Bioorganic & Medicinal Chemistry Letters, Vol.2, No.1, pp. 99-104, 1992
Printed in Great Britain

0960-894X/92 $3.00 + .00
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HMG-CoA REDUCTASE INHIBITORS 4. TETRAZOLE SERIES: CONFORMATIONAL CONSTRAINTS AND STRUCTURAL REQUIREMENTS AT THE HYDROPHOBIC DOMAIN.

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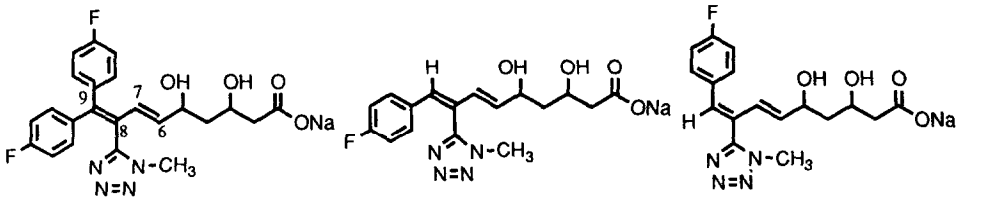
(*Received* 16 *October* 1991)

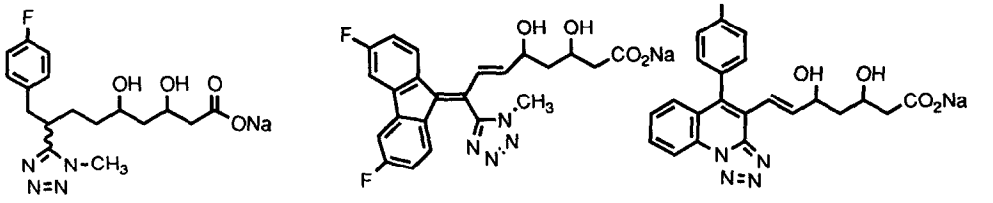
ABSTRACT: Synthetic analogs **2-5** were prepared and the inhibitory activity was evaluated in order to assess the conformational constraints and the minimal structural requirements associated with the hydrophobic terminus of these tetrazole-derived dihydroxy acids for optimum potency.

The concept of enzyme inhibitors as therapeutic agents for the intervention of diseases is becoming increasingly important in recent years. The enzyme 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA) is the key regulatory enzyme in the biosynthesis of cholesterol and is, therefore, a prime target for therapeutic intervention in atherosclerosis[1]. Natural products like Mevinolin and Pravastatin, known inhibitors of HMG-CoA reductase, have been approved as drugs for heart disease. In this context, we[2] and others[3] have been interested in developing synthetic inhibitors of this enzyme. Earlier, we reported the synthesis and biological activity of **1** (BMY 21950) and the SAR related to the C-8 substituents[2]. Compound **1** is currently in clinical trials. In this letter, we address the issue of minimal structural requirements and conformational constraints associated with it at the hydrophobic terminus for high potency. The following compounds (**2-5**; Scheme I) were prepared and evaluated for their activity against the enzyme HMG-CoA reductase.

Scheme I



1 (BMY 21950) 2a 2b



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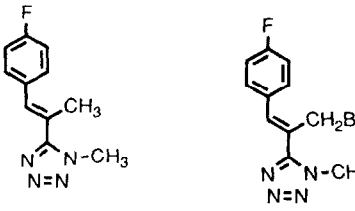
This paper is dedicated to Prof. Norman A. LeBel on the occasion of his 60th birthday.

The synthesis of the cis and trans tetrazole compounds **2a** and **2b** began with the addition of the anion generated from 5-ethyl-1-methyltetrazole (n-BuLi/THF/-78°C) to 4-fluorobenzaldehyde followed by dehydration (conc.H_2SO_4) to provide the olefin **6** as colorless crystals (80%; mp 59-61°C). Allylic bromination of **6** (NBS/AIBN/CCl_4) afforded the bromide **7** (49%; 89-91°C). Oxidation of **7** with 2-nitropropane in EtOH/EtONa furnished the aldehyde **8** as the major isomer[4] (89%). Double bond homologation with triphenyl phosphorylidine acetaldehyde (OHC-HC=PPh_3/benzene reflux) gave the aldehyde **9** in 51% yield. Aldol condensation with ethyl acetoacetate dianion yielded the hydroxy keto ester **10** as a gum (39%). Stereoselective reduction[5] (BEt_3/THF/$NaBH_4$/-78°C/MeOH) of **10** gave the erythro diol **11** as the major diastereomer. Base hydrolysis gave the sodium salt **2a**.



