2.79-2.69 (m, 4 H), 2.66-2.55 (m, 1 H), 1.98-1.95 (m, 2 H), 1.90-1.75 (m, 2 H), 1.35 (s, 9 H), 1.26 (t, *J* = 7.6 Hz, 3 H). LC-MS: m/z = 552.4 [M+H].

4-(3,4-Difluorophenyl)-*N*-[2-ethyl-6-(1-methylsulfonyl-4-piperidyl)imidazo[1,2-*a*]pyridin-3-yl]-*N*-methyl-thiazol-2-amine (29). Compound **54g** (105 mg, 0.20 mmol) reacted with methane sulfonyl chloride in presence of TEA according general procedure D to give after LC-MS purification 4-(3,4-difluorophenyl)-N-[2-ethyl-6-(1-methylsulfonyl-3,6-dihydro-2H-pyridin-4-yl)imidazo[1,2-a]pyridin-3-yl]-N-methyl-thiazol-2-amine as a yellow oil (58 mg, 55%). The latter compound (43 mg, 0.08 mmol) was submitted to general procedure H to afford **29** as a colorless oil (15 mg, 35%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.79-7.73 (m, 1 H), 7.63-7.53 (m, 3 H), 7.24-7.11 (m, 2 H), 6.70 (s, 1 H), 3.97-3.93 (m, 2 H), 3.62 (s, 3 H), 2.81 (s, 3 H), 2.79-2.69 (m, 4 H), 2.68-2.56 (m, 1 H), 2.03-1.95 (m, 2 H), 1.92-1.81 (m, 2 H), 1.35 (t, *J* = 7.6 Hz, 3 H). LC-MS: m/z = 532.2 [M+H].

4-(2,4-difluorophenyl)-*N*-[2-ethyl-6-(1-methylsulfonyl-4-piperidyl)imidazo[1,2-*a*]pyridin-3-yl]-*N*-methyl-thiazol-2-amine (30). Compound **51** was treated with 2-chloro-1-(2,4-difluorophenyl)ethanone according to general procedure G to afford **30** (42 mg, 48% over 2 steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.29-8.18 (m, 1 H), 7.66-7.56 (m, 2 H), 7.19 (dd, *J* = 9.3, 1.8 Hz, 1 H), 7.03-6.95 (m, 2 H), 6.95-6.89 (m, 1 H), 4.00-3.96 (m, 2 H), 3.62 (s, 3 H), 2.82 (s, 3 H), 2.82-2.71 (m, 4 H), 2.66-2.57 (m, 1 H), 2.01-1.94 (m, 2 H), 1.88-1.81 (m, 2 H), 1.35 (t, *J* = 7.6 Hz, 3 H). LC-MS: m/z = 532.2 [M+H].

4-(3,5-Difluorophenyl)-*N*-[2-ethyl-6-(1-methylsulfonyl-4-piperidyl)imidazo[1,2-*a*]pyridin-3-yl]-*N*-methyl-thiazol-2-amine (31). Compound **54h** (80 mg, 0.15 mmol) was submitted to general procedure H to afford 4-(3,5-difluorophenyl)-N-[2-ethyl-6-(4-piperidyl)imidazo[1,2-a]pyridin-3-yl]-N-methyl-thiazol-2-amine (56 mg, 70%) that was used without purification in the next step. This amine was submitted to general procedure D with TEA to give after LC-MS purification compound **31** as a yellow oil (22 mg, 40%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.64-7.61 (m, 2 H), 7.47-7.41 (m, 2 H), 7.19 (dd, *J* = 9.3, 1.8 Hz, 1 H), 6.83 (s, 1 H), 6.81-6.76 (m, 1 H), 4.00-3.96 (m, 2 H), 3.65 (s, 3 H), 2.85 (s, 3 H), 2.82-2.75 (m, 4 H), 2.69-2.61 (m, 1 H), 2.02-1.96 (m, 2 H), 1.92-1.81 (m, 2 H), 1.38 (t, *J* = 7.6 Hz, 3 H). LC-MS: m/z = 532.2 [M+H].

***N*-[2-ethyl-6-(1-methylsulfonyl-4-piperidyl)imidazo[1,2-*a*]pyridin-3-yl]-4-(4-fluoro-2-methyl-phenyl)-*N*-methyl-thiazol-2-amine (32).** *Step 1: 2-bromo-1-(4-fluoro-2-methyl-phenyl)ethanone.* 1-(4-fluoro-2-methyl-phenyl)ethanone was submitted to general procedure I to get a mixture of expected product/di-brominated intermediate that was used as such in the next step. *Step 2: N-[2-ethyl-6-(1-methylsulfonyl-4-piperidyl)imidazo[1,2-a]pyridin-3-yl]-4-(4-fluoro-2-methyl-phenyl)-N-methyl-thiazol-2-amine* (**32**). Compound **51** was treated with 2-bromo-1-(4-fluoro-2-methyl-phenyl)ethanone according to general procedure G to afford **32** (43 mg, 48%

over 2 steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.71-7.62 (m, 2 H), 7.58 (d, *J* = 9.3 Hz, 1 H), 7.15 (dd, *J* = 9.3, 1.9 Hz, 1 H), 7.01-6.91 (m, 2 H), 6.48 (s, 1 H), 3.99-3.95 (m, 2 H), 3.58 (s, 3 H), 2.83 (s, 3 H), 2.81-2.72 (m, 4 H), 2.72-2.61 (m, 1 H), 2.53 (s, 3 H), 2.04-1.94 (m, 2 H), 1.93-1.81 (m, 2 H), 1.39 (d, *J* = 7.6 Hz, 3 H). LC-MS: m/z = 528.0 [M+H].

[2-Ethyl-6-(1-methanesulfonyl-piperidin-4-yl)-imidazo[1,2-*a*]pyridin-3-yl]-(5-fluoro-8H-indeno[1,2-*d*]thiazol-2-yl)-methyl-amine (33). *Step 1: 2-bromo-6-fluoro-indan-1-one.* 6-Fluoro-indan-1-one was submitted to general procedure I to get a mixture of expected product/di-brominated intermediate that was used as such in the next step. *Step 2: [2-Ethyl-6-(1-methanesulfonyl-piperidin-4-yl)-imidazo[1,2-a]pyridin-3-yl]-(5-fluoro-8H-indeno[1,2-d]thiazol-2-yl)-methyl-amine* (**33**). Compound **51** was treated with 2-bromo-2,3-dihydro-6-fluoro-1H-inden-1-one according to general procedure G to afford the desired product **33** (41 mg, 64% yield over 2 steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.66-7.57 (m, 2 H), 7.39 (dd, *J* = 8.9, 2.4 Hz, 1 H), 7.35 (dd, *J*=8.2, 4.8 Hz, 1 H), 7.21-7.12 (m, 1 H), 6.89 (ddd, *J* = 9.3, 8.3, 2.6 Hz, 1 H), 3.96-3.92 (m, 2 H), 3.64 (s, 3 H), 3.62-3.60 (m, 2 H), 2.81 (s, 3 H), 2.80-2.70 (m, 4 H), 2.65-2.58 (m, 1 H), 2.00-1.92 (m, 2 H), 1.89-1.76 (m, 2 H), 1.36 (t, *J* = 7.6 Hz, 3 H). LC-MS: m/z = 526.3 [M+H].

