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Inhibitors of Acyl-CoA:Cholesterol *O*-Acyltransferase (ACAT) as Hypocholesterolemic Agents 14. Synthesis and Structure-Activity Relationships of a Novel Series of Sulfonamide Tetrazoles.

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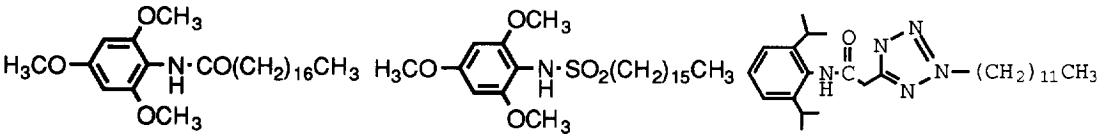
Abstract:

The syntheses and biological activities of a series of novel sulfonamide tetrazole derivatives are reported. The ability of these compounds to inhibit ACAT is described. Such agents may decrease the absorption of dietary cholesterol in the intestine and/or the secretion of VLDL by the liver and therefore provide a therapy for the treatment of hypercholesterolemia and atherosclerosis in man.

Introduction: Hypercholesterolemia is a well-known risk factor for the development of coronary heart disease.[1] As an approach for potential therapy, the inhibition of cholesterol absorption has generated considerable interest. Acyl-CoA:cholesterol acyltransferase (ACAT), an intracellular enzyme that catalyzes the reaction between cholesterol and CoA-activated fatty acids to form cholesteryl esters in all cells, is an attractive target for such an approach.[2] In the intestine ACAT is believed to play an important role in the absorption of dietary cholesterol.[3] In addition, liver ACAT is implicated in the secretion of hepatic very low density lipoprotein (VLDL), the precursor of the atherogenic low density lipoprotein (LDL) particle.[4] Its activity in the macrophages of the artery wall results in the accumulation of cholesteryl ester in arterial lesions.[5] It has been demonstrated that various inhibitors of ACAT can block the intestinal absorption of cholesterol in experimental animals, resulting in reduced total plasma cholesterol concentrations.[2] Furthermore, systemically available ACAT inhibitors, such as CI-976, have been shown to induce the regression of atherosclerotic lesions in cholesterol-fed rabbits.[6] Some of these agents have been evaluated clinically, however, efficacy in humans has not yet been demonstrated.[7]

We have previously reported that the biosteric replacement of the amide group in a long chain alkylamide by a sulfonamide group results in a decrease in *in vitro* potency.[8] For example, **1** was shown to be a potent inhibitor of ACAT (IC_{50} = 0.052 μM), while the closely related sulfonamide **2** was an order of magnitude less potent (IC_{50} = 0.69 μM). We have also recently disclosed a series of tetrazole amides which were potent inhibitors of ACAT (e.g. **3**, IC_{50} = 0.0026 μM),[9] and it became the focus of our research efforts to study the



1 2 3

corresponding sulfonamides. Accordingly, we prepared a series of sulfonamide tetrazoles (**12a-f**, **13a-b**, **16a-b** and **17**) and evaluated their ability to inhibit the enzyme ACAT.

Chemistry: The synthetic route for compounds represented by **12** and **13** is shown in Scheme **I**. The route for compounds **16** and **17** is shown in Scheme **II**.

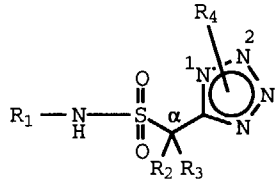
Esterification of sulfoacetic acid **4** in refluxing ethanol gave ethyl ester **5** (100%). Which was converted to the sulfonyl chloride **6** with $POCl_3$ at 125°C. Compound **6** was then treated with an aniline (trisubstituted or disubstituted) in the presence of Et_3N in THF to give the sulfonamide **7** (44%), which was hydrolyzed using KOH in aqueous ethanol and then reacted with $(COCl)_2$ in toluene to produce **8** (>95%). Treatment with gaseous ammonia gave amide **9** (100%),[10] which was dehydrated with tosyl chloride in pyridine to generate the key intermediate sulfonamidoacetonitrile **10** (57%). Cyclization with Bu_3SnN_3 in refluxing dioxane, followed by treatment with aqueous HCl gave tetrazole **11** (57%),[11] which was alkylated with different alkyl halides in refluxing CH_3CN, in the presence of Et_3N, to give predominately regioisomer **12** (45%) and a small amount of **13** (5%), which were readily separated by silica gel chromatography.

