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Graphical Abstract

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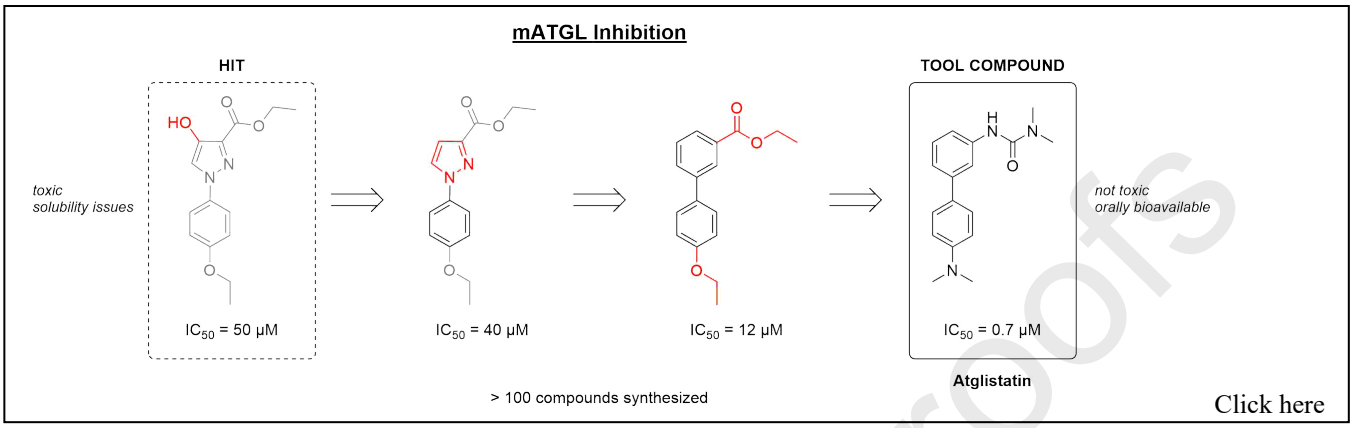

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Structure-activity relationship studies for the development of inhibitors of murine adipose triglyceride lipase (ATGL)

Nicole Mayer[a,#], Martina Schweiger[b,c,#], Elisabeth Fuchs[a], Anna K. Migglautsch[a], Carina Doler[a], Gernot F. Grabner[b], Matthias Romauch[b], Michaela-Christina Melcher[a], Rudolf Zechner[b,c], Robert Zimmermann[b,c,*], Rolf Breinbauer[a,c,*]

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ABSTRACT

High serum fatty acid (FA) levels are causally linked to the development of insulin resistance, which eventually progresses to type 2 diabetes and non-alcoholic fatty liver disease (NAFLD) generalized in the term metabolic syndrome. Adipose triglyceride lipase (ATGL) is the initial enzyme in the hydrolysis of intracellular triacylglycerol (TG) stores, liberating fatty acids that are released from adipocytes into the circulation. Hence, ATGL-specific inhibitors have the potential to lower circulating FA concentrations, and counteract the development of insulin resistance and NAFLD. In this article, we report about structure-activity relationship (SAR) studies of small molecule inhibitors of murine ATGL which led to the development of Atglistatin. Atglistatin is a specific inhibitor of murine ATGL, which has proven useful for the validation of ATGL as a potential drug target.

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1. I

Adipose tissue (AT) constitutes the body's largest energy store. The prevailing cell type of AT is the adipocyte which major function is to store and to liberate energy substrates in form of free fatty acids (FA) on demand. To fulfill their functions, adipocytes comprise a set of lipogenic and lipolytic enzymes and regulatory proteins, which are tightly regulated by feeding/fasting cycle of the organism. In the postprandial state a surplus of energy is stored as triacylglycerol within cytoplasmic lipid droplets. Upon fasting, adipose triglyceride lipase (ATGL) initiates the breakdown of stored TG to diacylglycerol (DG) and FA.[1] Subsequently the activities of hormone-sensitive lipase (HSL) and monoacylglycerol lipase (MGL) generate glycerol and two FA.[2] Adipocytes release the lipolytic products into the circulation to provide energy substrates for oxidative tissues. A constantly positive energy balance causes an increase in AT mass due to an increase in adipocyte number and adipocyte size, and obesity. However, the expandability of AT is not infinite. If the supply of energy substrates exceeds the storage capacity of AT, FA concentrations in the circulation increase and lipids are deposited in ectopic tissues, like muscle and liver. These ectopically stored lipids are responsible for the development of obesity associated insulin resistance and non-alcoholic fatty liver disease (NAFLD), finally leading to tissue dysfunction and early death.[3,4] It is suggested that an increased release of FA from hypertrophic adipocytes contributes to the elevation of plasma and ectopic lipid concentrations.[5] As ATGL is the initiating enzyme in the liberation of FA from TG stores of adipocytes, reducing FA in the circulation by inhibiting ATGL represents an attractive strategy to counteract the metabolic complications of obesity. Indeed, as mouse models of global as well as adipose tissue specific genetic ATGL deficiency showed, reduced plasma FA concentrations accompanied by a resistance to diet induced obesity and insulin resistance.[6,7]

These findings prompted us to develop and characterize small molecule inhibitors of ATGL. The versatile synthetic strategies we applied resulted in the development of Atglistatin. Atglistatin selectively, competitively, and transiently inhibits murine ATGL (mATGL) activity *in vitro*, in cultured cells, and *in vivo*.[8] In contrast to complete ATGL deficiency in mice and humans, long term Atglistatin treatment does not cause cardiac steatosis, cardiac failure or a premature death. Importantly, Atglistatin treatment reduced circulating plasma lipids and protected from diet induced obesity, insulin resistance and NAFLD in mice.[9] At present no 3D-structure of ATGL is available and any effort to identify inhibitors of ATGL has to rely on the traditional approach of synthesizing and testing compounds.[10] In this manuscript we describe the structure-activity relationship (SAR) studies which allowed us in

