Bioorganic & Medicinal Chemistry Letters, Vol.3, No.4, pp. 595-600, 1993
Printed in Great Britain

0960-894X/93 $6.00 + .00

AROMATIC ISOSTERES AS CONFORMATIONAL PROBES FOR AN ISOPRENYL SUBUNIT: APPLICATION TO INHIBITORS OF SQUALENE SYNTHASE

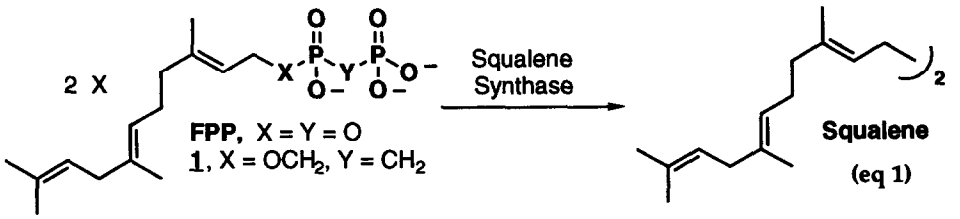
Scott A. Biller*, Jeffrey W. Abt, Andrew T. Pudzianowski, Lois C. Rich, Dorothy A. Slusarchyk and Carl P. Ciosek, Jr.

Bristol-Myers Squibb Pharmaceutical Research Institute
P. O. Box 4000, Princeton, New Jersey 08543

(*Received in USA* 21 *December* 1992)

Abstract. A series of aromatic isosteres of the farnesyl chain of potent squalene synthase inhibitor **1** were prepared and evaluated. The results are consistent with the local conformation indicated in structure **2**.

Squalene synthase catalyzes the conversion of farnesyl diphosphate (**FPP**) to squalene in the first committed step of cholesterol biosynthesis.[1] Due to its unique position in the sterol pathway, inhibition of squalene synthase may have advantages over interfering elsewhere in the pathway for the development of novel antihypercholesterolemic agents. We recently described the discovery of ether **1** (**eq 1**), the first potent inhibitor of squalene synthase (K_i = 37 nM against the rat liver microsomal enzyme).[2,3] We proposed that the large enhancement in enzyme-inhibitor binding energy observed for ether **1** relative to the substrate **FPP** (K_m = 12,700 nM) is a result of strong interaction(s) between the ether oxygen and the catalytic group(s) which promote the solvolysis of **FPP** to an allyl cation - inorganic diphosphate ion pair.[2] *A priori*, the farnesyl subunit of **1** is expected to possess a large degree of conformational freedom, some of which must be lost upon binding to the enzyme. This report describes our preliminary efforts to define the bound conformation of the isoprenyl subunit of these inhibitors through the synthesis and evaluation of conformationally restricted isosteres.



Examination of the crystal stucture of squalene[4] obtained at -110 °C reveals a repeating motif for each five carbon isoprene subunit. The most striking feature of the solid state conformation is the eclipsing of the allylic C-C bond on the disubstituted olefin terminus with the C=C bond, as indicated

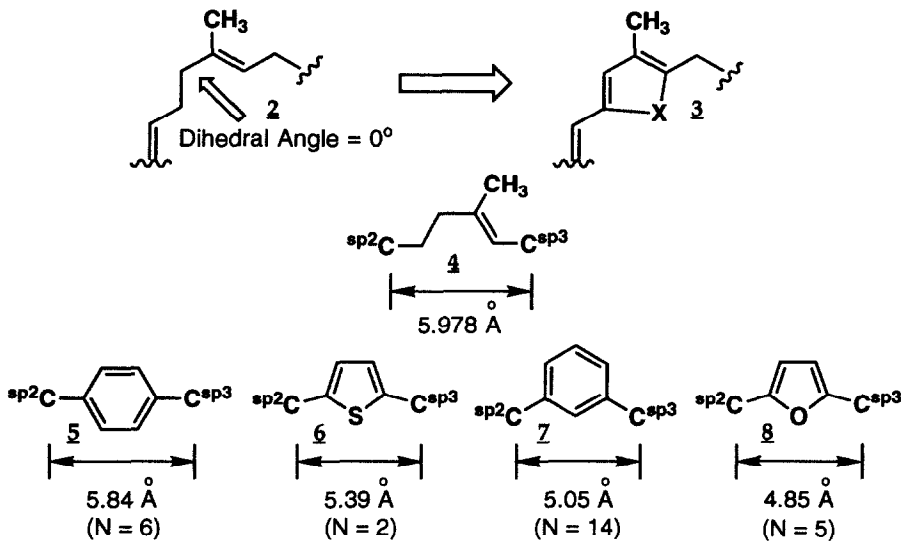
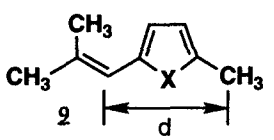