[2-Ethyl-6-(1-methanesulfonyl-piperidin-4-yl)-imidazo[1,2-*a*]pyridin-3-yl]-(6-fluoro-8H-indeno[1,2-*d*]thiazol-2-yl)-methyl-amine (34). *Step 1: 2-Bromo-5-fluoro-indan-1-one.* (a) 5-Fluoro-indan-1-one was submitted to general procedure I to get 2,2-dibromo-5-fluoro-indan-1-one (301 mg, 98%). (b) 2,2-Dibromo-5-fluoro-indan-1-one (255 mg, 0.83 mmol) was dissolved in THF (2 mL) and diethylphosphite (107 µL, 0.83 mmol) was added dropwise followed by a cooled solution of TEA (115 µL, 0.83 mmol) in THF (1 mL). The reaction was warmed to rt and stirred for 6 h, and solvents were concentrated *in vacuo*. The residue was taken up in water and

EtOAc, layers were separated and the aqueous layer was extracted twice with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated. The crude mixture was purified by chromatography on silica gel to obtain the desired compound (126 mg, 67%).^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.90 (dd, *J* = 8.4, 5.3 Hz, 1 H,), 7.21-7.15 (m, 2 H), 4.70 (dd, *J* = 7.5, 3.2 Hz, 1 H,), 3.87 (dd, *J* = 18.4, 7.5 Hz, 1 H), 3.45 (dd, *J* = 18.4, 3.2 Hz, 1 H,). *Step 2: [2-Ethyl-6-(1-methanesulfonyl-piperidin-4-yl)-imidazo[1,2-a]pyridin-3-yl]-(6-fluoro-8H-indeno[1,2-d]thiazol-2-yl)-methyl-amine* (**34**). Compound **51** was treated with 2-bromo-2,3-dihydro-5-fluoro-1H-inden-1-one according to general procedure G to afford the desired product **34** (11 mg, 48% yield over 2 steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.63-7.56 (m, 3 H), 7.17 (td, *J* = 8.5, 2.0 Hz, 2 H), 7.12-7.03 (m, 1 H), 3.97-3.92 (m, 2 H), 3.68-3.57 (m, 5 H), 2.81 (s, 3 H), 2.80-2.70 (m, 4 H), 2.63-2.58 (m, 1 H), 2.00-1.92 (m, 2 H), 1.90-1.75 (m, 2 H), 1.36 (t, *J* = 7.6 Hz, 3 H). LC-MS: m/z = 526.3 [M+H].

2-[2-[[2-ethyl-6-(1-methylsulfonyl-4-piperidyl)imidazo[1,2-a]pyridin-3-yl]-methyl-amino]thiazol-4-yl]-5-fluoro-benzonitrile (35). Compound **52** (50 mg, 0.08 mmol) was dissolved in pyridine (2.5 mL), CuCN (38 mg, 0.42 mmol) was added and the mixture was irradiated under microwaves at 150°C for 2 h. The reaction was quenched by addition of water and the aqueous phase was extracted with EtOAc 3 times. The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by chromatography on silica gel to afford the expected compound **35** (18 mg, 41%).^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.10 (dd, *J* = 8.9, 5.5 Hz, 1 H), 7.65 (br s, 1 H), 7.62 (d, *J* = 9.4 Hz, 1 H), 7.48 (1 H, dd, *J*=8.1, 2.7 Hz), 7.43-7.38 (m, 1 H), 7.21-7.19 (m, 2H), 4.02-3.96 (m, 2 H), 3.66 (s, 3 H), 2.86 (s, 3 H), 2.83-2.77 (m, 4 H), 2.71-2.63 (m, 1 H), 2.04-1.97 (m, 2 H), 1.93-1.81 (m, 2 H), 1.39 (t, *J* = 7.6 Hz, 3 H). LC-MS: m/z = 539.3 [M+H].

[2-Ethyl-6-(1-methanesulfonyl-piperidin-4-yl)-imidazo[1,2-*a*]pyridin-3-yl]-[4-(4-fluoro-phenyl)-5-methyl-thiazol-2-yl]-methyl-amine (36). Compound **51** was treated with 2-chloro-1-(4-fluoro-phenyl)-propan-1-one according to general procedure G to afford the desired product **36** (23 mg, 21% yield over 2 steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.71-7.65 (m, 2 H), 7.64 (s, 1 H), 7.57 (d, *J*=9.3 Hz, 1 H), 7.18-7.05 (m, 3 H), 3.99-3.95 (m, 2 H), 3.54 (s, 3 H), 2.83 (s, 3 H), 2.79-2.73 (m, 4 H), 2.69-2.56 (m, 1 H), 2.32 (s, 3 H), 2.04-1.93 (m, 2 H), 1.92-1.77 (m, 2 H), 1.36 (t, *J* = 7.6 Hz, 3 H). LC-MS: m/z = 528.2 [M+H].

2-[[2-Ethyl-6-(1-methylsulfonyl-4-piperidyl)imidazo[1,2-*a*]pyridin-3-yl]-methyl-amino]-4-(4-fluorophenyl)thiazole-5-carbonitrile (37). Compound **8** (50 mg, 0.1 mmol) was dissolved in DCM (1 mL) and bromine on polymer support (loading 1.2-1.8 mmol/g, 59 mg, 0.1 mmol) was added. The suspension was shaken for 4 h at 0°C. The resin was filtered, washed with DCM and MeOH and the filtrate was evaporated to afford a brominated intermediate. This latter compound (50 mg, 0.08 mmol) was dissolved in pyridine (2.5 mL), CuCN was added (38 mg, 0.42 mmol) and the resulting suspension was heated under microwave irradiation at 150°C for 2 h. The reaction medium was quenched with water and extracted twice with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered and concentrated *in vacuo*. The residue was purified by chromatography on silica gel to afford compound **37** (18 mg, 41%).^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.22-8.17 (m, 2 H), 7.70-7.62 (m, 2 H), 7.26-7.19 (m, 3 H), 4.02-3.97 (m, 2 H), 3.68 (s, 3 H), 2.86 (s, 3 H), 2.85-2.76 (m, 4 H), 2.73-2.65 (m, 1 H), 2.07-1.98 (m, 2 H), 1.94-1.82 (m, 2 H), 1.38 (t, *J* = 7.6 Hz, 3 H). LC-MS: m/z = 539.4 [M+H].