In order to alkylate **12** at the α-methylene position, it was necessary to protect sulfonamide nitrogen with a methoxymethylene (MOM) protecting group (Scheme **II**). The MOM protected sulfonamide tetrazole **14** (79%) was then treated with NaH and an alkyl halide to give a monoalkylated compound **15a** (96%). Similarly, a second equivalent of base may then be added, followed by an appropriate alkyl halide to give disubstituted analogs **15** (98%). Finally, the protecting group was removed by BBr_3 to give **16** (or **17**, 48%).

Biological Methods: ACAT inhibition *in vitro* was determined by incubation with [1-^{14}C]oleoyl-CoA and intestinal microsomes isolated from cholesterol-fed rabbits.[12] Results are reported as the micromolar concentration of the drug required to inhibit the enzymatic activity by 50% (IC_{50}). *In vivo* activity was assessed in rats by giving a single dose of compound in an aqueous suspension (1.5% carboxymethylcellulose, CMC/0.2% Tween 20) vehicle, at a dose of 30mg/kg and the rats were then fed a high fat , cholesterol-rich diet (5.5% peanut oil, 1.5% cholesterol and 0.5% cholic acid) overnight. Plasma cholesterol levels were measured and the results were expressed as the percent change from control animals given vehicle and diet only.

Result and Discussion: Data for the *in vitro* potency and *in vivo* efficacy of the sulfonamide tetrazole ACAT inhibitors are shown in Table **1**. Based on previous results.[8,12,13,14] we incorporated both the 2,6-diisopropylphenyl and 2,4,6-trimethoxyphenyl aniline moieties into the compounds to obtain optimal ACAT inhibitory activity. The data revealed that compound **12b** was 8 times more potent than compound **12c** (IC_{50} =