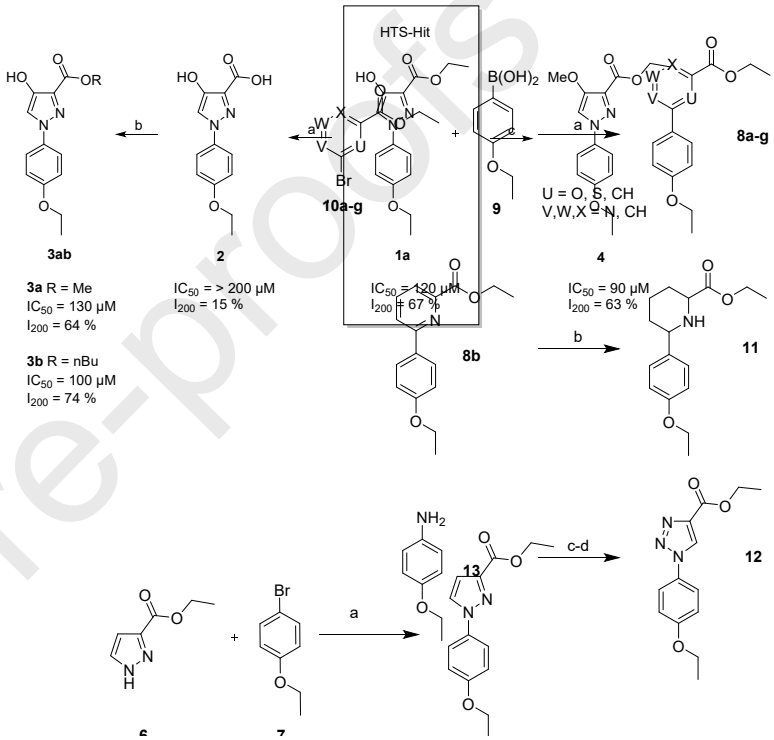
Scheme 1. HTS-hit and explorative modifications. Reagents and conditions: (a) 5 M NaOH, EtOH, 60 °C, 30 min, 41%; (b) R-OH, cat. conc. H_2SO_4, reflux; (c) 1.5 equiv K_2CO_3, DMF, overnight, 93%.

iterative rounds of optimization to develop biaryl hit compound **1** (IC_{50} (half maximal inhibitory concentration) = 120 µM, resulting from a high-throughput screening (HTS) campaign originally aimed to identify HSL inhibitors) to Atglistatin, which has proven to serve as a valuable tool compound to validate ATGL as a drug target. The presented SAR-data give valuable insight about the nature of the ATGL binding pocket and might provide opportunities for applying *in silico* methods to develop ATGL inhibitors.

2. Results and discussion

2.1. Preliminary experiments

the HTS hit compound **1a** to modify the substituents of the pyrazole moiety (Scheme 1). Saponification of the ethyl ester delivered carboxylic acid **2**, which did not show any substantial mATGL inhibitory activity anymore. The importance of the ester moiety was further corroborated by the decent inhibitory activities of the methyl and butyl esters **3a-b**. Alkylation of the parent HTS-hit compound **1a** with methyliodide produced methoxypyrazole **4**, which showed a significantly improved IC_{50} value. In addition to IC_{50} values, activity of new compounds was further determined as % of inhibition at a single concentration of 200 µM (I_{200}). Variation of the substitution pattern (**1b-p**) of the bottom ring of the parent hit structure **1a** led to activity loss (see SI). Further we tested if a substituent is necessary at all at the 4-position. The



Scheme 3. Introducing variations into the top ring fragments. Reagents and conditions: (a) 0.05 equiv $PdCl_2$(dppf)*DCM, 2.1 equiv CsF, DME, 80 °C; (b) 1.6 equiv PtO_2, 1 bar H_2, EtOH/DCM, RT, 27 h, 59%; (c) 1.5 equiv $NaNO_2$, H_2O, conc. HCl, 0 °C, 1 h, then 2.0 equiv NaN_3, H_2O, 0 °C to RT, 1 h, 84%; (d) 1.0 equiv ethyl propiolate, 0.2 equiv sodium ascorbate, 0.07 equiv $CuSO_4$*$5H_2O$, H_2O/ACN, RT, 15 h

desired compound **5** was prepared via CuI-catalyzed *N*-arylation of ethyl 3-pyrazolecarboxylate (**6**) and *p*-bromophenetole (**7**) (Scheme 2).[11] **5** exhibited the best inhibitory activity so far. Unfortunately, we also observed a considerable inhibitory activity of **5** against MGL (see SI, Figure S1).

2.2. Exchange of the top ring system

In order to improve lipase selectivity, the pyrazole ring was replaced by several different ring systems, while keeping the 1,3-arrangement of the 4-ethoxyphenyl and ethyl ester substituents constant (**8a-g**). 3-Bromoaryl esters **10a-g** were coupled with 4-ethoxyphenylboronic acid (**9**) in a Suzuki coupling reaction with $PdCl_2$(dppf)*DCM as catalyst and CsF as base in DME (Scheme 3).[12,13] In order to test if a non-planar compound could also fit into the binding pocket, piperidine derivative **11** was produced from pyridine ester **8b** via catalytic hydrogenation.[14] Triazole ester **12** was produced by converting aniline **13** via diazonium chemistry into the corresponding azide,[15] which was reacted with ethyl propiolate in a Cu(I)-catalyzed azide-alkyne 1,3-dipolar

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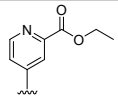
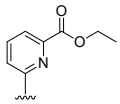
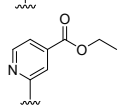
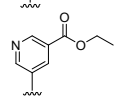
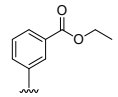
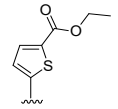
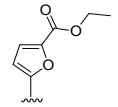
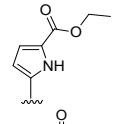
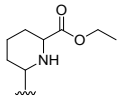
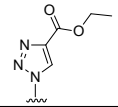
acetonitrile and water.[16]

