Discovery of BI-2545: A Novel Autotaxin Inhibitor That Significantly Reduces LPA Levels in Vivo

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S Supporting Information

ABSTRACT: In an effort to find new therapeutic interventions addressing the unmet medical need of patients with idiopathic pulmonary fibrosis, we initiated a program to identify new autotaxin (ATX) inhibitors. Starting from a recently published compound (PF-8380), we identified several highly potent ATX inhibitors with improved pharmacokinetic and safety profiles. Further optimization efforts resulted in the identification of a single-digit nanomolar lead compound (BI-2545) that shows substantial lowering of LPA in vivo and is therefore considered a valuable tool for further studies.

KEYWORDS: autotaxin, IPF, LPA, tool compound

Autotaxin is a therapeutic target for the treatment of fibrotic conditions such as idiopathic pulmonary disease (IPF), a disease characterized by a progressive scarring of lung tissue which leads to worsening lung function and is ultimately fatal within 3–5 years from the onset of symptoms.¹ There were no treatment options for IPF until 2014, when the FDA approved nintedanib (Ofev)^{2–5} and pirfenidone (Esbriet).⁶ Despite this significant advancement, there remains a need for additional medicines to treat IPF.

Patients with IPF have elevated levels of lysophosphatidic acid (LPA) in their bronchoalveolar lavage fluid (BALF) and exhaled breath condensate.^{7,8} LPAs are bioactive lysophospholipids that exert a variety of cellular responses including cell proliferation, cell motility and cell survival through their interaction with six G-protein-coupled receptors (GPCRs) known as LPAR1–6.^{9,10} LPAR1 has been identified to be the predominant LPA receptor in lung fibroblasts of IPF patients, responsible for enhanced fibroblast cell migration and vascular leak.⁷ It is therefore envisaged that LPAR1 antagonists will be a potential drug target for the treatment of IPF. In recent years, several LPAR1 antagonists have been reported,^{11–13} and some of these compounds are currently being evaluated in clinical trials for the treatment of IPF. Nevertheless, the pathophysiology of fibrotic disorders is complex and is likely to involve multiple cell types and LPARs driving fibrosis.^{7,14–17} Consequently, there is high interest in therapeutic approaches with the potential to inhibit the overactivation of multiple LPARs caused by elevated

LPA levels in disease. This may be achievable by controlling the rate of formation of LPA species, thereby inhibiting the LPAR1–*n* drivers of pathology. Indeed, inhibition of the enzyme autotaxin (ATX or ENPP2), which is responsible for the formation of LPA from lysophosphatidylcholine (LPC), is an attractive therapeutic concept.¹⁸ Autotaxin is an extracellular enzyme which was first isolated as a motility-stimulating protein from A2058 melanoma cells¹⁹ and that belongs to the family of ectonucleotide pyrophosphatase/phosphodiesterase (ENPP) proteins. The intense interest in discovering compounds capable of inhibiting ATX–LPA signaling is reflected in the substantial increase in publications and filed patents describing small-molecule ATX inhibitors from numerous pharma companies in recent years.^{18,20–27} The only ATX inhibitor which is currently in clinical development for the treatment of IPF is GLPG1690 (1) (Figure 1A).^{28,29}

Our ongoing efforts to find new therapeutic treatments for IPF motivated us to identify small-molecule ATX inhibitors. In addition to running a traditional high-throughput screen of our compound collection, we also utilized a tool compound published by Pfizer, PF-8380³⁰ (2) (Figure 1B), as a starting point for the design of new ATX inhibitors.

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Figure 1. (A) Structure of autotaxin inhibitor GLPG-1690. (B) Structure of autotaxin inhibitor PF-8380.

In order to support our optimization activities, we first evaluated the binding mode of **2** by solving the crystal structure in complex with ATX (detailed information about synthesis of **2**, crystallization and structure solution can be found in the SI). According to the crystal structure that we obtained (see Figures 2

Figure 2. Cocrystal structure of PF-8380 (**2**) bound to autotaxin.