2-[[2-Ethyl-6-(1-methylsulfonyl-4-piperidyl)imidazo[1,2-*a*]pyridin-3-yl]-methyl-amino]-4-(4-fluorophenyl)thiazole-5-carboxamide (38). Compound **37** (50 mg, 0.09 mmol) was dissolved in concentrated H_2SO_4 (208 µL) and stirred at rt overnight. The reaction medium was

diluted with water and basified with saturated aqueous $NaHCO_3$. The precipitate was filtered off, washed with water, dried and collected to afford compound **38** (15 mg, 29%). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.10 (s, 1 H), 7.77-7.74 (m, 2 H), 7.54 (d, *J* = 8.8 Hz, 1 H), 7.36 (d, *J* = 8.8 Hz, 1 H), 7.28-7.23 (m, 2 H), 3.72-3.65 (m, 2 H), 3.50 (s, 3 H), 3.30 (s, 2 H), 2.89 (s, 3 H), 2.82-2.71 (m, 3 H), 2.63 (q, *J* = 7.6 Hz, 2 H), 1.92-1.83 (m, 2 H), 1.81-1.71 (m, 2 H), 1.25 (t, *J* = 7.6 Hz, 3 H). LC-MS: m/z = 557.3 [M+H].

2-[[2-ethyl-6-(1-methylsulfonyl-4-piperidyl)imidazo[1,2-*a*]pyridin-3-yl]-methyl-amino]-4-(4-fluorophenyl)thiazole-5-carboxylic acid (39). Compound **53** (95 mg, 0.16 mmol) was suspended in THF / water (1 / 1 mL), LiOH was added (20 mg, 0.48 mmol) and the mixture was stirred at rt for 2 h. Then LiOH (47 mg, 1.12 mmol) was added again, the reaction was stirred at rt overnight and at 50°C for 2 h. Water (1 mL) was added and the mixture stirred at 50°C for 12 h. After cooling down to rt aqueous HCl (0.1 N, 3mL) was added and the precipitate filtered. The filtrate was extracted with EtOAc and the organic layer was concentrated *in vacuo*. The resulting residue and the former obtained precipitate were dissolved in DCM, combined and solvent was evaporated to afford **39** as a white solid (16 mg, 18%). ^{1}H NMR (400 MHz, methanol-d_4) δ ppm 8.02 (s, 1 H), 7.90-7.87 (m, 2 H), 7.62 (d, *J* = 9.3 Hz, 1 H), 7.51 (dd, *J* = 9.3, 1.5 Hz, 1 H), 7.19-7.14 (m, 2 H), 3.95-3.87 (m, 2 H), 3.65 (s, 3 H), 2.96-2.78 (m, 8 H), 2.05-1.99 (m, 2 H), 1.96-1.86 (m, 2 H), 1.41 (t, *J* = 7.6 Hz, 3 H). LC-MS: m/z = 558.0 [M+H].

2-[[2-Ethyl-6-[1-[2-(3-hydroxyazetidin-1-yl)-2-oxo-ethyl]-4-piperidyl]imidazo[1,2-*a*]pyridin-3-yl]-methyl-amino]-4-(4-fluorophenyl)thiazole-5-carbonitrile (40). Compound **22** (1.5 g, 2.73 mmol) was dissolved in DCM (60 mL) and bromine on polymer support (loading 1.2-1.8 mmol/g, 1.91 g, 2.87 mmol) was added. The suspension was shaken overnight. The resin was filtered, washed with DCM and MeOH and the filtrate was evaporated. The residue was

taken up in water and extracted with DCM twice. Then the combined organic phases were washed with brine, dried over Na_2SO_4, filtered and concentrated *in vacuo* to afford the expected brominated intermediate (1.6 g, 93%). This intermediate (1.34 g, 2.13 mmol) was dissolved in pyridine (10 mL), CuCN was added (953 mg, 10.64 mmol) and the resulting suspension was heated under microwave irradiation for 3.5 h at 125°C.The reaction mixture was quenched with water and saturated aqueous Na_2CO_3 and extracted twice with EtOAc. The aqueous phase was filtered to remove insoluble copper salts, and was then extracted again with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered and concentrated *in vacuo*. LC-MS purification of the residue afforded compound **40** (110 mg, 9%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.22-8.12 (m, 2 H), 7.63-7.54 (m, 2 H), 7.28-7.19 (m, 3 H), 4.74-4.66 (m, 1 H), 4.49-4.42 (m, 1 H), 4.29 (dd, *J* = 10.4, 7.0 Hz, 1 H), 4.16-4.09 (m, 1 H), 3.90 (dd, *J* = 10.4, 7.0 Hz, 1 H), 3.64 (s, 3 H), 3.08-2.99 (m, 4 H), 2.75 (q, *J* = 7.6 Hz, 2 H), 2.70-2.51 (m, 2 H), 2.23-2.14 (m, 2 H), 1.89-1.77 (m, 4 H), 1.36 (t, *J* = 7.6 Hz, 3 H). LC-MS: m/z = 574.4 [M+H].

2-Ethyl-3-formylamino-imidazo[1,2-*a*]pyridine-6-carboxylic acid methyl ester (42a). 6-Amino-nicotinic acid methyl ester was treated according to general procedure J to afford the desired product **42a** as a white solid (3.4 g, 64% yield over 2 steps). ^{1}H NMR (400 MHz, $CDCl_3$, presence of rotamers), δ (ppm) 8.73 (s, 1 H), 8.61-8.55 (m, 1 H), 8.22-8.15 (m, 1 H), 7.86-7.74 (m, 1 H), 7.57 (d, *J* = 9.3 Hz, 1 H), 3.97-3.95 (m, 3 H), 2.89-2.70 (m, 2 H), 1.42-1.29 (m, 3 H). LC-MS: m/z = 248.2 [M+H].

***N*-(6-Bromo-2-ethyl-imidazo[1,2-*a*]pyridin-3-yl)-formamide (42b).** 2-amino-5-bromopyridine was treated according to general procedure J to afford the desired product **42b** as a yellow powder (39 g, 56% yield over 2 steps). ^{1}H NMR (400 MHz, methanol-d_4) δ ppm 8.45

(s, 1 H), 8.22-8.20 (m, 1 H), 7.43-7.40 (m, 2 H), 2.70 (q, *J* = 7.6 Hz, 2 H), 1.28 (t, *J* = 7.6 Hz, 3 H). LC-MS: m/z = 267.9/269.9 [M+H].

