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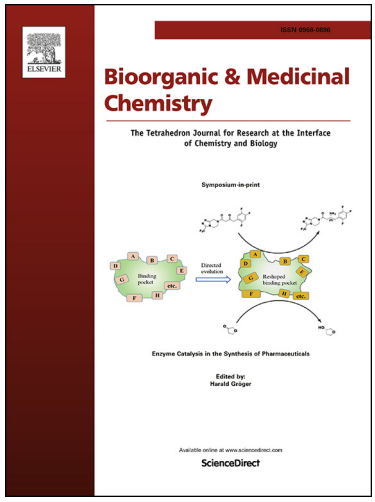

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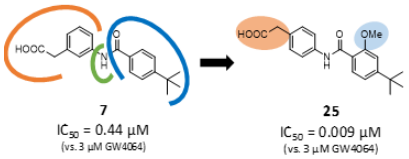

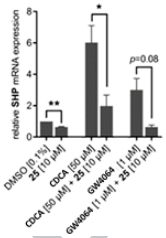

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Structural optimization and *in vitro* profiling of *N*-phenylbenzamide-based farnesoid X receptor antagonists

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ABSTRACT

Activation of the nuclear farnesoid X receptor (FXR) which acts as cellular bile acid sensor has been validated as therapeutic strategy to counter liver disorders such as non-alcoholic steatohepatitis by the clinical efficacy of obeticholic acid. FXR antagonism, in contrast, is less well studied and potent small molecule FXR antagonists are rare. Here we report the systematic optimization of a novel class of FXR antagonists towards low nanomolar potency. The most optimized compound antagonizes baseline and agonist induced FXR activity in a full length FXR reporter gene assay and represses intrinsic expression of FXR regulated genes in hepatoma cells. With this activity and a favorable toxicity-, stability- and selectivity-profile it appears suitable to further study FXR antagonism *in vitro* and *in vivo*.

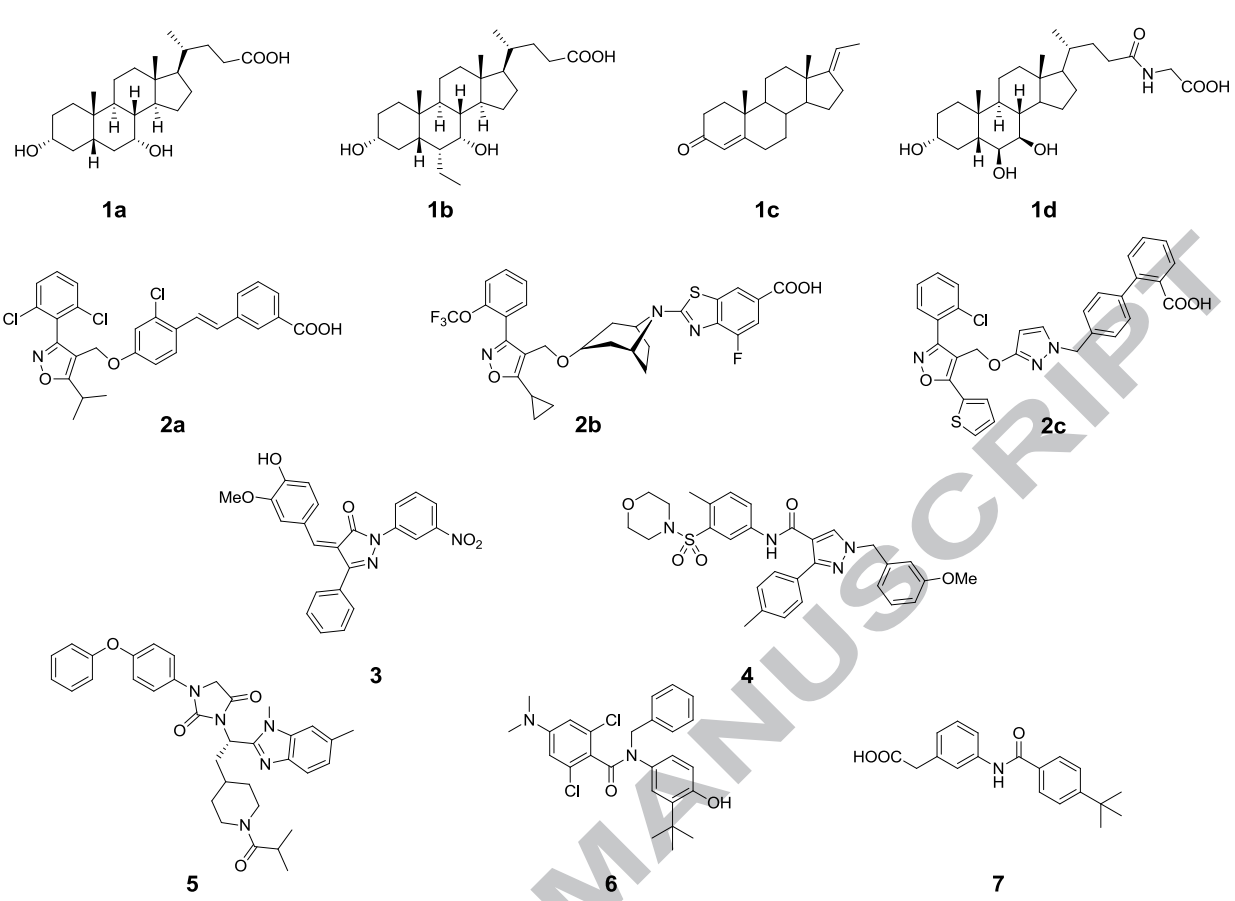
1. Introduction

The nuclear farnesoid X receptor (FXR) is a ligand-activated transcription factor and a key metabolic regulator that acts as cellular sensor for bile acids.[1–3] It takes part in the self-regulation of bile acid homeostasis with the result that bile acid synthesis is blocked and their catabolism is enhanced when high levels of toxic bile acids occur. Hence, FXR is an important liver protector.[4] Moreover, FXR is involved in glucose and lipid homeostasis regulation and seems to have anti-inflammatory effects. The most potent endogenous FXR activator is the bile acid chenodeoxycholic acid (**1a**, CDCA, Scheme 1).[3] Furthermore, a number of synthetic FXR agonists and antagonists were discovered in the past years.[5] Their diversity, however, is confined to few scaffolds. The 6α-ethyl derivate of **1a**, obeticholic acid (**1b**, OCA), was the first FXR agonist to gain market approval while the most widely used non-steroidal FXR agonist, the synthetic isoxazole GW4064 (**2a)**, is not suitable as drug due to toxicity and poor bioavailability.[6] However, replacement of the stilbene moiety and the di-chlorinated phenyl group led to Tropifexor (**2b**, LJN452), which has already reached phase 2 clinical trials for primary biliary cholangitis (PBC) and nonalcoholic steatohepatitis (NASH) treatment.[7] In addition to these full agonists, several partial agonists were developed to prevent hyperactivation and reduce side effects.[8–10]

Thus, FXR agonists are widely available and FXR activation is already validated as therapeutic strategy for several liver disorders and metabolic diseases. In contrast, antagonism on FXR needs further investigation to uncover its potential therapeutic value. FXR seems to be overexpressed in some cancer cells, especially in pancreatic and colon cancer,[11] Barett's Esophagus and adenoma suggesting therapeutic potential for FXR antagonism[12] and on the other hand it might be a treatment strategy for cholestasis. However, in hepatic cancer reduced FXR activity is connected with tumor growth and FXR antagonism might disturb adipogenesis.[13] As a consequence of impaired bile acid metabolism through FXR antagonism, high levels of bile acids could also lead to liver toxicity.

The most described FXR antagonist is Guggulsterone (**1c)**, which is extracted from the gum resin of *Commiphora mukul*. The extract contains a number of compounds, but the isolated stereoisomers E- and Z-Guggulsterone showed a decline of hepatic cholesterol in rodent models.[14] **1c** inhibits CDCA-induced FXR activation by blocking co-activator recruitment. Still, the precise mechanism of action of **1c** remains to be elucidated and the compound is known to modulate various other nuclear receptors limiting its use as tool compound. [15] According to recent studies, inhibition of intestinal FXR activity through glycine-β-muricholic acid (**1d**, Gly-β-MCA) reduced weight gain, non-alcoholic fatty liver disease (NAFLD) and insulin resistance in obese mice.[16] Intestinal FXR knockout abrogated these beneficial metabolic effects.[16,17] Altogether, the study suggests significant therapeutic value of FXR antagonism but only few non-steroidal FXR antagonist chemotypes were reported including 1,3,4-trisubstituted-pyrazolones (e.g. **3**),[18] pyrazole-4-carboxamides (e.g. **4**),[19] benzimidazoles (e.g. **5**)[20], NDB (**6**)[21] and GW4064-analogue **2c**[22] with *in vitro* potencies ranging from 0.04-12.2 µM. Therefore, novel potent, non-steroidal and selective FXR antagonists are required to further study FXR antagonism as potential therapeutic strategy.[5,13,22]

During structure activity relationship (SAR) studies on partial FXR agonists[9,10] we discovered marked FXR antagonistic potency of **7**. It robustly reduced baseline FXR activity in our bile salt export protein (BSEP)-based full-length FXR reporter gene assay with an IC_{50} value of 0.35±0.04 µM and competed with reference FXR agonist **2a** (3 µM) with an IC_{50} value of 0.44±0.15 µM (Figure 1A). However, a similar scaffold as **7** has been reported as potential firefly luciferase inhibitor[23] which might affect our reporter gene assay leading to false positive results. To exclude such activity, we conducted two control experiments. First, we fully induced firefly expression in our assay with **2a** (3 µM) and added **7** (10 µM) only one hour before cell lysis which had no effect on firefly activity (Figure 1B). If **7** would directly bind and inhibit the enzyme firefly luciferase, reduced firefly activity would be observable in this experiment. Second, we studied the effect of **7** on the expression of the FXR regulated gene small heterodimer partner (SHP). **7** diminished baseline SHP expression and GW4064-induced as well as CDCA-induced SHP expression (Figure 1C). Thus, the control experiments confirmed FXR mediated activity of **7** and we selected the compound as lead for FXR modulator development.



Scheme 1: *Natural and synthetic FXR ligands*: Steroidal agonists CDCA (**1a**) and OCA (**1b**), antagonists (Z)-Guggulsterone (**1c**) and Gly-β-MCA (**1d**). Synthetic agonists GW4064 (**2a**) and Tropifexor (**2b**) and antagonists **2c** & **3**-**6**. Antagonistic lead compound **7**.

2. Results

2.1. Chemistry

N-Phenylbenzamides **7**-**32** were prepared according to

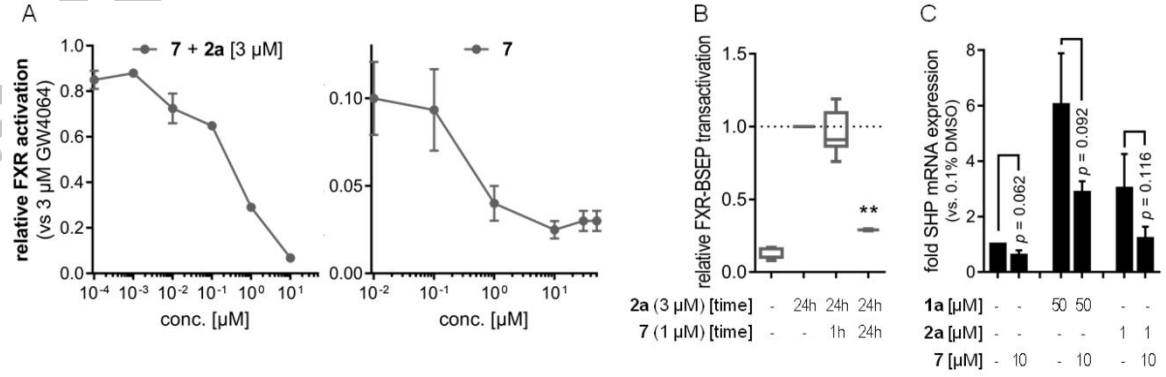
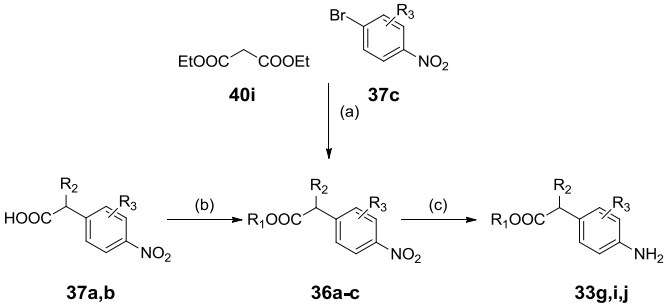


Figure 1: *Characterization of lead compound 7*: (A) Compound **7** antagonized GW4064-induced (left panel) and intrinsic (right panel) FXR activity in a bile salt export protein (BSEP)-based flFXR reporter gene assay (mean±SEM, n≥4). (B) When **7** (1 µM) was added one hour before lysis to cells treated with GW4064 (**2a**, 3 µM) for 23 hours in the reporter gene assay, no effect on firefly activity was observed (mean±SEM, n=6). Would **7** directly bind and inhibit firefly luciferase, the compound would also lower firefly activity in this setting. When **2a** and **7** were co-incubated for 24 h, significant (** $p < 0.01$) repression of firefly activity was observed confirming that **7** is not a firefly inhibitor but that its effects are FXR mediated. (C) Compound **7** suppressed intrinsic, GW4064- and CDCA-induced expression of FXR target gene small heterodimer partner (SHP) in HepG2 cells (mean±SEM, n=3).

Schemes 2-5.

