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Discovery of BI 99179, a potent and selective inhibitor of type I fatty acid synthase with central exposure

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ABSTRACT

Based on a high-throughput screen, cyclopentanecarboxanilides were identified as a new chemotype of non-covalent inhibitors of type I fatty acid synthase (FAS). Starting from initial hits we aimed at generating a tool compound suitable for the in vivo validation of FAS as a therapeutic target. Optimisation yielded BI 99179 which is characterised by high potency, remarkably high selectivity and significant exposure (both peripheral and central) upon oral administration in rats.

Mammalian type I fatty acid synthase (FAS) is a key enzyme for lipogenesis and highly expressed in lipogenic tissues. While most tissues (except liver and adipose tissue) have low levels of FAS expression and activity, FAS is over expressed in many cancers and has been suggested as a potential anticancer target.[1] More recently, accumulating evidence points towards FAS inhibition as a potential antiviral principle.[2] FAS is also expressed in the brain and targeted deletion of FAS in hypothalamic neurons produces hypophagic, lean mice.[3] Furthermore central nervous system administration of the non-specific irreversible FAS inhibitors Cerulenin and C75 in rodents decreases brain levels of orexigenic neuropeptides and produces hypophagia and weight loss, suggesting central FAS inhibition as a potential way to treat obesity.[4] Moreover, FAS is highly expressed in human sebocytes, the lipid producing cells of the sebaceous glands.[5] The pathogenesis of acne, the most common disorder involving the sebaceous gland, involves lipid overproduction by the sebaceous gland,[6] and it has been reported that inhibitors of FAS reduce the production of sebum in sebocytes,[7] suggesting topical FAS inhibition as a potential anti-acne approach.

A high throughput screen was set up at Boehringer Ingelheim with the aim of generating a tool compound suitable for the validation of FAS as a therapeutic target. Intended properties were: (a) a non-covalent mode of action, (b) sufficient potency (IC_{50} <1 μM), (c) high target selectivity and (d) suitability for in vivo evaluation of therapeutic potential, preferably via oral application to rats. Central exposure, though an ambitious goal, was envisaged as we were interested in investigations on the role(s) of FAS activity in the brain.

During the last years, several non-covalent inhibitors of type I FAS have been reported in the literature (Fig. 1): Compound **1** was described by Rivkin et al. as the most potent compound among a series of 3-aryl-4-hydroxyquinolinones,[8] and the symmetric urea derivative **2** was published by Vásquez et al. as GSK837149A.[9] Although literature IC_{50} values for both compounds are in the double digit nM range, potency in our FAS assay was considerably lower.[10] From a library of furanylmethylene-pyrimidinetriones, Richardson and Smith identified compound **3** as the most potent one with a calculated K_i value of 0.38 μM for the inhibition of the thioesterase domain of FAS.[11] For none of these compounds pharmacokinetic or in vivo data have been reported. In contrast, Butlin et al. and Wallenius et al. disclosed compound **4** as a type I FAS inhibitor (reported IC_{50} for rat FAS inhibition: 350 nM) with oral availability in rats.[12] To our knowledge, this is the first non-covalent inhibitor of type I FAS reported to be suitable for in vivo experiments. However, the authors state that no significant CNS exposure was measured for compounds from the series containing **4**.

Here, we wish to report the discovery and characterisation of BI 99179 (compound **13**) as a potent and selective inhibitor of human FAS with central exposure upon oral administration in rats.[13]

Based on a high throughput screen of our internal compound collection, we identified the cyclopentanecarboxanilide **5** with