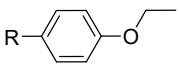
In the biological screening data (Table 1) we noticed a strong influence of the top ring. Replacing the pyrazole ring with a phenyl ring (**8e**) resulted in a similar IC_{50} value (50 µM) and inhibition

Entry	No.	R	IC_{50} [µM]	I_{200} [%]
1	**8a**		105	72
2	**8b**		60	79
3	**8c**		80	87
4	**8d**		130	70
5	**8e**		**50**	**87**
6	**8f**		70	81
7	**8g**		50	93
10	**8h**		> 200	44
8	**11**		> 200	48
9	**12***		> 200	43

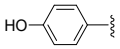
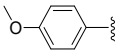
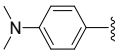
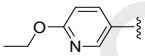
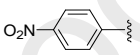
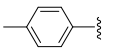
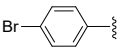
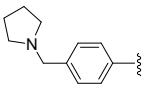
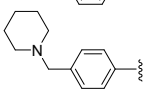
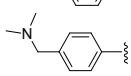
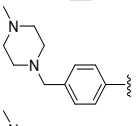
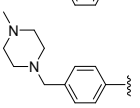
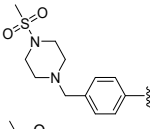
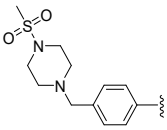
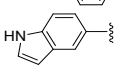
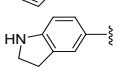
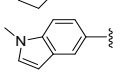
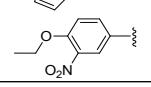
effect (87%). Also, the pyridine ring with the nitrogen in 2-position (**8b**) and the furan ring (**8g**) showed comparably good results. From these compounds we chose the presumably more drug-like structure **8e** for further studies. **8e** showed no cellular toxicity and very good selectivity in an *ex vivo* assay by taking tissue pieces of gonadal fat and incubating them with the inhibitor compound (see SI, Figure S2 and Figure S3). Gonadal fat is the tissue of choice to determine the release of lipolytic products as a measure of tissue lipolysis as it is a homogenous tissue consisting mostly of white adipocytes in contrast to other adipose tissue depots, like subcutaneous adipose tissue.[17,18]

Scheme 2. Synthesis of unsubstituted derivative **5**. Reagents and conditions: (a) 0.2 equiv CuI, 2 equiv Cs_2CO_3, MeCN/DMF, 120 °C, 65 h, 8%

with Cos-7 cells).



*reaction conditions: 2.0 equiv. K_3PO_4, 0.02 equiv. SPhos, 0.01 equiv. $Pd(OAc)_2$, toluene, 100 °C

Entry	No.	R	IC_{50} [µM]	I_{200} [%]
1	**16a**		100	77
2	**16b**		80	77
3	**16c**		200	52
4	**16d**		**40**	**87**
5	**16e**		130	68
6	**16f**		95	63
7	**16g**		> 200	40
8	**16h**		75	67
9	**16i**		90	55
10	**16j**		140	63
11	**16k***		> 200	34
12	**17a**		> 200	33
13	**17b**		75	68
14	**17c**		150	67
15	**17d**		> 200	26
16	**17d***		> 200	32
17	**17e**		100	68
18	**17e***		200	54
19	**18**		**60**	**80**
20	**19**		120	66
21	**20**		**75**	**84**
22	**21**		200	44

2.3. Variations of bottom ring substituents

investigate the SAR of this moiety by varying the bottom ring fragment. Again Pd-catalyzed cross-coupling provided efficient synthetic access to many of the test compounds (Scheme 4). Some others had to be synthesized by subsequent modification via Buchwald-Hartwig-amination, reductive amination, or alkylation of amines (see SI).

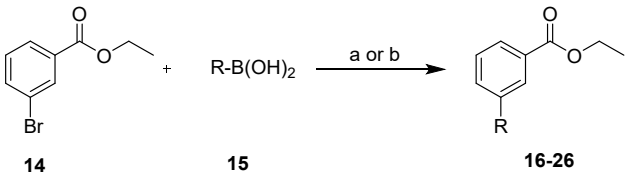
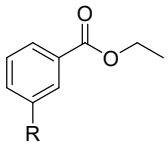
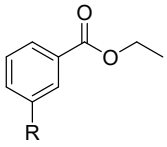


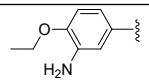
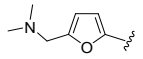
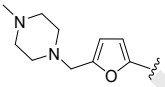
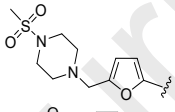
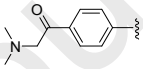
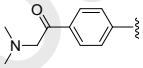
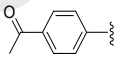
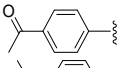
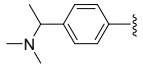
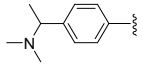
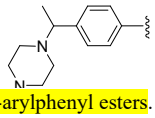
Table 2. Screening results of 3-arylphenyl esters (obtained in assays with Cos-7 cells).



*values measured with *E.coli* lysate

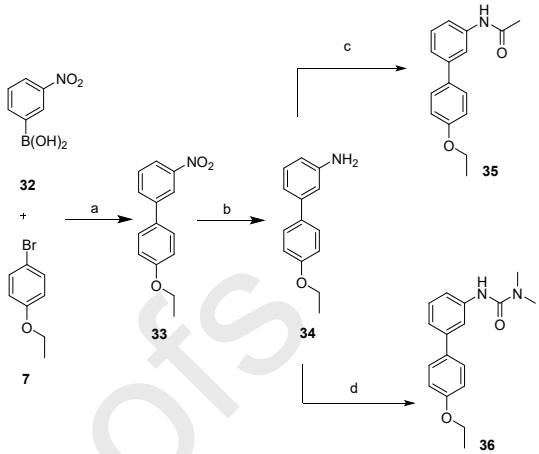
Table 2. Screening results of 3-arylphenyl esters (obtained in assays with Cos-7 cells), continued.