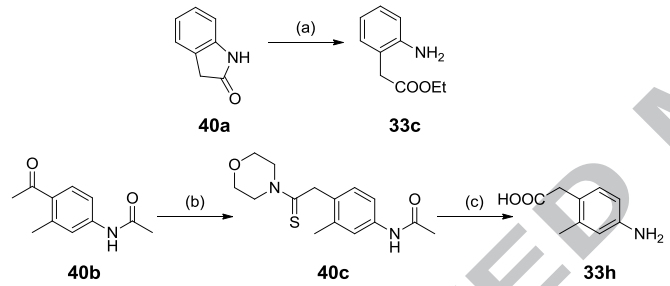
Anilines **33a,b,d-f** were commercially available, derivatives **33c,g-j** were synthesized. **36c** was prepared from bromo-nitrobenzene **37c** and diethyl malonate **40i** with NaH in DMF. Carboxylic acids **37a,b** were esterified with $SOCl_2$ in MeOH to **36a-c** before their nitro groups were reduced to amines using



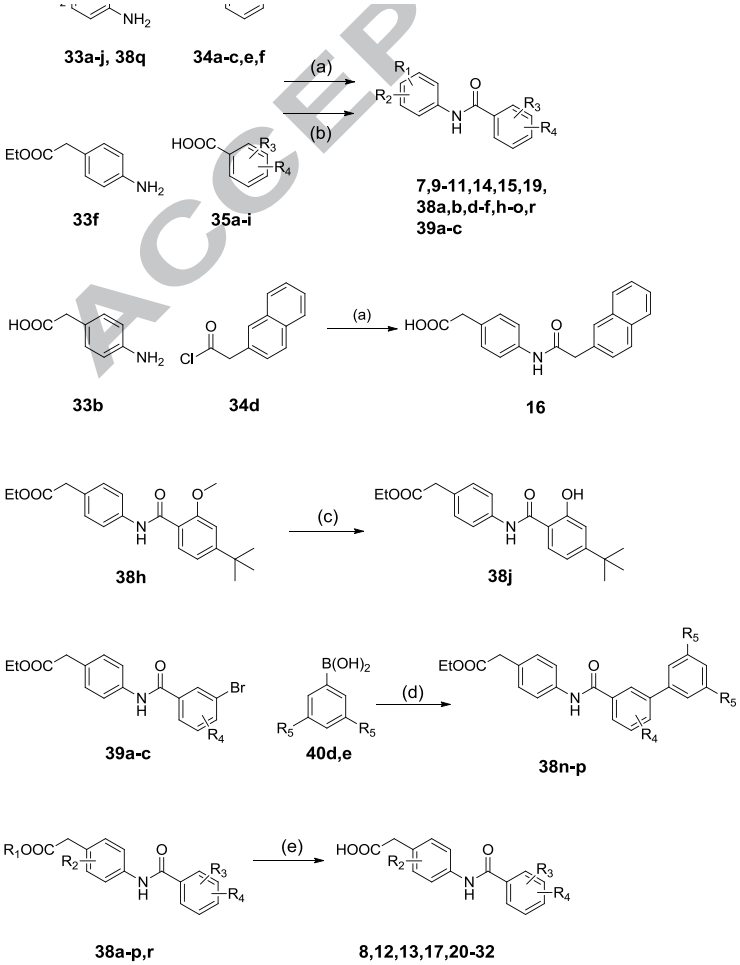
Scheme 2: *Reagents and conditions*: (a) NaH, DMF, 0 °C to 80 °C, 16 h (b) $SOCl_2$, MeOH, 0 °C to 80 °C, 2 h (c) Pd/C, H_2, MeOH, rt, 16 h.

Pd/C and H_2 to obtain the anilines **33g,i,j**.

Ester **33c** was synthesized by ring opening of lactam **40a** under acidic conditions. Precursor **33h** was obtained in a two-



Scheme 3: *Reagents and conditions*: (a) H_2SO_4, EtOH, 80 °C, 16 h (b) sulfur, morpholine, 135 °C, 6 h (c) KOH, EtOH, 80 °C, 6 h.

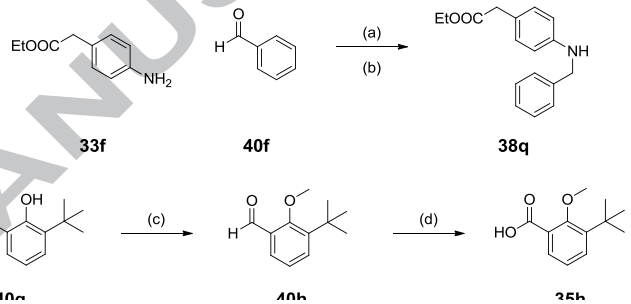


step Willgerodt-Kindler reaction starting with **40b**, sulfur and morpholine.

N-substituted derivative **38q** was available by reductive amination of ethyl 2-(4-aminophenyl)acetate (**33f**) and benzaldehyde (**40f**) with $NaBH(OAc)_3$. Benzoic acid **35h** was synthetized from salicyl aldehyde **40g** trough methylation of the alcohol with MeI followed by oxidation of the aldehyde using $KMnO_4$.

Building blocks **33a-j** and **38q** were fused with carbonyl chlorides **34a-f** in the presence of pyridine or by coupling with carboxylic acids **35a-i** in the presence of EDC and 4-DMAP leading to compounds **7, 9-11, 14, 15, 19**, esters **38a,b,d-f,h-o,r**, and **39a-c**. Bromides **39a-c** were coupled with boronic acids **40d,e** under Suzuki conditions to yield esters **38n-p**. The methoxy ether of **38h** was cleaved to a free hydroxyl function in **38j** by BBr_3. As final step, all esters **38a-p,r** were hydrolyzed under basic conditions to compounds **8, 12, 13, 17, 20-32** (scheme 5).

2.2. Biological evaluation



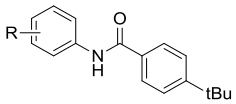
Scheme 5: *Reagents and conditions*: (a) pyridine, DMF, THF, rt, 2 h (b) EDC·HCl, DMAP, $CHCl_3$, 0 °C to 80 °C, 4 h (c) BBr_3, DCM, 0 °C to rt, 2 h (d) $Pd(PPh_3)_4$, Cs_2CO_3, EtOH, toluene, 80 °C, 16 h (e) LiOH, THF, H_2O, 60 °C, 16 h.

FXR antagonists **7-32** were characterized *in vitro* in a full-length FXR reporter gene assay relying on a FXR inducible firefly luciferase under the control of the human FXR response element from the promoter region of FXR target gene BSEP. The assay was conducted in HeLa cells that were transiently transfected by the calcium phosphate method with constitutive (CMV promoter) expression plasmids for the full-length human FXR and its heterodimer partner RXR, the FXR inducible firefly construct as reporter gene and a constitutively active *renilla* luciferase (SV40 promoter) for transfection efficiency normalization and toxicity control. For antagonistic characterization, dose-response curves were recorded for **7-32** in competition with FXR agonist **2a** at 3 µM.

2.3. Structure-activity relationship

With **7** as lead pharmacophore, we explored the structure activity relationship of this novel class of nonsteroidal FXR antagonists by systematically evaluating all molecular building blocks. We entered this FXR antagonist optimization with identifying the most suitable position and length of the acidic side chain (**7-11**, Table 1). Compared to 2-(3-phenyl)acetic acid in **7**, 2-(2-phenyl)acetic acid **8** and 2-(4-phenyl)acetic acid **9** improved FXR antagonistic activity with **9** as slightly more potent isomer. Chain elongation from 2-(4-phenyl)acetic acid **9** to 3-(4-phenyl)propionic acid **10** and 4-(4-phenyl)butyric acid **11** diminished potency rendering **9** as improved lead for further optimization.

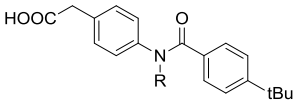
Table 1: FXR-antagonistic potency of compounds **7-11** in competition with **2a** (3 µM) *in vitro* (mean±SEM, n≥4).



ID	R =	IC_{50}(hFXR) [µM]
7	3-CH_2-COOH	0.4±0.2
8	2-CH_2-COOH	0.102±0.007
9	4-CH_2-COOH	0.076±0.009
10	4-CH_2-CH_2-COOH	0.63±0.03
11	4-CH_2-CH_2-CH_2-COOH	0.7±0.1

Using **9** as lead, we evaluated optimization potential of introducing substituents at the amine nitrogen (Table 2) but neither a small methyl group in **12** nor a bulky lipophilic benzyl substituent in **13** retained the nanomolar potency of **9**.

Table 2: FXR-antagonistic potency of compounds **12** and **13** in competition with **2a** (3 µM) *in vitro* (mean±SEM, n≥4).

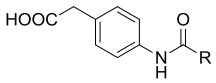


ID	R =	IC_{50}(hFXR) [µM]
9	-H	0.076±0.009
12	-CH_3	8.1±0.6
13	-CH_2-Ph	2.2±0.4

Further focusing on **9** as lead, we then replaced the 4-*tert*-butyl moiety by alternative bulky and lipophilic residues (Table 3). Both naphthyl isomers **14** and **15** were significantly less active and introduction of a methylene linker in **16** strongly diminished potency to intermediate micromolar values. Biphenyls **17**-**19** displayed a distinguished SAR with 2-biphenyl **17** being inactive, 3-biphenyl **18** being almost equally potent as **9** and 4-biphenyl **19** as intermediate FXR antagonist. In previous FXR modulator SAR studies,[24] we had observed potent antagonistic activity of a 3-(3,5-dichlorophenyl)pyridine scaffold which was compatible for combination with 3-biphenyl **18**. However, the SAR of this compound series turned out different as introduction of two chlorine atoms in **20** significantly diminished potency.

As no improvement in potency compared to **9** was achieved with alternative lipophilic backbones (**14**-**20**) or residues on the amide bond (**12, 13**), we analyzed the potential of introducing further substituents on **9** (Table 4). Systematic introduction of an additional methyl group in every free position of the acidic head group scaffold (**21**-**23**) only revealed comparable potency for 3-methyl derivative **22** suggesting that this position might offer an opportunity for optimization. However, even slight enlargement to a methoxy group (**24**) caused a significant loss in antagonistic activity. Both, α-methyl analogue **21** and 2-methyl derivative **23** were significantly less active than **9** and indicated that expansion in these positions was not tolerated. Thus, further variations in the 2-(4-aminophenyl)acetic acid head group failed to improve potency.

Table 3: FXR-antagonistic potency of compounds **14**-**20** in competition with **2a** (3 µM) *in vitro* (mean±SEM, n≥4).



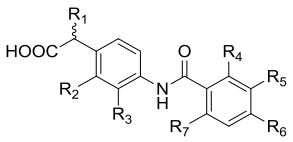
ID	R =	IC_{50}(hFXR) [µM]
9	4-*t*Bu-Ph	0.076±0.009
14	1-naphthyl	0.81±0.02
15	2-naphthyl	0.68±0.01
16	-CH_2-2-naphthyl	27±1
17	2-biphenyl	inactive at 30 µM
18	3-biphenyl	0.28±0.02
19	4-biphenyl	0.85±0.01
20	3‘,5‘-dichloro-3-biphenyl	9.1±0.2

Next, we focused on the 4-*tert*-butylbenzamide moiety as remaining molecular building block to be optimized. Shifting the *tert*-butyl residue from 4- (**9**) to 3-position (**28**) only slightly diminished potency and was superior to 3-biphenyl **18** suggesting that the *tert*-butyl substituent was already an optimal moiety for FXR antagonism. Following a similar strategy as for the 2-(4-aminophenyl)acetic acid group by introducing methoxy groups in the free positions of **9** produced a remarkable improvement in antagonistic activity for 4-*tert*-butyl-2-methoxybenzamide **25** which revealed a single-digit nanomolar IC_{50} value. The 3-methoxy isomer **26** was significantly less active. Replacing the 2-methoxy substituent (**25**) by a 2-hydroxy group (**27**) to eventually enhance polarity and solubility failed to retain high potency of **25**. In an attempt to combine our findings, we also studied the introduction of methoxy groups in the favorable 3-biphenyl (**18**) and 3-*tert*-butyl (**28**) derivatives but neither combination (**29**-**32**) revealed a comparable low nanomolar activity as **25** rendering 4-*tert*-butyl-2-methoxybenzamide **25** the most potent FXR modulator of this study.

2.4. In vitro pharmacological characterization

FXR modulator **25** was, therefore, characterized in more detail *in vitro*. **25** competed with **2a** (3 µM) in our assay with an IC_{50} value of 9.2±0.6 nM and antagonized intrinsic FXR activation with an IC_{50} value of 1.3±0.7 nM (Figure 2A) rendering the compound one of the most potent FXR antagonists in literature. Control experiments as described above for **7** excluded firefly inhibition by **25** confirming FXR mediated activity (Figure 2B). **25** turned out non-toxic in a WST-1 assay in HepG2 cells (used for gRT-PCR) up to 100 µM and in HeLa cells (used for the reporter gene assay) up to 50 µM whereas in HEK293T cells considerable toxicity was observable above 10 µM (Figure 2C). Moreover, antagonist **25** comprised favorable aqueous solubility (16 mg/L) and showed high metabolic stability with 95% of the compound remaining after 60 min incubation with rat liver microsomes (Figure 2D). Selectivity profiling on nuclear receptors related to FXR (Figure 2E and 2F) revealed no agonistic or antagonistic activity of **25** at 1 µM on retinoid X receptor α (RXRα), constitutive androstane receptor (CAR), peroxisome proliferator-activated receptor γ (PPARγ), or liver X receptor α (LXRα).

Table 4: FXR-antagonistic potency of compounds **21**-**32** in competition with **2a** (3 µM) *in vitro* (mean±SEM, n≥4).



ID	R_1	R_2	R_3	R_4	R_5	R_6	R_7	IC_{50}(hFXR) [µM]
9	H	H	H	H	H	*t*Bu	H	0.076±0.009
21	$-CH_3$	H	H	H	H	*t*Bu	H	0.97±0.04
22	H	$-CH_3$	H	H	H	*t*Bu	H	0.08±0.01
23	H	H	$-CH_3$	H	H	*t*Bu	H	1.5±0.3
24	H	$-OCH_3$	H	H	H	*t*Bu	H	3.7±0.2
25	H	H	H	$-OCH_3$	H	*t*Bu	H	0.0092±0.0006
26	H	H	H	H	$-OCH_3$	*t*Bu	H	0.22±0.01
27	H	H	H	-OH	H	*t*Bu	H	0.14±0.03
18	H	H	H	H	-Ph	H	H	0.28±0.02
28	H	H	H	H	*t*Bu	H	H	0.19±0.02
29	H	H	H	$-OCH_3$	*t*Bu	H	H	3.3±0.3
30	H	H	H	H	*t*Bu	H	$-OCH_3$	inactive at 30 µM
31	H	H	H	$-OCH_3$	-Ph	H	H	5±1
32	H	H	H	H	-Ph	H	$-OCH_3$	0.17±0.02

To further analyze FXR modulation by **25**, we studied its effects on FXR regulated gene expression in human hepatocytes (HepG2 cells) on mRNA level by qRT-PCR (Figure 3). **25** robustly diminished intrinsic expression (compared to 0.1% DMSO) of the FXR target genes small heterodimer partner (SHP) and BSEP but surprisingly had virtually no effect on organic solute transporter α (OSTα) expression indicating gene selective activity. Moreover, **25** strongly diminished FXR agonist (**1a** or **2a**) induced SHP expression which further confirmed its potent FXR antagonistic activity.

3. Discussion & Conclusion

FXR is experiencing remarkable interest as innovative drug target for the treatment of liver disorders and metabolic diseases. Particularly the high incidence of NAFLD and NASH as hepatic manifestation of the metabolic syndrome[25] is promoting FXR targeted drug discovery with OCA (**1b**) leading the NASH pipeline[26]. While the clinical efficacy of **1b** has validated FXR activation as therapeutic strategy to counter NAFLD, NASH and the metabolic syndrome, FXR antagonism is far less studied. In contrast to many potent FXR agonists reported in literature, the number and diversity of FXR antagonists are still limited. Several data point to a potential therapeutic value of FXR antagonism including liver protection in cholestais[13,27,28] and treatment of some cancers that are characterized by FXR overexpression[12,13,29]. Moreover, also promising metabolic effects of intestine-selective FXR antagonism have been reported[16] that need further evaluation and confirmation. To further study and validate FXR antagonism towards therapeutic applications, novel and more potent FXR antagonists are needed.

