Phenoxypropylamines: A New Series of Squalene Synthase Inhibitors

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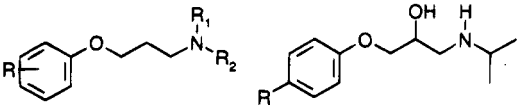
The lowering of plasma cholesterol levels in patients with coronary heart disease has been known[1] for some time to be beneficial. Recently, however, an unequivocal improvement in the survival time of patients with existing coronary heart disease has been demonstrated[2] in the 4S-Scandinavian Simvastatin Survival Study (where cholesterol lowering was achieved with an HMGCoA reductase inhibitor). This new development adds impetus to the search for novel agents with an even greater hypocholesterolaemic effect that might afford further improvements in the treatment of coronary disease. In this respect the potential advantages of a drug which interrupts cholesterol biosynthesis at the squalene synthase (SQS) step have been vigorously pursued, leading to the identification[3] of potent inhibitors *in vitro*. To date the most effective inhibitors of SQS *in vivo* are the polyanionic natural products[4] (termed squalestatins or zaragozic acids) and the substituted bisphosphonates.[5] Both of these inhibitor series have been reported[6] to have poor oral bioavailability in animals or to display potentially toxic actions. Thus alternative series of inhibitors have been sought, and we report our discovery that substituted phenoxypropylamines (PPAs, **1**) represent a new series of orally active SQS inhibitors.

In seeking a novel series of SQS inhibitors with good oral bioavailability, reduced toxic actions, and improved pharmaceutical stability, we sought to avoid polyanionic compounds, phosphorus compounds that might affect calcium metabolism, and heavily unsaturated substituents such as farnesyl groups. This aim was initially pursued by examining the Zeneca compound collection in a rat liver microsomal SQS assay[7] and the β-adrenergic receptor blocking drug, metoprolol **2** was identified as a weak (IC_{50} 14 μM) inhibitor. Further screening of known[8] analogues of **2** that retained the phenoxypropanolamine fragment, but had a range of different 4-alkyl or 4-alkyloxy substituents, revealed the more potent inhibitor **3** (IC_{50} 2 μM). It was realized that the observed enzyme inhibition found for **2** and **3** might have been a consequence of the nonspecific membrane-stabilizing actions known[9] to be a feature of certain β-blocking drugs, rather than being a genuine enzyme inhibition. This possibility was rejected however, because the more lipophilic and more potent membrane stabilising[9] β-blocking drug propranolol **4** did not inhibit SQS at a concentration of 25 μM in the microsomal assay. (This lack of activity for **4** is discussed below.)



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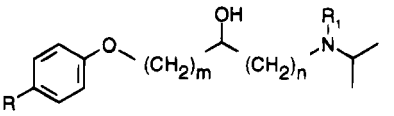
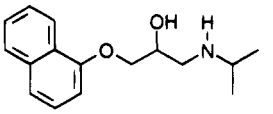
2 R = $MeOCH_2CH_2$
3 R = BuO



3a R = ^{n}BuO, m = 2, n = 1, R_1 = H
3b R = ^{n}BuO, m = 1, n = 2, R_1 = H
3c R = ^{n}BuO, m = 1, n = 1, R_1 = Me
3d R = ^{n}HeptO, m = 1, n = 1, R_1 = H