N-(2-Ethyl-6-iodo-imidazo[1,2-a]pyridin-3-yl)-formamide (42c). 2-Amino-5-iodopyridine was treated according to general procedure J to afford the desired product **42c** (20 g, 78% over 2 steps). ^{1}H NMR (400 MHz, DMSO-d_6, presence of rotamers) δ ppm 10.04 (s, 1 H), 8.41-8.38 (m, 1 H), 8.36 (s, 1 H), 8.29-8.24 (m, 1 H), 8.10 (s, 1 H), 7.55-7.22 (m, 2 H), 2.63 (q, *J* = 7.6 Hz, 2 H), 2.58 (q, *J* = 7.6 Hz, 2 H), 1.31 (t, *J* = 7.6 Hz, 3 H), 1.28 (t, *J* = 7.6 Hz, 3 H). LC-MS: m/z = 316.0 [M+H].

2-Ethyl-3-methylamino-imidazo[1,2-*a*]pyridine-6-carboxylic acid methyl ester (43a). Compound **42a** was treated with MeI according to general procedure K to afford the desired product **43a** as a yellow solid (5.4 g, 80% yield over 2 steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 9.22 (s, 1 H), 8.22-8.19 (m, 2 H), 4.04 (s, 3 H), 3.11-3.01 (m, 2 H), 2.98 (s, 3 H), 1.56-1.44 (m, 3 H). LC-MS: m/z = 234.2 [M+H].

(6-Bromo-2-ethyl-imidazo[1,2-*a*]pyridin-3-yl)-methyl-amine (43b). Compound **42b** was treated with MeI according to general procedure K to afford the desired product **43b** as a yellow solid (19 g, 67% yield over 2 steps). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 15.30 (s, 1 H), 8.96 (d, *J* = 1.6 Hz, 1 H), 7.92 (dd, *J* = 9.4, 1.6 Hz, 1 H), 7.80 (d, *J* = 9.4 Hz, 1 H), 7.41-6.54 (br. s, 1 H), 2.85 (q, *J* = 7.6 Hz, 2 H), 2.77 (s, 3 H), 1.30 (t, *J* = 7.6 Hz, 3 H). LC-MS: m/z = 254.0/256.0 [M+H].

(2-Ethyl-6-iodo-imidazo[1,2-a]pyridin-3-yl)-methyl-amine (43c). Compound **42c** was treated with MeI according to general procedure K to afford the desired product **43c** (19 g, 37% yield over 2 steps). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 15.19 (s, 1 H), 8.92-8.90 (m, 1 H),

7.98 (dd, *J* = 9.3, 1.5 Hz, 1 H), 7.65 (dd, *J* = 9.3, 0.9 Hz, 1 H), 2.83 (q, *J* = 7.6 Hz, 2 H), 2.76 (s, 3 H), 1.29 (t, *J* = 7.6 Hz, 3 H). LC-MS: m/z = 302.0 [M+H].

3-{[4-(4-Chloro-phenyl)-thiazol-2-yl]-methyl-amino}-2-ethyl-imidazo[1,2-*a*]pyridine-6-carboxylic acid methyl ester (44a). Compound **43a** was treated with 2-bromo-1-(4-chloro-phenyl)-ethanone according to general procedure G to afford the desired product **44a** (1.4 g, 27% yield over 2 steps). ^{1}H NMR (400 MHz, methanol-d_4) δ ppm 8.71-8.69 (m, 1 H), 7.96 (dd, *J* = 9.3, 1.3 Hz, 1 H), 7.91 (d, *J* = 8.8 Hz, 2 H) , 7.68 (d, *J* = 9.3 Hz, 1 H), 7.44 (d, *J* = 8.7 Hz, 2 H), 7.12(s, 1 H), 3.96 (s, 3 H), 3.70(s, 3 H), 2.82 (q, *J* = 7.6 Hz, 2H,), 1.36 (t, *J* = 7.6 Hz, 3 H). LC-MS: m/z = 427.0/429.0 [M+H].

(6-Bromo-2-ethyl-imidazo[1,2-*a*]pyridin-3-yl)-[4-(4-fluoro-phenyl)-thiazol-2-yl]-methyl-amine (44b). Compound **43b** was treated with 2-bromo-1-(4-fluoro-phenyl)-ethanone according to general procedure G to afford the desired product **44b** (7.4 g, 60% yield over 2 steps). ^{1}H NMR (400 MHz, methanol-d_4) δ ppm 8.75 (d, *J* = 0.8 Hz, 1 H), 7.99 (dd, *J* = 9.4, 1.7 Hz, 1 H), 7.84-7.75 (m, 3 H), 7.13 (s, 1 H), 7.12-7.03 (m, 2 H), 3.64 (s, 3 H), 2.87 (q, *J* = 7.6 Hz, 2 H), 1.36 (t, *J* = 7.6 Hz, 3 H). LC-MS: m/z = 431.0/433.0 [M+H].

(2-Ethyl-6-iodo-imidazo[1,2-*a*]pyridin-3-yl)-[4-(4-fluoro-phenyl)-thiazol-2-yl]-methyl-amine (44c). Compound **43c** was treated with 2-bromo-1-(4-fluoro-phenyl)-ethanone according to general procedure G to afford the desired product **44c** (1.9 g 62% yield over 2 steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.23 (s, 1 H), 8.00 (br. d, *J* = 7.1 Hz, 1 H), 7.81-7.77 (m, 3 H), 7.12-7.07 (m, 2 H), 6.81 (s, 1 H), 3.64 (s, 3 H), 2.91 (q, *J* = 7.6 Hz, 2 H), 1.46 (t, *J* = 7.6 Hz, 3 H). LC-MS: m/z = 479.0 [M+H].

([4-(4-tert-Butyl-phenyl)-thiazol-2-yl]-(2-ethyl-6-iodo-imidazo[1,2-*a*]pyridin-3-yl)-methyl-amine (44d). Compound **43c** was treated with 2-bromo-1-(4-*tert*-butyl-phenyl)-ethanone

according to general procedure G to afford the desired product **44d** (140 mg, 61% yield over 2 steps). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 7.88-7.85 (m, 1 H), 7.61-7.55 (m, 2 H), 7.24-7.16 (m, 4 H), 6.49 (s, 1 H), 3.39 (s, 3 H), 2.53 (q, *J* = 7.6 Hz, 2 H), 1.36 (t, *J* = 7.6 Hz, 3 H), 1.15 (s, 9 H). LC-MS: m/z = 517.3 [M+H].

***N*-(2-ethyl-6-iodo-imidazo[1,2-*a*]pyridin-3-yl)-*N*-methyl-4-[4-(trifluoromethyl)phenyl] thiazol-2-amine (44e).** Compound **43c** was treated with 2-bromo-1-[4-(trifluoro-methyl)phenyl]ethanone according to general procedure G to afford the desired product **44e** (254 mg, 74% yield over 2 steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.39-8.35 (m, 2 H), 8.06 (dd, *J* = 9.2, 1.5 Hz, 1 H), 7.92 (d, *J* = 8.2 Hz, 2 H), 7.69 (d, *J* = 8.2 Hz, 2 H), 7.09 (s, 1 H), 3.85 (s, 3 H), 3.69 (s, 3 H), 3.02 (q, *J* = 7.6 Hz, 2 H), 1.56 (t, *J* = 7.6 Hz, 3 H). LC-MS: m/z = 529.0 [M+H].