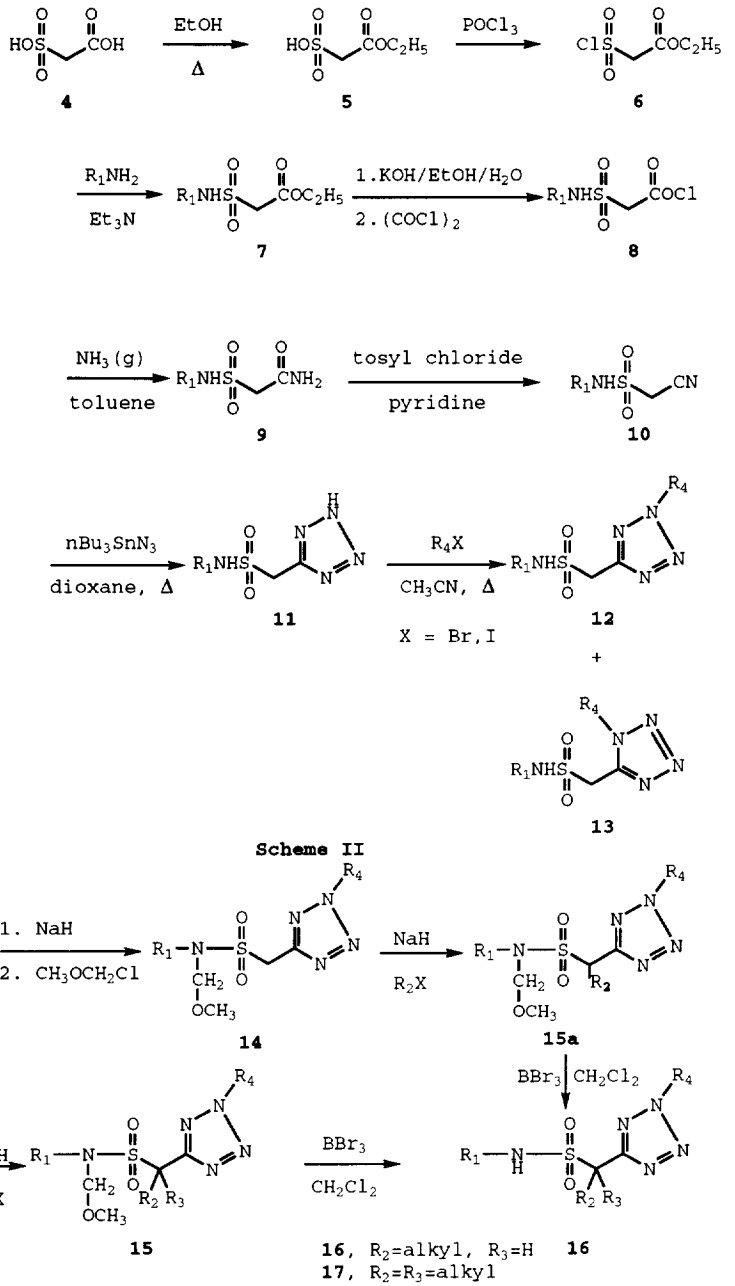
Table 1



cmpd	R_1	R_2	R_3	R_4	IC_{50} (μM)	%Δ TC
1					0.052	-11
2					0.69	c
3					0.0026	-64***
12a	2,6-di-i-Pr-Ph	H	H	2-$(CH_2)_7CH_3$	0.75	-14
12b	2,6-di-i-Pr-Ph	H	H	2-$(CH_2)_{11}CH_3$	0.022	-67***
13a	2,6-di-i-Pr-Ph	H	H	1-$(CH_2)_{11}CH_3$	1.2	-41*
12c	2,4,6-tri-OMe-Ph	H	H	2-$(CH_2)_{11}CH_3$	0.18	-60***
13b	2,4,6-tri-OMe-Ph	H	H	1-$(CH_2)_{11}CH_3$	3.7	-30*
12d	2,6-di-i-Pr-Ph	H	H	2-$(CH_2)_{13}CH_3$	0.15	-42*
12e	2,6-di-i-Pr-Ph	H	H	2-$(CH_2)_{15}CH_3$	0.6	-20
12f	2,6-di-i-Pr-Ph	H	H	2-$(CH_2)_{17}CH_3$	7.6	-31*
17	2,6-di-i-Pr-Ph	CH_3	CH_3	2-$(CH_2)_{11}CH_3$	0.04	-53***
16a	2,6-di-i-Pr-Ph	CH_3	H	2-$(CH_2)_{11}CH_3$	0.032	-67***
16b	2,6-di-i-Pr-Ph	CH_2Ph	H	2-$(CH_2)_{11}CH_3$	0.057	-27*

Statistical difference from control values: * p<.05; ** p<.01; *** p<.001. At 3 mg/kg the % ΔTC values for 1, 2, 3 and 12b are -31**, -31**, -62***, and -59** respectively. c Not tested.

Scheme I



Scheme II

16, R_2=alkyl, R_3=H **16**
17, R_2=R_3=alkyl

0.022μM vs IC_{50} = 0.18μM), but there was no significant difference in *in vivo* activity at the administered dose (ΔTC = -67% vs -60%). The data also showed that the length of the hydrocarbon chain on the tetrazole is a important factor for ACAT activity. It was demonstrated that 12 carbons were required for optimum activity; an increase or decrease in the chain length diminished the activity both *in vitro* and *in vivo*. When the chain length was extended to 18 carbons (**12f**), there was a significant loss in activity (IC_{50} = 7.6μM, ΔTC = -31%). When the chain length was reduced to 8 carbons as in compound **12a**, the efficacy was greatly reduced (IC_{50} = 0.75μM, ΔTC = -14%). The optimal position for the alkyl group on the tetrazole ring was shown to be at the N-2 position. Thus, compound **12b** was significantly more potent than compound **13a** *in vitro* (IC_{50} = 0.022μM vs 1.2μM) and more efficacious in *in vivo* (ΔTC = -67% vs -41%). The α-substituted compounds **16a**, **16b** and the α,α–disubstituted compound **17** were about 2 fold less potent *in vitro* (IC_{50} = 0.04, 0.032, 0.057μM, respectively) compared to **12b** (IC_{50} = 0.022μM). However, while those with the small substituents (**16a** and **17**) maintained good in *in vivo* efficacy (ΔTC = -53%, -67%, vs -61% (**12b**)), the benzyl analog (**16b**) showed a significant loss in *in vivo* activity (ΔTC = -27%).

In conclusion, the use of sulfonamide as a isosteric replacement of the amide group in the series of tetrazole amides results in comparable *in vivo* efficacy but lower *in vitro* potencies. Thus the best compound in the present series, **12b**, show similar *in vivo* efficacy as compared with the amide analog (**3**, ΔTC = -64%), although it is less potent *in vitro* .[9] This trend in *in vitro* potency is also found in the fatty acid anilide series, (e.g. **1**) when compared with its sulfonamide isostere (**2**). Within the present series, the position and the length of the alkyl substituents on the tetrazole ring had a marked effect on ACAT inhibitory activity. Small substituents in the α position apparently had a minor influence on activity except when the α substituent was a benzyl group, and a loss of efficacy was noted. Compound **12b**, showed good *in vitro* potency and *in vivo* efficacy and has prompted further pharmacological investigation.