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Thus the β-receptor blocking agent **3** was established as the initial lead compound, and compounds of greater potency were subsequently sought by systematic variation of the constituent groups of **3** to determine the key structural requirements for inhibitory activity. Almost all of these close synthetic analogues of **3** had significantly reduced enzyme inhibitory activity (IC_{50} > 25 μM). Thus SQS inhibition was diminished by modifications such as lengthening the alkanolamine carbon chain by placing a methylene group on either side of the CHOH moiety (**3a**, **3b**); increasing or decreasing the bulk of the *N*-alkyl group with mono- or dialkylation (e.g. **3c**); removing the basicity *via* acetylation of the NH group or methylation to the quaternary iodide; and altering the carbon chain length of the 4-alkoxy group (C_1 to C_7, e.g. **3d**) or removing it. By contrast other changes to **3** such as methylation of the OH group (**5**, Table 1), left the inhibitory potency relatively unchanged, and deletion of the OH group to give the substituted phenoxypropylamine (PPA) **6**, led to a 40-fold increase in potency (IC_{50} 56 nM in the rat liver microsomal SQS assay). The potent SQS inhibitor **6** is structurally quite dissimilar to the inhibitors reported above,[3-5] and to the known basic inhibitors; for example, the ammonium-substituted cyclopropyl polyene compounds reported[10] by Poulter: farnesylamines[11] and amidines.[12] All of these basic inhibitors more closely resemble in structure the intermediates in the biosynthetic pathway from farnesyl pyrophosphate to squalene than the PPA series. Work on substituted quinuclidine SQS inhibitors has appeared in numerous patents[13] and recently at a meeting.[14] The new PPA inhibitors are distinguished from the quinuclidine series of inhibitors by having a more flexible link between the aryl ring and the basic center and by being secondary as opposed to rigid tertiary amines.

The above work had revealed that the phenoxypropylamine moiety was optimal for inhibitory activity, and

† Deceased.

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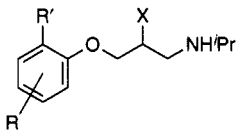
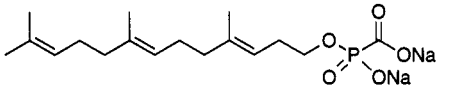
Table 1. Inhibition of Rat Microsomal SQS by Phenoxypropylamines





30 Squibb 32377

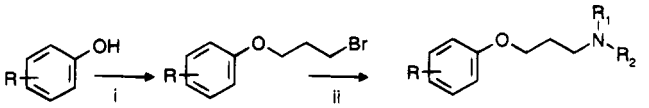
compd	R	R'	X	formula[a]	mp, °C	IC_{50} (μM)
5	4-^{n}BuO	H	OMe	$C_{17}H_{29}NO_3$	135–136[b]	3.3
6	4-^{n}BuO	H	H	$C_{16}H_{27}NO_2$	147–148[c]	0.056
7	4-H	allyl	OH	$C_{15}H_{23}NO_2$[d]	b	4.2
8	4-Ph	H	OH	$C_{18}H_{23}NO_2$[e]	b	3.0
9	4-EtCONH	benzyl	OH	$C_{22}H_{30}NO_3$[f]	–	0.9
10	4-H	benzyl	OH	$C_{19}H_{25}NO_2$[g]	b	8.8
11	4-EtOCO	H	OH	$C_{15}H_{23}NO_4$[h]	b	1.4
12	4-^{n}PrCO	allyl	OH	$C_{19}H_{29}NO_3$	163–165[i]	0.0045
13	4-H	allyl	H	$C_{15}H_{23}NO$	195–196[c]	0.056
14	4-Ph	H	H	$C_{18}H_{23}NO$	120–122[b]	0.17
15	4-EtCONH	benzyl	H	$C_{22}H_{30}N_2O_2$	227–228[b]	0.0006
16	4-H	benzyl	H	$C_{19}H_{25}NO$	136–137[c]	0.012
17	4-EtOCO	H	H	$C_{15}H_{23}NO_3$	151–152[b]	0.003
18	4-^{n}PrCO	allyl	H	$C_{19}H_{20}NO_2 \cdot 0.5H_2O$	114–115[i]	0.001
19	4-EtOCO	allyl	H	$C_{18}H_{27}NO_3$	103–104[b]	0.00005
20	4-^{n}BuO	allyl	H	$C_{19}H_{31}NO_2 \cdot 0.5H_2O$	oil	0.0006
21	4-Ph	allyl	H	$C_{21}H_{27}NO \cdot 0.5H_2O$	179–181[i]	0.000004
22	3-Ph	H	H	$C_{18}H_{23}NO$	147–149[b]	0.6
23	4-(3-pyridyl)	H	H	$C_{17}H_{22}N_2O$	228–231[b]	>2.5
24	4-H	geranyl	H	$C_{22}H_{35}NO$	oil	>2.5
25	4-MeCONH	allyl	H	$C_{17}H_{26}N_2O_2$	150–151[c]	0.065
26	4-MeCONH	H	H	$C_{14}H_{22}N_2O_2$	194–196[b]	>2.5
27	4-EtCONH	allyl	H	$C_{18}H_{28}N_2O_2$	133–135[c]	0.005
28	4-MeC=NOMe	allyl	H	$C_{18}H_{28}N_2O_2 \cdot 2.0H_2O$	126–128[b]	0.0002
29	4-NH_2	allyl	H	$C_{15}H_{24}NO \cdot 0.5H_2O$	150–151[c]	0.25
30	Squibb 32377					30[j]

