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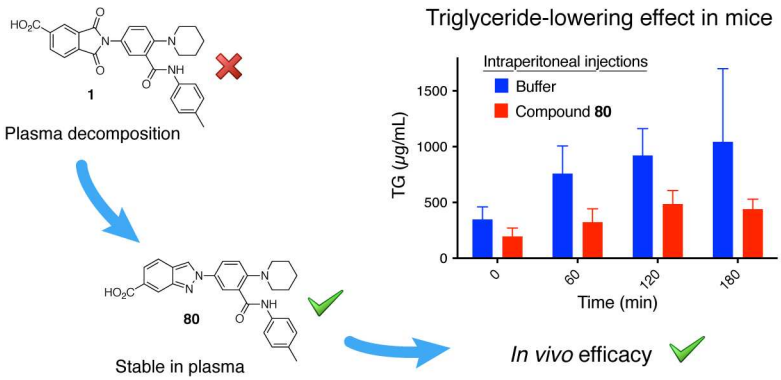

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Structure-activity relationships for lipoprotein lipase agonists that lower plasma triglycerides *in vivo*

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Abstract

The risk of cardiovascular events increases in individuals with elevated plasma triglyceride (TG) levels, therefore advocating the need for efficient TG-lowering drugs. In the blood circulation, TG levels are regulated by lipoprotein lipase (LPL), an unstable enzyme that is only active as a non-covalently associated homodimer. We recently reported on a N-phenylphthalimide derivative (**1**) that stabilizes LPL *in vitro*, and moderately lowers triglycerides *in vivo* (*Biochem. Biophys. Res. Commun.* **2014,** *450*, 1063). Herein, we establish structure-activity relationships of 51 N-phenylphthalimide analogues of the

screening hit **1**. *In vitro* evaluation highlighted that modifications on the phthalimide moiety were not tolerated and that lipophilic substituents on the central phenyl ring were functionally essential. The substitution pattern on the central phenyl ring also proved important to stabilize LPL. However, *in vitro* testing demonstrated rapid degradation of the phthalimide fragment in plasma which was addressed by replacing the phthalimide scaffold with other heterocyclic fragments. The *in vitro* potency was retained or improved and substance **80** proved stable in plasma and efficiently lowered plasma TGs *in vivo*.

Keywords: Lipoprotein Lipase, LPL, triglyceride, structure-activity relationship, agonist

1. Introduction

Coronary Artery Disease (CAD) is a medical term encompassing a set of conditions responsible for the narrowing and hardening of blood vessels. CAD is classified as the world's leading cause of death and, therefore, the prevention, control and treatment of its associated risk factors remain essential.[1, 2] For instance, the probability of developing CAD increase significantly with age,[3] family history,[4] obesity,[5] diabetes,[6] high blood pressure and/or high blood cholesterol levels.[7] Likewise and even though it has long been debated, hypertriglyceridemia is an independent risk factor of CAD.[8, 9] Abnormally elevated plasma triacylglycerol (TG) levels is a frequently encountered lipid disorder closely associated with other CAD risk factors.[10, 11]

Plasma TGs originate from exogenous and endogenous sources. Dietary TGs are packed into large lipoprotein particles (chylomicrons) in the intestine whereas endogenous TGs are assembled into very low-density lipoproteins (VLDLs) in the liver. After entering the blood circulation these TG enriched lipoproteins are processed by lipoprotein lipase (LPL), a TG specific hydrolase enzyme.[12-14] The released free fatty acids and monoglycerides are stored or directly utilized for energy production in adipose or muscle tissues, respectively.

The remnant lipoproteins are absorbed by the liver or, in the case of VLDL remnants, they are further processed into low-density lipoproteins (LDL).

LPL plays a central role in the regulation of plasma TG levels.[12, 15] The enzyme, which is only active as an unstable non-covalently associated homodimer, is produced and secreted from parenchymal cells. LPL is then transported and anchored to its site of action at the luminal surface of endothelial cells in capillaries.[14] Several endogenous factors such as angiopoietin-like protein (ANGPTL) 3 and 4 regulate LPL activity.[15, 16] ANGPTL3 is reported to inhibit LPL[17] whereas ANGPTL4 directly promotes the dissociation of the active homodimer into inactive monomeric species[18].

Causes for abnormally elevated plasma TG levels are gathered into two categories: familial and acquired hypertriglyceridemia.[10] Familial hypertriglyceridemia is believed to be a consequence of an accumulation of inherited deleterious alleles in genes regulating lipoprotein catabolism, including LPL.[19] The condition is said acquired when it is induced by environmental factors such as obesity, type 2 diabetes mellitus or high alcohol consumption. Lifestyle changes, e.g. low fat diet, physical activity and alcohol restriction usually lower plasma TG levels in hypertriglyceridemic patients; however, for patients for whom lifestyle changes have been ineffective, medication with fibrates, niacin and/or n-3 fatty acids may be necessary.[20]

Herein, we report on the design, synthesis and evaluation of small molecules that stabilize LPL in the presence of ANGPTL4 and, therefore, prevent the dissociation and inactivation of homodimeric LPL. As a result, a significant increase in LPL-mediated hydrolysis was seen *in vitro* with a number of compounds. Structure-activity relationships (SAR) based on the screening hit were established and the optimized compounds were found to be efficacious *in vivo*.

2. Results and discussion

High throughput screening of 17340 compounds provided the foundation for this medicinal chemistry program.[21] The screening hit **1** (Figure 1a), a substituted N-phenylphthalimide, was selected as starting point on the basis of its positive effects on LPL stability. Compound **1** preserved LPL activity at 37 °C or when incubated at room temperature with the enzyme in the presence of ANGPTL4. Chemical diversity around the N-phenylphthalimide scaffold (Figure 1b) can be achieved by starting from readily available building blocks. All compounds in the present study were investigated for effects on LPL activity and stability, in the presence of ANGPTL4, according to previously described protocols.[21]

The synthesis of **1** was straightforward and in good yields (Scheme 1). Compound **2** was converted to the corresponding acid chloride upon treatment with oxalyl chloride. Thereafter, coupling with *p*-toluidine provided **3** in 90% yield. Direct amination of **3** with piperidine under basic conditions yielded **4** (94%).[22] Subsequent reduction of the nitro group using tin dichloride in refluxing ethanol[23] or sodium borohydride and palladium on charcoal in methanol[24] afforded **5** in nearly quantitative yields. Finally, the condensation reaction between aniline **5** and a substituted phthalic anhydride (**6**) in refluxing glacial acetic acid gave **1** in 89% yields.[25]

2.1. Relevance of the carboxylic acid

Compound **7**, which only differs from the selected hit (**1**) by the absence of the carboxylic acid functional group, was also evaluated during the primary screen (Table 1).[21] Interestingly, it could not preserve LPL activity which indicates that a substituent on the phthalimide moiety (R^1 in Figure 1b) is essential. To probe the role of the carboxylic acid, a set of compounds in which the carboxylic acid present in **1** was moved or replaced with other functional groups was first investigated (Table 1).

The synthesis of the N-phenylphthalimide derivatives followed the synthetic route to **1** (Scheme 1), using the appropriate phthalic anhydride derivatives in the last step (83-97%

yields).[25] Some of the target compounds necessitated an additional transformation. The methyl ester **12** was conveniently obtained in high yield (97%) by reacting **1** with trimethylsilyldiazomethane.[26] The carboxamide derivative **11** was synthesized by treatment of **1** with oxalyl chloride to form the corresponding acid chloride and the activated carboxylic acid was then reacted with hexamethyldisilazane and provided **11** in 11% yield after recrystallization. Finally, microwave-assisted cyanation of **8** using copper cyanide as the nitrile source gave **10** in 24% yield.

The substances were subsequently evaluated for stabilization of LPL activity.[21] In brief, ANGPTL4 and LPL were added to a phosphate buffer solution containing the compounds previously dissolved in DMSO and a detergent. The mixtures were incubated for 10 min at 25 °C and the remaining LPL activity was determined by addition of a substrate mix containing Intralipid®. LPL-mediated hydrolysis was terminated after 45 min and the released free fatty acids were quantified using a titration kit (NEFA HR2 from Wako Chemicals). All enzymatic activity measurements were normalized to DMSO controls where 100% represent LPL incubated without ANGPTL4.

The effect of the compounds on LPL activity is summarized in Table 1. Solely **1** contributed to a significantly greater enzymatic activity. Indeed, a replacement of the carboxylic acid by another small functional group at the position 5 of the phthalimide (compounds **7-12**) resulted in the limited or absence of LPL-mediated hydrolysis. The low activity of **11** at 25 µM underlined the importance of having an acidic functional group (carboxylic acid in **1** vs. carboxamide in **11**). It also demonstrated that electron-withdrawing substituents have no direct effect on the compound potency. As expected from the initial screen, **7** was confirmed inactive. Similarly, moving the carboxylic acid to position 4 of the phthalimide (**13**) completely suppressed the effect observed for **1**. These results are conspicuous and evidence the need of having a carboxylic acid group at position 5 of the

phthalimide. Consequently, it was decided that newly designed compounds all should include a carboxylic acid functional group at this position.

2.2. Substituent scrambling

Efforts to optimize the screening hit then focused on the substituents on the central phenyl ring (R^2 and R^3 in Figure 1b). A series of seven analogues that present a scrambling of the two substituents around the central phenyl ring were synthesized (Table 2). In addition, three other substances where the 1-piperidinyl group in compound **23** was replaced by a chloride (**14**), a phenyl- or tolylamino derivative (**15** and **16**, respectively) were also analyzed.

Compounds **17-22** were prepared using the same synthetic route as in Scheme 1 with use of the appropriate starting materials. A different synthetic strategy was however adopted for the preparation of compounds **15**, **16** and **23**, using 3-bromo-5-aminobenozoic acid (**24**) as starting building block. During the amide coupling and the direct amination reactions (Scheme 2, steps a and b/c respectively), the unprotected amino group present in **24** would compete with the reacting amines and therefore a non-negligible amount of homo-coupling products would form. To circumvent this problem **24** was conveniently converted into the corresponding formamidine product upon reaction with the Vilsmeier reagent.[27] A subsequent amide coupling with *p*-toluidine formed **25** (60%), in a one-pot procedure. Compound **25** was subsequently reacted with aromatic or aliphatic amines under Buchwald coupling conditions[28, 29] and after formamidine cleavage in refluxing ethylenediamine,[27] compounds **28**, **29** and **30** were isolated in 53-73% yields. The condensation reaction between the resulting amines and **6** yielded the final products (50-62%).

Modification of the substitution pattern of **1** generally proved detrimental, or at best non-beneficial, for LPL stabilization (Tables 1 and 2). Thus, displacing the *p*-tolylcarbamoyl substituent to position 2 of the central phenyl ring (**19**) did not improve the hit potency.

Moving the 1-piperidinyl group to position 5 (**23**) or 6 (**20**) of the ring considerably or slightly reduced LPL activity, respectively. Interestingly, reversing the substituents (**21**) resulted in a compound with low activity. Sterically congested compounds **18** and **22** were also demonstrated poorly active. Finally, **17** showed a reduced potency compared to the screening hit. As a result, the substitution pattern of **1** was concluded as the pattern of choice for LPL stabilization.

The set of modified derivatives of **23** (**14**, **15** and **16**) also provided valuable information. While **14** proved inactive, the replacement of 1-piperidinyl by a phenylamino group (**15**) improved the potency of the compound in comparison to **23**. However, elongation of the phenylamino substituent of **15** with a methyl group (**16**) caused a dramatic loss of activity, thus suggesting that larger groups are not tolerated.

2.3. Substituent variation.

A set of 33 compounds with the 1,3,4 pattern on the central phenyl ring (cf. Figure 1) was designed and synthesized (Table 3). N-phenylphthalimide (**31**), despite the absence of a carboxylic acid functional group, was added to this series on the basis of its previously reported hypolipidemic activity in mice.[30] Challenging **31** in our *in vitro* assay would indicate a similar or different mode of action than our analogues. For comparison **32**, the carboxylated version of **31**, was also evaluated. The target compounds were synthesized as described in Scheme 1 using of the appropriate building blocks. For compound **54**, O-alkylation was achieved in 75% yield by treating **3** with the alcohol derivative under basic conditions in DMSO.[31]

Table 3 presents the effect on LPL activity of one or a combination of two modifications at the central phenyl ring in respect to **1** (Table 1). Importantly, the previously reported **31** and **32** proved completely inactive under our experimental conditions, thus suggesting that **1** and the other active analogues might preserve LPL activity via a different mechanism.

The introduction of a more polar substituent (**51**-**54** and **63**) resulted in a complete loss of LPL activity. Similarly, **55**, **56** and **64** that have an electron donating or electron withdrawing group on the central fragment were not active. It was not surprising to find that the combination of two unfavorable modifications (**45** and **46**) resulted in inactive analogues. Also, the replacement of the methyl group on the carbamoyl substituent with a methyl-ether (**34**) decreased the compound potency.

Continuing the analysis on the carbamoyl substituent, the addition of a chloride (**48**) caused a significant increase in potency. However, the corresponding fluoride analogue **50** did not exhibit any enhanced potency in comparison to the screening hit. Likewise, trifluoromethyl group in **35** and **49** did not contribute to LPL stabilization. Benzylic (**44**) and aliphatic (**47**) substituents were found inactive and less potent than **1**, respectively. Removal of the methyl group (**33**) significantly reduced LPL-mediated hydrolysis whereas its shift (**36**) decreased LPL activity. Interestingly, increasing the bulkiness of the substituent (**37**, **39**-**40** and **43**) proved favorable at 6.25 μM; however, the efficacy of the compounds remained relatively low (≤ 71% of control) at higher concentrations regardless to the size of the substituent.

Substitution of the 1-piperidinyl moiety with a phenylamino group (**57**) was confirmed as a beneficial transformation for LPL activity. Compounds **38**, **41**-**42** and **58** showed similar potencies as **57** and, therefore cumulative effects resulting from two favorable modifications were not observed. Finally, the aliphatic derivatives (**59**-**62**) provided valuable information. Short aliphatic chains (**59** and **62**) showed a detrimental effect on LPL activity whereas a longer chain (**60**) enhanced the compound's potency to stabilize LPL. However, further elongation of the tail (**61**) almost turned the compound inactive, thus, probing the importance of the length of the aliphatic chain.

Several compounds e.g. **41**, **42**, **58**, reach a plateau at the two or three highest concentrations tested. This could possibly be due to low solubility or that these compounds act as partial agonists.

2.4. Evaluation of the *in vitro* plasma stability.

Screening hit **1** was selected as model compound for the absorption, distribution, metabolism and excretion (ADME) profiling of the N-phenylphthalimide derivatives. Unfortunately, **1** rapidly degraded during the initial plasma protein binding study (2% remaining after 4 h; see experimental section for details). To verify the poor stability, compound **1** was incubated and sampled over 24 hours in human plasma which showed a short half-life ($t_{1/2}$ = 1.5 h). The degradation mechanism was investigated by LC-MS/MS and the first and second metabolites were identified as the partially (**65** and **66**) and fully (**5**) hydrolyzed N-phenylphthalimide derivatives (Table 4). Thus, **65** was formed predominantly (51% after 24 h) while **66** and **5** were produced in lower quantities (24% and 23% after 24 h, respectively).

In order to establish whether the metabolites could stabilize LPL, they were synthesized and evaluated in our enzymatic assay (Table 4). Regioisomers **65** and **66** were obtained by addition of **5** to anhydride **6** in acetic acid. After completion of the reaction, the two regioisomers were separated using HPLC. Despite a mild effect on LPL activity at 25 µM, compounds **65** and **66** remained less potent than **1**. The second metabolite (**5)** was found completely inactive in our *in vitro* assay. Although these results highlight the beneficial effect of the rigid bicyclic scaffold over the ring-opened structures, investigations on the N-phenylphthalimide compounds were discontinued as a consequence of their poor plasma stability. The previously reported plasma TG lowering-effect of compound **1** in mice should probably be reconsidered as the effect of a cocktail of substances (**1** + metabolites) rather

than the effect of **1** alone.[21] Consequently, the intrinsic potency of compound **1** was probably underestimated *in vivo*.

2.5. Design and synthesis of compounds stable in plasma

A set of compounds (Table 5) where the hydrolysis-prone phthalimide fragment was replaced by other bicyclic scaffolds was designed and synthesized. The selected heterocyclic rings included benzimidazole **71**, benzofuran **77**, indole **78** and indazole **80**-**82** derivatives.

Compound **71** was synthesized in four steps starting from intermediate **5** (Scheme 3). The aniline derivative was converted into aryl bromide **67** (90%) via a Sandmeyer reaction.[32] Halogen exchange using a copper-catalyzed procedure provided aryl iodide **68** in excellent yields.[33] Benzimidazole **70** was prepared in moderate yields by direct arylation of **69** with **68**.[34] Subsequent methyl ester hydrolysis under basic conditions yielded the target compound (**71**, 90%).

Benzofuran and indole derivatives were prepared using the synthetic route displayed in Scheme 4. Cross-coupling reaction between aryl bromide **67** and trimethylsilylacetylene gave **72** in 60% yields after cleavage of the trimethylsilyl group.[35] A cascade reaction involving a Sonogashira coupling between aryl iodide **73** or **74** and acetylene **72** followed by an intramolecular ring-closure yielded benzofuran (**75**, 67%) and indole (**76**, 51%) derivatives, respectively.[36] In this reaction the trifluoroacetyl leaving group in **74** is needed for the ring-closure to occur during the coupling. Subsequent methyl ester hydrolysis yielded the targeted benzofuran **77** (80%) and indole **78** (77%).

2*H*-Indazole derivatives **80**-**82** were synthesized according to a previously reported one-pot three-component procedure (Scheme 5).[37] With our substrates, higher yields were however obtained when the imine intermediate was synthesized separately. The imine was prepared by refluxing **5** and **79**[38] in ethanol.

The newly synthesized compounds were tested for stabilization of LPL (Table 5). Compound **71** was evaluated as the weakest LPL stabilizer of the series with a moderate effect on LPL activity at 25 µM. At low concentrations, the benzofuran scaffold in **77** proved beneficial over the phthalimide fragment in **1** and the indole derivative **78** appeared potent. However, a drop in LPL activity was observed for both compounds at 25 µM. The underlying reason is most likely precipitation of the substances due to poor aqueous solubility. Indazole isomers **80** and **81** were found to have similar potency as **1**. Interestingly, the shift of the carboxylic acid from position 5 (**81**) to position 6 (**80**) did not affect the compound ability to stabilize LPL. In order to verify the importance of the carboxylic acid group in this compound class, **82** was investigated and found inactive in agreement with the previous SAR.

The results depicted in Table 5 clearly demonstrate that heterocyclic fragments can efficiently replace the phthalimide scaffold. In order to validate our optimization strategy, the drug-likeness of compounds **77** and **80** was subjected to ADME profiling *in vitro* (Table 6). Unlike **1**, the substances proved stable in human plasma (> 93% stability). Both compounds strongly bound to plasma proteins with < 0.01% of estimated unbound fraction (see experimental section for details). The high protein binding indicates a limited tissue exposure, which could be advantageous since LPL is operating in plasma. The kinetic solubility of compounds **77** and **80** was tested in phosphate buffered saline solution at neutral and acidic pH (Table 6). Because of the presence of lipophilic substituents on their core structure, **77** and **80** were expected to be poorly soluble in aqueous solution. The experimental measurements confirmed this hypothesis. Ionization of the acid function at pH 7.4 (< 2 µM for both substances) or the apparent protonation of the different basic functions at pH 1.2 (7 µM for **77**; < 2 µM for **80**) did not significantly improve the solubility.

In vitro permeability was measured from transport rates across Caco-2 cell monolayers as previously reported.[39] Compounds **77** (P_{app} value = 1.2×10^{-6} cm/s) and **80** (P_{app} value = 23

$\times 10^{-6}$ cm/s) had a moderate and high intestinal permeability in the absorptive, apical to basolateral direction (a-b), respectively. Comparable permeability coefficients were obtained in the secretory, basolateral to apical (b-a) direction. Calculation of the efflux ratios (*E* = b-a P_{app} / a-b P_{app}) indicated that no apparent active efflux interfered with the transport of either **77** (*E* = 1.5) or **80** (*E* = 1.4). Incubation in human liver microsomes showed very high metabolic stability for **77** ($t_{1/2}$ > 80 h, CL_{int} < 17 µL/min/mg) and **80** ($t_{1/2}$ > 80 h, CL_{int} < 17 µL/min/mg) which thus indicates a very low risk for oxidative metabolism in the liver.[40, 41] Compounds **77** and **80** were also tested in freshly prepared human hepatocytes, where additional drug metabolizing enzymes are present and where **77** ($t_{1/2}$ = 40 min, CL_{int} = 35 µL/min/10^6 cells) showed a higher degree of metabolism. In contrast, **80** ($t_{1/2}$ > 2 h, CL_{int} = 12 µL/min/10^6 cells) proved stable also under the *in vivo*-like conditions in the human hepatocytes. In summary, the lead optimization was successful. Further, the compounds are drug-like with regards to the predicted oral drug space.[42]

Compound **80**, its regiosiomer **81** and the screening hit **1** were then further investigated *in vitro* by dose-response analysis and determination of EC_{50} values (see the Supporting Information for dose-response curves). EC_{50} values were found to be 6.0 µM (**80**), 5.0 µM (**81**) and 6.6 µM (**1**) showing that the compounds have similar potencies and reach full efficacy.

2.6. Previously reported hypolipidemic agents.

Our approach to the discovery of new and potent hypotriglyceridemic agents offers the advantage to assess the *in vitro* evaluation of the target compounds. LPL is unstable and difficult to handle. Therefore, *in vivo* studies are often privileged. We wanted to investigate whether previously known lipid lowering compounds could stabilize LPL in our enzymatic assay. As discussed above, N-phenylphthalimide **31** and the carboxylated analogue **32** (Table 3), which belongs to the same class of compounds as our screening hit, were inefficient in our

assay thus suggesting that **1** and the other active analogues preserve LPL activity via a different mechanism than **31**. Other LPL activators (Figure 2), including Ibrolipim[43, 44], also known as NO-1886, some 5-fluoro-*1H*-indole-2-carboxamides derivatives[45] and C10 that was recently reported as an LPL agonist preserving the enzyme from inhibition by ANGPTL4[46] were tested in our system. None of these substances proved active at 25 µM in our *in vitro* assay, confirming that the LPL-activating mechanisms are different compared to the stabilizing effects of the compounds described in this study.

2.7. *In vivo* investigation.

In order to compare the *in vivo* efficacy with the *in vitro* findings on our compounds, **1**, **71**, **77**, **78**, **80**, **81** and vehicle only were administered through intraperitoneal (i.p.) injections to mice (n = 8/group), once daily for 4 days (10 mg/kg body weight per injection). Before the last injection the mice were fasted overnight. On the next morning they were given a last injection and after 60 min they were challenged with an oral olive oil gavage to monitor postprandial lipid clearance. In the fasting state **80** was the only compound that caused significantly lower TG levels than the vehicle control (Figure 3). However, 60 min after the gavage all animals but those receiving compound **1** had significantly lower plasma TG levels than the vehicle control. The low plasma stability of **1**, described above, is most likely the reason for the modest efficacy of this compound.[21] At 120 min, a similar pattern was seen compared to vehicle control. At 180 min, the TG values for most compounds were significantly lower than for compound **1**. These data demonstrate that compounds **71**, **77**, **78**, **80** and **81** are superior to the previously reported compound **1** for accelerating postprandial lipid clearance. In early studies, a single i.p. injection was found to be less efficient than the repeated injections for 4 days in lowering TG levels (data not shown).

Potentially the TG lowering effect could the result of transcriptional activation.[47] To address this we profiled compound **80** for agonist activity against a set of nuclear receptors

(see Experimental section for details). For all receptors the EC_{50} values exceeded 100 μM. These results supported our theory that the efficacy *in vivo* of the compounds is due to a direct effect on LPL.

The most commonly used drugs for lowering TG are niacin, fibrates and fish oil. We have not benchmarked these drugs against our compounds, mainly because their mode of action is different. All three currently approved drugs affect TG levels by lowering VLDL synthesis to some extent,[48, 49] while our compounds appear to increase the clearance of plasma TG. In support of this we detected increased LPL activity in homogenates of subcutaneous white adipose tissue for the most potent compounds at 3 h after the lipid gavage (preliminary data, not shown).

3. Conclusions

Compounds with greater potencies and stability than the screening hit (**1**) were designed, synthesized and evaluated *in vitro*. The SAR analysis illustrated the necessity of having a carboxylic acid functional group at the position 5 (or 6) of the bicyclic ring. On the central phenyl ring, we confirmed the substitution pattern and evidenced the need of having lipophilic substituents. Larger substituents at the carbamoyl group were probed as beneficial for the potency (**43**). At position 4 of the central fragment, cyclic and more rigid substituents were not obligatory, and thus, an *n*-hexylamino group (**60**) added a favorable effect. However, the length of the aliphatic tail was demonstrated as highly important (**61** and **62**). Unfortunately, additive effect of favorable modifications could not be evidenced. The metabolic stability of the screening hit was also significantly improved by replacing the hydrolysis-prone phthalimide scaffold by other heterocyclic fragments. In addition to the SAR analysis, we challenged previously reported hypotriglyceridemic agents in our test system and, consequently, we demonstrated that our compounds have a different mode of action on LPL activity than those previously reported. Injections of the optimized lead

compounds (**71**, **77**, **78**, **80** and **81**) to mice demonstrated that all compounds lowered plasma TG levels after a lipid gavage compared to mice treated with vehicle or with the previously identified, but unstable, compound **1**.

