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# ONO-8430506: A Novel Autotaxin Inhibitor That Enhances the Antitumor Effect of Paclitaxel in a Breast Cancer Model

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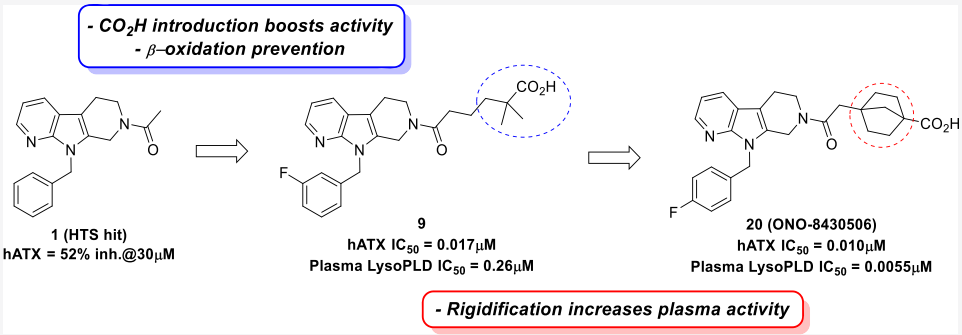


**ABSTRACT:** Lysophosphatidic acid (LPA) is a bioactive lipid mediator that elicits a number of biological functions, including smooth muscle contraction, cell motility, proliferation, and morphological change. LPA is endogenously produced by autotaxin (ATX) from extracellular lysophosphatidylcholine (LPC) in plasma. Herein, we report our medicinal chemistry effort to identify a novel and highly potent ATX inhibitor, ONO-8430506 (**20**), with good oral availability. To enhance the enzymatic ATX inhibitory activity, we designed several compounds by structurally comparing our hit compound with the endogenous ligand LPC. Further optimization to improve the pharmacokinetic profile and enhance the ATX inhibitory activity in human plasma resulted in the identification of ONO-8430506 (**20**), which enhanced the antitumor effect of paclitaxel in a breast cancer model.

**KEYWORDS:** *Autotaxin, ENPP2, LPA, β-oxidation, in vivo isomerization*

Autotaxin (ATX) is an extracellular enzyme[1] that has lysophospholipase D (LysoPLD) activity to produce lysophosphatidic acid (LPA) from lysophosphatidylcholine (LPC) in circulating blood.[2,3] The primary structure is identical to the ectonucleotide pyrophosphatase/phosphodiesterase 2 (ENPP2).[4] Many reports have suggested the involvement of the LPA-producing enzyme ATX in the formation of blood vessels,[5] chronic inflammation,[6] and pain generation.[7] Additionally, multiple studies have reported on the function of the ATX-LPA system in cancer. It has been suggested that the anticancer effects of taxanes, doxorubicin, and radiation may be controlled by ATX-LPA.[8−10] The use of ATX inhibitors is expected to ameliorate the disease for patients whose cancer cells are resistant to the effects of these anticancer treatments.

Owing to its attractiveness as a novel medicinal target, intensive efforts on the discovery of potent ATX inhibitors have been conducted. As reviewed by Kokotos et al., many researchers in the pharmaceutical industry have discovered novel ATX inhibitors in the past several years.[11,12] Among them, GLPG1690 is currently in phase 3 clinical trials for idiopathic pulmonary fibrosis (IPF).[13] Herein, we report our efforts to find a novel, potent and orally available ATX inhibitor.

To identify novel ATX inhibitors, we performed high-throughput screening (HTS) of our in-house compound library and discovered compound **1** as a hit compound (Figure 1). Although **1** showed very weak enzymatic inhibitory activity toward recombinant human ATX (hATX assay, 51.6% inhibition at 30 $\mu$M), its modular structure encouraged us to investigate the structure−activity relationship (SAR) to increase the activity.

