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3-Aryl-4-hydroxyquinolin-2(1*H*)-one derivatives as type I fatty acid synthase inhibitors

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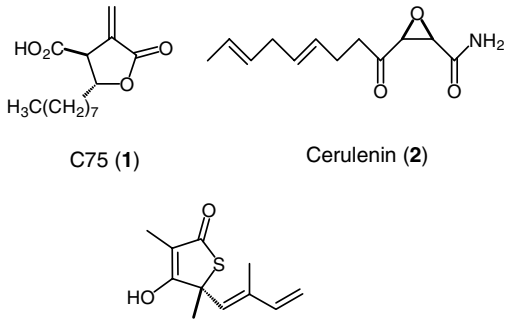
Abstract—A series of 3-aryl-4-hydroxyquinolin-2(1*H*)-ones with fatty acid synthase inhibitory activity was prepared. Starting from a derivative with an IC_{50} = 1.4 μM, SAR studies led to compounds with more than 70-fold increase in potency (IC_{50} < 20 nM).

Inhibition of human fatty acid synthase (FAS) has been shown to induce apoptosis in a variety of cancer cells, and consequently to be a potential therapeutic target for the treatment of cancer.[1] Expression and activity of FAS is significantly higher in a variety of tumor cells than in normal tissue, which creates the potential for a large therapeutic index.[2] To date, only a few inhibitors of FAS have been reported and most of them are covalent inhibitors.[1d,e] Of these the most commonly studied are C75 (**1**) and cerulenin (**2**) which are covalent inhibitors that have been shown to be efficacious in xenograft studies at micromolar levels (Fig. 1).[1d] Recently, several derivatives of the naturally occurring (5*R*)-thiolactomycin (**3**) were shown to be reversible FAS inhibitors in the micromolar range.[3]

FAS catalyzes the synthesis of long chain fatty acids (primarily palmitate) from acetyl-CoA and malonyl-CoA. The enzyme is a homodimer, each with seven catalytic domains that in concert catalyze the reactions leading to palmitate synthesis.[4] The combination of structural complexity and until recently the lack of X-ray crystallography data of mammalian FAS creates a significant challenge in the development of small molecule inhibitors for human FAS.[5]

Herein, we report the synthesis and SAR studies of 3-aryl-4-hydroxyquinolin-2(1*H*)-one analogues as a novel class of FAS inhibitors. As part of our screening campaign, we identified **4**, an interesting, albeit weak, lead (IC_{50} value of 1.4 μM (human)). We reasoned that the enol functionality in both **3** and **4** may be mimicking the enol intermediate that occurs during the enzymatic condensation reaction of



C75 (**1**) Cerulenin (**2**)

(5,*R*)-Thiolactomycin (**3**)

Figure 1. Literature reported FAS inhibitors.

Keywords: Fatty acid synthase; Cancer; Apoptosis.
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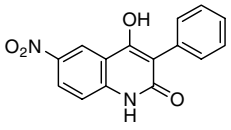
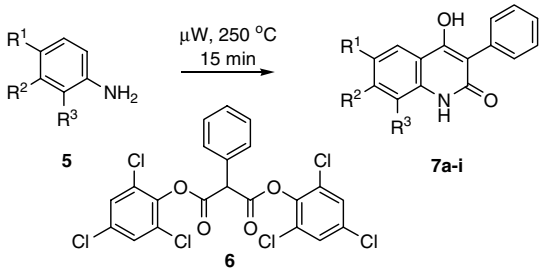
acetyl-CoA and malonyl-CoA by the β-ketoacyl synthase domain of FAS. We subsequently initiated SAR studies with **4** as our lead structure with the goal of improving potency (see Fig. 2).

The general synthetic approaches to 3-aryl-4-hydroxyquinolin-2(1*H*)-one and variants are shown in Schemes 1–3. To explore the structure–activity relationship of the quinolin-2(1*H*)-one core, we developed an efficient and general solvent-free microwave cyclocondensation reaction that is shown in Scheme 1.[6] Irradiation of a variety of electron-deficient or electron-rich anilines **5** with activated arylmalonate **6** led to the corresponding products **7a–i** in moderate to high yields. These compounds were quickly purified by precipitation with diethyl ether and filtration. Using this methodology we were able to survey a variety of substituents on the quinolin-2(1*H*)-one core.