In conclusion, we have developed novel TG lowering compounds that preserve the activity of LPL via a mechanism based upon stabilization of the dimeric enzyme from inactivation by ANGPTL4. Preliminary experiments in mice revealed that these compounds can blunt a postprandial elevation of plasma TG levels *in vivo*.

4. Experimental section

4.1. General Chemical Procedures

LC-MS was carried out with a Waters LC system equipped with an Xterra C18 column (50 × 19 mm, 5 μm, 125 Å), eluted with a linear gradient of acetonitrile in water, both of which contained formic acid (0.2 %). A flow rate of 1.5 mL/min was used and detection was performed at 254 nm. Mass spectra were obtained on a Water micromass ZQ 2000 using positive and negative electrospray ionization. ^{1}H NMR and ^{13}C NMR spectra were recorded with a Bruker DRX-400 or DRX-360 spectrometer. NMR experiments were conducted at 298 K in DMSO-d_6 (residual solvent peak = 2.50 ppm (δH) and 39.52 (δC)). Compounds **9**, **13**, **14**, **27**, **33**, **34**, **36**, **37**, **44**, **51**, **55**, **56**, **57**, **59**, **64** and **C10** were purchased from ChemBridge Corp. or Sunblochem HB, analyzed with LC-MS, and used without further purification. All target compounds were ≥ 95% pure according to HPLC UV-traces, except the low-active compounds **9**, **13** and **65** that were ≥ 90% pure.

4.2. Synthetic Procedures

*Procedure A: Synthesis of an acyl chloride and coupling to an aniline derivative (Exemplified by **3**)*

A catalytic amount of DMF was added to a stirred solution of 2-chloro-5-nitrobenzoic acid (**2**) (4.00 g, 19.85 mmol) and oxalyl chloride (8.65 mL, 99.19 mmol) in DCM (50 mL) at 0 °C under nitrogen atmosphere. After 20 min, the reaction was allowed to warm to room temperature and to proceed for a further 2 h. The reaction mixture was concentrated, re-dissolved in DCM and concentrated again. Triethylamine (13.83 mL, 99.22 mmol) and *p*-toluidine (2.13 g, 19.85 mmol) were added to the crude product dissolved in DCM (50 mL). The reaction was stirred for 5 h at room temperature under nitrogen atmosphere. After completion, the reaction mixture was diluted with DCM and successively washed with HCl (aq., 1M), $NaHCO_3$ (aq., sat.), water and brine. The organic phase was dried over $MgSO_4$, filtered, and concentrated to dryness. The crude amide was purified by column chromatography (SiO_2, EtOAc/n-heptane, 7:3 -> 9:1) to give **3** in 90% yield.

*Procedure B: Synthesis of an aromatic amide prepared from 3-aminobenzoic acid derivatives (Exemplified by **25**)*

Preparation of the Vilsmeier reagent: oxalyl chloride (404 µL, 4.63 mmol) was added dropwise to a stirred solution of DMF (358 µL, 4.63 mmol) in DCM (5 mL) at 0 °C under nitrogen atmosphere (note: a white foam is forming). The reaction was allowed to warm to room temperature and to proceed for 30 min. 3-Amino-5-bromobenzoic acid (500 mg, 2.31 mmol) in DCM (5 mL) was added to the Vilsmeier reagent at 0 °C and the reaction was stirred for 1 h. Pyridine (561 µL, 6.95 mmol) was added to the mixture at 0 °C and the reaction was allowed to warm to room temperature. After 30 min, *p*-toluidine (248 mg, 2.31 mmol) was added and the reaction was stirred for a further 2 h. The mixture was then diluted with DCM and successively washed with $NaHCO_3$, water and brine. The organic phase was dried over $MgSO_4$ and concentrated to dryness. The crude amide was purified by column chromatography (SiO_2, DCM/methanol, 5:1) to give **25** in 60% yield.

*Procedure C: Direct amination of aryl chlorides with amino compounds (Exemplified by **4**)*

Piperidine (665 µL, 6.72 mmol) and triethylamine (937 µL, 6.72 mmol) were simultaneously added to a stirred solution of **3** (1.70 g, 5.85 mmol) in ethanol (15 mL). The mixture was heated at reflux temperature for 5 h. If the reaction was not completed, additional amine (e.g. 2 eq.) was added and the reaction was allowed to proceed overnight. After completion, the mixture was slowly cooled to room temperature. The product was filtered off, washed with cold ethanol and dried under vacuum to give **4** as a yellow crystalline powder in 94% yield.

*Procedure D: Direct amination of aryl bromides with aniline derivatives (Exemplified by **27**)*

An oven-dried microwave tube was charged with $Pd(OAc)_2$ (1 mol%, 0.6 mg) and xPhos (3 mol%, 4.0 mg). The vessel was evacuated and backfilled with nitrogen (this process was repeated 3 times). Degassed 1,4-dioxane (0.5 mL) and degassed H_2O (4 mol%, 0.2 µL) were successively added and the solution was heated at 80 °C for 1.5 min (dark green color). A second oven-dried microwave tube was charged with NaO*t*-Bu (37.4 mg, 0.39 mmol), **25** (100 mg, 0.28 mmol) and *p*-toluidine (35.7 mg, 0.33 mmol). The vessel was evacuated and backfilled with nitrogen (this process was repeated 3 times). The activated catalyst solution was transferred from the first microwave tube into the second one using a degassed syringe. Additional 1,4-dioxane (0.5 mL) was then added in order to ease the stirring. The solution was heated at 80-90 °C for 5 h. After completion, the reaction mixture was cooled down to room temperature, diluted with EtOAc, washed with water and brine, dried over $MgSO_4$ and concentrated to dryness. The crude product was purified by column chromatography (SiO_2, DCM/methanol, 95:5) to give **27** in 79% yield.

*Procedure E: Direct amination of aryl bromides with piperidine (Exemplified by **28**)*

25 (113 mg, 0.31 mmol), Copper (I) iodide (5 mol%, 3.18 mg), (R)-BINOL (20 mol%, 18 mg) and tripotassium phosphate (133.5 mg, 0.63 mmol) were charged in an oven-dried

microwave tube. The vessel was evacuated and backfilled with nitrogen (this process was repeated 3 times) and piperidine (311 µL, 3.15 mmol) was added via a degassed syringe. The reaction was heated, solvent-free, at 110 °C for 1 day. The crude product was directly used in the next step (formamidine cleavage) without further purification.

*Procedure F: Formamidine cleavage (Exemplified by **30**)*

27 (81 mg, 0.21 mmol) was dissolved in ethanol (5 mL) and ethylenediamine (63 µL, 0.94 mmol) was added to the mixture. The reaction was heated at reflux temperature for 6 h before being concentrated. The crude product was purified by column chromatography (SiO_2, EtOAc/n-heptane, 1:1) to give **30** in 93% yield.

*Procedure G: O-Alkylation of aryl chlorides and alcohol (Exemplified by the synthesis of **54**)*

3 (300 mg, 1.03 mmol) and potassium hydroxide (85.3 mg, 1.55 mmol) were dissolved in DMSO (3 mL) and the mixture was stirred at room temperature under nitrogen atmosphere. 2-(2-Methoxyethoxy)ethanol (147 µL, 1.24 mmol) was then added and the reaction was heated at 60 °C for 1 day. After cooling to room temperature, the reaction mixture was acidified with HCl (aq., 5%). The precipitate was filtered off, washed with water and dried under vacuum to give pure product as a yellow powder in 75% yield.

*Procedure H: Reduction of nitro compounds, method 1 (Exemplified by **5**)*

4 (800 mg, 2.36 mmol) and Pd/C (31 mg) was dissolved/suspended in methanol (20 mL) and stirred vigorously. $NaBH_4$ (222.8 mg, 5.89 mmol) was added portion wise over a period of 30 min and the reaction was allowed to perform until hydrogen gas release ended. If the reaction was not completed, additional $NaBH_4$ was added. The solution was filtered through a pad of Celite® and concentrated. The crude product was purified by column chromatography (SiO_2, EtOAc/n-heptane, 1:9 -> 1:1) to give **5** in 90% yield.

*Procedure I: Reduction of nitro compounds, method 2 (Exemplified by **5**)*

$SnCl_2$ (18.07 g, 80.09 mmol) was added to a solution of **4** (5.44 g, 16.05 mmol) in ethanol (100 mL) and the mixture was heated for 3 h at reflux temperature. After completion, the reaction mixture was allowed to cool down to room temperature. The mixture was then diluted with water and, after removal of the white solid using a Celite® pad, the product was extracted with ethyl acetate. Pure product was obtained by crystallization.

*Procedure J: Phthalamido compound synthesis by cyclocondensation of phthalic anhydride derivatives and amino compounds (Exemplified by **1**)*

5 (500 mg, 1.62 mmol) and 1,2,4-benzenetricarboxylic anhydride (**6**, 311 mg, 1.62 mmol) were refluxed in glacial acetic acid (2.5 mL). After 5 h, the reaction mixture was allowed to cool down to rt. The product was filtered off, washed abundantly with n-heptane and dried under vacuum to give **1** as a yellow powder in 89% yield.

*Procedure K: Methyl ester synthesis (Exemplified by **12**)*

1 (50 mg, 0.10 mmol) was dissolved in a DCM/methanol mixture (1.6:0.2 mL) and the mixture was stirred at room temperature and under nitrogen atmosphere. Trimethylsilyldiazomethane (2M in hexane; 62 µL, 0.12 mmol) was added dropwise over a period of 10 min. After the nitrogen gas release ended, the reaction mixture was diluted in DCM. The organic phase was successively washed with $NaHCO_3$, water and brine, dried over $MgSO_4$ and concentrated to dryness to give **12** as a white powder in 97% yield.

*Procedure L: Carboxamide synthesis (Exemplified by **11**)*

A catalytic amount of DMF was added to a stirred solution of **1** (50 mg, 0.10 mmol) and oxalyl chloride (45 µL, 0.52 mmol) in THF (1 mL) at 0 °C and under nitrogen atmosphere. After 30 min, the reaction was allowed to warm to room temperature and to perform for a further 4 h. Then, the reaction mixture was concentrated, dissolved in DCM and concentrated

again. The crude acyl chloride was dissolved in DCM (1 mL) and the mixture was stirred at 0 °C and under nitrogen atmosphere. Hexamethyldisilazane (65 µL, 0.31 mmol) in cold DCM (0 °C, 0.5 mL) was rapidly added to the acyl chloride solution and the reaction was allowed to warm to room temperature overnight. After removal of the solvent, the crude product was dissolved in acetone. The remaining solids were filtered off and the filtrate was concentrated. Recrystallization in DCM gave **11** in 11% yield.

*Procedure M: Cyanation of aryl bromides (Exemplified by **10**)*

8 (100 mg, 0.19 mmol) and Copper (I) cyanide (25.9 mg, 0.29 mmol) were charged in a microwave tube and NMP (0.2 mL) was added. The vessel was sealed and the reaction, under nitrogen atmosphere, was heated at 175 °C for 20 h. The reaction was allowed to cool down to room temperature and the mixture was diluted in ethyl acetate before being filtered through a Celite® pad and concentrated. The product was purified on column chromatography (SiO_2, EtOAc/n-heptane, 1:5 -> 1:0) to give **10** as a white solid in 29% yield.

*Procedure N: Synthesis of amides using phthalic anhydride derivatives and amino compounds (Exemplified by **65** and **66**)*

5 (1.1 equiv.) and 1,2,4-benzenetricarboxylic anhydride (**6**, 29 mg, 0.15 mmol) were stirred in glacial acetic acid (2 mL) for 20 h at room temperature. After removal of the solvent, **65** and **66** were separated by reverse phase HPLC (Acetonitrile/Water: 4:6 -> 1:1, with 0.005% of formic acid). The fractions containing pure compound **65** or **66** were combined and lyophilized to give the regioisomers in 7% and 9% yields, respectively.

*Procedure O: Synthesis of aryl bromides via Sandmeyer reaction (Exemplified by **67**)*

A concentrated HBr solution (0.5 mL, 8 equiv.) was added dropwise at 5 °C to a suspension of **5** (350 mg, 1.13 mmol) in acetonitrile (3.5 mL); a pink pale precipitate formed. Then, a cold aqueous solution of sodium nitrite (1 equiv., 0.35 M) was added dropwise while the

temperature was kept below 5 °C. A cold solution of copper (I) bromide (340.6 mg, 2.1 equiv.) in HBr (0.5 mL, 8 equiv.) was added dropwise to the formed diazonium salt. The reaction was stirred at room temperature for 1 h and then heated to reflux temperature for an additional hour. The reaction mixture was diluted with DCM and washed with brine. The organic phase was dried over $MgSO_4$, filtered, and concentrated to dryness. The crude was purified by column chromatography (SiO_2, gradient EtOAc/n-heptane) to give **67** in 90% yield.

*Procedure P: Halogen exchange in aryl halides (Exemplified by **68**)*

67 (600 mg, 1.6 mmol), copper (I) iodide (30.6 mg, 0.1 equiv.) and sodium iodide (482 mg, 2 equiv.) were charged in an oven-dried microwave tube. The sealed vessel was evacuated and backfilled with nitrogen (this process was repeated 3 times) and N,N'-dimethylethylenediamine (34 µL, 0.2 equiv.) followed by degassed 1,4-dioxane (2.1 mL) were added via a degassed syringe. The reaction was heated at 110 °C for 1 day. After cooling down to room temperature, the reaction mixture was diluted with DCM and successively washed with water and brine. The organic phase was dried over $MgSO_4$, filtered and concentrated to dryness. The crude was purified by column chromatography (SiO_2, gradient EtOAc/n-heptane) to give **68** in 94% yield.

*Procedure Q: Direct arylation of methyl 1H-benzimidazole-5-carboxylate (Exemplified by **70**)*

Methyl 1H-benzimidazole-5-carboxylate (**69**, 30 mg, 0.17 mmol, 1 equiv.), **68** (143.2 mg, 2 equiv.), copper (I) iodide (65 mg, 2 equiv.), and palladium (II) acetate (1.9 mg, 0.05 equiv.) were charged in an oven-dried microwave vial. The vessel was evacuated and backfilled with nitrogen (this process was repeated 3 times) and dry DMF (0.8 mL) was added via a degassed syringe. The reaction was heated at 140 °C for 4 days. After cooling down to room temperature, the reaction mixture was diluted with ethyl acetate and successively washed

with water and brine. The organic phase was dried over $MgSO_4$, filtered, and concentrated to dryness. The crude was purified by column chromatography (SiO_2, DCM/methanol, 95:5) to give **70** in 56% yield.

Procedure R: Methyl ester hydrolysis

An aqueous solution of potassium hydroxide (37 mM) was added to an ethanolic solution of methyl ester derivative (25 mM). The reaction mixture was heated to reflux temperature for 1 day. After cooling down to room temperature and the pH of the solution was set to 2-3 using HCl (aq., 1 M). The precipitate was filtered off, washed with cold water and dried under vacuum.

*Procedure S: Sonogashira coupling reaction of alkyne derivatives with aryl bromides (Exemplified by **72**)*

67 (0.11 mmol, 1 equiv.), copper (I) iodide (0.1 equiv.) and bis(triphenylphosphine) palladium(II) chloride (0.05 equiv.) were charged in an oven-dried microwave tube. The vessel was evacuated and backfilled with nitrogen (this process was repeated 3 times) and dry DMF (1.2 mL) followed by triethylamine (2.0 equiv.) were added via a degassed syringe. The reaction was allowed to perform for 5 min at room temperature and trimethylsilylacetylene (3.0 equiv.) was added. The reaction was heated at 60 °C for 2 days. After cooling down to room temperature, the reaction mixture was diluted with ethyl acetate and washed with brine. The organic phase was dried over $MgSO_4$, filtered, and concentrated to dryness. The crude was dissolved in a methanol/THF mixture (1:4) and potassium carbonate (2 equiv.) was added. After overnight stirring, the reaction mixture was diluted with ethyl acetate and washed with brine. The organic phase was dried over $MgSO_4$, filtered, and concentrated to dryness. The crude was purified by column chromatography to yield **72** in 60% yield.

*Procedure T: Synthesis of Benzofuran and Indole derivatives via Sonogashira coupling reaction of alkyne with aryl iodides (Exemplified by **72**)*

73 (0.11 mmol, 1 equiv.), **72** (2 equiv.), copper (I) iodide (0.1 equiv.) and bis(triphenylphosphine) palladium(II) chloride (0.05 equiv.) were charged in an oven-dried microwave tube. The vessel was evacuated and backfilled with nitrogen (this process was repeated 3 times) and dry DMF (1.2 mL) followed by triethylamine (2.7 equiv.) were added via a degassed syringe. The reaction was heated under microwave irradiation conditions at 110 °C for 20 min. After cooling to room temperature, the reaction mixture was diluted with ethyl acetate and washed with brine. The organic phase was dried over $MgSO_4$, filtered, and concentrated to dryness. The crude was purified by column chromatography to give **75** in 67% yield.

*Procedure U: Synthesis of 2H-Indazole derivatives (Exemplified by **80**)*

5 (3 mmol, 1 equiv.) and **79** (1 equiv.) were dissolved in ethanol (70 mL) and the mixture was refluxed for 20 min. After cooling down to room temperature, the formed precipitate was filtered off, washed carefully with cold ethanol and dried under vacuum. The preformed imine (1.77 mmol. 1 equiv.) and cesium carbonate (1.0 equiv.) were dissolved in dry, degassed DMSO (80 mL) and the mixture was heated at 100 °C. A freshly prepared DMSO solution (4 mL) of copper iodide (0.2 equiv.) and TMEDA (0.2 equiv.) was added to the reaction vessel. Sodium azide (2.0 equiv.) was then added in small portions and the reaction was stirred at 120 °C for 24 h. The brown mixture was allowed to reach room temperature, diluted in ethyl acetate and successively washed with HCl (1 M), brine, dried over Na_2SO_4, filtered and concentrated to give a brown solid. The crude solid was triturated with ethanol and then ethanol was pipetted off. The solids were dissolved in 50 ml of hot ethanol and the product was precipitated with 200 ml of heptane. After cooling to ca. 10 °C, the solids were vacuum filtered to give **80** in 56% yield. (Note: The reaction proved more efficient when the

imine was preformed. A one-pot procedure where all the reactants were added together also provided the desire product, albeit a lower reaction efficiency.)

4.3. Tabulated compounds

*1,3-Dioxo-2-(4-(piperidin-1-yl)-3-(p-tolylcarbamoyl)phenyl)isoindoline-5-carboxylic acid (**1**).* **1** was synthesized by procedures A, C, H (or I) and J.

^{1}H-NMR (400 MHz, DMSO-d_6): δ 13.80 (bs, 1H), 11.43 (s, 1H), 8.41 (dd, *J* = 1.5, 7.7 Hz, 1H), 8.30 (d, *J* = 1.4 Hz, 1H), 8.05 (d, *J* = 7.7 Hz, 1H), 7.88 (d, *J* = 2.6 Hz, 1H), 7.66 (d, *J* = 8.3 Hz, 2H), 7.56 (dd, *J* = 2.6, 8.5 Hz, 1H), 7.43 (d, *J* = 8.7 Hz, 1H), 7.18 (d, *J* = 8.6 Hz, 2H), 2.95-3.05 (m, 4H), 2.28 (s, 3H), 1.62-1.73 (m, 4H), 1.48-1.58 (m, 2H).

^{13}C-NMR (100.5 MHz, DMSO-d_6): δ 166.36, 166.35, 165.84, 163.92, 151.41, 136.40, 136.36, 135.41, 135.03, 132.49, 132.16, 130.57, 129.38, 129.07, 126.81, 123.77, 123.33, 120.80, 119.32, 53.70, 25.95, 23.29, 20.50.

ESI-MS *m/z* calcd for $C_{28}H_{25}N_3O_5$ $(M+H)^+$ 484.18; found: 484.21.

*5-(1,3-dioxoisoindolin-2-yl)-2-(piperidin-1-yl)-N-p-tolylbenzamide (**7**).* **7** was synthesized by procedures A, C, H and J.

^{1}H-NMR (400 MHz, DMSO-d_6): δ 11.46 (s, 1H), 7.94-8.00 (m, 2H), 7.88-7.94 (m, 2H), 7.86 *(d, J = 2.5 Hz, 1H), 7.66 (d, J = 8.3 Hz, 2H), 7.55 (dd, J = 2.6, 8.5 Hz, 1H), 7.43 (d, J = 8.6 Hz,* 1H), 7.18 (d, *J* = 8.4 Hz, 2H), 2.95-3.07 (m, 4H), 2.28 (s, 3H), 1.62-1.74 (m, 4H), 1.48-1.59 (m, 2H).

ESI-MS m/z calcd for $C_{27}H_{25}N_3O_3$ $(M+H)^+$ 440.19; found: 439.97.

*5-(5-bromo-1,3-dioxoisoindolin-2-yl)-2-(piperidin-1-yl)-N-p-tolylbenzamide (**8**).* **8** was synthesized by procedures A, C, H and J.

^{1}H-NMR (360 MHz, DMSO-d_6): δ 11.43 (s, 1H), 8.17 (d, *J* = 1.8 Hz, 1H), 8.1 (dd, *J* = 1.8, 7.9 Hz, 1H), 7.9 (d, *J* = 7.9 Hz, 1H), 7.85 (d, *J* = 2.5 Hz, 1H), 7.66 (d, *J* = 8.1 Hz, 2H), 7.54

(dd, *J* = 2.7, 8.4 Hz, 1H), 7.43 (d, *J* = 8.7 Hz, 1H), 7.18 (d, *J* = 8.4 Hz, 2H), 3.01 (t, *J* = 4.8 Hz, 4H), 2.28 (s, 3H), 1.61-1.73 (m, 4H), 1.48-1.58 (m, 2H).

ESI-MS *m/z* calcd for $C_{27}H_{24}BrN_3O_3$ $(M+H)^+$ 518.10; found: 518.11.

*5-(5-amino-1,3-dioxoisoindolin-2-yl)-2-(piperidin-1-yl)-N-p-tolylbenzamide (**9**).*

^{1}H-NMR (400 MHz, DMSO-d_6): δ 11.53 (s, 1H), 7.80 (d, *J* = 2.6 Hz, 1H), 7.65 (d, *J* = 8.4 Hz, 2H), 7.58 (d, *J* = 8.3 Hz, 1H), 7.50 (dd, *J* = 2.6, 8.5 Hz, 1H), 7.40 (d, *J* = 8.7 Hz, 1H), 7.18 (d, *J* = 8.3 Hz, 2H), 7.00 (d, *J* = 2.0 Hz, 1H), 6.86 (dd, *J* = 2.0, 8.3 Hz, 1H), 6.56 (bs, 2H), 2.95-3.03 (m, 4H), 2.28 (s, 3H), 1.62-1.74 (m, 4H), 1.49-1.58 (m, 2H).

ESI-MS *m/z* calcd for $C_{27}H_{26}N_4O_3$ $(M+H)^+$ 455.20; found: 455.02.

*5-(5-cyano-1,3-dioxoisoindolin-2-yl)-2-(piperidin-1-yl)-N-p-tolylbenzamide (**10**).* **10** was synthesized by procedure M.

^{1}H-NMR (400 MHz, DMSO-d_6): δ 11.41 (s, 1H), 8.51 (s, 1H), 8.37 (dd, *J* = 1.4, 7.7 Hz, 1H), 8.14 (d, *J* = 7.8 Hz, 1H), 7.87 (d, *J* = 2.6 Hz, 1H), 7.65 (d, *J* = 8.1 Hz, 2H), 7.55 (dd, *J* = 2.6, 8.5 Hz, 1H), 7.44 (d, *J* = 8.6 Hz, 1H), 7.18 (d, *J* = 8.1 Hz, 2H), 2.98-3.05 (m, 4H), 2.28 (s, 3H), 1.63-1.72 (m, 4H), 1.50-1.58 (m, 2H).

ESI-MS *m/z* calcd for $C_{28}H_{24}N_4O_3$ $(M+H)^+$ 465.18; found: 465.38.

*1,3-dioxo-2-(4-(piperidin-1-yl)-3-(p-tolylcarbamoyl)phenyl)isoindoline-5-carboxamide (**11**).* **11** was synthesized by procedure L.

^{1}H-NMR (360 MHz, DMSO-d_6): δ 11.44 (s, 1H), 8.33-8.43 (m, 3H), 8.05 (d, *J* = 7.8 Hz, 1H), 7.87 (d, *J* = 2.6 Hz, 1H), 7.79 (bs, 1H), 7.66 (d, *J* = 8.3 Hz, 2H), 7.56 (dd, *J* = 2.7, 8.6 Hz, 1H), 7.43 (d, *J* = 8.7 Hz, 1H), 7.18 (d, *J* = 8.7 Hz, 2H), 2.98-3.04 (m, 4H), 2.28 (s, 3H), 1.64-1.72 (m, 4H), 1.50-1.58 (m, 2H).