As an initial strategy, we focused on the structures of compound **1** and an endogenous substrate of ATX,

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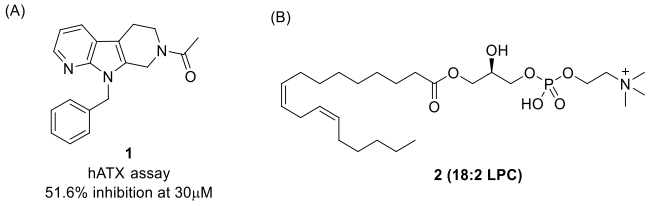
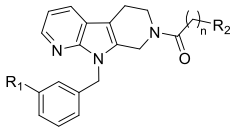



**Figure 1.** Structures of hit compound (**1**) and 18:2 LPC (**2**).

lysophosphatidylcholine (**2**, 18:2 LPC). We considered the possibility of both compounds structurally overlapping each other with the fatty-acid side chain of LPC occupying the same binding pocket as the 9-benzyl tetrahydro-$\beta$-carboline moiety of compound **1**. Based on this hypothesis, we supposed that compound **1** lacked a polar group such as a phosphate group, which is thought to be important for the activity. Indeed, Nishimasu et al. reported that the phosphate group of LPA interacts with $Zn^{2+}$ and other highly conserved residues among the ENPP family in the crystal structure of ATX bound with LPAs having different side chain lengths and saturations.[14] Therefore, we tried to introduce a polar group with various lengths of methylene linker into the acetyl part of compound **1**.

As shown in Table 1, we employed a carboxyl group as the introduced polar group and various carboxylic acid analogues

**Table 1. Structure–Activity Relationship of Carboxylic Acid Analogues**



| compd. | $R_1$ | $R_2$ | n | hATX assay[a] $IC_{50}$ ($\mu$M) |
|---|---|---|---|---|
| **1** | H | Me | 0 | 51.6% inhibition at 30 $\mu$M |
| **3** | H | $CO_2H$ | 2 | 5.4 |
| **4** | H | $CO_2H$ | 3 | 0.83 |
| **5** | H | $CO_2H$ | 4 | 0.24 |
| **6** | H | $CO_2H$ | 5 | 0.25 |
| **7** | F | $CO_2H$ | 4 | 0.050 |

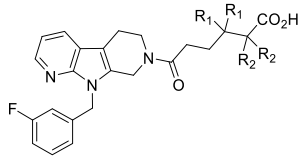
[a]The inhibitory activity of the compound toward recombinant human ATX was evaluated using 16:0 LPC as a substrate.

were investigated. Our studies revealed that compounds **3** and **4**, which have ethylene (C-2) and propylene (C-3) linkers between the amide moiety and carboxyl group, were less potent in the hATX assay (**3**, $IC_{50}$ = 5.4 $\mu$M; **4**, $IC_{50}$ = 0.83 $\mu$M). Conversely, compounds **5** and **6** with butylene (C-4) and pentylene (C-5) linkers, respectively, demonstrated significantly increased potency (**5**, $IC_{50}$ = 0.24 $\mu$M; **6**, $IC_{50}$ = 0.25 $\mu$M). In particular, **5** showed an approximately 100-fold increase in $IC_{50}$ relative to **1** and was considered to be a suitable compound for further exploration. This SAR illustrated that the existence of a carboxyl group at the appropriate position plays an important role in providing strong ATX inhibitory activity in this chemical series. Substitutions at the benzene ring were also quickly explored, considering the potential metabolic instability of the unsubstituted benzene ring. This exploration showed that lipophilic groups were well tolerated and gave the 3-fluoro substituted analogue **7** as a more potent compound.

With the potent compound (**7**) in hand, pharmacokinetic profiles of **7** were evaluated to confirm its potential as an orally available drug (Table 2). Single-dose rat pharmacokinetic studies (oral dosing at 3 mg/kg and intravenous dosing at 1 mg/kg) revealed that **7** demonstrated relatively low plasma clearance (CL = 7.5 mL/min/kg) and moderate oral bioavailability ($\%F_{rat}$ = 30%), which are thought to be acceptable values as a tool compound for *in vivo* study. However, more optimization was needed to improve its pharmacokinetic profiles and the ATX inhibitory activity toward a clinical candidate.

When we worked on improving the pharmacokinetic profiles of compound **7**, we considered that **7** has no substituents at the $\alpha$ and $\beta$ carbon positions of the carboxyl group. Therefore, this part of the molecule was susceptible to $\beta$-oxidation. In fact, the metabolic stability in the hepatocyte of **7** was very low as shown in Table 2 (remaining % in hepatocyte, human: 29%, rat: 10%). While several approaches have been reported to prevent $\beta$-oxidation,[15,16] we decided to introduce a dimethyl group adjacent to the carboxyl group in **7** considering its low measured LogD value ($LogD_{7.4}$ = 0.51). Introduction of dimethyl group at the $\alpha$ and $\beta$ carbon positions of the carboxyl group in **7** afforded compounds **8** and **9**, respectively, which exhibited improved metabolic stability in hepatocytes with an improvement in the hATX inhibitory activity (**8**, $IC_{50}$ = 0.024 $\mu$M; **9**, $IC_{50}$ = 0.017 $\mu$M). Indeed, an evaluation of the pharmacokinetic profiles of compounds **8** and **9** revealed that both compounds displayed decreased plasma clearance (**8**, CL = 3.8 mL/min/kg; **9**, CL = 3.1 mL/min/kg). Furthermore, the oral bioavailability was also improved (**8**, $\%F_{rat}$ = 75%; **9**, $\%F_{rat}$ = 82%) presumably because of the improvement in the membrane permeability with

