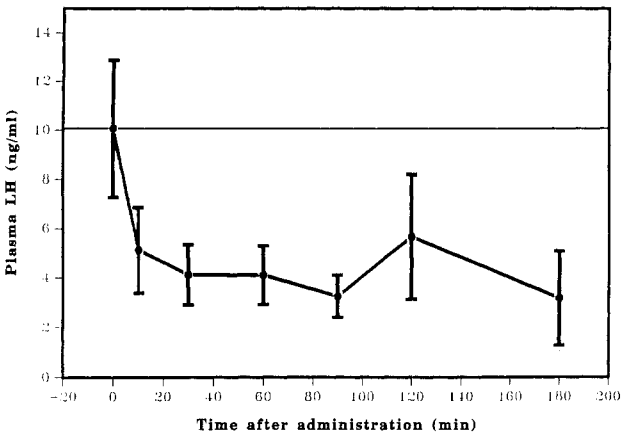
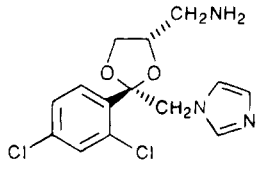


Figure 2. Three male Sprague–Dawley rats (approximately 300-g body weight) that were castrated 4 weeks before experimentation were treated with ketoconazole (100 mg/kg in 1 mL/kg 20% propylene glycol in 0.9% NaCl solution), administered as a single bolus dose by the intraduodenal route. Blood samples (0.5 mL) were obtained under light ether anesthesia by jugular venipuncture before dosing and at 10, 30, 60, 90, 120, and 180 min. Plasma luteinizing hormone (LH) concentration was determined by radioimmunoassay. Immunoaffinity-purified anti-rat-LH IgG was obtained from Dr. P. M. Conn of the University of Iowa. The standard curve was constructed with the national standard rat LH preparation (NIH-RP2) obtained from A. Parlow. 45 μL of rat plasma sample was diluted to 100 μL with 1% normal rabbit serum in Dulbeccos phosphate buffered saline (PBS; pH 7.4) and combined with 100 μL of [^{125}I]-rat-LH (Chemicon; 20 000 cpm) and 100 μL of antibody. The mixture was incubated for 24 h at 4 °C. Goat-antirabbit antibody (50 μL) was added to the mixture, and after 4 h at 4 °C, antibody-bound [^{125}I]LH was precipitated by centrifugation and counted by γ spectroscopy. Each sample was assayed in triplicate. The sensitivity of the assay was 50 pg per tube (1.1 ng/mL of plasma).

without further separation. In all cases, only L-amino acids were used.



11

Pharmacology

The affinities of **1–10** were tested in a rat pituitary LH-RH receptor competitive binding assay,[14] using [^{125}I]leuprolide as the radioligand. Ketoconazole (**8**) possessed micromolar affinity for LH-RH receptors. The interaction was competitive, because increasing concentrations of [^{125}I]leuprolide from 15 pM to 1.5 nM tested in the absence or presence of 3.16, 10, and 31.6 μM **8** demonstrated that only the affinity of radioligand but not the apparent binding site density was affected by **8** (Figure 1). Interstingly enough, itraconazole (**7**), a close relative of ketoconazole, failed to show any significant affinity even at a concentration as high as 30 μM. This may be due to the fact that the piperazine side chain of ketoconazole occupies a specific area with strict structural requirements while binding to the LH-RH receptor. A requirement for specific features of the ketoconazole side chain was further demonstrated by the lack of binding by analogues **1–5**. Compounds **6**, **9**, and **10**, which possess structural similarities to the amino terminal pyroGlu-His region of LH-RH, also interacted with the LH-RH receptor. Analogues **8** and **10** antagonized the leuprolide-induced release of LH from dispersed pituitary cells in culture,[15] with antagonist potencies (pA_2s; -log M) of 4.19 and 5.34, respectively. In castrated (30 days before the experiment began) male rats (n = 3, control LH 10.07 ng/mL, Figure 2), 100 mg/kg of **8** given intraduodenally produced a maximum 68% decrease in plasma LH concentration, sustained throughout a 6-h experimental period. Bhasin et al.[10] conducted a similar set of experiments where two groups of castrated male Sprague–Dawley rats were treated with 25 mg of ketoconazole in oil or oil alone by im injection every 8 h for 3 days. Plasma samples were collected at the end of a 3-day study and no significant change in either LH or FSH levels were seen between the groups. As stated earlier, our protocol was different in the sense that we used a 100 mg/kg bolus dose and sequentially sampled blood immediately after administration.