ESI-MS *m/z* calcd for $C_{28}H_{26}N_4O_4$ $(M+H)^+$ 483.20; found: 483.27.

*Methyl-1,3-dioxo-2-(4-(piperidin-1-yl)-3-(p-tolylcarbamoyl)phenyl)isoindoline-5-carboxylate (**12**).* **12** was synthesized by procedure K.

^{1}H-NMR (360 MHz, DMSO-d_6): δ 11.42 (bs, 1H), 8.43 (d, *J* = 7.8 Hz, 1H), 8.32 (s, 1H), 8.33 (s, 1H), 8.11 (d, *J* = 7.8 Hz, 1H), 7.88 (d, *J* = 2.5 Hz, 1H), 7.66 (d, *J* = 8.1 Hz, 2H), 7.56 (dd, *J* = 2.5, 8.5 Hz, 1H), 7.43 (d, *J* = 8.6 Hz, 1H), 7.18 (d, *J* = 8.1 Hz, 2H), 3.95 (s, 3H), 2.97-3,05 (m, 4H), 2.28 (s, 3H), 1.62-1,72 (m, 4H), 1.49-1.58 (m, 2H).

ESI-MS *m/z* calcd for $C_{29}H_{27}N_3O_5$ $(M+H)^+$ 498.20; found: 498.07.

*1,3-dioxo-2-(4-(piperidin-1-yl)-3-(p-tolylcarbamoyl)phenyl)isoindoline-4-carboxylic acid (**13**).*

^{1}H-NMR (400 MHz, DMSO-d_6): δ 13.69 (bs, 1H), 11.43 (s, 1H), 7.90-8.10 (m, 3H), 7.85 (d, *J* = 2.6 Hz, 1H), 7.66 (d, *J* = 8.3 Hz, 2H), 7.55 (dd, *J* = 2.6, 8.6 Hz, 1H), 7.42 (d, *J* = 8.6 Hz, 1H), 7.18 (d, *J* = 8.6 Hz, 2H), 2.98-3.06 (m, 4H), 2.28 (s, 3H), 1.63-1.72 (m, 4H), 1.49-1.59 (m, 2H).

ESI-MS *m/z* calcd for $C_{28}H_{25}N_3O_5$ $(M+H)^+$ 484.18; found: 483.97.

*2-(3-chloro-5-(p-tolylcarbamoyl)phenyl)-1,3-dioxoisoindoline-5-carboxylic acid (**14**).*

^{1}H-NMR (400 MHz, DMSO-d_6): δ 13.80 (bs, 1H), 10.37 (s, 1H), 8.44 (dd, *J* = 1.4, 7.8 Hz, 1H), 8.34 (s, 1H), 8.15 (t, *J* = 2.22 Hz, 1H), 8.13 (d, *J* = 7.7 Hz, 1H), 8.02 (t, *J* = 1.7 Hz, 1H), 7.82 (t, *J* = 1.9 Hz, 1H), 7.64 (d, *J* = 8.5 Hz, 2H), 7.16 (d, *J* = 8.7 Hz, 2H), 2.29 (s, 3H).

ESI-MS *m/z* calcd for $C_{23}H_{15}ClN_2O_5$ $(M+H)^+$ 435.07; found: 434.90.

*1,3-dioxo-2-(3-(phenylamino)-5-(p-tolylcarbamoyl)phenyl)isoindoline-5-carboxylic acid (**15**).* **15** was synthesized by procedures B, D, F and J.

^{1}H-NMR (360 MHz, DMSO-d_6): δ 13.76 (bs, 1H), 10.20 (s, 1H), 8.64 (s, 1H), 8.42 (dd, *J* = 1.1, 7.4 Hz, 1H), 8.32 (s, 1H), 8.09 (d, *J* = 7.8 Hz, 1H), 7.63 (m, 3H), 7.42 (t, *J* = 1.5 Hz, 1H),

7.37 (t, *J* = 1.5 Hz, 1H), 7.29 (t, *J* = 7.48 Hz, 2H), 7.19 (d, *J* = 8.3 Hz, 2H), 7.15 (d, *J* = 8.3 Hz, 2H), 6.91 (t, *J* = 7.3 Hz, 1H), 2.28 (s, 3H).

ESI-MS *m/z* calcd for $C_{29}H_{21}N_3O_5$ $(M+H)^+$ 492.15; found: 492.28.

*1,3-dioxo-2-(3-(p-tolylamino)-5-(p-tolylcarbamoyl)phenyl)isoindoline-5-carboxylic acid (**16**).* **16** was synthesized by procedures B, D, F and J.

^{1}H-NMR (360 MHz, DMSO-d_6): δ 13.02 (bs, 1H), 10.18 (s, 1H), 8.49 (s, 1H), 8.42 (d, *J* = 7.8 Hz, 1H), 8.32 (s, 1H), 8.08 (d, *J* = 7.8 Hz, 1H), 7.62 (d, *J* = 8.1 Hz, 2H), 7.55 (t, *J* = 1.3 Hz, 1H), 7.36 (s, 1H), 7.28 (s, 1H), 7.06-7.17 (m, 6H), 2.27 (s, 3H), 2.24 (s, 3H).

ESI-MS *m/z* calcd for $C_{30}H_{23}N_3O_5$ $(M+H)^+$ 506.16; found: 505.82.

*1,3-dioxo-2-(5-(piperidin-1-yl)-2-(p-tolylcarbamoyl)phenyl)isoindoline-5-carboxylic acid (**17**).* **17** was synthesized by procedures A, C, I and J.

^{1}H-NMR (400 MHz, DMSO-d_6): δ 13.72 (bs, 1H), 10.33 (s, 1H), 8.39 (dd, *J* = 1.5, 7.7 Hz, 1H), 8.26 (s, 1H), 8.03 (d, *J* = 7.7 Hz, 1H), 7.44 (d, *J* = 8.3 Hz, 2H), 7.31 (d, *J* = 8.8 Hz, 1H), 7.25 (d, *J* = 2.7 Hz, 1H), 7.19 (dd, *J* = 2.5, 8.4 Hz, 1H), 7.06 (d, *J* = 8.0 Hz, 2H), 3.28-3.38 (m, 4H), 2.22 (s, 3H), 1.54-1.71 (m, 6H).

ESI-MS *m/z* calcd for $C_{28}H_{25}N_3O_5$ $(M+H)^+$ 484.18; found: 484.04.

*1,3-dioxo-2-(2-(piperidin-1-yl)-6-(p-tolylcarbamoyl)phenyl)isoindoline-5-carboxylic acid (**18**).* **18** was synthesized by procedures A, C, I and J.

^{1}H-NMR (400 MHz, DMSO-d_6): δ 13.71 (bs, 1H), 10.38 (s, 1H), 8.41 (d, *J* = 7.6 Hz, 1H), 8.30 (s, 1H), 8.06 (d, *J* = 7.8 Hz, 1H), 7.60 (t, *J* = 7.9 Hz, 1H), 7.40-7.51 (m, 4H), 7.06 (d, *J* = 8.0 Hz, 2H), 2.69-2.78 (m, 4H), 2.22 (s, 3H), 1.24-1.41 (m, 6H).

ESI-MS *m/z* calcd for $C_{28}H_{25}N_3O_5$ $(M+H)^+$ 484.18; found: 483.93.

*1,3-dioxo-2-(4-(piperidin-1-yl)-2-(p-tolylcarbamoyl)phenyl)isoindoline-5-carboxylic acid (**19**).*

^{1}H-NMR (400 MHz, DMSO-d_6): δ 13.65 (bs, 1H), 10.33 (s, 1H), 8.38 (dd, *J* = 1.0, 7.7 Hz, 1H), 8.25 (s, 1H), 8.01 (d, *J* = 7.7 Hz, 1H), 7.44 (d, *J* = 8.3 Hz, 2H), 7.31 (d, *J* = 8.9 Hz, 1H), 7.25 (d, *J* = 2.7 Hz, 1H), 7.19 (dd, *J* = 2.7, 8.8 Hz, 1H), 7.06 (d, *J* = 8.4 Hz, 2H), 3.30-3.40 (m, 4H), 2.22 (s, 3H), 1.55-1.73 (m, 6H).

ESI-MS *m/z* calcd for $C_{28}H_{25}N_3O_5$ $(M+H)^+$ 484.18; found: 483.97.

*1,3-dioxo-2-(2-(piperidin-1-yl)-5-(p-tolylcarbamoyl)phenyl)isoindoline-5-carboxylic acid (**20**).* **20** was synthesized by procedures A, C, I and J.

^{1}H-NMR (400 MHz, DMSO-d_6): δ 13.79 (bs, 1H), 11.21 (s, 1H), 8.43 (dd, *J* = 1.5, 7.8 Hz, 1H), 8.32 (s, 1H), 8.09 (d, *J* = 7.8 Hz, 1H), 7.87 (d, *J* = 8.2 Hz, 1H), 7.67 (d, *J* = 8.4 Hz, 2H), 7.38 (d, *J* = 2.0 Hz, 1H), 7.26 (dd, *J* = 1.8, 8.4 Hz, 1H), 7.18 (d, *J* = 8.5 Hz, 2H), 2.93-3.03 (m, 4H), 2.29 (s, 3H), 1.60-1.71 (m, 4H), 1.45-1.55 (m, 2H).

ESI-MS *m/z* calcd for $C_{28}H_{25}N_3O_5$ $(M+H)^+$ 484.18; found: 483.97.

*1,3-dioxo-2-(3-(piperidin-1-yl)-4-(p-tolylcarbamoyl)phenyl)isoindoline-5-carboxylic acid (**21**).* **21** was synthesized by procedures A, C, I and J.

^{1}H-NMR (400 MHz, DMSO-d_6): δ 13.79 (bs, 1H), 10.10 (s, 1H), 8.45 (dd, *J* = 1.4, 7.7 Hz, 1H), 8.36 (s, 1H), 8.14 (d, *J* = 7.8 Hz, 1H), 8.06 (dd, *J* = 2.3, 8.5 Hz, 1H), 7.94 (d, *J* = 2.2 Hz, 1H), 7.63 (d, *J* = 8.4 Hz, 2H), 7.33 (d, *J* = 8.5 Hz, 1H), 7.15 (d, *J* = 8.6 Hz, 2H), 2.75-2.88 (m, 4H), 2.27 (s, 3H), 1.26-1.43 (m, 6H).

ESI-MS *m/z* calcd for $C_{28}H_{25}N_3O_5$ $(M+H)^+$ 484.18; found: 483.97.

*1,3-dioxo-2-(2-(piperidin-1-yl)-3-(p-tolylcarbamoyl)phenyl)isoindoline-5-carboxylic acid (**22**).* **22** was synthesized by procedures A, C, I and J.

^{1}H-NMR (400 MHz, DMSO-d_6): δ 13.79 (bs, 1H), 10.45 (s, 1H), 8.46 (dd, *J* = 1.5, 7.7 Hz, 1H), 8.36 (s, 1H), 8.14 (d, *J* = 7.8 Hz, 1H), 7.63 (d, *J* = 8.4 Hz, 2H), 7.46-7.54 (m, 2H), 7.31

(7, *J* = 7.7 Hz, 1H), 7.14 (d, *J* = 8.5 Hz, 2H), 2.76-2.90 (m, 4H), 2.27 (s, 3H), 1.06-1.28 (m, 6H).

ESI-MS *m/z* calcd for $C_{28}H_{25}N_3O_5$ $(M+H)^+$ 484.18; found: 484.02.

*1,3-dioxo-2-(3-(piperidin-1-yl)-5-(p-tolylcarbamoyl)phenyl)isoindoline-5-carboxylic acid (**23**).* **23** was synthesized by procedures B, E, F and J.

^{1}H-NMR (400 MHz, DMSO-d_6): δ 13.75 (bs, 1H), 10.15 (s, 1H), 8.43 (dd, *J* = 1.5, 7.8 Hz, 1H), 8.32 (s, 1H), 8.09 (d, *J* = 7.8 Hz, 1H), 7.62 (d, *J* = 8.4 Hz, 2H), 7.52 (t, *J* = 1.6 Hz, 1H), 7.33 (t, *J* = 1.8 Hz, 1H), 7.21 (t, *J* = 2.2 Hz, 1H), 7.16 (d, *J* = 8.8 Hz, 2H), 3.25-3.31 (m, 4H), 2.28 (s, 3H), 1.62-1.68 (m, 4H), 1.54-1-62 (m, 2H).

ESI-MS *m/z* calcd for $C_{28}H_{25}N_3O_5$ $(M+H)^+$ 484.18; found: 484.21.

*2-phenylisoindoline-1,3-dione (**31**).* **31** was synthesized by procedure J.

^{1}H-NMR (400 MHz, DMSO-d_6): δ 7.88- 8.02 (m, 4H), 7.40-7.57 (m, 5H).

ESI-MS *m/z* calcd for $C_{14}H_9NO_2$ $(M+H)^+$ 224.06; found: 224.19.

*1,3-dioxo-2-phenylisoindoline-5-carboxylic acid (**32**).* **32** was synthesized by procedure J.

^{1}H-NMR (400 MHz, DMSO-d_6): δ 13.76 (bs, 1H), 8.41 (dd, *J* = 1.4, 7.7 Hz, 1H), 8.31 (s, 1H), 8.09 (d, *J* = 7.7 Hz, 1H), 7.42-7.59 (m, 5H).

ESI-MS *m/z* calcd for $C_{15}H_9NO_4$ $(M-H)^-$ 266.05; found: 266.02.

*1,3-dioxo-2-(3-(phenylcarbamoyl)-4-(piperidin-1-yl)phenyl)isoindoline-5-carboxylic acid (**33**).*

^{1}H-NMR (400 MHz, DMSO-d_6): δ 13.77 (bs, 1H), 11.54 (s, 1H), 8.41 (dd, *J* = 1.5, 7.7 Hz, 1H), 8.30 (s, 1H), 8.07 (d, *J* = 7.8 Hz, 1H), 7.90 (d, *J* = 2.6 Hz, 1H), 7.78 (d, *J* = 7.7 Hz, 2H), 7.57 (dd, *J* = 2.5, 8.5 Hz, 1H), 7.44 (d, *J* = 8.8 Hz, 1H), 7.38 (dd, *J* = 7.5, 8.5 Hz, 2H), 7.10 (dd, *J* = 7.0, 7.7 Hz, 1H), 2.96-3.09 (m, 4H), 2.58 (q, *J* = 7.6 Hz, 2H), 1.62-1.74 (m, 4H), 1.49-1.60 (m, 2H), 1.18 (t, *J* = 7.6 Hz, 3H).

ESI-MS *m/z* calcd for $C_{27}H_{23}N_3O_5$ $(M+H)^+$ 470.16; found: 469.98.

*2-(3-(4-methoxyphenylcarbamoyl)-4-(piperidin-1-yl)phenyl)-1,3-dioxoisoindoline-5-carboxylic acid (**34**).*

^{1}H-NMR (400 MHz, DMSO-d_6): δ 13.73 (bs, 1H), 11.40 (s, 1H), 8.41 (dd, *J* = 1.4, 7.8 Hz, 1H), 8.30 (s, 1H), 8.08 (d, *J* = 7.7 Hz, 1H), 7.88 (d, *J* = 2.4 Hz, 1H), 7.69 (d, *J* = 8.6 Hz, 2H), 7.56 (dd, *J* = 2.5, 8.6 Hz, 1H), 7.43 (d, *J* = 8.6 Hz, 1H), 6.96 (d, *J* = 8.6 Hz, 2H), 3.75 (s, 3H), 2.93-3.07 (m, 4H), 1.61-1.74 (m, 4H), 1.49-1.58 (m, 2H).

ESI-MS *m/z* calcd for $C_{28}H_{25}N_3O_6$ $(M+H)^+$ 500.17; found: 499.90.

*1,3-dioxo-2-(4-(piperidin-1-yl)-3-(4-(trifluoromethyl)phenylcarbamoyl)phenyl)isoindoline-5-carboxylic acid (**35**).* **35** was synthesized by procedures A, C, H and J.

^{1}H-NMR (400 MHz, DMSO-d_6): δ 13.75 (bs, 1H); 11.66 (s, 1H); 8.42 (dd, *J* = 1.3, 7.8 Hz, 1H); 8.31 (s, 1H); 8.08 (d, *J* = 7.7 Hz, 1H); 7.99 (d, *J* = 8.5 Hz, 2H); 7.85 (d, *J* = 2.5 Hz, 1H); 7.76 (d, *J* = 8.7 Hz, 2H); 7.59 (dd, *J* = 2.5, 8.7 Hz, 1H); 7.44 (d, *J* = 8.6 Hz, 1H); 3.03 (t, *J* = 4.6 Hz, 4H); 1.61-1.70 (m, 4H); 1.49-1.57 (m, 2H).

ESI-MS *m/z* calcd for $C_{28}H_{22}F_3N_3O_5$ $(M+H)^+$ 538.15; found: 538.21.

*1,3-dioxo-2-(4-(piperidin-1-yl)-3-(m-tolylcarbamoyl)phenyl)isoindoline-5-carboxylic acid (**36**).*

^{1}H-NMR (400 MHz, DMSO-d_6): δ 13.74 (bs, 1H), 11.44 (s, 1H), 8.42 (dd, *J* = 1.5, 7.7 Hz, 1H), 8.31 (s, 1H), 8.08 (d, *J* = 7.7 Hz, 1H), 7.87 (d, *J* = 2.6 Hz, 1H), 7.66 (s, 1H), 7.57 (dd, *J* = 2.6, 8.6 Hz, 1H), 7.50 (d, *J* = 7.6 Hz, 1H), 7.44 (d, *J* = 8.7 Hz, 1H), 7.26 (t, *J* = 7.8 Hz, 1H), 6.93 (d, *J* = 7.4 Hz, 1H), 2.97-3.07 (m, 4H), 2.32 (s, 3H), 1.61-1.74 (m, 4H), 1.49-1.60 (m, 2H).

ESI-MS *m/z* calcd for $C_{28}H_{25}N_3O_5$ $(M+H)^+$ 484.18; found: 483.93.

*2-(3-(4-ethylphenylcarbamoyl)-4-(piperidin-1-yl)phenyl)-1,3-dioxoisoindoline-5-carboxylic acid (**37**).*

^{1}H-NMR (400 MHz, DMSO-d_6): δ 13.76 (bs, 1H), 11.47 (s, 1H), 8.41 (dd, *J* = 1.4, 7.8 Hz, 1H), 8.30 (s, 1H), 8.08 (d, *J* = 7.7 Hz, 1H), 7.88 (d, *J* = 2.5 Hz, 1H), 7.68 (d, *J* = 8.1 Hz, 2H), 7.56 (dd, *J* = 2.5, 8.5 Hz, 1H), 7.44 (d, *J* = 8.7 Hz, 1H), 7.22 (d, *J* = 8.1 Hz, 2H), 2.96-3.07 (m, 4H), 2.58 (q, *J* = 7.6 Hz, 2H), 1.62-1.74 (m, 4H), 1.48-1.59 (m, 2H), 1.18 (t, *J* = 7.6 Hz, 3H).

ESI-MS *m/z* calcd for $C_{29}H_{27}N_3O_5$ $(M+H)^+$ 498.20; found: 497.99.

*2-(3-(Biphenyl-2-ylcarbamoyl)-4-(phenylamino)phenyl)-1,3-dioxoisoindoline-5-carboxylic acid (**38**).* **38** was synthesized by procedures A, C, H and J.

^{1}H-NMR (360 MHz, DMSO-d_6): δ 13.81 (bs, 1H), 10.02 (s, 1H), 9.13 (s, 1H), 8.43 (dd, *J* = 1.6, 7.7 Hz, 1H), 8.34 (s, 1H), 8.11 (d, *J* = 7.8 Hz, 1H), 7.7 (d, *J* = 2.3 Hz, 1H), 7.55 (d, *J* = 7.6 Hz, 1H), 7.27-7.44 (m, 11H), 7.2 (t, *J* = 6.9 Hz, 1H), 7.13 (d, *J* = 7.7 Hz, 2H), 7.02 (t, *J* = 7.3 Hz, 1H).

^{13}C-NMR (100.5 MHz, DMSO-d_6): δ 166.84, 166.48, 166.46, 165.83, 144.00, 141.15, 138.99, 137.87, 136.61, 135.62, 134.77, 134.47, 131.92, 131.22, 130.22, 129.43, 128.48, 128.34, 128.28, 127.86, 127.57, 127.11, 126.73, 123.87, 123.41, 122.31, 121.96, 119.85, 115.73.

ESI-MS *m/z* calcd for $C_{34}H_{23}N_3O_5$ $(M-H)^-$ 552.16; found: 552.26.

*2-(3-(4-isopropylphenylcarbamoyl)-4-(piperidin-1-yl)phenyl)-1,3-dioxoisoindoline-5-carboxylic acid (**39**).* **39** was synthesized by procedures A, C, H and J.

^{1}H-NMR (400 MHz, DMSO-d_6): δ 13.75 (bs, 1H), 11.51 (s, 1H), 8.42 (dd, *J* = 1.5, 7.8 Hz, 1H), 8.31 (s, 1H), 8.08 (d, *J* = 7.8 Hz, 1H), 7.89 (dd, *J* = 2.4, 3.2 Hz, 1H), 7.68 (d, *J* = 8.7 Hz, 2H), 7.57 (dd, *J* = 2.6, 8.5 Hz, 1H), 7.45 (dd, *J* = 2.7, 8.8 Hz, 1H), 7.25 (d, *J* = 8.5 Hz, 2H),

2.98-3.06 (m, 4H), 2.87 (hept, *J* = 6.9 Hz, 1H), 1.64-1.74 (m, 4H), 1.51-1.60 (m, 2H), 1.21 (d, *J* = 6.9 Hz, 6H).

^{13}C-NMR (100.5 MHz, DMSO-d_6): δ 166.35, 165.83, 163.83, 151.41, 143.60, 136.60, 136.36, 135.40, 135.02, 132.15, 130.59, 129.09, 129.01, 126.89, 126.71, 123.76, 123.31, 120.91, 119.46, 53.72, 25.96, 23.95, 23.27.

ESI-MS *m/z* calcd for $C_{30}H_{29}N_3O_5$ $(M+H)^+$ 512.21; found: 512.31.

*2-(3-(3-ethylphenylcarbamoyl)-4-(piperidin-1-yl)phenyl)-1,3-dioxoisoindoline-5-carboxylic acid (**40**).* **40** was synthesized by procedures A, C, H and J.

^{1}H-NMR (400 MHz, DMSO-d_6): δ 13.74 (bs, 1H), 11.53 (s, 1H), 8.42 (dd, *J* = 1.5, 7.8 Hz, 1H), 8.31 (s, 1H), 8.08 (d, *J* = 7.8 Hz, 1H), 7.89 (dd, *J* = 2.4, 3.2 Hz, 1H), 7.66 (s, 1H), 7.57 (dt, *J* = 2.5, 8.5 Hz, 2H), 7.45 (dd, *J* = 2.9, 8.7 Hz, 1H), 7.28 (t, *J* = 7.8 Hz, 1H), 6.96 (d, *J* = 7.59 Hz, 1H), 2.97-3.10 (m, 4H), 2.62 (q, *J* = 7.5 Hz, 2H), 1.64-1.76 (m, 4H), 1.50-1.60 (m, 2H), 1.20 (t, *J* = 7.6 Hz, 3H).

^{13}C-NMR (100.5 MHz, DMSO-d_6): δ 166.36, 165.85, 164.01, 151.46, 144.53, 138.89, 136.44, 135.43, 135.01, 132.15, 130.64, 129.07, 129.00, 128.91, 126.93, 123.79, 123.34, 123.11, 120.96, 118.71, 116.84, 53.75, 28.28, 25.96, 23.29, 15.48.

ESI-MS *m/z* calcd for $C_{29}H_{27}N_3O_5$ $(M+H)^+$ 498.20; found: 498.26.

*2-(3-(4-ethylphenylcarbamoyl)-4-(phenylamino)phenyl)-1,3-dioxoisoindoline-5-carboxylic acid (**41**).* **41** was synthesized by procedures A, C, H and J.

^{1}H-NMR (400 MHz, DMSO-d_6): δ 13.77 (bs, 1H), 10.30 (s, 1H), 9.24 (s, 1H), 8.43 (dd, *J* = 1.5, 7.7 Hz, 1H), 8.33 (s, 1H), 8.10 (d, *J* = 7.8 Hz, 1H), 7.81 (d, *J* = 2.2 Hz, 1H), 7.59 (d, *J* = 8.4 Hz, 2H), 7.39-7.47 (m, 2H), 7.30-7.37 (m, 2H), 7.23 (dd, *J* = 1.1, 8.6 Hz, 2H), 7.17 (d, *J* = 8.3 Hz, 2H), 7.01 (t, *J* = 7.5 Hz, 1H), 2.57 (q, *J* = 7.6 Hz, 2H), 1.16 (t, *J* = 7.6 Hz, 3H).

^{13}C-NMR (100.5 MHz, DMSO-d_6): δ 166.57, 166.54, 165.83, 144.05, 141.20, 139.44, 136.61, 136.29, 136.00, 134.80, 131.96, 131.24, 129.46, 128.41, 127.80, 123.85, 123.39, 122.33, 121.92, 120.77, 120.51, 119.96, 115.76, 27.67, 15.71.