**Table 2. Introduction of the Dimethyl Group Adjacent to the Carboxylic Group**



| | | | | | metabolic stability in hepatocyte[a] (%) | | *in vivo* PK (rat) | |
|---|---|---|---|---|---|---|---|---|
| compd. | $R_1$ | $R_2$ | hATX assay $IC_{50}$ ($\mu$M) | $logD_{7.4}$ | human | rat | CL[b] (mL/min/kg) | F[c] (%) |
| **7** | H | H | 0.050 | 0.51 | 29 | 10 | 7.5 | 30 |
| **8** | Me | H | 0.024 | 1.54 | 42 | 66 | 3.8 | 75 |
| **9** | H | Me | 0.017 | 1.71 | 92 | 47 | 3.1 | 82 |

[a]The values show the remaining % 2 h after hepatocyte incubation. [b]Plasma clearance (CL) was calculated based on an intravenous dose of 1 mg/kg ($n$ = 3). [c]Bioavailability (F) was calculated based on an intravenous dose of 1 mg/kg and an oral dose of 3 mg/kg ($n$ = 3).



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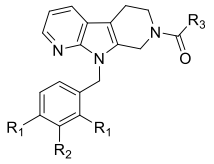
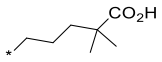
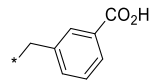
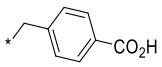
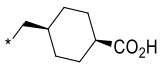
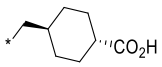
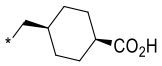
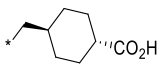
ACS Med. Chem. Lett. 2020, 11, 1335–1341

**Table 3. Rigidification around Carboxyl Group**



| compd. | $R_1$ | $R_2$ | $R_3$ | hATX assay $IC_{50}$ (μM) | human plasma LysoPLD assay[a] $IC_{50}$ (μM) | $logD_{7.4}$ | plasma protein binding (%) human |
|---|---|---|---|---|---|---|---|
| **9** | H | F |  | 0.017 | 0.26 | 1.71 | >99.9 |
| **10** | H | F |  | 0.10 | NT | NT | 98.9 |
| **11** | H | F |  | 0.028 | NT | 0.67 | 99.0 |
| **12** | H | F |  | 0.029 | NT | 1.45 | 99.6 |
| **13** | H | F |  | 0.017 | NT | 1.07 | 99.3 |
| **14** | F | H |  | 0.019 | 0.016 | 1.36 | 99.4 |
| **15** | F | H |  | 0.014 | 0.0073 | 1.15 | 98.4 |

[a]The LysoPLD inhibitory activity of the compound in human plasma was evaluated using 16:0 LPC as a substrate.

increased LogD values. As compound **9** showed better pharmacokinetic profiles, especially in the metabolic stability in human hepatocyte, compound **9** was selected for further profiling.

We evaluated the ATX inhibitory activity under physiological conditions in plasma as the LysoPLD activity. As shown in Table 3, the LysoPLD inhibitory activity in human plasma of compound **9** was weakened by 15-fold compared with that evaluated using recombinant human ATX (human plasma LysoPLD assay; $IC_{50}$ = 0.26 μM vs hATX assay; $IC_{50}$ = 0.017 μM). One plausible reason for this result may be derived from its high plasma protein binding (>99.9% in human). Therefore, our design strategy was focused toward reducing plasma protein binding.