[a] All new compounds analyzed correctly (±0.4%) for (CHN) and afforded ^{1}H-NMR data consistent with the structures assigned. [b] HCl salt. [c] HBr salt. [d] Brandstrom, A.; Corrodi, H.; Jurggren, U.; Jonson, T. E. *Acta Pharm. Suec.* **1966**, *3*, 303–310. [e] Cox, M. T.; Jaggers, S. E. *J. Med. Chem.* **1978**, *21*, 182–188. [f] Howe, R.; Smith, L. H. U. K. Pat. Appl. 1199037; 1970; *Chem. Abstr.* **1970**, *73*, 109526. [g] Casagrande, C.; Ferrari, G.; Ferrini, R. *Boll. Chim. Farm.* **1968**, *107*, 234–248. [h] Orzalesi, G.; Selleri, R.; Caldini, O.; Innocenti, F.; Magli, M. *Boll. Chim. Farm.* **1975**, *114*, 445–449. [i] Monooxalate salt. [j] Squibb 32377 used as standard SQS inhibitor (lit.[3b] IC_{50} = 9 μM).

attention was turned to optimization of the phenyl ring of **6**. The OH or OMe groups at the 2-position of the propyl chain of the PPAs clearly disrupted binding to the enzyme compared to having no substituent in this position such as in **6**, but notwithstanding this, as a working hypothesis we assumed that the β-blocker inhibitors, e.g. **3** and the PPA analogue **6**, were inhibiting the enzyme in a similar manner. This enabled us to use the large collection of Zeneca β-blocking agents for screening for SQS inhibitory activity, in order to find more favorable phenyl ring substitution patterns that could be incorporated into the PPA series. This testing showed that while most of the β-blocker compounds were inactive (like propranolol **4**), a few (**7**–**12**, Table 1) were found to be inhibitors, although still in the micromolar range (IC_{50} < 10 μM). The inactivity found for **4** at 25 μM as opposed to the weak inhibitory activity of **7** and **10** may relate to the lack in **4** of either the pendant 2-substituent on the phenyl ring (in **7** and **10**) or a 4-substituent (as in **2**).

The PPA analogues **13**–**18** corresponding to these active β-blocker analogues were synthesized uneventfully (Scheme 1) by alkylation of the appropriately substituted phenol with 1,3-dibromopropane and subsequent reaction with an amine in 2-propanol at 70 °C. Other compounds in Table 1 were made in a similar manner from the appropriate phenol, e.g. **25** was prepared from 4-acetamidophenol allyl ether, which was thermally rearranged to give 4-acetamido-2-allylphenol as a startling material. Hydrolysis of **25** with sodium hydroxide solution afforded **29**. The phenol required for the preparation of **24** is known.[15]

Scheme 1[a]



[a] (i) $Br(CH_2)_3Br$, K_2CO_3, butan-2-one, 80 °C; (ii) R_1R_2NH, ^{i}PrOH, 70 °C.

