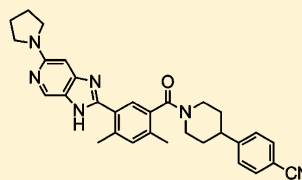# Imidazopyridine-Based Fatty Acid Synthase Inhibitors That Show Anti-HCV Activity and in Vivo Target Modulation

Johan D. Oslob,* Russell J. Johnson, Haiying Cai, Shirley Q. Feng, Lily Hu, Yuko Kosaka, Julie Lai, Mohanram Sivaraja, Samnang Tep, Hanbiao Yang, Cristiana A. Zaharia, Marc J. Evanchik, and Robert S. McDowell

3-V Biosciences, Inc., 1050 Hamilton Court, Menlo Park, California 94025, United States

Ⓢ *Supporting Information*

ABSTRACT: Potent imidazopyridine-based inhibitors of fatty acid synthase (FASN) are described. The compounds are shown to have antiviral (HCV replicon) activities that track with their biochemical activities. The most potent analogue (compound **19**) also inhibits rat FASN and inhibits de novo palmitate synthesis in vitro (cell-based) as well as in vivo.

IC_{50} (hFAS): 17 nM
EC_{50} (de novo palmitate synthesis): 12 nM
EC_{50} (HCV replicon): 18 nM
In vivo inhibition of *de novo* palmitate synthesis at 50 mg/kg (PO)

KEYWORDS: FASN, inhibitor, antiviral, HCV, structure–activity relationship, in vivo, target modulation, reversible

Human fatty acid synthase (hFASN) is a key enzyme in the de novo synthesis of fatty acids (FAs) including palmitate.[1,2] While the putative involvement of FASN in cancer has been well documented,[3–5] other potential therapeutic uses of FASN inhibitors are beginning to emerge including cardiovascular disease and diabetes.[6–8] Furthermore, it has recently been shown that FASN is up-regulated during hepatitis C virus (HCV) infection and that reduction of FASN levels using siRNA leads to diminished replication of the virus.[9] Infection by HCV has become a serious health issue with a significant portion (~3%) of the world's population affected.[10,11] While the majority of the newer HCV treatments are direct-acting antivirals (DAAs) that target viral proteins,[10] it has been proposed that blocking the infectious cycle by modulating cellular factors such as FASN may result in a higher barrier to the emergence of resistance.[12,13]
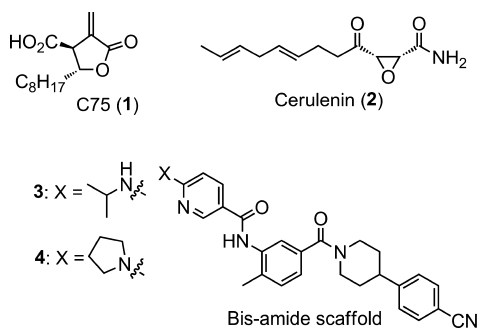
Although a number of inhibitors of FASN have been reported, the majority of them have had limited utility due to modest inhibitory activity or low cell permeability or were irreversible binders; the latter include the widely used tool compounds C75 and Cerulenin (Chart 1).[5,14–16] The goal of the current work was two-fold. First, we wished to identify potent, small-molecule inhibitors of FASN with a reversible mode of inhibition. Second, to enable studies in cell-based assays, good cell permeability was essential. Compounds with these characteristics were expected to be useful tools for evaluating the potential of FASN as a small-molecule drug target for the treatment of HCV.[17] Furthermore, to pave the way for subsequent drug discovery efforts, the compounds needed to have good activity against both human and rodent FASN, along with reasonable rodent pharmacokinetic (PK) profiles, enabling pharmacodynamic (PD) studies.[18]

In spite of having very limited existing structure–activity relationships (SAR) or published data, compound **3** (Chart 1) and its close analogue **4** were among the more promising starting points at the inception of our explorations.[14,19] However, while these molecules were potent biochemical inhibitors of human FASN (hFASN, Scheme 1), members of this class were found to undergo cleavage of the secondary amide moiety in rodent plasma resulting in the formation of a potentially toxic primary aniline. Furthermore, the weak activities of **3** and **4** against rat FASN (rFASN) precluded their use in preclinical in vivo studies. A search for modified structures, devoid of these issues, was therefore initiated (Scheme 1).

