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Design and synthesis of a series of bioavailable fatty acid synthase (FASN) KR domain inhibitors for cancer therapy

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ABSTRACT

We designed and synthesized a new series of fatty acid synthase (FASN) inhibitors with potential utility for the treatment of cancer. Extensive SAR studies led to highly active FASN inhibitors with good cellular activity and oral bioavailability, exemplified by compound **34**. Compound **34** is a potent inhibitor of human FASN (IC_{50} = 28 nM) that effectively inhibits proliferation of A2780 ovarian cells (IC_{50} = 13 nM) in lipid-reduced serum (LRS). This cellular activity can be rescued by addition of palmitate, consistent with an on-target effect. Compound **34** is also active in many other cell types, including PC3M (IC_{50} = 25 nM) and LnCaP-Vancouver prostate cells (IC_{50} = 66 nM), and is highly bioavailable (F 61%) with good exposure after oral administration. In a pharmacodynamics study in H460 lung xenograft-bearing mice, oral treatment with compound **34** results in elevated tumor levels of malonyl-CoA and decreased tumor levels of palmitate, fully consistent with the desired target engagement.

Human Fatty Acid Synthase (FASN) is a complex homodimeric enzyme (552-kDa) that carries out *de novo* fatty acid (palmitate) synthesis from acetate and malonate in mammalian cells in the presence of NADPH.[1–3] Studies have recently associated FASN with a variety of human diseases and adverse health conditions including obesity, inflammation, cardiovascular disease, and especially cancer.[4–8] FASN is up-regulated in cancer cells, providing fatty acid building blocks for rapid cell growth and cell division. Increased FASN expression is correlated with disease progression and poor prognosis in many malignancies including prostate, breast, ovary, colon, and lung cancers.[9–12] FASN has been demonstrated to play an important role in carcinogenesis by protecting cells from apoptosis; inhibition with the non-specific FASN inhibitors orlistat or cerulenin prevents tumor growth in various mouse xenograft models.[13,14] Thus, FASN is an attractive emerging target for cancer drug discovery.

Recent publications disclose several FASN inhibitor chemotypes that share a common pharmacophore involving an aromatic group and an acylated cyclic amine attached to a central scaffold (e.g. 1H-imidazo[4,5-*c*]pyridine).[15–17] We postulated that a 5,5-disubstituted-spirocyclic imidazolinone core would be an acceptable and drug-like scaffold, inspired by the precedent of irbesartan, an approved antihypertensive drug in which a spirocyclopentyl-imidazolinone core replaces the substituted imidazole ring of losartan, an older approved agent from the same drug class (Fig. 1). Working from this hypothesis we have discovered a new class of spirocyclic imidazolinone based FASN inhibitors.

General synthesis of the new series of imidazolinone FASN inhibitors is outlined in Scheme 1 starting from, as an example, 1-amino-cyclopropane-1 carboxamide (**I**). First, the aminoamide was coupled with a substituted bromophenylcarboxylic acid (**II**)

Abbreviations: FASN, Fatty acid synthase; LRS, lipid-reduced serum; NADPH, nicotinamide adenine dinucleotide phosphate; KR, ketoreductase domain; ER, enoylreductase domain; ψME, pseudo-methyltransferase domain; ψKR, pseudo-ketoreductase domain.

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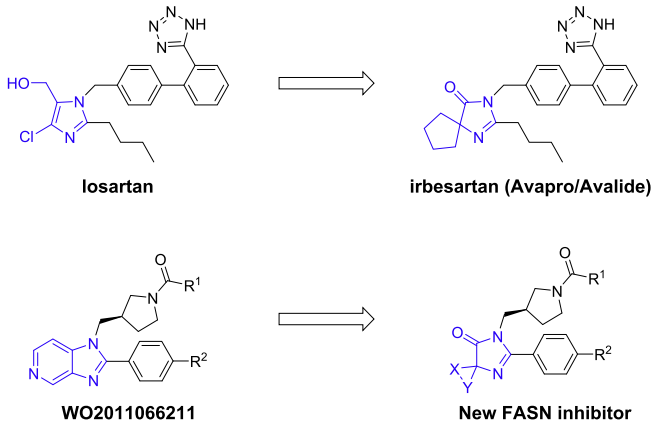
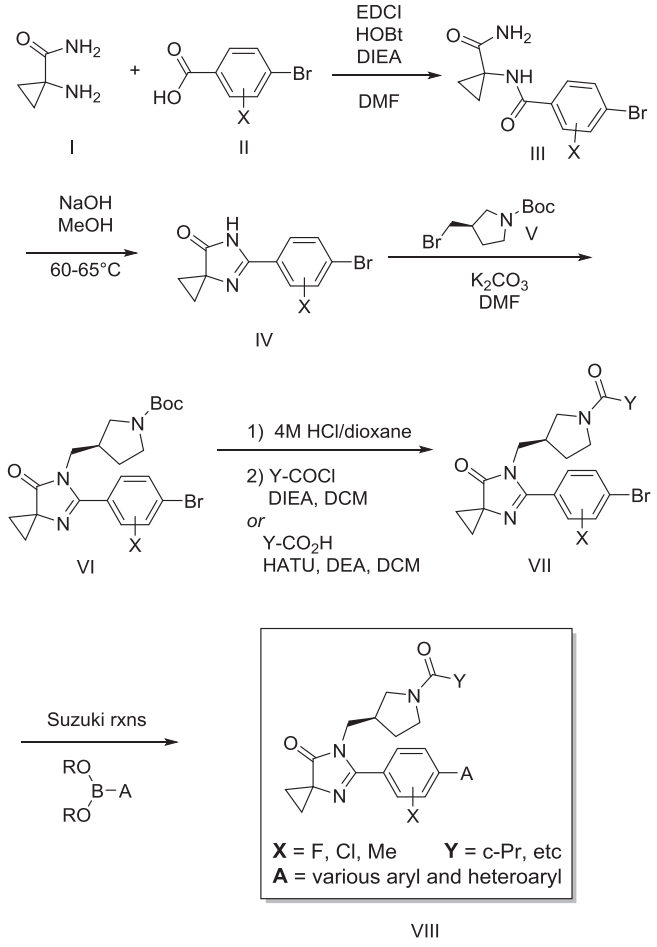