Entry	No.	R	IC_{50} [µM]	I_{200} [%]
23	**22**		140	59
24	**23a**		190	53
25	**23b**		120	67
26	**23c**		100	66
27	**24**		200	20
28	**24***		200	4
29	**25**		200	36
30	**25***		200	17
31	**26a**		200	50
32	**26a***		200	9
33	**26b**		90	69

Scheme 4. Synthesis of 3-arylphenyl esters. Reagents and conditions: (a) 0.05 equiv $PdCl_2$(dppf)*DCM, 2.1 equiv CsF, DME, 80 °C, (b) 2.0 equiv. K_3PO_4, 0.02 equiv. SPhos, 0.01 equiv. $Pd(OAc)_2$, toluene, 100 °C.

In order to increase reproducibility, to improve the yield of recombinant protein and to reduce background activity, we switched the ATGL-overexpression system from eukaryotic



Scheme 7. Synthesis of 4'-ethoxybiphenyl acetamido and urea derivatives. Reagents and conditions: (a) 2 equiv K_3PO_4, 0.01 equiv $Pd(OAc)_2$, 0.02 equiv S-Phos, toluene, 100 °C, overnight, 68 %; (b) 10% Pd/C, 1 bar H_2, EtOAc, RT, overnight, 85 %; (c) 1.2 equiv. Ac_2O, DCM, RT, 2 h 74 %; (d) 1.2 equiv. Me_2NCOCl, 1 equiv. Et_3N, EtOAC, 0 °C to reflux, overnight, 27 %.

Cos-7 cells to bacteria (*E.coli*). In the following screenings (Tables 2-4) SAR studies marked with ***** have been performed using *E.coli* lysates containing recombinant mATGL. The screening results (Table 2) showed a preference for electron-rich biarylic systems with electron donating substituents, such as dimethylamine **16d**, and indoles (compounds **18** and **19**). By introducing substituents bridged with a CH_2-group to the biaryl (Table 2, **17a-e** and **23a-c**) or keto- (Table 2, **24-25**) and methine-moieties (Table 2, **26a-b**) the biological activity was decreased suggesting a negative influence of either increased flexibility or steric bulk. The best results were achieved for **16d** and **18** with IC_{50} values from 40-60 µM. Compound **16d** was further characterized for its effect on ATGL and HSL activity *in vitro*, on the release of FA from fat explants from WT and ATGL-ko mice, and cultured 3T3-L1 adipocytes (see SI, Figure S3, S4 and S5) in which **16d** showed

Scheme 6. Synthesis of 4'-ethoxybiphenyl esters and amides. Reagents and conditions: (a) 14 equiv ROH, 3 equiv H_2SO_4, reflux, 3-5 h; (b) 15 equiv $SOCl_2$, 2 drops DMF, reflux, 23 h, quant.; (c) 1 equiv ROH, 2 equiv pyridine, DCM, reflux; (d) 1.5 equiv amine, 1.2 eq. EDC, THF, 0 °C to RT, 24 h.

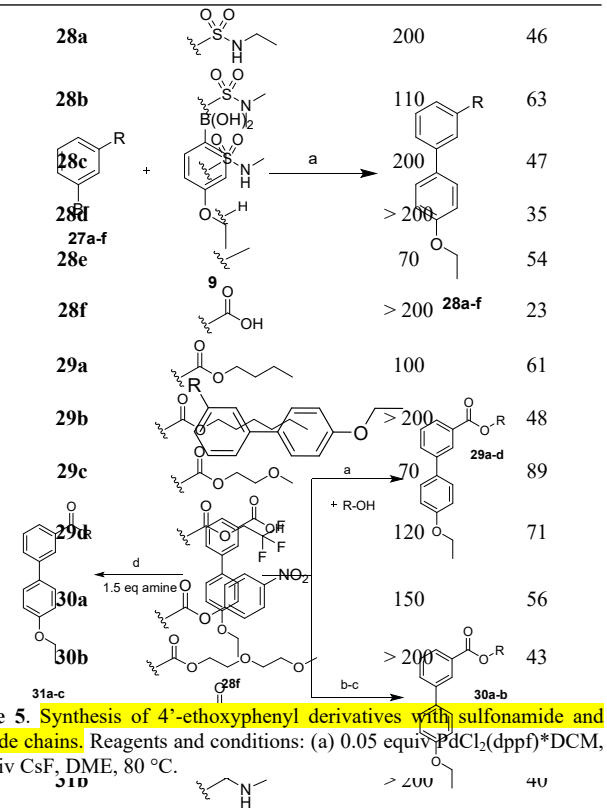
improved selectivity compared to **8e.**

In orienting experiments, the inhibitor **16d** was injected intraperitoneally into mice (with a concentration of 200 µM) and plasma FA concentration was determined after 2, 4 and 8 h to measure FA release from adipose tissue. We found, that FA release was decreased by 40% 8 h after inhibitor injection (see SI; Figure S6). The inhibition of lipolysis by the use of **16d** could be increased to 40% *in vivo*, which was an encouraging result considering the maximum inhibition of lipolysis by ~60% in ATGL-ko-mice.[19]

2.4. Variations of top ring side chain

In a next step to further improve the activity and selectivity of the inhibitors, the 4'-ethoxybiphenyl moiety was decorated with sulfonamides or alkyl groups (Scheme 5), different ester and amide groups (Scheme 6), and acetamido and urea derivatives (Scheme 7).

Entry				
1	**28a**		200	46
2	**28b**		110	63
3	**28c**		200	47
4	**28d**		> 200	35
5	**28e**		70	54
6	**28f**		> 200	23
7	**29a**		100	61
8	**29b**		> 200	48
9	**29c**		70	89
10	**29d**		120	71
11	**30a**		150	56
12	**30b**		> 200	43
14	**31b**		> 200	40
15	**31c**		90	62
16	**33**		> 200	35
17	**34**		100	65
18	**35**		200	52
19	**36**		70	68
20	**36***		9	83



Scheme 5. Synthesis of 4'-ethoxyphenyl derivatives with sulfonamide and alkyl side chains. Reagents and conditions: (a) 0.05 equiv $PdCl_2(dppf)$*DCM, 2.1 equiv CsF, DME, 80 °C.