In an attempt to prevent amide cleavage, the parity of the amide was initially reversed, resulting in an equally active hFASN compound, **5** (Scheme 1), with good plasma stability ($t_{1/2}$ > 10 h). However, the activity against rFASN was poor, and initial attempts to optimize this new series met with limited success. Hence, additional scaffold modifications were explored (Scheme 1). Cyclizing the reversed amide (**5**) yielded the
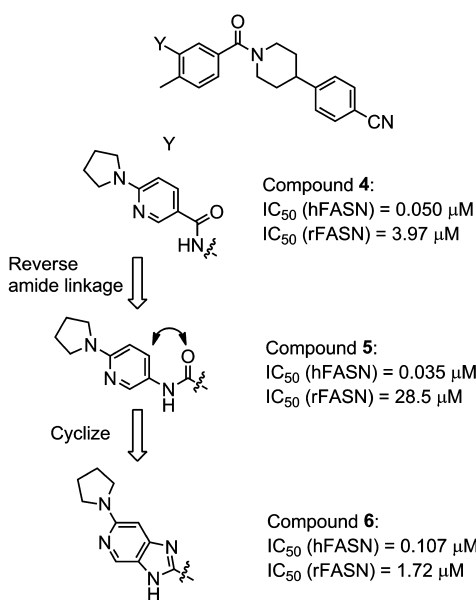
Chart 1. Inhibitors of FASN

C75 (**1**)

Cerulenin (**2**)

3: X =

4: X =

Bis-amide scaffold

Scheme 1. Compound Progression Leading to Imidazopyridine Motif

corresponding imidazopyridine derivative **6**. The new derivative, devoid of the secondary amide motif, had good plasma stability ($t_{1/2}$ > 10 h). It also displayed promising biochemical activities against both hFASN and rFASN. The scaffold was therefore selected for further profiling and optimization. Compound **6** had good antiviral activity in a cell-based HCV replicon assay with a minor shift from the biochemical assay and an acceptable selectivity index (SI = EC_{50}/CC_{50}, where CC_{50} is cell viability EC_{50}, Table 1). These observations are consistent with good cell permeability as confirmed in an Madin−Darby canine kidney (MDCK) permeability assay (P_{app} = 8.0 × 10^{-6} cm/s, efflux ratio = 2.5). Next, a methyl-walk around the central ring was conducted (Table 1). Replacing the R1-methyl with a hydrogen resulted in modest loss of human and rodent activities (compound **6** vs **7**), whereas a methyl at R2 (compound **8**) completely abolished biochemical and antiviral activities. On the other hand, replacing the R3-hydrogen with a methyl yielded an analogue (**9**) that had good activity against hFASN and also improved activity against the rodent enzyme. Finally, introducing a methyl at R4 again

yielded a compound (**10**) devoid of activity against hFASN and rFASN. Overall, as shown previously, the antiviral activities track with biochemical inhibition of hFASN.[17,20]

A preliminary survey of the imidazopyridine portion was undertaken next (Table 2). While replacing the pyrrolidine moiety in **6** with a hydrogen resulted in modest loss in potency against hFASN, a more significant decrease in activity was observed against the rat enzyme (compound **6** vs **11**, Table 2). The corresponding unsubstituted benzimidazole analogue **12** showed further loss of activity.