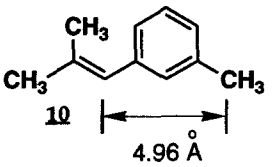
Figure 1. Conformationally Restricted Aromatic Templates: The indicated bridging distances are derived from crystal structures from the Cambridge Structural Database[7] for the aromatics (N = number of structures averaged), and from the crystal structure of squalene[4] for the isoprene.

in **2** (**Figure 1**). Experimental[5] and molecular modeling[6] studies on related olefinic systems suggest that this is a low energy conformation. To probe the acceptability of this local conformation in the context of the ether inhibitors, we proposed to enforce conformation **2** in a series of aromatic isosteres **3**. The bridging distance of 5.978 Å in **4**, derived from the squalene crystal structure,[4] was utilized as a yardstick for the fit of the aromatic templates with the natural isoprene. As illustrated in **Figure 1**, the degree of fit decreases as one proceeds through the series from **5** to **8**. The bridging distances for the substructures **9** and **10** (**Table 1**) obtained from PM3 calculations[8] are consistent with those in **Figure 1** derived from crystal structures.[7]

Table 1. Bridging Distance as Estimated from PM3 Calculations[8]



X	d (Å)
HC=CH	5.74
S	5.34
O	4.83



Aromatic isosteres 11 - 13 and 15 - 17 were synthesized *(vide infra)* and evaluated as inhibitors of rat liver microsomal squalene synthase[9] in comparison to the acyclic parents 1 and 14 (**Figure 2**). In the initial series (11, 12 & 13), which all contain the natural "vinylic" methyl group, there is both a lack of correlation between the inhibitory activity and the bridging distance, and a profound loss of activity relative to the acyclic parent. In contrast, the des-methyl series (15, 16 & 17) does exhibit a correlation between inhibitory potency and the bridging distance. The most potent aromatic isostere, the para-disubstituted phenyl analog 15, possesses similar activity to the acyclic analogs 1 and 14. The discrepancy remains, however, between the methyl and des-methyl series: inhibitors 14 and 15 are equipotent whereas 11 is 250-fold less potent than 1. Our working hypothesis rests on the fact that the para-phenylene substructure is axially symmetric and bridges the required distance whether or not the olefinic plane of 1/14 and the aromatic plane of 11/15 bind in a coincident manner. The structure of the active site may force the aromatic ring to rotate about this axis as a result of steric interactions between the indicated carbons (•) and the enzyme. In the case of 11, this would cause the methyl group to rotate out of its binding pocket, perhaps into a sterically costly environment (**Figure 3**).

