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Discovery of potent inhibitors of the *lyso*phospholipase autotaxin

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ABSTRACT

The autotaxin–*lyso*phosphatidic acid (ATX–LPA) axis has been implicated in several disease conditions including inflammation, fibrosis and cancer. This makes ATX an attractive drug target and its inhibition may lead to useful therapeutic agents. Through a high throughput screen (HTS) we identified a series of small molecule inhibitors of ATX which have subsequently been optimized for potency, selectivity and developability properties. This has delivered drug-like compounds such as **9v** (CRT0273750) which modulate LPA levels in plasma and are suitable for in vivo studies. X-ray crystallography has revealed that these compounds have an unexpected binding mode in that they do not interact with the active site zinc ions but instead occupy the hydrophobic LPC pocket extending from the active site of ATX together with occupying the LPA 'exit' channel.

Autotaxin (ATX), also known as ectonucleotide pyrophosphatase/phosphodiesterase family member 2 (ENPP2), is a secreted lysophospholipase D (lysoPLD) that cleaves choline from *lyso*phosphatidylcholine (LPC) forming *lyso*phosphatidic acid (LPA), a mitogen and motility factor that has been implicated in the pathophysiology of cancer[1–3] and many other biological processes such as vascular development, lymphocyte homing and inflammation.[4–6] LPA consists of a single fatty acyl chain, a glycerol backbone and a free phosphate group. Several structurally diverse forms of LPA exist with different acyl-chain lengths and saturations. LPA acts through a number of LPA receptors and the great variety of cellular and biological actions of LPA is explained by the fact that the six known LPA receptors show broad tissue expression and can couple to at least three distinct G proteins, which, in turn, feed into multiple effector systems[7] (Fig. 1).

The ATX–LPA axis has been implicated in several disease states including inflammation,[8] pulmonary fibrosis,[9] and tumour progression.[10] ATX is processed along the classical export pathway and secreted as a catalytically active glycoprotein. ATX's major lipid substrate, LPC, is secreted by the liver and is abundantly present in plasma and interstitial fluids. Potent and selective inhibitors of ATX would clearly be of value to elucidate the biology of this target and an overview of the patent and primary literature describing the development of novel ATX inhibitors has recently been published.[11] Clinical trials have recently been initiated with GLPG1690 which is the first autotaxin inhibitor to enter the clinic and is under evaluation for the treatment of idiopathic pulmonary fibrosis.[12] Herein we report the discovery of a new class of small molecule inhibitors of ATX which have been optimized to deliver compounds suitable for in vivo studies.

A high throughput biochemical screen using the FS-3 assay[13] was run using the CRT compound collection of 87,865 compounds. The screen was run at 30 μM and the confirmed hit rate, defined as giving >50% inhibition at 30 μM, was 1.2%. After IC_{50} determination in the FS-3 assay, a number of distinct chemical series were identified. Compounds from each series were then screened in the enzyme coupled (EC) biochemical assay which uses the more physiologically relevant substrate LPC C16:0 and measures the release of choline.[14] The absolute potencies of the compounds in the enzyme coupled biochemical assay were generally lower and this helped prioritise between the chemical series so that chemistry effort could be focused on the most promising scaffolds. Based