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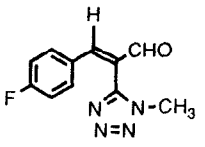

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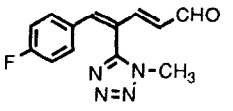

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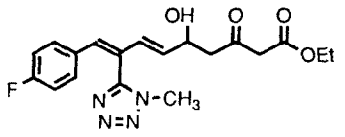

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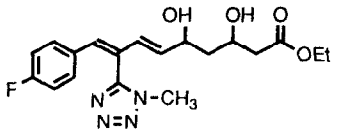

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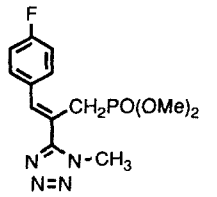

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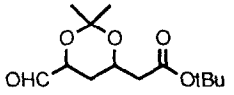

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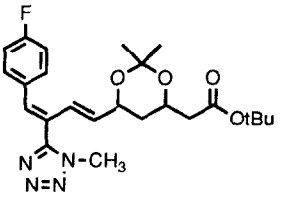

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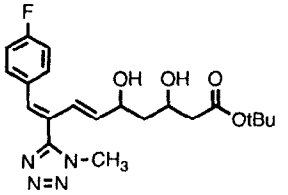

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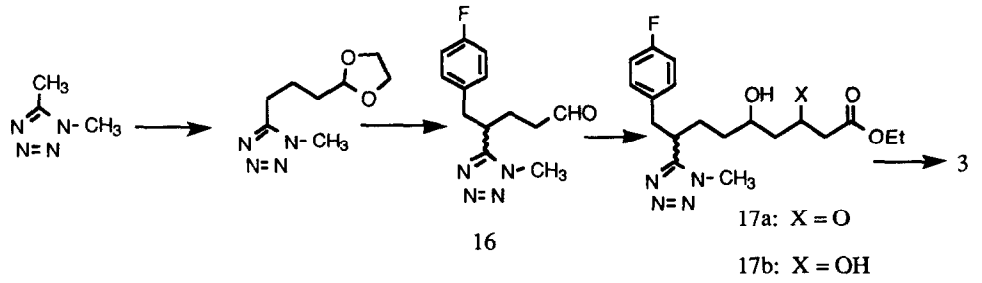
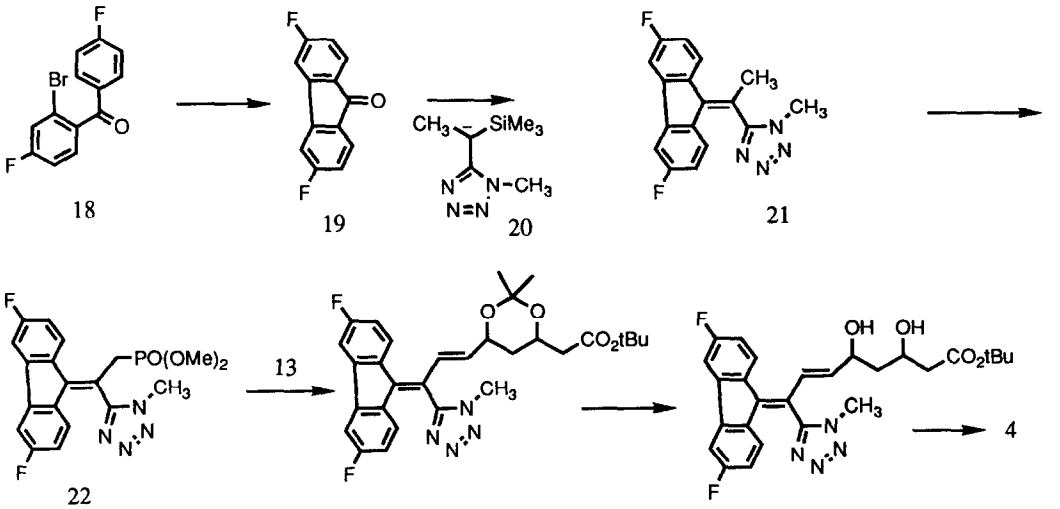
Bromide **7** served as the common intermediate for the synthesis of **2a** and **2b**. Thus, phosphonate **12** was prepared from **7** under standard Arbuzov conditions [$P(OMe)_3$/100°C/2h] in almost quantitative yield. Coupling of the phosphonate **12** with the aldehyde **13**[6] (n-BuLi/THF/-78°C) proceeded smoothly to give the diene ester **14** in 72% yield as a foam. Deprotection of the acetonide moiety under acidic conditions

(pTSA/MeOH) gave the erythro diol **15**. Hydrolysis and lactonization produced the sodium salt **2b** and the corresponding lactone.



