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INHIBITORS OF ACYL-CoA:CHOLESTEROL *O*-ACYL TRANSFERASE (ACAT) AS HYPOCHOLESTEROLEMIC AGENTS. 13. DESIGN, SYNTHESIS AND BIOLOGICAL EVALUATION OF TETRAZOLE ANILIDES AS POTENT INHIBITORS OF ACAT IN VITRO AND HYPOCHOLESTEROLEMIC AGENTS IN VIVO.

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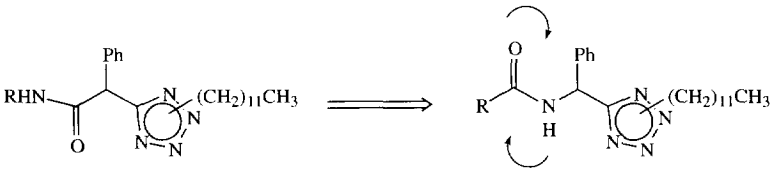
Abstract The syntheses and biological activities for anilides derived from 2-phenyl-2-(dodecyl-2H-tetrazol-5-yl)acetic acid are described. Evidence is provided that one of these compounds, **(+)-8b**, stereoselectively inhibits ACAT *in vitro* and possesses superior efficacy *in vivo* compared to **(-)-8b** or the racemic mixture **(±)-8b**.

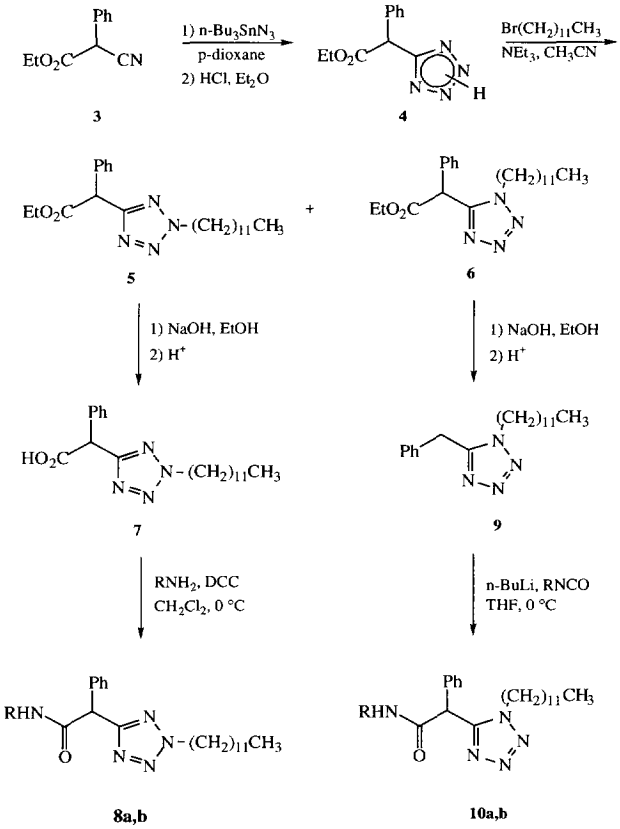
We have recently reported on a series of benzamide and nicotinamide derivatives (**1**) that potently inhibit ACAT *in vitro* and are efficacious in lowering plasma total cholesterol *in vivo*.[1] Evidence was provided that the (*N*-dodecyltetrazol-5-yl)-benzyl moiety in **1** is necessary for potent inhibition, since replacing this functionality with an oleyl side chain or varying the tetrazole chain length, resulted in a marked reduction in inhibitory activity. In a related study however, oleic acid anilides, substituted with electron donating groups on the 2,6- or 2,4,6-aryl positions, potently inhibit ACAT *in vitro* with IC_{50}'s in the 7 to 700 nM range.[2] Since fatty acid anilides have been shown to be significantly more potent than the corresponding benzamide isosteres,[1,3] we sought to improve the *in vitro* activity of the tetrazole derivatives by combining optimal structural features from both series, by replacing the benzamide bond of **1** with 2,6-diisopropyl- and 2,4,6-trimethoxy substituted anilide bioisosteres (**2**). Of the compounds prepared, a trimethoxyphenyl analog of **2** was resolved into individual enantiomers, in order to assess whether the biological activity observed for **2** resided in one particular enantiomeric form. In this paper, we will describe the syntheses and biological results for this novel series of tetrazole amide ACAT inhibitors.