(2-Ethyl-6-iodo-imidazo[1,2-*a*]pyridin-3-yl)-[4-(4-methoxy-phenyl)-thiazol-2-yl]-methyl-amine (44f). Compound **43c** was treated with 2-bromo-1-(4-methoxy-phenyl)-ethanone according to general procedure G to afford the desired product **44f** as a white powder (298 mg, 62% yield over 2 steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.36-8.31 (m, 2 H), 8.04-7.97 (m, 1 H), 7.71 (d, *J* = 8.8 Hz, 2 H), 6.93 (d, *J* = 8.8 Hz, 2 H), 6.79 (s, 1 H), 3.85 (s, 3 H), 3.64 (s, 3 H), 2.98 (q, *J* = 7.6 Hz, 2 H), 1.42 (t, *J* = 7.6 Hz, 3 H). LC-MS: m/z = 491.0 [M+H].

4-(3,4-difluorophenyl)-*N*-(2-ethyl-6-iodo-imidazo[1,2-*a*]pyridin-3-yl)-*N*-methyl-thiazol-2-amine (44g). Compound **43c** was treated with 2-bromo-1-(3,4-difluoro-phenyl)-ethanone according to general procedure G to afford the desired product **44g** (120 mg, 40% yield over 2 steps). LC-MS: m/z = 497.0 [M+H].

4-(3,5-difluorophenyl)-*N*-(2-ethyl-6-iodo-imidazo[1,2-*a*]pyridin-3-yl)-*N*-methyl-thiazol-2-amine (44h). Compound **43c** was treated with 2-bromo-1-(3,5-difluoro-phenyl)-ethanone

according to general procedure G to afford the desired product **44h** (280 mg, 74% yield over 2 steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.40-8.35 (m, 2 H), 8.07 (dd, *J* = 9.3, 1.4 Hz, 1 H), 7.37-7.32 (m, 2 H), 7.01 (s, 1 H), 6.84-6.79 (m, 1 H), 3.69 (s, 3 H), 3.01 (q, *J* = 7.6 Hz, 2 H), 1.46 (t, *J* = 7.6 Hz, 3 H). LC-MS: m/z = 497.0 [M+H].

[2-Ethyl-6-(1,2,3,6-tetrahydro-pyridin-4-yl)-imidazo[1,2-*a*]pyridin-3-yl]-[4-(4-fluoro-phenyl)-thiazol-2-yl]-methyl-amine hydrochloride (45). Compound **44b** reacted with *N*-boc-1,2,3,6-tetrahydropyridine-4-boronic acid pinacol ester according to general procedure B, using $Pd(PPh_3)_4$, to afford the 4-(2-ethyl-3-{[4-(4-fluoro-phenyl)-thiazol-2-yl]-methyl-amino}-imidazo[1,2-*a*]pyridin-6-yl)-3,6-dihydro-*2H*-pyridine-1-carboxylic acid *tert*-butyl ester as a yellow foam. The latter (1.19 g, 2.23 mmol) was dissolved in a 1.25 N solution of HCl in MeOH (15 mL). The reaction mixture was stirred at room temperature for 16 h, and concentrated to dryness to afford the expected compound **45** used as such in the next step (1.10 g, quantitative yield over 2 steps). ^{1}H NMR (400 MHz, methanol-d_4) δ ppm 8.51 (s, 1 H), 8.25 (d, *J* = 9.3 Hz, 1 H), 8.00 (d, *J* = 9.3 Hz, 1 H), 7.77 (dd, *J* = 8.7, 5.5 Hz, 2 H), 7.21 (s, 1 H), 7.09 (t, *J* = 8.7 Hz, 2 H), 6.41 (br. s, 1 H.), 3.90 (br. s, 2 H), 3.71 (s, 3 H), 3.50-3.46 (m, 2 H), 2.94 (q, *J* = 7.6 Hz, 2 H), 2.88-2.80 (m, 2 H), 1.40 (t, *J* = 7.6 Hz, 3 H). LC-MS: m/z = 434.0 [M+H].

(2-Ethyl-6-piperidin-4-yl-imidazo[1,2-*a*]pyridin-3-yl)-[4-(4-fluoro-phenyl)-thiazol-2-yl]-methyl-amine (46). Compound **44b** was treated with *N*-boc-1,2,3,6-tetrahydropyridine-4-boronic acid pinacol ester according to general procedure B, using $Pd(PPh_3)_4$, to afford 4-(2-ethyl-3-{[4-(4-fluoro-phenyl)-thiazol-2-yl]-methyl-amino}-imidazo[1,2-a]pyridin-6-yl)-3,6-dihydro-*2H*-pyridine-1-carboxylic acid *tert*-butyl ester as a yellow foam. To the latter compound (905 mg, 1.70 mmol) in solution in DCM (20 mL) and EtOH (30 mL) was added palladium(II) hydroxide (181 mg) and the reaction mixture was stirred at 40°C under H_2 atmosphere for 4 h,

and at room temperature overnight. The reaction mixture was filtered on Celpure® P65 and the filtrate was concentrated to dryness. The residue was purified by column chromatography to give *tert*-butyl 4-[2-ethyl-3-[[4-(4-fluorophenyl)thiazol-2-yl]-methyl-amino]imidazo[1,2-*a*]pyridin-6-yl]piperidine-1-carboxylate (685 mg, 76% yield over 2 steps). This latter intermediate (280 mg, 0.52 mmol) was dissolved in a 1.25 N solution of HCl in MeOH (15 mL). The reaction mixture was stirred at room temperature for 16 h and concentrated to dryness to afford the expected compound **46** as a pale yellow foam (305 mg, quantitative yield). ^{1}H NMR (400 MHz, methanol-d_4) δ ppm 8.47 (s, 1 H), 8.12-8.07 (m, 1 H), 7.99 (d, J = 9.4 Hz, 1 H), 7.84-7.70 (m, 2 H), 7.20 (s, 1 H), 7.10 (t, J = 8.8 Hz, 2 H), 3.71 (s, 3 H), 3.59-3.45 (m, 2 H), 3.25-3.10 (m, 3 H), 2.90 (q, J = 7.6 Hz, 2 H), 2.18-1.98 (m, 4 H), 1.40 (t, J = 7.6 Hz, 3 H). LC-MS: m/z = 436.0 [M+H].