In summary, we found ketoconazole (**8**) to be an antagonist of LH-RH receptors both in vitro and in vivo, with micromolar affinity. We have also uncovered a novel series of peptidomimetics having submicromolar affinities.

(14) Marian, J.; Cooper, R. L.; Conn, P. M. *Mol. Pharmacol.* **1981**, *19*, 399.

(15) Jinnah, H. A.; Conn, P. M. *Endocrinology* **1986**, *118*, 2599.

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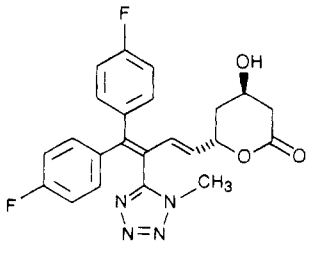
A Potent, Tissue-Selective, Synthetic Inhibitor of HMG-CoA Reductase

Sir:

Hypercholesterolemia (elevated levels of serum cholesterol) is a primary risk factor for coronary artery disease and atherosclerosis, which are major causes of death in western countries.[1] The enzyme 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase) is the key regulatory enzyme in the biosynthesis of cholesterol and is, therefore, a prime target for therapeutic intervention in atherosclerosis.[2] Fungal metabolites, compactin and mevinolin, and synthetic analogues have been shown to be potent inhibitors of this enzyme.[3,4] Herein, we report a novel, tissue-selective, synthetic inhibitor of HMG-CoA reductase, **1** (BMY 22089).



1

As part of our synthetic effort, we have designed and prepared compounds having a substituted butadiene unit

(1) (a) Kannel, W. B.; Castelli, W. P.; Gordon, T.; McNamara, P. M. *Ann. Intern. Med.* **1971**, *74*, 1. (b) Endo, A. *J. Med. Chem.* **1985**, *28*, 401 and references therein.
(2) Brown, M. S.; Goldstein, J. L. *Science* **1986**, *232*, 34.

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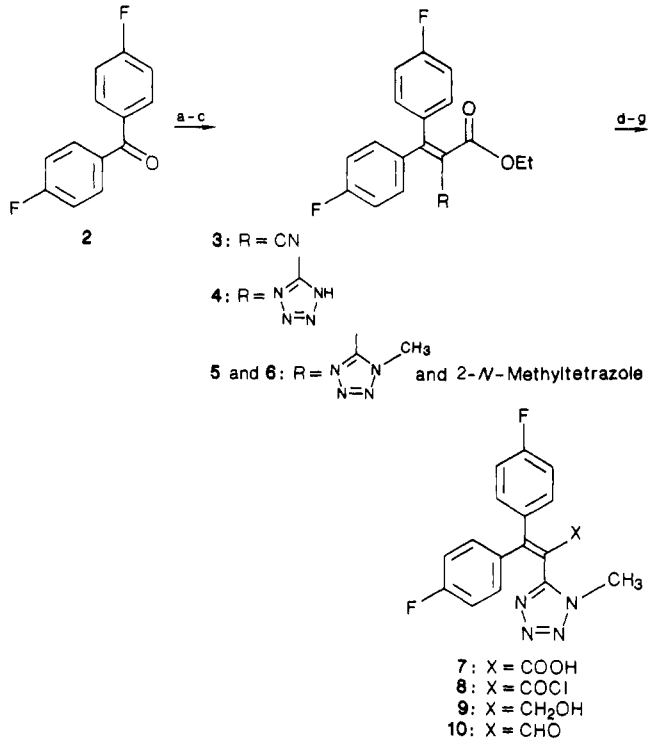
Scheme I[a]



[a] (a) Ethyl cyanoacetate, β-alanine/azeotropic distillation in $HOAc/C_6H_6$. (b) Tri-*n*-butyltin azide, neat/110–120 °C. (c) MeI/NaH/THF followed by fractional crystallization. (d) $LiOH/H_2O$ followed by HCl. (e) Oxalyl chloride/CH_2Cl_2. (f) $LiAlH_4$ in THF/−78 °C. (g) PCC, CH_2Cl_2.