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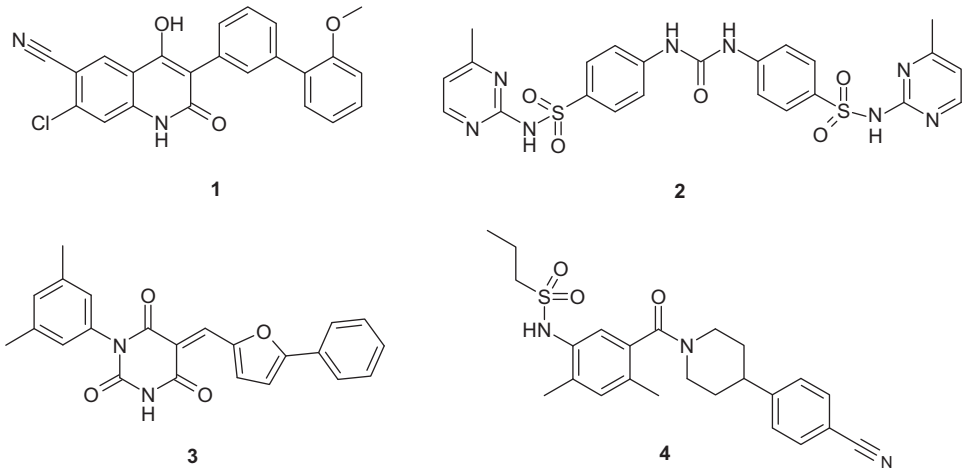


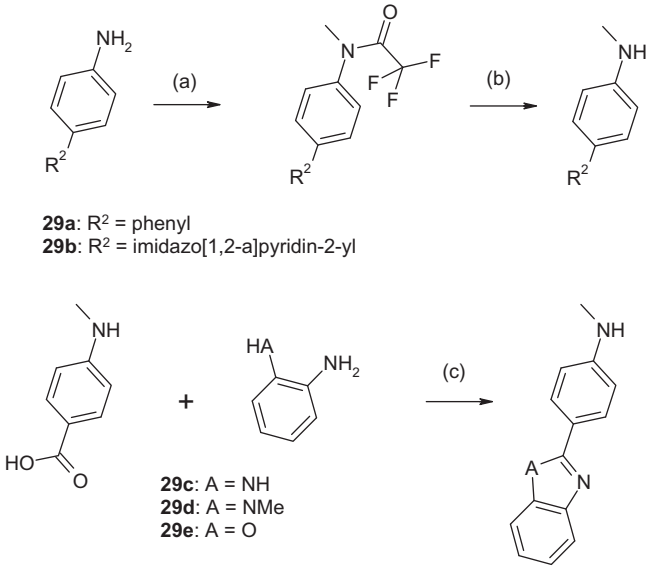
Figure 1. Noncovalent inhibitors of type I FAS reported in the literature.

submicromolar IC_{50} in our FAS assay.[10] Interestingly, like the structurally diverse competitors' compounds **1**–**4**,[14] compound **5** and the other potent cyclopentanecarboxanilides described herein are quite rigid structures with less than five rotatable bonds.[15]

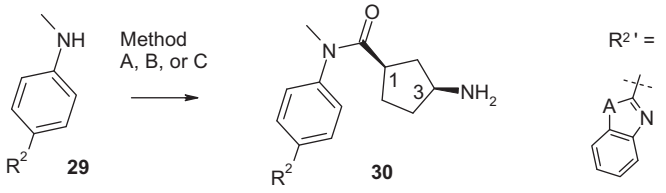
The general synthetic approach employed to prepare the cyclopentanecarboxanilides is outlined in Schemes 1–4.[16] N-Methylation of the respective anilines or benzimidazole/benzoxazole formation starting from 4-methylamino-benzoic acid furnishes the required *N*-methylanilines (Scheme 1). These are acylated using FMOC-3-aminocyclopentane-carboxylic acids under coupling conditions outlined in Scheme 2. Removal of the FMOC moiety (Scheme 2) is followed by the final diversifying acylation (Scheme 3). Alternatively, the need for a protecting group can be avoided by coupling preformed 3-acylamino-cyclopentane-carboxylic acids to *N*-methylanilines as outlined in Scheme 4.

A first series of R^1 variations intended to explore SAR of the right-hand side acyl moiety yielded the slightly more potent propionyl compound **6** along with less potent analogues some of which are listed in table 1 (compounds **7**–**9**).