Rigidified molecules are often used to increase activity, reduce lipophilicity, and improve metabolic stability.[17−19] We considered the possibility that rigidification around the polar carboxyl group would reduce lipophilicity and plasma protein binding. The result is shown in Table 3. First, we synthesized benzoic acid analogues **10** (*meta*) and **11** (*para*) to determine the preferred position of the carboxyl group, and demonstrated that putting the carboxyl group at the *para* position was preferred (hATX assay; **11**, $IC_{50}$ = 0.028 μM vs **10**, $IC_{50}$ = 0.10 μM). Next, the replacement of benzoic acid with cyclohexanecarboxylic acid was attempted to give **12** (*cis* isomer) and **13** (*trans* isomer). Fortunately, the *trans* isomer **13** showed strong enzymatic ATX inhibitory activity ($IC_{50}$ = 0.017 μM) and reduced plasma protein binding (99.3% in human). Our preliminary explorations revealed that lipophilic groups were well tolerated as substitutions at the benzene ring, and minor modifications on the substituents could be used to modulate the overall properties (e.g., ATX inhibitory activity and DMPK profiles). With these observations in mind, we examined several substitutions for compound **13** and found the difluoro-substituted analogue **15** as a more advanced compound. As we expected, compound **15** demonstrated improved activity in the human plasma LysoPLD assay (human plasma LysoPLD assay; $IC_{50}$ = 0.0073 μM vs hATX assay; $IC_{50}$ = 0.014 μM). Notably, the effect of rigidification on increasing the plasma LysoPLD inhibitory activity was supported by the reduced plasma protein binding and measured logD value.

However, as shown in Figure 2, a detailed evaluation of the pharmacokinetic profile revealed that compound **15** was isomerized *in vivo* to the corresponding *cis* isomer **14** upon oral administration in a rat (34% of the *trans* isomer **15** was converted to the *cis* isomer **14** on an area under the curve (AUC) basis). We also evaluated the metabolic stability of the compounds using hepatocytes from several species to confirm

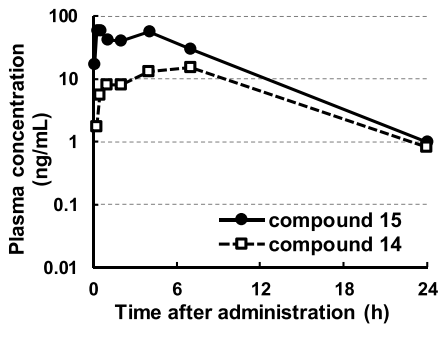



**Figure 2.** Pharmacokinetic profiles of compound **15**.



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whether there was any possibility of causing this isomerization in other species (Table 4). As a result, the isomerization was

**Table 4. Hepatocyte Incubation Study of Compound 15**

| conversion ratio in hepatocyte incubation study[a] (% of concentration, **14**/**14** + **15**) | | | |
|---|---|---|---|
| human | rat | dog | monkey |
| 29.8 | 14.4 | 9.0 | 21.4 |

[a]Conversion ratio was defined as the ratio of concentration 2 h after hepatocyte incubation.

observed in all evaluated species (human, rat, dog and monkey), indicating the possibility of making clinical studies complicated. He et al. also reported on a similar *in vivo* isomerization.[20] Therefore, further modification to prevent this isomerization was needed.

Our first approach to prevent the isomerization was to introduce a methyl group at the $\alpha$ position of the carboxyl group to produce compound **16**. Although this compound exhibited slightly decreased activity (human plasma LysoPLD assay; $IC_{50}$ = 0.025 $\mu$M), its good pharmacokinetic profiles prompted us to pursue further modification. Our next approach involved the use of bicyclic systems as in compounds **17** and **18**. Bicyclic systems contribute toward keeping the molecules in the biologically active conformation and prevent isomerization. As expected, compounds **17** and **18** displayed single-digit nanomolar potency in the human plasma LysoPLD assay (**17**, $IC_{50}$ = 0.0081 $\mu$M; **18**, $IC_{50}$ = 0.0079 $\mu$M). Thus, a minor modification of the substituents at the benzene ring was conducted again, while retaining the bicyclic systems as in compounds **17** and **18**. Ultimately, compound **20** (**ONO-8430506**) was selected as a preclinical candidate based on its strong potency, good pharmacokinetic profiles, and other overall profiles (Table 5).

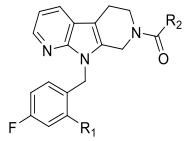
Table 6 shows the pharmacokinetic profiles of compound **20** in several preclinical species. Compound **20** exhibited a relatively low plasma clearance and long half-life. Additionally, an allometric scaling prediction showed that the human clearance would be much lower (predicted human clearance = 2.1 mL/min/kg).