ESI-MS *m/z* calcd for $C_{30}H_{23}N_3O_5$ $(M+H)^+$ 506.16; found: 506.20.

*2-(3-(4-isopropylphenylcarbamoyl)-4-(phenylamino)phenyl)-1,3-dioxoisoindoline-5-carboxylic acid (**42**).* **42** was synthesized by A, C, H and J.

^{1}H-NMR (400 MHz, DMSO-d_6): δ 13.77 (bs, 1H), 10.30 (s, 1H), 9.24 (s, 1H), 8.43 (dd, *J* = 1.5, 7.7 Hz, 1H), 8.33 (s, 1H), 8.10 (d, *J* = 7.7 Hz, 1H), 7.81 (d, *J* = 2.2 Hz, 1H), 7.59 (d, *J* = 8.5 Hz, 2H), 7.39-7.48 (m, 2H), 7.34 (t, *J* = 7.4 Hz, 2H), 7.23 (dd, *J* = 1.0, 8.6 Hz, 2H), 7.21 (d, *J* = 8.6 Hz, 2H), 7.02 (t, *J* = 7.3 Hz, 1H), 2.86 (hept, *J* = 6.9 Hz, 1H), 1.19 (d, *J* = 6.9 Hz, 6H).

^{13}C-NMR (100.5 MHz, DMSO-d_6): δ 166.53, 165.82, 144.08, 144.04, 141.19, 136.67, 136.36, 135.58, 134.76, 131.94, 131.22, 129.44, 128.39, 126.29, 123.82, 123.38, 122.31, 121.91, 120.78, 120.49, 119.95, 115.73, 32.91, 23.94.

ESI-MS *m/z* calcd for $C_{31}H_{25}N_3O_5$ $(M+H)^+$ 520.18; found: 520.31.

*2-(3-(biphenyl-2-ylcarbamoyl)-4-(piperidin-1-yl)phenyl)-1,3-dioxoisoindoline-5-carboxylic acid (**43**).* **43** was synthesized by procedures A, C, H and J.

^{1}H-NMR (360 MHz, DMSO-d_6): δ 11.56 (bs, 1H), 8.42 (d, *J* = 7.8 Hz, 1H), 8.32 (s, 1H), 8.25 (d, *J* = 8.3 Hz, 1H), 8.13-8.08 (m, 2H), 7.59 (dd, *J* = 8.6, 2.7 Hz, 1H), 7.47 (d, *J* = 4.7 Hz, 4H), 7.45-7.36 (m, 4H), 7.29 (t, *J* = 7.5 Hz, 1H), 2.70 (m, 4H), 1.25 (m, 2H), 1.02 (m, 4H).

^{13}C-NMR (100.5 MHz, DMSO-d_6): δ 166.34, 165.83, 163.38, 151.77, 138.42, 136.38, 135.43, 135.03, 134.67, 132.91, 132.16, 131.22, 130.67, 129.65, 129.01, 128.94, 128.03, 127.79, 127.70, 127.59, 125.08, 123.82, 123.35, 121.34, 54.18, 24.94, 22.71, 21.06.

ESI-MS *m/z* calcd for $C_{33}H_{27}N_3O_5$ $(M+H)^+$ 546.20; found: 546.46.

*2-(3-(benzylcarbamoyl)-4-(piperidin-1-yl)phenyl)-1,3-dioxoisoindoline-5-carboxylic acid (**44**).*

^{1}H-NMR (400 MHz, DMSO-d_6): δ 13.77 (bs, 1H), 9.99 (t, *J* = 5.8 Hz, 1H), 8.41 (d, *J* = 7.8 Hz, 1H), 8.30 (s, 1H), 8.08 (d, *J* = 7.8 Hz, 1H), 7.89 (d, *J* = 2.4 Hz, 1H), 7.52 (dd, *J* = 2.5, 8.4 Hz, 1H), 7.24-7.44 (m, 6H), 4.54 (d, *J* = 5.7 Hz, 2H), 2.84-2.93 (m, 4H), 1.34-1.52 (m, 6H).

ESI-MS *m/z* calcd for $C_{28}H_{25}N_3O_5$ $(M+H)^+$ 484.18; found: 483.94.

*2-(4-Chloro-3-(pyridin-3-ylmethylcarbamoyl)phenyl)-1,3-dioxoisoindoline-5-carboxylic acid (**45**).* **45** was synthesized by procedures A, H and J.

^{1}H-NMR (400 MHz, DMSO-d_6): δ 13.78 (bs, 1H), 9.21 (t, *J* = 5.9 Hz, 1H), 8.58 (d, *J* = 2.3 Hz, 1H), 8.47 (dd, *J* = 1.9, 4.7 Hz, 1H), 8.42 (dd, *J* = 1.5, 7.8 Hz, 1H), 8.32 (s, 1H), 8.09 (d, *J* = 7.8 Hz, 1H), 7.76 (ddd, *J* = 1.7, 1.9, 8.0 Hz, 1H), 7.71 (d, *J* = 8.6 Hz, 1H), 7.60 (d, *J* = 2.3 Hz, 1H), 7.57 (dd, *J* = 2.4, 8.6 Hz, 1H), 7.38 (dd, *J* = 4.8, 7.8 Hz, 1H), 4.49 (d, *J* = 5.9 Hz, 2H).

ESI-MS *m/z* calcd for $C_{22}H_{14}ClN_3O_5$ $(M+H)^+$ 436.06; found: 436.22.

*1,3-dioxo-2-(4-(pyridin-3-ylmethylamino)-3-(pyridin-3-ylmethylcarbamoyl)phenyl)isoindoline-5-carboxylic acid (**46**).* **46** was synthesized by procedures A, C, H and J.

^{1}H-NMR (400 MHz, DMSO-d_6): δ 8.99 (t, *J* = 5.7 Hz, 1H), 8.60 (s, 1H), 8.55 (s, 1H), 8.38-8.52 (m, 4H), 8.29 (s, 1H), 8.06 (d, *J* = 7.7 Hz, 1H), 7.76 (d, *J* = 8.0 Hz, 1H), 7.72 (d, *J* = 8.0 Hz, 1H), 7.67 (s, 1H), 7.33-7.41 (m, 2H), 7.29 (d, *J* = 9.1 Hz, 1H), 6.79 (d, *J* = 8.9 Hz, 1H), 4.52 (d, *J* = 5.8 Hz, 2H), 4.46 (d, *J* = 5.9 Hz, 2H).

ESI-MS *m/z* calcd for $C_{28}H_{21}N_5O_5$ $(M+H)^+$ 508.15; found: 508.34.

*2-(4-(hexylamino)-3-(hexylcarbamoyl)phenyl)-1,3-dioxoisoindoline-5-carboxylic acid (**47**).* **47** was synthesized by procedures A, C, H and J.

^{1}H-NMR (400 MHz, DMSO-d_6): δ 8.42 (dd, *J* = 1.4, 7.8 Hz, 1H), 8.30 (s, 1H), 8.25 (t, *J* = 5.6 Hz, 1H), 8.07 (d, *J* = 7.8 Hz, 1H), 8.03 (t, *J* = 5.5 Hz, 1H), 7.57 (d, *J* = 2.4 Hz, 1H), 7.3 (dd, *J* = 2.3, 8.8 Hz, 1H), 6.78 (d, *J* = 8.97 Hz, 1H), 3.10-3.24 (m, 4H), 1.54-1.63 (m, 2H), 1.43-1.52 (m, 2H), 1.35-1.42 (m, 2H), 1.22-1.34 (m, 10H), 0.82-0.91 (m, 6H).

ESI-MS *m/z* calcd for $C_{28}H_{35}N_3O_5$ $(M+H)^+$ 494.26; found: 493.97.

*2-(3-(3-chloro-4-methylphenylcarbamoyl)-4-(piperidin-1-yl)phenyl)-1,3-dioxoisoindoline-5-carboxylic acid (**48**).* **48** was synthesized by procedures A, C, H and J.

^{1}H-NMR (400 MHz, DMSO-d_6): δ 11.40 (s, 1H), 8.41 (dd, *J* = 1.4, 7.7 Hz, 1H), 8.31 (s, 1H); 8.07 (d, *J* = 7.8 Hz, 1H), 8.02 (d, *J* = 2.0 Hz, 1H), 7.82 (d, *J* = 2.5 Hz, 1H), 7.57 (dd, *J* = 2.6, 8.5 Hz, 1H), 7.49 (dd, *J* = 2.0, 8.2 Hz, 1H), 7.41 (d, *J* = 8.8 Hz, 1H), 7.35 (d, *J* = 8.1 Hz, 1H), 2.96-3.06 (m, 4H), 2.31 (s, 3H), 1.60-1.70 (m, 4H), 1.49-1.57 (m, 2H).

^{13}C-NMR (100.5 MHz, DMSO-d_6): δ 166.42, 166.36, 165.83, 164.64, 151.37, 138.04, 136.44, 136.36, 135.41, 133.17, 132.15, 131.42, 130.71, 130.17, 128.97, 128.85, 126.53, 123.75, 123.31, 120.56, 119.28, 118.01, 53.56, 25.92, 23.28, 18.99.

ESI-MS *m/z* calcd for $C_{28}H_{24}ClN_3O_5$ $(M+H)^+$ 518.14; found: 518.42.

*2-(3-(4-methyl-3-(trifluoromethyl)phenylcarbamoyl)-4-(piperidin-1-yl)phenyl)-1,3-dioxoisoindoline-5-carboxylic acid (**49**).* **49** was synthesized by procedures A, C, H and J.

^{1}H-NMR (400 MHz, DMSO-d_6): δ 13.75 (bs, 1H), 11.52 (s, 1H), 8.42 (dd, *J* = 1.3, 7.6 Hz, 1H), 8.31 (s, 1H), 8.25 (d, *J* = 2.2 Hz, 1H), 8.08 (d, *J* = 7.7 Hz, 1H), 7.79-7.85 (m, 2H), 7.57 (dd, *J* = 2.6, 8.7 Hz, 1H), 7.45 (d, *J* = 8.5 Hz, 1H), 7.42 (d, *J* = 8.9 Hz, 1H), 3.03 (t, *J* = 4.6 Hz, 4H), 2.42 (s, 3H), 1.61-1.69 (m, 4H), 1.49-1.56 (m, 2H).

ESI-MS *m/z* calcd for $C_{29}H_{24}F_3N_3O_5$ $(M+H)^+$ 552.17; found: 552.19.

*2-(3-(3-fluoro-4-methylphenylcarbamoyl)-4-(piperidin-1-yl)phenyl)-1,3-dioxoisoindoline-5-carboxylic acid (**50**).* **50** was synthesized by procedures A, C, H and J.

^{1}H-NMR (400 MHz, DMSO-d_6): δ 13.75 (bs, 1H), 11.46 (s, 1H), 8.42 (d, *J* = 7.8 Hz, 1H), 8.31 (s, 1H), 8.08 (d, *J* = 7.8 Hz, 1H), 7.84 (d, *J* = 2.6 Hz, 1H), 7.74 (dd, *J* = 2.0, 12.2 Hz, 1H), 7.57 (dd, *J* = 2.6, 8.6 Hz, 1H), 7.42 (d, *J* = 8.6 Hz, 1H), 7.35 (dd, *J* = 1.9, 8.3 Hz, 1H), 7.28 (app t, *J* = 8.5 Hz, 1H), 3.02 (t, *J* = 4.5 Hz, 4H), 2.21 (s, 3H), 1.60-1.71 (m, 4H), 1.48-1.58 (m, 2H).

ESI-MS *m/z* calcd for $C_{28}H_{24}FN_3O_5$ $(M+H)^+$ 502.17; found: 502.23.

*2-(4-morpholino-3-(p-tolylcarbamoyl)phenyl)-1,3-dioxoisoindoline-5-carboxylic acid (**51**).*

^{1}H-NMR (400 MHz, DMSO-d_6): δ 13.75 (bs, 1H), 10.92 (s, 1H), 8.42 (dd, *J* = 1.5, 7.7 Hz, 1H), 8.31 (s, 1H), 8.08 (d, *J* = 7.7 Hz, 1H), 7.77 (d, *J* = 2.5 Hz, 1H), 7.67 (d, *J* = 8.5 Hz, 2H), 7.56 (dd, *J* = 2.6, 8.6 Hz, 1H), 7.39 (d, *J* = 8.7 Hz, 1H), 7.18 (d, *J* = 8.5 Hz, 2H), 3.65-3.76 (m, 4H), 2.99-3.09 (m, 4H), 2.28 (s, 3H).

ESI-MS *m/z* calcd for $C_{27}H_{23}N_3O_6$ $(M+H)^+$ 486.16; found: 485.93.

*2-(4-(4-methylpiperazin-1-yl)-3-(p-tolylcarbamoyl)phenyl)-1,3-dioxoisoindoline-5-carboxylic acid (**52**).* **52** was synthesized by procedures A, C, H and J.

^{1}H-NMR (400 MHz, DMSO-d_6): δ 10.97 (s, 1H), 8.41 (d, *J* = 7.8 Hz, 1H), 8.31 (s, 1H), 8.05 (d, *J* = 7.8 Hz, 1H), 7.78 (s, 1H), 7.66 (d, *J* = 8.0 Hz, 2H), 7.56 (d, *J* = 8.6 Hz, 1H), 7.40 (d, *J* = 8.6 Hz, 1H), 7.18 (d, *J* = 7.9 Hz, 2H), 3.06-3.16 (m, 4H), 2.57-2.71 (m, 4H), 2.32 (s, 3H), 2.28 (s, 3H).

ESI-MS *m/z* calcd for $C_{28}H_{26}N_4O_5$ $(M+H)^+$ 499.19; found: 498.92.

*2-(4-(2-methoxyethylamino)-3-(p-tolylcarbamoyl)phenyl)-1,3-dioxoisoindoline-5-carboxylic acid (**53**).* **53** was synthesized by procedures A, C, H and J.

^{1}H-NMR (400 MHz, DMSO-d_6): δ 13.77 (bs, 1H), 10.01 (s, 1H), 8.43 (dd, *J* = 1.1, 8.0 Hz, 1H), 8.32 (s, 1H), 8.09 (d, *J* = 7.7 Hz, 1H), 7.74 (m, 2H), 7.54 (d, *J* = 8.1 Hz, 2H), 7.39 (dd, *J* = 2.3, 8.9 Hz, 1H), 7.13 (d, *J* = 8.0 Hz, 2H), 6.89 (d, *J* = 9.0 Hz, 1H), 3.57 (t, *J* = 5.2 Hz, 2H), 3.33-3.40 (m, 2H), 3.31 (s, 3H), 2.27 (s, 3H).

ESI-MS *m/z* calcd for $C_{26}H_{23}N_3O_6$ $(M+H)^+$ 474.16; found: 474.20.

*2-(4-(2-(2-methoxyethoxy)ethoxy)-3-(p-tolylcarbamoyl)phenyl)-1,3-dioxoisoindoline-5-carboxylic acid (**54**).* **54** was synthesized by procedures A, G, H and J.

^{1}H-NMR (400 MHz, DMSO-d_6): δ 10.16 (s, 1H), 8.42 (dd, *J* = 1.4, 7.8 Hz, 1H), 8.31 (s, 1H), 8.08 (d, *J* = 7.8 Hz, 1H), 7.91 (d, *J* = 2.6 Hz, 1H), 7.56-7.68 (m, 3H), 7.39 (dd, *J* = 8.9 Hz, 1H), 7.16 (d, *J* = 8.1 Hz, 2H), 4.39 (bt, *J* = 4.2 Hz, 2H), 3.89 (bt, *J* = 4.1 Hz, 2H), 3.62 (dd, *J* = 4.4, 5.6 Hz, 2H), 3.39 (dd, *J* = 4.4, 5.0 Hz, 2H), 3.15 (s, 3H), 2.28 (s, 3H).

ESI-MS *m/z* calcd for $C_{28}H_{26}N_2O_8$ $(M+H)^+$ 518.17; found: 518.89.

*2-(4-methoxy-3-(p-tolylcarbamoyl)phenyl)-1,3-dioxoisoindoline-5-carboxylic acid (**55**).*

^{1}H-NMR (400 MHz, DMSO-d_6): δ 13.76 (bs, 1H), 10.15 (s, 1H), 8.41 (dd, *J* = 1.4, 7.8 Hz, 1H), 8.30 (s, 1H), 8.08 (d, *J* = 7.7 Hz, 1H), 7.70 (d, *J* = 2.6 Hz, 1H), 7.62 (d, *J* = 8.1 Hz, 2H), 7.58 (dd, *J* = 2.7, 8.9 Hz, 1H), 7.34 (d, *J* = 8.9 Hz, 1H), 7.15 (d, *J* = 8.1 Hz, 2H), 3.96 (s, 3H), 2.27 (s, 3H).

ESI-MS *m/z* calcd for $C_{24}H_{18}N_2O_6$ $(M+H)^+$ 431.12; found: 430.97.

*2-(4-chloro-3-(p-tolylcarbamoyl)phenyl)-1,3-dioxoisoindoline-5-carboxylic acid (**56**).*

^{1}H-NMR (400 MHz, DMSO-d_6): δ 13.79 (bs, 1H), 10.57 (s, 1H), 8.43 (dd, *J* = 1.5, 7.7 Hz, 1H), 8.33 (s, 1H), 8.11 (d, *J* = 7.7 Hz, 1H), 7.76 (d, *J* = 8.6 Hz, 1H), 7.68 (d, *J* = 2.5 Hz, 1H), 7.56-7.64 (m, 3H), 7.16 (d, *J* = 8.4 Hz, 2H), 2.27 (s, 3H).

ESI-MS *m/z* calcd for $C_{23}H_{15}ClN_2O_5$ $(M+H)^+$ 435.07; found: 434.87.

*1,3-dioxo-2-(4-(phenylamino)-3-(p-tolylcarbamoyl)phenyl)isoindoline-5-carboxylic acid (**57**).*

^{1}H-NMR (400 MHz, DMSO-d_6): δ 13.77 (bs, 1H), 10.29 (s, 1H), 9.23 (s, 1H), 8.44 (dd, *J* = 1.5, 7.7 Hz, 1H), 8.33 (s, 1H), 8.10 (d, *J* = 7.8 Hz, 1H), 7.81 (d, *J* = 2.1 Hz, 1H), 7.56 (d, *J* = 8.4 Hz, 2H), 7.38-7.48 (m, 2H), 7.34 (dd, *J* = 7.3, 8.3 Hz, 2H), 7.23 (d, *J* = 7.6 Hz, 2H), 7.14 (d, *J* = 8.6 Hz, 2H), 7.02 (t, *J* = 7.2 Hz, 1H), 2.27 (s, 3H).

^{13}C-NMR (100.5 MHz, DMSO-d_6): δ 166.55, 166.52, 165.81, 165.33, 144.02, 141.19, 136.57, 136.10, 135.58, 134.80, 132.94, 131.95, 131.21, 129.43, 128.97, 128.42, 123.83, 123.37, 122.30, 121.91, 120.65, 120.52, 119.94, 115.74, 20.49.

ESI-MS *m/z* calcd for $C_{29}H_{21}N_3O_5$ $(M+H)^+$ 492.15; found: 491.97.

*2-(3-(3-ethylphenylcarbamoyl)-4-(phenylamino)phenyl)-1,3-dioxoisoindoline-5-carboxylic acid (**58**).* **58** was synthesized by procedures A, C, H and J.

^{1}H-NMR (400 MHz, DMSO-d_6): δ 13.77 (bs, 1H), 10.30 (s, 1H), 9.21 (s, 1H), 8.44 (dd, *J* = 1.5, 7.7 Hz, 1H), 8.33 (s, 1H), 8.1 (d, *J* = 7.8 Hz, 1H), 7.82 (d, *J* = 2.3 Hz, 1H), 7.55 (t, *J* = 2.0 Hz, 1H), 7.51 (dd, *J* = 2.2, 8.2 Hz, 1H), 7.39-7.48 (m, 2H), 7.34 (t, *J* = 7.8 Hz, 2H), 7.21-7.28 (m, 3H), 7.02 (t, *J* = 7.3 Hz, 1H), 6.96 (d, *J* = 7.6 Hz, 1H), 2.58 (q, *J* = 7.6 Hz, 2H), 1.16 (t, *J* = 7.6 Hz, 3H).

^{13}C-NMR (100.5 MHz, DMSO-d_6): δ 166.62, 166.54, 166.52, 165.80, 144.13, 144.05, 141.19, 138.63, 136.57, 135.58, 134.79, 131.94, 131.25, 129.43, 128.47, 123.83, 123.45, 123.37, 122.32, 121.90, 120.53, 120.01, 118.06, 115.76, 28.26, 15.55.

ESI-MS *m/z* calcd for $C_{30}H_{23}N_3O_5$ $(M+H)^+$ 506.16; found: 506.24.

*2-(4-(diethylamino)-3-(p-tolylcarbamoyl)phenyl)-1,3-dioxoisoindoline-5-carboxylic acid (**59**).*

^{1}H-NMR (400 MHz, DMSO-d_6): δ 13.72 (bs, 1H), 12.50 (s, 1H), 8.42 (dd, *J* = 1.5, 7.7 Hz, 1H), 8.32 (s, 1H), 8.09 (d, *J* = 7.6 Hz, 1H), 8.07 (d, *J* = 2.6 Hz, 1H), 7.53-7.66 (m, 4H), 7.18 (d, *J* = 8.6 Hz, 2H), 3.19 (q, *J* = 7.2 Hz, 4H), 2.29 (s, 3H), 1.03 (t, *J* = 7.1 Hz, 6H).

ESI-MS *m/z* calcd for $C_{27}H_{25}N_3O_5$ $(M+H)^+$ 472.18; found: 471.95.

*2-(4-(Hexylamino)-3-(p-tolylcarbamoyl)phenyl)-1,3-dioxoisoindoline-5-carboxylic acid (**60**).* **60** was synthesized by procedures A, C, H and J.

^{1}H-NMR (400 MHz, DMSO-d_6): δ 13.78 (bs, 1H), 10.00 (s, 1H), 8.43 (dd, *J* = 1.5, 7.7 Hz, 1H), 8.31 (s, 1H), 8.09 (d, *J* = 7.7 Hz, 1H), 7.74 (d, *J* = 2.4 Hz, 1H), 7.68 (t, *J* = 5.0 Hz, 1H), 7.54 (d, *J* = 8.5 Hz, 2H), 7.38 (dd, *J* = 2.4, 8.8 Hz, 1H), 7.13 (d, *J* = 8.7 Hz, 2H), 6.85 (d, *J* = 9.0 Hz, 1H), 3.15-3.24 (m, 2H), 2.27 (s, 3H), 1.61 (quint, *J* = 7.0 Hz, 2H), 1.25-1.45 (m, 6H), 0.88 (t, *J* = 7.0 Hz, 3H).

^{13}C-NMR (100.5 MHz, DMSO-d_6): δ 167.14, 166.76, 166.73, 165.81, 149.30, 136.52, 136.24, 135.56, 134.78, 132.66, 132.00, 131.91, 129.01, 128.93, 128.91, 128.16, 123.76, 123.31, 120.77, 120.75, 117.89, 114.91, 111.38, 42.28, 30.99, 28.48, 26.25, 22.08, 20.47, 13.91.

ESI-MS *m/z* calcd for $C_{29}H_{29}N_3O_5$ $(M+H)^+$ 500.21; found: 500.34.

*2-(4-(decylamino)-3-(p-tolylcarbamoyl)phenyl)-1,3-dioxoisoindoline-5-carboxylic acid (**61**).* **61** was synthesized by procedures A, C, H and J.

^{1}H-NMR (400 MHz, DMSO-d_6): δ 13.74 (bs, 1H), 9.99 (s, 1H), 8.43 (dd, *J* = 1.5, 7.7 Hz, 1H), 8.31 (s, 1H), 8.09 (d, *J* = 7.8 Hz, 1H), 7.74 (d, *J* = 2.4 Hz, 1H), 7.69 (t, *J* = 4.8 Hz, 1H), 7.54 (d, *J* = 8.4 Hz, 2H), 7.38 (dd, *J* = 2.3, 8.8 Hz, 1H), 7.13 (d, *J* = 8.4 Hz, 2H), 6.84 (d, *J* = 9.0 Hz, 1H), 3.13-3.24 (m, 2H), 2.27 (s, 3H), 1.54-1.65 (m, 2H), 1.15-1.45 (m, 14H), 0.85 (t, *J* = 6.4 Hz, 3H).

ESI-MS *m/z* calcd for $C_{33}H_{37}N_3O_5$ $(M+H)^+$ 556.27; found: 555.98.

*1,3-dioxo-2-(4-(propylamino)-3-(p-tolylcarbamoyl)phenyl)isoindoline-5-carboxylic acid (**62**).* **62** was synthesized by procedures A, C, H and J.