connected to a strategically positioned 4-hydroxy-2*H*-pyran-2-one moiety. At the initial phase of our program, structural comparison of known inhibitors[3g-k] indicated that the substituents at the position corresponding to C8 of the nonadienoic acid were primarily hydrophobic. However, the compound having a cyano substitution[5] at that carbon did show activity against the enzyme (IC_{50} = 8 μM), indicating that hydrophilic substitution (log P_{CN} = −1.27) is also tolerated. Also, consideration of molecular models of mevinolin and compactin suggested that an increase in steric bulk at that position would be desireable. These considerations led us to the choice of alkylated tetrazoles as target structures. In this preliminary report, we would like to disclose that one of these compounds, **1**, is a promising drug candidate for further development. Compound **1** was originally synthesized by derivatizing aldehyde intermediate **10**, which was readily available by the sequence of transformations depicted in Scheme I.

Cyanoester **3** was prepared (56%, mp 114–6 °C) by a classical Knoevenagel condensation. The dipolar cycloaddition to the cyano functionality of **3** was carried out as described in the literature[6] to give quantitative yields of **4** after acidic workup. The free tetrazole derivative **4** was alkylated (MeI/NaH/THF) to furnish a 2:1 mixture (separable by fractional crystallization) of 1-methyl- and 2-methyltetrazole isomers (**5** and **6**, respectively). The pure 1-methyl isomer (52%, mp 144–5 °C) was converted (four steps) into the key aldehyde **10** in 74% overall yield (mp 141–2 °C). This crucial aldehyde (**10**) could also be synthesized by a number of other routes (Scheme II). For instance, addition of the anion generated from 1,5-dimethyltetrazole[7] (*n*-BuLi/THF/−78 °C) to 4,4′-difluorobenzophenone followed by dehydration of the resulting carbinol (PTSA/toluene/reflux) provided olefin **11** as colorless crystals (75%; mp 169–71 °C). Deprotonation of **11** (*n*-BuLi/THF/−78 °C) afforded a dark red anion which reacted smoothly with ethyl formate to furnish aldehyde **10** (70%; mp 135–7 °C). However, this formylation was not reliable on a large scale. More notably, the use of 5-ethyl-1-methyltetrazole[7] in the above sequence gave the corresponding olefin **12** (58%, mp 146–7 °C). Allylic bromination of **12** (NBS/AIBN/CCl_4/reflux/2 h) afforded **13** (93%, mp 159–60 °C) and subsequent oxidation using 2-nitropropane in EtOH/EtONa furnished aldehyde **10** in 91% yield.[8] Chain extension with (triphenylphosphoranylidene) acetaldehyde provided homologated aldehyde **14** (85%, mp 164–5 °C). Alternatively, aldehyde **14** can also be obtained directly from olefin **11** (65%) by reaction with 3-methoxyacrolein. Aldol condensation with acetoacetic ester dianion yielded hydroxy keto ester **15** (72%). Stereoselective reduction (BEt_3/THF/$NaBH_4$/−78 °C/MeOH)[9] gave *erythro*-dihydroxy ester **16** (75%), which was hydrolyzed (aqueous NaOH/EtOH) and lactonized to provide lactone **1** (89%, mp 146–8 °C) (Scheme II).

Bromide **13** served as a common intermediate for both the linear and convergent syntheses. Thus, phosphonate **18** was prepared from **13** under standard Arbuzov condi-

(3) (a) Lee, T. J. *Trends Pharm. Sci.* **1987**, *8*, 442 and references therein. (b) Brown, A. G.; Smale, T. C.; King, T. J.; Hasenkamp, R.; Thompson, R. H. *J. Chem. Soc., Perkin Trans. 1* **1976**, 1165. (c) Endo, A.; Kuroda, M.; Tsujita, Y. *J. Antibiot.* **1976**, *29*, 1346. (d) Alberts, A. W.; Chen, J.; Kuron, G.; Hunt, V.; Hoffman, C.; Rothrock, J.; Lopez, M.; Joshua, H.; Harris, E.; Patchett, A.; Monaghan, R.; Currie, S.; Stapley, E.; Alberschonberg, G.; Hensens, O.; Hirshfield, J.; Hoogsteen, K.; Liesch, J.; Springer, J. *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 3959. (e) Endo, A. *J. Antibiot.* **1979**, *32*, 852. (f) Boader, E.; Baitmann, W.; Beck, G.; Bergmann, A.; Jendialla, H.; Kessler, K.; Wess, G.; Schnbert, W.; Granzer, E.; Kerekjarto, B. V.; Krause, R. *Tetrahedron Lett.* **1988**, *29*(8), 929. (g) Hoffman, W. F.; Alberts, A. W.; Anderson, P. S.; Chen, J. S.; Smith, R. L.; Willard, A. K. *J. Med. Chem.* **1986**, *29*, 849. (h) Stokker, G. E.; Alberts, A. W.; Anderson, P. S.; Cragoe, E. J., Jr.; Deana, A. A.; Bilfillan, J. L.; Hirshfield, J.; Holtz, W. J.; Hoffman, W. F.; Huff, J. W.; Novello, F. C.; Prugh, J. D.; Rooney, C. S.; Smith, R. L.; Willard, A. K. *J. Med. Chem.* **1986**, *29*, 170. (i) Stokker, G. E.; Hoffman, W. F.; Alberts, A. W.; Cragoe, E. J., Jr.; Deana, A. A.; Gilfillan, J. L.; Huff, J. W.; Novello, F. C.; Prugh, J. D.; Smith, R. L.; Willard, A. K. *J. Med. Chem.* **1985**, *28*, 347. (j) Kathawala, F. G. World Patent WO 84/02131. (k) Endo, A. *J. Med. Chem.* **1985**, *28*, 401.

