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Design, synthesis, and in vivo activity of novel inhibitors of delta-5 desaturase for the treatment of metabolic syndrome

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ABSTRACT

The synthesis, SAR, and in vivo activity of inhibitors of delta-5 desaturase are described. Ring-constraint of the initial series provided access to a variety of in vitro active chemotypes, from which the indazole was selected. Examples from the indazole series displayed in vivo activity in reducing the enzymatic activity of liver delta-5 desaturase.

Metabolic syndrome is associated with cardiovascular disease as well as type 2 diabetes—in particular insulin resistance, high blood pressure, central obesity, decreased high-density lipoprotein (HDL) cholesterol, and elevated triglycerides.[1–3] A correlation has been observed between the level of activity of desaturases and various disease states, including obesity.[4,5] For instance, regulation of both delta-5 desaturase [also known as fatty acid desaturase (FADS1)] and delta-6 desaturase activity has been associated with changes in body weight in mice, humans and other animals. Desaturases are a family of enzymes that are involved in the production of poly-unsaturated fatty acids, beginning with the desaturation of palmitic acid, and are named according to the type of fatty acid they generate (delta-5, delta-6, and delta-9 desaturase).[6] Our interest in the desaturase enzymes came about through the production of almost 5000 lines of knock out (KO) mice.[7] In our hands, delta-5 desaturase KO mice had decreased body fat, improved glucose tolerance, and lower fasting serum levels of insulin, cholesterol and triglycerides when compared to wild type littermate control mice.[8] With our knowledge of the high degree of homology between murine and human delta-5 desaturase, and as delta-5 desaturase is one of the four desaturases present in humans, we initiated an effort to discover inhibitors of this target for the treatment of body composition disorders, such as obesity and diabetes.

To date, there have been a structurally diverse array of small molecule inhibitors of delta-5 desaturase previously reported in the literature, including compounds such as Sesamin **1**,[9] *para*-isopentoxyaniline **2**,[10] fused-pyrimidones **3**,[11] dibenzoazepine **4**,[12] and amide **5**,[12] Figure 1.

We identified compound **5** as an interesting small molecule lead. It has previously been disclosed as a delta-5 desaturase inhibitor that was selective over delta-6 and delta-9 desaturase.[12] Obukowicz et al.[12] investigated compound **5** with a view to its potential anti-inflammatory effects. From this starting point, we first investigated variations in the linker between the two aromatic rings, and the amide linkage proved to be the optimal one. It was found that the reverse amide, reduction of the amide to an amine, and methylation of the amide nitrogen all removed inhibitory activity.

The synthesis of our derivatives was carried out following the synthetic methods shown in Scheme 1. The appropriate aniline was reacted with a functionalized isatoic anhydride or the necessary benzoic acid to yield amides 5–12.

We initially set out to survey changes upon the right-hand aromatic ring. Under our assay conditions,[8] compound **5** showed an

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0960-894X/

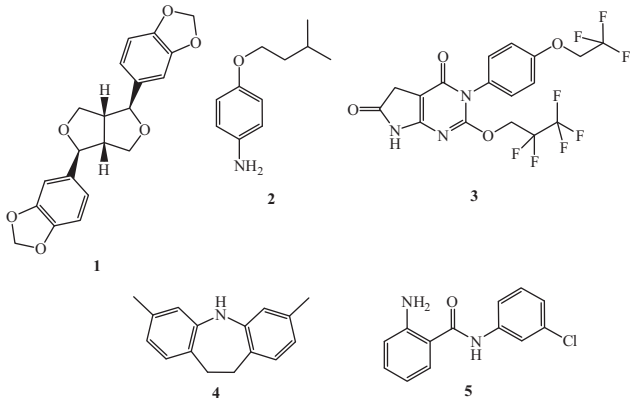
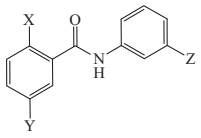


Figure 1. Representative literature described delta-five desaturase inhibitors.