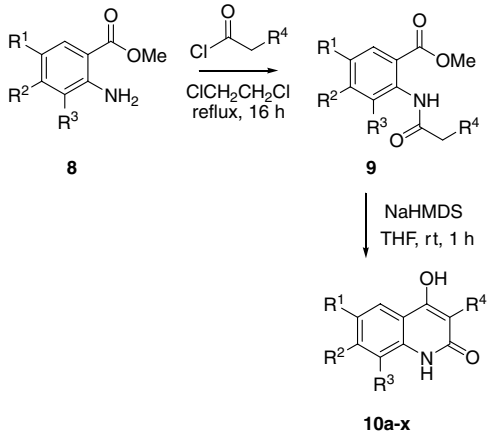
4

Figure 2. Lead FAS inhibitor.



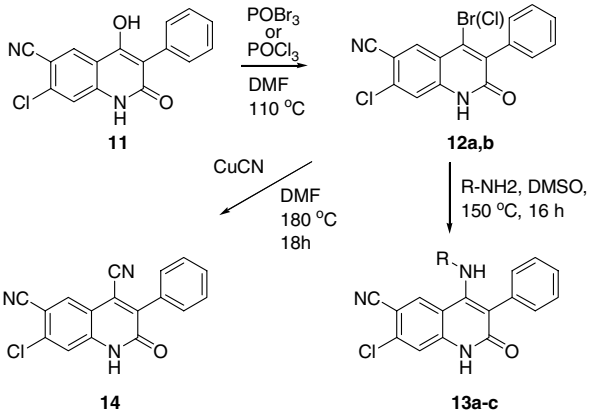
5 **7a-i** **6**

Scheme 1. Microwave assisted synthesis of 3-aryl-4-hydroxyquinolin-2(1*H*)-ones using activated aryl malonates.



8 **9** **10a-x**

Scheme 2. Traditional synthesis of 3-aryl-4-hydroxyquinolin-2(1*H*)-ones.



11 **12a,b** **14** **13a-c**

Scheme 3. Synthetic modifications of enol **11**.

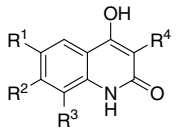
To investigate the structure–activity relationship of the phenyl moiety of **4**, we utilized the traditional synthetic approach to 3-aryl-4-hydroxyquinolin-2(1*H*)-one analogues as shown in Scheme 2.[7] Starting with methyl 2-aminobenzoates **8**, acylation with the appropriate acid chloride in refluxing 1,2-dichloroethane gave the desired corresponding phenylacetamides **9** in high yield. Intramolecular cyclization of the phenylacetamides **9** with sodium *bis*(trimethylsilyl)amide in THF afforded the desired 3-aryl-4-hydroxyquinolin-2(1*H*)-one analogues **10a–x** in high yield. The hydroxy group could then be converted to the corresponding bromide or chloride as shown in Scheme 3. The bromide can then be displaced with a variety of primary amines to provide the corresponding enamines **13** or reacted with CuCN to give the corresponding nitrile **14** (Scheme 3).

The results of our initial SAR studies with rat fatty acid synthase are shown in Table 1. The removal of the nitro group led to complete loss of activity and migration of the nitro group ortho to the nitrogen led to a 6-fold decrease in activity. Addition of a chloro or fluoro substituent ortho to the nitro group led to a remarkable 10-fold improvement in potency (**10b** + **10c**).

We then turned our attention toward exploring the SAR profile of the phenyl group (**10e** → **10n**) in hopes of finding a permissive region. The removal of the phenyl group led to complete loss of activity. Introduction of fluoro or chloro substituents into the phenyl ring led to a minor loss of activity. In contrast, replacement of the phenyl group with 2-naphthyl or a variety of heterocycles led to a substantial loss of activity. Finally, substitution of the 4-position with a phenyl or benzyloxy groups gave equally potent compounds.