The inhibitory activity of these PPA congeners did indeed parallel that of the SQS-inhibiting β-blocking agents, but to somewhat differing extents (Table 1), possibly because the presence or absence of the OH group would have a differing effect on interaction with the enzyme when in combination with the other binding substituents present.

As the isopropylamino group (with a pK_a of ca. 10.5) of the PPA inhibitors would be protonated at physiological pH, the PPA inhibitors could be acting as carbocation mimics of the postulated cyclopropyl cation intermediate[10] in the rearrangement pathway to squalene and associating with phosphate/pyrophosphate to form an ion pair in a manner similar to that suggested for other basic inhibitors.[10–12] Analysis of how the lipophilic substituents of the phenyl ring of the PPA compounds might interact with the enzyme was based initially on consideration of the range of lipophilic groups in the bisphosphonate[5] and phosphinylmethyl phosphonate[15] series of SQS inhibitors. In this latter

series Biller[16] has shown that a 4-biphenylyl-substituted inhibitor is more potent by a factor of 40 compared to the analogous 3-biphenylyl compound. In the PPA series, however, the difference in inhibitory potency found for the 4-biphenylyl analogue **14** and the corresponding 3-biphenylyl analogue **22** was slight, indicating that both groups may lie in a lipophilic binding region of the enzyme. In addition, consideration of the poor inhibitory potency of 4-(3-pyridyl)phenyl compound **23**, in which the more polar pyridine ring replaces the phenyl ring of **14**, led us to postulate that the 4-phenyl substituent of **14** (and by implication, the lipophilic substituents of the other potent PPA inhibitors) binds to one of the SQS farnesyl group binding regions in the active site of SQS. The *ortho* and *para* substituents of the phenyl ring in the most potent PPA inhibitors may be locating into two discrete areas within the lipophilic binding region of the inhibitor site since their contribution to binding was shown to be co-operative. For example, while the 2-allyl analogue **13** (IC_{50} 56 nM) and the 4-carbethoxy analogue **17** (IC_{50} 3 nM) were both very potent, analogue **19** with both of these substituents, had a dramatically reduced IC_{50} of 0.05 nM. Similarly, the 4-*n*-butoxy-2-allyl analogue **20** (IC_{50} 0.6 nM) was considerably more potent than either of the analogues with the two substituents alone (**6**, **13**), and further evidence for co-operativity of binding came from a comparison of the 2-allyl-4-phenyl analogue **21** with the singly substituted compounds **13** and **14**. If the 4-substituted phenyl moiety were binding at one of the sites occupied by the two substrate farnesyl chains, it may be that the additional inhibitory potency conferred by the 2-allyl and 2-benzyl substituents on the PPAs, could be due to occupation of part of the other farnesyl substrate site. The inactivity found for the 2-geranyl analogue **24** appears inconsistent with this view (because it might be expected to gain additional binding over the allyl compound **13** at a farnesyl site), but here the longer geranyl group may be extending beyond or lying away from the lipophilic binding region.