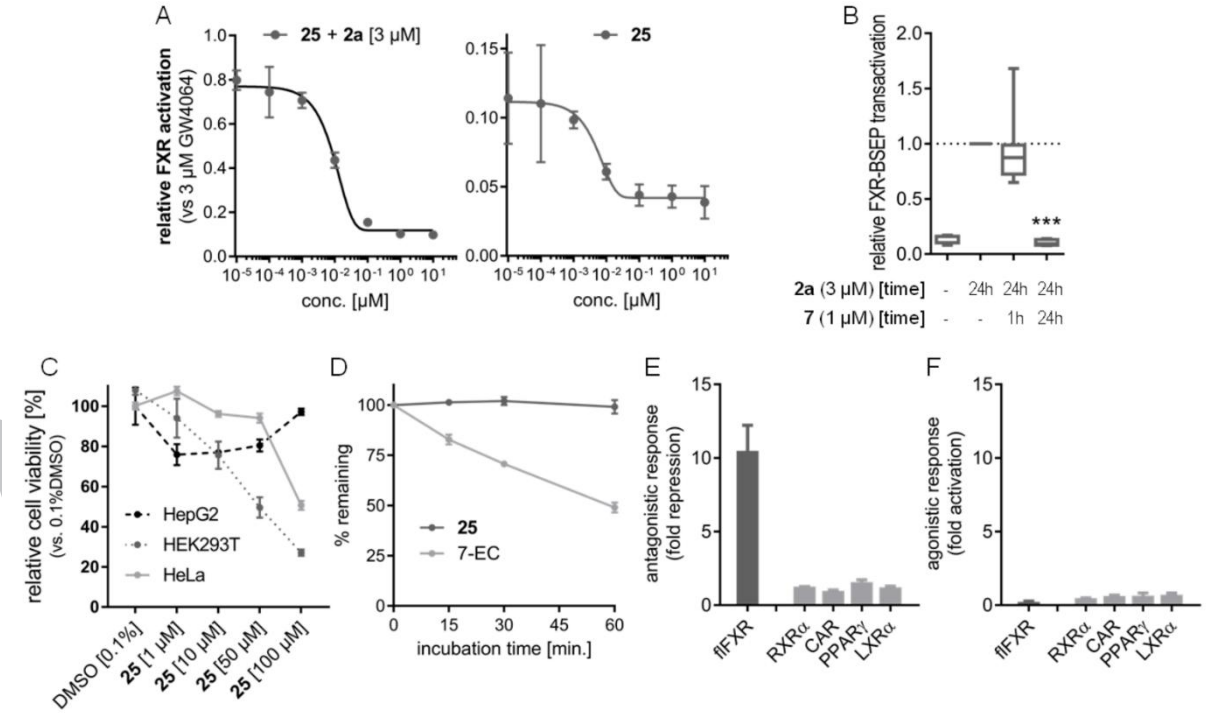


Figure 2: *In vitro profiling of FXR antagonist **25***: (A) **25** repressed intrinsic FXR activity (light grey) and antagonized GW4064-induced FXR activity (dark grey) in the full-length FXR reporter gene assay with low nanomolar IC_{50} values (mean±SEM, *n*=4). (B) Control experiments revealed no direct influence on firefly luciferase activity (incubation with **25** for 1 h) whereas 24 h co-incubation significantly (*** p<0.001) repressed firefly activity confirming FXR-mediated effects of **25** (mean±SEM, *n*=6). (C) **25** possessed no cytotoxicity in HepG2 cells up to 100 µM or in HeLa cells up to 50 µM while HEK293T cells were more sensitive and revealed marked toxic effects above 10 µM (mean±SEM, *n*=4). (D) In vitro metabolism studies revealed high microsomal stability of **25** with >95% parent compound remaining after 60 min incubation (7-EC: 7-ethoxycoumarin as control; mean±SEM, *n*=3). (E/F) Selectivity profiling of **25** on related nuclear receptors revealed no antagonistic (E) or agonistic (F) activity at 1 µM concentration (mean±SEM, *n*=3; reference compounds bexarotene (RXRα), CITCO (CAR), pioglitazone (PPARγ) and T0901317 (LXRα) were used at 1 µM concentration).

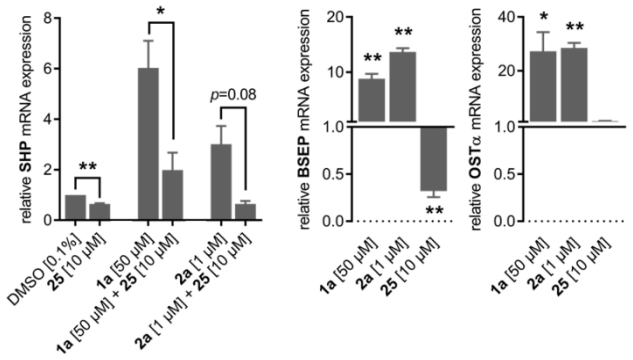


Figure 3: *Effects of **25** on FXR regulated gene expression:* FXR antagonist **25** significantly diminishes intrinsic expression of FXR target genes small heterodimer partner (SHP) and bile salt export protein (BSEP) but does not affect organic solute transporter α (OSTα) expression. Values are mean±SEM of mRNA expression compared to DMSO (0.1%) treated cells. n=3. * p<0.05; ** p<0.01.

Here we report a new class of FXR antagonists that was systematically optimized to **25** comprising low nanomolar potency. Of note, **25** competitively antagonized FXR activation by the reference agonist **2a** but also repressed intrinsic FXR activity in a full-length FXR reporter gene assay and in hepatoma cells indicated by reduced expression of FXR regulated genes SHP and BSEP. Furthermore, FXR antagonist **25** revealed low cytotoxicity, displayed high metabolic stability against microsomal degradation and was selective over related nuclear receptors rendering it a valuable tool to study FXR antagonism *in vitro* and *in vivo.*

4. Experimental

4.1. General:

All chemicals and solvents were of reagent grade and used without further purification unless otherwise specified. All reactions were conducted in oven-dried glassware under argon atmosphere and in absolute solvents. NMR spectra were recorded on a Bruker AV 400, Bruker AV 300, Bruker am250xp, or a Bruker AV 500 spectrometer (Bruker Corporation, Billerica, MA, USA). Chemical shifts (δ) are reported in ppm relative to tetramethylsilane (TMS) as reference. Multiplicity is reported: s, singlet; d, doublet; dd, doublet of doublets; t, triplet; dt, doublet of triplets; m, multiplet. Approximate coupling constants (*J*) are shown in hertz (Hz). Mass spectra were obtained on a VG Platform II (Thermo Fischer Scientific, Inc., Waltham, MA, USA) using electrospray ionization (ESI). High resolution mass spectra were recorded on a MALDI LTQ ORBITRAP XL instrument (Thermo Fisher Scientific). Compound purity was analyzed on a Varian ProStar HPLC (SpectraLab Scientific Inc., Markham, ON, Canada) equipped with a MultoHigh100 phenyl-5 µ 240 mm + 4 mm column (CS-Chromatographie Service GmbH, Langerwehe, Germany) using a gradient (H_2O/MeOH 80:20 + 0.1% formic acid isocratic for 5 min to MeOH + 0.1% formic acid after additional 45 min and MeOH + 0.1% formic acid for additional 10 min) at a flow rate of 1 mL/min and UV detection at 245 and 280 nm. All final compounds for biological evaluation had a purity of ≥95%.

4.2. Synthesis:

General procedure A

An aminobenzoic acid or a corresponding ester (**33a-e,g-j, 38q**, 1.0 eq) was dissolved in THF (abs., 20 mL/mmol), and DMF (abs., 1 mL/mmol) and pyridine (3.0 eq) were added. The respective benzoyl chloride (**34a-c,e,f**, 1.3 eq) was then added dropwise at room temperature. The mixture was stirred for two hours at room temperature. 5% aqueous hydrochloric acid (equal volume as THF) and EtOAc (equal volume as THF) were added, phases were separated, and the aqueous layer was extracted twice with EtOAc (equal volume as THF). The combined organic layers were dried over Na_2SO_4 and the solvents were removed in vacuum. Further purification was performed by column chromatography or crystallization.

General procedure B

Ethyl 2-(4-aminophenyl)acetate (**33f**, 1.3 eq) was dissolved in $CHCl_3$ (abs., 25 mL/mmol) and cooled to 0 °C. 4-(Dimethylamino)pyridine (0.10 eq), 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (1.1 eq) and the respective benzoic acid (**35a-i**, 1.0 eq) were added. The mixture was warmed to room temperature and afterwards stirred overnight at 80 °C under reflux. 5% aqueous hydrochloric acid (equal volume as $CHCl_3$) was added, phases were separated, and the aqueous layer was extracted three times with EtOAc (equal volume as $CHCl_3$). The combined organic layers were dried over Na_2SO_4 and the solvents were removed in vacuum. Further purification was performed by column chromatography or crystallization.

General procedure C

Ester (**38a-p,r**, 1.0 eq) was dissolved in THF (20 mL). H_2O (2 mL) and LiOH (6.0 eq) were added and the mixture was stirred for 18 h at 60 °C. 5% aqueous hydrochloric acid (20 mL) and EtOAc (20 mL) were added, phases were separated, and the aqueous layer was extracted with EtOAc (2x 20 mL). The combined organic layers were dried over Na_2SO_4 and the solvents were removed in vacuum. Further purification was performed by column chromatography or crystallization.

2-(3-(4-*tert*-Butylbenzamido)phenyl)acetic acid (7)

Preparation according to general procedure A using 2-(3-aminophenyl)acetic acid (**33a**) and 4-*tert*-butylbenzoyl chloride (**34a**). Further purification was performed by column chromatography with hexane/EtOAc/acetic acid (74:24:2) as mobile phase. Yield 0.15 g, 50%. R_f(hexane/EtOAc/acetic acid = 74:24:2) = 0.13. ^{1}H-NMR (400,13 MHz, DMSO-d_6): δ= 1.32 (s, 3H), 3.55 (s, 2H), 6.98-7.00 (d, J = 7.63 Hz, 1H), 7.26-7.30 (t, J = 7.84 Hz, 1H), 7.53-7.55 (d, J = 8.51 Hz, 2H), 7.66-7.71 (m, 2H), 7.88-7.90 (d, J = 8.49 Hz, 2H), 10.15 (s, 1H), 12.28 (bs, 1H). ^{13}C-NMR (100,61 MHz, DMSO-d_6): δ= 30.92, 34.65, 40.97, 118.62, 121.10, 124.60, 125.10, 127.49, 128.41, 132.21, 135.40, 139.22, 154.36, 165.41, 171.95, 172.57. HRMS (MALDI): *m/z* calculated 312.15941 for $C_{19}H_{22}NO_3$, found 312.15942 ($[M+H]^+$).

2-(2-(4-*tert*-Butylbenzamido)phenyl)acetic acid (8)

Preparation according to general procedure C using **38a**. Crystallization was performed in hexane/EtOAc. Yield 0.20 g, 47%. ^{1}H-NMR (500 MHz, DMSO-d_6): δ= 9.93 (s, 1H), 7.89-7.87 (m, 2H), 7.55-7.52 (m, 2H), 7.46 (d, J= 7.5 Hz, 1H), 7.34-7.27 (m, 2H), 7.20 (td, J= 7.5, 1.2 Hz, 1H), 3.65 (s, 2H), 1.32 (s, 9H). ^{13}C-NMR (126 MHz, DMSO-d_6): δ= 173.26, 165.67, 154.95, 131.45, 131.01, 127.93, 127.69, 126.71, 126.16, 125.86, 125.67, 38.10, 35.14, 31.40. HRMS (MALDI): *m/z* calculated 312.15942 for $C_{19}H_{22}NO_3$, found 312.15979 ($[M+H]^+$).

2-(4-(4-*tert*-Butylbenzamido)phenyl)acetic acid (9)

Preparation according to general procedure A using 2-(4-aminophenyl)acetic acid (**33b**) and 4-*tert*-butylbenzoyl chloride (**34a**). Further purification was performed by column chromatography with hexane/EtOAc/acetic acid (74:24:2) as mobile phase. Yield 0.18 g, 58%. R_f(hexane/EtOAc/acetic acid = 74:24:2) = 0.01. ^{1}H-NMR (400,13 MHz, DMSO-d_6): δ= 1.31 (s,

3H), 3.53 (s, 2H), 7.21-7.23 (d, *J* = 8.51 Hz, 2H), 7.53-7.55 (d, *J* = 8.51 Hz, 2H), 7.69-7.71 (d, *J* = 8.51 Hz, 2H), 7.86-7.89 (d, *J* = 8.50 Hz, 2H), 10.13 (s, 1H), 12.29 (bs, 1H). ^{13}C-NMR (100,61 MHz, DMSO-d_6): δ= 30.92, 34.65, 120.18, 125.11, 127.47, 129.45, 130.14, 132.25, 137.78, 154.34, 165.35, 172.77. HRMS (MALDI): *m/z* calculated 312.15942 for $C_{19}H_{22}NO_3$, found 312.15973 ([M+H]$^+$).

3-(4-(4-*tert*-Butylbenzamido)phenyl)propionic acid (10)
Preparation according to general procedure A using 3-(4-aminophenyl)propionic acid (**33d**) and 4-*tert*-butylbenzoyl chloride (**34a**). Further purification was performed by column chromatography with hexane/EtOAc/acetic acid (74:24:2) as mobile phase. Yield 0.32 g, 98%. R_f(hexane/EtOAc/acetic acid = 74:24:2) = 0.31. ^{1}H-NMR (400,13 MHz, DMSO-d_6): δ= 1.32 (s, 3H), 2.52-2.54 (m, 2H), 2.78-2.81 (t, *J* = 7.56 Hz, 2H), 7.18-7.20 (d, *J* = 8.49 Hz, 2H), 7.52-7.54 (d, *J* = 8.49 Hz, 2H), 7.65-7.68 (d, *J* = 8.52 Hz, 2H), 7.86-7.88 (d, *J* = 8.51 Hz, 2H), 10.09 (s, 1H), 12.12 (bs, 1H). ^{13}C-NMR (100,61 MHz, DMSO-d_6): δ= 29.85, 30.92, 34.64, 35.38, 120.29, 125.09, 127.44, 128.28, 132.31, 136.02, 137.24, 154.28, 165.29. HRMS (MALDI): *m/z* calculated 326.17507 for $C_{20}H_{24}NO_3$, found 326.17484 ([M+H]$^+$).

4-(4-(4-*tert*-Butylbenzamido)phenyl)butyric acid (11)
Preparation according to general procedure A using 4-(4-aminophenyl)butyric acid (**33e**) and 4-*tert*-butylbenzoyl chloride (**34a**). Further purification was performed by column chromatography with hexane/EtOAc (50:50) as mobile phase. Yield 0.22 g, 64%. R_f(hexane/EtOAc = 50:50) = 0.11. ^{1}H-NMR (500 MHz, DMSO-d_6): δ= 7.88-7.85 (m, 2H), 7.66 (d, *J*= 8.5 Hz, 2H), 7.55-7.51 (m, 2H), 7.15 (d, *J*= 8.5 Hz, 2H), 2.56 (t, *J*= 7.6 Hz, 2H), 2.21 (t, *J*= 7.4 Hz, 2H), 1.83-1.71 (m, 2H), 1.31 (s, 9H). ^{13}C-NMR (126 MHz, DMSO-d_6): δ= 174.47, 165.49, 154.45, 137.19, 136.86, 132.40, 128.52, 127.57, 125.25, 120.49, 34.77, 33.93, 33.11, 31.04, 26.45. HRMS (MALDI): *m/z* calculated 340.19072 for $C_{21}H_{26}NO_3$, found 340.19093 ([M+H]$^+$).