Systematic modifications of all other parts of the molecule revealed generally steep SAR except for R^1 and R^2 (data not shown). We concluded that the *N*-methylcarboxanilide scaffold of **5** constitutes an optimum and potency can be improved only through modifications of R^1 and R^2. Furthermore, the optical antipode of **5**, compound **10** was found to be inactive. Therefore, we next explored R^2 more extensively, also aiming at improved solubility through more polar and/or more flexible replacements of the phenyl group. While most modifications significantly reduced potency



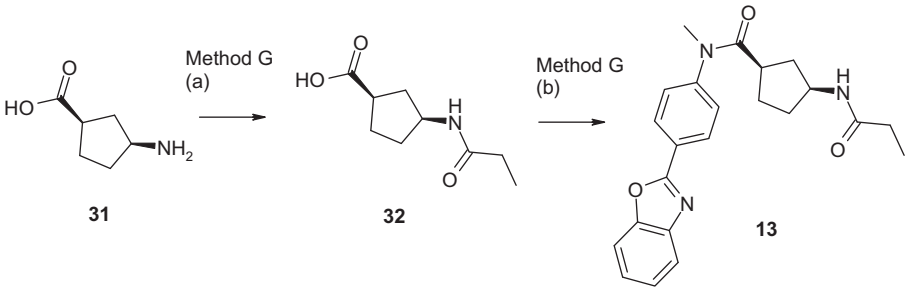
Scheme 1. Synthesis of *N*-methylanilines **29**. Reagents and conditions: (a) (1) $(TFA)_2O$, TEA, DCM, 0 °C to rt; (2) MeI, K_2CO_3/acetone (for **29a**) or NaH/DMF (for **29b**), rt; (b) K_2CO_3, MeOH/H_2O, 50 °C; (c) polyphosphoric acid, 200–210 °C (4 h), then H_2O (80 °C to rt) over night.



Educt	Product	R^2	Method applied	Stereochemistry
29a	30a	phenyl	A	(1R,3S)
29c	30c	$R^{2'}$; A = NH	B	(1R,3S)
29d	30d	$R^{2'}$; A = NMe	B	(1R,3S)
29e	30e	$R^{2'}$; A = O	C	(1R,3S)
29a	30f	phenyl	A	(1S,3R)
29e	30g	$R^{2'}$; A = O	C	(1S,3R)

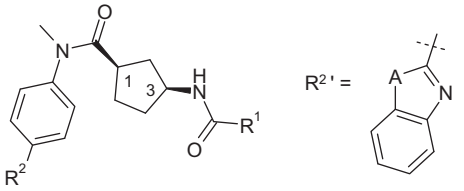
Scheme 2. Preparation of 3-aminocylopentanecarboxanilides **30**. Reagents and conditions: Method A: (1) *N*-FMOC-3-aminocyclopentane-1-carboxylic acid (respective diastereomer), TBTU, DIPEA, DMF, rt, over night; (2) diethylamine, DCM, rt, 2 h. Method B: (1) *N*-FMOC-3-aminocyclopentane-1-carboxylic acid (respective diastereomer), diphenylphosphinic chloride, TEA, THF (10 min, rt) then **29**, 60 °C, over night; (2) piperidine/THF, rt, over night; Method C: (1) *N*-FMOC-3-aminocyclopentane-1-carboxylic acid (respective diastereomer), 1-chloro-*N*,*N*,2-trimethylpropenyldiamine, DCM, (rt, 2 h) then **29**, 2,4,6-collidine, DCM, over night; (2) diethyl amine/THF, 40 °C, 2 h.

Scheme 3. Final acylation step. Reagents and conditions: Method D: R^1COCl, TEA, DMF, rt; Method E: ethyl isocyanate, TEA, DMF, rt; Method F: 6-dimethylamino hexanoic acid hydrobromide (for **9**, **18**, **20**, **27**) or difluoroacetic acid (for **23**), TEA, TBTU, DMF, rt.



Scheme 4. Alternative synthesis of propionamides as exemplified for **13**. Reagents and conditions (Method G): (a) propionyl chloride added drop wise to **31** in THF/aq NaOH; (b) (1) 1-chloro-*N*,*N*,2-trimethylpropenyldiamine, DCM, rt; (2) addition to **29e**, 2,4,6-collidine, DCM, rt.