Replacing the pyrrolidine moiety in **6** with polar substituents (compounds **13** and **14**) rendered more soluble analogues but with a concomitant loss of rodent activity. In an attempt to regain some activity against rFASN (see above, compound **9**, Table 1), the R3-methyl was introduced to yield piperazine derivative **15**, which indeed diplayed improved activity against the rodent enzyme as compared to its R3 = hydrogen counterpart, compound **14**. Therefore, subsequent analogues were prepared with the R3 = Me substitution pattern. While the corresponding unsubstituted piperazine analogue **16** did not show any improvement in activity, the noncharged morpholine and methyl ether derivatives (**17** and **18**) displayed good activities across the assays, including inhibition of rFASN and antiviral activity. Combining the original pyrrolidine moiety with the R3 = Me substitution pattern (compound **19**) resulted in potent inhibition of hFASN and HCV replication. The activity against the rodent enzyme was also improved. While the corresponding azetidine derivative (**20**) showed a modest loss of activity, introduction of a methyl-sulfone in place of the pyrrolidne led to a more significant decrease, in particular against rFASN. Consistent with the SAR shown above, changing the position of the nitrogen in the imidazopyridine moiety resulted in minor changes in activities (compounds **20** vs **23** and **21** vs **22**), whereas the effect of moving the X substituent is more pronounced (compound **20** vs **24**). As noted above and previously, the cell-based antiviral activities are found to track well with the human biochemical activities.[20] Further characterization using dialysis and washout studies indicated that compounds from this series are reversible inhibitors of FASN, both biochemically as well as in cell-based assays.[17,18]
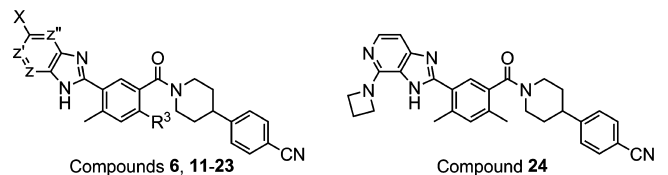
The analogues described above were prepared using a general route exemplified by the preparation of compound **19** in Scheme 2. Treatment of 2,4-dimethylbenzoic acid (**25**) with

Table 1. Preliminary SAR around Compound 6: Methyl-Walk, Antiviral, and FASN Inhibitory Activities

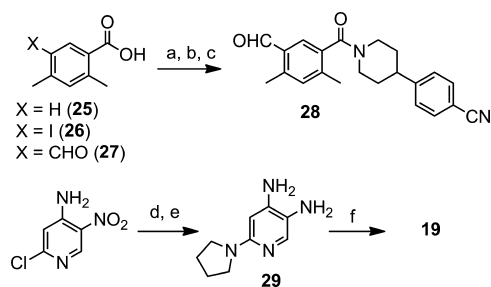| compd	R^1	R^2	R^3	R^4	hFASN[a] (IC_{50}, μM)	rFASN[b] (IC_{50}, μM)	HCV[c] (EC_{50}, μM)	CC_{50}[d] (IC_{50}, μM)	SI[e]
6	Me	H	H	H	0.107	1.72	0.175	>20	>114
7	H	H	H	H	0.130	2.95	0.550	>3.0	>5.0
8	H	Me	H	H	>50	>50	>10	>10	N/A
9	H	H	Me	H	0.060	0.635	0.150	8.6	57
10	H	H	H	Me	>50	>50	>10	1.9	<0.19

[a]Biochemical inhibition of human FASN. [b]Biochemical inhibition of rat FASN. [c]Inhibition of HCV RNA in the replicon system. Telaprevir was used as a positive control. [d]Inhibition of cell viability. [e]SI = EC_{50}/CC_{50}; $n \geq 2$, and CV ≤ 50%.