2 **1**

Chemistry: The racemic anilides **8a,b** and **10a,b** were prepared employing the synthetic route illustrated in Scheme 1. The tetrazole intermediate **4** was synthesized in 66% yield by treating (±)-ethyl phenylcyanoacetate **3** with *n*-tributyltin azide in refluxing *p*-dioxane, with subsequent cleavage of tributyltin from the tetrazole moiety with ethereal HCl. Alkylation of **4** with 1-bromododecane in refluxing acetonitrile provided a 2.5:1 mixture of regioisomers **5** (52%) and **6** (21%).[4] This isomeric mixture could be easily separated by silica gel chromatography, or taken on to the next step as a crude mixture. Alternatively, **5** can be prepared by isomerizing the mixture by heating neat in iodododecane at 140 °C.[5] Saponification of **5** gives the expected carboxylic acid

Scheme 1



derivative **7** (90%). However, **6** decarboxylates to **9** quantitatively upon treatment with sodium hydroxide in ethanol. This proved to be an effective method of separating the 1- from the 2-regioisomer without the use of chromatography, since **9** is insoluble in aqueous base, whereas the sodium salt of **7** is highly soluble. Filtration of **9** followed by acidification of the filtrate, gave **7** exclusively as the 2-regioisomer. Amides **8a,b** were then prepared by coupling an appropriately substituted aniline with **7** in dichloromethane using DCC as the coupling agent (60%). To prepare the 1-regioisomers **10a,b**, the benzylic tetrazole **9** was deprotonated with *n*-butyllithium in THF, with subsequent quenching of the anion with 2,6-diisopropylphenyl- or 2,4,6-trimethoxyphenyl isocyanate (40%).

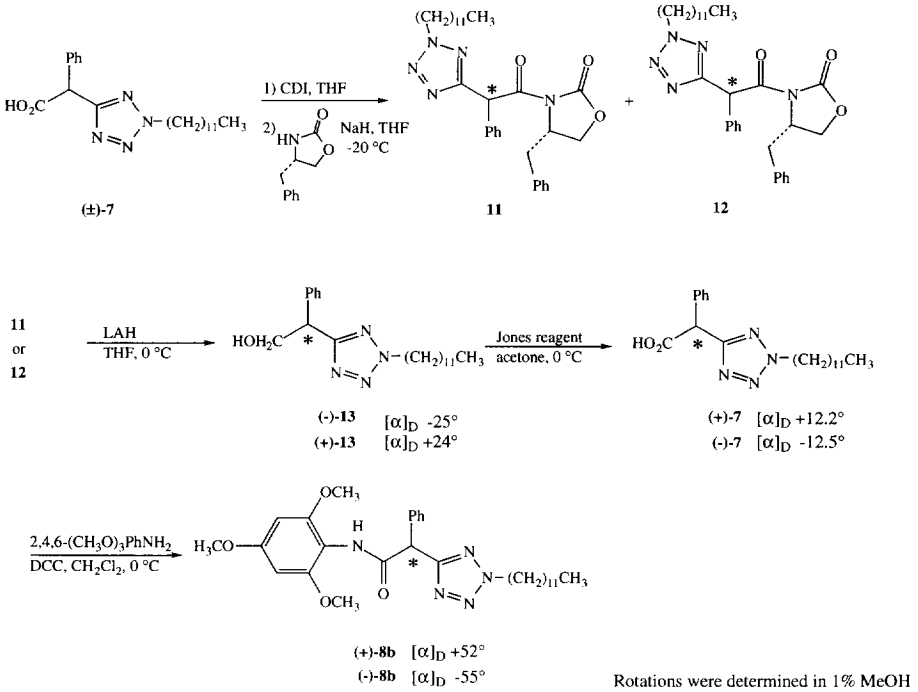
The enantiomers of **8b** were initially isolated by chiral preparative HPLC. The racemate, **8b**, was dissolved in a solution of 80:20 2-propanol:hexane and injected onto a 500 x 20.0 mm Chiralcel OG® preparative column at a flow rate of 8.0 ml/minute. The first enantiomer to elute, **(-)-8b**, was found to be 98% enantiomerically pure ($[\alpha]_D$=-58.0 °, c=1% MeOH) by HPLC. The second enantiomer isolated, **(+)-8b**, was 96.3% pure ($[\alpha]_D$= +55.1°, c=1% MeOH), but contained 3.7% of **(-)-8b**. After 19 injections, 1.85 grams of **(±)-8b** yielded 708 mg of **(-)-8b** and 727 mg of **(+)-8b**.