^{1}H-NMR (400 MHz, DMSO-d_6): δ 13.74 (bs, 1H), 10.00 (s, 1H), 8.43 (dd, *J* = 1.4, 7.8 Hz, 1H), 8.32 (s, 1H), 8.09 (d, *J* = 7.7 Hz, 1H), 7.75 (d, *J* = 2.4 Hz, 1H), 7.71 (t, *J* = 5.5 Hz, 1H), 7.54 (d, *J* = 8.4 Hz, 2H), 7.38 (dd, *J* = 2.3, 8.9 Hz, 1H), 7.13 (d, *J* = 8.1 Hz, 2H), 6.86 (d, *J* = 9.0 Hz, 1H), 3.13-3.21 (m, 2H), 2.27 (s, 3H), 1.57-1.69 (m, 2H), 0.98 (t, *J* = 7.4 Hz, 3H).

ESI-MS *m/z* calcd for $C_{26}H_{23}N_3O_5$ $(M+H)^+$ 458.16; found: 457.87.

*1,3-dioxo-2-(4-(piperidin-1-yl)-3-(pyridin-3-ylmethylcarbamoyl)phenyl)isoindoline-5-carboxylic acid (**63**).* **63** was synthesized by procedures A, C, H and J.

^{1}H-NMR (400 MHz, DMSO-d_6): δ 9.89 (t, *J* = 5.9 Hz, 1H), 8.62 (s, 1H), 8.48 (d, *J* = 4.7 Hz, 1H), 8.4 (d, *J* = 7.5 Hz, 1H), 8.28 (s, 1H), 7.95 (d, *J* = 7.6 Hz, 1H), 7.77-7.84 (m, 2H), 7.51 (dd, *J* = 2.6, 8.8 Hz, 1H), 7.33-7.42 (m, 2H), 4.56 (d, *J* = 5.6 Hz, 2H), 2.83-2.93 (m, 4H), 1.38-1.54 (m, 6H).

ESI-MS *m/z* calcd for $C_{27}H_{24}N_4O_5$ $(M+H)^+$ 485.17; found: 485.41.

*2-(4-bromo-3-(p-tolylcarbamoyl)phenyl)-1,3-dioxoisoindoline-5-carboxylic acid (**64**).*

^{1}H-NMR (400 MHz, DMSO-d_6): δ 13.78 (bs, 1H), 10.54 (s, 1H), 8.43 (d, *J* = 7.8 Hz, 1H), 8.32 (s, 1H), 8.10 (d, *J* = 7.8 Hz, 1H), 7.90 (d, *J* = 8.5 Hz, 1H), 7.65 (d, *J* = 2.4 Hz, 1H), 7.59 (d, *J* = 8.1 Hz, 2H), 7.53 (dd, *J* = 2.3, 8.5 Hz, 1H), 7.16 (d, *J* = 8.0 Hz, 2H), 2.28 (s, 3H).

ESI-MS *m/z* calcd for $C_{23}H_{15}BrN_2O_5$ $(M+H)^+$ 479.02; found: 478.81.

*2-((4-(piperidin-1-yl)-3-(p-tolylcarbamoyl)phenyl)carbamoyl)terephthalic acid (**65**).* **65** was synthesized by procedures A, C, H and N.

^{1}H-NMR (400 MHz, DMSO-d_6): δ 13.06 (bs, 1H), 12.20 (s, 1H), 10.62 (bs, 1H), 8.25 (d, *J* = 2.7 Hz, 1H), 8.10 (dd, *J* = 1.9, 7.8 Hz, 1H), 8.05 (s, 1H), 7.95 (d, *J* = 8.0 Hz, 1H), 7.87 (dd,

J = 2.6, 8.8 Hz, 2H), 7.66 (d, *J* = 8.4 Hz, 2H), 7.39 (d, *J* = 8.7 Hz, 1H), 7.19 (d, *J* = 8.5 Hz, 2H), 2.89-2.98 (m, 4H), 2.29 (s, 3H), 1.66-1.76 (m, 4H), 1.51-1.61 (m, 2H).

ESI-MS m/z calcd for $C_{28}H_{27}N_3O_6$ $(M+H)^+$ 502.19; found: 501.92.

*4-((4-(piperidin-1-yl)-3-(p-tolylcarbamoyl)phenyl)carbamoyl)isophthalic acid (**66**)*. **66** was synthesized by procedures A, C, H and N.

^{1}H-NMR (400 MHz, DMSO-d_6): δ 12.20 (s, 1H), 8.34 (s, 1H), 8.24 (d, *J* = 2.7 Hz, 1H), 8.05 (d, *J* = 7.3 Hz, 1H), 7.88 (dd, *J* = 2.7, 8.8 Hz, 1H), 7.77 (d, *J* = 8.0 Hz, 1H), 7.66 (d, *J* = 8.4 Hz, 2H), 7.38 (d, *J* = 8.8 Hz, 1H), 7.19 (d, *J* = 8.3 Hz, 2H), 2.90-2.98 (m, 4H), 2.29 (s, 3H), 1.66-1.75 (m, 4H), 1.51-1.61 (m, 2H).

ESI-MS m/z calcd for $C_{28}H_{27}N_3O_6$ $(M+H)^+$ 502.19; found: 501.96.

*2-(4-(piperidin-1-yl)-3-(p-tolylcarbamoyl)phenyl)-1H-benzo[d]imidazole-5-carboxylic acid (**71**)*. **71** was synthesized by procedures A, C, H, O, P, Q and R.

^{1}H-NMR (400 MHz, DMSO-d_6): δ 10.63 (s, 1H), 8.44 (d, *J* = 2.4Hz, 1H), 8.29 (dd, *J* = 2.4, 8.9 Hz, 1H), 8.24 (s, 1H), 8.03 (d, *J* = 8.6 Hz, 1H), 7.81 (d, *J* = 8.9 Hz, 1H), 7.66 (d, *J* = 8.1 Hz, 2H), 7.41 (d, *J* = 8.4 Hz, 1H), 7.19 (d, *J* = 8.1 Hz, 1H), 5.73 (s, 1H), 3.08-3.16 (m, 4H), 2.29 (s, 3H), 1.59-1.68 (m, 4H), 1.49-1.57 (m, 2H).

^{13}C-NMR (100.5 MHz, DMSO-d_6): δ 167.05, 165.45, 154.17, 151.28, 136.76, 136.52, 133.63, 132.87, 130.69, 129.92, 129.46, 128.55, 127.27, 126.01, 119.58, 119.48, 115.71, 115.47, 114.01, 52.43, 25.61, 23.43, 20.62.

ESI-MS m/z calcd for $C_{27}H_{26}N_4O_3$ $(M+H)^+$ 455.20; found: 455.24.

*2-(4-(piperidin-1-yl)-3-(p-tolylcarbamoyl)phenyl)benzofuran-5-carboxylic acid (**77**)*. **77** was synthesized by procedures A, C, H, O, S, T and R.

^{1}H-NMR (400 MHz, DMSO-d_6): δ 12.89 (bs, 1H), 11.15 (s, 1H), 8.25 (dd, *J* = 2.3, 6.1 Hz, 1H), 8.01 (dd, *J* = 2.2, 8.6 Hz, 1H), 7.91 (dd, *J* = 2.0, 8.6 Hz, 1H), 7.73 (d, *J* = 8.6 Hz, 1H),

7.68 (d, *J* = 8.5 Hz, 2H), 7.52 (s, 1H), 7.37 (d, *J* = 8.6 Hz, 1H), 7.19 (d, *J* = 8.3 Hz, 2H), 2.99-3.06 (m, 4H), 2.29 (s, 3H), 1.61-1.69 (m, 4H), 1.50-1.56 (m, 2H).

^{13}C-NMR (100.5 MHz, DMSO-d_6): δ 167.42, 164.67, 156.52, 156.03, 152.13, 136.50, 132.52, 129.38, 129.14, 127.92, 126.49, 125.91, 123.51, 122.91, 120.55, 119.37, 111.04, 101.66, 53.55, 25.84, 23.35, 20.53.

ESI-MS m/z calcd for $C_{28}H_{26}N_2O_4$ $(M+H)^+$ 455.19; found: 455.30.

*2-(4-(piperidin-1-yl)-3-(p-tolylcarbamoyl)phenyl)-1H-indole-5-carboxylic acid (**78**).* **78** was synthesized by procedures A, C, H, O, S, T and R. (Note: methyl 3-iodo-4-(2,2,2-trifluoroacetamido)benzoate (**76**) was used instead of **75** in procedure T)

^{1}H-NMR (400 MHz, DMSO-d_6): δ 12.39 (bs, 1H), 11.98 (s, 1H), 11.36 (s, 1H), 8.29 (d, *J* = 2.4 Hz, 1H), 8.21 (s, 1H), 7.99 (dd, *J* = 2.4, 8.3 Hz, 1H), 7.66-7.75 (m, 3H), 7.43 (d, *J* = 8.6 Hz, 1H), 7.40 (d, *J* = 8.6 Hz, 1H), 7.20 (d, *J* = 8.3 Hz, 2H), 7.03 (d, *J* = 1.4 Hz, 1H), 2.96-3.09 (m, 4H), 2.29 (s, 3H), 1.62-1.73 (m, 4H), 1.46-1.59 (m, 2H).

^{13}C-NMR (100.5 MHz, DMSO-d_6): δ 168.48, 164.80, 150.73, 139.77, 138.59, 136.53, 132.72, 129.51, 128.99, 128.58, 128.35, 127.00, 126.74, 122.93, 122.73, 122.05, 121.08, 119.56, 111.13, 99.74, 53.88, 25.95, 23.29, 20.61.

ESI-MS m/z calcd for $C_{28}H_{27}N_3O_3$ $(M+H)^+$ 454.21; found: 454.20.

*2-(4-(piperidin-1-yl)-3-(p-tolylcarbamoyl)phenyl)-2H-indazole-6-carboxylic acid (**80**).* **80** was synthesized by procedures A, C, I and U.

^{1}H-NMR (400 MHz, DMSO-d_6): δ 13.04 (s, 1H), 11.37 (s, 1H), 9.25 (s, 1H), 8.47 (d, *J* = 2.8 Hz, 1H), 8.37 (s, 1H), 8.21 (dd, *J* = 2.9, 8.6 Hz, 1H), 7.85 (d, *J* = 8.7 Hz, 1H), 7.70 (d, *J* = 8.4 Hz, 2H), 7.62 (dd, *J* = 1.2, 8.8 Hz, 1H), 7.48 (d, *J* = 8.8 Hz, 1H), 7.20 (d, *J* = 8.4 Hz, 2H), 2.99-3.07 (m, 4H), 2.29 (s, 3H), 1.62-1.72 (m, 4H), 1.49-1.59 (m, 2H).

^{13}C-NMR (100.5 MHz, DMSO-d_6): δ 167.62, 163.86, 151.38, 148.17, 136.35, 134.85, 132.61, 129.65, 129.50, 129.38, 124.26, 123.30, 122.06, 121.57, 121.45, 121.07, 120.29, 119.39, 53.58, 25.87, 23.26, 20.49.

ESI-MS *m/z* calcd for $C_{27}H_{26}N_4O_3$ $(M+H)^+$ 455.20; found: 455.14.

*2-(4-(piperidin-1-yl)-3-(p-tolylcarbamoyl)phenyl)-2H-indazole-5-carboxylic acid (**81**).* **81** was synthesized by procedures A, C, I and U.

^{1}H-NMR (400 MHz, DMSO-d_6): δ 12.84 (s, 1H), 11.38 (s, 1H), 9.35 (s, 1H), 8.49 (s, 1H), 8.46 (d, *J* = 2.8 Hz, 1H), 8.20 (dd, *J* = 2.8, 8.8 Hz, 1H), 7.82 (dd, *J* = 1.6, 9.0 Hz, 1H), 7.77 (d, *J* = 9.3 Hz, 1H), 7.69 (d, *J* = 8.4 Hz, 2H), 7.49 (d, *J* = 8.9 Hz, 1H), 7.20 (d, *J* = 8.6 Hz, 2H), 2.99-3.08 (m, 4H), 2.30 (s, 3H), 1.63-1.73 (m, 4H), 1.49-1.58 (m, 2H).

^{13}C-NMR (100.5 MHz, DMSO-d_6): δ 167.46, 163.86, 151.18, 149.88, 136.32, 134.81, 132.63, 129.60, 129.37, 126.39, 125.08, 124.49, 124.24, 123.28, 121.96, 121.88, 121.53, 119.42, 117.26, 53.63, 25.84, 23.20, 20.49.

ESI-MS *m/z* calcd for $C_{27}H_{26}N_4O_3$ $(M+H)^+$ 455.20; found: 455.08.

*5-(2H-indazol-2-yl)-2-(piperidin-1-yl)-N-(p-tolyl)benzamide (**82**).* **82** was synthesized by procedures A, C, I and U.

^{1}H-NMR (400 MHz, DMSO-d_6): δ 11.50 (s, 1H), 9.12 (s, 1H), 8.48 (d, *J* = 2.8 Hz, 1H), 8.18 (dd, *J* = 2.9, 8.8 Hz, 1H), 7.74 (dd, *J* = 8.5, 16.8 Hz, 2H), 7.69 (d, *J* = 8.5 Hz, 2H), 7.47 (d, *J* = 8.8 Hz, 1H), 7.31 (dd, *J* = 6.6, 8.9 Hz, 1H), 7.20 (d, *J* = 8.8 Hz, 2H), 7.10 (dd, *J* = 6.6, 8.5 Hz, 1H), 2.96-3.06 (m, 4H), 2.29 (s, 3H), 1.62-1.73 (m, 4H), 1.48-1.58 (m, 2H).

^{13}C-NMR (100.5 MHz, DMSO-d_6): δ 163.82, 151.01, 148.92, 136.38, 135.28, 132.59, 129.62, 129.41, 126.76, 123.05, 122.53, 122.07, 121.80, 121.59, 121.45, 120.89, 119.38, 117.38, 53.68, 25.92, 23.26, 20.51.

ESI-MS *m/z* calcd for $C_{26}H_{26}N_4O$ $(M+H)^+$ 411.21; found: 411.22.

4.4. Enzymatic activity assay

All LPL activity measurements were performed using Intralipid® as substrate as described previously.[21] Compounds (1 µL in DMSO) or pure DMSO (in controls) were added to 49 µL of assay buffer (50 mM potassium phosphate pH 7.4 containing 0.15 M NaCl and 0.01% (v/v) Triton X-100). The N-terminal part (a.a. 26-184) of recombinant human ANGPTL4 (5 µL of 900 nM ANGPTL4 in assay buffer yielding a final concentration of 75 nM) and bovine LPL (5 µL of 180 nM LPL in assay buffer yielding a final concentration of 15 nM) were then added. Addition of Triton X-100 to the assay buffer was a derivative of our initial HTS campaign to prevent the rate of frequent hitters as recommended by Feng and Shoichet.[50] In addition the detergent served to prevent precipitation of the compounds and also increased the potency of ANGPTL4 in terms of inactivating LPL—thus lowering the tight-binding limit of the assay. The mixtures were incubated for 10 min at 25 °C in 96-well microtiter plates (Nunc, Cat No. 236108) on an orbital shaker (600 rpm). The remaining LPL activity was then determined by addition of 90 µL of substrate mix with the following final concentrations: 0.15 M Tris, pH 8.5, 0.1 M NaCl, 6% BSA, 5% heat-inactivated rat serum, 16.7 units of heparin/mL, and Intralipid® corresponding to 2 mg TG/mL. The LPL-mediated hydrolysis was stopped after 45 min by addition of Triton X-100 to a final concentration of 2.5% (v/v). The released fatty acids were quantified using the kit NEFA HR2 (Wako Chemicals). The activity is reported as normalized percent of control, ((sample mean)-(mean negative control))/((mean positive control)-(mean negative control))*100 where the negative control is DMSO+LPL+ANGPTL4 and the positive control is DMSO+LPL only. In the dose-response analysis the ratio between LPL and the new batch of ANGPTL4 was adjusted to achieve the same level of inactivation as in previous experiments.

4.5. Plasma protein binding

We utilized Rapid Equilibrium Dialysis (RED) device inserts which allow for short dialysis times (2-4h) as compared to traditional methods (> 8h) and minimal drug consumption in a 96-well format. Pooled human plasma was provided by Uppsala Academic Hospital and was collected from two male and two female donors (non-smoking). In brief, 0.2 ml of the plasma test solution with typically 10µM final compound concentration was transferred to the membrane tube in the RED insert. Then 0.35 ml isotonic phosphate buffer pH 7.4 was added to the other side of the membrane. The 96-well base plate was then sealed with an adhesive plastic film (Scotch Pad) to prevent evaporation. The plate was incubated with rapid rotation (□ 900 rpm) on a Kisker rotational incubator at 37 □C for 4 h to achieve equilibrium.

A stability test of the test solution was also prepared (to allow detection of drug degradation). For this, 100 µL of the plasma test solution (in a plastic vial or on a sealed plate) was incubated at 37 □C for 4 h (as long as the dialysis time). The plasma test solution was frozen directly after the administration to prevent degradation. After incubation, the contents of each plasma and buffer compartment were removed and immediately frozen until analysis. Prior to LC-MS/MS analysis the samples were mixed with equal volumes of control buffer or plasma as appropriate to maintain matrix similarity for analysis. Plasma proteins were then precipitated by the addition of methanol (1:4) containing Warfarin as analytical internal standard. The plate was then sealed, centrifuged and the supernatant was analyzed by mass spectrometry (LC-MS/MS). The following system was used: Waters XEVO TQ triple-quadrupole mass spectrometer (electrospray ionization, ESI) coupled to a Waters Acquity UPLC (Waters Corp.). For chromatographic separation a general gradient was used (1% mobile phase B to 90% over 2 min total run) on a C18 BEH 1.7µm column 2 x 50mm (Waters Corp.). The mobile phase A consisted of 5% acetonitrile 0.1% formic acid and the mobile phase B 100% acetonitrile 0.1% formic acid. The flow rate was 0.5 ml/min. Five µL of the sample were injected and run with the mass spectrometric settings reported in table 1.

For analyses of plasma protein binding or Caco-2 permeability a standard curve between 1-1000 nM was prepared. No detailed method validation was performed and an accurate limit of detection is therefore not known. The method sensitivity was however very good with an estimated limit of detection < 1 nM. Analysis of chemical/metabolic stability does not require a standard curve since it is quantified on a relative basis. Chemical/metabolic stability in plasma was quantified on a relative basis.

4.6. Kinetic solubility

All testing was made from a 10 mM DMSO stock solution and analysis with LC-MS/MS as described below. Studies on solubility were performed in PBS (pH 7.4) and in an HCl-adjusted PBS to low pH, approximately pH 1.2. The study was performed by addition of 1 µl DMSO stock per 100 µl buffer, (1 % DMSO, theoretical maximal detectable solubility = 0.1 mM) and incubation at 37 °C for 2 h in sealed glass vials. After the incubation an aliquot of the solution was transferred to a glass vial insert and centrifuged at 10000 x g, 37 °C, for 20 min.

4.7. Caco-2 permeability

The Caco-2 study was performed in accordance with published protocols.[39] Caco-2 cell monolayers (passage 94-105) were grown on permeable filter support and used for transport study on day 21 after seeding. Prior to the experiment a drug solution of 10 µM was prepared and warmed to 37 □C. The Caco-2 filters were washed with pre-warmed Hank's balance salt measurement (HBSS) prior to the experiment, and thereafter the experiment was started by applying the donor solution on the apical side. The transport experiments were carried out at pH 6.5 in the apical chamber, reflecting the pH of the intestinal lumen, and pH 7.4 in the basolateral chamber reflecting the pH of the blood. The experiments were performed at 37 □C and with a stirring rate of 500 rpm. The receiver compartment was sampled at 15, 30 and

60 minutes, and at 60 minutes also a final sample from the donor chamber was taken in order to calculate the mass balance of the compound.

Directly after the termination of the experiment the filter inserts were washed with pre-warmed HBSS and the membrane integrity was checked. This was performed by transepithelial electrical resistance (TEER) measurement and by measurement of Mannitol permeability, which is a paracellular marker used for integrity measurements.

4.8. Metabolic stability

The microsomal metabolic stability assay utilized pooled human or animal species, liver microsomes (LM) with supplemented cofactor (NADPH) to facilitate CYP reactivity against target compound. Human hepatocytes (HHep) were freshly prepared at UDOPP (liver from surgery at the academic hospital in Uppsala on the same day). For incubation, target compound (1μM in the incubation) and LM (0.5 mg/ml) or hepatocytes (0.5 x 10^6 cells/ml) were diluted in 0.1M phosphate buffer pH 7.4 (LM) or Williams medium E (HHep). The incubation volume was 150 μL. The reaction with LM was initiated with addition of NADPH (1mM incubation concentration) in buffer or by the addition of cells with HHep. The incubation times were 0, 5, 15, 40 min (in duplicate for LM) or 0, 10, 30, 60 min (in duplicate for HHep) and the reaction was quenched, at each time point, by addition of 100 μL acetonitrile containing Warfarin as the analytical internal standard. The plate was then sealed, centrifuged and frozen at -20 □C until LC-MS/MS analysis.

4.9. Animal procedures and in vivo experiments

Female C57B16/J mice (Taconic, Denmark) aged 8-9 weeks with an average weight of 18 g were used for the *in vivo* experiments (n = 8/group). The mice were housed at room temperature with free access to tap water and standard rodent diet at 12:12h light cycle with the light off at 18.00-06.00. The mice were treated for 3 consecutive days with 0.5 ml

intraperitoneal injections of PBS (pH 7.4), DMSO (3%, v/v) and Triton X-100 (0.2%, v/v), containing 1 mM compound. On the 3rd day the mice were fasted 8 h from midnight and injected a final 4th time in the morning. One hour after the final injection the mice were given a 0.2 ml oral olive oil gavage. Blood samples were collected from the tail vein in EDTA coated capillary tubes at baseline and for every 60 min until 180 min. The blood was immediately centrifuged (+4 □C for 10 min at 1500 RCF) and the plasma was stored at -80 □C. After the final blood sample, the mice were sacrificed.

Plasma was analyzed with Trig/BG (Roche/Hitachi, Mannheim, Germany) in micro titer plates according to manufacturer instructions.

The local animal ethics committee at Umeå University approved all animal experiments (case number: A54/11).

Statistics were calculated using GraphPad Prism (GraphPad Software, Inc, La Jolla, CA) or Student's t-test for repeated measures unless otherwise stated.

5. Nuclear receptor profiling

Compound **80** was profiled at ten concentrations against the nuclear receptors FXR, PPARα, PPARγ, and RXRα for agonist activity using the Life Technologies' SelectScreen® Profiling Service (www.lifetechnologies.com). For all receptors EC_{50} values exceeded 100 μM.

Appendix A. Supplementary data.

Supplementary data associated with this article can be found, in the online version, at www.sciencedirect.com.

Notes

ML, SKN, GO, PAE, and ME are shareholders in Lipigon Pharmaceuticals AB that has filed a patent application that includes the compounds in this article and their use. This does not alter the authors' adherence to policies on sharing data and materials.

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Abbreviations

ADME, absorption, distribution, metabolism and excretion; ANGPTL, angiopoietin-like protein; apo-, apolipoprotein; BINOL, 1,1'-Bi-2-naphthol; BSA, bovine serum albumin; CAD, Coronary Artery Disease; EC_{50}, effective concentration 50%; LPL, lipoprotein lipase; SAR, structure activity relationship; TG, triacylglycerol; VLDL, very low density lipoprotein.

References

[1] M. Langlois, Laboratory approaches for predicting and managing the risk of cardiovascular disease: postanalytical opportunities of lipid and lipoprotein testing, Clinical chemistry and laboratory medicine : CCLM / FESCC, 50 (2011) 1169-1181.

[2] U. Khot, M. Khot, C. Bajzer, S. Sapp, E. Ohman, S. Brener, S. Ellis, A. Lincoff, E. Topol, Prevalence of conventional risk factors in patients with coronary heart disease, J. Am. Med. Assoc., 290 (2003) 898-904.

[3] P. Jousilahti, E. Vartiainen, J. Tuomilehto, P. Puska, Sex, Age, Cardiovascular Risk Factors, and Coronary Heart Disease : A Prospective Follow-Up Study of 14 786 Middle-Aged Men and Women in Finland, Circulation, 99 (1999).

[4] E. Barrett-Connor, K. Khaw, Family history of heart attack as an independent predictor of death due to cardiovascular disease, Circulation, 69 (1984).

[5] H.B. Hubert, M. Feinleib, P.M. McNamara, W.P. Castelli, Obesity as an independent risk factor for cardiovascular disease: a 26- year follow-up of participants in the Framingham Heart Study, Circulation, 67 (1983).

[6] W. Kannel, D. McGee, Diabetes and glucose tolerance as risk factors for cardiovascular disease: the Framingham study, Diabetes care, 2 (1979) 120-126.

[7] P.W.F. Wilson, R.B.D. Agostino, D. Levy, A.M. Belanger, H. Silbershatz, W.B. Kannel, Prediction of Coronary Heart Disease Using Risk Factor Categories, Circulation, 97 (1998).

[8] P. Cullen, Evidence that triglycerides are an independent coronary heart disease risk factor, Am. J. Cardiol., 86 (2000) 943-949.

[9] A. Boullart, J. de Graaf, A. Stalenhoef, Serum triglycerides and risk of cardiovascular disease, Biochim. Biophys. acta, 1821 (2012) 867-875.

[10] G. Yuan, K. Al-Shali, R. Hegele, Hypertriglyceridemia: its etiology, effects and treatment, Can. Med. Assoc. J., 176 (2007) 1113-1120.