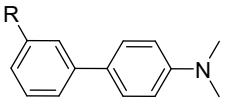
While the amides (Table 3, **31a-c**) and sulfonamides (Table 3, **28a-c**) resulted in inferior activity, various ester derivatives (e.g. **29c**) performed quite well. To our delight, the urea compound **36** (Table 3) showed quite promising results.

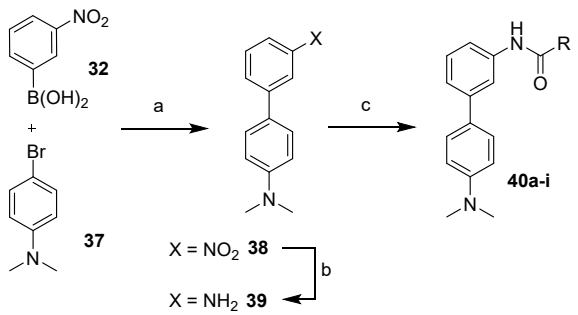
Table 3. Screening results 3-(4'-ethoxyphenyl)-substituted phenyl derivatives

2.5. Combination of best results

Hoping for an additive effect we decided to combine the best features of the optimization of the top ring, bottom ring substituent and the top ring side chain proposing 3-(4`-dimethylamino)biphenyl-3-yl)-1,1-dimethylurea (Table 4, **40a**) as a target structure (Scheme 8). The synthesis of this final compound starts with the known Suzuki coupling reaction between 3-nitrophenylboronic acid (**32**) and 4-bromo-*N,N*-dimethylaniline (**37**) using $PdCl_2(dppf)$*DCM catalyst and CsF in DME. After reaction overnight biphenyl **38** could be isolated in 90% yield after purification via column chromatography. The nitro group was reduced by hydrogenation with 10% Pd/C and hydrogen stream[20] overnight furnishing 95% 4`-(1,1-dimethylamino)biphenyl-3-amine (**39**). Amine **39** was directly converted to the urea without further purification. It was reacted with 1.5 eq dimethylcarbamoyl chloride and 1.0 equiv. triethylamine in DCM[21] at 50 °C for 5 d. After reaching full conversion the final inhibitor compound could be isolated and purified via column chromatography yielding urea **40a** in 54% yield. In addition to the previously tested dimethylurea, also other related side chains were incorporated (Table 4, **40b-i, 42, 43**). However, **40a** proved to be the most potent tested compound and was named Atglistatin®.

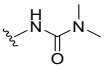
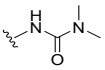
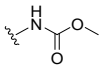
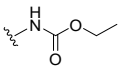
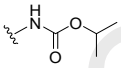
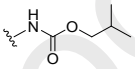
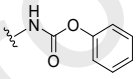
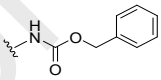
Table 4. Screening results of 3-(4'-dimethylaminophenyl)-substituted phenyl derivatives





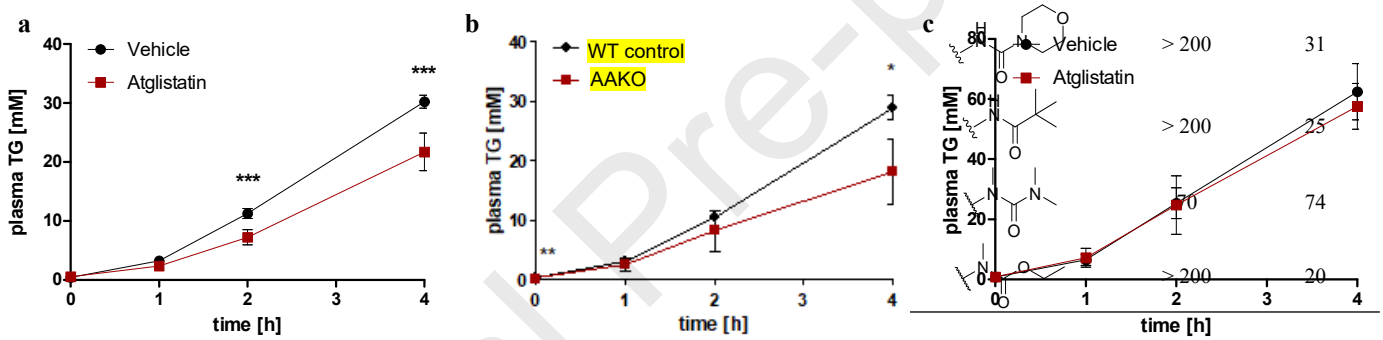
Scheme 8. Synthesis of Atglistatin and related derivatives. Reagents and conditions: (a) 0.05 equiv. $PdCl_2(dppf)$*DCM, 2.1 equiv. CsF, DME, 80 °C, overnight, 98%; (b) 10% Pd/C, 1 atm H_2, EtOH, EtOAc, RT, overnight, 95%; (c) 1.5 equiv. RCOCl, Et_3N, DCM or THF.