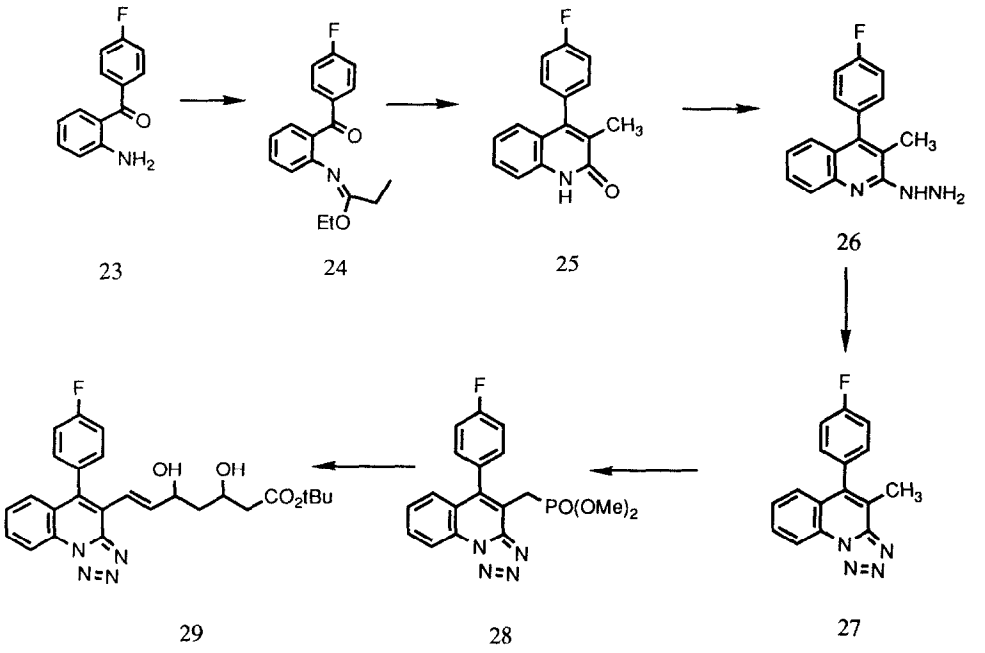
Sequential alkylation of 1,5 dimethyltetrazole with the bromopropionaldehyde acetal and p-fluorobenzyl bromide furnished the aldehyde **16** after hydrolysis in 39% overall yield. Addition of ethyl acetoacetate dianion to **16** gave the inseparable diasteromers of the keto-ester **17a.** Stereoselective reduction of the hydroxy-keto ester as described earlier gave the dihydroxy ester **17b** as the major isomer (erythro diol) after chromatographic separation. This was hydrolized to the sodium salt **3** (55%) and evaluated for the enzyme inhibition as a diastereoisomeric mixture.

Scheme II



The synthesis of fused ring analog **4** is outlined in Scheme II. Friedel-Crafts acylation[7a] of 3-bromofluorobenzene with 4-fluorobenzoyl chloride afforded the known benzophenone **18**. Palladium catalyzed coupling[7b] of the two aryl rings was achieved using $Pd(OAc)_2$ in dimethylacetamide to give **19** (81%), which reacted with the tetrazolyl anion **20** to furnish the olefin **21** via the Peterson olefination (42%; mp 231-232°C). Phosphonate **22**, obtained from a bromination / Arbuzov sequence (49%; mp 210-211°C), was coupled with the six carbon synthon **13** to give the sodium salt **4** (overall 37%) after functional group manipulation. Scheme III shows the synthesis of the fused tetrazoloquinoline analog **5**. The aminobenzophenone **23** was heated with triethyl orthopropionate to give the intermediate imine **24**, which on treatment with Na_2CO_3/DMF afforded the pyridone **25** (30%; mp 250-251°C) after hydrolysis[7c]. Conversion to the hydrazine **26** (79%; mp 207-208°C) was readily achieved by sequential chlorination, followed by heating with hydrazine. Cyclization[7d] to the tetrazolo[1,5-a]quinoline **27** occurred spontaneously on diazotization of **26** with $NaNO_2$/AcOH. Following standard procedures, sodium salt **5** was prepared via the intermediate phosphonate **28**.