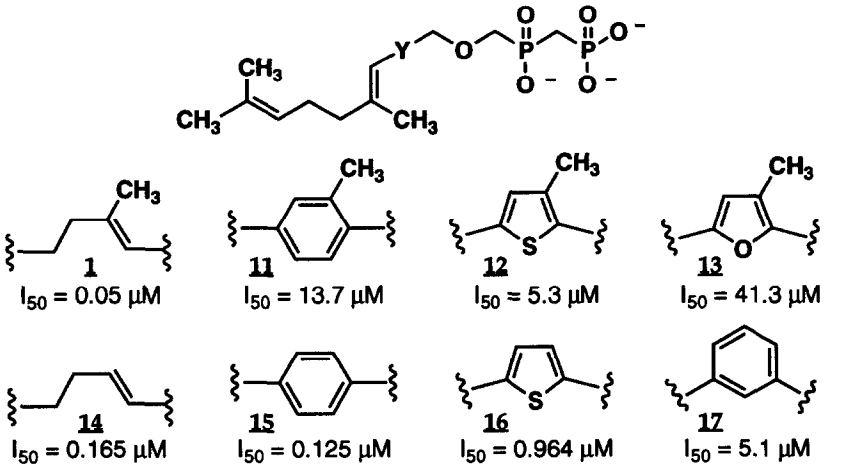


Figure 2. Inhibition of Rat Liver Microsomal Squalene Synthase

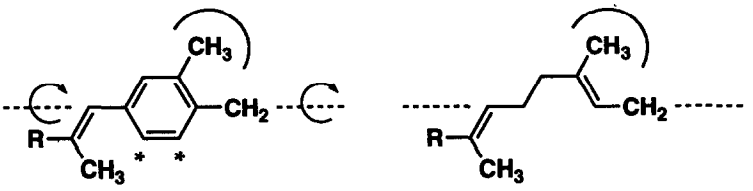


Figure 3.

The synthesis of the aromatic isosteres capitalizes on the palladium-catalyzed cross-coupling[10] of the vinyl zinc derived from iodide **18**[11] with a series of iodoesters **19a-e.**[12] This procedure[13] affords **20a-e** in a highly stereoselective manner[14] (**Scheme I**, **Table 2**). For the preparation of inhibitor **12**, the coupling was performed with iodothiophene **22**[15] already containing the ether linked phosphonate to afford **23f** in 71 % yield (**eq 2**). After reduction of esters **20a-e** (LAH, ether, 0 °C, > 90 %) to the corresponding modified farnesols **21a-e**, the syntheses of the inhibitors were completed utilizing modifications of our published methodology.[2,3,16,17] The corresponding lithium alkoxides (n-BuLi or $LiN(TMS)_2$, THF) were coupled with $CF_3SO_3CH_2PO(O\text{-}iPr)_2$[18] (THF, - 78 to 0 °C, 55-73 %) to provide phosphonates **23a-e**, which, along with **23f**, were hydrolyzed to the corresponding monoacids **24a-f** (**Scheme II**). The phosphonic acids were activated either as acid chlorides (Method A), *p*-nitrophenyl esters (Method B), or acid fluorides (Method C)[19] prior to coupling with $LiCH_2PO_3Me_2$ to afford triesters **26a-f** (37-71 % overall from **23a-f**). Methods B and C were particularly preferred when the intermediates were acid sensitive. Finally, the triesters were deprotected with TMSBr[20] (2,4,6,-collidine, CH_2Cl_2; NaOH, 58-91 %) to provide the tri-sodium salts in **Figure 2**.

Scheme I

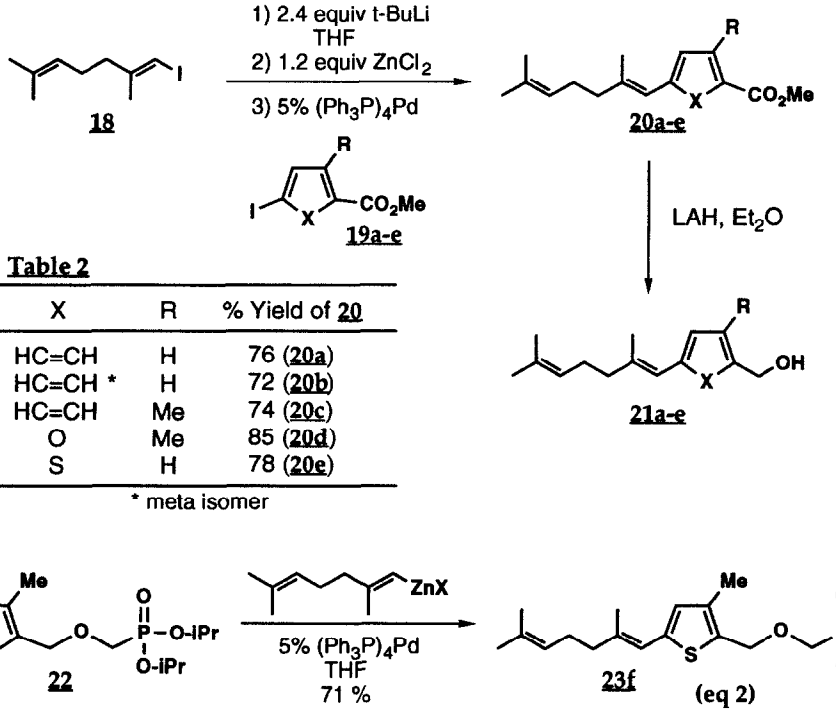
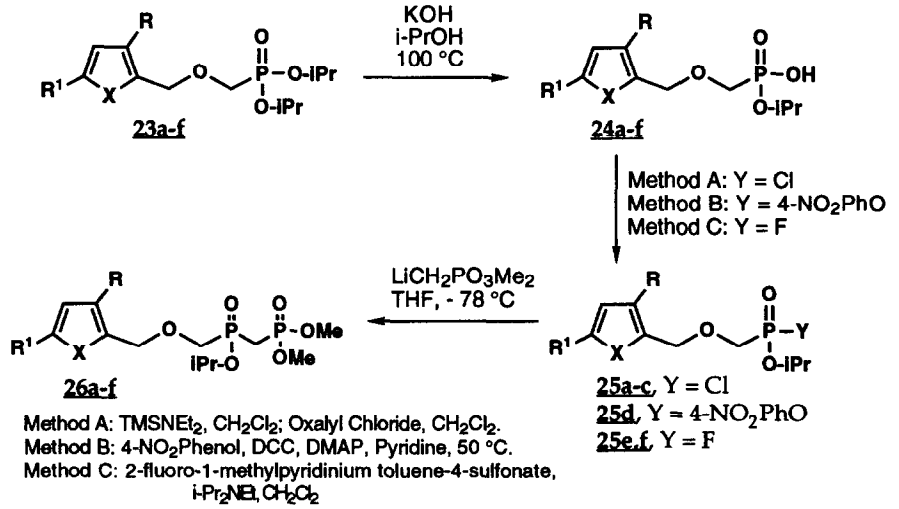