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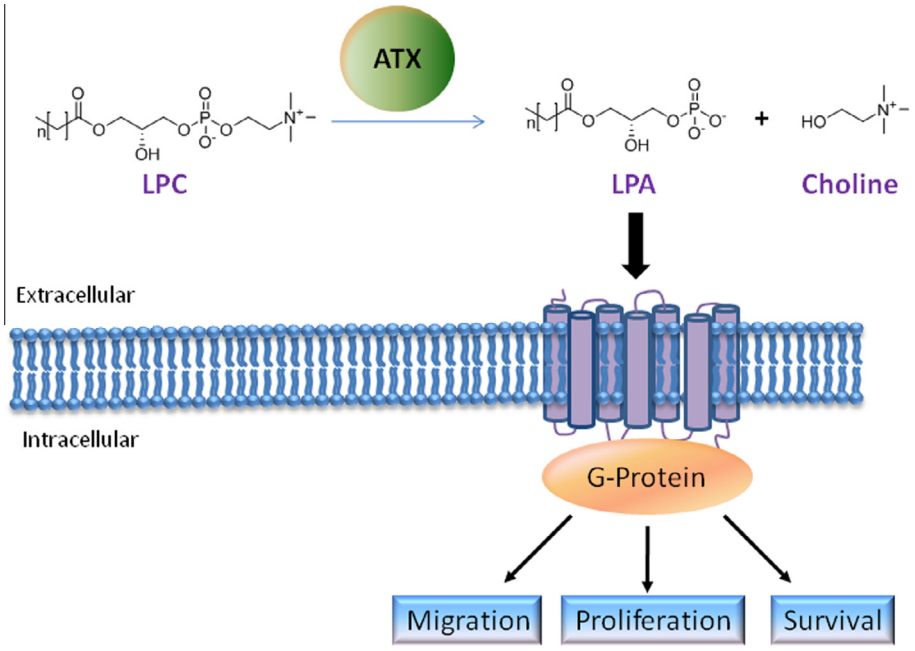
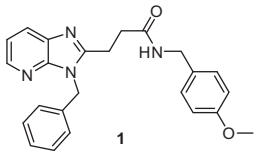


Figure 1. The autotaxin–LPA axis showing conversion of LPC to LPA mediated by autotaxin and subsequent activation of LPA receptors.

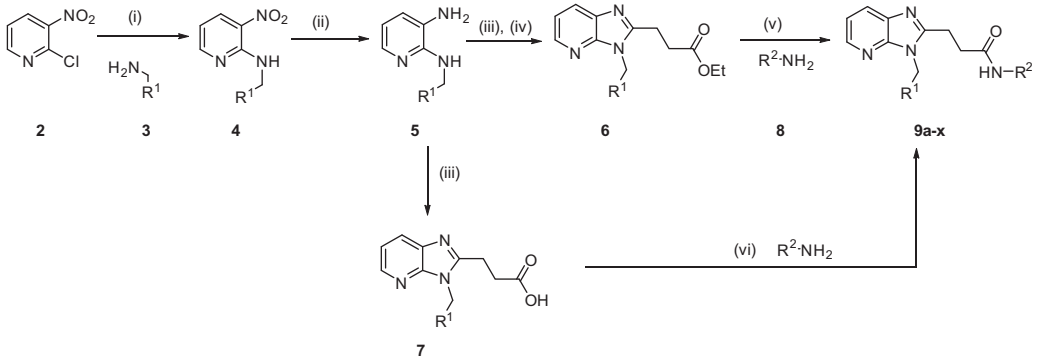
Table 1
Profile of hit compound **1** selected for further optimisation



1

FS-3 IC_{50}[a] (μM)	EC IC_{50}[b] (μM)	LE[c]	*c*Log*P*[d]	PSA[e]	M wt
0.040	0.473	0.29	3.1	69	400

[a] The FS-3 biochemical assay utilizes a doubly labelled analogue of LPC wherein the fluorophore is quenched through intramolecular energy transfer. Hydrolysis of the substrate produces an increase in fluorescence.
[b] EC IC_{50}—this assay utilises LPC C16:0 as substrate and measures the release of choline.
[c] LE = ligand efficiency = (1.36*pIC_{50})/HAC, HAC = heavy atom count,
[d] *c*Log*P* is the octanol/water partition coefficient calculated using Stardrop™.
[e] PSA = polar surface area calculated using Stardrop™.



Scheme 1. Synthesis of imidazo[4,5-*b*]pyridine derivatives **9**. Reagents and conditions: (i) Cs_2CO_3, $R^1CH_2NH_2$, MeOH reflux, 61–100%; (ii) H_2, 10% Pd/C, EtOH, 1 atm or cHCl, Fe powder, EtOH, 88–100%; (iii) succinic anhydride, dioxane, reflux, 52–81%; (iv) cH_2SO_4, EtOH, 10–13% combined yield for steps (iii) and (iv); (v) KO^{t}Bu, R^2NH_2, MW, 4–42%; (vi) HBTU, Et_3N, R^2NH_2, 35–79%.