Scheme III



Our synthetic analogs and their IC_{50} values against the enzyme HMG-CoA reductase are summarized in Table I. The participation of "hydrophobic anchors" in the binding of several inhibitors has been well demonstrated in the literature[8]. Thus, compounds **2a** and **2b** were designed to explore the minimal structural

requirements for high affinity interaction at the hydrophobic binding site. The inhibitory activities of these compounds were surprisingly low given the high activity of **1**. This low activity may be explained by insufficient hydrophobic interaction to better stabilize the enzyme-inhibitor complex. Compounds **3, 4** and **5** were designed to probe the tolerance of the enzyme for the conformational constraints posed on the inhibitor **1**. Not surprisingly, the conformationally flexible analog **3**[9] displayed poor inhibitory activity. Similarly, when the bis-aryl moiety or the aryl-tetrazole portion were locked into a fixed conformation, a drop in the inhibitory potencies was also observed. This may be due to the apparent specificity requirement for binding at the hydrophobic domain . This is consistent with the proposal of Abeles et.al. that the high affinity for inhibitors like **1** is due to simultaneous interaction at two separate binding areas: the mevalonate domain at the active site and the hydrophobic domain outside the active site[10].

Table I

Compound[a]	IC_{50} (μM)[b]	relative potency[c]
1	0.043	1
2a	210	2.04×10^{-4}
2b	>250	$<1.72 \times 10^{-4}$
3	>250	$<1.72 \times 10^{-4}$
4	4.1	1.0×10^{-2}
5	4.6	9.3×10^{-3}

[a] All compounds tested were racemic. [b] Standard rat liver microsomal HMG-CoA reductase inhibition assay; see ref.3. [c] Value relative to compound 1.

In conclusion, our data demonstrate that the conformational relationship of the bis-aryl and the tetrazole moieties is an important component of the inhibitor binding to the enzyme. Further, the minimum structural requirements at the hydrophobic terminus in this nonadienoic acid series for high affinity binding with the enzyme HMG-CoA reductase include the C9- bis aryl substituents and the C8-1N-methyl tetrazole group, held in appropriate spatial orientation relative to one another. This data also suggest that the specificity requirement for interaction with the hydrophobic domain is equally important to that of the active site region.

Acknowledgement: We thank Charles M. Combs and Randy D. Rutkowske of the Analytical Department, Bristol-Myers Squibb for their assistance with the NMR experiments.

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Catt, J. D.; Brown, P. J.; Harte, W. E.; Thompson, M. D.; Wright, J. J. *J. Med. Chem.* **1990**, *33*, 2982.

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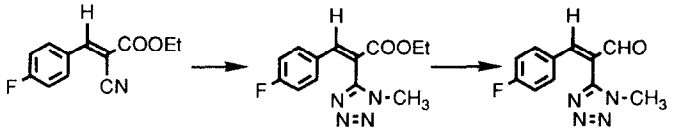
4. The stereochemical assignments were determined by NOESY experiments on a 300 MHz Varian spectrometer; also by independent synthesis from the known cyano ester



5. Stereospecific synthesis of the dihydroxy side chain has been a subject of several publications, for example, Rosen, T.; Heathcock, C. H. *Tetrahedron* **1986**, *42*, 4909 and references therein. In most cases, we obtained atleast a ratio of 93: 7 of syn : anti diol. The major diasteromer was separated by flash chromatography.

6. For the preparation of this aldehyde, see ref.2. Wittig-Horner type coupling of **13** with the phosphonates **12**, **22**, **28** invariably produced the ***E***-olefin as the only isolable product. Vinyl-and aryl-stabilized phosphonate reagents of this type are known in theliterature and generally show robust ***E*** selectivity. For a review see Maryanoff, B. ; Reitz, M. *Chemical Reviews* **1989**, *89*,908.

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9. All compounds gave satisfactory IR, NMR, MS ,elemental analysis and/or high resolution mass spectra.

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