Figure 3. Cocrystal structure of PF-8380 (**2**) bound to autotaxin. Carbon atoms of **2** are shown in dark green; Zn ions and water molecules are depicted as brown and red spheres, respectively. Figures showing structural data have been prepared with PyMOL.

and **3**), the benzoxazolidinone of **2** ($pK_a = 7.6$) occupies the Zn binding pocket and coordinates to the Zn^{2+} ion in the proximity to Asp³¹¹ and His⁴⁷⁴. The oxygen of the carbonyl group coordinates a water molecule that is involved in a strong hydrogen-bonding network. Through the linker region, PF-8380 elongates in the direction of the hydrophobic pocket (Leu²¹³, Leu²¹⁶, Ala²¹⁷, Ile¹⁶⁷, Ser¹⁶⁹, Trp²⁶⁰, Phe²⁷³) where finally the dichlorobenzene is situated. There are few direct interactions of the linker region between the benzoxazolidinone and the dichlorobenzene. The piperazine-1-carboxylate is partially sandwiched between Tyr³⁰⁶ and Phe²⁷⁴. The carbonyl group of the carbamate forms a weak hydrogen bond with the backbone nitrogen of Trp²⁷⁵. There is no ionic interaction of the positively charged nitrogen. Phe²⁷⁴ flanks the opening of a hydrophobic channel (bottom Tyr²¹⁴, Trp²⁶⁰). In addition, within the crystal ten atoms of polyethylene-glycol from the crystallization buffer are visible in the hydrophobic channel.

The reported in vitro potency (IC_{50}) of PF-8380 (**2**) is 3 nM in an enzyme assay, using recombinant ATX and the artificial substrate surrogate FS-3.³⁰ We confirmed this potency of PF-8380 in our recently developed mass-spectrometry-based ATX assay using the natural substrate 18:1 LPC.³¹ In addition to that, we determined an IC_{50} of 307 nM for PF-8380 in rat whole blood (rWB). However, additional profiling of PF-8380 revealed liabilities such as low metabolic stability in human liver microsomes (70% of hepatic blood flow) and a rather prominent inhibition of the hERG channel with an IC_{50} of 480 nM (for details, see the SI). Based on these undesirable properties, our goal was to develop a compound with comparable or improved potency but at the same time a clean hERG profile and ultimately improved pharmacokinetic properties.

We tested the hypothesis that removing a basic center in **2** could dial out the off-target activity. Therefore, the basic piperazine nitrogen of **2** (marked in magenta in Figure 4) was

Figure 4. Replacement of the basic piperazine core in PF-8380 as a strategy to dial out hERG activity and topology of **3** for structure-activity relationship studies.

removed and in addition the core region of the molecule was rigidified by the incorporation of a [3.1.0]-bicycle. The resulting compound **3** displayed an IC_{50} of 3 nM in the LPA enzyme assay and a 4-fold higher potency (IC_{50} 56 nM) in rat whole blood. In line with our hypothesis, it had a clean hERG profile (no inhibitory effect up to a concentration of 10 μ M) and also had increased metabolic stability (41% of hepatic blood flow in human liver microsomes) compared to PF-8380.

With **3** as a potent starting point in hand, we began to explore SAR of the different regions of the inhibitor (see Figure 4). We began by introducing additional modifications in the linker region of the molecule to see if the carbamate present in PF-8380 was the optimal linker between the [3.1.0]-bicyclic core and the residue binding to the hydrophobic pocket. A variety of linker groups were evaluated at this position, and the results are summarized in Table 1. Replacing the carbamate present in **3** with a cinnamic acid derived amide (**4**) did not affect the potency in our enzymatic assay but led to a dramatic drop in rat whole blood potency, accompanied by increased hERG inhibition. The corresponding saturated analog of **4**, compound **5**, suffered from very poor stability in liver microsomes which precluded further profiling of this compound. In order to further rigidify the backbone of our compounds, we also introduced a cyclopropyl linker. For the resulting compound **6**, despite having desirable potency in our primary assay, we observed a dramatic drop in rat whole blood potency in addition to unfavorable hERG inhibition. Changing the carbamate to an urea linker resulted in a compound (**7**) that not only exhibited decreased potency in the enzymatic assay but also low permeability and high efflux. Further modifications of the linker region such as increased steric bulk