[11] S.M. Grundy, Hypertriglyceridemia, Insulin Resistance, and the Metabolic Syndrome, Am. J. Cardiol., 83 (1999) 25F-29F.

ACCEPTED MANUSCRIPT

[12] H. Wang, R. Eckel, Lipoprotein lipase: from gene to obesity, Am. J. Physiol. A, 297 (2009) E271-288.

[13] G. Olivecrona, T. Olivecrona, Triglyceride lipases and atherosclerosis, Curr. Opin. Lipidol., 21 (2010) 409-415.

[14] S.G. Young, R. Zechner, Biochemistry and pathophysiology of intravascular and intracellular lipolysis, Genes Dev., 27 (2013) 459-484.

[15] W. Dijk, S. Kersten, Regulation of lipoprotein lipase by Angptl4, Trends Endocrinol. Metab., 25 (2014) 146-155.

[16] C. Li, Genetics and regulation of angiopoietin-like proteins 3 and 4, Curr. Opin. Lipidol, 17 (2006) 152-156.

[17] T. Shimizugawa, M. Ono, M. Shimamura, K. Yoshida, Y. Ando, R. Koishi, K. Ueda, T. Inaba, H. Minekura, T. Kohama, H. Furukawa, ANGPTL3 decreases very low density lipoprotein triglyceride clearance by inhibition of lipoprotein lipase, J. Biol. Chem., 277 (2002) 33742-33748.

[18] V. Sukonina, A. Lookene, T. Olivecrona, G. Olivecrona, Angiopoietin-like protein 4 converts lipoprotein lipase to inactive monomers and modulates lipase activity in adipose tissue, Proceedings of the National Academy of Sciences of the United States of America, 103 (2006) 17450-17455.

[19] C.T. Johansen, S. Kathiresan, R.A. Hegele, Thematic Review Series: Genetics of Human Lipid Diseases: Genetic determinants of plasma triglycerides, J. Lipid. Res., 52 (2011) 189-206.

ACCEPTED MANUSCRIPT

[20] L. Berglund, J.D. Brunzell, A.C. Goldberg, I.J. Goldberg, F. Sacks, M.H. Murad, A.F.H. Stalenhoef, Evaluation and Treatment of Hypertriglyceridemia: An Endocrine Society Clinical Practice Guideline, J. Clin. Endocrinol. Metab., 97 (2012) 2969-2989.

[21] M. Larsson, R. Caraballo, M. Ericsson, A. Lookene, P.-A. Enquist, M. Elofsson, S.K. Nilsson, G. Olivecrona, Identification of a small molecule that stabilizes lipoprotein lipase in vitro and lowers triglycerides in vivo, Biochem. Biophys. Res. Commun., 450 (2014) 1063-1069.

[22] M. Esteves, I. Fragiadaki, R. Lopes, E. Scoulica, M. Cruz, Synthesis and biological evaluation of trifluralin analogues as antileishmanial agents, Bioorg. Med. Chem., 18 (2010) 274-281.

[23] C. Suoding, H.-M. Fernando, R. René, Scope and Applications of "Active and Latent" Thioglycosyl Donors. Part 4, J. Carbohydr. Chem., 17 (1998).

[24] A. Eastwood, A. Blum, N. Zacharias, D. Dougherty, A selenide-based approach to photochemical cleavage of peptide and protein backbones at engineered backbone esters, J. Org. Chem., 74 (2009) 9241-9244.

[25] E.J. Carcache de-Blanco, B. Pandit, Z. Hu, J. Shi, A. Lewis, P.-K. Li, Inhibitors of NF-kappaB derived from thalidomide, Bioorg. Med. Chem. Lett., 17 (2007) 6031-6035.

[26] N. Hashimoto, T. Aoyama, T. Shioiri, New methods and reagents in organic synthesis. 0.14. A simple efficient preparation of methyl-esters with trimethylsilyldiazomethane (TMSCHN2) and its application to gas-chromatographic analysis of fatty acids., Chem. Pharm. Bull., 29 (1981) 1475-1478.

[27] P. Zhichkin, L. Peterson, C. Beer, W. Rennells, The use of formamidine protection for the derivatization of aminobenzoic acids, J. Org. Chem., 73 (2008) 8954-8959.

[28] B. Fors, P. Krattiger, E. Strieter, S. Buchwald, Water-mediated catalyst preactivation: an efficient protocol for C-N cross-coupling reactions, Org. Lett., 10 (2008) 3505-3508.

[29] F. Kwong, A. Klapars, S. Buchwald, Copper-catalyzed coupling of alkylamines and aryl iodides: an efficient system even in an air atmosphere, Org. Lett., 4 (2002) 581-584.

[30] J.M. Chapman, Jr, P.J. Voorstad, G.H. Cocolas, I.H. Hall, Hypolipidemic Activity of Phthalimide Derivatives. 2. N-Phenylphthalimide and derivatives, J. Med. Chem., 26 (1983) 237-243.

[31] T. Woltering, G. Adam, A. Alanine, J.r. Wichmann, F.d.r. Knoflach, V. Mutel, S. Gatti, Synthesis and characterization of 8-ethynyl-1,3-dihydro-benzo[b][1,4]diazepin-2-one derivatives: new potent non-competitive metabotropic glutamate receptor 2/3 antagonists. Part 1, Bioorg. Med. Chem. Lett., 17 (2007) 6811-6815.

[32] P.H. Fries, D. Imbert, Parallel NMR based on solution magnetic-susceptibility differences. Application to isotopic effects on self-diffusion, J. Chem. Eng. Data, 55 (2010) 2048-2054.

[33] A. Klapars, S. Buchwald, Cooper-catalyzed halogen exchange in aryl halides: an aromatic Finkelstein reaction, J. Am. Chem. Soc., 124 (2002) 14844-14845.

[34] F. Bellina, C. Calandri, S. SCauteruccio, R. Rossi, Efficient and highly regioselective direct C-2 arylation of azoles, including free (NH)-imidazole, -benzimidazole and -indole, with aryl halides, Tetrahedron, 63 (2007) 1970-1980.

[35] Y. Nakamura, S. Inomata, M. Ebine, Y. Manabe, I. Iwakura, M. Ueda, “Click-made” biaryl-linker improving efficiency in protein labelling for the membrane target protein of a bioactive compound, Org. Biomol. Chem., 9 (2011) 83-85.

[36] S. Harper, B. Pacini, S. Avolio, M. Di Filippo, G. Migliaccio, R. Laufer, R. De Francesco, M. Rowley, F. Narjes, Development and preliminary optimization of indole-N-acetamide inhibitors of hepatitis C virus NS5B polymerase, J. Med. Chem., 48 (2005) 1314-1317.

[37] M.R. Kumar, A. Park, N. Park, S. Lee, Consecutive condensation, C-N and N-N bond formation: a copper-catalyzed one-pot three-component synthesis of 2H-indazole, Org. Lett., 13 (2011) 3542-3545.

[38] A.D. Burrows, C.G. Frost, M.F. Mahon, C. Richardson, Post-synthetic modification of tagged metal-organic frameworks, Angew. Chem. Int. Ed., 47 (2008) 8482-8486.

[39] I. Hubatsch, E.G.E. Ragnarsson, P. Artursson, Determination of drug permeability and prediction of drug absorption in Caco-2 monolayers, Nat. Protoc., 2 (2007) 2111-2119.

[40] J.B. Houston, Utility of in-vitro drug-metabolism data in predicting in-vivo metabolic-clearance, Biochem. Pharmacol., 47 (1994) 1469-1479.

[41] R.S. Obach, Prediction of human clearance of twenty-nine drugs from hepatic microsomal intrinsic clearance data: an examination of in vitro half-life approach and nonspecific binding to microsomes., Drug Metab. Dispos., 27 (1999) 1350-1359.

[42] P. Matsson, C.A.S. Bergström, N. Nagahara, S. Tavelin, U. Norinder, P. Artursson, Exploring the Role of Different Drug Transport Routes in Permeability Screening, J. Med. Chem., 48 (2005) 604-613.

[43] W. Yin, K. Tsutsumi, Lipoprotein lipase activator NO-1886, Cardiovascular drug reviews, 21 (2003) 133-142.

[44] K. Tsutsumi, Y. Inoue, A. Shima, K. Iwasaki, M. Kawamura, T. Murase, The novel compound NO-1886 increases lipoprotein lipase activity with resulting elevation of high density lipoprotein cholesterol, and long-term adminstration inhibits atherogenises in the coronary arteries of rats with experimental atherosclerosis., J. Clin. Invest., 92 (1993) 411-417.

[45] G. Shattat, R. Al-Qirim, Y. Al-Hiari, G. Sheikha, T. Al-Qirim, W. El-Huneidi, M. Shahwan, Synthesis and anti-hyperlipidemic: evaluation of N-(benzoylphenyl)-5-fluoro-1H-indole-2-carboxamide derivatives in Triton WR-1339-induced hyperlipidemic rats, Molecules, 15 (2010) 5840-5849.

[46] W.J. Geldenhuys, D. Aring, P. Sadan, A novel lipoprotein lipase (LPL) agonist rescues the enzyme from inhibition by angiopoietin-like 4 (ANGPTL4), Bioorg. Med. Chem. Lett., 24 (2014) 2163-2167.

[47] J.Y. Chang, Nuclear receptor regulation of lipid metabolism: potential therapeutics for dyslipidemia, diabetes, and chronic heart and liver diseases, Curr. Opin. Investig. Drugs, 6 (2005) 994-1001.

[48] B. Staels, J. Dallongeville, J. Auwerx, K. Schoonjans, E. Leitersdorf, J.-C. Fruchart, Mechanism of Action of Fibrates on Lipid and Lipoprotein Metabolism, Circulation, 98 (1998) 2088-2093.

[49] M.M. Aye, E.S. Kilpatrick, P. Afolabi, S.A. Wootton, A.S. Rigby, A.M. Coady, D.D. Sandeman, S.L. Atkin, Postprandial effects of long-term niacin/laropiprant use on glucose and lipid metabolism and on cardiovascular risk in patients with polycystic ovary syndrome, Diabetes Obes. Metab., 16 (2014) 545-552.

[50] B.Y. Feng, B.K. Shoichet, A detergent-based assay for the detection of promiscuous inhibitors, Nat. Protoc., 1 (2006) 550-553.

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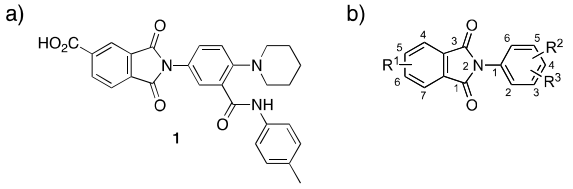

Figure 1. a) Screening hit **1**[21] and b) Chemical diversity on the N-phenylphthalimide scaffold investigated in this study.

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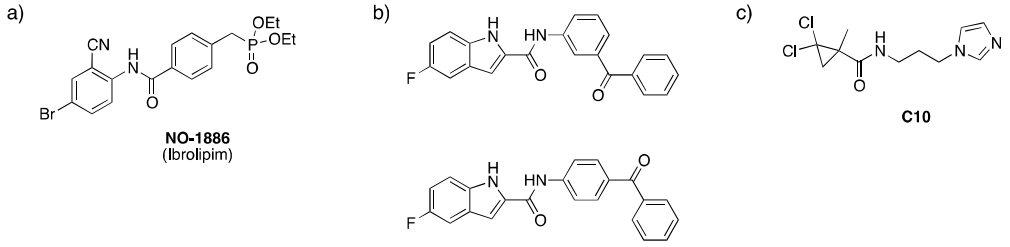

Figure 2. Previously reported LPL activators tested in our enzymatic assays a) Ibrolipim (NO-1886); b) 5-fluoro-*1H*-indole-2-carboxamides and c) LPL agonist C10.

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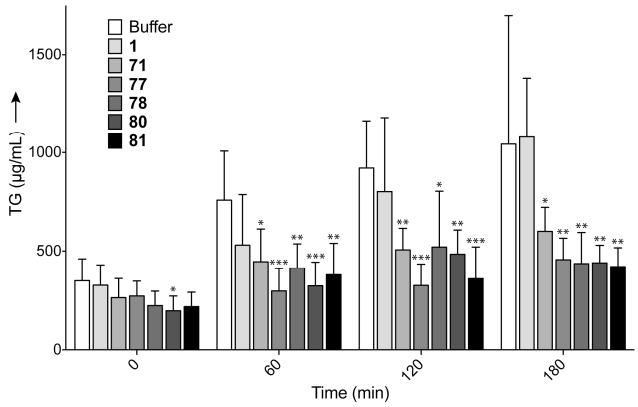

Figure 3. Oral lipid gavage on wild type mice. Mice (n = 8/group) were treated as indicated once daily for 4 days with i.p. injections. One hour after the last injection mice were given a 200 µl oral lipid gavage. Plasma TG levels were investigated at the indicated time points. Data for TG are mean values with standard deviation. A one-way ANOVA Tukey's multiple comparison test was used for statistical analysis. *, ** and *** correspond to p-values of 0.05, 0.01 and 0.001, respectively.

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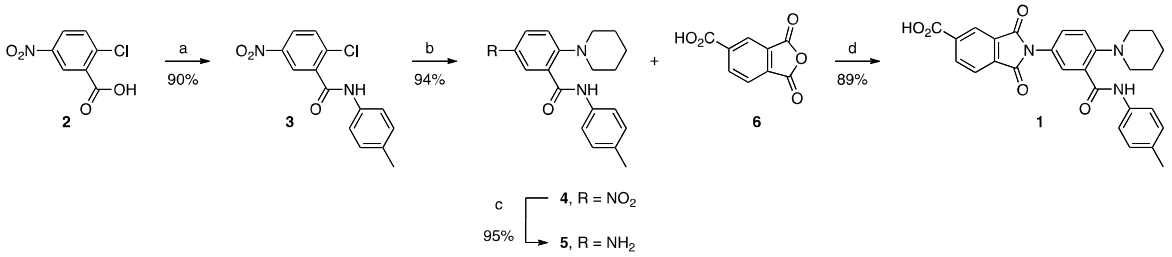

Reagents and conditions: (a) *i*: $(COCl)_2$, DMF (cat.), CH_2Cl_2, 0 °C to rt, 2 h, *ii*: *p*-toluidine, TEA, CH_2Cl_2, 5 h. (b) piperidine, TEA, EtOH, reflux, 5 h. (c) $SnCl_2$, EtOH, reflux, 3 h. (d) AcOH, reflux, 5 h.

Scheme 1. Synthesis of **1**.

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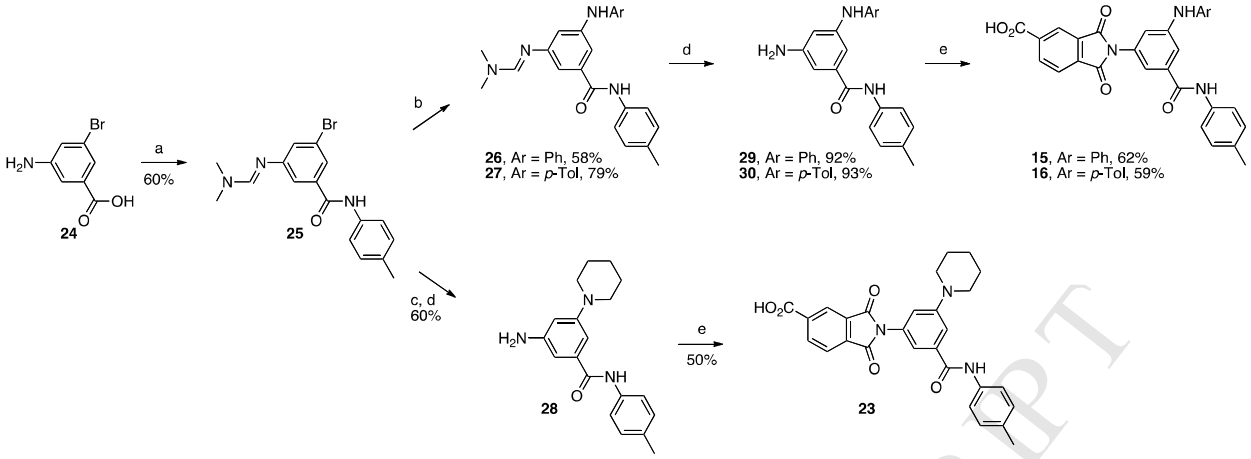
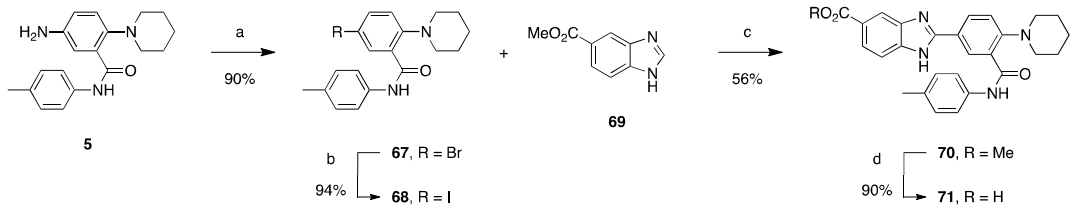


Reagents and conditions: (a) *i*: DMF/$(COCl)_2$, CH_2Cl_2, 0 °C to rt, 1 h, *ii*: Py, 0 °C to rt, 30 min *iii*: *p*-toluidine, rt, 2 h. (b) $ArNH_2$, $Pd(OAc)_2$, xPhos, NaO*t*-Bu, H_2O, 1,4-dioxane, 80 °C, 24 h. (c) piperidine, CuI, R-BINOL, K_3PO_4, 110 °C, 24 h. (d) ethylenediamine, EtOH, reflux, 6 h. (e) **6**, AcOH, reflux, 5 h.

Scheme 2. Synthesis of **15**, **16** and **23**.

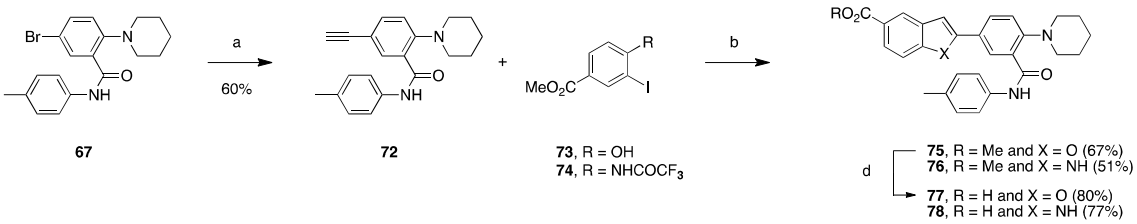
ACCEPTED MANUSCRIPT



Reagents and conditions: (a) *i*: HBr, CH_3CN, 5 °C, *ii*: $NaNO_2$ (aq.), 5 °C, *iii*: CuBr, HBr, 5 °C to rt, 1 h, reflux, 1 h. (b) CuI, $NaNO_2$, N,N'-dimethylethylenediamine, 1,4-dioxane, 110 °C (MW), 1 d. (c) $Pd(OAc)_2$, CuI, DMF, 140 °C, 4 d. (d) KOH, $EtOH/H_2O$, reflux, 1 d.

Scheme 3. Synthesis of **71**.

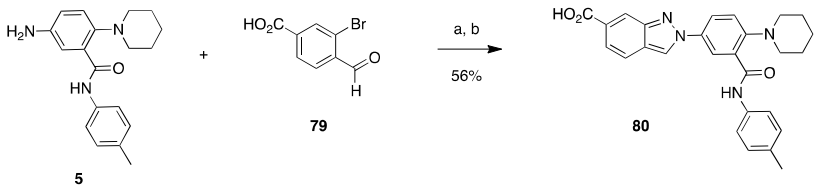
ACCEPTED MANUSCRIPT



Reagents and conditions: (a) *i*: $PdCl_2(PPh_3)_2$, CuI, TMSA, TEA, DMF, 60 °C, 18h, *ii*: K_2CO_3, MeOH, 12 h. (b) $PdCl_2(PPh_3)_2$, CuI, TEA, DMF, 110 °C, 10 min (MW). (b) KOH, EtOH/ H_2O, reflux, 1 d.

Scheme 4. Synthesis of **77** and **78**.[a]

ACCEPTED MANUSCRIPT

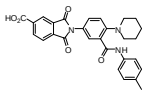
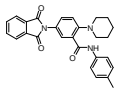
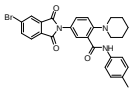
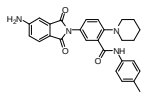
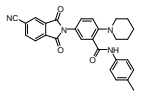
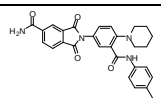
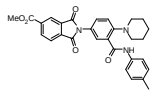
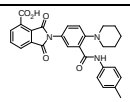


Reagents and conditions: (a) EtOH, reflux, 20 min. (b) *i*: Cs_2CO_3, CuI, TMEDA, DMSO, 100 °C, 30 min (MW). *ii*: NaN_3, 120 °C, 1 d.

Scheme 5. Synthesis of **80**.

ACCEPTED MANUSCRIPT

Table 1. Effect of carboxylic acid substitution on LPL stabilization

ID	Structure	% of control[a] (C in μM)			
		3.12	6.25	12.5	25
1		NA	NA	90.0 ± 16.0	93.8 ± 16.3
7		NA	NA	NA	NA
8		NA	NA	NA	NA
9		NA	NA	NA	11.4 ± 15.8
10		NA	NA	NA	NA
11		NA	NA	NA	3.5 ± 0.6
12		NA	NA	NA	NA
13		NA	NA	NA	NA

[a] See experimental section for details. NA: not active.

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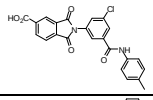
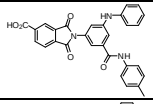
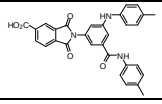
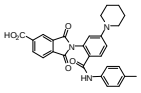
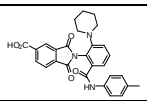
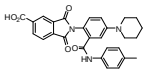
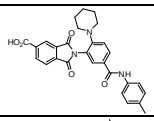
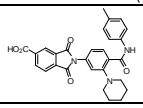
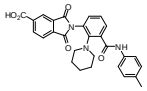
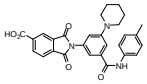
Table 2. Effect of substituent scrambling on LPL stabilization

ID	Structure	% of control[a] (C in μM)			
		3.12	6.25	12.5	25
14		NA	NA	NA	NA
15		NA	4.2 ± 0.8	73.1 ± 2.3	89.4 ± 3.2
16		NA	5.0 ± 0.8	7.0 ± 1.2	24.6 ± 3.5
17		NA	NA	NA	96.7 ± 5.5
18		NA	NA	NA	7.3 ± 2.6
19		NA	NA	55.1 ± 4.5	36.3 ± 3.7
20		NA	NA	81.3 ± 5.7	79.0 ± 4.0
21		NA	NA	NA	14.1 ± 2.9
22		NA	NA	NA	13.5 ± 2.7
23		NA	NA	NA	32.6 ± 1.4

[a] See experimental section for details. NA: not active.