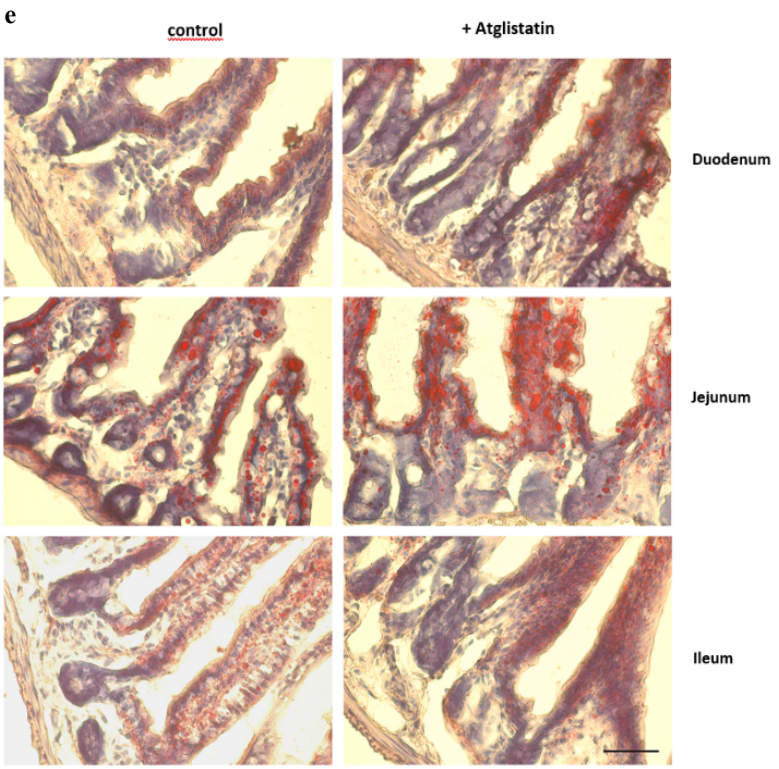
*values measured with *E.coli* lysate

Entry	No.	R	IC_{50} [µM]	I_{200} [%]
1	**38**		> 200	21
2	**39**		100	70
3	**40a**		**< 5**	**98**
4	**40a***		**0.7**	**99**
5	**40b**		5	95
6	**40c**		3	93
7	**40d**		82	69
8	**40e**		22	90
9	**40f**		73	75
10	**40g**		> 200	50

2.6. The Biological Data

The results of the *in vitro* screening of Atglistatin were comparable in both Cos-7 and *E.coli* lysates (Table 4). With an IC_{50} value below 5 µM we could achieve up to 98% inhibition of the enzyme by applying an inhibitor concentration of 100 µM instead of 200 µM as for all compounds before. To further explore its *in vivo* activity, we administered Atglistatin at a final concentration of 200 µM via oral gavage to *ad libitum* fed or 12 h fasted mice and measured circulating TG, FA and glycerol 8 h later (Fig. 1d). In *ad libitum* fed mice, FA and glycerol concentrations were reduced by 27% and 23% (not significant) after Atglistatin gavage, respectively. In fasted mice Atglistatin gavage reduced FA and glycerol concentrations by 27%, and 26%, respectively - again validating Atglistatin as a tool compound to reduce plasma lipids. Interestingly, plasma TG content was 2-fold increased in the fed and 37% decreased in the fasted animals in response to Atglistatin gavage. To investigate whether reduced TG plasma content is due to reduced synthesis and/or secretion of lipoproteins from the liver or intestine we treated mice with Atglistatin and poloxamer to inhibit lipoprotein lipase (LPL) activity, and determined plasma TG concentrations. Mice injected with Atglistatin showed significantly reduced TG concentrations in the fasted state (Fig 1a)





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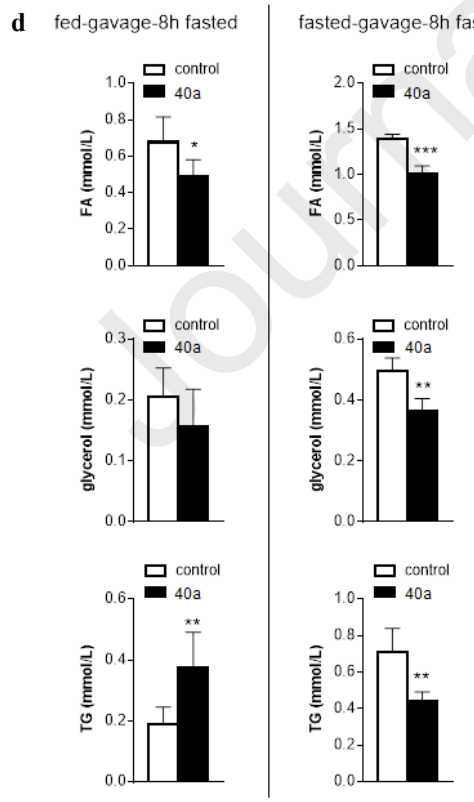
Same results were obtained in mice specifically lacking ATGL in adipose tissues (AAKO) (Fig. 1b), indicating that reduced VLDL synthesis is due to a reduced flux of FA from adipose tissue to the liver. No difference in plasma TG concentration was observed after an oral lipid load (200 µL olive oil) indicating that chylomicron synthesis is not affected by Atglistatin (Fig 1c). However, but in line with a recent report on intestine specific ATGL/CGI-58 deficient animals, gavage of Atglistatin in olive oil led to an accumulation of intestinal lipids, primarily in the duodenum and jejunum indicating that ATGL is rather responsible for the degradation of lipid stores that are not directed to lipoprotein synthesis (Fig 1e).[22]

The data from this report and our previous studies[9] indicate that Atglistatin exerts its main metabolic activity by the inhibition of ATGL specifically in adipose tissue. With regard to non-adipocyte cells it has been shown that ATGL inhibition leads to the accumulation of arachidonic acid thereby preventing an inflammatory response in immune cells.[23] Moreover, pharmacological inhibition of ATGL decreased the regeneration of corneal nerves after injury, presumably by reducing the release of DHA.[24] Interestingly, while it is reported that Atglistatin does not inhibit human ATGL,[9] a recent study showed that the compound reduces TG hydrolase activity as well as TG rich lipoprotein secretion from human hepatoma cells.[25] Further studies are needed, to evaluate the potential off target effects of Atglistatin in non-adipocyte cells. In contrast to ATGL deficiency, Atglistatin treatment protected from cardiac and hepatic steatosis on a high fat diet (HFD).[9] In this regard recent studies demonstrated that Atglistatin rescues mice from heart failure by the inhibition of ATGL in adipocytes.[26] Moreover, inhibition of ATGL by Atglistatin reduced immune cell abundance in adipose tissue and completely blocked the IL-6 mediated induction of lipolysis. Hence, the unfavorable immunometabolic phenotypes associated with the complete absence of ATGL activity are not observed by Atglistatin treatment. This is most likely due to a transient and not a permanent inhibition of ATGL, and the preference of the drug to