Table 1
SAR of amide analogs as inhibitors of delta-5 desaturase



Compound	X	Y	Z	IC_{50} (nM)
5	NH_2	H	Cl	58
6	NH_2	H	F	516
7	NH_2	H	*i*-Pr	843
8	OH	H	Cl	174
9	NH_2	F	Cl	67
10	NH_2	Cl	Cl	34
11	OH	OCH_3	Cl	15
12	OH	OCH_2CH_3	Cl	925

IC_{50} of 58 nM (see Table 1). Moving the chlorine atom of compound **5** from the meta position to either the *ortho*- or *para*-position gave compounds with markedly lower potency (39% and 36% enzyme inhibition, respectively, at 1 μM compound concentration), thus our work focused primarily on changes at the meta position. Examples of the functional group variations that we employed included changing the chlorine atom to a fluorine or isopropyl group, Table 1, compounds **6** and **7**, both of which resulted in significantly reduced activity. Di-substituted right-hand side derivatives of compound **5** were also pursued, however they too resulted in reduced activity—for example, 3,5-dichloro (245 nM), and 3-chloro-4-fluoro (155 nM).

Maintaining the preferred amide linker and right-hand side aromatic ring, we next made changes to the left-hand ring. Moving from X = amino to X = hydroxyl (**5** vs **8**) showed a slight drop in activity. Attempts to replace the amino or hydroxyl with other functionalities such as methyl, cyano, and chloro all provided derivatives with significantly lower activity, and moving the amino group around the left-hand ring demonstrated that the optimal position for the amino group was at the 2-position (for instance, the 3-amino derivative showed only 54% enzyme inhibition at 1 μM compound concentration). Addition of the Y-substituent at the 5-position resulted in increased potency (**10** and **11**). Steric effects were also found, with a drastic loss in potency seen when changing from 5-methoxy to 5-ethoxy (**11** vs **12**).

After exploring the SAR with amide based derivatives, we found that they possessed poor metabolic stability and pharmacokinetics. Since we believed that the activity of the compounds was related to a six-membered hydrogen bond between the amide carbonyl and the anilinic/phenolic functionality.[13,14] we next focused on making cyclized versions of these derivatives.

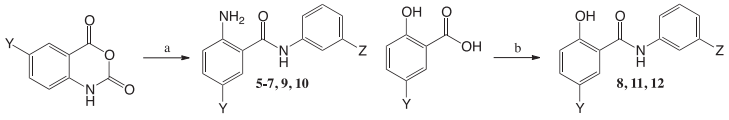
The syntheses of the initial ring-constrained analogs were carried out by following one of the four methods shown in Scheme 2. The compounds were either synthesized by S_NAr displacement of an aryl halide, Buchwald–Hartwig aryl amination, copper(II) acetate mediated coupling of an aryl amine (Chan–Lam coupling), or by reaction neat of the chloro-indazole with the aniline hydrochloride.[8]

As can be seen from Table 2, a variety of different bicyclic systems as ring-constraints were well tolerated. Quinoline, quinazoline, and naphthalene derivatives **12**–**14**, all showed good potency, whereas the furo[3,2-*c*]pyridyl derivative **15** was much less active. Thieno[3,2-*d*]pyrimidine **16** showed good activity, whereas the regioisomeric thieno[2,3-*d*]pyrimidine **17** was substantially less potent. The indane, compound **18**, was reasonably potent as the racemate, and the indazole, compound **19**, also showed good potency. From these, as well as other ring-constrained analogs not shown, the indazole derivative **19** was selected for further investigation. With the previous amide and phenol series' showing improved activity upon substitution upon the left-hand aromatic ring, we next set about producing substituted analogs of **19**.