2-chloro-1-(3-hydroxy-azetidin-1-yl)-ethanone (47). To a suspension of potassium carbonate (13.9 g, 100 mmol) in water (33.5 mL) was added hydroxyazetidine hydrochloride (5 g, 45.6 mmol). The reaction mixture was stirred at room temperature until complete dissolution. The obtained solution was diluted with 33.5 mL of DCM and chloroacetyl chloride was introduced dropwise at 0°C over 30 min. After 2 h stirring at room temperature, the reaction mixture was filtered and the layers were separated. The aqueous phase was extracted with a mixture EtOAc / *n*-BuOH 1:1 (6 × 16 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated *in vacuo*. The residue was suspended in acetone (48 mL) and stirred vigorously for 20 min, filtered and the filtrate was concentrated *in vacuo* to afford compound **47** as a white solid (4.74g, 70%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 4.80-4.67 (m, 1 H), 4.56-4.46 (m, 1 H), 4.38-4.29 (m, 1 H), 4.16 (ddd, J = 9.5, 4.2, 1.1 Hz, 1 H), 3.95 (dd, J = 11.1, 3.6 Hz, 1 H), 3.91 (s, 2 H), 2.56 (d, J = 5.9 Hz, 1 H).

(2-Ethyl-6-piperazin-1-yl-imidazo[1,2-*a*]pyridin-3-yl)-[4-(4-fluoro-phenyl)-thiazol-2-yl]-methyl-amine (48). Compound **44a** was treated with *N*-boc-piperazine according to general procedure C, using Davephos and sodium *tert*-butoxide in toluene, to afford the expected boc-protected amine as a yellow oil. To the latter intermediate (201 mg, 0.36 mmol) in solution in MeOH (0.5 mL) was added at 0°C a 4N solution of HCl in dioxane (1.5 mL). The reaction mixture was allowed to warm up to room temperature and was stirred at this temperature for 1 h. It was then concentrated to dryness and taken in Et_2O. The pink precipitate was filtered, rinsed with Et_2O and DCM and dried under suction to afford the expected product **48** (180 mg, 79% yield over 2 steps). ^{1}H NMR (MHz, methanol-d_4) δ ppm 8.04 (dd, J = 9.8, 2.3 Hz, 1 H), 7.93-7.87 (m, 2 H), 7.83-7.77 (m, 2 H), 7.19 (s, 1 H), 7.11 (t, J = 8.8 Hz, 2 H), 3.69 (s, 3 H), 3.60-3.47 (m, 4 H), 3.46-3.36 (m, 4 H), 2.90 (q, J = 7.6 Hz, 2 H), 1.38 (t, J = 7.6 Hz, 3 H). LC-MS: m/z = 437.0 [M+H].

2-chloro-1-morpholino-ethanone (49). To a solution of morpholine (663 µL, 7.53 mmol) in DCM (25 mL) and TEA (1.75 mL, 12.55 mmol) at 0°C was added chloroacetyl chloride (500 µL, 6.28 mmol). The reaction was stirred at 0°C for 1 h and then water and 1 N aqueous HCl were added. The solvents were concentrated *in vacuo* to afford **49** that was used as such in the next step.

***N*-[2-Ethyl-6-(1-methanesulfonyl-1,2,3,6-tetrahydro-pyridin-4-yl)-imidazo[1,2-*a*]pyridin-3-yl]-*N*-methyl-formamide (50).** NaH 60% (6.41 g, 160 mmol) was washed twice with heptane and suspended in DMF (300 mL). A solution of compound **42b** (35.8 g, 134 mmol) in DMF (300 mL) was added dropwise. The reaction mixture was stirred at room temperature for 1 h and methyl iodide (11.64 mL, 187 mmol) was added dropwise. The reaction mixture was allowed to stir at room temperature for 16 h, then was quenched with water and concentrated *in vacuo*. The

residue was diluted with water and DCM. The aqueous layer was extracted twice with DCM and twice with EtOAc. The DCM- and EtOAc layers were separately washed with water and brine, then combined, dried over Na_2SO_4, filtered and concentrated *in vacuo*. The oily residue was triturated with DIPE and the formed precipitate was filtered. The solid was rinsed with DIPE, water, DIPE again and dried. The mother liquor was separated, the DIPE phase was dried over Na_2SO_4, filtered, concentrated *in vacuo*, and submitted to a second crystallization process. The precipitates of the 2 crystallization processes were combined to afford *N*-(6-bromo-2-ethyl-imidazo[1,2-*a*]pyridin-3-yl)-*N*-methyl-formamide. A fraction of the latter intermediate was treated with 1-methanesulfonyl-4-(tetramethyl-1,3,2-dioxaborolan-2-yl)-1,2,3,6-tetrahydropyridine according to general procedure B, using $Pd(PPh_3)_4$, to afford the expected compound **50** (6.6 g, 80% yield over 2 steps). ^{1}H NMR (MHz, $CDCl_3$) δ ppm 8.21 (s, 1 H), 7.68-7.65 (m, 1 H), 7.55 (d, *J* = 9.4 Hz, 1 H), 7.35 (dd, *J* = 9.4, 1.8 Hz, 1 H), 6.21-6.02 (m, 1 H), 4.02-3.98 (m, 2 H), 3.57-3.53 (m, 2 H), 3.26 (s, 3 H), 2.88 (s, 3 H), 2.72 (d, *J* = 7.6 Hz, 1 H), 2.66-2.62 (m, 2 H), 1.33 (t, *J* = 7.6 Hz, 3 H). LC-MS: m/z = 363.3 [M+H].

[2-Ethyl-6-(1-methanesulfonyl-piperidin-4-yl)-imidazo[1,2-*a*]pyridin-3-yl]-methyl-amine (51). Compound **50** (3.0 g, 8.28 mmol) was dissolved in MeOH (30 mL) and THF (30 mL) and platinum dioxide (113 mg, 0.414 mmol) was added. The mixture was degassed, saturated with H_2 and stirred at room temperature under 1 atm H_2 for 18 h. Additional platinum dioxide (113 mg, 0.414 mmol) was added and the mixture was stirred at room temperature under 1 atm H_2 for 40 h. The mixture was filtered over celite, rinsed with MeOH and DCM and the filtrate was concentrated *in vacuo*. The residue was purified by chromatography on silica gel to afford *N*-[2-ethyl-6-(1-methanesulfonyl-piperidin-4-yl)-imidazo[1,2-*a*]pyridin-3-yl]-N-methyl-formamide.

To the latter compound (1.96 g, 5.38 mmol), dissolved in MeOH (100 mL) was added a 4 N

solution of HCl in dioxane (5.38 mL, 21.51 mmol). The reaction mixture was stirred at room temperature for 1 h. Additional 4 N solution of HCl in dioxane was added (5 mL) and the reaction mixture was stirred at room temperature until reaction completion. The solvents were evaporated *in vacuo*. The residue was triturated with chloroform, filtered and dried to afford compound **51** as hydrochloride salt (2.0 g, 65% yield over 2 steps). LC-MS: m/z = 337.2 [M+H].