Table 2. Imidazopyridine SAR, Antiviral, and FASN Inhibitory Activities

Compounds **6, 11-23**   Compound **24**

Cmpnd	X	R^3	Z	Z'	Z"	hFASN[a] (IC50, μM)	rFASN[b] (IC50, μM)	HCV[c] (EC50, μM)	CC50[d] (IC50, μM)	SI[e]
6	pyrrolidinyl	H	CH	N	CH	0.107	1.72	0.175	>20	>114
11	H	H	CH	N	CH	0.590	>50			
12	H	H	CH	CH	CH	0.980	>50			
13	HO-azetidinyl	H	CH	N	CH	0.300	31.0			
14	N-methylpiperazinyl	H	CH	N	CH	0.99	>50	0.83	15	18
15	N-methylpiperazinyl	Me	CH	N	CH	0.43	3.84	0.52	>20	>38
16	piperazinyl	Me	CH	N	CH	0.87	3.07			
17	morpholinyl	Me	CH	N	CH	0.040	0.316	0.040	>3	>75
18	methoxy-azetidinyl	Me	CH	N	CH	0.060	0.316	0.084	>20	>238
19	pyrrolidinyl	Me	CH	N	CH	0.017	0.160	0.018	>3	>166
20	azetidinyl	Me	CH	N	CH	0.045	0.397	0.091	>20	>219
21	MeSO$_2$-	Me	CH	N	CH	0.085	2.43			
22	MeSO$_2$-	Me	N	CH	CH	0.070	3.85	0.130	>20	>153
23	azetidinyl	Me	CH	CH	N	0.070	0.325	0.31	>3	>9
24						0.48	0.51			

[a]Biochemical inhibition of human FASN. [b]Biochemical inhibition of rat FASN. [c]Inhibition of HCV RNA in the replicon system, typically determined only for more active compounds. See the Supporting Information for correlations across a larger number of analogues. Telaprevir was used as a positive control. [d]Inhibition of cell viability. [e]SI = EC$_{50}$/CC$_{50}$; $n \geq 2$, and CV $\leq$ 50%.

Scheme 2. Preparation of Compound 19[a]

[a]Reagents and conditions: (a) NaIO$_4$, I$_2$, H$_2$SO$_4$, AcOH. (b) n-BuLi, THF, −78 °C, then DMF. (c) 4-(Piperidine-4-yl)benzonitrile, HBTU, DIEA, DMF. (d) Pyrrolidine, K$_2$CO$_3$, MeCN, 70 °C (91%). (e) H$_2$, Pd/C, MeOH (94%). (f) Compound **28**, Na$_2$S$_2$O$_5$, DMF, 100 °C.

iodine and sodium periodate in a mixture of sulfuric acid and acetic acid furnished the iodinated aryl compound **26** in 82% yield. Lithium-halogen exchange followed by addition of *N,N*-dimethylformamide (DMF) gave the corresponding aldehyde derivative (**27**) in good yield (74%). Amide coupling with 4-(piperidin-4-yl)benzonitrile[21] was then achieved using HBTU in DMF to afford intermediate **28** (84%). Subsequent imidazole formation was accomplished by reacting aldehyde

28 with diamino-pyridine derivative **29** under oxidative conditions to give target compound **19** in 73% yield.

In the proton NMR spectrum of **19**, the signal for the R^3-methyl was split ($\Delta\delta \approx$ 40 Hz, d_6-DMSO), suggesting a possible hindered rotation around the phenyl-carbonyl bond. Preliminary estimates of rotational barriers in compound **19** were determined using VT-NMR.[22,23] At a moderately elevated temperature (Tc < 323 K), the R^3-methyl peaks coalesce, suggesting a fast interconversion between rotamers (<0.01 s at 37 °C).[22−27] In a similar analysis of the rotation around the carbonyl-nitrogen bond, the coalescence temperature of piperidine protons (Tc ≈ 357 K, $\Delta\delta \approx$ 65 Hz, d_6-DMSO) implies a rotamer half-life of ~0.1 s at 37 °C. Taken together, these preliminary observations suggest that **19** does not represent a mixture of discrete atropisomers.

Having identified potent inhibitors of human and rat FASN, we next studied the relationship between exposure and target modulation in vivo using **19** as a sentinel compound. De novo synthesis of palmitate was selected as a PD marker since palmitate is directly produced by FASN.[1,18] As expected, **19** was a potent inhibitor of palmitate synthesis in both rat and human cells (Table 3). While compound **19** had moderate iv clearance and a short half-life in the rat (Table 4), its oral bioavailability and exposure over time were considered acceptable for an initial assessment of in vivo target modulation.