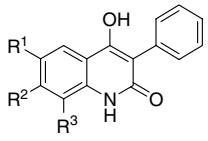
Our SAR studies continued with human fatty acid synthase (Table 2). Interestingly, in comparing the potency of **7a** and **10b** against human and rat FAS, we observed a moderate shift in in vitro potency. We were gratified to find that the replacement of the nitro group with a cyano group led to an improvement in activity, since the nitro group represented a potential liability for toxicity. Introduction of a chloride ortho to the cyano led to a 10-fold increase in activity and the migration of the chloride to

Table 1. Rat fatty acid synthase inhibition data



Compound	R^1	R^2	R^3	R^4	FAS inhibition[8] IC_{50} (nM)
7a	NO_2	H	H	Ph	550
7b	H	H	H	Ph	>15,000
10a	H	H	NO_2	Ph	3000
10b	NO_2	Cl	H	Ph	73
10c	NO_2	F	H	Ph	50
10d	NO_2	H	H	H	>15,000
10e	NO_2	Cl	H	2,4-Dichlorophenyl	443
10f	NO_2	Cl	H	2,4-Difluorophenyl	173
10g	NO_2	Cl	H	3,5-Dichlorophenyl	134
10h	NO_2	Cl	H	3,5-Difluorophenyl	172
10i	NO_2	Cl	H	2-Naphthyl	6000
10j	NO_2	Cl	H	2-Thienyl	700
10k	NO_2	Cl	H	1*H*-Tetrazol-5-yl	2600
10l	NO_2	Cl	H	3-Methylisoxazol-5-yl	3400
10m	NO_2	Cl	H	4-Biphenyl	122
10n	NO_2	Cl	H	4-(Benzyloxy)phenyl	86

Table 2. Rat and Human fatty acid synthase inhibition data



Compound	R^1	R^2	R^3	Human FAS inhibition[8] IC_{50} (nM)	Rat FAS inhibition[8] IC_{50} (nM)
7a	NO_2	H	H	1403	550
10b	NO_2	Cl	H	207	73
7c	CN	H	H	1294	NA
7d	CN	Cl	H	136	NA
7e	CN	H	Cl	402	NA
7f	CF_3	H	H	>15,000	>15,000
7g	$MeSO_2$	H	H	>15,000	>15,000
7h	CF_3SO_2	H	H	>15,000	>15,000
7i	CO_2H	H	H	NA	>15,000

NA, not available.

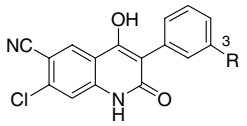
the meta position led to a 4-fold loss in activity. Substitution of the nitro group with other electron-withdrawing groups such as trifluoromethyl, methyl sulfone, trifluoromethyl sulfone, and carboxylic acid led to inactive compounds.

We then focused our efforts toward delineating the SAR around the phenyl moiety of **7d**. Interestingly, introduction of a methylsulfonyl group in the 3-position led to 40-fold loss in activity, whereas introduction of the phenyl group in the same position led to a 3-fold improvement in activity (**10o** vs **10p**). This result suggested that the 3-position occupies a hydrophobic region of the binding site. Further substitution of the phenyl group was well tolerated (**10q**–**10x**). The most favorable substitution was a 2-methoxyphenyl group at the 3-position (**10v**, IC_{50} = 19 nM), which represents a 74-fold improvement in activity over the lead **4** (IC_{50} = 1.4 μM) (see Table 3).

In the final phase of our SAR studies we focused our efforts on the substitution of the enol functionality (Table 4). Replacement of the 4-hydroxy group with a bromide, chloride, amine, benzylamine, and nitrile group led to complete loss of activity, which suggests that the 4-hydroxy group is essential for activity.

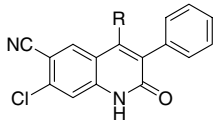
In conclusion, we have discovered, through our SAR studies, several 3-aryl-4-hydroxyquinolin-2(1*H*)-ones that are potent human FAS inhibitors (IC_{50} = <100 nM) against the human enzyme in vitro. These SAR studies are highlighted by the successful replacement of the nitro with nitrile moiety, which eliminated potential liability for toxicity. In addition, this work led to the identification of a permissive region in the 3-phenyl group, which led to the discovery of some of our most potent inhibitors.