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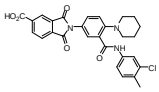
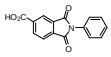
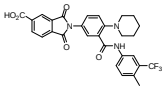
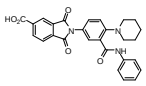
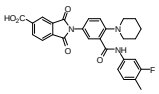
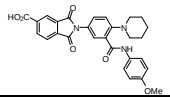
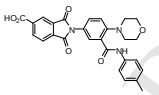
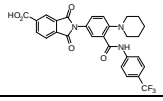
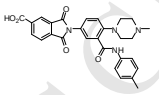
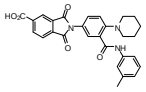
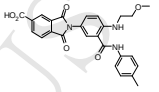
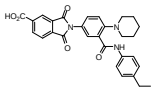
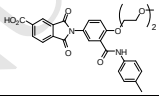
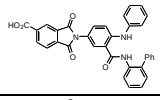
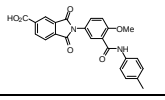
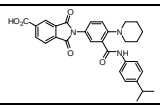
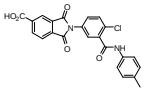
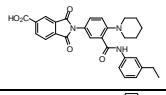
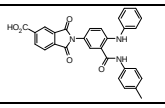
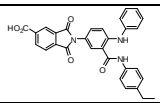
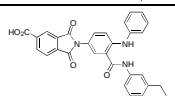
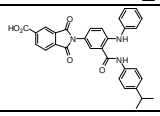
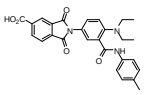
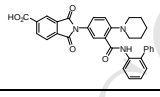
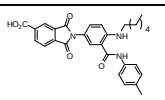
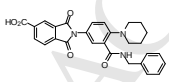
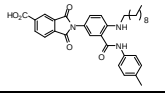
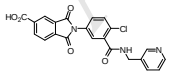
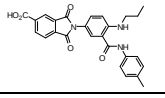
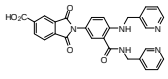
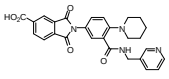
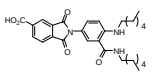
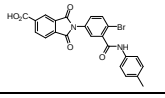
Table 3. Effect of substitutions at the core fragment on LPL stabilization

ID	Structure	% of control[a] (C in μM) 3.12	6.25	12.5	25	ID	Structure	% of control[a] (C in μM) 3.12	6.25	12.5	25
31		NA	NA	NA	NA	**48**		NA	66.8 ± 4.8	73.0 ± 4.3	69.8 ± 5.4
32		NA	NA	NA	NA	**49**		NA	19.4 ± 0.4	42.2 ± 0.7	65.8 ± 1.7
33		NA	NA	NA	57.0 ± 2.6	**50**		NA	NA	53.3 ± 1.2	72.1 ± 1.1
34		NA	NA	NA	43.8 ± 6.8	**51**		NA	NA	NA	NA
35		NA	NA	54.6 ± 0.7	81.6 ± 1.1	**52**		NA	NA	NA	NA
36		NA	NA	38.6 ± 5.2	56.6 ± 5.2	**53**		NA	NA	NA	NA
37		NA	32.4 ± 2.2	36.5 ± 2.8	47.0 ± 2.3	**54**		NA	NA	NA	NA
38		NA	45 ± 2.3	61.5 ± 3.8	73.8 ± 3.6	**55**		NA	NA	NA	NA
39		NA	55.6 ± 3.8	71.0 ± 3.9	53.1 ± 2.5	**56**		NA	NA	NA	NA
40		NA	9.2 ± 1.9	63.4 ± 1.6	71.0 ± 1.7	**57**		NA	57.2 ± 8.6	80.1 ± 8.2	94.4 ± 11.1
41		NA	37.6 ± 2.2	60.3 ± 3.8	60.0 ± 2.2	**58**		NA	44.3 ± 1.2	63.9 ± 3.7	52.2 ± 6.6
42		NA	44.1 ± 3.5	59.1 ± 4.0	52.5 ± 3.2	**59**		NA	NA	NA	49.5 ± 4.7
43		NA	60.1 ± 2.3	63.3 ± 2.7	68.5 ± 3.4	**60**		NA	60.6 ± 4.5	47.1 ± 3.8	67.2 ± 4.3
44		NA	NA	NA	NA	**61**		13.6 ± 5.8	5.9 ± 1.3	10.0 ± 1.6	19.2 ± 1.6
45		NA	NA	NA	NA	**62**		NA	NA	6.2 ± 0.4	39.7 ± 1.8
46		NA	NA	NA	NA	**63**		NA	NA	NA	NA
47		4.0 ± 0.6	19.3 ± 3.2	31.8 ± 11.5	67.8 ± 4.4	**64**		NA	NA	NA	NA

[a] See experimental section for details. NA: not active.

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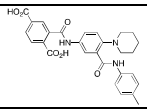
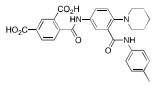
Table 4. Effect of the firsts and second metabolites on LPL stabilization

ID	Structure	% of control[a] (C in µM)			
		3.12	6.25	12.5	25
5		NA	NA	NA	NA
65		NA	NA	NA	38.8 ± 6.6
66		NA	NA	NA	30.6 ± 15.5

[a] See experimental section for details. NA: not active.

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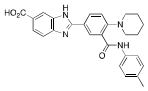
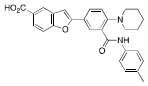
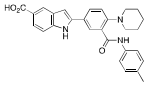
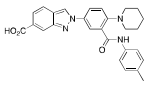
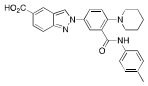
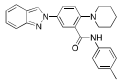
Table 5. Effect of the new heterocyclic compounds on LPL stabilization

ID	Structure	% of control[a] (C in µM)			
		3.12	**6.25**	**12.5**	**25**
71		NA	NA	NA	49.0 ± 1.7
77		NA	49.8 ± 2.5	76.0 ± 3.5	67.9 ± 3.3
78		35.0 ± 3.7	100.8 ± 2.2	85.5 ± 5.7	24.5 ± 2.8
80		NA	16.0 ± 1.9	75.6 ± 6.1	97.0 ± 6.2
81		NA	11.2 ± 1.2	90.6 ± 6.2	89.7 ± 6.4
82		NA	NA	NA	NA

[a] See experimental section for details. NA: not active.

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Table 6. *In vitro* ADME parameters of hit compound **1** and optimized hits **77** and **80**

ID	Solubility (μM)		Caco-2 P_{app}[a] (x 10^{-6} cm/s)		Efflux	PPB[a] (%)		CL_{int}[a] (μl/min/mg)		
	pH 1.2	pH 7.4	(a-b)	(b-a)	$E = \frac{(b-a)}{(a-b)}$	fu[a]	Stability	HLM[a]	MLM[a]	HHEP[a]
1	-	80	12	31	2.7	-	2	-	-	-
77	7	< 2	1.2	1.8	1.5	< 0.01	93	< 17	-	35
80	< 2	< 2	23	31	1.4	< 0.01	96	< 17	< 12	< 10

[a] Abbreviations: P_{app} (apparent permeability); PPB (plasma protein binding); fu (fraction unbound); CL_{int} (intrinsic clearance); HLM/MLM (human/mouse liver microsomes); HHEP (human hepatocytes).

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- Design and synthesis of novel compounds that preserve LPL activity.
- Structure-activity relationships from a previously identified screening hit.
- Pharmacodynamic and pharmacokinetic optimization.
- Several compounds efficiently lowered plasma triglycerides *in vivo*.

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Structure-activity relationships for lipoprotein lipase agonists that lower plasma triglycerides *In Vivo*

Rémi Caraballo,[a] Mikael Larsson,[b] Stefan K. Nilsson,[b] Madelene Ericsson,[b] Weixing Qian,[c] Nam Phuong Nguyen Tran,[a] Tomas Kindahl,[a] Richard Svensson,[d] Valeria Saar,[b] Per Artursson,[d] Gunilla Olivecrona,[b] Per-Anders Enquist[c] and Mikael Elofsson[a, c, *]

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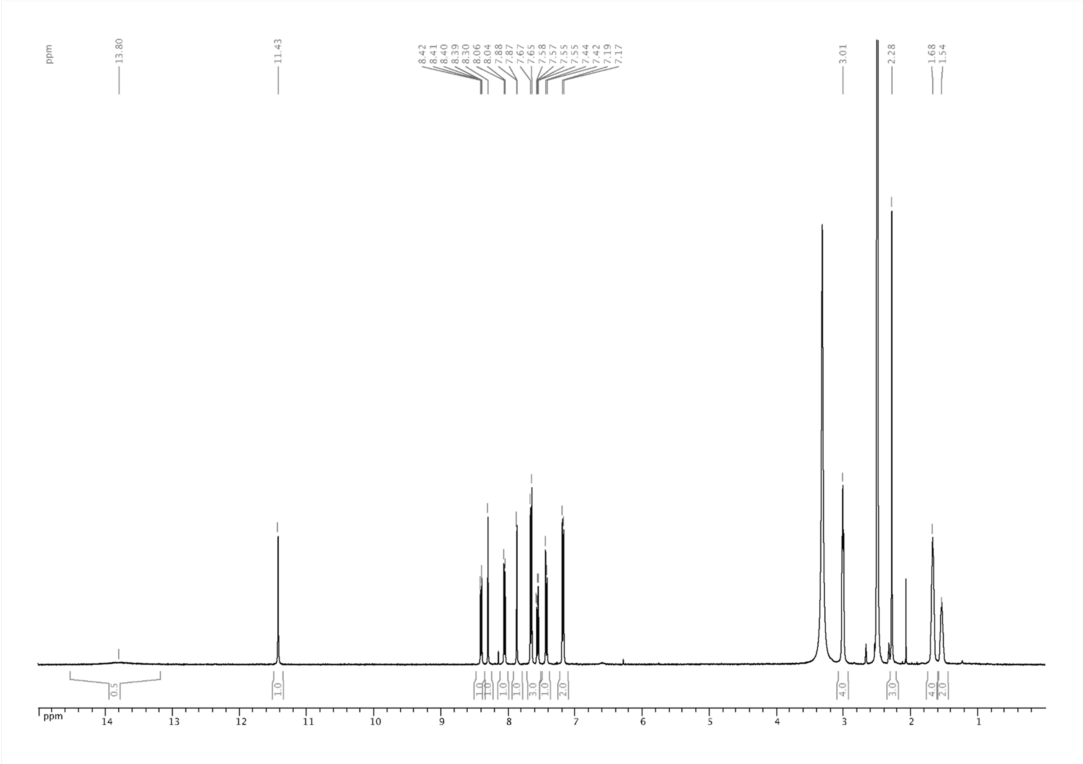
[*] Corresponding author; Email: mikael.elofsson@umu.se; Phone: +46-90-7869328; Fax: +46-90-7867655

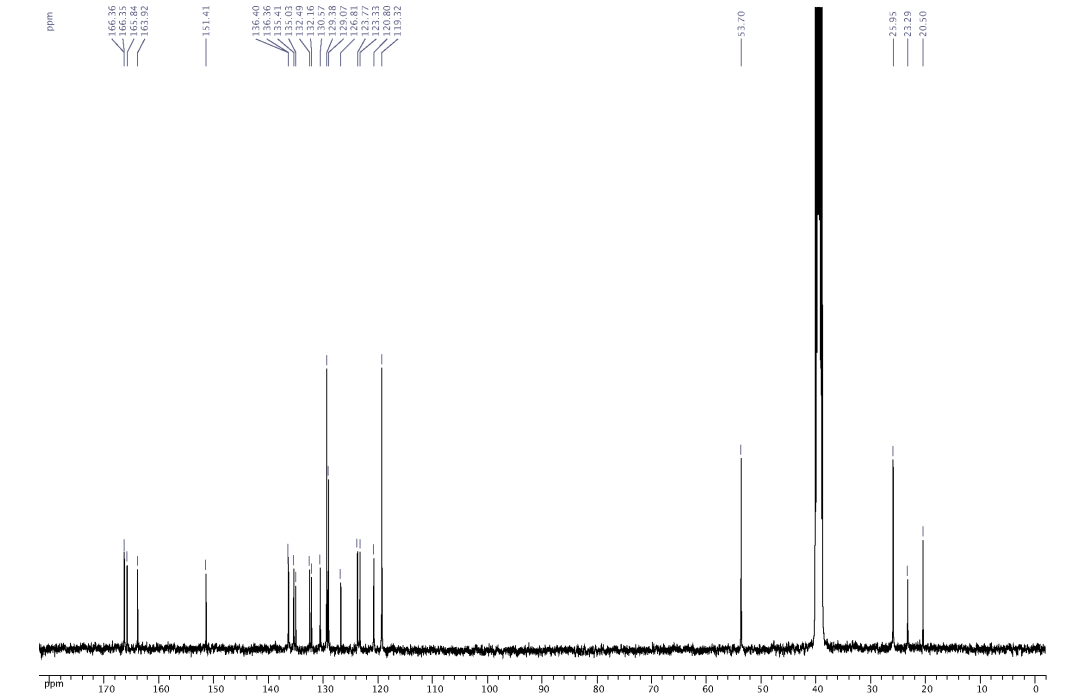
Contents:

Compound **1**

^{1}H-NMR (400 MHz, DMSO-d_6)



^{13}C-NMR (100.5 MHz, DMSO-d_6)



Compound **71**

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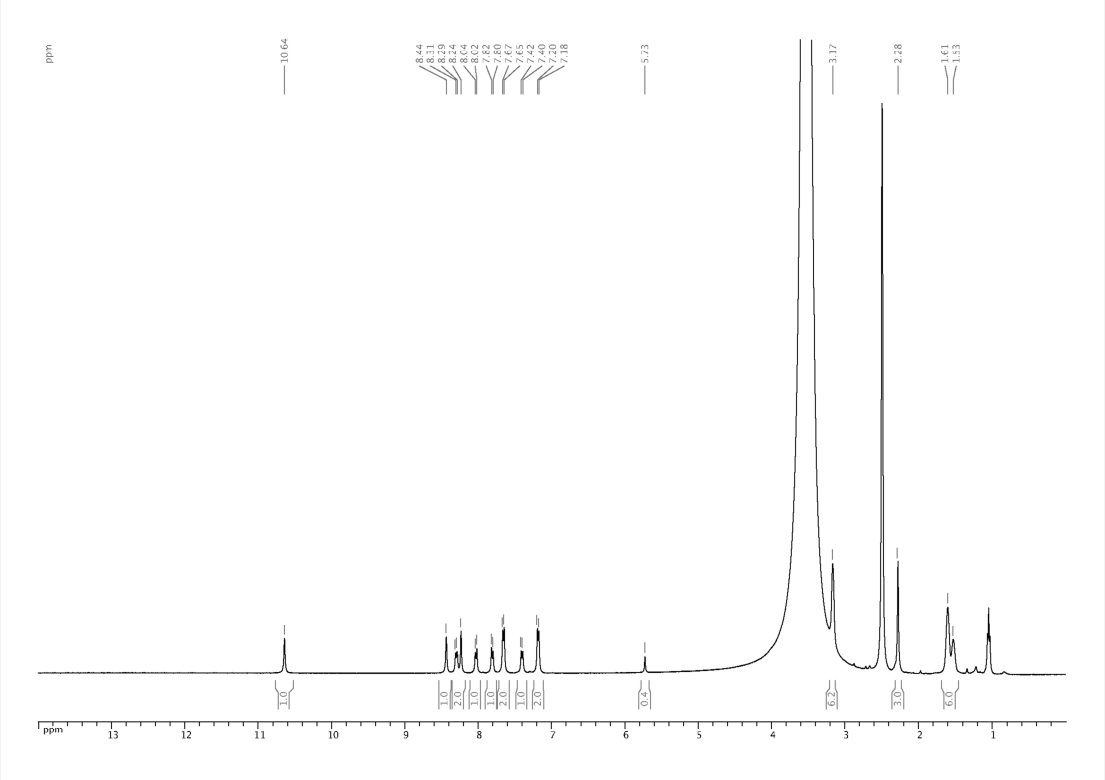

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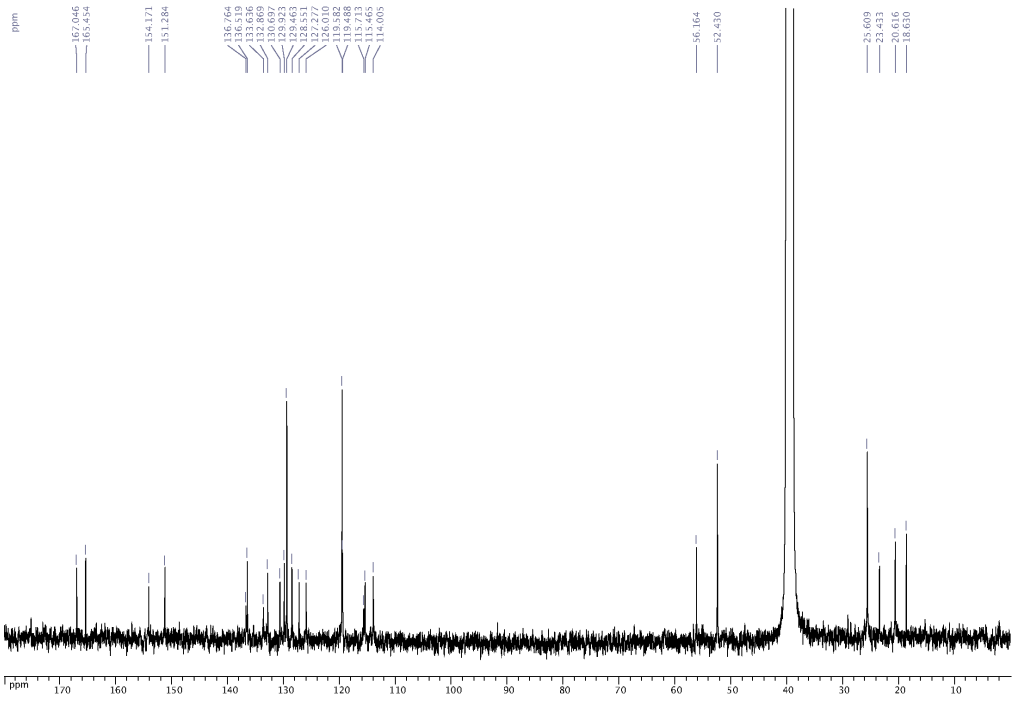

Compound **77**

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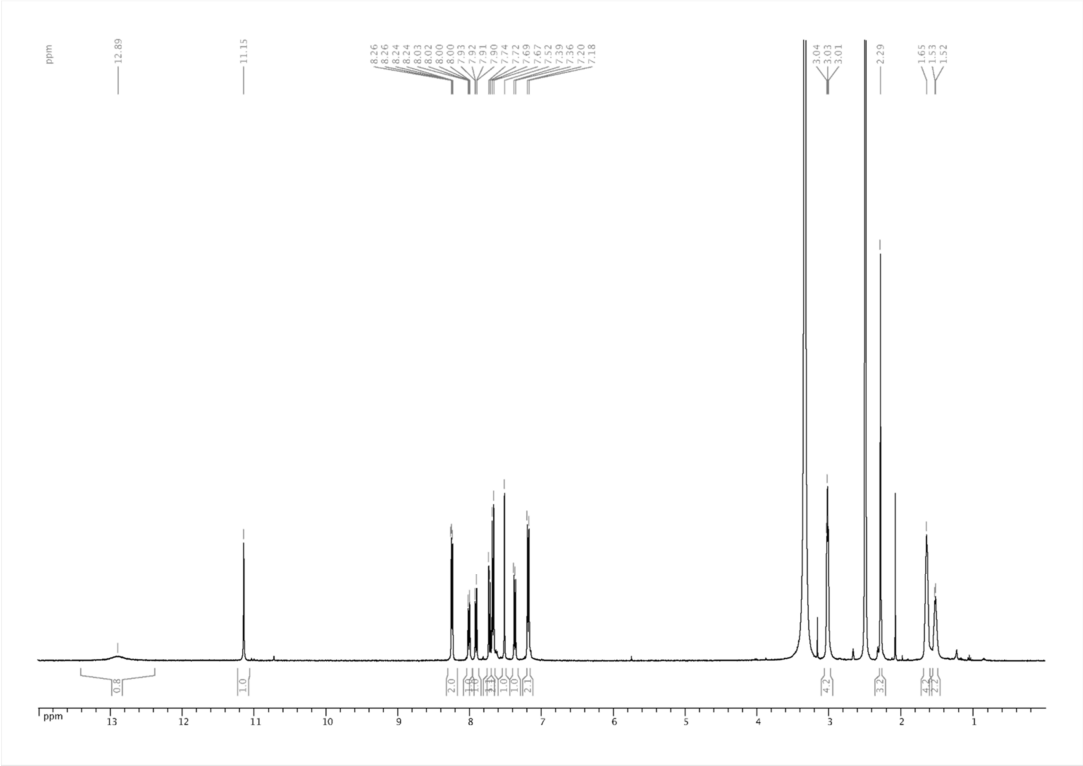

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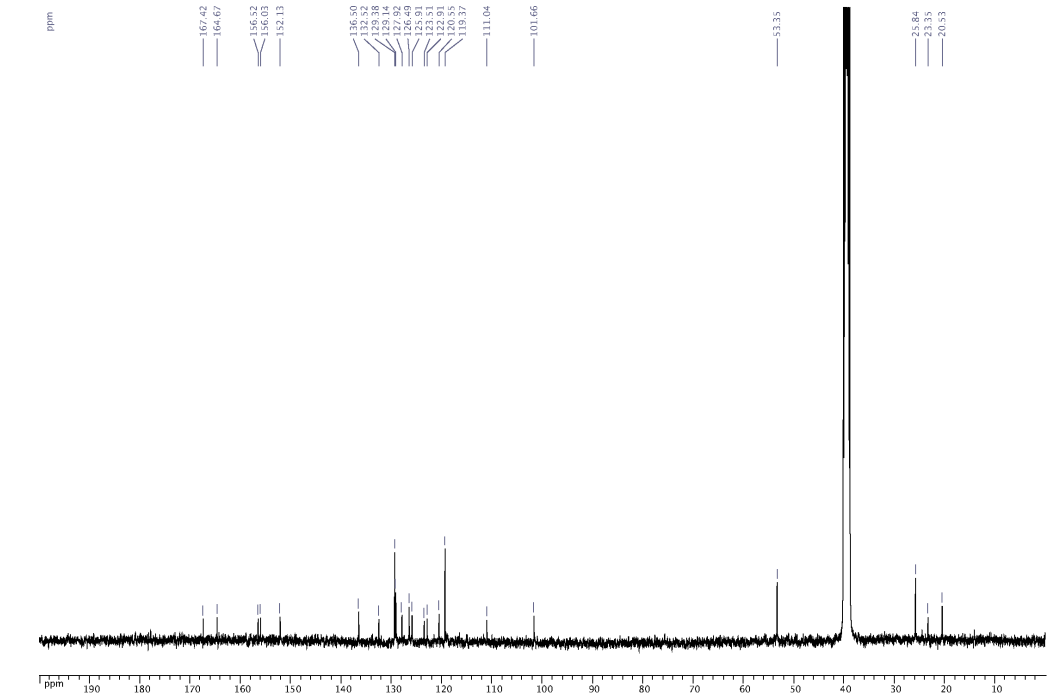

Compound **78**

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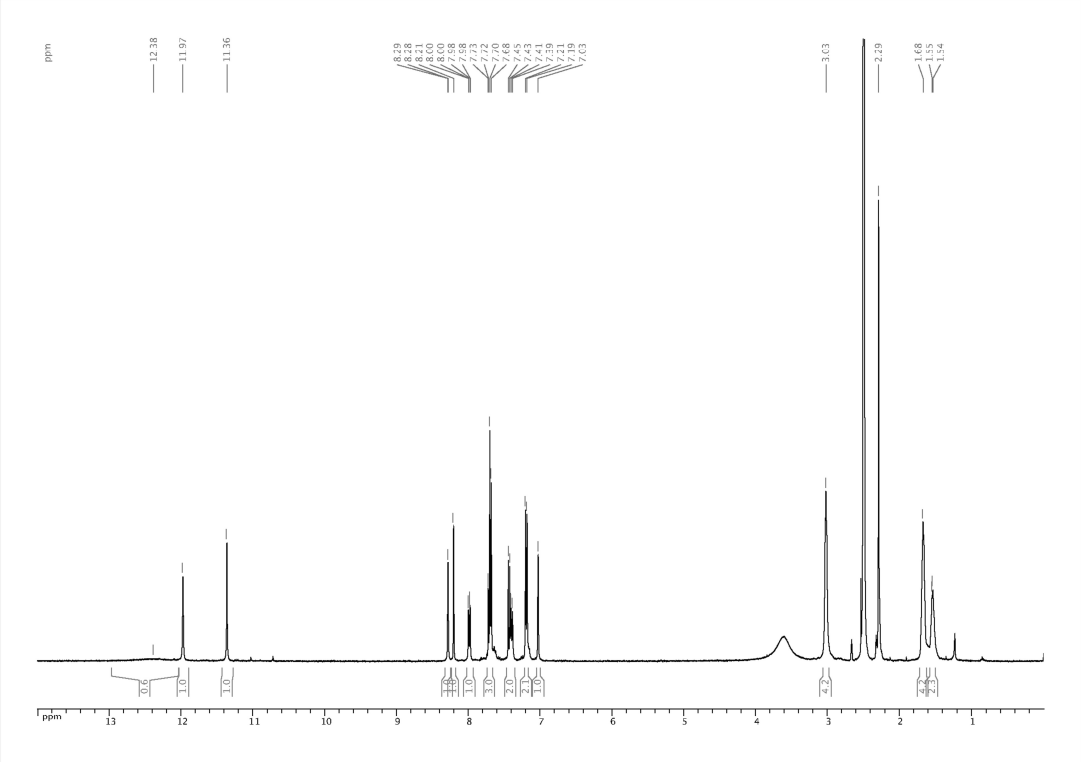
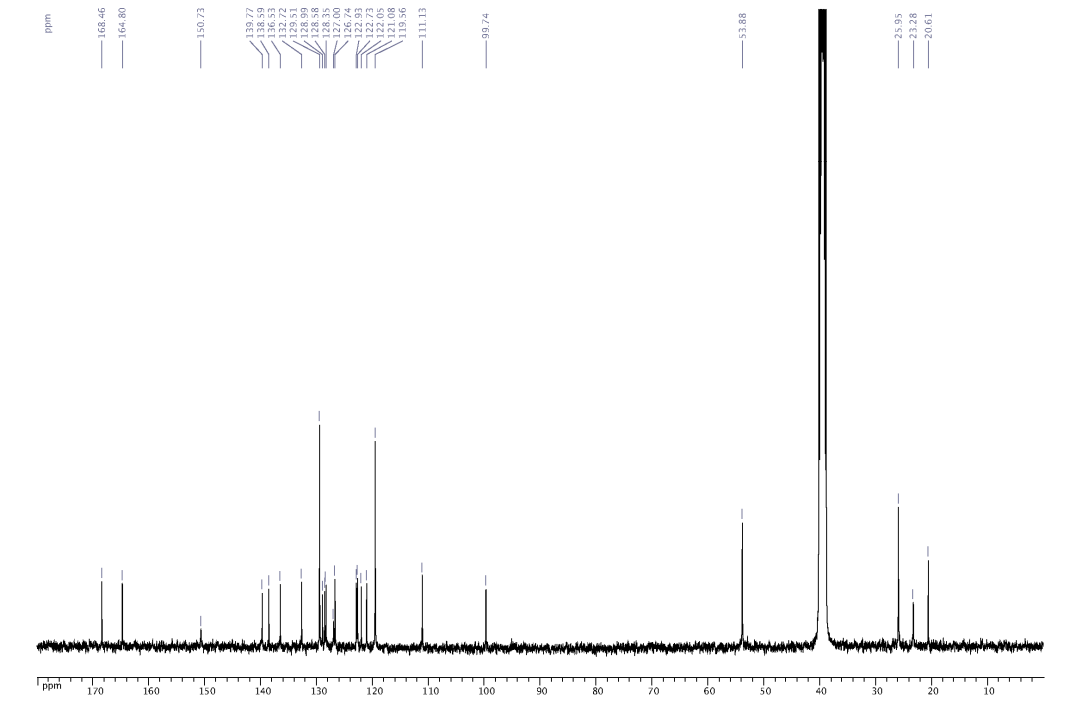