With the enantiomers in hand, we performed a solution stability study to determine the racemization potential for the respective isomers at 37 °C. Compounds **(+)-8b** and **(-)-8b** were evaluated at pH's of 1.0, 7.4 and 8.5 over a 25-hour period by chiral HPLC. In an acidic media, no racemization was observed. At a physiological pH of 7.4 however, the half-life of racemization is 56 hours for **(+)-8b** and 62 hours for **(-)-8b**. The rate of racemization is significantly increased (11 and 6 hours respectively) at pH 8.5.

In order to prepare quantities of **(+)-** and **(-)-8b** sufficient for *in vivo* biological testing, several resolution strategies were examined. Attempt to resolve **8** (Scheme 1) using classical resolution techniques were ineffective. As shown in Scheme 2, however, utilizing Evan's oxazolidone chiral auxiliary,[6] we were successful in preparing **8b** in either enantiomeric form. Thus, racemic acid **7** was coupled to the sodium salt of (4*S*)-benzyl-2-oxazolidone using CDI in THF to give a 1:1 mixture of diastereomers **11** and **12**. These compounds were separated pure (>99%), but in low yield (35%), using silica gel chromatography (elution with hexane/ THF (5:1) gave **11** and **12** respectively). Reduction of the imide functionality in **12** with LAH in THF provided the resolved alcohol **(+)-13**[7] (78%), followed by Jones oxidation at 0 °C to the penultimate acid **(-)-7** in 42% yield. Treatment of **(-)-7** with 2,4,6-trimethoxyaniline and DCC in dichloromethane yielded the target compound (50%), **(-)-8b,** in 93% enantiomeric excess.[8]

Identical reaction conditions were used converting **11** to **(+)-8b.**

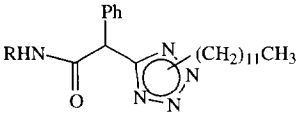
Scheme 2



Results and Discussion: The biological activity associated with the isosteric modification of **1** with 2,6-diisopropyl- and 2,4,6-trimethoxy-substituted anilides is shown in Table I.[9] The initial compounds prepared for this study, **8a** and **8b**, are considerably more potent than the corresponding benzamide tetrazoles **1** previously reported (compare IC_{50}'s of 10 and 24 nM to 810 and 1700 nM respectively).[1] This enhancement in *in vitro* potency correlates well with the *in vivo* efficacy obtained in the APCC screen, since both **8a** and **8b** significantly lower plasma cholesterol (-46%, -65%) at a dose (3 mg/kg) whereas the corresponding benzamide derivatives are ineffective. Positioning of the tetrazole alkyl chain in these compounds favors the 2-position, since the 1-regioisomers, **10a** and **10b**, were less active than **8a** and **8b** both *in vitro* and *in vivo*. A further assessment of hypocholesterolemic activity for **8a** and **8b** was measured in the CPCC

screen. In this model, **8b** was considerably more efficacious than **8a**, lowering plasma total cholesterol by 59% at 10 mg/kg, whereas the reduction in plasma total cholesterol with **8a** did not achieve statistical significance at doses as high as 30 mg/kg. In this series of biological assays, **8b** also demonstrated superior *in vitro* potency and efficacy *in vivo* compared to CI-976.