Table 1. Structure–Activity Relationship for Linker Region

Cmpd	R	hATX LPA IC ₅₀ (nM) ^a	Rat WB IC ₅₀ (nM) ^a	clogP	hERG IC ₅₀ (μM)	Solubility [μg/ml] at pH 2.2/4.5/6.8	Metabolic stability hLM/rLM [%Qh]	Caco-2 a-b 10 ⁻⁶ [cm/s]/Efflux
3		3.1±2.3 (3)	56±22 (2)	3.8	>10	<1/<1/<1	41/64	137/1
4		1.7±1.0 (3)	1904±698 (2)	2.7	4.2	<1/<1/<1	25/<22	38/4
5		2.3±0.9 (3)	7±4.4 (2)	1.9	8.1	<1/<1/<1	94/88	18/7
6		4.8±2.0 (3)	2399±185 (2)	1.9	4.7	<1/<1/<1	-/<22	64/2
7		13.9±7.0 (3)	77±30 (2)	2.3	>10	<1/<1/<1	36/35	2/38
8		66.9±24.3 (3)	208±79 (2)	4.2	8.5	<1/<1/<1	57/70	118/1
9		82.6±6.4 (3)	n.d.	3.1	n.d.	<1/<1/<1	-/53	47/3

Table 2. Structure–Activity Relationship for Hydrophobic Pocket Residues

Cmpd	R	hATX LPA IC ₅₀ (nM) ^a	Rat WB IC ₅₀ (nM) ^a	clogP	hERG IC ₅₀ (μM)	Solubility [μg/ml] at pH 2.2/4.5/6.8	Metabolic stability hLM/rLM [%Qh]	Caco-2 a-b 10 ⁻⁶ [cm/s]/ Efflux
10		2.3±0.2 (3)	4.5±6.0 (2)	4.2	5.2	<1/<1/<1	<23/47	108/1
11		1.9±1.3 (3)	94±n.d. (1)	3.2	>10	<1/<1/<1	48/56	44/3
12		49.0±1.4 (3)	6.9±3.2 (2)	2.2	>10	48/36/25	98/97	6/21
13		1530±n.d. (1)	1607±880 (2)	1.4	>10	>92/66/56	25/<22	1/68
14		1674±1800 (3)	n.d.	0.7	>10	>86/>93/63	<23/<22	2/30

in the α -position of the carbamate (see methyl derivative **8**) or cyclization to a bicyclic linker as present in **9** led to 30–40 fold loss in ATX inhibitory potency. Based on these results, we decided to keep the carbamate linker present in **3**.

Subsequently, we explored the hydrophobic pocket SAR of ATX (see Table 2). Interestingly, within a clogP range of 2.5–4, a fairly broad variety of substituted benzylic residues was tolerated and led to moderately to highly potent ATX inhibitors (more than 30 analogues were prepared with potencies in IC₅₀ range between 1 and 100 nM in enzymatic assay, not shown). The most lipophilic representative of the selected compounds shown in Table 2, ATX inhibitor **10** displayed excellent potency in our rat whole blood assay, favorable metabolic stability in human liver microsomes, and excellent permeability but also inhibited the hERG channel with an IC₅₀ of 5.2 μM. While less potent in our rat whole blood assay, compound **11** was devoid of hERG

inhibition and showed a moderate overall profile. When more polar residues were introduced, solubility could be improved but potency could not be maintained, as illustrated with selected examples **12**–**14**. Based on the data, we decided to keep the 3-(trifluoromethoxy)benzonitrile residue for subsequent SAR studies of the Zn-binding motif.

We next investigated a number of potential Zn-binding groups as an alternative for the benzoxazolone (Table 3). We found that by changing the benzoxazolone present in **11** to a benzotriazole (compound **15**), both potency in rat whole blood and metabolic stability could be improved. Unfortunately, **15** still displayed very poor solubility which led us to consider more polar groups. When using a 1*H*,4*H*,5*H*,6*H*,7*H*-[1,2,3]triazolo[4,5-*c*]pyridine as a Zn-binding residue, the resulting compound **16** displayed improved potency in our rWB assay and increased solubility at pH 2.2 and 4.5 compared to our starting point **11**. We could also increase