2-(4-(4-*tert*-Butyl-*N*-methylbenzamido)phenyl)acetic acid (12)
38b (0.39 g, 1.2 mmol, 1.0 eq) was dissolved in THF (abs., 3 mL) and ^{t}BuONa (0.13 g, 1.4 mmol, 1.2 eq) was added. The mixture was stirred for 15 minutes at room temperature. Subsequently iodomethane (0.09 mL, 1.4 mmol, 1.2 eq) was added and the mixture was stirred for 12 hours at room temperature. Afterwards H_2O (3 mL) and EtOAc (3 mL) were added, phases were separated, and the aqueous layer was extracted with EtOAc (2x 3 mL). The combined organic layers were dried over Na_2SO_4 and the solvents were removed in vacuum. Further purification was performed by column chromatography with hexane/EtOAc/HOAc (74:24:2) as mobile phase (R_f(hexane/EtOAc/HOAc = 74:24:2) = 0.26). The residue was then dissolved in THF (20 mL), H_2O (2 mL) and LiOH (0.03 g, 12.7 mmol, 1.1 eq) were added and the mixture was stirred for 16 hours at 60 °C. Afterwards the solution was treated with a 5% aqueous hydrochloric acid. The phases were separated, and the aqueous layer was extracted three times with EtOAc (3x 20 mL). The combined organic layers were dried over Na_2SO_4 and the solvents were removed in vacuum. No further purification was performed. Yield 0.06 g, 16%. ^{1}H-NMR (500 MHz, DMSO-d_6): δ= 7.73-7.66 (m, 6H), 7.56 (d, *J*= 8.4 Hz, 2H), 4.03 (s, 2H), 3.85 (s, 3H), 1.68 (s, 9H). ^{13}C-NMR (126 MHz, DMSO-d_6): δ= 182.18, 180.12, 163.01, 154.73, 144.31, 143.60, 140.66, 139.18, 137.37, 134.99, 50.27, 48.32, 44.87, 41.07, 33.00, 24.03. HRMS (MALDI): *m/z* calculated 326.17507 for $C_{20}H_{24}NO_3$, found 326.17530 ([M+H]$^+$).

2-(4-(4-*tert*-Butyl-*N*-benzylbenzamido)phenyl)acetic acid (13)
Preparation according to general procedure C using **38r**. Further purification was performed by column chromatography with hexane/EtOAc/acetic acid (78:20:2) as mobile phase. Yield 0.04 g, 29%. Rf (hexane/EtOAc/acetic acid (78:20:2)) = 0.32. ^{1}H-NMR (500 MHz, DMSO-d_6): δ= 12.32 (s, 1H), 7.32-7.22 (m, 9H), 7.08 (d, *J*= 8.3 Hz, 2H), 7.00 (d, *J*= 8.3 Hz, 2H), 5.06 (s, 2H), 3.45 (s, 2H), 1.20 (s, 9H). ^{13}C-NMR (126 MHz, DMSO-d_6): δ= 172.37, 169.54, 152.32, 141.68, 137.66, 133.18, 133.15, 130.01, 128.39, 127.59, 127.11, 127.04, 124.61, 53.10, 34.46, 30.88. HRMS (MALDI): *m/z* calculated 402.20637 for $C_{26}H_{28}NO_3$, found 402.20600 ([M+H]$^+$).

2-(4-(Naphthalen-1-ylamido)phenyl)acetic acid (14)
Preparation according to general procedure A using 2-(4-aminophenyl)acetic acid (**33b**) and 1-napthoyl chloride (**34b**). Further purification was performed by column chromatography with toluene/EtOAc/acetic acid (88:0:2) as mobile phase. Yield 0.36 g, 55%. Rf (toluene/EtOAc/acetic acid (88:10:2)) = 0.12. ^{1}H-NMR (500 MHz, DMSO-d_6): δ= 10.41 (s, 1H), 8.58 (s, 1H), 8.10-7.99 (m, 4H), 7.75 (d, *J*= 8.4 Hz, 2H), 7.67-7.60 (m, 2H), 7.25 (d, *J*= 8.4 Hz, 2H), 3.54 (s, 2H). ^{13}C-NMR (100,61 MHz, DMSO-d_6): δ= 172.90, 165.50, 137.73, 134.28, 132.28, 132.11, 130.56, 129.58, 128.98, 128.03, 127.95, 127.84, 127.70, 126.88, 124.48, 120.31. HRMS (MALDI): *m/z* calculated 328.09441 for $C_{19}H_{15}NO_3Na$, found 328.09443 ([M+Na]$^+$).

2-(4-(Naphthalen-2-ylamido)phenyl)acetic acid (15)
Preparation according to general procedure A using 2-(4-aminophenyl)acetic acid (**33b**) and 2-napthoyl chloride (**34c**). Further purification was performed by column chromatography with toluene/EtOAc/acetic acid (88:10:2) as mobile phase. Yield 0.47 g, 73%. Rf (toluene/EtOAc/acetic acid (88:10:2)) = 0.13. ^{1}H-NMR (500 MHz, DMSO-d_6): δ= 12.35 (s, 1H), 10.42 (s, 1H), 8.58 (s, 1H), 8.11-7.99 (m, 4H), 7.76 (d, *J*= 8.3 Hz, 2H), 7.67-7.60 (m, 2H), 7.26 (d, *J*= 8.4 Hz, 2H), 3.55 (s, 2H). ^{13}C-NMR (100,61 MHz, DMSO-d_6): δ= 172.86, 165.48, 137.76, 134.27, 132.27, 132.10, 130.40, 129.58, 128.97, 128.01, 127.95, 127.82, 127.69, 126.86, 124.47, 120.31. HRMS (MALDI): *m/z* calculated 306.11247 for $C_{19}H_{16}NO_3$, found 306.11236 ([M+H]$^+$).

2-(4-(2-(Naphthalen-2-yl)acetamido)phenyl)acetic acid (16)
Preparation according to general procedure A using 2-(4-aminophenyl)acetic acid (**33b**) and 2-(naphthalen-2-yl)acetyl chloride (**34d**). Further purification was performed by column chromatography with toluene/EtOAc/acetic acid (74:24:2) as mobile phase. Yield 0.16 g, 24%. Rf (toluene/EtOAc/acetic acid (74:24:2)) = 0.41. ^{1}H-NMR (500 MHz, DMSO-d_6): δ= 12.29 (s, 1H), 10.22 (s, 1H), 7.88 (dd, *J*= 7.6, 5.2 Hz, 3H), 7.83 (s, 1H), 7.54 (d, *J*= 8.5 Hz, 2H), 7.52-7.45 (m, 3H), 7.18 (d, *J*= 8.5 Hz, 2H), 3.81 (s, 2H), 3.49 (s, 2H). ^{13}C-NMR (100,61 MHz, DMSO-d_6): δ= 172.82, 168.96, 137.76, 133.72, 133.01, 131.85, 129.87, 129.65, 127.73, 127.68, 127.51, 127.45, 127.43, 126.15, 125.62, 119.09, 43.40. HRMS (MALDI): *m/z* calculated 320.12812 for $C_{20}H_{18}NO_3$, found 320.12790 ([M+H]$^+$).

2-(4-([1,1'-Biphenyl]-2-carboxamido)phenyl)acetic acid (17)
Preparation according to general procedure C using **38d**. Further purification was performed by column chromatography with hexane/EtOAc/acetic acid (78:20:2) as mobile phase. Yield 0.16 g, 42%. R_f(hexane/EtOAc/acetic acid = 78:20:2) = 0.10. ^{1}H-NMR (500 MHz, DMSO-d_6): δ= 12.30 (s, 1H), 10.19 (s, 1H), 7.57 (dd, *J*= 11.1, 4.4 Hz, 2H), 7.50-7.35 (m, 8H), 7.30 (t, *J*= 7.3 Hz, 1H), 7.14 (d, *J*= 8.4 Hz, 2H), 3.48 (s, 2H). ^{13}C-NMR (126 MHz, DMSO-d_6): δ= 172.82, 167.72, 140.04, 139.19, 137.60, 137.14, 130.17, 129.97, 129.75, 129.50, 128.30, 128.28,

127.80, 127.29, 127.22, 119.50. HRMS (MALDI): *m/z* calculated 354.11006 for $C_{21}H_{17}NO_3Na$, found 354.11088 ($[M+Na]^+$).

2-(4-([1,1'-Biphenyl]-3-carboxamido)phenyl)acetic acid (18)
Preparation according to general procedure A using 2-(4-aminophenyl)acetic acid (**33b**) and [1,1'-Biphenyl]-3-carbonyl chloride (**34e**). Further purification was performed by column chromatography with hexane/EtOAc/acetic acid (74:24:2) as mobile phase. Yield 0.04 g, 29%. Rf (hexane/EtOAc/acetic acid (74:24:2)) = 0.14. ^{1}H-NMR (500 MHz, DMSO-d_6): δ= 12.43 (s, 1H), 10.32 (s, 1H), 8.22 (s, 1H), 7.91 (dd, *J*= 29.0, 8.0 Hz, 2H), 7.79-7.69 (m, 4H), 7.62 (t, *J*= 7.7 Hz, 1H), 7.52 (t, *J*= 7.7 Hz, 2H), 7.42 (t, *J*= 7.4 Hz, 1H), 7.25 (d, *J*= 8.5 Hz, 2H), 3.53 (s, 2H). ^{13}C-NMR (126 MHz, DMSO-d_6): δ= 172.92, 165.29, 140.31, 139.55, 137.57, 135.63, 130.72, 129.75, 129.54, 129.14, 129.05, 127.86, 126.96, 126.85, 125.81, 120.35. HRMS (MALDI): *m/z* calculated 332.12812 for $C_{21}H_{18}NO_3$, found 332.12804 ($[M+H]^+$).

2-(4-([1,1'-Biphenyl]-4-carboxamido)phenyl)acetic acid (19)
Preparation according to general procedure A using 2-(4-aminophenyl)acetic acid (**33b**) and [1,1'-Biphenyl]-4-carbonyl chloride (**34f**). Further purification was performed by column chromatography with DCM/methanol/acetic acid (97:1:2) as mobile phase. Yield 0.04 g, 7%. Rf (DCM/methanol/acetic acid (97:1:2)) = 0.50. ^{1}H-NMR (500 MHz, DMSO-d_6): δ= 12.35 (s, 1H), 10.28 (s, 1H), 8.06 (d, *J*= 8.3 Hz, 2H), 7.84 (d, *J*= 8.3 Hz, 2H), 7.75 (dd, *J*= 13.4, 8.0 Hz, 4H), 7.51 (t, *J*= 7.6 Hz, 2H), 7.43 (t, *J*= 7.3 Hz, 1H), 7.24 (d, *J*= 8.4 Hz, 2H), 3.54 (s, 2H). ^{13}C-NMR (126 MHz, DMSO-d_6): δ= 172.87, 165.05, 143.08, 139.13, 137.73, 133.71, 130.38, 129.55, 129.09, 128.37, 128.17, 126.94, 126.60, 120.30. HRMS (MALDI): *m/z* calculated 332.12812 for $C_{21}H_{18}NO_3$, found 332.12809 ($[M+H]^+$).

2-(4-(3',5'-Dichloro-[1,1'-biphenyl]-3-carboxamido)phenyl)acetic acid (20)
Preparation according to general procedure C using **38p**. Yield 0.04 g, 58%. Crystallization was performed in hexane/EtOAc. ^{1}H-NMR (500 MHz, DMSO-d_6): δ= 12.28 (s, 1H), 10.32 (s, 1H), 8.27 (s, 1H), 8.01-7.96 (m, 2H), 7.88 (d, *J*= 1.8 Hz, 2H), 7.72 (d, *J*= 8.5 Hz, 2H), 7.65 (dt, *J*= 15.6, 4.8 Hz, 2H), 7.26 (d, *J*= 8.5 Hz, 2H), 3.55 (s, 2H). ^{13}C-NMR (126 MHz, DMSO-d_6): δ= 172.82, 164.99, 143.06, 137.55, 137.30, 135.71, 134.79, 130.49, 130.15, 129.59, 129.36, 128.20, 127.28, 126.03, 125.72, 120.51, 30.43. HRMS (MALDI): *m/z* calculated 400.05018 for $C_{20}H_{24}NO_4$, found 400.04981 ($[M+H]^+$).

2-(4-(4-*tert*-butylbenzamido)phenyl)propionic acid (21)
Preparation according to general procedure C using **38e**. Further purification was performed by column chromatography with hexane/EtOAc/acetic acid (88:10:2) as mobile phase. Yield 0.46 g, 64%. Rf (hexane/EtOAc/acetic acid (88:10:2)) = 0.09. ^{1}H-NMR (500 MHz, DMSO-d_6): δ= 12.26 (s, 1H), 10.15 (s, 1H), 7.88 (d, *J*= 8.5 Hz, 2H), 7.71 (d, *J*= 8.6 Hz, 2H), 7.54 (d, *J*= 8.5 Hz, 2H), 7.25 (d, *J*= 8.6 Hz, 2H), 3.64 (q, *J*= 7.1 Hz, 1H), 1.36 (d, *J*= 7.1 Hz, 3H), 1.32 (s, 9H). ^{13}C-NMR (126 MHz, DMSO-d_6): δ= 175.48, 165.38, 154.40, 137.94, 136.38, 132.24, 127.54, 127.51, 125.16, 120.37, 44.16, 34.69, 30.95, 18.52. HRMS (MALDI): *m/z* calculated 326.17485 for $C_{20}H_{24}NO_3$, found 326.17541 ($[M+H]^+$).

2-(4-(4-*tert*-Butylbenzamido)-2-methylphenyl)acetic acid (22)
Preparation according to general procedure A using **33h** and 4-*tert*-butylbenzoyl chloride (**34a**). Further purification was performed by column chromatography with hexane/EtOAc/acetic acid (74:24:2) as mobile phase. Yield 0.04 g, 51%. Rf (hexane/EtOAc/acetic acid (74:24:2)) = 0.39. ^{1}H-NMR (500 MHz, DMSO-d_6): δ= 10.07 (s, 1H), 7.90-7.86 (m, 2H), 7.58-7.51 (m, 4H), 7.13 (d, *J*= 8.2 Hz, 1H), 3.54 (s, 2H), 2.23 (s, 3H), 1.32 (s, 9H). ^{13}C-NMR (126 MHz, DMSO-d_6): δ= 172.72, 172.09, 165.34, 154.37, 137.91, 136.82, 132.30, 130.42, 129.19, 127.51, 125.18, 121.82, 117.84, 30.98, 21.12, 19.43. HRMS (MALDI): *m/z* calculated 326.17485 for $C_{20}H_{24}NO_3$, found 326.17528 ($[M+H]^+$).