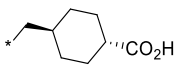
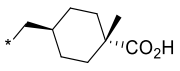
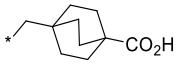
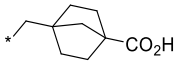
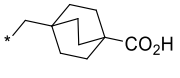
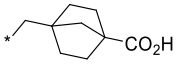
Previously, we found that compound **20** (**ONO-8430506**) suppressed LPA formation in plasma and decreased rat intraurethral pressure in a dose-dependent manner during the 20 min after intraduodenal administration *in vivo*.[21] We considered examining the long-term effects of the compound after oral administration. Because it was reported that the antitumor effects of paclitaxel (PTX) were attenuated by LPA stimulation *in vitro*,[8] we confirmed this concept using our compound *in vivo*.

We evaluated the antitumor effects of compound **20** in combination with PTX in a mouse model of the subcutaneously implanted human breast cancer cell line MDA-MB-231, as shown in Figure 3. Specifically, the enhancing effect of the compound on the antitumor effects of monotherapy PTX at the maximum tolerated dose was evaluated. PTX was administered intraperitoneally at 20 mg/kg (the maximum tolerated dose) three times weekly for 5 weeks.

Considering the pharmacokinetic profiles of compound **20** in the mouse model, the compound was administered orally at doses of 30 or 100 mg/kg twice daily for 32 days. In the PTX monotherapy group, the tumor volume was significantly lower than that in the vehicle group with TGI (tumor growth inhibition)$_{median}$ at 81.5%. Conversely, there were no significant effects on the tumor volume in the "compound **20** at 100 mg/kg" monotherapy group with $TGI_{median}$ at 14.7%. In the "PTX and compound **20** combination" group, the tumor volume was significantly lower at all doses in comparison with that in the PTX monotherapy group. This observation was dependent on

**Table 5. Attempts to Prevent *in vivo* Isomerization**




| compd. | $R_1$ | $R_2$ | hATX assay $IC_{50}$ (μM) | human plasma LysoPLD assay $IC_{50}$ (μM) | plasma protein binding (%) human | plasma protein binding (%) rat | In vivo PK rat AUC[a] (ng·h/mL, 0.5mg/kg) |
|---|---|---|---|---|---|---|---|
| **15** | F |  | 0.014 | 0.0073 | 98.4 | 97.9 | NT |
| **16** | F |  | 0.022 | 0.025 | 99.1 | 96.1 | 677 |
| **17** | F |  | 0.0090 | 0.0081 | 99.1 | 95.7 | 472 |
| **18** | F |  | 0.011 | 0.0079 | 99.2 | 93.9 | 500 |
| **19** | H |  | 0.012 | 0.0061 | 98.6 | 94.6 | 420 |
| **20** | H |  | 0.010 | 0.0055 | 99.0 | 95.1 | 842 |

[a]The values show AUC after 0.5 mg/kg oral administration (cassette dosing) for all compounds.



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ACS Med. Chem. Lett. 2020, 11, 1335−1341

**Table 6. Pharmacokinetic Profiles of Compound 20**

| species | route | dose (mg/kg) | Cmax[a] (ng/mL) | $V_{ss}$[a] (mL/kg) | $T_{1/2}$[a] (h) | CL[a] (mL/min/kg) | F[b] (%) |
|---|---|---|---|---|---|---|---|
| mouse | po | 1 | 124 ± 49 | | 5.4 ± 2.1 | | |
| rat | iv | 0.3 | | 1474 ± 153 | 3.4 ± 0.9 | 8.2 ± 2.3 | |
| | po | 1 | 261 ± 107 | | 2.5 ± 0.3 | | 51.6 |
| dog | iv | 0.3 | | 1863 ± 878 | 8.9 ± 5.3 | 4.7 ± 0.7 | |
| | po | 1 | 1670 ± 610 | | 5.9 ± 2.0 | | 71.1 |
| monkey | iv | 0.3 | | 2275 ± 235 | 7.9 ± 1.0 | 5.8 ± 0.6 | |
| | po | 1 | 63 ± 38 | | NC | | 30.8 |

[a]The values are expressed as the mean ± standard deviation ($n$ = 3), NC = not calculated. [b]F (%) was calculated using the mean of AUC (po) and the mean of AUC (iv).