References:

1. a. McCarthy, P. A. *Med. Res. Rev.* **1993**,*13*, 139.
   b. Davies, M. J.; Woolf, N. *Br. Heart J.* **1993**, 69 (Supplement): S3.
   c. Cobbe, S. M.; Shepherd, J. *ibid,* S63.
2. a. O'Brien, P. M.; Sliskovic, D. R. *Current Opinion in Therapeutic Patents*, **1992**, *2*, 507.
   b. Sliskovic, D. R.; White, A. D. *Trends. Pharmacol. Sci.* **1991**,*12*, 194.
3. Grundy, S. M.; Metzger, A. L. *Gastroenterology,* **1972**, *62*, 1200
4. Krause, B. R.; Anderson, M.; Bisgaier, C. L.; Bocan, T.; Bousley, R.; DeHart, P.; Essenburg, A.; Hamelehle, K.; Homan, R.; Kieft, K.; McNally, W.; Stanfield, R.; Newton, R. S. *J. Lipid Res.* **1993**, *34*, 279.
5. Brown, M. S.; Goldstein, J. L. *Ann. Rev. Biochem.* **1983**, *52*, 223.
6. Bocan, T. M. A.; Mueller, S. B.; Uhlendorf, P. D.; Newton, R. S.; Krause, B. R. *Arteriosclerosis and*

Thrombosis, **1991**, *11*, 1830.

7. Harris, W. S.; Dujovne, C. A.; von Bergmann, K.; Neal, J.; Akester, J.; Windsor, S. L.; Greene, D.; Look, Z. *Clin. Pharmacol. Ther.* **1990**, *48*, 189.
8. Roark, W. H.; Roth, B. D.; Holmes, A.; Trivedi, B. K.; Kieft, K. A.; Essenburg, A. D.; Krause, B. R.; Stanfield, R. L. *J. Med. Chem.* **1993**, *36*, 1662.
9. O'Brien, P. M.; Picard, J. P.; Purchase, C. F.; Both, B. D.; Sliskovic, D. R.; White, A. D. Eur. Patent 92 917 230.2-, 1994.
10. a. Beck, M. L. *J. Org. Chem.* **1968**, *33*, 897.
    b. Hinman, R. L.; Locatell, L. Jr. *J. Am. Chem. Soc.* **1959**, *81*, 5655.
11. Bernstein, P. R.; Vacek, E. P. *Synthesis*, **1987**, 1133.
12. Roth, B. D.; Blankley, C. J.; Hoefle, M. L.; Holmes, A.; Roark, W. H.; Trivedi, B. K.; Essenburg, A. D.; Kieft, K. A.; Krause, B. R.; Stanfield, R. L. *J. Med. Chem.* **1992**, *35*, 1609.
13. a. Trivedi, B. K.; Stoeber, T. L.; Stanfield, R. L.; Essenburg, A. D.; Hamelehle, K. L.; Krause, B. R. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 259.
    b. Trivedi, B. K.; Holmes, A.; Stoeber, T. L.; Blankley, C. J.; Roark, W. H.; Picard, J. A.; Shaw, M. K.; Essenburg, A. D.; Stanfield, R. L.; Krause, B. R. *J. Med. Chem.* **1993**, *36*, 3300.
14. a. Augelli-Szafran, C. E.; Blankley, C. J.; Roth, B. D.; Trivedi, B. K.; Bousley, R. F.; Essenburg, A. D.; Hamelehle, K. L.; Krause, B. R.; Stanfield, R. L. *J. Med. Chem.* **1993**, *36*, 2943.
    b. Roark, W. H.; Minton, L. L.; Bousley, R. F.; Stanfield, R. L. Abstracts of Papers, American Chemical Society, 204th National ACS Meeting, Washington D.C., **1992**, MEDI 37.
    c. Sliskovic, D. R.; Picard, J. A.; Roark, W. H.; Essenburg, A. D.; Krause, B. R.; Minton, L. L.; Stanfield, R. L. Abstracts of Papers, American Chemical Society, 204th National ACS Meeting, Washington D.C., **1992**, MEDI 38.

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