2-(4-(4-*tert*-Butylbenzamido)-3-methylphenyl)acetic acid (23)
Preparation according to general procedure C using **38f**. Yield 0.28 g, 86%. Crystallization was performed in hexane/EtOAc. ^{1}H-NMR (500 MHz, DMSO-d_6): δ= 12.30 (s, 1H), 9.76 (s, 1H), 7.91 (d, *J*= 8.5 Hz, 2H), 7.58-7.52 (m, 2H), 7.26 (d, *J*= 8.0 Hz, 1H), 7.14 (d, *J*= 1.4 Hz, 1H), 7.09 (dd, *J*= 8.0, 1.8 Hz, 1H), 3.53 (s, 2H), 2.20 (s, 3H), 1.32 (s, 9H). ^{13}C-NMR (126 MHz, DMSO-d_6): δ= 172.84, 165.25, 154.40, 135.07, 133.49, 132.61, 131.83, 131.29, 127.53, 127.01, 126.47, 125.22, 34.71, 30.99, 17.89. HRMS (MALDI): *m/z* calculated 326.17485 for $C_{20}H_{24}NO_3$, found 326.17507 ($[M+H]^+$).

2-(4-(4-*tert*-Butylbenzamido)-2-methoxyphenyl)acetic acid (24)
Preparation according to general procedure C using **38g**. Further purification was performed by column chromatography with hexane/EtOAc/acetic acid (78:20:2) as mobile phase. Yield 0.07 g, 42%. Rf (hexane/EtOAc/acetic acid (78:20:2)) = 0.03. ^{1}H-NMR (500 MHz, DMSO-d_6): δ= 10.14 (s, 1H), 7.89 (d, *J*= 8.5 Hz, 2H), 7.54 (d, *J*= 8.5 Hz, 2H), 7.50 (d, *J*= 1.9 Hz, 1H), 7.32 (dd, *J*= 8.1, 1.9 Hz, 1H), 7.11 (d, *J*= 8.2 Hz, 1H), 3.75 (s, 3H), 3.46 (s, 2H), 1.32 (s, 9H). ^{13}C-NMR (126 MHz, DMSO-d_6): δ= 172.77, 172.16, 165.46, 157.23, 154.49, 139.44, 132.29, 130.81, 127.54, 125.24, 118.83, 111.85, 103.30, 55.36, 35.12, 34.75, 31.00. HRMS (MALDI): *m/z* calculated 342.16998 for $C_{20}H_{24}NO_4$, found 342.16982 ($[M+H]^+$).

2-(4-(4-*tert*-Butyl-2-methoxybenzamido)phenyl)acetic acid (25)
Preparation according to general procedure C using **38h**. Yield 0.22 g, 86%. Crystallization was performed in EtOAc. ^{1}H-NMR (500 MHz, DMSO-d_6): δ= 12.28 (s, 1H), 10.03 (s, 1H), 7.66 (d, *J*= 8.5 Hz, 2H), 7.60 (d, *J*= 8.0 Hz, 1H), 7.21 (d, *J*= 8.5 Hz, 2H), 7.13-7.06 (m, 2H), 3.93 (s, 3H), 3.52 (s, 2H), 1.32 (s, 9H). ^{13}C-NMR (126 MHz, DMSO-d_6): δ= 172.86, 164.24, 156.42, 155.53, 137.69, 130.04, 129.63, 129.60, 122.00, 119.54, 117.48, 109.08, 55.88, 34.97, 30.96. HRMS (MALDI): *m/z* calculated 342.16998 for $C_{20}H_{24}NO_4$, found 342.17051 ($[M+H]^+$).

2-(4-(4-*tert*-Butyl-3-methoxybenzamido)phenyl)acetic acid (26)
Preparation according to general procedure C using **38i**. Yield 0.24 g, 88%. Crystallization was performed in EtOAc. ^{1}H-NMR (500 MHz, DMSO-d_6): δ= 12.29 (s, 1H), 10.13 (s, 1H), 7.69 (d, *J*= 8.3 Hz, 2H), 7.49 (d, *J*= 7.1 Hz, 2H), 7.35 (d, *J*= 8.2 Hz, 1H), 7.23 (d, *J*= 8.3 Hz, 2H), 3.91 (s, 3H), 3.54 (s, 2H), 1.36 (s, 9H). ^{13}C-NMR (126 MHz, DMSO-d_6): δ= 172.83, 165.22, 157.98, 140.92, 137.71, 134.07, 130.24, 129.52, 126.16, 120.35, 119.64, 110.98, 55.43, 34.72, 29.42. HRMS (MALDI): *m/z* calculated 342.16998 for $C_{20}H_{24}NO_4$, found 342.16997 ($[M+H]^+$).

2-(4-(4-*tert*-Butyl-2-hydroxybenzamido)phenyl)acetic acid (27)
Preparation according to general procedure C using **38j**. Yield 0.06 g, 33%. Crystallization was performed in hexane/DCM. ^{1}H-NMR (500 MHz, DMSO-d_6): δ= 12.30 (s, 1H), 11.87 (s, 1H), 10.30 (s, 1H), 7.93 (d, *J*= 8.4 Hz, 1H), 7.62 (d, *J*= 8.4 Hz, 2H),

7.25 (d, *J*= 8.4 Hz, 2H), 7.01 (dd, *J*= 8.4, 1.5 Hz, 1H), 6.95 (d, *J*= 1.5 Hz, 1H), 3.55 (s, 2H), 1.28 (s, 9H). ^{13}C-NMR (126 MHz, DMSO-d_6): δ= 172.76, 166.75, 158.80, 157.24, 136.66, 130.85, 129.66, 128.55, 120.97, 116.40, 114.17, 113.90, 34.66, 30.72. HRMS (MALDI): *m/z* calculated 328.15433 for $C_{19}H_{22}NO_4$, found 328.15427 ($[M+H]^+$).

2-(4-(3-*tert*-Butylbenzamido)phenyl)acetic acid (28)
Preparation according to general procedure C using **38k**. No further purification was performed. Yield 0.10 g, 60%. ^{1}H-NMR (500 MHz, DMSO-d_6): δ= 12.07 (s, 1H), 10.19 (s, 1H), 7.92 (t, *J*= 1.7 Hz, 1H), 7.77 (dd, *J*= 6.6, 1.4 Hz, 1H), 7.69 (d, *J*= 8.5 Hz, 2H), 7.62 (ddd, *J*= 7.8, 1.8, 1.0 Hz, 1H), 7.45 (t, *J*= 7.8 Hz, 1H), 7.23 (d, *J*= 8.5 Hz, 2H), 3.54 (s, 2H), 1.34 (s, 9H). ^{13}C-NMR (126 MHz, DMSO-d_6): δ= 172.86, 165.82, 150.94, 137.72, 134.75, 130.33, 129.53, 128.54, 128.14, 124.85, 124.35, 120.49, 34.64, 31.10. HRMS (MALDI): *m/z* calculated 312.15942 for $C_{19}H_{22}NO_3$, found 312.15985 ($[M+H]^+$).

2-(4-(3-*tert*-Butyl-2-methoxybenzamido)phenyl)acetic acid (29)
Preparation according to general procedure C using **38l**. No further purification was performed. Yield 0.14 g, 99%. ^{1}H-NMR (500 MHz, DMSO-d_6): δ= 12.21 (s, 1H), 10.31 (s, 1H), 7.67 (d, *J*= 8.5 Hz, 2H), 7.40 (dd, *J*= 7.9, 1.7 Hz, 1H), 7.32 (dd, *J*= 7.5, 1.7 Hz, 1H), 7.22 (d, *J*= 8.5 Hz, 2H), 7.09 (t, *J*= 7.7 Hz, 1H), 3.74 (s, 3H), 3.53 (s, 2H), 1.37 (s, 9H). ^{13}C-NMR (126 MHz, DMSO-d_6): δ= 172.83, 166.34, 156.78, 141.99, 137.84, 130.78, 130.24, 129.66, 128.26, 127.60, 122.56, 119.58, 60.87, 34.85, 30.50, 30.43. HRMS (MALDI): *m/z* calculated 342.16998 for $C_{20}H_{24}NO_4$, found 342.16989 ($[M+H]^+$).

2-(4-(5-*tert*-Butyl-2-methoxybenzamido)phenyl)acetic acid (30)
Preparation according to general procedure C using **38m**. Crystallization was performed in EtOAc. Yield 0.07 g, 50%. ^{1}H-NMR (500 MHz, DMSO-d_6): δ= 12.29 (s, 1H), 10.07 (s, 1H), 7.66 (dd, *J*= 13.7, 5.5 Hz, 3H), 7.52 (dd, *J*= 8.7, 2.6 Hz, 1H), 7.22 (d, *J*= 8.4 Hz, 2H), 7.11 (d, *J*= 8.8 Hz, 1H), 3.89 (s, 3H), 3.53 (s, 2H), 1.29 (s, 9H). ^{13}C-NMR (126 MHz, DMSO-d_6): δ= 172.83, 164.59, 154.38, 142.73, 137.65, 130.08, 129.61, 128.84, 126.24, 124.06, 119.55, 111.78, 55.98, 33.88, 31.22. HRMS (MALDI): *m/z* calculated 342.16998 for $C_{20}H_{24}NO_4$, found 342.17016 ($[M+H]^+$).

2-(4-(2-Methoxy-[1,1'-biphenyl]-3-carboxamido)phenyl)acetic acid (31)
Preparation according to general procedure C using **38n**. Crystallization was performed in hexane/EtOAc. Yield 0.15 g, 87%. ^{1}H-NMR (500 MHz, DMSO-d_6): δ= 12.26 (s, 1H), 10.33 (s, 1H), 7.68 (d, *J*= 8.5 Hz, 2H), 7.60-7.45 (m, 6H), 7.44-7.36 (m, 1H), 7.31 (t, *J*= 7.6 Hz, 1H), 7.23 (d, *J*= 8.5 Hz, 2H), 3.53 (s, 2H), 3.44 (s, 3H). ^{13}C-NMR (126 MHz, DMSO-d_6): δ= 172.83, 165.21, 154.29, 137.71, 137.47, 134.87, 132.52, 131.76, 130.24, 129.67, 128.83, 128.47, 128.31, 127.53, 124.19, 119.49, 61.29. HRMS (MALDI): *m/z* calculated 362.13868 for $C_{22}H_{20}NO_4$, found 362.13873 ($[M+H]^+$).

2-(4-(4-Methoxy-[1,1'-biphenyl]-3-carboxamido)phenyl)acetic acid (32)
Preparation according to general procedure C using **38o**. Crystallization was performed in hexane/EtOAc. Yield 0.13 g, 75%. ^{1}H-NMR (500 MHz, DMSO-d_6): δ= 12.26 (s, 1H), 10.16 (s, 1H), 7.89 (d, *J*= 2.4 Hz, 1H), 7.81 (dd, *J*= 8.6, 2.5 Hz, 1H), 7.73-7.64 (m, 4H), 7.50-7.42 (m, 2H), 7.39-7.19 (m, 4H), 3.94 (s, 3H), 3.54 (s, 2H). ^{13}C-NMR (126 MHz, DMSO-d_6): δ= 172.82, 164.29, 156.08, 139.12, 137.66, 132.42, 130.16, 129.89, 129.63, 129.01, 127.59, 127.13, 126.29, 125.57, 119.58, 112.67, 56.12. HRMS (MALDI): *m/z* calculated 362.13868 for $C_{22}H_{20}NO_4$, found 362.13868 ($[M+H]^+$).

Ethyl 2-(2-aminophenyl)acetate (33c)
Oxindole (**40a**, 2.53 g, 19.0 mmol, 1.0 eq) was dissolved in EtOH (abs., 19 mL) and 3.8 mL 98% sulfuric acid were added at room temperature. The mixture was stirred at 80 °C for 16 hours. Afterwards the solution was brought to pH 8 with an aqueous solution of Na_2CO_3. EtOAc (20 mL) was added, phases were separated, and the aqueous layer was extracted twice with EtOAc (2x 20 mL). The combined organic layers were dried over Na_2SO_4 and the solvents were removed in vacuum. Further purification was performed by column chromatography with hexane/EtOAc (60:40) as mobile phase. Yield 1.37 g, 40%. R_f(hexane/EtOAc = 60:40) = 0.70. ^{1}H NMR (250 MHz, $CDCl_3$) δ= 7.12-7.03 (m, 2H), 6.78-6.65 (m, 2H), 4.13 (dd, *J* = 14.3, 7.1 Hz, 4H), 3.55 (s, 2H), 1.24 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ= 172.00, 145.63, 131.23, 128.60, 119.74, 119.06, 116.67, 61.19, 38.68, 14.25.

Methyl 2-(4-aminophenyl)propionate (33g)
36a (1.0 g, 4.8 mmol, 1.0 eq) was dissolved in methanol (abs., 48 mL) and palladium on carbon (10%, 0.51 g, 0.05 mmol, 0.01 eq) was added. The mixture was then set under hydrogen atmosphere and stirred for 16 hours at room temperature. Afterwards the solution was filtered through celite and the solvent was removed in vacuum. Further purification was performed by column chromatography with hexane/EtOAc (75:25) as mobile phase. Yield 0.44 g, 50%. R_f(hexane/EtOAc = 75:25) = 0.13. ^{1}H NMR (250 MHz, DMSO-d_6) δ= 6.90 (d, *J*= 8.4 Hz, 2H), 6.50 (d, *J*= 8.5 Hz, 2H), 4.96 (s, 2H), 3.55 (s, 3H), 1.30 (d, *J*= 7.1 Hz, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ= 174.94, 147.62, 127.72, 127.49, 113.90, 51.52, 43.54, 18.62.

2-(4-Amino-2-methylphenyl)acetic acid (33h)
40c (0.76 g, 2.6 mmol, 1.0 eq) was dissolved in aqueous KOH solution (50% w/w, 2.0 mL), ethanol (3.6 mL) was added and the mixture was stirred for 6 hours at 80 °C. The mixture was then concentrated in vacuum, 50 °C warm water was added, and the mixture was filtered. The filtrate was brought to an acidic pH by addition of 36% aqueous hydrochloric acid and the precipitated product was filtered off. Yield 0.04 g, 9%. ^{1}H NMR (250 MHz, DMSO-d_6) δ= 10.18 (s, 4H), 7.28 (d, *J*= 8.8 Hz, 2H), 7.21-7.05 (m, 4H), 3.62 (s, 5H), 2.25 (s, 6H). ^{13}C NMR (75 MHz, DMSO-d_6) δ= 172.21, 138.61, 134.01, 131.52, 130.41, 124.33, 120.51, 19.11.

Methyl 2-(4-amino-3-methylphenyl)acetate (33i)
36b (0.42 g, 2.0 mmol, 1.0 eq) was dissolved in MeOH (abs., 20 mL), palladium on carbon (10%, 0.21 g, 0.02 mmol, 0.01 eq) was added, and the mixture was set under hydrogen atmosphere. The mixture was stirred for 16 hours at room temperature, was then filtered through celite and the solvent was removed in vacuum. Further purification was performed by column chromatography. Yield 0.20 g, 55%. R_f(hexane/EtOAc = 75:25) = 0.12. ^{1}H NMR (250 MHz, DMSO-d_6) δ= 6.77 (d, *J*= 12.2 Hz, 2H), 6.53 (d, *J*= 7.9 Hz, 1H), 4.72 (s, 2H), 3.57 (s, 3H), 3.41 (s, 2H), 2.02 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ= 172.36, 145.40, 130.78, 127.26, 121.40, 121.05, 113.94, 51.51, 39.59, 17.41.