(4) (a) For a recent synthesis of compactin and mevinolin, see: Clive, D. L. J.; Keshava Murthy, K. S.; Wee, A. G. H.; Siva Prasad, J.; da Silva, G. V. J.; Majewski, M.; Anderson, P. C.; Haugen, R. D.; Heerze, L. D. *J. Am. Chem. Soc.* **1988**, *110*, 6914 and references therein. (b) For a review, see: Rosen, T.; Heathcock, C. H. *Tetrahedron* **1986**, *42*, 4909.

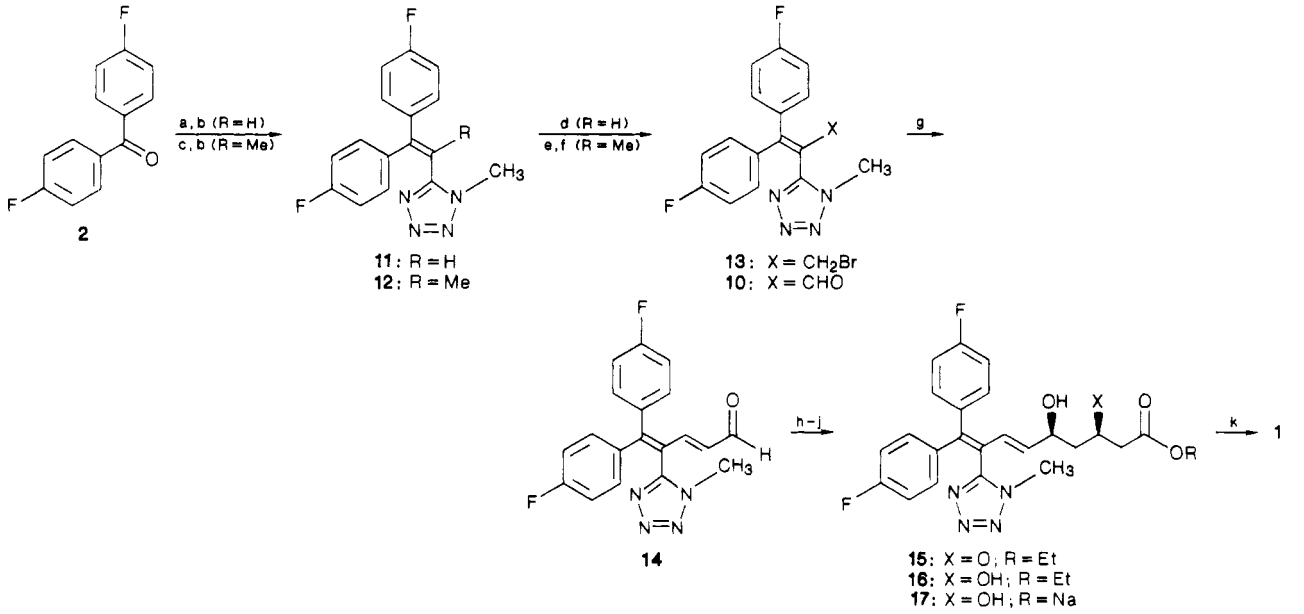
(5) (a) Sit, S. Y.; Parker, R. A.; Brown, P. J.; Balasubramanian, N.; Catt, J. D.; Harte, W. E.; Motoc, L.; Wright, J. J. Presented at the 196th National Meeting of the American Chemical Society, Los Angeles, CA, September 26–30, 1988; paper MEDI 108. (b) Balasubramanian, N.; Brown, P. J.; Catt, J. D.; Han, W. T.; Harte, W. E.; Sit, S. Y.; Wright, J. J. Presented at the 196th National Meeting of the American Chemical Society, Los Angeles, CA, September 26–30, 1988; paper MEDI 109. (c) Parker, R. A.; Sit, S. Y.; Wright, J. J.; Clark, R. W.; Grosso, R. A.; Keely, S. L.; Antonaccio, M. J. *Arteriosclerosis* **1988**, *8*, 634a.