Table 3. Human fatty acid synthase inhibition data



Compound	R	FAS inhibition[8] IC_{50} (nM)
7d	H	136
10o	Phenyl	52
10p	Methylsulfonyl	4164
10q	4-Fluorophenyl	54
10r	3,4-Difluorophenyl	68
10s	3,5-Difluorophenyl	728
10t	2-Naphthyl	127
10u	1-Benzothien-3-yl	101
10v	2-Methoxyphenyl	19
10w	1-Methyl-1*H*-pyrazol-4-yl	734
10x	Pyridin-3-yl	194

Table 4. Human fatty acid synthase inhibition data



Compound	R	FAS inhibition[8] IC_{50} (nM)
12a	Br	>15,000
12b	Cl	>15,000
13a	NH_2	>15,000
13b	*p*-MeO-Benzylamine	>15,000
13c	Benzylamine	>15,000
14	CN	>15,000

References and notes

1. (a) Kuhajda, F. P.; Jenner, K.; Wood, F. D.; Hennigar, R. A.; Jacobs, L. B.; Dick, J. D.; Pasternack, G. R. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 6379; (b) Zhou, W.; Simpson, J. P.; McFadden, J. M.; Townsend, C. A.; Medghalchi, S. M.; Vadlamudi, A.; Pinn, M. L.; Ronnett, G. V.; Kuhajda, F. P. *Cancer Res.* **2003**, *63*, 7330; (c) Knowles, L. M.; Axelrod, F.; Browne, C. D.; Smith, J. W. A. *J. Biol. Chem.* **2004**, *279*, 30540; (d) Pizer, E. S.; Thupari, J.; Han, W. F.; Pinn, M. L.; Chrest, F. J.; Frehywot, G. L.; Townsend, C. A.; Kuhajda, F. P. *Cancer Res.* **2000**, *60*, 213; (e) Kuhajda, F. P.; Pizer, E.; Li, J. N.; Mani, N. S.; Frehywot, G. L.; Townsend, C. A. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 3450; (f) De Schrijver, E.; Brusselmans, K.; Heyns, W.; Verhoeven, G.; Swinnen, J. V. *Cancer Res.* **2003**, *63*, 3799.
2. (a) Pizer, E. S.; Lax, S. F.; Kuhajda, D. P.; Pasternack, G. R.; Kurman, R. J. *Cancer* **1998**, *83*, 528; (b) Rashid, A.; Pizer, E. S.; Moga, M.; Milgraum, L. Z.; Zahurak, M.; Pasternack, G. R.; Kuhajda, F. P.; Hamilton, S. R. *Am. J. Pathol.* **1997**, *150*, 201; (c) Milgraum, L. Z.; Witters, L. A.; Pasternack, G. R.; Kuhajda, F. P. *Clin. Cancer Res.* **1997**, *3*, 2115.
3. McFadden, J. M.; Medghalchi, S. M.; Thupari, J. N.; Pinn, M. L.; Vadlamudi, A.; Miller, K. I.; Kuhajda, F. P.; Townsend, C. A. *J. Med. Chem.* **2005**, *48*, 946.
4. Asturias, F. J.; Chadick, J. Z.; Cheung, I. K.; Stark, H.; Witkowski, A.; Joshi, A. K.; Smith, S. *Nat. Struct. Mol. Biol.* **2005**, *12*, 225.
5. Maier, T.; Jenni, S.; Ban, N. *Science* **2006**, *311*, 1258.
6. Rivkin, A.; Adams, B. *Tetrahedron Lett.* **2006**, *47*, 2395.
7. (a) DeVita, R. J.; Hollings, D. D.; Goulet, M. T.; Wyvratt, M. J.; Fisher, M. H.; Lo, J. L.; Yang, Y. T.; Cheng, K.; Smith, R. G. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 2615; (b) DeVita, R. J.; Goulet, M. T.; Wyvratt, M. J.; Fisher, M. H.; Lo, J. L.; Yang, Y. T.; Cheng, K.; Smith, R. G. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 2621.
8. In vitro enzymatic activity of FAS was determined by monitoring the production of free coenzyme A (CoA-SH) from malonyl- and acetyl-CoA during the FAS enzymatic reaction. CoA-SH levels were determined using the thiol-reactive dye 7-diethylamino-3-(4-maleimidophenyl)-4-methylcoumarin (CPM). Unreacted CPM is nonfluorescent, whereas the CPM–CoA adduct shows a peak fluorescence emission at 475 nM after excitation at 380 nM.