Table 1
Structure and in vitro FAS inhibitory properties for compounds **5**–**28**



Compound	Final acylation step		R^1	R^2	Absolute stereochemistry	IC_{50}[b] (μM)
	Starting material	Method applied[a]				
5	**30a**	D	Methyl	Phenyl	(1*R*,3*S*)	0.48
6	**30a**	D	Ethyl	Phenyl	(1*R*,3*S*)	0.30
7	**30a**	D	2-Propyl	Phenyl	(1*R*,3*S*)	0.42[c]
8	**30a**	E	NH-Ethyl	Phenyl	(1*R*,3*S*)	1.5
9	**30a**	F	$-(CH_2)_5-NMe_2$	Phenyl	(1*R*,3*S*)	2.0[d]
10	**30f**	D	Methyl	Phenyl	(1S,3R)	>10[d]
11	**30c**	D	Ethyl	$R^{2\prime}$; A = NH	(1*R*,3*S*)	0.19
12	**30d**	D	Ethyl	$R^{2\prime}$; A = NMe	(1*R*,3*S*)	0.31
13	**30e**	D	Ethyl	$R^{2\prime}$; A = O	(1*R*,3*S*)[e]	0.079
14	**29b**[f]	G	Ethyl	Imidazo[1,2-*a*]pyridin-2-yl	(1*R*,3*S*)	0.29
15	**30c**	D	Methyl	$R^{2\prime}$; A = NH	(1*R*,3*S*)	1.3[d]
16	**30c**	D	2-Propyl	$R^{2\prime}$; A = NH	(1*R*,3*S*)	1.5[d]
17	**30c**	E	NH-Ethyl	$R^{2\prime}$; A = NH	(1*R*,3*S*)	0.40[d]
18	**30c**	F	$-(CH_2)_5-NMe_2$	$R^{2\prime}$; A = NH	(1*R*,3*S*)	0.70
19	**30d**	E	NH-Ethyl	$R^{2\prime}$; A = NMe	(1*R*,3*S*)	1.4[d]
20	**30d**	F	$-(CH_2)_5-NMe_2$	$R^{2\prime}$; A = NMe	(1*R*,3*S*)	2.4
21	**30e**	D	Methyl	$R^{2\prime}$; A = O	(1*R*,3*S*)	0.20[d]
22	**30e**	D	*n*-Propyl	$R^{2\prime}$; A = O	(1*R*,3*S*)	0.42[d]
23	**30e**	F	CHF_2	$R^{2\prime}$; A = O	(1*R*,3*S*)	0.27
24	**30e**	D	$-CH_2-O-CH_3$	$R^{2\prime}$; A = O	(1*R*,3*S*)	0.91[d]
25	**30e**	E	NH-Ethyl	$R^{2\prime}$; A = O	(1*R*,3*S*)	0.53
26	**30e**	D	NMe_2	$R^{2\prime}$; A = O	(1*R*,3*S*)	0.28[d]
27	**30e**	F	$-(CH_2)_5-NMe_2$	$R^{2\prime}$; A = O	(1*R*,3*S*)	0.58
28	**30g**	D	Ethyl	$R^{2\prime}$; A = O	(1S,3*R*)[e]	>3

[a] For a description of methods D, E, F, and G see Schemes 3 and 4.
[b] Unless stated otherwise, median values of at least three independent IC_{50} determinations are given. For a description of the assay see Ref. 10.
[c] Data point from a single IC_{50} determination.
[d] Median from two independent IC_{50} determinations.
[e] Specific rotation for **13**: $[\alpha]_D^{20}$ (*c* 0.3, MeOH) = +23; for **28**: $[\alpha]_D^{20}$ (*c* 0.3, MeOH) = −24).
[f] Compound **14** was prepared by method G (Scheme 4) replacing **29e** by **29b** as starting material.

(data not shown), we found that a range of heterobicyclic aromatic systems could replace the phenyl moiety in **5** (compounds **11**–**14**). Gratifyingly the benzoxazole **13** which proved to be the most potent compound has a significantly lower *c* log *P* value as compared to **5** (*c* log *P* = 3.1 vs 3.6, respectively).[17] We then further explored the R^1/R^2 matrix including some close analogues of compound **13** (compounds **15**–**28**). Data from this series indicate linear SAR (i.e., the influence of R^1 on potency was found to be independent on the nature of R^2 and vice versa) and confirmed the inactivity of the optical antipodes (compound **28**). With respect to both potency and polarity, **13** remained the most interesting compound.