Table 2

X	R	% Yield of **20**
HC=CH	H	76 (**20a**)
HC=CH *	H	72 (**20b**)
HC=CH	Me	74 (**20c**)
O	Me	85 (**20d**)
S	H	78 (**20e**)

* meta isomer

Scheme II



In summary, we have generated a series of aromatic isosteres of the potent squalene synthase inhibitor **1**, in order to probe the local conformation of the isoprene subunit adjacent to the ether function. The results are consistent with conformation **2**, or a closely related conformer, as suggested by experimental and molecular modeling studies, but the aromatic ring may not be able to bind in a manner that is coincident with the plane of the olefin of the acyclic analogs.

Acknowledgements. The authors would like to acknowledge the support and comments of Dr. Eric M. Gordon.

References and Notes

1. Review: Poulter, C. D.; Rilling, H. C. In *Biosynthesis of Isoprenoid Compounds*; Porter, J. W.; Spurgeon, S. L. Eds.; Wiley: NY, 1981; Vol. 1, Chapter 8.
2. Biller, S. A.; Sofia, M. J.; DeLange, B.; Forster, C.; Gordon, E. M.; Harrity, T.; Rich, L. C.; Ciosek, C. P., Jr. *J. Am. Chem. Soc.* **1991**, *113*, 8522-8524.
3. Biller, S. A.; Sofia, M. J.; Abt, J. W.; DeLange, B.; Dickson, J. K., Jr.; Forster, C.; Gordon, E. M.; Harrity, T.; Magnin, D. R.; Marretta, J.; Rich, L. C.; Ciosek, C. P., Jr. "Potent, Rationally Designed Inhibitors of Squalene Synthase" in "Regulation of Isopentenoid Metabolism", Nes, W. D, Parish, E. J. and Trzaskos, J. M. eds., ACS Symposium Series # 497, 1992, Chapter 7, pages 65-80.
4. Ernst, J.; Furhop, J.-H. *Liebigs Ann. Chem.* **1979**, 1635-1642.
5. Shimanouchi, T.; Abe, Y. *J. Polym. Sci.: Part A-2* **1968**, *6*, 1419-1434.
6. Murgolo, N. J.; Patel, A.; Stivala, S. S.; Wong, T. K. *Biochemistry* **1989**, *28*, 253-260. Broecker,

J. L.; Hoffmann, R. W.; Houk, K. N. *J. Am. Chem. Soc.* **1991**, *113*, 5006-5017. Berg, U.; Sandström, J. *Adv. Phys. Org. Chem.* **1989**, *25*, 1-97. Wiberg, K. *Adv. Mol. Model.* **1988**, *1*, 101-134.

7. Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, U.K.