Table 3. In Vitro and in Vivo Inhibition of de Novo Palmitate Synthesis by Compound 19

human IC$_{50}$ (HeLa, μM)	rat IC$_{50}$ (NMU, μM)	
	in vitro	
0.012	0.062	
rat no.	[19]$_{liver}$[b] (μM)	% inhibition[c]
	in vivo: 15 mg/kg[a]	
1	1.7	33
2	0.16	2
3	0.13	9
4	2.9	35
5	8.1	70
	in vivo: 50 mg/kg[a]	
1	23.6	91
2	14.4	96
3	12.6	52
4	9.8	100
5	3.4	40

[a]Oral dose. [b]Liver level of **19** at 8 h. [c]% inhibition of de novo palmitate synthesis at 8 h as compared to vehicle group.

Table 4. Rat PK Profile of Compound 19

route	Cl[c] (mL/min/kg)	V[d] (L/kg)	t$_{1/2}$[e] (h)	AUC[f] (h ng/mL)	% F[g]
iv[a]	36	2.0	0.7	460	
po[b]			3.2	18200	88

[a]1 mg/kg. [b]50 mg/kg. [c]Clearance. [d]Volume of distribution. [e]Half-life. [f]Area under the curve. [g]Oral bioavailability.

The in vivo PD response was assessed in the liver since hepatocytes are the site of HCV infection.[11] Livers were harvested 8 h after treating animals with oral doses of compound **19** delivered as a methylcellulose suspension (15 mg/kg, 50 mg/kg, or vehicle only), and the levels of compound and newly synthesized palmitate were determined. As shown in Table 3, the extent of palmitate synthesis inhibition 8 h after dosing is dose- and exposure-dependent. Strong inhibition of hepatic palmitate synthesis is observed at the higher dose (50 mg/kg), and a weaker response occurs at 15 mg/kg. The data in Table 3 indicate that the degree of suppression is correlated to the level of compound in the liver.

In summary, the present study outlines the discovery of a potent FASN inhibitor that blocks palmitate synthesis in the liver. While compound **19** has desirable attributes including good cell permeability (MDCK P_{app} = 8.3 × 10^{-6} cm/s) and notable antiviral activity, its highly lipophilic nature (clogP = 4.99, cLipE = 2.75)[28−30] and relatively high molecular weight (M_w = 504.6 g/mol, LE = 15.21)[31,32] pose challenges to further development.[33] Subsequent manuscripts will present our efforts to improve these properties while maintaining desired biological activities using the results and SAR presented in the current study as a foundation.

ASSOCIATED CONTENT

S **Supporting Information**

Biological assays and experimental procedures. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*E-mail: johan.oslob@3vbio.com.

ABBREVIATIONS

BuLi, butyl lithium; DIEA, *N,N*-diisopropylethylamine; DMF, *N,N*-dimethylformamide; FASN, fatty acid synthase; HCV, hepatitis C virus; MDCK, Madin−Darby canine kidney; PD, pharmacodynamic; PK, pharmacokinetic; SAR, structure−activity relationships; SI, selectivity index

REFERENCES

(1) Chirala, S. S.; Wakil, S. J. Structure and function of animal fatty acid synthase. *Lipids* **2004**, 39 (11), 1045−1053.

(2) Liu, H.; Liu, J. Y.; Wu, X.; Zhang, J. T. Biochemistry, molecular biology, and pharmacology of fatty acid synthase, an emerging therapeutic target and diagnosis/prognosis marker. *Int. J. Biochem. Mol. Biol.* **2010**, 1 (1), 69−89.

(3) Kuhajda, F. P. Fatty-acid synthase and human cancer: new perspectives on its role in tumor biology. *Nutrition* **2000**, 16 (3), 202−208.

(4) Menendez, J. A.; Lupu, R. Fatty acid synthase and the lipogenic phenotype in cancer pathogenesis. *Nat. Rev. Cancer* **2007**, 7 (10), 763−777.