Cellular activity (i.e., inhibition of ^{14}C-acetate incorporation) of our compounds was determined in the mouse hypothalamic N-42 cell line.[18] To confirm that reduced ^{14}C-acetate incorporation is not due to any cytotoxic effect, we determined cytotoxicity in a standard LDH-release assay.[19] Again, compound **13** proved to be the most potent derivative with an IC_{50} of 0.6 μM in the mouse N-42 cellular assay. Notably, **13** showed no significant LDH release in the cytotoxicity assay up to 30 μM compound concentration. This clearly indicates that (i) the cellular activity is not due to a cytotoxic effect and (ii) compound **13** (as other cyclopentanecarboxanilides tested) potently inhibit mouse FAS in addition to the human enzyme applied in our biochemical assay.

To further evaluate the usefulness of **13** as a tool compound for the validation of FAS as a potential therapeutic target, it was subject to extensive selectivity testing: When screened against a panel of 30 diverse targets, **13** showed less than 20% inhibition at a concentration of 10 μM in all cases, indicating IC_{50} values far above 10 μM.[16] Also for the major human P450 isoenzymes (CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4) IC_{50} values were determined to be higher than 10 μM. Compound **13** has been incorporated into Boehringer Ingelheim's HTS compound collection and results available to date from a double digit number of screens against diverse targets confirm the very clean selectivity profile.

Favourable in vitro PK parameters of **13** (CaCo-2 permeability: 94×10^{-6} cm/s with no indication for the involvement of efflux transporters; high metabolic stability in rat and human microsomes: <27% and <26% of the respective liver-blood-flow Q_H; rat plasma protein binding: 97.6 ± 0.5%) appear to translate into a low clearance (CL = 8.2 ml/min/kg) and a moderate volume of distribution (V_{ss} = 1.6 L/kg) following intravenous administration to male Han/Wistar rats. Furthermore, and crucial for the envisaged in vivo studies, the compound proved to be well suitable for oral application as seen from the data shown in table 2. Notably similarly high compound levels in plasma and brain are seen upon oral administration (the ratio C_{CSF}: C_{brain} of approx. 4% can be rationalized by protein binding which was determined to be 97.6% with rat plasma).

Clearly, the relatively low aqueous solubility of **13** (5 μg/ml @ pH 7.4) does not compromise the compound's good PK properties. Nevertheless, for experiments where high aqueous solubility is key rather than cellular activity and favourable PK properties, compounds **18** and **27** with submicromolar potency in the enzymatic assay, bearing a basic moiety designed to improve aqueous solubility may be the tools or choice.

In conclusion, cyclopentanecarboxanilides were identified from a high throughput screen of Boehringer Ingelheim's compound collection for FAS inhibitors. Optimisation of the initial hits lead to the discovery of BI 99179 (**13**) which is characterised by high potency, remarkably high selectivity and significant exposure (both peripheral and central) upon oral administration in rats. We are confident that selective tool compounds suitable for in vivo studies like BI 99179 will help to better understand the role of FAS in physiological as well as diseased conditions and to evaluate the potential of selective non-covalent FAS inhibitors as therapeutic agents. Results from first acute and subchronic in vivo pharmacology studies with BI 99179 in rodents will be published elsewhere.

Table 2
Pharmacokinetic parameters of BI 99179 (**13**) in male Han/Wistar rats (fasted) upon oral application of 4 mg/kg

$t_{1/2}$ (h)	t_{max} (h)	C_{max} (nM)	$AUC_{0\text{-inf}}$ (nM h)	F (%)	$C_{brain,2h}$ (nM)	$C_{CSF,2h}$ (nM)
3.0	0.5	2110	9350	46	1300	50

Acknowledgements

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.07.083.