Diethyl 4-amino-2-methoxyphenylmalonate (33j)

36c (0.21 g, 0.7 mmol, 1.0 eq) was dissolved in MeOH (abs., 7 mL), palladium on carbon (10%, 0.07 g, 0.007 mmol, 0.01 eq) was added, and the mixture was set under hydrogen atmosphere. The mixture was stirred for 16 hours at room temperature. The solution was then filtered through celite and the solvent was removed in vacuum. No further purification was performed. Yield 0.18 g, 93%. ^{1}H NMR (250 MHz, DMSO-d_6) δ= 6.80 (d, *J*= 8.2 Hz, 2H), 6.22 (d, *J*= 2.0 Hz, 2H), 6.13 (dd, *J*= 8.2, 2.0 Hz, 2H), 5.17 (s, 4H), 4.76 (s, 2H), 4.11 (q, *J*= 7.1 Hz, 11H), 3.66 (s, 6H), 1.16 (t, *J*= 7.1 Hz, 16H). ^{13}C NMR (75 MHz, DMSO-d_6) δ= 168.55, 157.39, 150.02, 129.28, 108.31, 105.85, 97.03, 60.92, 55.21, 50.24, 13.92.

3-*tert*-Butyl-2-methoxybenzoic acid (35h)
40h (0.43 g, 2.26 mmol, 1.0 eq) and $KMnO_4$ (0.89 g, 5.64 mmol, 2.5 eq) were dissolved in a mixture of acetone (20 mL) and H_2O (20 mL) and the mixture was stirred for 18 hours at room temperature. Afterwards the reaction mixture was filtered through celite and acetone was removed in vacuum. The aqueous layer was brought to alkaline pH by addition of aqueous NaOH solution (3 M, 10 mL) and washed with EtOAc (15 mL). The aqueous layer was then acidified by addition of 36% aqueous hydrochloric acid and extracted with EtOAc (3x 15 mL). The combined organic layers were dried over Na_2SO_4 and the solvent was removed in vacuum. No further purification was performed. Yield 0.20 g, 42%. ^{1}H NMR (250 MHz, DMSO-d_6) δ= 12.38 (s, 1H), 7.46 (ddd, *J*= 16.0, 7.7, 1.7 Hz, 2H), 7.05 (t, *J*= 7.7 Hz, 1H), 3.76 (s, 3H), 1.34 (s, 9H). ^{13}C NMR (75 MHz, DMSO-d_6) δ= 172.03, 168.48, 158.95, 142.38, 129.78, 128.98, 125.83, 122.37, 61.53, 34.80, 30.39, 21.06.

Methyl 2-(4-nitrophenyl)propionate (36a)
2-(4-Nitrophenyl)propionic acid (**37a**, 1.0 g, 5.1 mmol, 1.0 eq) was dissolved in MeOH (abs., 21 mL) and cooled to 0 °C. Thionyl chloride (1.1 mL, 15.4 mmol, 3.0 eq) was added and the mixture was stirred at 70 °C for 2 hours. After cooling to room temperature, H_2O (20 mL) and EtOAc (20 mL) were added, phases were separated, and the aqueous layer was extracted with EtOAc (3x 20 mL). The combined organic layers were washed with brine, dried over Na_2SO_4, and the solvents were removed in vacuum. Further purification was performed by column chromatography with hexane/EtOAc (90:10) as mobile phase. Yield 1.01 g, 94%. R_f(hexane/EtOAc = 90:10) = 0.31. ^{1}H NMR (250 MHz, DMSO-d_6) δ= 8.20 (d, *J*= 8.9 Hz, 2H), 7.58 (d, *J*= 8.6 Hz, 2H), 4.04 (q, *J*= 7.1 Hz, 1H), 3.61 (s, 3H), 1.44 (d, *J*= 7.2 Hz, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ= 173.35, 148.25, 146.58, 128.95, 123.69, 52.09, 44.14, 18.21.

Methyl 2-(3-methyl-4-nitrophenyl)acetate (36b)
2-(3-Methyl-4-nitrophenyl)acetic acid (**37b**, 0.41 g, 2.56 mmol, 1.0 eq) was dissolved in MeOH (abs., 10 mL) and cooled to 0 °C. Thionyl chloride (0.6 mL, 7.7 mmol, 3.0 eq) was added and the mixture was stirred at 80 °C for 2 hours. After cooling to room temperature, H_2O (10 mL) and EtOAc (10 mL) were added, phases were separated, and the aqueous layer was extracted with EtOAc (3x 10 mL). The combined organic layers were washed with brine, dried over Na_2SO_4, and the solvents were removed in vacuum. Further purification was performed by column chromatography with hexane/EtOAc (90:10) as mobile phase. Yield 0.43 g, 79%. R_f(hexane/EtOAc = 90:10) = 0.26. ^{1}H NMR (250 MHz, DMSO-d_6) δ= 7.96 (d, *J*= 8.3 Hz, 1H), 7.43-7.33 (m, 2H), 3.82 (s, 2H), 3.65 (s, 3H), 2.52 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ= 170.84, 147.67, 140.33, 133.78, 132.83, 128.40, 124.55, 51.92, 39.46, 19.61.

Diethyl (2-methoxy-4-nitrophenyl)malonate (36c)
2-Bromo-5-nitroanisol (**37c**, 0.40 g, 1.72 mmol, 1.0 eq) was dissolved in DMF (abs., 10 mL) and cooled to 0 °C before NaH (0.91 g, 5.7 mmol, 3.3 eq) was added. The mixture was warmed to room temperature and stirred for 90 min. Then, the reaction was cooled to 0 °C again and diethyl malonate (**40i**, 0.87 mL, 5.7 mmol, 3.3 eq) was added. The mixture was stirred overnight at 80 °C. The mixture was then poured on ice, EtOAc (15 mL) was added, phases were separated, and the aqueous layer was extracted with EtOAc (3x 15 mL). The combined organic layers were dried over Na_2SO_4 and the solvents were removed in vacuum. Further purification was performed by column chromatography with hexane/EtOAc (90:10) as mobile phase. Yield 0.21 g, 39%. R_f(hexane/EtOAc = 90:10) = 0.13. ^{1}H NMR (250 MHz, DMSO-d_6) δ= 7.94-7.78 (m, 2H), 7.51 (d, *J*= 8.4 Hz, 1H), 5.12 (s, 1H), 4.17 (q, *J*= 7.1 Hz, 4H), 3.92 (s, 3H), 1.18 (t, *J*= 7.1 Hz, 7H). ^{13}C NMR (75 MHz, DMSO-d_6) δ= 166.97, 157.33, 148.39, 130.36, 129.12, 115.59, 106.01, 61.69, 56.61, 51.45, 13.86.

Ethyl 2-(2-(4-*tert*-butylbenzamido)phenyl)acetate (38a)
Preparation according to general procedure A using **33c** and 4-*tert*-butylbenzoyl chloride (**34a**). Further purification was performed by column chromatography with hexane/EtOAc/acetic acid (74:24:2) as mobile phase. Yield 0.37 g, 99%. R_f(hexane/EtOAc/acetic acid = 74:24:2) = 0.67. ^{1}H NMR (250 MHz, DMSO-d_6) δ= 9.88 (s, 1H), 7.89 (d, *J* = 8.4 Hz, 2H), 7.59-7.49 (m, 2H), 7.43 (d, *J* = 7.9 Hz, 1H), 7.33 (t, *J* = 7.4 Hz, 2H), 7.23 (t, *J* = 7.3 Hz, 1H), 3.98 (q, *J* = 7.1 Hz, 2H), 3.74 (s, 2H), 1.34 (s, 9H), 1.04 (t, *J* = 7.1 Hz, 3H). ^{13}C-NMR (75 MHz, DMSO-d_6): δ= 171.22, 165.39, 154.36, 137.97, 132.24, 129.48, 129.42, 127.48, 125.12, 120.25, 60.19, 34.65, 30.92, 14.07.

Ethyl 2-(4-(4-*tert*-butylbenzamido)phenyl)acetate (38b)
Preparation according to general procedure A using ethyl 2-(4-aminophenyl)acetate (**33f**) and 4-*tert*-butylbenzoyl chloride (**34a**). Further purification was performed by column chromatography with hexane/EtOAc (80:20) as mobile phase. Yield 0.49 g, 96%. R_f(hexane/EtOAc = 80:20) = 0.36. ^{1}H-NMR (250 MHz, DMSO-d_6): δ= 10.15 (s, 2H), 7.88 (d, *J* = 8.4 Hz, 4H), 7.71 (d, *J* = 8.5 Hz, 4H), 7.54 (d, *J* = 8.4 Hz, 4H), 7.23 (d, *J* = 8.5 Hz, 4H), 4.08 (q, *J* = 7.1 Hz, 4H), 3.62 (s, 4H), 1.32 (s, 17H), 1.18 (t, *J* = 7.1 Hz, 6H). ^{13}C-NMR (75 MHz, DMSO-d_6): δ= 171.22, 165.39, 154.36, 137.97, 132.24, 129.48, 129.42, 127.48, 125.12, 120.25, 60.19, 34.65, 30.92, 14.07.

Ethyl 2-(4-([1,1'-biphenyl]-2-carboxamido)phenyl)acetate (38d)
Preparation according to general procedure B using ethyl 2-(4-aminophenyl)acetate (**33f**) and [1,1'-Biphenyl]-2-carboxylic acid acid (**35a**). Further purification was performed by column chromatography with hexane/EtOAc (80:20) as mobile phase. Yield 0.42 g, 95%. R_f(hexane/EtOAc = 80:20) = 0.18. ^{1}H NMR (250 MHz, DMSO-d_6) δ= 10.21 (s, 1H), 7.61-7.25 (m, 13H), 7.15 (d, *J*= 8.5 Hz, 2H), 4.12-3.99 (m, 2H), 3.58 (s, 2H), 1.17 (td, *J*= 7.1, 3.0 Hz, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ= 171.22, 167.75, 140.03, 139.20, 137.78, 137.09, 129.97, 129.74, 129.48, 128.27, 127.78, 127.27, 127.20, 119.61, 60.21, 39.52, 14.07.

Methyl 2-(4-(4-*tert*-butylbenzamido)phenyl)propionate (38e)
Preparation according to general procedure A using **33g** and 4-*tert*-butylbenzoyl chloride (**34a**). Further purification was performed by column chromatography with hexane/EtOAc (90:10) as mobile phase. Yield 0.46 g, 56%. R_f(hexane/EtOAc = 90:10) = 0.24. ^{1}H NMR (250 MHz, DMSO-d_6) δ= 10.15 (s, 1H), 7.88 (d, *J*= 8.5 Hz, 2H), 7.71 (t, *J*= 5.4 Hz, 2H), 7.54 (d, *J*= 8.5 Hz, 2H), 7.25 (d, *J*= 8.6 Hz, 2H), 3.77 (q, *J*= 7.1 Hz, 1H),

3.59 (s, 3H), 1.39 (d, *J*= 7.1 Hz, 3H), 1.32 (s, 8H). ^{13}C NMR (75 MHz, DMSO-d_6) δ= 174.85, 165.88, 154.88, 138.61, 136.17, 132.67, 129.67, 127.98, 125.62, 120.93, 52.22, 44.33, 35.15, 31.41, 18.95.

Methyl 2-(4-(4-*tert*-butylbenzamido)-3-methylphenyl)acetate (38f)

Preparation according to general procedure A using **33i** and 4-*tert*-butylbenzoyl chloride (**34a**). Further purification was performed by column chromatography with hexane/EtOAc (75:25) as mobile phase. Yield 0.16 g, 65%. R_f(hexane/EtOAc = 75:25) = 0.31. ^{1}H NMR (250 MHz, DMSO-d_6) δ= 9.75 (s, 1H), 7.91 (d, *J*= 8.6 Hz, 2H), 7.54 (d, *J*= 5.0 Hz, 2H), 7.28 (d, *J*= 8.0 Hz, 1H), 7.18-7.06 (m, 2H), 3.65 (s, 2H), 3.62 (s, 3H), 2.20 (s, 3H), 1.32 (s, 9H). ^{13}C NMR (75 MHz, DMSO-d_6) δ= 172.53, 172.18, 167.75, 165.70, 156.29, 154.86, 135.73, 134.09, 132.35, 131.70, 129.67, 127.96, 127.44, 127.02, 125.85, 125.65, 52.18, 35.14, 31.42, 21.53, 18.31.

Diethyl 4-(4-*tert*-butylbenzamido)-2-methoxyphenylmalonate (38g)

Preparation according to general procedure A using **33j** and 4-*tert*-butylbenzoyl chloride (**34a**). No further purification was performed. Yield 0.20 g, 95%. ^{1}H NMR (400 MHz, DMSO-d_6) δ= 10.29 (s, 1H), 7.87 (d, *J*= 8.6 Hz, 6H), 7.50 (d, *J*= 8.4 Hz, 6H), 4.93 (s, 1H), 4.18-4.12 (m, 4H), 3.77 (s, 3H), 1.31 (s, 9H), 1.19-1.17 (m, 6H). ^{13}C NMR (75 MHz, DMSO-d_6) δ= 170.34, 168.00, 167.27, 165.55, 156.70, 155.81, 154.55, 140.53, 132.11, 129.23, 128.11, 127.61, 125.37, 125.17, 116.53, 112.12, 103.51, 61.24, 59.78, 55.67, 50.79, 34.78, 30.95, 30.88, 20.77, 13.93.

Ethyl 2-(4-(4-*tert*-butyl-2-methoxybenzamido)phenyl)acetate (38h)

Preparation according to general procedure B using ethyl 2-(4-aminophenyl)acetate (**33f**) and 4-*tert*-butyl-2-methoxybenzoic acid (**35b**). Further purification was performed by column chromatography with hexane/EtOAc (80:20) as mobile phase. Yield 0.28 g, 67%. R_f(hexane/EtOAc = 80:20) = 0.31. ^{1}H NMR (250 MHz, DMSO-d_6) δ= 10.03 (s, 1H), 7.63 (dd, *J*= 16.7, 8.2 Hz, 3H), 7.21 (d, *J*= 8.5 Hz, 2H), 7.14-7.06 (m, 2H), 4.08 (q, *J*= 7.1 Hz, 2H), 3.94 (s, 3H), 3.61 (s, 2H), 1.32 (s, 9H), 1.18 (t, *J*= 7.1 Hz, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ= 171.26, 164.25, 156.41, 155.53, 137.83, 129.56, 129.40, 121.96, 119.62, 117.46, 109.07, 60.23, 55.87, 34.95, 30.94, 14.10.