4-(2-bromo-4-fluoro-phenyl)-*N*-[2-ethyl-6-(1-methylsulfonyl-4-piperidyl)imidazo[1,2-*a*]pyridin-3-yl]-*N*-methyl-thiazol-2-amine (52). *Step 1: 2-bromo-1-(2-bromo-4-fluoro-phenyl)ethanone.* 1-(2-Bromo-4-fluoro-phenyl)ethanone was submitted to general procedure I to get a mixture of starting material, monobrominated and dibrominated intermediate that was used as such in the next step. *Step 2: 4-(2-bromo-4-fluoro-phenyl)-N-[2-ethyl-6-(1-methylsulfonyl-4-piperidyl)imidazo[1,2-a]pyridin-3-yl]-N-methyl-thiazol-2-amine* (**52**). Compound **51** was treated with 2-bromo-1-(4-fluoro-2-bromo-phenyl)ethanone according to general procedure G to afford **52** (63 mg, 30% yield over 2 steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.97 (dd, *J* = 8.8, 6.3 Hz, 1 H), 7.66 (br. s, 1 H), 7.62 (d, *J* = 8.8 Hz, 1 H), 7.45 (dd, *J* = 8.4, 2.6 Hz, 1 H), 7.20-7.13 (m, 2 H), 7.06 (s, 1 H), 4.02-3.97 (m, 2 H), 3.62 (s, 3 H), 2.86 (s, 3 H), 2.84-2.77 (m, 4 H), 2.71-2.63 (m, 1 H), 2.06-1.97 (m, 2 H), 1.93-1.83 (m, 2 H), 1.40 (t, *J* = 7.6 Hz, 3 H). LC-MS: m/z = 592.1/594.1 [M+H].

Methyl 2-[[2-ethyl-6-(1-methylsulfonyl-4-piperidyl)imidazo[1,2-a]pyridin-3-yl]-methyl-amino]-4-(4-fluorophenyl)thiazole-5-carboxylate (53). *Step 1: Methyl 2-bromo-3-(4-fluorophenyl)-3-oxo-propanoate.* Methyl 3-(4-fluorophenyl)-3-oxo-propanoate was submitted to general procedure I to get the expected brominated intermediate. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.10 - 8.05 (2 H, m), 7.25 - 7.19 (2 H, m), 5.66 (1 H, s), 3.88 (3 H, s). *Step 2: Methyl 2-[[2-ethyl-6-(1-methylsulfonyl-4-piperidyl)imidazo[1,2-a]pyridin-3-yl]-methyl-amino]-4-(4-*

*fluorophenyl)thiazole-5-carboxylate (**53**).* Compound **51** was treated with methyl 2-bromo-3-(4-fluorophenyl)-3-oxo-propanoate according to general procedure G to afford **53** (95 mg, 74% yield over 2 steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.93-7.88 (m, 2 H), 7.76-7.68 (m, 1 H), 7.65 (s, 1 H), 7.29-7.25 (m, 1 H), 7.19-7.13 (m, 2 H), 4.03-3.99 (m, 2 H), 3.72 (s, 3 H), 3.63 (s, 3 H), 2.87 (s, 3 H), 2.84-2.77 (m, 4 H), 2.74-2.66 (m, 1 H), 2.07-1.98 (2 H, m), 1.94-1.84 (m, 2 H), 1.43 (t, *J* = 7.6 Hz, 3 H). LC-MS: m/z = 572.1 [M+H].

***N*-[2-ethyl-6-(1,2,3,6-tetrahydropyridin-4-yl)imidazo[1,2-*a*]pyridin-3-yl]-*N*-methyl-4-[4-(trifluoromethyl)phenyl]thiazol-2-amine (54e).** Compound **44e** (100 mg, 0.19 mmol) reacted with *N*-boc-1,2,3,6-tetrahydropyridine-4-boronic acid pinacol ester according to general procedure B to afford *tert*-butyl 4-[3-[[4-(4-(trifluoromethyl)phenyl)thiazol-2-yl]-methyl-amino]-2-ethyl-imidazo[1,2-*a*]pyridin-6-yl]-3,6-dihydro-2H-pyridine-1-carboxylate (95 mg, 86%). The latter intermediate was dissolved in MeOH (2 mL) and HCl 4 N in dioxane was added (3 mL). The mixture was stirred at rt for 4 h and solvents were concentrated to dryness to get compound **54e** as hydrochloride salt used as such in the next step.

***N*-[2-ethyl-6-(1,2,3,6-tetrahydropyridin-4-yl)imidazo[1,2-*a*]pyridin-3-yl]-4-(4-methoxyphenyl)-*N*-methyl-thiazol-2-amine (54f).** Compound **44f** (85 mg, 0.17 mmol) reacted with *N*-boc-1,2,3,6-tetrahydropyridine-4-boronic acid pinacol ester according to general procedure B to afford *tert*-butyl 4-[3-[[4-(4-methoxy)thiazol-2-yl]-methyl-amino]-2-ethyl-imidazo[1,2-*a*]pyridin-6-yl]-3,6-dihydro-2H-pyridine-1-carboxylate as a yellow oil (86 mg, 91%). The latter compound (85 mg, 0.156 mmol) was dissolved in MeOH (2 mL) and a 4 N solution of HCl in dioxane (2 mL) was added. The reaction mixture was stirred at rt for 16h and concentrated to dryness to afford the expected deprotected amine **54f** as hydrochloride salt used as such in the next step (76 mg, 94%).

4-(3,4-difluorophenyl)-N-[2-ethyl-6-(1,2,3,6-tetrahydropyridin-4-yl)imidazo[1,2-a]pyridin-3-yl]-N-methyl-thiazol-2-amine (54g). Compound **44g** (120 mg, 0.24 mmol) reacted with *N*-boc-1,2,3,6-tetrahydropyridine-4-boronic acid pinacol ester according general procedure B to afford *tert*-butyl 4-[3-[[4-(3,4-difluorophenyl)thiazol-2-yl]-methyl-amino]-2-ethyl-imidazo[1,2-a]pyridin-6-yl]-3,6-dihydro-2H-pyridine-1-carboxylate (125 mg, 94%). The latter compound was dissolved in MeOH (2 mL) and HCl 4 N in dioxane was added (3 mL). The mixture was stirred at rt for 4 h and solvents were concentrated to dryness to get compound **54g** as hydrochloride salt used as such in the next step (105 mg, 90%). LC-MS: m/z = 452.2 [M+H].