40a

taken into consideration for future drug development.[27]

3. Conclusion

In this manuscript we have described how a classic medicinal chemistry approach led us to the development of the first inhibitor of mATGL, Atglistatin (**40a**), which – despite efforts to modify its structure[28,29] – remains the reference tool compound for studying ATGL function in adipocytes, hepatocytes, corneocytes, cardiomyocytes, cancer cells, *in vitro* and *in vivo*. The data gained with Atglistatin in mice demonstrate that the pharmacological intervention of ATGL function offers an opportunity to develop drugs against several diseases, such as diabetes type 2, non-alcoholic fatty liver disease, heart failure and infectious disease. As Atglistatin does not inhibit human ATGL,[30] it will be necessary to develop a new class of compounds which target the human enzyme. Efforts in this direction are currently actively pursued in our labs and will be reported in due course.

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Declaration of Competing Interest

The authors declare no conflict of interest.

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Supplementary Material

Supplementary data to this article can be found online at xy

References

1. Zimmermann R, Strauss JG, Haemmerle G, et al. Fat mobilization in adipose tissue is promoted by adipose triglyceride lipase. *Science*. 2004;306(5700):1383-1386. doi:10.1126/science.1100747.
2. Schweiger M, Schreiber R, Haemmerle G, et al. Adipose triglyceride lipase and hormone-sensitive lipase are the major enzymes in adipose tissue triacylglycerol catabolism. *J Biol Chem*. 2006;281(52):40236-40241. doi:10.1074/jbc.M608048200.
3. Unger RH, Clark GO, Scherer PE, Orci L. Lipid homeostasis, lipotoxicity and the metabolic syndrome. *Biochim Biophys Acta*. 2010;1801(3):209-214. doi:10.1016/j.bbalip.2009.10.006.
4. Ebbert JO, Jensen MD. Fat depots, free fatty acids, and dyslipidemia. *Nutrients*. 2013;5(2):498-508. doi:10.3390/nu5020498.
5. Samuel VT, Shulman GI. Mechanisms for insulin resistance: common threads and missing links. *Cell*. 2012;148(5):852-871. doi:10.1016/j.cell.2012.02.017.
6. Schoiswohl G, Stefanovic-Racic M, Menke MN, et al. Impact of Reduced ATGL-Mediated Adipocyte Lipolysis on Obesity-Associated Insulin Resistance and Inflammation in Male Mice. *Endocrinology*. 2015;156(10):3610-3624. doi:10.1210/en.2015-1322.
7. Schreiber R, Hofer P, Taschler U, et al. Hypophagia and metabolic adaptations in mice with defective ATGL-mediated lipolysis cause

Figure 1. (a) Plasma TG of fasted mice treated with Atglistatin. (b) Plasma TG of fasted WT control and adipose tissue specific ATGL-k.o. (AAKO) mice. (c) Plasma TG of fed mice treated with Atglistatin. (d) Plasma TG, FA and glycerol after oral gavage of Atglistatin in mice. n = 4-5. Data represent mean ± s.d. Statistical significance was determined by unpaired Student's *t*-test (*P ≤ 0.05, **P ≤ 0.01; ***P ≤ 0.001). (e) Neutral lipid accumulation in small intestine of mice. Mice received Atglistatin by oral gavage and were sacrificed after 4 h. Cryo-sections of duodenum, jejunum, and ileum were stained for neutral lipids with OilRedO. Nuclei were counterstained with hematoxylin. Bar = 50µm.