Table 3. Structure–Activity Relationship for Various Zn-Binding Residues

Cmpd	R	hATX LPA IC ₅₀ (nM) ^a	Rat WB IC ₅₀ (nM) ^a	clogP	hERG IC ₅₀ (μM)	Solubility [μg/mL] at pH 2.2/4.5/6.8	Metabolic stability hLM/rLM [%Qh]	Caco-2 a-b 10 ⁻⁶ [cm/s]/ Efflux
15		1.5±0.1 (3)	18±5.4 (2)	3.9	>10	<1/<1/<1	29/28	8/17
16		1.3±0.3 (3)	33±15 (2)	2.3	>10	32/26/5	44/43	7/16
17		5.5±1.4 (3)	65±10 (2)	2.2	>10	75/70/66	28/47	3/12
18		10±1.5 (3)	6±n.d. (1)	2.9	>10	<1/<1/>113	<23/<22	0/17

^aFor Tables 1–3, values are reported as arithmetic means with standard deviations. Number of replicates in parentheses.

solubility at neutral pH while retaining favorable potencies when the benzoxazolone was replaced by a triazole that was linked to our [3.1.0] core via a C2- (not shown) or C3-linker (compound 17). However, this also led to reduced Caco-2 permeability. ATX inhibitor 18, which displayed the highest solubility at pH 6.8 contained a 4*H*,5*H*,6*H*,7*H*-[1,2]oxazolo[5,4-*c*]pyridin-3-ol. This fairly acidic moiety is an interesting Zn-binding moiety that led to a compound with favorable metabolic stability. Unfortunately, permeability was low so that we did not further follow up on this residue. Notably, all compounds shown in Table 3 had a clean hERG profile.

Disappointingly, when ATX inhibitors 15 and 16 were further evaluated in an in vivo rat PK study, both compounds showed high clearance (for further details, see the SI), and consequently, we shifted our attention to the bis-trifluoromethyl residue, as this residue had resulted in superior potency in rat whole blood and metabolic stability. When combining this residue with a benzotriazole as the Zn-binding group, we obtained compound 19 (BI-2545), which had the best overall profile (for the structure of BI-2545, see Table 4). Since BI-2545 (19) not only displayed good potency in the LPA and rat whole blood assay but also had favorable ADME properties (see Table 4), it was further evaluated in a rat PK/PD model.

BI-2545 (19) proved to be stable in human and moderately stable in rat liver microsomes. In vitro, 19 showed high Caco-2 permeability and low efflux. Consequently, low clearance was observed in vivo in rats, and in vivo oral dosing to rats resulted in good oral exposure. Under these conditions, the sum of the plasma LPA species was reduced up to 90% (see Figure 5), demonstrating high and sustained in vivo efficacy in reducing LPAs. Furthermore, we achieved a higher in vivo potency compared to PF-8380 (see Table 4, SI). As the most attractive candidate from this chemical series, 19 was profiled against a broad panel of in vitro pharmacology assays. At a concentration of 10 μM (~4500-fold the IC₅₀ for ATX), it was significantly, but incompletely, cross-reactive in only four assays: 5-HT2a (55%), L-type Calcium channel (80%), Na⁺ channel site 2 (66%), and norepinephrine transporter (61%; see Table 5, SI for the full profile). These data confirm the selectivity of BI-2545 and, together with its in vitro and in vivo profiles, highlight its suitability as an ATX inhibitor tool compound.

A representative synthesis of our ATX inhibitors (exemplified by compound 11) is shown in Scheme 1. Commercially available Boc-protected primary amine 20 was coupled with 2-oxo-3*H*-

Table 4. In Vitro and in Vivo Profile of BI-2545 (19)^a

parameter	in vitro	in vivo	i.v.	p.o.
IC ₅₀ potency on human/rat ATX ^b (nM)	2.2 ± 1.0 (3)/ 3.4 ± 2.1 (2)			
IC ₅₀ potency on ATX in human/rat whole blood ^b (nM)	29 ± 5.0 (2)/ 96 ± 32.6 (2)			
solubility (μg/mL) at pH 2.2/4.5/6.8	<1/<1/<1			
hERG IC ₅₀ (μM)	>10			
metabolic stability hLM/rLM (% Qh)	23/32			
Caco permeability (× 10 ⁻⁶ cm s ⁻¹)	9			
Caco efflux ratio	1.4			
PPB (h/r)	>99.8/>99.8			
cLogP	4.9			
	in vivo			
dose (mg·kg ⁻¹)	1			10
dose normalized AUC (nM·h)	2320			675
clearance (% Qh)	10			
% QH (%)	10			
V _{ss} (L·kg ⁻¹)	0.9			
dose-normalized c _{max} (nM)				149
T _{max} (h)				1.7
t _{1/2} (h)		1.8		3.4
F (%)				30
IC ₅₀ potency on ATX (nM)				100