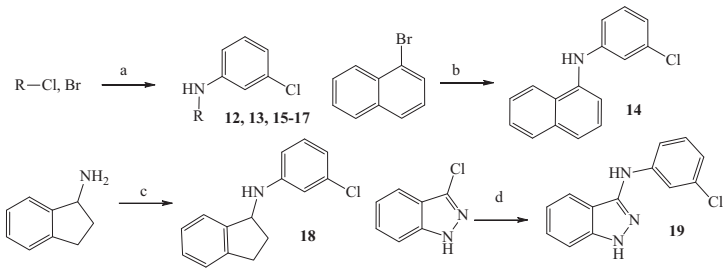
The substituted indazole derivatives were synthesized either via displacement of the 3-halo indazole with the appropriate aniline, coupling of the requisite 3-amino indazole with the appropriate aryl boronic acid in the presence of copper(II) acetate, or by palladium catalyzed aryl amination, Scheme 3.[8]

In some cases, replacement of the *Z* chloro substituent with fluorine did still produce active molecules, such as **20**, but this was not general, and thus chloro was the *Z* group of choice, Table 3. As expected from our previous work, substitutions at the 4-, 6-, and 7-position of the indazole ring with groups larger than fluorine were almost exclusively detrimental to the activity against the target, with a notable exception being the 7-chloro derivative of compound **19** which showed an IC_{50} of 33 nM. Hence, we focused on variations at the 5-position. Introduction of fluorine at the 5-position, compound **21**, maintained potency, whereas dichloro derivative **22** showed a substantial improvement in activity, and brominated compound **23** resulted in an appreciable increase in activity compared to compound **19**. Methyl derivative **24** was highly potent, whilst the trifluoromethyl variation **25** resulted in diminished activity. Methoxy substituted **26** was also very active, whilst trifluoromethoxy-containing **27** was not.

From these indazole derivatives, four compounds, **19**, **20**, **21** and **26** were selected to be tested in an in vivo liver enzyme inhibition experiment—delta-5 desaturase is highly expressed in the mammalian liver.[6] Each compound was given orally along with vehicle control, using a PEG/Solutol (20:80) dosing vehicle, to mice ($n = 4$) at a dose of 30 or 60 mg/kg, then 30 min later ^{14}C-di-homo-gamma-linolenic acid (DGLA) was given using an intraperitoneal

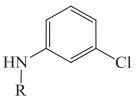


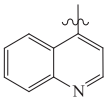
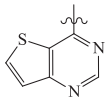
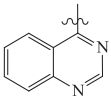
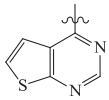
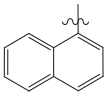
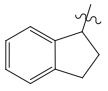
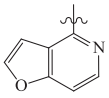
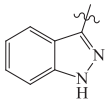
Scheme 1. Reagents and conditions: (a) aniline, EtOH, 100 °C; (b) aniline, PCl_3, PhMe, 120 °C. For lists of Y and Z see Table 1.



Scheme 2. Reagents and conditions: (a) 3-chloroaniline, *i*-PrOH, HCL (cat.), 60 °C; (b) 3-chloroaniline, $Pd_2(dba)_3$, BINAP, NaO^tBu, 120 °C; (c) 3-chlorophenyl boronic acid, $Cu(OAc)_2$, 2,6-lutidine, PhMe, air, 20 °C; (d) 3-chloroaniline. HCl, 180 °C. For lists of R see Table 2.

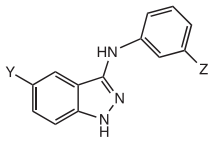
Table 2
SAR of bicyclic analogs as inhibitors of delta-5 desaturase



Compound	R	IC_{50} (nM)	Compound	R	IC_{50} (nM)
12		18	**16**		50
13		11	**17**		260
14		17	**18**		173
15		371	**19**		84

injection.[8] Plasma was removed from the mice 0.5 h post compound dose, and was bioanalyzed to determine compound concentration.[15] After 2.5 h the livers of the animals were collected, and the ^{14}C labeled products were evaluated by thin-layer chromatography (TLC), using quantitative phosphoimaging to analyze for the delta-5 desaturase mediated conversion of ^{14}C DGLA to ^{14}C Arachidonic acid, and comparing those levels to vehicle control.[8] The results from these experiments are summarized in Table 4. The 30 mg/kg doses of compounds **19**, **20**, **21**, and **26** produced a range of concentrations in the plasma at 0.5 h, and factoring in the potencies of the compounds, it can be seen that the compound concentrations are 151–652 fold over the IC_{50} values. This in turn manifests itself in the inhibition of delta-5 desaturase in the liver in the experiments, showing inhibition of the conversion of DGLA to Arachidonic acid from 18% to 75%. Compound **26** was also dosed at 60 mg/kg, and at this dose the compound plasma concentration at 0.5 h is 1753 fold above the IC_{50} value, which achieves 97%