TABLE I



Compound[a]	R	Isomer	IC_{50} (μM)[b]	APCC (%ChangeTC)[c] 30 mg/kg	3 mg/kg	CPCC (%ChangeTC)[d] 10 mg/kg	3 mg/kg
CI-976			0.110	-57*	-15	-17	-12
8a	2,6-iPr_2Ph	2	0.010	-49*	-46*	-38[e]	-
8b	2,4,6-$(CH_3O)_3$Ph	2	0.024	-63*	-65*	-59*	-23
8b-(+)	2,4,6-$(CH_3O)_3$Ph	2	0.014	-68*	-66*	-60*	-55*
8b-(-)	2,4,6-$(CH_3O)_3$Ph	2	0.330	-64*	-47*	-11	+7
10a	2,6-iPr_2Ph	1	0.079	-44*	-	-24[e]	-
10b	2,4,6-$(CH_3O)_3$Ph	1	0.052	-38*	-	-	-

[a] Analytical results are within ± 0.4% of the theoretical values. [b] ACAT inhibition in vitro, liver microsomes isolated from cholesterol-fed rats. Each determination performed in triplicate. See reference 9 for complete protocol. [c] Denotes percent change in total cholesterol in cholic acid (0.5%)-cholesterol (1.5%)-peanut oil (5.5%)-fed rats administered a single dose of test compound . See reference 9 for the complete protocol. [d] Denotes percent change in total cholesterol using the diet as in footnote *c*, dosing over a period of 7 days. See reference 9 for the complete protocol. [e] Denotes the compound was tested at 30 mg/kg. * Denotes significantly different from control, $p < 0.05$ using analysis of variance followed by Fisher's multiple range test.

Due to the excellent activity of **8b**, we resolved the racemic mixture into its individual enantiomers. The *in vitro* evaluation of these isomers suggested that **(+)-8b**, may stereoselectively inhibit ACAT, since it is nearly twice as potent as the racemate and greater than 20 fold more potent than **(-)-8b** (14 nM vs 330 nM). It is noted that the 2% of **(+)-8b** present in **(-)-8b**, could account for its residual potency (IC_{50}= 330 nM). This stereoselectivity is in agreement with a communication recently published by McCarthy et al., demonstrating that (*S*)-*N*-(2,4-bis(methylthio)-methylpyridin-3-yl)-2-(hexylthio)-decanoic acid amide is 7-fold more potent than the corresponding *R*-stereoisomer (IC_{50}= 22 and 160 nM respectively).[10] Although **(+)-8b** is the preferred stereoisomer *in vitro*, it is equiefficacious with **(-)-8b** and the racemate when administered in a single dose to cholesterol-fed rats. Based on these results, we assumed the asymmetric center epimerizes *in vivo*. However, the enantiomers could be differentiated in the chronic in vivo assay. In this assay, **(-)-8b** was shown to be inactive at 10 and 3 mg/kg whereas

(+)-8b lowers plasma total cholesterol by 60 and 55% respectively. The racemate was also less effective than **(+)-8b** in this study.

In summary, we have identified 2-phenyl-2-(dodecyl-2H-tetrazol-5-yl)acetic acid as a fatty acid mimetic, that when coupled with 2,6-diisopropyl- and 2,4,6-trimethoxyaniline, yields novel bioisosteric replacements for **1** that are extremely potent *in vitro* and efficacious *in vivo*. Of the compounds prepared, **8b**, is significantly more potent and efficacious than the fatty acid amides previously reported,[2] including CI-976. We have demonstrated that the majority of the ACAT inhibition observed for **8b** resides in the (+)-enantiomer, **(+)-8b**, since the (-)-enantiomer **(-)-8b**, was significantly less potent *in vitro* and ineffective when administered to hypercholesterolemic rats. Further extensions of this study will be the topic of future communications from this laboratory.

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4. The isomeric mixture was evaluated by ^{1}HNMR prior to chromatography. Assignments were made based on the chemical shift of the methylene protons attached to the tetrazole ring (see: Scott, F. L.; Tobin, J. C. *J. Chem. Soc. C* **1971**, 703).
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7. The enantiomeric purity was assessed by ^{1}HNMR using Mosher's acid choride. Both **(-)-13** and **(+)-13** were considered isomerically pure by this method.
8. The enantiomeric excess was determined using a Chiracel OG 10 micron (4.6 x 250mm) column with a mobile phase of 80:20 hexane:IPA and a flow rate of 1ml/min. Retention time for **8b-(-)** is 15.2 min and **8b-(+)** is 17.8 min.
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