(6) Sisido, K.; Nabiko, K.; Isida, T. *Organomet. Chem.* **1971**, *33*, 337.

(7) 1,5-Dimethyltetrazole **29** is purchased from commercial sources. 5-Ethyl-1-methyltetrazole was prepared from **29** in one step (LDA/−78 °C/THF/CH_3I); on a larger scale a small amount of dialkylation product was observed.

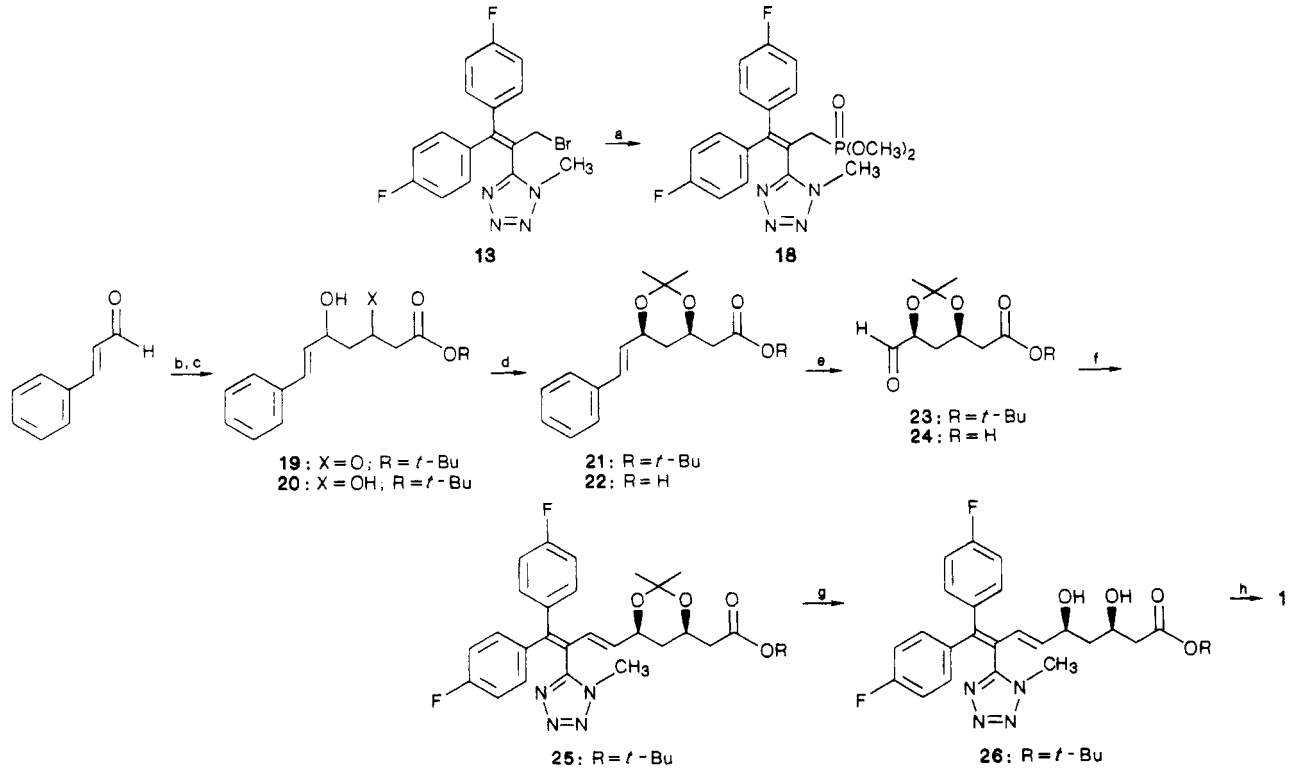
(8) (a) Direct allylic oxidation of ($CH_3 \rightarrow CHO$) olefin **12** was always accompanied with unreacted starting material or other unwanted side products. (b) Hass, H. B.; Bender, M. L. *Organic Syntheses*; Wiley: New York, 1963; Collect. Vol. IV, p 932. Large-scale synthesis of **10** from **29** was developed by the Chemical Process Development Group, Bristol Myers, Evansville, IN.