Figure 1. Design principle for new FASN inhibitors.

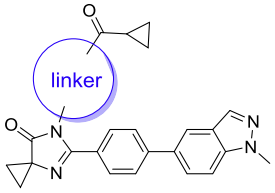


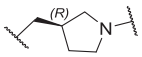
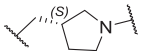
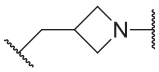
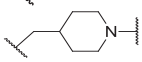
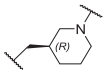
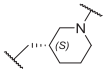
Scheme 1. General synthetic procedure.

under standard peptide coupling conditions. The resulting product **III** was then cyclized under basic conditions (NaOH in methanol) to give the imidazolinone (**IV**), which in turn was allowed to react with an appropriate alkylating reagent such as (*R*)-*tert*-butyl 3-(bromomethyl)pyrrolidine-1-carboxylate (**V**) to produce product **VI**. Boc de-protection and acylation provided **VII**, and final Suzuki coupling gave the desired products **VIII**. Alternatively, the Suzuki coupling was performed first, followed by Boc de-protection and acylation, to prepare the final products **VIII**.

Using the spirocyclopropane-imidazolinone core substituted with (4-(1-methyl-1*H*-indazol-5-yl)phenyl) at the 2-position as a

Table 1
SAR (full length FASN enzyme) of the linker between the imidazolinone and acyl group.



Compound #	Linker	FASN IC_{50} (nM)
1		30
2		250
3		69
4		1380
5		8510
6		>10,000

starting point, we began structure–activity relationship (SAR) studies of the linker between the imidazolinone and the terminal acyl group (Table 1, see Supplementary Data for assay conditions). The most potent linker is *(R)*-pyrrolidine (**1**), with an IC_{50} of 30 nM. *(S)*-pyrrolidine (**2**) is 8-fold less potent, whereas azetidine (**3**) is only 2-fold less potent. 4-Piperidine (**4**) and (*S*)-3-piperidine (**5**) are 45-fold and 280-fold less potent than **1**, respectively, and *(R)*-3-piperidine (**6**) is completely inactive at >10 μM.

We also explored the SAR at the 5-position of the imidazolinone (Table 2). Compared to the benchmark spirocyclopropyl analogue **1**, spirocyclopentyl (**7**), 5,5-dimethyl (**8**), and 5-phenyl-5-methyl (**10**) are less potent and 5-methyl-5-methoxymethyl (**9**) is much less potent. Incorporation of either an oxygen or nitrogen atom into the spirocycle (**11**–**15**) reduces potency by at least 6-fold. The weaker potency of *N-i*-propylpiperidine (**12**) compared to cyclopropanecarbonylpiperidine (**13**) suggests that the 5-substituent interacts with a region of FASN that does not tolerate positive charge. This observation will be discussed further below in the context of an X-ray crystal structure of compound **1**.

Investigation of the 2-substituent on the core was conducted in two parts. First, SAR for the central aryl ring between the imidazolinone and terminal aryl was explored (Table 3). Comparing again to benchmark compound **1** having a central phenyl ring (IC_{50} = 30 nM), fluorophenyl (**16**) and methylphenyl (**17**) with substitution *ortho* to the imidazolinone have similar potency (IC_{50} = 22 and 51 nM, respectively), while fluorophenyl (**18**) and methylphenyl (**19**) with substitution *ortho* to the terminal aryl group show decreased potency (IC_{50} = 130 and 300 nM respectively). Pyridinyl replacements (**20** and **21**) for phenyl are at least 15-fold less potent, and pyrimidinyl (**22** and **23**) or thiophenyl (**24**) analogues are dramatically less potent.

The right-hand terminus of the 5-subustutent (Table 4) tolerates a variety of aryl and heteroaryl rings. Bicyclic heteroaryls including indole (**36**), benzothioazole (**27**), indazole (**25**, **26**), naphthalene (**30**), quinoline (**29**) and isoquinoline (**28**) all show good activity, with potency comparable to or slightly better than **1**. Biaryls such as 1-methyl-1H-pyrazol-4-ylphenyl (**34**) and