4-(3,5-Difluorophenyl)-*N*-[2-ethyl-6-(1,2,3,6-tetrahydropyridin-4-yl)imidazo[1,2-*a*]pyridin-3-yl]-N-methyl-thiazol-2-amine (54h). Compound **44h** (150 mg, 0.30 mmol) was submitted to the general procedure B with *N*-boc-1,2,3,6-tetrahydropyridine-4-boronic acid pinacol ester to afford *tert*-butyl 4-[3-[[4-(3,5-difluorophenyl)thiazol-2-yl]-methyl-amino]-2-ethyl-imidazo[1,2-*a*]pyridin-6-yl]-3,6-dihydro-2H-pyridine-1-carboxylate (132 mg, 79%). The latter compound was dissolved in MeOH (5 mL) and HCl 4 N in dioxane was added (3 mL). The mixture was stirred overnight at rt and solvents were concentrated to dryness to get compound **54h** as hydrochloride salt (130 mg, quantitative yield) used as such in the next step.

Biochemical assay with FS-3 substrate. Starting from 20 µM highest concentration, 10 µL of a dilution series of compound, 1/5 dilution, was added to the wells. Glycosylated human ATX protein (see supporting information) was used at a final concentration of 0.4 µg/mL or 0.64 µg/mL. The enzyme was diluted in 50 mM Tris-HCl (2-amino-2-(hydroxymethyl)-1,3-propanediol hydrochloride) pH 8.0, 250 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 0.1% fatty acid free BSA in a total volume of 20 µL. The enzyme mixture was added to compounds and the resulting mixture was incubated for 30 minutes at room temperature under

shaking. The reaction was started by the addition of 20 µL of 0.75 µM FS-3 diluted in the same buffer as described above. Fluorescence was read on an Envision apparatus (Perkin Elmer) after 30 minutes incubation at room temperature (excitation 485nm, emission 520 nM).

Biochemical assay with LPC 16:0 substrate. Starting from 20 µM highest concentration, 5 µL of a dilution series of compound (1/5 dilution), was added to the wells. Glycosylated human ATX protein (see supporting information) was used at a final concentration of 1 µg/mL or 3 µg/mL. The enzyme was diluted in 50 mM Tris-HCl pH 8.5, 500 mM NaCl, 5 mM KCl, 10 mM $CaCl_2$, 0.1% fatty acid free BSA in a total volume of 10 µL. The reaction was started by the addition of 10 µL of 150 µM LPC 16:0 diluted in the same buffer as described above and the mixture was incubated at 37°C for 30 minutes. The reaction was terminated and choline quantified by the addition of a 25 µL of a mixture containing 0.6 U/mL of choline oxidase, 0.6 U/mL of horseradish peroxidase (HRP), 1.8 mM TOOS (N-ethyl-N-(2-hydroxy-3-sulfopropyl)-3-methylaniline, sodium salt dihydrate), 1.2 mM 4-aminoantipyrine, 20 mM EGTA (ethylene glycol-bis(2-aminoethylether)-N,N,N′,N′-tetraacetic acid, stop-developer solution) diluted in the buffer described above. Luminescence was read on an Envision apparatus (Perkin Elmer) after 30 minutes incubation at room temperature (excitation 555 nm, excitation light = 70%).

Rat plasma assay. Rat plasma was thawed on ice and added into a plate containing a dose range of compound to be tested. After 2 h incubation at 37°C plasma proteins from a 10 µL aliquot were precipitated with an excess of methanol containing LPA 17:0 as internal standard. After centrifugation, the corresponding supernatant was diluted and injected on a C18 column. Analytes were eluted out of the column under isocratic conditions. No calibration curve was prepared for LPA 18:2 and all quantifications were performed based on peak area ratios (LPA 18:2 / LPA 17:0). For each concentration of compound LPA data were expressed as percentage

of reduction (% reduction) using the formula: 100-[((LPA ratio)/(LPA ratio in control sample))*100].

Rat PK/PD with compound 40. Male Sprague Dawley rats were maintained in controlled environment and dosed at 5 mg/kg with compound **40** formulated in 10% (2-hydroxypropyl)-β-cyclodextrin with pH adjusted to 3 with citric acid (1 mg/mL of **40**). Blood samples were collected at the jugular vein via a catheter according to protocols approved by the GALAPAGOS Ethical Committee for animals welfare with the agreement of the Ministère de l'Enseignement Supérieur et de la Recherche and the Direction Départementale de la Protection des Populations, at the following time points: 0.5, 1, 3 h after dosing and placed into tubes containing Li-heparin as anticoagulant. LPA 18:2 plasma peak areas and compound **40** plasma concentrations were assayed by LC-MS/MS. Plasma concentrations of compound **40** were measured against a calibration curve consisting of eight levels with a 3-Log amplitude. Back-calculated values of the QCs (three levels prepared in duplicate) were used for accepting or rejecting the whole batch. The lower limit of quantification was 4 ng/mL for compound **40**, using a plasma volume of 25 µL. Plasma proteins were precipitated with an excess of methanol containing the internal standard and the corresponding supernatant was injected on a C18 column. Analytes were eluted out the HPLC system by increasing the percentage of the organic mobile phase. Pharmacokinetic parameters were calculated after averaging individual plasma concentrations, by non-compartmental analysis using WinNonlin® software (Pharsight, version 5.2). Plasma levels were compiled and average of the plasma levels of the 3 rats at each sampling time was used. For the analysis of LPA 18:2 plasma peak areas, plasma proteins from a 10 µL aliquot were precipitated with an excess of methanol containing the internal standard, LPA 17:0. After centrifugation, the corresponding supernatant was diluted and injected on a C18 column. Analytes were eluted out

of the column under isocratic conditions. No calibration curve was prepared for LPA 18:2 and all quantifications were performed based on peak area ratios (LPA 18:2 / LPA 17:0). LPA data were finally expressed as percentage of reduction (% reduction) using the formula: 100-[((LPA value at time point t)/(mean of LPA value at the same time point t, in vehicle group))*100].

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Notes

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ABBREVIATIONS

ATX, autotaxin; Cl, clearance; Davephos, 2-dicyclohexylphosphino-2′-(N,N-dimethylamino)biphenyl; dba, dibenzylideneacetone; DIPE, diisopropyl ether; dppf, 1,1′-ferrocenediyl-bis(diphenylphosphine); iv, intravenous; JohnPhos, (2-biphenyl)di-*tert*-butylphosphine; LC-MS, liquid chromatography mass spectrometry; LPA, lysophosphatidic acid; LPC, lysophosphatidyl choline; po, *per os*;; $t_{1/2}$, half-life; xantphos, 4,5-bis(diphenylphosphino)-9,9-dimethylxanthene.

ASSOCIATED CONTENT

Supporting Information

Preparation, characterization of ATX protein and co-crystallization protocol. This material is available free of charge via the Internet at http://pubs.acs.org.

Co-crystal structure of compound **9** with ATX (PDB ID: 5M7M): authors will release the atomic coordinates and experimental data upon article publication.

Molecular Formula Strings.

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