^{13}C-NMR (100.5 MHz, DMSO-d_6)



Compound **80**

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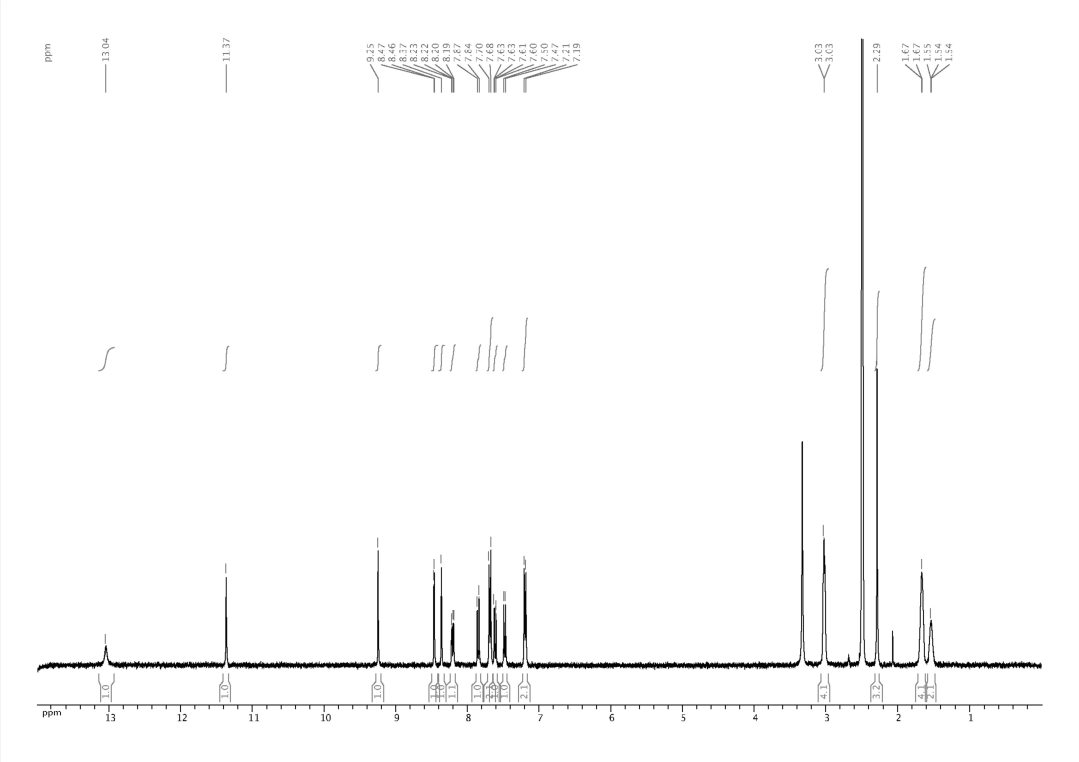
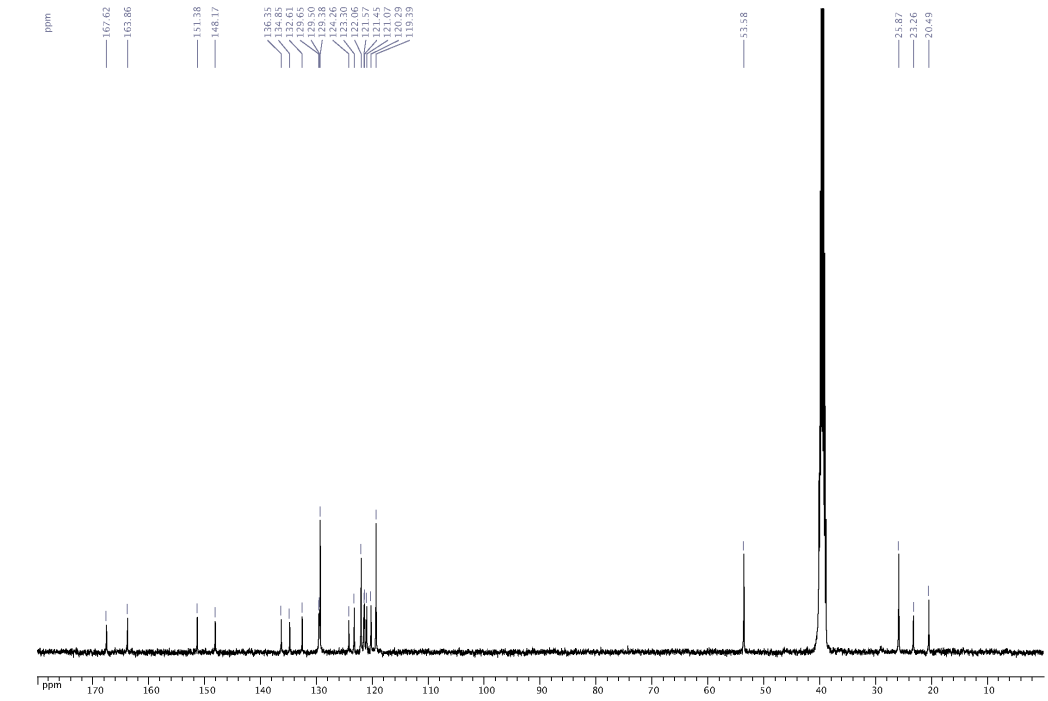


^{13}C-NMR (100.5 MHz, DMSO-d_6)



Compound **81**

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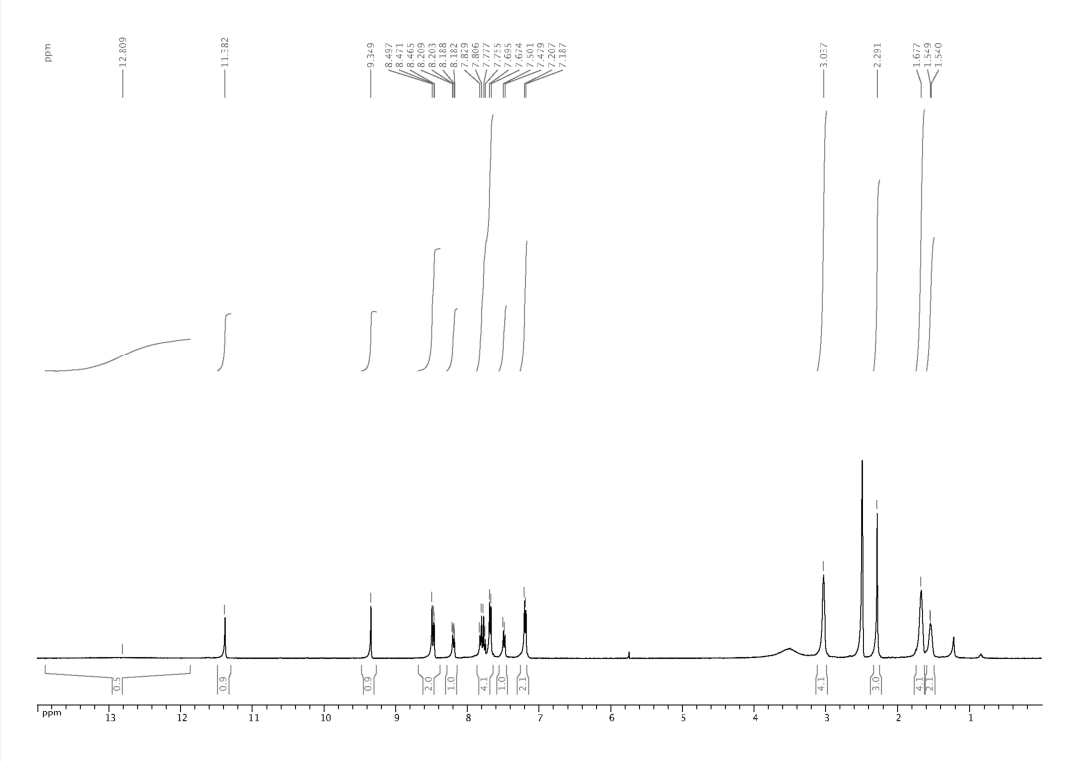
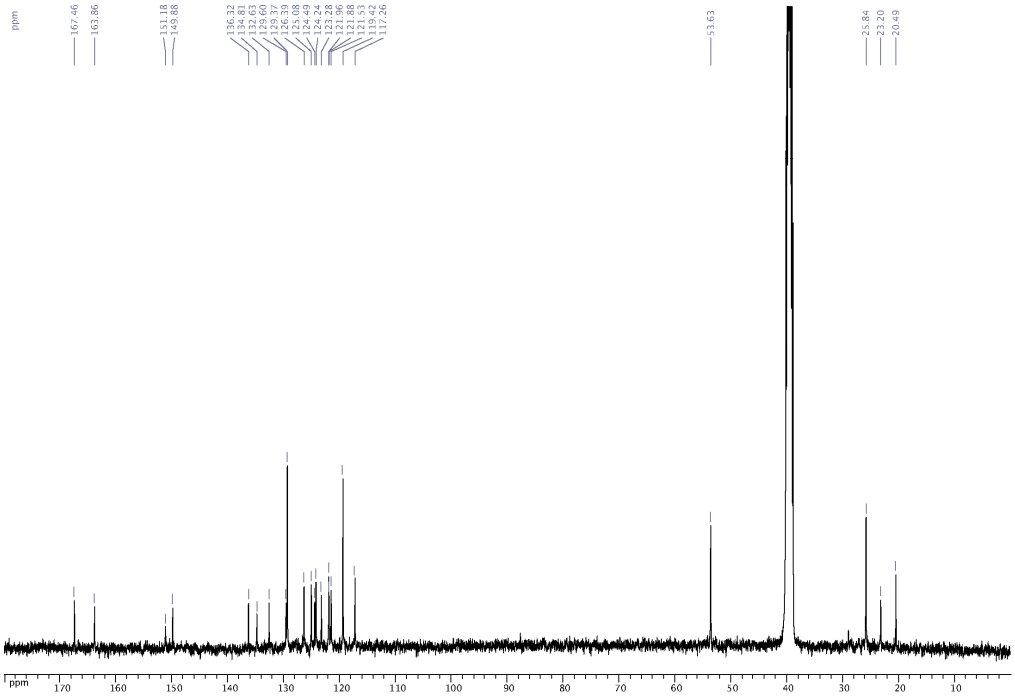
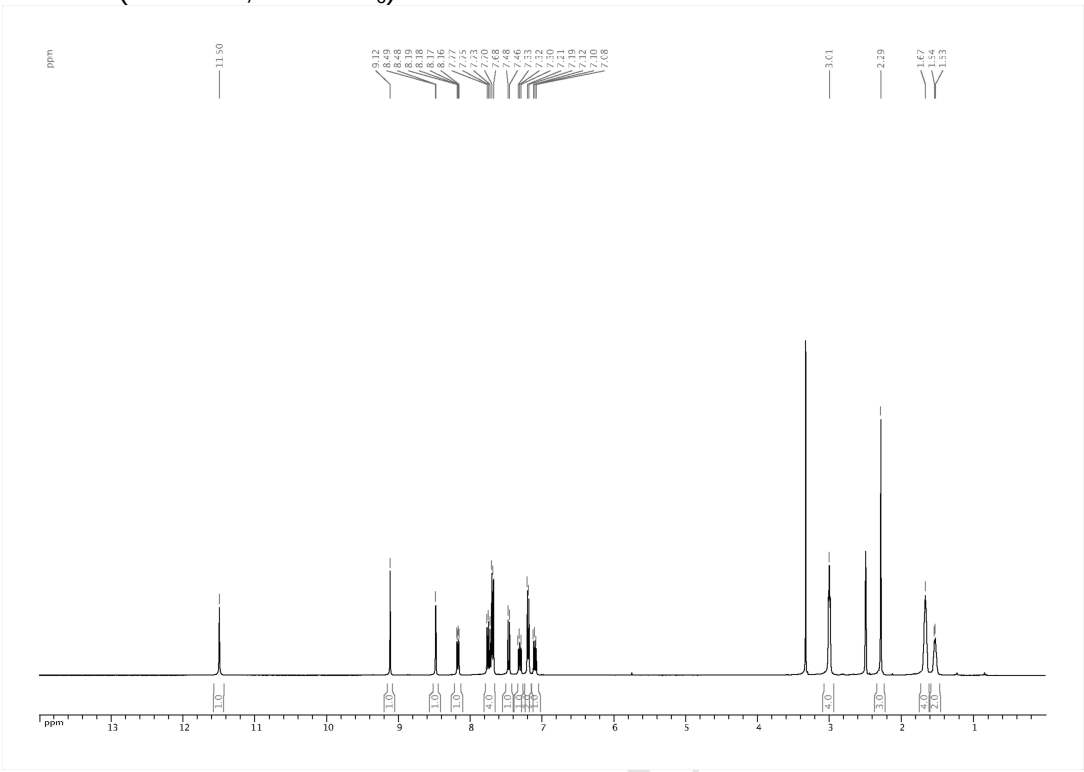


^{13}C-NMR (100.5 MHz, DMSO-d_6)

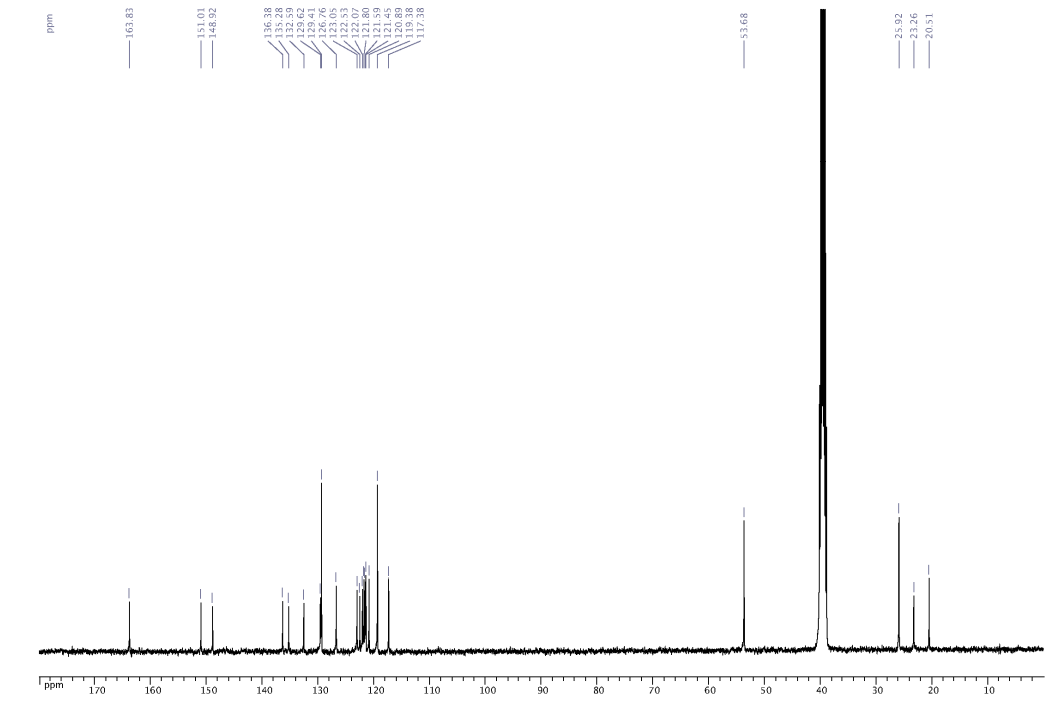


Compound **82**

^{1}H-NMR (400 MHz, DMSO-d_6)



^{13}C-NMR (100.5 MHz, DMSO-d_6)



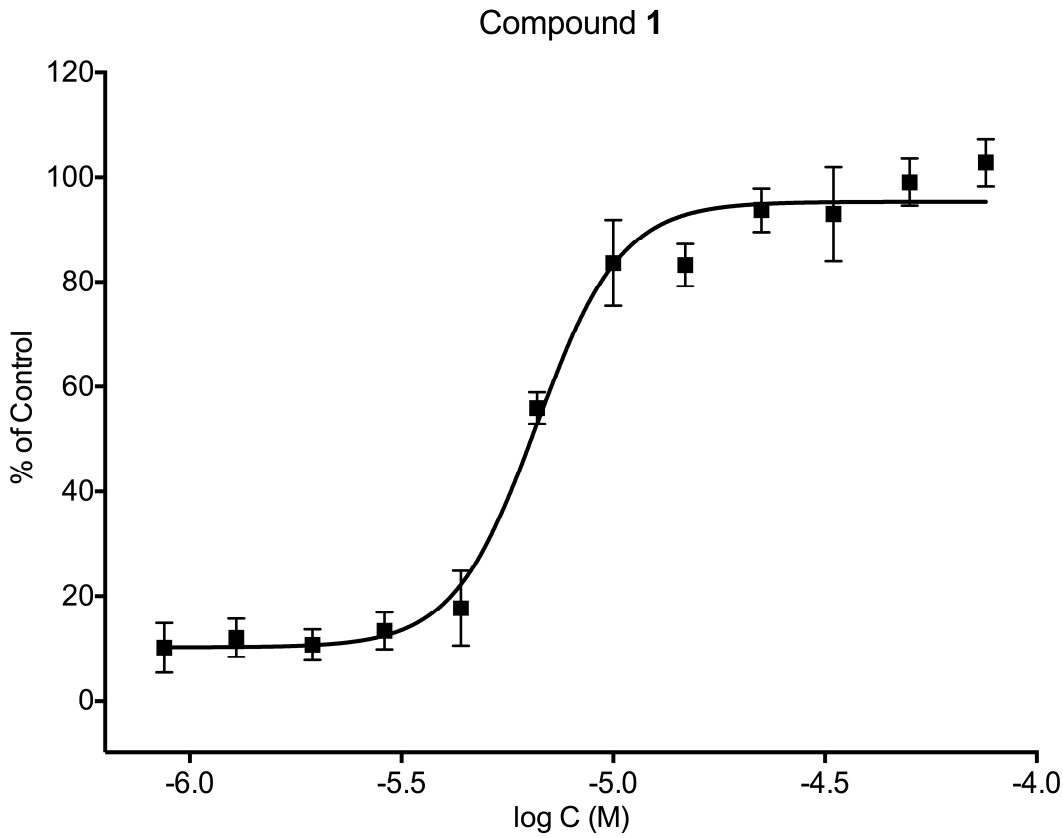


Figure S1. LPL activity in response to compound **1** under inactivating conditions

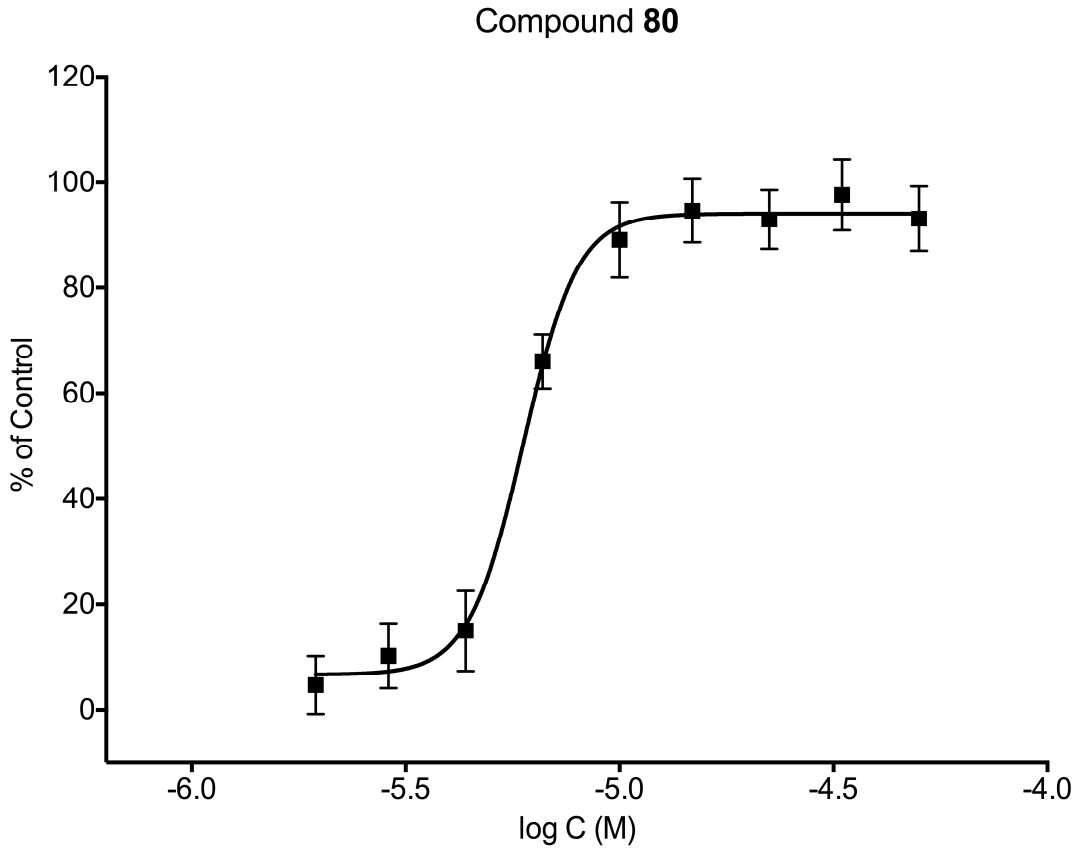


Figure S2. LPL activity in response to compound **80** under inactivating conditions

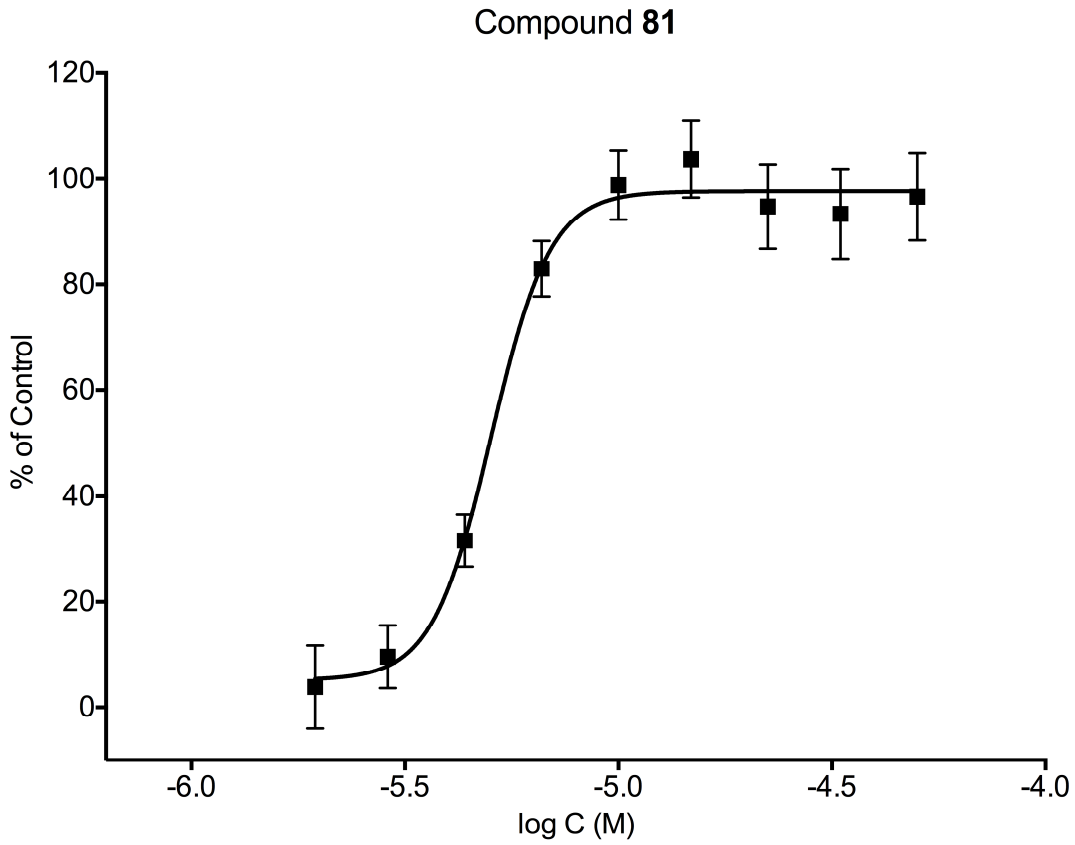


Figure S3. LPL activity in response to compound **81** under inactivating conditions

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