8. PM3: Stewart, J. J. P. *J. Comput. Chem.* **1989**, *10*, 209-220. Computations used version 6 of the *MOPAC* program: Stewart, J. J. P. *QCPE* **455** (1983).

9. Biller, S. A.; Forster, C.; Gordon, E. M.; Harrity, T.; Scott, W. A.; Ciosek, C. P., Jr. *J. Med. Chem.* **1988**, *31*, 1869-1871.

10. Negishi, E. *Acc. Chem. Res.* **1982**, *15*, 340-348. Negishi, E.; Luo, F.-T.; Frisbee, R.; Matsushita, H. *Heterocycles* **1982**, *18*, 117-122. Negishi, E.; Takahashi, T.; Baba, S.; Van Horn, D. E.; Okukado, N. *J. Am. Chem. Soc.* **1987**, *109*, 2393-2401. Kalinin, V. N. *Synthesis* **1992**, 413-432.

11. Negishi, E.; Van Horn; D. E.; Yoshida, T. *J. Am. Chem. Soc.* **1985**, *107*, 6639-6647.

12. Iodide **19c** was prepared according to the published method: Sato, T.; Oki, M.. *Bull. Chem. Soc. Jpn* **1957**, *30*, 958-961. Furan **19d** was synthesized from 3-methylfuran-2-carboxylic acid *via* iodination of the dianion: (a) 2.2 equiv LDA, THF, -78 to 0 °C; 2.2 equiv I_2, 0 °C, 100 %; (b) MeI, K_2CO_3, DMF, 75 % (Knight, D. W.; Nott, A. P. *J. Chem. Soc., Perkin Trans. I* **1981**, 1125-1131). Thiophene **19e** was prepared in a similar manner from thiophen-2-carboxylic acid: (a) 2 equiv n-BuLi, THF, -78 °C; 1 equiv N-iodosuccinimide, 0 °C, 96 %; (b) MeI, K_2CO_3, DMF, 75 % (Knight, D. W.; Nott, A. P. *J. Chem. Soc., Perkin Trans. I* **1983**, 791-794).

13. **Representative Procedure.** Preparation of **20e**: To 20 mL of THF under argon at -78 °C was added 11.0 mL (18.8 mmol) of 1.7 M t-butyllithium in pentane, followed by 1.96 g (7.83 mmol) of **18** in 10 mL of THF added dropwise over 5 min. After 0.5 h at -78 °C and 0.5 h at 0 °C, 1.28 g (9.40 mmol) of zinc chloride (fuse-dried under vacuum) in 25 mL of THF was added to give a pale yellow solution, which was allowed to stir at 0 °C for 1 h. This solution was added *via* cannula to 323 mg (0.28 mmol) of $Pd(Ph_3P)_4$ and 1.5 g (5.59 mmol) of **19e** in 30 mL of THF at 0 °C. After 1 h at RT, the mixture was diluted with ether, washed with 1N HCl, water, saturated $NaHCO_3$ and brine, dried ($MgSO_4$) and evaporated to give 2.03 g of orange oil with solids. Chromatography on 200 g of silica gel eluted with 1:1 hexane/toluene provided 1.15 g (78%) of **20e** as a clear colorless oil.

14. Nuclear-Overhauser enhancement studies confirmed the stereochemistry of **20a**.

15. The litium alkoxide from 5-iodo-3-methylthiophen-2-methanol (Klaus, M. J.; Pawson, B. A. U. S. Patent 4,256,878) was coupled with $CF_3SO_3CH_2PO(O\text{-}iPr)_2$[18] (THF, - 78 to 0 °C, 73 %) to provide iodophosphonate **22**.

16. Details for the preparation of **1** can be found in the supplementary material to reference 2.

17. Biller, S. A.; Forster, C. *Tetrahedron* **1990**, *46*, 6645-6658.

18. Phillion, D. P.; Andrew, S. S. *Tetrahedron Lett.* **1986**, *27*, 1477-1480.

19. Godfrey, J; Mueller, R. H., unpublished results. Watanabe, Y.; Hyodo, N.; Ozaki, S. *Tetrahedron Lett.* **1988**, *29*, 5763-5764.

20. McKenna, C. E.; Higa, M. T.; Cheung, N. H.; McKenna, M. C. *Tetrahedron Lett.* **1977**, 155-158.