^a%QH: normalized clearance based on liver blood flow. V_{ss}: volume of distribution at steady state. C_{max}: maximal concentration. T_{max}: Time point at C_{max}. t_{1/2}: half-life. F: bioavailability; ^bValues are reported as arithmetic means with standard deviations. Number of replicates in parentheses.

1,3-benzoxazole-6-carboxylic acid to give 21. After acidic deprotection of the Boc group, intermediate 22 was reacted with 3-(hydroxymethyl)-5-(trifluoromethoxy)benzonitrile to afford the corresponding carbamate 11.

We could confirm the binding mode of BI-2545 (19) into ATX with a crystal structure (see Figure 6). Compound 19 superimposes almost perfectly onto 2 (PF-8380) with minor deviations in the 3.1.0-bicycle and the linker between that moiety

Figure 5. PK profile of BI-2545 (**19**) and the plasma LPA level reduction of the respective LPA species. Error bars represent standard error of the mean ($N = 3$).

Scheme 1. Synthesis of Compound **11**^a

^aReagents and conditions: (a) 2-oxo-2,3-dihydrobenzoxazole-6-carboxylic acid, TBTU, Et₃N, DMF, then **20**, rt, 12 h, 71%; (b) 4 N HCl in dioxane, rt, 12 h, 69%; (c) 3-(hydroxymethyl)-5-(trifluoromethoxy)benzonitrile, CDI, THF, then **22**, 50 °C, 12 h, 56%.

Figure 6. Cocystal structure of BI-2545 (**19**) bound to autotaxin and overlaid with PF-8380.

and the benzotriazole. The space filling of the hydrophobic pocket is optimized by the two trifluoromethyl groups compared to the dichlorophenyl of **2**. The linker between the [3.1.0]-bicycle and the benzotriazole is shifted by 1.2 Å compared to the position of the linker between the piperazine and the benzoxazolidinone in **2**. Due to the flexibility of the linker, this does not translate into a major rearrangement of the two Zn-binding moieties. The benzotriazole-ring is tilted by 14° with respect to the benzoxazolidinone, but the position of the Zn-coordinating oxygen and nitrogen atoms remains unchanged. All other hydrogen bonds are also preserved among the two compounds.

In summary, by applying knowledge-based design on the recently disclosed ATX inhibitor PF-8380 (**2**), we identified BI-2545 (**19**), which inhibits ATX in the single-digit nanomolar region and translates into good potency in human whole blood. Our ATX inhibitor BI-2545 (**19**) is orally available in rodents,

devoid of hERG channel inhibition, and shows an excellent PK/target engagement relationship. It is therefore considered a valuable tool for further in vivo studies.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmchemlett.7b00312.

Procedures for the preparation of **2–19** (including intermediates), biological and in vitro ADME assays, crystallization and structural determination, PK data, and pharmacology profile of BI-2545 (PDF)

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Notes

The authors declare no competing financial interest.

BI-2545 is available free of charge with no strings attached on [openMe](http://openme.com), the new Boehringer Ingelheim Open Innovation Platform (www.openme.com).

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■ ABBREVIATIONS

ATX, autotaxin; LPA, lysophosphatidic acid; IPF, idiopathic pulmonary fibrosis; hERG, human ether-a-go-go related gene; SAR, structure–activity relationship; TBTU, O-(benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium tetrafluoroborate; DMF, dimethylformamide; CDI, 1,1'-carbonyldiimidazole; CDT, 1,1'-carbonyl-di(1,2,4-triazole); THF, tetrahydrofuran; DIPEA, N,N-diisopropylethylamine; rt, room temperature; ADME, absorption distribution metabolism excretion; PK, pharmacokinetics; PD, pharmacodynamics

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