Ethyl 2-(4-(4-*tert*-butyl-3-methoxybenzamido)phenyl)acetate (38i)

Preparation according to general procedure B using ethyl 2-(4-aminophenyl)acetate (**33f**) and 4-*tert*-butyl-3-methoxybenzoic acid (**35c**). Further purification was performed by column chromatography with hexane/EtOAc (80:20) as mobile phase. Yield 0.30 g, 71%. R_f(hexane/EtOAc = 80:20) = 0.33. ^{1}H NMR (250 MHz, DMSO-d_6) δ= 10.13 (s, 1H), 7.70 (d, *J*= 8.5 Hz, 2H), 7.49 (d, *J*= 5.7 Hz, 2H), 7.35 (d, *J*= 8.5 Hz, 1H), 7.24 (d, *J*= 8.5 Hz, 2H), 4.08 (q, *J*= 7.1 Hz, 2H), 3.91 (s, 3H), 3.63 (s, 2H), 1.36 (s, 9H), 1.19 (t, *J*= 7.1 Hz, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ= 171.25, 165.25, 157.98, 140.96, 137.87, 134.05, 129.61, 129.46, 126.15, 120.43, 119.64, 110.99, 60.23, 55.43, 34.71, 29.41, 14.09.

Ethyl 2-(4-(4-*tert*-butyl-2-hydroxybenzamido)phenyl)acetate (38j)

38i (0.15 g, 0.41 mmol, 1.0 eq) was dissolved in DCM (abs., 33 mL) and cooled to 0 °C. BBr_3 in DCM (1 M, 4.1 mL, 4.06 mmol, 10.0 eq) was added. The mixture was warmed to room temperature and stirred for two hours. 30 mL ice/water was then added and phases were separated. The aqueous layer was brought to pH 4 with $NaHCO_3$ and extracted with EtOAc (3x 15 mL). The combined organic layers were dried over Na_2SO_4 and the solvents were removed in vacuum. No further purification was performed. Yield 0.09 g, 62%. ^{1}H NMR (250 MHz, DMSO-d_6) δ= 11.83 (s, 1H), 10.30 (s, 1H), 7.92 (d, *J*= 8.1 Hz, 1H), 7.63 (d, *J*= 8.5 Hz, 2H), 7.26 (d, *J*= 8.5 Hz, 2H), 7.04-6.92 (m, 2H), 4.13-4.05 (m, 2H), 3.64 (s, 2H), 1.28 (s, 9H), 1.18 (dd, *J*= 7.1, 4.2 Hz, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ= 171.20, 166.72, 158.80, 157.22, 136.86, 130.20, 129.60, 128.58, 121.01, 116.35, 114.23, 113.91, 60.25, 34.65, 30.71, 14.09.

Ethyl 2-(4-(3-*tert*-butylbenzamido)phenyl)acetate (38k)

Preparation according to general procedure B using ethyl 2-(4-aminophenyl)acetate (**33f**) and 3-*tert*-butyl-benzoic acid (**35g**). No further purification was performed. Yield 0.18 g, 96%. ^{1}H NMR (250 MHz, DMSO-d_6) δ= 10.25 (s, 1H), 7.99 (t, *J*= 1.7 Hz, 1H), 7.83 (d, *J*= 7.7 Hz, 1H), 7.76 (d, *J*= 8.5 Hz, 2H), 7.72-7.64 (m, 1H), 7.51 (t, *J*= 7.7 Hz, 1H), 7.30 (d, *J*= 8.5 Hz, 2H), 4.14 (q, *J*= 7.0 Hz, 2H), 3.69 (s, 2H), 1.40 (s, 8H), 1.25 (t, *J*= 7.1 Hz, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ= 171.24, 165.81, 150.91, 137.87, 134.71, 129.64, 129.43, 128.50, 128.10, 124.81, 124.31, 120.54, 60.22, 34.60, 31.06, 14.08.

Ethyl 2-(4-(3-*tert*-butyl-2-methoxybenzamido)phenyl)acetate (38l)

Preparation according to general procedure B using ethyl 2-(4-aminophenyl)acetate (**33f**) and **35h**. Further purification was performed by column chromatography with hexane/EtOAc (80:20) as mobile phase. Yield 0.15 g, 43%. R_f(hexane/EtOAc = 80:20) = 0.52. ^{1}H NMR (250 MHz, DMSO-d_6) δ= 10.30 (s, 1H), 7.68 (d, *J*= 8.4 Hz, 2H), 7.40 (dd, *J*= 7.8, 1.5 Hz, 1H), 7.32 (dd, *J*= 7.5, 1.5 Hz, 1H), 7.23 (d, *J*= 8.4 Hz, 2H), 7.09 (t, *J*= 7.6 Hz, 1H), 4.13-4.04 (m, 2H), 3.75 (s, 3H), 3.62 (s, 2H), 1.37 (s, 9H), 1.19 (t, *J*= 7.1 Hz, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ= 171.21, 166.33, 156.75, 141.97, 137.97, 130.73, 129.59, 129.56, 128.24, 127.56, 122.52, 119.64, 60.86, 60.21, 34.81, 30.47, 14.08.

Ethyl 2-(4-(5-*tert*-butyl-2-methoxybenzamido)phenyl)acetate (38m)

Preparation according to general procedure B using ethyl 2-(4-aminophenyl)acetate (**33f**) and 5-*tert*-butyl-2-methoxybenzoic acid (**35i**). Further purification was performed by column chromatography with hexane/EtOAc (80:20) as mobile phase. Yield 0.16 g, 87%. R_f(hexane/EtOAc = 80:20) = 0.27. ^{1}H NMR (250 MHz, DMSO-d_6) δ= 10.06 (s, 1H), 7.66 (dd, *J*= 7.3, 5.6 Hz, 3H), 7.52 (dd, *J*= 8.7, 2.6 Hz, 1H), 7.22 (d, *J*= 8.5 Hz, 2H), 7.10 (d, *J*= 8.8 Hz, 1H), 4.08 (q, *J*= 7.1 Hz, 2H), 3.88 (s, 3H), 3.62 (s, 2H), 1.29 (s, 9H), 1.18 (t, *J*= 7.1 Hz, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ= 171.26, 154.39, 142.75, 137.80, 129.56, 129.46, 126.25, 124.01, 119.67, 111.80, 60.23, 55.99, 33.87, 31.21, 14.09.

Ethyl 2-(4-(2-methoxy-[1,1'-biphenyl]-3-carboxamido)phenyl)acetate (38n)

39b (0.27 g, 0.70 mmol, 1.0 eq), phenylboronic acid (**40d**, 0.09 g, 0.70 mmol, 1.0 eq) and Cs_2CO_3 (0.57 g, 1.75 mmol, 2.5 eq) were dissolved in a mixture of toluene (abs., 7 mL) and EtOH (abs., 0.7 mL). The mixture was stirred for 30 min at room temperature before tetrakis(triphenylphosphine)palladium(0) (0.08 g, 0.07 mmol, 0.1 eq) was added. The mixture was then stirred at 80 °C for 5 hours. After cooling to room temperature, H_2O (10 mL) was added, phases were separated, and the aqueous layer was extracted with EtOAc (3x 10 mL). The combined organic layers were dried over Na_2SO_4 and the solvents were removed in

vacuum. Further purification was performed by column chromatography with hexane/EtOAc (80:20) as mobile phase. Yield 0.18 g, 67%. R_f(hexane/EtOAc = 80:20) = 0.47. ^{1}H NMR (250 MHz, DMSO-d_6) δ= 10.35 (s, 1H), 7.69 (d, *J*= 8.5 Hz, 2H), 7.60-7.39 (m, 7H), 7.31 (t, *J*= 7.6 Hz, 1H), 7.24 (d, *J*= 8.5 Hz, 2H), 4.14-4.04 (m, 2H), 3.62 (s, 2H), 3.44 (s, 3H), 1.19 (t, *J*= 7.1 Hz, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ= 171.38, 165.36, 154.39, 137.91, 137.54, 134.96, 132.67, 131.69, 129.71, 128.90, 128.56, 128.40, 127.63, 124.30, 119.74, 61.39, 60.37, 14.17.

Ethyl 2-(4-(4-methoxy-[1,1'-biphenyl]-3-carboxamido)phenyl)acetate (38o)

39c (0.27 g, 0.70 mmol, 1.0 eq), phenylboronic acid (**40d**, 0.09 g, 0.70 mmol, 1.0 eq) and Cs_2CO_3 (0.57 g, 1.75 mmol, 2.5 eq) were dissolved a mixture of toluene (abs., 7 mL) and EtOH (abs., 0.7 mL). The mixture was stirred for 30 min at room temperature before tetrakis(triphenylphosphine)palladium(0) (0.08 g, 0.07 mmol, 0.1 eq) was added. The mixture was then stirred at 80 °C for 5 hours. After cooling to room temperature, H_2O (10 mL) and EtOAc (10 mL) were added, phases were separated, and the aqueous layer was extracted with EtOAc (3x 10 mL). The combined organic layers were dried over Na_2SO_4 and the solvents were removed in vacuum. Further purification was performed by column chromatography. Yield 0.17 g, 63%. R_f(hexane/EtOAc = 80:20) = 0.19. ^{1}H NMR (250 MHz, DMSO-d_6) δ= 10.17 (s, 1H), 7.89 (d, *J*= 2.4 Hz, 1H), 7.81 (dd, *J*= 8.6, 2.4 Hz, 1H), 7.68 (t, *J*= 7.3 Hz, 4H), 7.46 (t, *J*= 7.4 Hz, 2H), 7.34 (t, *J*= 7.3 Hz, 1H), 7.25 (t, *J*= 8.9 Hz, 3H), 4.14-4.02 (m, 2H), 3.94 (s, 3H), 3.63 (s, 2H), 1.19 (t, *J*= 7.1 Hz, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ= 171.28, 156.11, 139.13, 137.81, 132.47, 129.96, 129.60, 129.03, 127.63, 127.16, 126.30, 125.50, 119.73, 112.71, 60.26, 56.15, 14.11.

Ethyl 2-(4-(3',5'-dichloro-[1,1'-biphenyl]-3-carboxamido)phenyl)acetate (38p)

39a (0.27 g, 0.74 mmol, 1.0 eq), 3,5-dichlorophenylboronic acid (**40e**, 0.14 g, 0.74 mmol, 1.0 eq) and Cs_2CO_3 (0.6 g, 1.86 mmol, 2.5 eq) were dissolved in toluene (abs., 8 mL) and EtOH (abs., 0.8 mL) was added. The mixture was stirred for 30 min at room temperature. Tetrakis(triphenylphosphine)palladium(0) (0.09 g, 0.07 mmol, 0.1 eq) was added and the mixture was stirred at 80 °C for 5 hours. After cooling to room temperature, H_2O (10 mL) was added, phases were separated, and the aqueous layer was extracted with EtOAc (3x 10 mL). The combined organic layers were dried over Na_2SO_4 and the solvents were removed in vacuum. Further purification was performed by column chromatography with hexane/EtOAc (80:20) as mobile phase. Yield 0.08 g, 26%. R_f(hexane/EtOAc = 80:20) = 0.02. ^{1}H NMR (250 MHz, DMSO-d_6) δ= 10.32 (s, 1H), 8.27 (s, 1H), 7.98 (t, *J*= 7.0 Hz, 2H), 7.87 (d, *J*= 1.8 Hz, 2H), 7.73 (d, *J*= 8.4 Hz, 2H), 7.68-7.60 (m, 2H), 7.26 (d, *J*= 8.4 Hz, 2H), 4.09 (q, *J*= 7.1 Hz, 2H), 3.64 (s, 2H), 1.22-1.16 (m, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ= 171.37, 165.16, 143.12, 137.75, 137.38, 135.74, 134.88, 130.22, 130.00, 129.62, 129.48, 128.26, 127.35, 126.07, 125.76, 120.72, 60.37, 14.17.

Ethyl 2-(4-benzylaminophenyl)acetate (38q)

Ethyl 2-(4-aminophenyl)acetate (**33f**, 0.30 g, 1.67 mmol, 1.0 eq) and benzaldehyde (**40f**, 0.14 mL, 1.84 mmol, 1.1 eq) were dissolved in dichloroethane (abs., 12 mL). Acetic acid (0.19 mL, 3.35 mmol, 2.0 eq) was added and the mixture was stirred for 2 h at room temperature before $NaBH(OAc)_3$ (0.50 g, 2.34 mmol, 1.4 eq) was added. The mixture was then stirred overnight at room temperature. NaOH-solution (1 M, 12 mL) was added and the mixture was stirred for another 30 minutes. Phases were then separated and the aqueous layer was extracted with Et_2O (3x 20 mL). The combined organic layers were dried over Na_2SO_4 and the solvents were removed in vacuum. Further purification was performed by column chromatography with hexane/EtOAc (90:10) as mobile phase. Yield 0.25 g, 55%. R_f(hexane/EtOAc = 90:10) = 0.24. ^{1}H NMR (250 MHz, DMSO-d_6) δ= 7.38-7.26 (m, 4H), 7.21 (dd, *J*= 10.6, 4.3 Hz, 1H), 6.91 (d, *J*= 8.4 Hz, 2H), 6.51 (d, *J*= 8.5 Hz, 2H), 6.17 (t, *J*= 6.1 Hz, 1H), 4.24 (d, *J*= 6.1 Hz, 2H), 4.03 (q, *J*= 7.1 Hz, 2H), 3.41 (s, 2H), 1.15 (t, *J*= 7.1 Hz, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ= 147.49, 140.30, 129.63, 128.24, 127.13, 126.56, 121.23, 114.46, 112.20, 59.97, 57.54, 46.49, 14.09.

Ethyl 2(4-(4-*tert*-butyl-*N*-benzylbenzamido)phenyl)acetate (38r)

Preparation according to general procedure A using **38q** and 4-*tert*-butylbenzoyl chloride (**34a**). Further purification was performed by column chromatography with hexane/EtOAc (75:25) as mobile phase. Yield 0.15 g, 48%. R_f(hexane/EtOAc = 75:25) = 0.39. ^{1}H-NMR (250 MHz, DMSO-d_6): δ= 7.31-7.20 (m, 9H), 7.08 (d, *J*= 8.5 Hz, 2H), 7.04-6.96 (m, 2H), 5.06 (s, 2H), 4.42 (d, *J*= 4.1 Hz, 2H), 4.03-3.96 (m, 2H), 3.53 (s, 2H), 1.19 (d, *J*= 1.7 Hz, 9H), 1.09 (t, *J*= 5.0 Hz, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ= 170.98, 169.77, 152.52, 141.93, 137.66, 133.25, 132.69, 130.03, 128.52, 127.76, 127.43, 127.22, 124.71, 62.23, 60.36, 53.16, 34.56, 30.97, 30.81, 25.55, 18.62, 14.10.

Ethyl 2-(4-(3-bromobenzamido)phenyl)acetate (39a)

Preparation according to general procedure A using ethyl 2-(4-aminophenyl)acetate (**33f**) and 3-bromobenzoic acid (**35d**). No further purification was performed. Yield 0.27 g, 99%. ^{1}H NMR (250 MHz, DMSO-d_6) δ= 10.32 (s, 1H), 8.14 (t, *J*= 1.7 Hz, 1H), 8.00-7.90 (m, 1H), 7.82-7.75 (m, 1H), 7.70 (d, *J*= 8.5 Hz, 2H), 7.50 (t, *J*= 7.9 Hz, 1H), 7.25 (d, *J*= 8.5 Hz, 2H), 4.08 (q, *J*= 7.1 Hz, 2H), 3.63 (s, 2H), 1.19 (t, *J*= 7.1 Hz, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ= 171.20, 163.86, 137.57, 137.05, 134.26, 130.65, 130.20, 129.93, 129.50, 126.83, 121.66, 120.43, 60.22, 14.08.