2015;112(45):13850-13855. doi:10.1073/pnas.1516004112.
8. Mayer N, Schweiger M, Romauch M, et al. Development of small-molecule inhibitors targeting adipose triglyceride lipase. *Nat Chem Biol*. 2013;9(12):785-787. doi:10.1038/nchembio.1359.
9. Schweiger M, Romauch M, Schreiber R, et al. Pharmacological inhibition of adipose triglyceride lipase corrects high-fat diet-induced insulin resistance and hepatosteatosis in mice. *Nat Commun*. 2017;8:14859. doi:10.1038/ncomms14859.
10. Mayer N, Schweiger M, Melcher M-C, et al. Structure-activity studies in the development of a hydrazone based inhibitor of adipose-triglyceride lipase (ATGL). *Bioorg Med Chem*. 2015;23(12):2904-2916. doi:10.1016/j.bmc.2015.02.051.
11. Zhu L, Guo P, Li G, Lan J, Xie R, You J. Simple copper salt-catalyzed N-arylation of nitrogen-containing heterocycles with aryl and heteroaryl halides. *J Org Chem*. 2007;72(22):8535-8538. doi:10.1021/jo0712289.
12. Peters M, Trobe M, Breinbauer R. A modular synthesis of teraryl-based α-helix mimetics, part 2: Synthesis of 5-pyridine boronic acid pinacol ester building blocks with amino acid side chains in 3-position. *Chemistry*. 2013;19(7):2450-2456. doi:10.1002/chem.201203006.
13. Peters M, Trobe M, Tan H, Kleineweischede R, Breinbauer R. A modular synthesis of teraryl-based α-helix mimetics, part 1: Synthesis of core fragments with two electronically differentiated leaving groups. *Chemistry*. 2013;19(7):2442-2449. doi:10.1002/chem.201203005.
14. Mach UR, Hackling AE, Perachon S, et al. Development of novel 1,2,3,4-tetrahydroisoquinoline derivatives and closely related compounds as potent and selective dopamine D3 receptor ligands. *Chembiochem*. 2004;5(4):508-518. doi:10.1002/cbic.200300784.
15. Chan PY, Ong SY, Zhu P, Zhao C, Phillips DL. Transient Resonance Raman and Density Functional Theory Investigation of 4-Methoxyphenylnitrenium and 4-Ethoxyphenylnitrenium Ions †. *J Phys Chem A*. 2003;107(40):8067-8074. doi:10.1021/jp0224261.
16. Barral K, Moorhouse AD, Moses JE. Efficient conversion of aromatic amines into azides: a one-pot synthesis of triazole linkages. *Org Lett*. 2007;9(9):1809-1811. doi:10.1021/ol070527h.
17. Schweiger M, Eichmann TO, Taschler U, Zimmermann R, Zechner R, Lass A. Measurement of lipolysis. *Meth Enzymol*. 2014;538:171-193. doi:10.1016/B978-0-12-800280-3.00010-4.
18. Cinti S. Adipose Organ Development and Remodeling. *Compr Physiol*. 2018;8(4):1357-1431. doi:10.1002/cphy.c170042.
19. Haemmerle G, Lass A, Zimmermann R, et al. Defective lipolysis and altered energy metabolism in mice lacking adipose triglyceride lipase. *Science*. 2006;312(5774):734-737. doi:10.1126/science.1123965.
20. Halbert SM, Michaud E, Thompson SK, Veber DF, inventors. U.S. Pat. Appl. Publ. U.S. Pat. Appl. Publ. 200220049316A1.
21. Kuhn B, Mohr P, Stahl M. Intramolecular Hydrogen Bonding in Medicinal Chemistry. *J Med Chem*. 2010;53(6):2601-2611. doi:10.1021/jm100087s.
22. Korbelius M, Vujic N, Sachdev V, et al. ATGL/CGI-58-Dependent Hydrolysis of a Lipid Storage Pool in Murine Enterocytes. *Cell Rep*. 2019;28(7):1923-1934.e4. doi:10.1016/j.celrep.2019.07.030.
23. Schlager S, Goeritzer M, Jandl K, et al. Adipose triglyceride lipase acts on neutrophil lipid droplets to regulate substrate availability for lipid mediator synthesis. *J Leukoc Biol*. 2015;98(5):837-850. doi:10.1189/jlb.3A0515-206R.
24. Pham TL, He J, Kakazu AH, Jun B, Bazan NG, Bazan HEP. Defining a mechanistic link between pigment epithelium-derived factor, docosahexaenoic acid, and corneal nerve regeneration. *J Biol Chem*. 2017;292(45):18486-18499. doi:10.1074/jbc.M117.801472.
25. Taxiarchis A, Mahdessian H, Silveira A, Fisher RM, Van't Hooft FM. PNPLA2 influences secretion of triglyceride-rich lipoproteins by human hepatoma cells. *J Lipid Res*. 2019;60(6):1069-1077. doi:10.1194/jlr.M090928.
26. Parajuli N, Takahara S, Matsumura N, et al. Atglistatin ameliorates functional decline in heart failure via adipocyte-specific inhibition of adipose triglyceride lipase. *Am J Physiol Heart Circ Physiol*. 2018;315(4):H879-H884. doi:10.1152/ajpheart.00308.2018.
adipose tissue inflammation to hepatic insulin resistance and type 2 diabetes. *Cell*. 2015;160(4):745-758. doi:10.1016/j.cell.2015.01.012.
28. Roy P-P, D'Souza K, Cuperlovic-Culf M, Kienesberger PC, Touaibia M. New Atglistatin closely related analogues: Synthesis and structure-activity relationship towards adipose triglyceride lipase inhibition. *Eur J Med Chem*. 2016;118:290-298. doi:10.1016/j.ejmech.2016.04.021.
29. Jin J, Huang S, Wang L, Leng Y, Lu W. Design and synthesis of Atglistatin derivatives as adipose triglyceride lipase inhibitors. *Chem Biol Drug Des*. 2017;90(6):1122-1133. doi:10.1111/cbdd.13029.
30. Iglesias J, Lamontagne J, Erb H, et al. Simplified assays of lipolysis enzymes for drug discovery and specificity assessment of known inhibitors. *J Lipid Res*. 2016;57(1):131-141. doi:10.1194/jlr.D058438.

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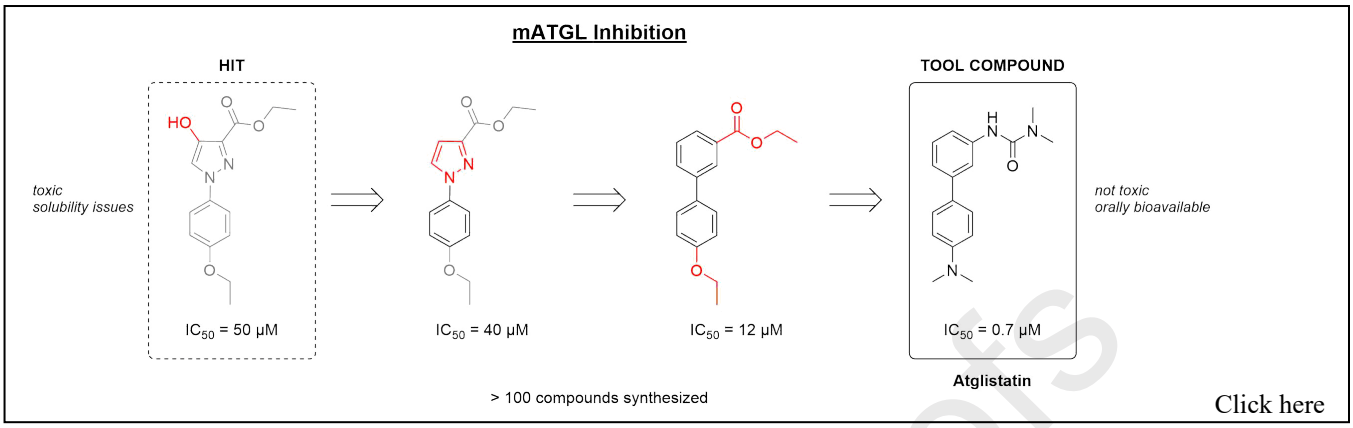

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