(5) Abramson, H. N. The lipogenesis pathway as a cancer target. *J. Med. Chem.* **2011**, 54 (16), 5615−5638.

(6) Menendez, J. A.; Vazquez-Martin, A.; Ortega, F. J.; Fernandez-Real, J. M. Fatty acid synthase: Association with insulin resistance, type 2 diabetes, and cancer. *Clin. Chem.* **2009**, 55 (3), 425−38.

(7) Wakil, S. J.; Abu-Elheiga, L. A. Fatty acid metabolism: target for metabolic syndrome. *J. Lipid Res.* **2009**, 50 (Suppl.), S138−S143.

(8) Wu, M.; Singh, S. B.; Wang, J.; Chung, C. C.; Salituro, G.; Karanam, B. V.; Lee, S. H.; Powles, M.; Ellsworth, K. P.; Lassman, M. E.; Miller, C.; Myers, R. W.; Tota, M. R.; Zhang, B. B.; Li, C. Antidiabetic and antisteatotic effects of the selective fatty acid synthase (FAS) inhibitor platensimycin in mouse models of diabetes. *Proc. Natl. Acad. Sci. U.S.A.* **2011**, 108 (13), 5378−5383.

(9) Yang, W.; Hood, B. L.; Chadwick, S. L.; Liu, S.; Watkins, S. C.; Luo, G.; Conrads, T. P.; Wang, T. Fatty acid synthase is up-regulated during hepatitis C virus infection and regulates hepatitis C virus entry and production. *Hepatology* **2008**, 48 (5), 1396−1403.

(10) Melnikova, I. Hepatitis C—Pipeline update. *Nat. Rev. Drug Discovery* **2011**, 10 (2), 93−94.

(11) Lemon, S. M.; Walker, M. C.; Alter, M. J.; Yi, M. Hepatitis C Virus. In *Fields Virology*; Knipe, D. M., Howley, P. M., Griffin, D. E., Lamb, R. A., Martin, M. A., Roizman, B., Straus, S. E., Eds.; Lippincott Williams & Wilkins: Philadelphia, 2006; Vol. 1, pp 1253−1304.

(12) Prussia, A.; Thepchatri, P.; Snyder, J. P.; Plemper, R. K. Systematic Approaches towards the Development of Host-Directed Antiviral Therapeutics. *Int. J. Mol. Sci.* **2011**, 12 (6), 4027−52.

(13) Schwegmann, A.; Brombacher, F. Host-directed drug targeting of factors hijacked by pathogens. *Sci. Signal* **2008**, 1 (29), re8.

(14) Flavin, R.; Peluso, S.; Nguyen, P. L.; Loda, M. Fatty acid synthase as a potential therapeutic target in cancer. *Future Oncol.* **2010**, 6 (4), 551−562.

(15) When tested in our biochemical assays, C75 and Cerulenin had weak activities (>5 μM) against human and rat fatty acid synthase.

(16) For a recent example of a novel inhibitor scaffold, see Abdel-Magid, A. F. Fatty Acid Synthase Inhibitors as Possible Treatment for Cancer. *ACS Med. Chem. Lett.* **2012**, 3 (8), 612−613.

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dx.doi.org/10.1021/ml300335r | *ACS Med. Chem. Lett.* 2013, 4, 113−117

(17) Kemble, G.; McDowell, R.; Moese, S.; Oslob, J. D.; Patick, A. K.; Yendluri, S.; Parsey, M. Potent Hepatitis C Antiviral Activity by Inhibiting Fatty Acid Synthase. *The 63rd Annual Meeting of the American Association for the Study of Liver Diseases*, Boston, MA, **2012**.