References and notes

1. For recent reviews see: (a) Flavin, R. et al *Future Oncol.* **2010**, *6*, 551; (b) Kridel, S. J.; Lowther, W. T.; Pemble, C. W. *Expert Opin. Invest. Drugs* **2007**, *16*, 1817.
2. (a) Heaton, N. S. et al *Proc. Natl. Acad. Sci. U.S.A.* **2010**, *107*, 17345; (b) Munger, J. et al *Nat. Biotechnol.* **2008**, *26*, 1179; (c) Yang, W. et al *Hepatology* **2008**, *48*, 1396.
3. Chakravarthy, M. V. et al *J. Clin. Invest.* **2007**, *117*, 2539.
4. Loftus, T. M. et al *Science* **2000**, *288*, 2379.
5. Kusakabe, T. et al *J. Histochem. Cytochem.* **2000**, *48*, 613.
6. Smith, K. R.; Thiboutot, D. M. *J. Lipid Res.* **2008**, *49*, 271.
7. D'Arcangelis, A. D., et al. U.S. Patent 53631, 2005.
8. Compound '**10v**' from: Rivkin, A. et al *Bioorg. Med. Chem. Lett.* **2006**, *16*, 4620.
9. Vásquez, M. J. et al *FEBS J.* **2008**, *275*, 1556.
10. *Human FAS enzyme assay*: Human type I fatty acid synthase is a large multiunit protein that harbours seven enzymatic activities catalysing the reductive synthesis of long chain fatty acids from acetyl CoA and malonyl CoA. In the reductive steps NADPH is consumed forming NADP, measured by analysing the decrease in fluorescence.
    Compounds diluted in 0.1% DMSO, 326 μM NADPH, 60 μM acetyl CoA (from Sigma, cat.-no. A-2181) in phosphate buffer (100 mM KH_2PO_4/K_2HPO_4, 1 mM EDTA, 1 mM DTT, pH 7.0), and FAS enzyme (prepared from HeLa cells) diluted in enzyme buffer (20 mM KH_2PO_4/K_2HPO_4, 1 mM EDTA, 1 mM DTT, 5% glycerol, pH 7.4) were incubated for 60 min at 37 °C. The reaction was then started by the addition of 200 μM malonyl CoA. The optical density was determined at 340 nm over 10 minutes and the slope (i.e., V_{max}) was calculated.
    The IC_{50} value of the known covalent FAS inhibitor Cerulenin was determined to be 5.8 μM in this assay.
11. Richardson, R. D.; Smith, J. W. *Mol. Cancer Ther.* **2007**, *6*, 2120.
12. Butlin, R. J., et al. Poster presented at the 15th RSC-SCI Medicinal Chemistry Symposium, Cambridge, UK, September 2009; Wallenius, K., et al.; Poster presented at the American Diabetes Association: 68th Scientific Sessions, San Francisco, CA, Poster 58-LB, June 2008; Compound **4** is also found as example 117 in: Butlin, R. J., et al. PCT Int. Appl. WO 2008/075070, 2008.
13. BI 99179 and other cyclopentanecarboxanilides are claimed in: Kley, J., et al. PCT Int. Appl. WO 2011/048018, 2011.
14. In addition to structural diversity, the compounds may also bind to different domains of FAS. From preliminary data (not shown) we conclude that the cyclopentanecarboxanilides disclosed herein probably bind to the ketoacyl reductase (KAR) domain of FAS.
15. The number of rotatable bonds was calculated using an in-house algorithm. Introduction of the flexible dimethylaminopentyl side chain (compounds **9**, **18**, **20**, and **27** with a higher number of rotatable bonds) significantly decreases potency as shown in Table 1.
16. For details see Supplementary data.
17. *c* log *P* values were calculated using Program CLOGP, Version 4.83, Daylight Chemical Information Systems, Inc. Los Altos, CA. (http://www.daylight.com) Chou, J. T.; Jurs, P. C. *J. Chem. Inf. Comput. Sci.* **1979**, *19*, 172.
18. ^{14}C-Acetate incorporation in N-42 cells: N-42 cells are immortalized clonal neuronal mouse hypothalamic cells from CELLutions Biosystems Inc. N-42 cells were incubated first with the compound for 60 minutes at 37 °C, then with ^{14}C-acetate dilution (10^6 dpm) for 4 h at 37 °C. After chloroform/methanol extraction, the lower fraction was vaporized and incorporated radioactivity was quantified by scintillation counting.
19. *Cytotoxicity assay*: Measurement of LDH release from U937 cells after 20 h of compound incubation (CytoTox-ONE™ Homogeneous Membrane Integrity Assay, Promega, G7890).