Methyl 2-(4-(3-bromo-2-methoxybenzamido)phenyl)acetate (39b)

Preparation according to general procedure B using ethyl 2-(4-aminophenyl)acetate (**33f**) and 3-bromo-2-methoxybenzoic acid (**35f**). No further purification was performed. Yield 0.18 g, 98%. ^{1}H NMR (250 MHz, DMSO-d_6) δ= 10.37 (s, 1H), 7.77 (dd, *J*= 8.0, 1.6 Hz, 1H), 7.66 (d, *J*= 8.5 Hz, 2H), 7.53 (dd, *J*= 7.6, 1.6 Hz, 1H), 7.27-7.15 (m, 3H), 4.08 (q, *J*= 7.1 Hz, 2H), 3.82 (s, 3H), 3.62 (s, 2H), 1.19 (t, *J*= 7.1 Hz, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ= 171.30, 164.27, 153.66, 137.67, 134.92, 133.00, 129.94, 129.70, 128.68, 125.76, 119.79, 116.97, 61.91, 60.33, 14.14.

Ethyl 2-(4-(5-bromo-2-methoxybenzamido)phenyl)acetate (39c)

Preparation according to general procedure B using ethyl 2-(4-aminophenyl)acetate (**33f**) and 5-bromo-2-methoxybenzoic acid (**35e**). No further purification was performed. Yield 0.83 g, 98%. ^{1}H NMR (250 MHz, DMSO-d_6) δ= 10.16 (s, 1H), 7.73-7.61 (m, 4H), 7.19 (dd, *J*= 18.4, 8.6 Hz, 3H), 4.14-4.01 (m, 2H), 3.88 (s, 3H), 3.62 (s, 2H), 1.18 (t, *J*= 7.1 Hz, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ= 171.27, 163.02, 155.79, 137.56, 134.25, 131.64, 129.81, 129.63, 127.27, 119.79, 114.56, 111.82, 60.28, 56.31, 14.12.

***N*-(3-Methyl-4-(2-(4-morpholinyl)-2-thioxoethyl)phenyl)acetamide (40c)**

4-Acetamido-2-methylacetophenone (**40b**, 0.96 g, 5.0 mmol, 1.0 eq) was dissolved in morpholine (0.9 mL) and sulfur (0.32 g,

10.0 mmol, 2.0 eq) were added. Afterwards the mixture was stirred at 135 °C for 6 hours. The reaction was stopped by pouring the warm mixture in warm ethanol (2 mL). After cooling to 0 °C for 16 hours, a precipitate was formed, which was filtered off and recrystallized in cold ethanol. Yield 0.76 g, 52%. ^{1}H NMR (250 MHz, DMSO-d_6) δ= 9.82 (s, 1H), 7.37 (dd, *J*= 13.4, 5.1 Hz, 2H), 6.96 (d, *J*= 8.3 Hz, 1H), 4.33-4.26 (m, 3H), 4.13 (s, 2H), 3.75-3.68 (m, 2H), 3.60 (dd, *J*= 8.4, 4.1 Hz, 3H), 3.55-3.49 (m, 3H), 2.20 (s, 3H), 2.03 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ= 199.43, 168.32, 137.73, 136.27, 129.70, 127.34, 120.77, 116.82, 65.85, 65.80, 56.15, 50.53, 49.73, 46.30, 24.01, 19.52, 18.60.

3-*tert*-Butyl-2-methoxybenzaldehyd (40h)

3-*tert*-Butylsalicylaldehyde (**40g**, 0.50 mL, 2.92 mmol, 1.0 eq) and K_2CO_3 (1.2 g, 8.75 mmol, 3.0 eq) were dissolved in DMF (5 mL) and stirred for 45 min before methyl iodide (0.27 g, 4.38 mmol, 1.5 eq) was added. The mixture was then stirred at room temperature for 6 hours. 10% aqueous hydrochloric acid (5 mL) and DCM (5 mL) were subsequently added, phases were separated, and the aqueous layer was extracted with DCM (3x 5 mL). The combined organic layers were dried over Na_2SO_4 and the solvents were removed in vacuum. No further purification was performed. Yield 0.43 g, 77%. ^{1}H NMR (250 MHz, DMSO-d_6) δ= 10.27 (d, *J*= 0.5 Hz, 1H), 7.63 (dt, *J*= 7.3, 1.8 Hz, 2H), 7.26-7.17 (m, 1H), 3.91 (s, 3H), 1.38 (s, 9H). ^{13}C NMR (75 MHz, DMSO-d_6) δ= 190.41, 162.70, 162.28, 143.08, 133.21, 129.44, 127.89, 123.79, 65.94, 35.75, 34.78, 30.73, 30.52.

4.3. Biological evaluation

Data analysis

All experiments were conducted with a minimum of two technical and three independent biological replicates. Reported values represent the mean±SEM. Statistical significance between two samples was analyzed by unpaired, two-sided student's t-test assuming different variances of the samples. IC_{50} values of all compounds were calculated from dose-response data by SigmaPlot 10.0 (Systat Software GmbH, Erkrath, Germany) using the mean±SD of individual concentrations and a four parameter logistic regression.

Full length FXR transactivation assay

Plasmids: pcDNA3-hFXR contains the sequence of human FXR and was already published elsewhere.[30] pGL3basic (Promega Corporation, Fitchburg, WI, USA) was used as a reporter plasmid, with a shortened construct of the promotor of BSEP cloned into the SacI/NheI cleavage site in front of the luciferase gene.[31] pRL-SV40 (Promega) was transfected as a control for normalization of transfection efficiency and cell growth. pSG5-hRXR was already published elsewhere as well.[32]

Assay procedure: HeLa cells were grown in DMEM high glucose supplemented with 10% FCS, sodium pyruvate (1 mM), penicillin (100 U/mL) and streptomycin (100 µg/mL) at 37 °C and 5% CO_2. 24 h before transfection, HeLa cells were seeded in 96-well plates with a density of 8000 cells per well. 3.5 h before transfection, medium was changed to DMEM high glucose, supplemented with sodium pyruvate (1 mM), penicillin (100 U/mL), streptomycin (100 µg/mL) and 0.5% charcoal-stripped FCS. Transient transfection of HeLa cells with BSEP-pGL3, pRL-SV40 and the expression plasmids pcDNA3-hFXR and pSG5-hRXR was carried out using calcium phosphate transfection method. 16 h after transfection, medium was changed to DMEM high glucose, supplemented with sodium pyruvate (1 mM), penicillin (100 U/mL), streptomycin (100 µg/mL) and 0.5% charcoal-stripped FCS. 24 h after transfection, medium was changed to DMEM without phenol red, supplemented with sodium pyruvate (1 mM), penicillin (100 U/mL), streptomycin (100 µg/mL), L-glutamine (2 mM) and 0.5% charcoal-stripped FCS, now additionally containing 0.1% DMSO and the respective test compound or 0.1% DMSO alone as untreated control. Each concentration was tested in triplicate wells and each experiment was repeated independently at least three times. Following 24 h incubation with the test compounds, cells were assayed for luciferase activity using Dual-Glo™ Luciferase Assay System (Promega) according to the manufacturer's protocol. Luminescence was measured with a Tecan Infinite M200 luminometer (Tecan Deutschland GmbH, Crailsheim, Germany). Normalization of transfection efficiency and cell growth was done by division of firefly luciferase data by renilla luciferase data multiplied by 1000 resulting in relative light units (RLU). Fold activation was obtained by dividing the mean RLU of the tested compound at a respective concentration by the mean RLU of untreated control. Relative activation was obtained by dividing the fold activation of the tested compound at a respective concentration by the fold activation of FXR full agonist GW4064 (**2a**) at 3 µM. EC_{50} and standard error of the mean values were calculated with the mean relative activation values of at least three independent experiments by SigmaPlot 10.0 (Systat Software GmbH, Erkrath, Germany) using a four parameter logistic regression. The assay was validated with FXR agonists **1a** (EC_{50}=18±1 µM, 88±3% rel. max. act.), **1b** (EC_{50}=0.16±0.02 µM, 87±3% rel. max. act.) and **2a** (EC_{50}=0.51±0.16 µM, 3 µM defined as 100%).[10]

Hybrid reporter gene assays for nuclear receptors PPARγ, LXRα, CAR and RARα

Plasmids: The Gal4-fusion receptor plasmids pFA-CMV-hPPARγ-LBD[33], pFA-CMV-hLXRα-LBD[24], pFA-CMV-hRARα-LBD[34], pFA-CMV-hCAR-LBD[34] containing the hinge region and ligand binding domain (LBD) of the respective nuclear receptor were constructed by integrating cDNA fragments obtained from PCR amplification of human monocytes into the SmaI/XbaI cleavage site of the pFA-CMV vector (Stratagene, La Jolla, CA, USA). Frame and sequence of the fusion receptors were verified by sequencing. pFR-Luc (Stratagene) was used as reporter plasmid and pRL-SV40 (Promega) for normalization of transfection efficiency and cell growth.

Assay procedure: HEK293T cells were grown in DMEM high glucose, supplemented with 10% FCS, sodium pyruvate (1 mM), penicillin (100 U/mL) and streptomycin (100 µg/mL) at 37 °C and 5% CO_2. The day before transfection, HEK293T cells were seeded in 96-well plates ($2.5 \cdot 10^4$ cells/well). Before transfection, medium was changed to Opti-MEM without supplements. Transient transfection was carried out using Lipofectamine LTX reagent (Invitrogen) according to the manufacturer's protocol with pFR-Luc (Stratagene), pRL-SV40 (Promega) and pFA-CMV-hRXRα-LBD. 5 h after transfection, medium was changed to Opti-MEM supplemented with penicillin (100 U/mL), streptomycin (100 µg/mL), now additionally containing 0.1% DMSO and the respective test compound or 0.1% DMSO alone as untreated control. Each concentration was tested in triplicates and each experiment was repeated independently at least three times. Following overnight (12-14 h) incubation with the test compounds, cells were assayed for luciferase activity using Dual-Glo™ Luciferase Assay System (Promega) according to the manufacturer's protocol. Luminescence was measured with an Infinite M200 luminometer (Tecan Deutschland GmbH). Normalization of transfection efficiency and cell growth was done by division of firefly luciferase data by renilla luciferase data and multiplying the value by 1000 resulting in relative light units (RLU). Fold activation was obtained by dividing the mean

RLU of a test compound at a respective concentration by the mean RLU of untreated control. Relative activation was obtained by dividing the fold activation of a test compound at a respective concentration by the fold activation of a respective reference agonist at 1 µM (PPARγ: pioglitazone; LXRα: T0901317; RARα: tretinoin; CAR: CITCO. All hybrid assays were validated with the above mentioned reference agonists which yielded values in agreement with literature.

FXR target gene quantification (quantitative real-time PCR)

FXR target gene quantification was performed as described previously.[10] In brief, HepG2 cells were incubated with test compound **25** (10 µM) or **1a** (50 µM) or **2a** (1 µM) or 0.1% DMSO alone as untreated control for 24 h, harvested, washed with cold phosphate buffered saline (PBS) and then directly used for RNA extraction. Two micrograms of total RNA were extracted from HepG2 cells by the Total RNA Mini Kit (R6834-02, Omega Bio-Tek, Inc., Norcross, GA, USA). RNA was reverse-transcribed into cDNA using the High-Capacity cDNA Reverse Transcription Kit (4368814, Thermo Fischer Scientific, Inc.) according to the manufacturer's protocol. FXR target gene expression was evaluated by quantitative real time PCR analysis with a StepOnePlus™ System (Life Technologies, Carlsbad, CA, USA) using PowerSYBRGreen (Life Technologies; 12.5 µL per well). The primers have been described previously.[10] Each sample was set up in duplicates and repeated in at least three independent experiments. The expression was quantified by the comparative ΔΔCt method and glycerinealdehyde 3-phosphate dehydrogenase (GAPDH) served as reference gene. Results (expressed as mean fold activation±SEM; n=3).

WST-1 assay

WST-1 assay (Roche Diagnostics International AG, Rotkreuz, Schweiz) was performed according to manufacturer's protocol and as described previously.[9] In brief, HepG2 cells were seeded in DMEM high glucose, supplemented with SP (1 mM), penicillin (100 U/mL), streptomycin (100 µg/mL) and 10% FCS in 96-well plates ($3 \cdot 10^4$ cells/well). After 24 h, medium was changed to DMEM high glucose, supplemented with penicillin (100 U/mL), streptomycin (100 µg/mL) and 1% charcoal stripped FCS and cells were incubated with **7** and **25** (final concentration 100 µM), Revlotron as positive control, and DMEM/1% DMSO as negative control. After 48 h, WST reagent (Roche Diagnostics International AG) was added to each well according to manufacturer's instructions. After 45 min incubation, absorption (450 nm/ reference: 620 nm) was determined with a Tecan Infinite M200 (Tecan Deutschland GmbH). Each experiment was repeated at least three times in duplicates. Results (expressed as mean percent of untreated control±SEM; n=3; DMSO=100%) **8**: 100 µM: 118±1%; **26**: 100 µM: 112±1%.

Metabolism assay

The solubilized test compound **25** (5 µL, final concentration 10 µM in phosphate buffer (0.1 M, pH 7.4)) was preincubated at 37 °C in 432 µL of phosphate buffer (0.1 M, pH 7.4) together with a 50 µL NADPH regenerating system (30 mM glucose-6-phosphate, 4 U/mL glucose-6-phosphate dehydrogenase, 10 mM NADP, 30 mM $MgCl_2$). After 5 min, the reaction was started by the addition of 13 µL of microsome mix from the liver of Sprague−Dawley rats (Invitrogen; 20 mg protein/mL in 0.1 M phosphate buffer) in a shaking water bath at 37 °C. The reaction was stopped by addition of 250 µL of ice-cold methanol at 0, 15, 30 and 60 min. The samples were diluted with 250 µL of DMSO and centrifuged at 10000 g for 5 min at 4 °C. The supernatants were analyzed and test compound was quantified by HPLC: mobile phase: MeOH 83%/H_2O 17%/formic acid 0.1%; flow-rate: 1 mL/min; stationary phase: MultoHigh Phenyl phase, 5 µm, 250×4, precolumn, phenyl, 5 µm, 20×4; detection wavelength: 330 and 254 nm; injection volume: 50 µL. Control samples were performed to check the stability of **25** in the reaction mixture: first control was without NADPH, which is needed for the enzymatic activity of the microsomes, second control was with inactivated microsomes (incubated for 20 min at 90 °C), third control was without test compound **25** (to determine the baseline). The amounts of the test compound **25** were quantified by an external calibration curve, where data are expressed as means±SEM of single determinations obtained in three independent experiments. The metabolism experiment showed the following results (expressed as mean percent of remaining compound±SEM; n=3): **25**: 0 min: 100±1%, 15 min: 98±1%, 30min: 96±1%, 60 min: 97±1%.

References and notes

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