(18) Evanchik, M.; Cai, H.; Feng, Q. S.; Hu, L.; Johnson, R.; Kemble, G.; Kosaka, Y.; Lai, J.; Oslob, J. D.; Sivaraja, M.; Tep, S.; Yang, H.; Zaharia, C. A.; McDowell, R. TVB-2640, a Novel Anti-HCV Agent, Safely Causes Sustained Host-Target Inhibition In Vivo. *The 63rd Annual Meeting of the American Association for the Study of Liver Diseases*, Boston, MA, **2012**.

(19) Wallenius, L.; Butlin, R.; Kjellstedt, A.; Lofgren, L.; Oakes, N. A Novel Fatty Acid Synthase Inhibitor Suppresses De Novo Lipogenesis but induces Hepatic Steatosis, Dermatitis and does not enhance Insulin Sensitivity in Obese Zucker Rats. *American Diabetes Association 68th Scientific Sessions*, San Francisco, CA, **2008**.

(20) See Graph S1 in the Supporting Information.

(21) Pieper, H.; Linz, G.; Himmelsbach, F.; Austel, V.; Muller, T.; Weisenberger, J.; Guth, B. Carboxylic acid derivatives, pharmaceutical compositions containing these compounds and processes for preparing them. U.S. 5442064, 1995.

(22) Sandström, J. *Dynamic NMR Spectroscopy*; Academic Press: London, New York, 1982.

(23) Friebolin, H., *Basic One- and Two-Dimensional NMR Spectroscopy*; Wiley-VCH: Weinheim, New York, 1998.

(24) Clayden, J.; Moran, W. J.; Edwards, P. J.; LaPlante, S. R. The challenge of atropisomerism in drug discovery. *Angew. Chem., Int. Ed. Engl.* **2009**, *48* (35), 6398−6401.

(25) Guile, S. D.; Bantick, J. R.; Cooper, M. E.; Donald, D. K.; Eyssade, C.; Ingall, A. H.; Lewis, R. J.; Martin, B. P.; Mohammed, R. T.; Potter, T. J.; Reynolds, R. H.; St-Gallay, S. A.; Wright, A. D. Optimization of monocarboxylate transporter 1 blockers through analysis and modulation of atropisomer interconversion properties. *J. Med. Chem.* **2007**, *50* (2), 254−263.

(26) LaPlante, S. R.; Edwards, P. J.; Fader, L. D.; Jakalian, A.; Hucke, O. Revealing atropisomer axial chirality in drug discovery. *ChemMedChem* **2011**, *6* (3), 505−513.

(27) Laplante, S. R.; Fader, L. D.; Fandrick, K. R.; Fandrick, D. R.; Hucke, O.; Kemper, R.; Miller, S. P.; Edwards, P. J. Assessing atropisomer axial chirality in drug discovery and development. *J. Med. Chem.* **2011**, *54* (20), 7005−7022.

(28) Leeson, P. D.; Springthorpe, B. The influence of drug-like concepts on decision-making in medicinal chemistry. *Nat. Rev. Drug Discovery* **2007**, *6* (11), 881−90.

(29) Ryckmans, T.; Edwards, M. P.; Horne, V. A.; Correia, A. M.; Owen, D. R.; Thompson, L. R.; Tran, I.; Tutt, M. F.; Young, T. Rapid assessment of a novel series of selective CB(2) agonists using parallel synthesis protocols: A Lipophilic Efficiency (LipE) analysis. *Bioorg. Med. Chem. Lett.* **2009**, *19* (15), 4406−4409.

(30) Here, cLipE = p(HCV EC$_{50}$) − clogP.

(31) Perola, E. An analysis of the binding efficiencies of drugs and their leads in successful drug discovery programs. *J. Med. Chem.* **2010**, *53* (7), 2986−2997.

(32) Here, LE = p(hFAS IC$_{50}$)/Mw × 1000.

(33) Edwards, M. P.; Price, D. A. Role of Physicochemical Properties and Ligand Lipophilicity Efficiency in Addressing Drug Safety Risks. In *Annual Reports in Medicinal Chemistry*; Macor, J. E., Ed.; Academic Press: Amsterdam, Boston, 2010; Vol. *45*, Chapter 23, pp 380−391.