Scheme II[a]



[a] (a) 1,5-Dimethyltetrazole/*n*-BuLi/THF/−78 °C. (b) PTSA/toluene/reflux. (c) 5-Ethyl-1-methyltetrazole/*n*-BuLi/THF/−78 °C. (d) *n*-BuLi/THF/−78 °C, 40 min, ethyl formate. (e) NBS/AIBN/CCl_4/reflux/2 h. (f) 2-Nitropropane/NaOEt/EtOH/2 h. (g) Ph_3P=CHCHO/benzene/reflux. (h) Ethyl acetoacetate/NaH/*n*-BuLi/THF/−20 °C. (i) BEt_3/$NaBH_4$/THF, MeOH/−78 °C. (j) NaOH/EtOH. (k) (i) HCl, (ii) 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-*p*-toluenesulfonate/CH_2Cl_2.

Scheme III[a]



[a] (a) $P(OMe)_3$, 100 °C, 5 min. (b) *tert*-Butyl acetoacetate/NaH, *n*-BuLi/THF/−20 °C. (c) BEt_3/$NaBH_4$/−78 °C/MeOH, THF. (d) $Me_2C(OMe)_2$, H^+. (e) O_3/CH_2Cl_2/−78 °C, Me_2S. (f) **18**/*n*-BuLi/THF/−78 °C to room temperature. (g) PTSA/MeOH. (h) (i) NaOH, (ii) 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-*p*-toluenesulfonate/CH_2Cl_2.

tions [$P(OMe)_3$/100 °C/3 min] in almost quantitative yield (mp 140–41 °C). Protected dihydroxy aldehyde **23**[9] was obtained from cinnamaldehyde following a four-step sequence: (1) addition of the dianion of acetoacetic ester, (2) stereoselective reduction[9] followed by (3) protection of the diol as the acetonide, and (4) ozonolysis of the double bond (Scheme III). Coupling of phosphonate **18** and aldehyde **23** (*n*-BuLi/THF/−78 °C) proceeded smoothly to give diene ester **25** in 70% yield (mp 159–60 °C). Deprotection of the acetonide (PTSA/MeOH) gave *erythro*-diol **26** (98%) and hydrolysis/lactonization as described above provided racemic lactone **1**.

Resolution of acid **22** into its 3*R*,5*S* and 3*S*,5*R* enantiomers using (1*S*,2*R*)-ephedrine and (2*S*,1*R*)-ephedrine followed by ozonolysis afforded the corresponding optically pure aldehydes **24**. The absolute configuration of one of the resolved acids [from (1*S*,2*R*)-ephedrine] was determined by the conversion into a known lactone, **28**.[10]

Table I.[a] Ratio of IC_{50} (for Sterol-Synthesis Inhibition) for Various Rat Tissues Relative to Hepatocytes[12]

cell type		BMY 22089 (1) (salt form) (*n*)		mevinolin (salt form) (*n*)
hepatocytes	(IC_{50} = 21 nM)	1 (7)	(IC_{50} = 39 nM)	1 (6)
spleen		149 (5)		1.2 (5)
testes		86 (5)		1.3 (5)
adrenal		76 (1)		1 (1)

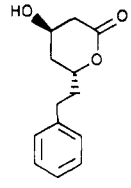
[a] Isolated cell dispersions were preincubated with inhibitors for 15 min followed by cholesterol synthesis assay by [^{14}C]acetate incorporation for 60 min. The table gives the ratio of the mean IC_{50} (nM) for sterol-synthesis inhibition for each tissue relative to hepatocyte expressed as mean values from *n* replicate determinations. For the biological assay/test procedures and preliminary in vivo data, see: (a) reference 3d. (b) Reference 5c. (c) Parker, R. A.; Sit, S. Y.; Clark, R. W.; Grosso, R. A.; Wright, J. J.; Keely, S. L. *FASEB J.* **1989**, *3A*, 644. (d) Clark, R. W.; Grosso, R. A.; Parker, R. A. *FASEB J.* **1989**, *3A*, 953.