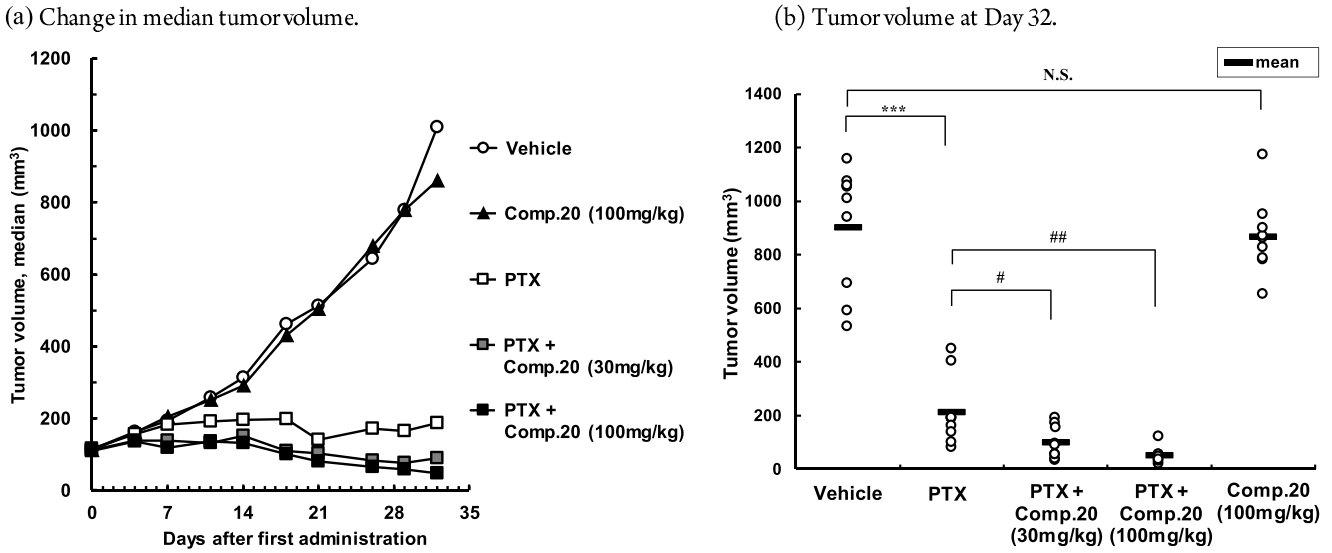


**Figure 3.** Antitumor effect of compound **20** in MDA-MB-231 human breast cancer-bearing mouse model. (a) Change in median tumor volume. (b) Tumor volume at day 32. (b) ***$p$ < 0.001. N.S.: not significant by Student $t$ test. #$p$ < 0.05, ##$p$ < 0.01 by Student $t$ test using a closed testing procedure from the high dose side. ($n$ = 8–9).

the dose of compound **20**. The TGI medians were 91.2% and 95.5% for the PTX and compound **20** at 30 and 100 mg/kg combination groups, respectively. We also evaluated LysoPLD activity from plasma at 18 h following the final administration of compound **20**. Inhibition rates of LysoPLD activity were 97.1% for the "PTX and compound **20** at 30 mg/kg combination" group and 99.3% for the "PTX and compound **20** at 100 mg/kg combination" group. The comparable inhibition of LysoPLD activity was achieved at 30 mg/kg once daily in rat models,[21] indicating that a lower dose could be expected in humans.

In summary, we developed a novel and highly potent ATX inhibitor that enhances the antitumor effect of PTX in a mouse model of a breast cancer cell line. To increase the enzymatic ATX inhibitory activity, a carboxyl group was successfully incorporated by structurally comparing our hit compound with an endogenous ligand LPC. Further optimization focused on improving the pharmacokinetic profiles and plasma LysoPLD inhibitory activity, leading to compound **20** (ONO-8430506). The detailed pharmacokinetic and pharmacodynamic profiles demonstrated that compound **20** (ONO-8430506) is a suitable candidate for further clinical development.

## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsmedchemlett.0c00200.

Experimental procedures and characterization data for all compounds. Experimental conditions of the biological and DMPK evaluations. (PDF)

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### Notes

The authors declare no competing financial interest.

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## ■ ABBREVIATIONS

ATX, autotaxin; LysoPLD, lysophospholipase D; LPA, lysophosphatidic acid; LPC, lysophosphatidylcholine; ENPP2, ectonucleotide pyrophosphatase/phosphodiesterase 2; PTX, paclitaxel; TGI, tumor growth inhibition

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