A representative range of compounds (**14**–**29**, including the most potent *in vitro* inhibitors) were dosed orally to rats at 50 mg/kg, and the inhibition of cholesterol biosynthesis from mevalonate was determined.[17] Oral activity was not significant (cholesterol synthesis inhibition < 50%) for PPAs bearing a single phenyl ring substituent (**16**, **17**, **22**, **23**) nor in the very potent *in vitro* inhibitors **18**–**21**, with inhibitory activity derived from the co-operative binding of lipophilic *ortho* and *para* substituents; e.g. for the very potent inhibitor **20**, inhibition was recorded as 13% at 50 mg/kg. The finding that the very potent and lipophilic inhibitors were not active at this dose level led to the examination of **20** (CLOG P 5.1) by iv dosing in the rat at 20 mg/kg when the inhibition was not significant at 2%. Further analogue synthesis in the 2-allylphenyl series was then aimed at compounds of lower CLOG P, with the expectation that the consequent lower lipophilicity of the compounds would lead to better oral activity. This analogue synthesis led to the identification of **25**, **27**, and **29** (CLOG P range 2.4–3.2) as orally active compounds in the rat with an ED_{50} range of 12–17 mg/kg for the inhibition of cholesterol biosynthesis from mevalonate. This finding of inhibition of cholesterol biosynthesis following oral dosing contrasts with the lack of oral activity in some early[10] basic SQS inhibitors. Recently, however, potent quinuclidine SQS inhibitors have been reported,[13,14] and these exhibit significant activity *in vivo* after oral dosing. Although the oxime ether **28** (CLOG P 4.7) which is isomeric with the propionamide **27** was also orally active (ED_{50} 13 ± 4 mg/kg, IC_{50} 0.2 nM), it is much more lipophilic than compounds **25**, **27**, and **29**. While oral activity was first found in compounds designed to have a lower log *P*, it would appear that a low log *P* is not an essential criteria for finding oral activity in this series.

In summary, we have identified a new series of highly potent *in vitro* SQS inhibitors of relatively simple structure which (unlike the main classes of inhibitors listed in the introduction) does not contain isoprene units, chiral centers, or groups which might potentially affect calcium metabolism. Consideration of how the PPAs were interacting at the enzyme suggested that they were acting as carbocation mimics, in which case they would be among the most potent *in vitro* SQS inhibitors with this mechanism of action. Compounds within the PPA series have been shown to inhibit cholesterol biosynthesis in the rat following an oral dose, and thus further exploitation of this series may afford a novel class of hypocholesterolaemic agents.

Supporting Information Available: General experimental procedures and spectral data are given for PPA preparation, including examples of the synthesis of 2-allyl and 2-benzyl compounds (2 pages). Ordering information is given on any current masthead page.

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(7) *In vitro* test: SQS inhibition was measured by incubation of test compounds (25 μM) at 37 °C with rat liver microsomal protein (20 μg) in a buffered solution of potassium phosphate (50 mM), magnesium chloride (4.95 mM), potassium fluoride (9.9 mM), and NADPH (0.9 mM). 1-[^{3}H]Farnesyl pyrophosphate was added to a final concentration of 20 μM, and the reaction stopped after 15 min with the addition of 4% KOH (50 μL). Reaction products

were separated on a C18 octadecyl 1 cm^3 Bond Elut column, and the column eas eluted with KOH (0.1 M, 250 μL) solution and ethyl acetate/hexane (1:9, 1 mL) to give the labeled squalene fraction. The difference in the radioactivity observed for this squalene fraction, in the absence and presence of test compound was used to find the percentage inhibition and establish an IC_{50} for inhibition of SQS. Dose–response data included two estimates for each concentration of inhibitor together with six or eight estimates of the control rate. IC_{50} values were calculated by using the "Origin" curve-fitting program supplied by MicroCal Software Inc. with a tight binding background.

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(17) *In vivo* test: Inhibition of cholesterol biosynthesis was determined in fed female rats (125–150 g) housed in reversed lighting conditions. Test compounds were dosed by oral gavage suspended in 10% DMSO in 0.1% HPMC with control animals receiving vehicle alone. After 1 h, rats were injected ip with [2-^{3}H]mevalonolactone (2.5 μCi) in saline (0.25 mL). After a further 1 h, rats were terminally anaesthetized with halothane, and a liver section was removed. Livers were saponified with ethanolic KOH (2 mL, 1 part 33% KOH/9 parts EtOH) at 75 °C for 2 h. The mixture was diluted with an equal volume of water and extracted twice with hexane (5 mL). The hexane was evaporated and the residue dissolved in EtOH for radioactivity measurements. Radioactivity was corrected for weight of liver, and the results were expressed as the percentage inhibition against the vehicle-dosed controls.

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