(1*S*,2*R*)-Ephedrine salt of **22** [41%, mp 170–171 °C, $[\alpha]^{25}_D$ +5.5° (*c* = 1, $CHCl_3$)] was hydrogenated [100%, H_2(1 atm)/Pd/C/EtOAc] and acidified to give free saturated acid **27**, which was lactonized by TFA to give lactone **28** [69%, $[\alpha]^{24}_D$ +24° (*c* = 0.07 M, $CHCl_3$) (lit.[10] $[\alpha]^{24}_D$ +48.8° (*c* = 0.20 M, $CHCl_3$))]. In this manner, the synthesis of both enantiomers of **1** was realized by Wittig-type coupling of the lithium salts of **24** with phosphonate **18**.[11]

Racemic lactone **1** inhibits HMG-CoA reductase noncompetitively with respect to the cofactor, NADPH, and competitively with respect to the substrate, HMG-CoA. Under conditions in which K_m for s-HMG-CoA was 28 ± 3.1 μM, the open chain salt form of **1** inhibited with K_i = 8.6 nM.[12] The salt form inhibited rat liver microsomal HMG-CoA reductase activity in vitro with IC_{50} = 43 ± 2.5 nM. The inhibitory activity is inherent in the 4*R*,6*S* enantiomer of **1** (IC_{50} = 19 nM) as the other enantiomer is nearly devoid of activity.

While suppression of sterol synthesis outside of the liver contributes little to plasma cholesterol lowering, perturbation of nonhepatic isoprenoid metabolism may lead to undesirable consequences. Examination of the tissue specificity of HMG-CoA reductase inhibitors may be a valuable criterion for long-term safety in the pharmacological profile of this important class of lipid-lowering agents. As shown in Table I, compound **1** shows remarkable selectivity for hepatic cells in vitro, unlike mevinolin. The clinical significance of this finding remains to be established.[13]

The synthesis of analogues of **1**, associated structure–activity relationships, and molecular modeling studies will be the subject of future publications.



28

(9) (i) Synthon for the lactone portion: Roth, B. D.; Roark, W. H. *Tetrahedron Lett.* **1988**, *29*, 1255 and references therein. (ii) Reduction of β-hydroxy ketones: (a) Narasaka, K.; Pai, F. C. *Tetrahedron* **1984**, *40*, 2233. (b) Kathawala, F. G.; Prager, B.; Prasad, K.; Repic, O.; Shapiro, M. J.; Stabler, R. S.; Widler, L. *Helv. Chim. Acta* **1986**, *69*, 803. (c) Chen, K.; Hartmann, G.; Prasad, K.; Repic, O.; Shapiro, M. J. *Tetrahedron Lett.* **1987**, 155. In general we obtained a 95:5 syn/anti mixture from these reactions. The unwanted isomer is separated by chromatography or crystallization in this and subsequent steps.

(10) Majewski, M.; Clive, D. L. J.; Anderson, P. *Tetrahedron Lett.* **1984**, *25* (*20*), 2102.

(11) 4*R*,6*S* isomer (+1): mp 172–3 °C, $[\alpha]^{25}_D$ +239.8° (*c* = 1.08, $CHCl_3$). 4*S*,6*R* isomer (−1): mp 160–2 °C, $[\alpha]^{25}_D$ −242.6° (*c* = 1.32, $CHCl_3$).

(12) Sterol synthesis assayed by [^{14}C]acetate and [^{3}H]water incorporation into cholesterol in rat hepatocytes. IC_{50} for the racemic, Na salt of BMY 22089 (**1**) = 21 nM; that for mevinolin Na salt = 39 nM. The complete experimental details and pharmacological evaluation of BMY 22089 will be reported elsewhere.

(13) (a) Tsujita, Y.; Kuroda, M.; Shimada, Y.; Tanzawa, K.; Arai, M.; Taneko, I.; Tanaka, M.; Masuda, H.; Tarumi, C.; Watanabe, Y.; Fujii, S. *Biochem. Biophys. Acta* **1986**, *877*, 50. (b) Germershausen, J. I.; Hunt, V. M.; Bostedor, R. G.; Bailey, P. J.; Karas, J. D.; Alberts, A. W. *Biochem. Biophys. Res. Commun.* **1989**, *158*, 667.

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