Table 3
SAR of indazole analogs as inhibitors of delta-5 desaturase

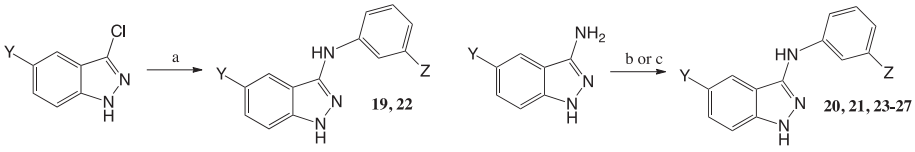


Compound	Y	Z	IC_{50} (nM)
19	H	Cl	84
20	H	F	59
21	F	Cl	58
22	Cl	Cl	5
23	Br	Cl	21
24	CH_3	Cl	6
25	CF_3	Cl	254
26	OCH_3	Cl	8
27	OCF_3	Cl	>3000

Table 4
Enzyme inhibition of delta-5 desaturase in the mouse liver by selected compounds

Compound	IC_{50} (nM)	Dose (mg/kg)	Plasma concentration 0.5 h (nm)	Plasma concentration 0.5 h/IC_{50}	% Liver delta-5 desaturase inhibition relative to vehicle control
19	84	30	12,699	151	42
20	59	30	9448	160	18
21	58	30	14,072	242	49
26	8	30	5215	652	75
26	8	60	14,025	1753	97

enzyme inhibition-displaying the dose dependent inhibition of delta-5 desaturase of compound **26**. Analysis of the ratio of plasma concentration for a range of structurally related analogs with respect to their respective in vitro IC_{50} values shows a very good correlation with the in vivo inhibition of delta-5 desaturase in the liver, Figure 2, showing the strong relationship between pharmacokinetics and pharmacodynamics in these experiments.



Scheme 3. Reagents and conditions: (a) Ar-NH_2. HCl, 180 °C; (b) Ar-$B(OH)_2$, $Cu(OAc)_2$, MeOH, air, 20 °C; (c) Ar-I, $Pd(bda)_2$, BINAP, Cs_2CO_3, PhME, MeCN, 140 °C. For lists of Y and Z Table 3.

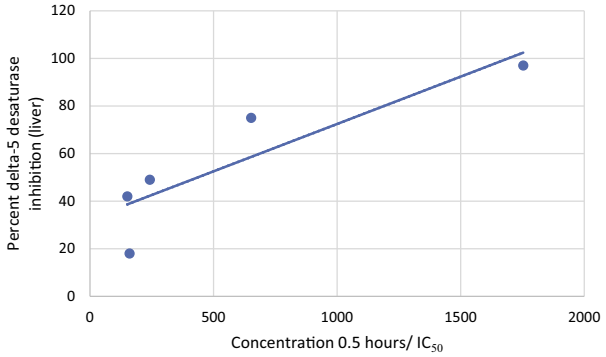


Figure 2. Correlation of pharmacokinetics and pharmacodynamics in the in vivo experiments.

In summary, we have developed a series of novel inhibitors of delta-5 desaturase, and four of these have displayed in vivo efficacy in reducing the enzymatic activity of delta-5 desaturase in the liver.

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15. The plasma fraction was immediately separated by centrifugation at 4 °C, and then stored at −20 °C until sample analysis. To quantify compound concentrations, plasma samples were extracted with acetonitrile–water–formic acid (80:20:0.1) containing verapamil as internal standard at a concentration of 1 μM. Extracted samples were analysed by LC/MS–MS. Compound levels were calculated by normalizing the area counts of compound to the area counts of the internal standard (relative area counts).